



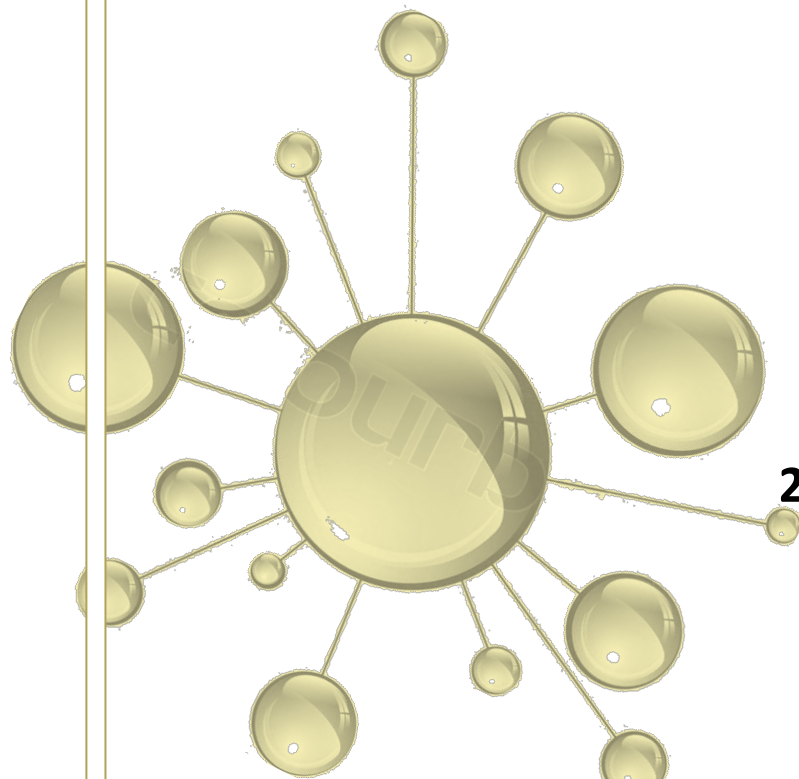
*Official Methods of Analysis<sup>SM</sup> (OMA)*

**AOAC EXPERT REVIEW PANEL  
FOR MICROBIOLOGY FOR FOODS AND ENVIRONMENTAL SURFACES**

**FRIDAY, JUNE 10, 2016**

**8:30AM – 12: 00PM  
Meeting Room: Boardroom**

**AOAC INTERNATIONAL  
2275 Research Blvd, Suite 300  
Rockville, Maryland 20850  
[www.aoac.org](http://www.aoac.org)**









## **AOAC OFFICIAL METHODS OF ANALYSIS<sup>SM</sup>**

The *Official Methods of Analysis<sup>SM</sup>* (OMA) program is AOAC INTERNATIONAL's premier methods program. The program evaluates chemistry, microbiology, and molecular biology methods. It also evaluates traditional benchtop methods, instrumental methods, and proprietary, commercial, and/or alternative methods. In 2011, AOAC augmented the *Official Methods<sup>SM</sup>* program by including an approach to First Action *Official Methods<sup>SM</sup>* status that relies on gathering the experts to develop voluntary consensus standards, followed by collective expert judgment of methods using the adopted standards.

The OMA program has undergone a series of transitions in support of AOAC's collaborations, evolving technology, and evolving technical requirements. Methods approved in this program have undergone rigorous scientific and systematic scrutiny such that analytical results by methods in the *Official Methods of Analysis of AOAC INTERNATIONAL* are deemed to be highly credible and defensible.

On September 7, 2012, AOAC INTERNATIONAL further clarified the AOAC *Official Methods<sup>SM</sup>* program by transitioning the conformity assessment component of the *Official Methods<sup>SM</sup>* program into the AOAC Research Institute. The AOAC Research Institute now administers the AOAC *Official Methods<sup>SM</sup>* program for all proprietary, single and sole source methods. Methods submitted through the PTM-OMA harmonized process also will be reviewed through the AOAC Research Institute. All methods in the AOAC *Official Methods<sup>SM</sup>* program are now reviewed by Expert Review Panels for First Action AOAC *Official Methods of Analysis<sup>SM</sup>* status.

## **EXPERT REVIEW PANEL (ERP)**

The AOAC Expert Review Panels (ERPs) are a key part of AOAC INTERNATIONAL's Method Approval Process. AOAC ERPs are authorized to adopt candidate methods as First Action *Official Methods* and to recommend adoption of these methods to Final Action *Official Methods* status. Scientists are recruited to serve on ERPs in a variety of ways. Normally, a call for experts is published at the same time as a call for methods is posted. Interested scientists are invited to submit their *curriculum vitae* (CV) for consideration. Advisory panel, stakeholder panel, and working group members may make recommendations to AOAC for ERP members. All CVs are reviewed and evaluated for expertise by the AOAC Chief Scientific Officer (CSO) and then to the AOAC Official Methods Board for formal review. The composition of the ERP must be fulfilled with qualified subject matter experts representing various perspectives. Please refer to our Call for Experts on the AOAC homepage for further information.

**AOAC INTERNATIONAL**  
2275 Research Blvd, Suite 300  
Rockville, Maryland 20850  
Phone: (301) 924-7077





**AOAC Official Methods of Analysis<sup>SM</sup> (OMA)**  
**Expert Review Panel for Microbiology for Foods and Environmental Surfaces**

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## EXPERT REVIEW PANEL (ERP) FOR MICROBIOLOGY FOR FOOD AND ENVIRONMENTAL SURFACES

AOAC INTERNATIONAL Headquarters  
2275 Research Blvd, Suite 300  
Rockville, Maryland 20850

Friday, June 10, 2016  
8:30AM – 12:00PM (EST)  
Meeting Room: Boardroom

### MEETING AGENDA

**Expert Review Panel Co-Chairs: Wendy McMahon, Silliker, Inc. and Michael Brodsky, Brodsky Consultants**

- I. **Welcome and Introductions**  
Expert Review Panel Co-Chairs
- II. **Review of AOAC Volunteer Policies & Expert Review Panel Process Overview and Guidelines**  
Deborah McKenzie, Senior Director, Standards Development and Method Approval Processes, AOAC INTERNATIONAL and AOAC Research Institute
- III. **Review of Methods**  
For each method the assigned ERP members will present a review of the proposed collaborative study manuscript, after which the ERP will discuss the method and render a decision on the status for each method.
  - 1) **OMAMAN-29: Evaluation Of 3M™ Molecular Detection Assay 2 - *Listeria* For The Detection Of *Listeria* In Selected Foods And Environmental Surfaces: Collaborative Study**  
Co-Study Directors: Lisa Monteroso, 3M Food Safety, 3M Center, Building 260-06B-01, St. Paul, MN 55144 and Erin Crowley, Q Laboratories, 1400 Harrison Avenue, Cincinnati, OH 45214
  - 2) **OMAMAN-30: Evaluation Of 3M™ Molecular Detection Assay 2 - *Listeria Monocytogenes* For The Detection Of *Listeria Monocytogenes* In Selected Foods And Environmental Surfaces: Collaborative Study**  
Co-Study Directors: Lisa Monteroso, 3M Food Safety, 3M Center, Building 260-06B-01, St. Paul, MN 55144 and Erin Crowley, Q Laboratories, 1400 Harrison Avenue, Cincinnati, OH 45214
- IV. **Discuss Final Action Requirements for First Action Official Methods** (if applicable)  
ERP will discuss, review and track First Action methods for 2 years after adoption, review any additional information (i.e., additional collaborative study data, proficiency testing, and other feedback) and make recommendations to the Official Methods Board regarding Final Action status.
- V. **Adjournment**







## **Expert Review Panel for Microbiology in Food and Environmental Surfaces**

**Michael Brodsky, Co-Chair**  
Brodsky Consultants

**Wendy McMahon, Co-Chair**  
Silliker Inc.

**Maya Achen, Member**  
Abbott Nutrition

**Patrice Arbault, Member**  
Nexidia

**Mark Carter, Member**  
MC2E

**Yi Chen, Member**  
FDA - CFSAN

**Peyman Fatemi, Member**  
The Acheson Group LLC

**Maria Fernandez, Member**  
University Of Buenos Aires

**Thomas Hammack, Member**  
FDA - CFSAN

**Anthony Hitchins, Member**  
FDA - CFSAN (Retired)

**Yvonne Salfinger, Member**  
Association Of Public Health Laboratories





*The Scientific Association Dedicated to Analytical Excellence®*

**AOAC INTERNATIONAL**  
**POLICY AND PROCEDURES ON**  
**VOLUNTEER CONFLICT OF INTEREST**

**Statement of Policy**

While it is not the intention of AOAC INTERNATIONAL (AOAC) to restrict the personal, professional, or proprietary activities of AOAC members nor to preclude or restrict participation in Association affairs solely by reason of such activities, it is the sense of AOAC that conflicts of interest or even the appearance of conflicts of interest on the part of AOAC volunteers should be avoided. Where this is not possible or practical under the circumstances, there shall be written disclosure by the volunteers of actual or potential conflicts of interest in order to ensure the credibility and integrity of AOAC. Such written disclosure shall be made to any individual or group within the Association which is reviewing a recommendation which the volunteer had a part in formulating and in which the volunteer has a material interest causing an actual or potential conflict of interest.

AOAC requires disclosure of actual or potential conflicts of interest as a condition of active participation in the business of the Association. The burden of disclosure of conflicts of interest or the appearance of conflicts of interest falls upon the volunteer.

A disclosed conflict of interest will not in itself bar an AOAC member from participation in Association activities, but a three-fourths majority of the AOAC group reviewing the issue presenting the conflict must concur by secret ballot that the volunteer's continued participation is necessary and will not unreasonably jeopardize the integrity of the decision-making process.

Employees of AOAC are governed by the provision of the AOAC policy on conflict of interest by staff. If that policy is in disagreement with or mute on matters covered by this policy, the provisions of this policy shall prevail and apply to staff as well.

**Illustrations of Conflicts of Interest**

1. A volunteer who is serving as a committee member or referee engaged in the evaluation of a method or device; who is also an employee of or receiving a fee from the firm which is manufacturing or distributing the method or device or is an employee of or receiving a fee from a competing firm.
2. A volunteer who is requested to evaluate a proposed method or a related collaborative study in which data are presented that appear detrimental (or favorable) to a product distributed or a position supported by the volunteer's employer.
3. A referee who is conducting a study and evaluating the results of an instrument, a kit, or a piece of equipment which will be provided gratis by the manufacturer or distributor to one or more of the participating laboratories, including his or her own laboratory, at the conclusion of the study.

4. Sponsorship of a collaborative study by an interest (which may include the referee) which stands to profit from the results; such sponsorship usually involving the privilege granted by the investigator to permit the sponsor to review and comment upon the results prior to AOAC evaluation.
5. A volunteer asked to review a manuscript submitted for publication when the manuscript contains information which is critical of a proprietary or other interest of the reviewer.

The foregoing are intended as illustrative and should not be interpreted to be all-inclusive examples of conflicts of interest AOAC volunteers may find themselves involved in.

### **Do's and Don'ts**

Do avoid the appearance as well as the fact of a conflict of interest.

Do make written disclosure of any material interest which may constitute a conflict of interest or the appearance of a conflict of interest.

Do not accept payment or gifts for services rendered as a volunteer of the Association without disclosing such payment or gifts.

Do not vote on any issue before an AOAC decision-making body where you have the appearance of or an actual conflict of interest regarding the recommendation or decision before that body.

Do not participate in an AOAC decision-making body without written disclosure of actual or potential conflicts of interest in the issues before that body.

Do not accept a position of responsibility as an AOAC volunteer, without disclosure, where the discharge of the accepted responsibility will be or may appear to be influenced by proprietary or other conflicting interests.

### **Procedures**

Each volunteer elected or appointed to an AOAC position of responsibility shall be sent, at the time of election or appointment, a copy of this policy and shall be advised of the requirement to adhere to the provisions herein as a condition for active participation in the business of the Association. Each volunteer, at the time of his or her election or appointment, shall indicate, in writing, on a form provided for this purpose by AOAC, that he or she has read and accepts this policy.

Each year, at the spring meeting of the AOAC Board of Directors, the Executive Director shall submit a report certifying the requirements of this policy have been met; including the names and positions of any elected or appointed volunteers who have not at that time indicated in writing that they have accepted the policy.

Anyone with knowledge of specific instances in which the provisions of this policy have not been complied with shall report these instances to the Board of Directors, via the Office of the Executive Director, as soon as discovered.

\* \* \* \* \*

Adopted: March 2, 1989  
Revised: March 28, 1990  
Revised: October 1996

**AOAC INTERNATIONAL**  
**ANTITRUST POLICY**  
**STATEMENT AND GUIDELINES**

**Introduction**

It is the policy of AOAC INTERNATIONAL (AOAC) and its members to comply strictly with all laws applicable to AOAC activities. Because AOAC activities frequently involve cooperative undertakings and meetings where competitors may be present, it is important to emphasize the on-going commitment of our members and the Association to full compliance with national and other antitrust laws. This statement is a reminder of that commitment and should be used as a general guide for AOAC and related individual activities and meetings.

**Responsibility for Antitrust Compliance**

The Association's structure is fashioned and its programs are carried out in conformance with antitrust standards. However, an equal responsibility for antitrust compliance \_\_ which includes avoidance of even an appearance of improper activity \_\_ belongs to the individual. Even the appearance of improper activity must be avoided because the courts have taken the position that actual proof of misconduct is not required under the law. All that is required is whether misconduct can be inferred from the individual's activities.

Employers and AOAC depend on individual good judgment to avoid all discussions and activities which may involve improper subject matter and improper procedures. AOAC staff members work conscientiously to avoid subject matter or discussion which may have unintended implications, and counsel for the Association can provide guidance with regard to these matters. It is important for the individual to realize, however, that the competitive significance of a particular conduct or communication probably is evident only to the individual who is directly involved in such matters.

**Antitrust Guidelines**

In general, the U.S. antitrust laws seek to preserve a free, competitive economy and trade in the United States and in commerce with foreign countries. Laws in other countries have similar objectives. Competitors (including individuals) may not restrain competition among themselves with reference to the price, quality, or distribution of their products, and they may not act in concert to restrict the competitive capabilities or opportunities of competitors, suppliers, or customers.

Although the Justice Department and Federal Trade Commission generally enforce the U.S. antitrust laws, private parties can bring their own lawsuits.

Penalties for violating the U.S. and other antitrust laws are severe: corporations are subject to heavy fines and injunctive decrees, and may have to pay substantial damage judgments to injured competitors, suppliers, or customers. Individuals are subject to criminal prosecution, and will be punished by fines and imprisonment.

Under current U.S. federal sentencing guidelines, individuals found guilty of bid rigging, price fixing, or market allocation must be sent to jail for at least 4 to 10 months and must pay substantial minimum fines.

Since the individual has an important responsibility in ensuring antitrust compliance in AOAC activities, everyone should read and heed the following guidelines.

1. Don't make any effort to bring about or prevent the standardization of any method or product for the purpose or intent of preventing the manufacture or sale of any method or product not conforming to a specified standard.
2. Don't discuss with competitors your own or the competitors' prices, or anything that might affect prices such as costs, discounts, terms of sale, distribution, volume of production, profit margins, territories, or customers.
3. Don't make announcements or statements at AOAC functions, outside leased exhibit space, about your own prices or those of competitors.
4. Don't disclose to others at meetings or otherwise any competitively sensitive information.
5. Don't attempt to use the Association to restrict the economic activities of any firm or any individual.
6. Don't stay at a meeting where any such price or anti\_competitive talk occurs.
7. Do conduct all AOAC business meetings in accordance with AOAC rules. These rules require that an AOAC staff member be present or available, the meeting be conducted by a knowledgeable chair, the agenda be followed, and minutes be kept.
8. Do confer with counsel before raising any topic or making any statement with competitive ramifications.
9. Do send copies of meeting minutes and all AOAC\_related correspondence to the staff member involved in the activity.
10. Do alert the AOAC staff to any inaccuracies in proposed or existing methods and statements issued, or to be issued, by AOAC and to any conduct not in conformance with these guidelines.

### **Conclusion**

Compliance with these guidelines involves not only avoidance of antitrust violations, but avoidance of any behavior which might be so construed. Bear in mind, however, that the above antitrust laws are stated in general terms, and that this statement is not a summary of applicable laws. It is intended only to highlight and emphasize the principal antitrust standards which are relevant to AOAC programs. You must, therefore, seek the guidance of either AOAC counsel or your own counsel if antitrust questions arise.

\* \* \* \* \*

Adopted by the AOAC Board of Directors: September 24, 1989  
Revised: March 11, 1991  
Revised October 1996





**AOAC INTERNATIONAL**  
**POLICY ON THE USE OF THE**  
**ASSOCIATION NAME, INITIALS,**  
**IDENTIFYING INSIGNIA, LETTERHEAD, AND BUSINESS CARDS**

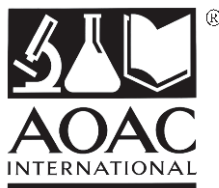
**Introduction**

The following policy and guidelines for the use of the name, initials, and other identifying insignia of AOAC INTERNATIONAL have been developed in order to protect the reputation, image, legal integrity and property of the Association.

The name of the Association, as stated in its bylaws, is "AOAC INTERNATIONAL". The Association is also known by its initials, AOAC, and by its logo, illustrated below, which incorporates the Association name and a representation of a microscope, book, and flask. The AOAC logo is owned by the Association and is registered with the U.S. Patent and Trademark Office.



The full Association insignia, illustrated below, is comprised of the logo and the tagline, "The Scientific Association Dedicated to Analytical Excellence," shown below. The typeface used is Largo. The AOAC tagline is owned by the Association and is registered with the U.S. Patent and Trademark office.



*The Scientific Association Dedicated to Analytical Excellence®*

### **Policy**

Policy on the use of the Association's name and logo is established by the AOAC Board of Directors as follows:

“The Board approves and encourages reference to the Association by name, either as AOAC INTERNATIONAL or as AOAC; or reference to our registered trademark, AOAC®, in appropriate settings to describe our programs, products, etc., in scientific literature and other instances so long as the reference is fair, accurate, complete and truthful and does not indicate or imply unauthorized endorsement of any kind.

The insignia (logo) of AOAC INTERNATIONAL is a registered trade and service mark and shall not be reproduced or used by any person or organization other than the Association, its elected and appointed officers, sections, or committees, without the prior written permission of the Association. Those authorized to use the AOAC INTERNATIONAL insignia shall use it only for the purposes for which permission has been specifically granted.

The name and insignia of the Association shall not be used by any person or organization in any way which indicates, tends to indicate, or implies AOAC official endorsement of any product, service, program, company, organization, event or person, endorsement of which, has not been authorized by the Association, or which suggests that membership in the Association is available to any organization.”

The Executive Director, in accordance with the above stated policy, is authorized to process, approve, fix rules, and make available materials containing the Association name and insignia.

It should be noted that neither the Association's name nor its insignia nor part of its insignia may be incorporated into any personal, company, organization, or any other stationery other than that of the Association; nor may any statement be included in the printed portion of such stationery which states or implies that an individual, company, or other organization is a Member of the Association.

### **Instructions**

1. Reproduction or use of the Association name or insignia requires prior approval by the Executive Director or his designate.
2. Association insignia should not be altered in any manner without approval of the Executive Director or his designate, except to be enlarged or reduced in their entirety.
3. Artwork for reproducing the Association name or insignia, including those incorporating approved alterations, will be provided on request to those authorized to use them (make such requests to the AOAC Marketing Department). Examples of the types of alterations that would be approved are inclusion of a section name in or the addition of an officer's name and address to the letterhead insignia.

4. When the Association name is used without other text as a heading, it should, when possible, be set in the Largo typeface.
5. Although other colors may be used, AOAC blue, PMS 287, is the preferred color when printing the AOAC insignia, especially in formal and official documents. It is, of course, often necessary and acceptable to reproduce the insignia in black.
6. Do not print one part of the logo or insignia in one color and other parts in another color.
7. The letterhead of AOAC INTERNATIONAL shall not be used by any person or organization other than the Association, its elected and appointed officers, staff, sections, or committees; except by special permission.

Correspondence of AOAC official business should be conducted using AOAC letterhead. However, those authorized to use AOAC letterhead shall use it for official AOAC business only.

Copies of all correspondence using AOAC letterhead or conducting AOAC official business, whether on AOAC letterhead or not, must be sent to the appropriate office at AOAC headquarters.

8. AOAC INTERNATIONAL business cards shall not be used by any person or organization other than the Association, its staff, and elected officials, except by special permission.

Those authorized to use AOAC business cards shall use them for official AOAC business only and shall not represent themselves as having authority to bind the Association beyond that authorized.

#### Sanctions

1. Upon learning of any violation of the above policy, the Executive Director or a designate will notify the individual or organization that they are in violation of AOAC policy and will ask them to refrain from further misuse of the AOAC name or insignia.
2. If the misuse is by an Individual Member or Sustaining Member of the Association, and the misuse continues after notification, the Board of Directors will take appropriate action.
3. If continued misuse is by a nonmember of the Association or if a member continues misuse in spite of notification and Board action, ultimately, the Association will take legal action to protect its property, legal integrity, reputation, and image.

\* \* \* \* \*





## ***Official Methods of Analysis<sup>SM</sup>* (OMA) Expert Review Panel MEETING AND METHOD REVIEW GUIDANCE**

The AOAC Research Institute administers AOAC INTERNATIONAL's premier methods program, the AOAC *Official Methods of Analysis<sup>SM</sup>* (OMA). The program evaluates chemistry, microbiology, and molecular biology methods. It also evaluates traditional benchtop methods, instrumental methods, and proprietary, commercial, and/or alternative methods and relies on gathering the experts to develop voluntary consensus standards, followed by collective expert judgment of methods using the adopted standards. The *Official Methods of Analysis of AOAC INTERNATIONAL* is deemed to be highly credible and defensible.

All Expert Review Panel (ERP) members are vetted by the AOAC Official Methods Board (OMB) and serve at the pleasure of the President of AOAC INTERNATIONAL. In accordance to the AOAC Expert Review Panel Member and Chair Volunteer Role Description all Expert Review Panel members are expected to 1) serve with the highest integrity, 2) perform duties and method reviews, and 3) adhere to review timelines and deadlines.

To assist the ERP Chair and its members, please note the following in preparation for Expert Review Panel meetings and method reviews.

### **Pre-Meeting Requirements**

1. Confirm availability and plan to be present to ensure a quorum of the ERP.  
(Please refer to page 25, Quorum Guidelines, [Expert Review Panel Information Packet](#))
2. Ensure that your laptop, CPU or mobile device can access online web documentation.
3. Be prepared for the meeting by reviewing all relevant meeting materials and method documentation.

### **In-Person Meeting and Teleconference Conduct**

1. Arrive on time.
2. Advise the Chair and ERP members of any potential Conflicts of Interest at the beginning of the meeting.
3. Participation is required from all members of the ERP. All members have been deemed experts in the specific subject matter areas.
4. The ERP Chair will moderate the meeting to ensure that decisions can be made in a timely manner.
5. Follow Robert's Rules of Order for Motions.
6. Speak loud, clear, and concise so that all members may hear and understand your point of view.
7. Due to the openness of our meetings, it is imperative that all members communicate in a respectful manner and tone.
8. Refrain from disruptive behavior. Always allow one member to speak at a time. Please do not interrupt.
9. Please note that all methods reviewed and decisions made during the Expert Review Panel process are considered confidential and should not be discussed unless during an Expert Review Panel meeting to ensure transparency.

### **Reviewing Methods**

Prior to the Expert Review Panel meeting, ERP members are required to conduct method reviews. All methods are reviewed under the following criteria, technical evaluation, general comments, editorial criteria, and recommendation status. These methods are being reviewed against their collaborative study protocols as provided in the supplemental documentation. *Note: The method author(s) will be present during the Expert Review Panel session to answer any questions.*



## **Official Methods of Analysis<sup>SM</sup> (OMA) Expert Review Panel MEETING AND METHOD REVIEW GUIDANCE**

### **Reviewing Methods (Cont'd)**

- Reviewers shall conduct in-depth review of method and any supporting information.
- In-depth reviews are completed electronically via the method review form. The method review form must be completed and submitted by the deadline date as provided.
- All reviews will be discussed during the Expert Review Panel meeting.
- Any ERP member can make the motion to adopt or not to adopt the method.
- If the method is adopted for AOAC First Action status, Expert Review Panel members must track and present feedback on assigned First Action *Official Methods*.
- Recommend additional feedback or information for Final Action consideration.

Here are some questions to consider during your review based on your scientific judgment:

1. Does the method sufficiently follow the collaborative study protocol?
2. Is the method scientifically sound and can be followed?
3. What are the strengths and weaknesses of the method?
4. How do the weaknesses weigh in your recommendation for the method?
5. Will the method serve the community that will use the method?
6. What additional information may be needed to further support the method?
7. Can this method be considered for AOAC First Action OMA status?

### **Reaching Consensus during Expert Review Panel Meeting**

1. Make your Motion.
2. Allow another member to Second the Motion.
3. The Chair will state the motion and offer the ERP an option to discuss the motion.
4. The Chair will call a vote once deliberations are complete.
5. Methods must be adopted by unanimous decision of ERP on first ballot, if not unanimous, negative votes must delineate scientific reasons. Negative voter(s) can be overridden by 2/3 of voting ERP members after due consideration.
6. All other motions will require 2/3 majority for vote to carry.



3M MDA Listeria and L. mono. Collaborative Study  
OMA-2016-MONTH-XXX

**AOAC INTERNATIONAL**  
**Official Methods of Analysis<sup>SM</sup> Program**

**Detection of *Listeria* Species in Selected Foods and Environmental Surfaces by the  
3M<sup>TM</sup> Molecular Detection Assay 2 - *Listeria***

**and**

**Detection of *Listeria monocytogenes* in Selected Foods and Environmental Surfaces by the  
3M<sup>TM</sup> Molecular Detection Assay 2 - *Listeria monocytogenes***

**Collaborative Study Protocol**

OMA 2016-MONTH-XXX

AOAC Research Institute  
Expert Review Panel Use Only

3M MDA 2 *Listeria* species and *L. monocytogenes* Collaborative Study  
OMA-2016-MONTH-XXX

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3M MDA 2 *Listeria* species and *L. monocytogenes* Collaborative Study  
OMA-2016-MONTH-XXX

## 1.0 INTRODUCTION

The goal of this collaborative study is to validate the 3M™ MDA 2(MDA2)- *Listeria* and the 3M™ MDA 2 -*Listeria monocytogenes* using deli turkey (125g) and raw chicken breast (25g). Test portions from 3 different contamination levels, including negative controls, will be sent to approximately 12 laboratories who will perform a comparison study between the 3M MDA2 methods and the USDA/FSIS Microbiological Laboratory Guidebook Chapter 8.09 Revision 09 “Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry and Egg Products, and Environmental Samples” (deli turkey and raw chicken breast). Data will be evaluated using probability of detection (POD) analysis and the difference in the POD at 3 contamination levels for both the candidate and reference method.

### 1.1 Description of the 3M MDAs

#### 3M MDA2- *Listeria*

3M™ MDA2 - *Listeria* is used with the 3M™ Molecular Detection System (MDS) for the rapid and specific detection of *Listeria* species in enriched food and environmental samples.

The 3M MDAs use loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations.

The 3M MDA2 - *Listeria* is intended for use in a laboratory environment by professionals trained in laboratory techniques. 3M has not documented the use of this product in industries other than food or beverage. For example, 3M has not documented this product for testing water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M 2 -*Listeria* has not been evaluated with all possible testing protocols or with all possible strains of bacteria.

#### 3M MDA2 -*Listeria monocytogenes*

3M™ MDA2 - *Listeria monocytogenes* is used with the 3M™ MDS for the rapid and specific detection of *Listeria* species in enriched food and environmental samples.

The 3M MDAs use loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations.

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The 3M MDA 2 -*Listeria monocytogenes* is intended for use in a laboratory environment by professionals trained in laboratory techniques. 3M has not documented the use of this product in industries other than food or beverage. For example, 3M has not documented this product for testing water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M MDA 2 -*Listeria monocytogenes* has not been evaluated with all possible testing protocols or with all possible strains of bacteria.

1.2 Summary of 3M MDA 2 -*Listeria monocytogenes* and 3M MDA 2 -*Listeria* PTM and Precollaborative Studies

**3M MDA2 -*Listeria***

The 3M MDA2 -*Listeria* PTM validation study was performed in 2015. The aim of this study was to demonstrate that the 3M MDA2 -*Listeria* method could detect *Listeria* species from selected environmental foods and surfaces as claimed by the manufacturer: hot dogs (25g & 125g), salmon (25g), deli turkey (25g & 125g), cottage cheese (25g), vanilla ice cream (25g), queso fresco (25g), spinach (25g), melon (whole), raw chicken leg pieces (25g), raw chicken fillet (25g), concrete (sponge, 225 mL & 100 mL), stainless steel (sponge, 225 mL), plastic (Enviroswab, 10 mL).

There was no significant difference between the 3M MDA2 -*Listeria* method and the reference method for any of the matrices tested using the probability of detection (POD) analysis. All other PTM parameters (inclusivity, exclusivity, ruggedness, stability and lot to lot) showed no significant differences. Approval is pending for this study.

**3M MDA2 -*Listeria monocytogenes***

The 3M MDA2 -*Listeria monocytogenes* PTM validation study was performed in 2015. The aim of this study was to demonstrate that the 3M MDA2 -*Listeria monocytogenes* method could detect *Listeria monocytogenes* from selected foods and environmental surfaces as claimed by the manufacturer: hot dogs (25g & 125g), salmon (25g), deli turkey (25g & 125g), cottage cheese (25g), chocolate milk (25 mL), vanilla ice cream (25g), queso fresco (25g), romaine lettuce (25g), melon (whole), raw chicken leg pieces (25g), concrete (sponge, 225 mL & 100 mL), stainless steel (sponge, 225 mL), plastic (Enviroswab, 10 mL).

There was no significant difference between the 3M MDA2 - *Listeria monocytogenes* method and the reference method for any of the matrices tested using the probability of detection (POD) analysis. All other PTM parameters (inclusivity, exclusivity, ruggedness, stability and lot to lot) showed no significant differences. Approval is pending for this study.

1.3 Study Directors for Collaborative Study

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## 2.0 Collaborators

A total of 15-16 collaborators will be solicited to participate in this study. A minimum of 10 laboratories, with acceptable data, will be needed for the successful completion of this study. Laboratories currently using the 3M MDS or those who will be thoroughly trained by a 3M representative, with a clear understanding of the reference methods, will act as collaborators for this study.

A sample letter to collaborators can be found in Appendix 9.1. This letter will be sent to each collaborator prior to the start of the study.

## 3.0 Collaborative Study Design

3.1 This collaborative study is designed according to the AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (2012).

3.2 In this study, two food types will be analyzed:

3.2.1 Deli turkey (125g) – 3M MDA 2 -*Listeria* and 3M MDA 2 - *Listeria monocytogenes* compared to USDA/FSIS MLG 8.09<sup>1</sup>

3.2.2 Raw chicken breast (25g) – 3M MDA 2 -*Listeria* and 3M MDA 2 - *Listeria monocytogenes* compared to USDA/FSIS MLG 8.09

3.3 Collaborators will receive test portions for each matrix:

3.3.1 **Raw chicken breast** – 36 x 60g samples. There will be 12 uninoculated control, 12 low level (target 0.2 – 2.0 cfu/test portion), and 12 high level (target 2 – 5 cfu/test portion) samples. Each sample will be divided into two separate 25g test portions: one will be tested using the 3M MDA 2 test kits (*Listeria* and *Listeria monocytogenes*) and the other will be tested by the reference method.

3.3.2 **Deli turkey** – 72 x 125g samples. There will be 24 uninoculated control, 24 low level (target 0.2 – 2.0 cfu/test portion), and 24 high level (target 2 – 5 cfu/test portion) samples. Thirty-six 125g test portions (12 at each level)

<sup>1</sup><http://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES>

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will be tested using the 3M MDA 2 test kits (*Listeria* and *Listeria monocytogenes*) and thirty-six 125g test portions (12 at each level) will be tested by the reference method.

3.3.3 Each laboratory will test all test portions. Each food will be inoculated with a different *Listeria monocytogenes* strain as listed in Table 1 and will be randomized and blind-coded for analysis.

3.4 Study Schedule

3.4.1 One matrix will be tested each week of the study. All data should be sent to the Study Director by the 4<sup>th</sup> week of study.

3.4.2 The contract laboratory will prepare the test portions and ship the test portions refrigerated via overnight courier to the collaborators to arrive on a Friday. Test portion processing and analysis will begin on Monday. Data forms will be completed and faxed or emailed back to the co-Study Director by the following Friday.

**Table 1. Overview of Study Design**

MATRIX	Inoculating organism	Target <i>L. monocytogenes</i> Levels	Test Portion Codes	Reference Method
<b>Raw chicken breast</b> 25g – 3M MDA2 25g – USDA/FSIS MLG	<i>L. monocytogenes</i> TBD	0 cfu/test portion	Random number between 200-299	USDA/FSIS MLG 8.09 Rev. 09 (5/1/2013)
		0.2-2 cfu/test portion		
		2-5 cfu/test portion		
<b>Deli turkey</b> 125g – 3M MDA2 125g – USDA/FSIS MLG	<i>L. monocytogenes</i> TBD	0 cfu/test portion	Random number between 300-399	USDA/FSIS MLG 8.09 Rev. 09 (5/1/2013)
		0.2-2 cfu/test portion		
		2-5 cfu/test portion		

### 3.0 Test Portion Preparation

- 3.1 **Preparation of inoculum.** Deli turkey should be inoculated with heat-stressed organisms (e.g. 50°C for 10 min). The degree of injury caused by heat-stressing should be demonstrated by plating the inoculum in triplicate on selective and non-selective agars. The degree of injury is calculated as:

$$\left(1 - \frac{n_{select}}{n_{nonselect}}\right) \times 100$$

where  $n_{select}$  = mean number of colonies on selective agar and  $n_{nonselect}$  = mean number of colonies on nonselective agar. Raw chicken breast should be inoculated with a fresh, overnight culture diluted to the appropriate concentration.

- 3.2 Products will be obtained from local retail outlets. All food types will be screened for the presence of *Listeria* using the reference method. Any lots that screen positive will be confirmed out to the species level as per the specified reference method for that food type. Naturally contaminated test portions will be used if available at sufficient levels. Alternatively, the test portions will be artificially inoculated to meet the targets of 0.2-2.0 cfu/test portion for the low and 2 – 5 cfu/test portion for the high with the appropriate concentration of a wet inoculum.

- 3.3 **Preparation of test portions.** Each food type will be chopped into small pieces or divided and a liquid inoculum will be added drop-wise to a bulk quantity of food for each inoculation level. The bulk quantity will then be mixed to achieve equal distribution of analyte throughout. The test portions will be stored at 2-8°C for 48-72 h prior to testing.

- 3.4 **Shipment of test portions.** Test portions will be packaged in leak-proof, insulated containers and shipped (according to the Dangerous Goods Regulations IATA for Infections Substances) refrigerated via overnight courier to the collaborators to arrive on a Friday. Test portion processing and analysis will begin on Monday. All refrigerated test portions will be packed with ice packs to maintain a temperature of <7°C during transport. A temperature control sample will be included with each shipment to verify temperature of sample upon receipt. Upon arrival, the test portions will be stored at 2-8°C until they are analyzed.

- 3.5 **Microbiological analysis.** Each collaborator will receive 36 x 60g raw chicken breast test portions (to be divided) and 72 x 125g deli turkey test portions. They will be instructed to weigh 25g raw chicken breast for the 3MMDA2 methods and another 25g for the reference methods. For deli turkey, they will be instructed to test one 125g test portion for the 3M MDA2 -*Listeria monocytogenes* method and the other 125g test portion for the USDA/FSIS MLG 8.09 reference method. All test portions assayed by the 3M MDA2 methods will be confirmed following the reference method for confirmation of *Listeria monocytogenes*. The reference method confirmation procedures following the USDA/FSIS MLG 8.09 should be



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performed starting with the secondary enrichment steps. The recovered isolates will be identified by the API *Listeria* Identification System or the VITEK 2 GP (AOAC OMA 2012.02).

One additional control (negative) test portion will be provided to each collaborator for each matrix to determine the total plate count on the day of analysis.

- 3.6 **Most Probable Number (MPN) Analysis.** To determine the level of *Listeria* spp. in the inoculated food test portions on the day of initiation of analysis, a 5-tube MPN determination is performed by the coordinating laboratory following the appropriate reference method. The level of *Listeria* will be determined by Most Probable Number (MPN) on the day of analysis by analyzing 5 x 50 g, 20 x 25 g (reference method test portions) and 5 x 10 g inoculated test samples for raw chicken breast. For the 125 g deli turkey test portion analysis, 5 x 250 g, 20 x 125 g (reference method test portions) and 5 x 60 g test portions will be analyzed. Each test portion will be enriched with the appropriate reference method enrichment broth at a 1:10 dilution and analyzed by the reference method procedure. The number of positives from the three test levels will be used to calculate the MPN using the MPN calculator. (<http://www.lcfltd.com/customer/LCfMPNCalculator.exe>)[1]. Report MPN as MPN/test portion.

4.0 3M MDA2 -*Listeria* species

4.1 *Applicability*

For the detection of *Listeria* species from:

- 4.1.1 **Beef hot dogs** (25 g and 125 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 4.1.2 **Cold smoked salmon** (25 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 4.1.3 **Deli turkey** (25 g and 125 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 4.1.4 **Full fat cottage cheese** (25 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 4.1.5 **Queso fresco** (25 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 4.1.6 **Vanilla ice cream** (25 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 4.1.7 **Bagged raw spinach** (25 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 4.1.8 **Cantaloupe** (whole melon): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 4.1.9 **Raw chicken leg pieces** (25 g): 28-32 hours of enrichment in Demi Fraser broth at 37°C

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- 4.1.10 **Raw chicken fillet** (25 g): 28-32 hours of enrichment in Demi Fraser broth at 37°C
- 4.1.11 **Stainless steel**: sponge in 225 mL with 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 4.1.12 **Concrete**: sponge in 100 mL and 225 mL and sponge in 225 mL with 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 4.1.13 **Plastic**: Enviroswab in 10 mL 24-30 hours of enrichment in Demi Fraser broth at 37°C

**Caution:** The 3M MDA2 -*Listeria* is intended for use in a laboratory environment by professionals trained in laboratory techniques. The user should read, understand and follow all safety information in the instructions for the 3M MDS and the 3M MDA2 -*Listeria* and retain the safety instructions for future reference.

The 3M MDA 2 -*Listeria* method may generate *Listeria monocytogenes* to levels sufficient to cause stillbirths and fatalities in pregnant women and the immuno compromised, if exposed. The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices, ISO 17025, or ISO 7218.

Follow all instructions carefully. Failure to do so may lead to inaccurate results. The 3M Molecular Detection Instrument is intended for use with samples that have undergone heat treatment during the assay lysis step, which is designed to destroy organisms present in the sample. Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M MDS (instrument).

Store the 3M MDA2 -*Listeria* at 2-8°C. Do not freeze. Keep kit away from light during storage. After opening the kit, check that the foil pouch is undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes should always be stored in the resealable pouch with the desiccant inside to maintain stability of the lyophilized reagents. Store resealed pouches at 2-8°C for no longer than 1 month. Do not use 3M MDA2 -*Listeria* past the expiration date. Expiration date and lot number are noted on the outside label of the box.

After use, the enrichment medium and the 3M MDA2 -*Listeria* tubes can potentially contain pathogenic materials. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the local regulations for disposal.

- 4.2 *Test Kit Reagents* - 3M MDA 2 -*Listeria*- 96 tests
  - 4.2.1 *Lysis Solution (LS) tubes*. – 96 (12 strips of 8 tubes)
  - 4.2.2 *Listeria Reagent tubes*. – 96 (12 strips of 8 tubes)

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- 4.2.3 *Extra caps.* – 96 (12 strips of 8 caps)
- 4.2.4 *Reagent Control (RC).* – 16 (2 pouches of 8)
- 4.2.5 *Quick Start Guide*
  
- 4.3 *Additional Supplies and Reagents*
  - 4.3.1 3M Molecular Detection System (instrument), power supply, cord, USB cable and software
  - 4.3.2 3M Molecular Detection Speed Loader Tray
  - 4.3.3 3M Molecular Detection Chill Block Tray and Chill block insert
  - 4.3.4 3M Molecular Detection Heat Block Insert
  - 4.3.5 3M Molecular Detection Cap/Decap Tool [Reagent]
  - 4.3.6 3M Molecular Detection Cap/Decap Tool [Lysis]
  - 4.3.7 Empty lysis tube rack
  - 4.3.8 Empty reagent tube rack
  - 4.3.9 Dey-Engley (D/E) Neutralizing Broth
  - 4.3.10 Demi-Fraser broth
  
- 4.4 *Additional media*
  - 4.4.1 Oxford Agar (OX)
  - 4.4.2 Sheep blood agar (SBA)
  
- 4.5 *Apparatus*
  - 4.5.1 *Pipettes.* – capable of 20 $\mu$ L
  - 4.5.2 *Multi-channel pipette.* – capable of 20 $\mu$ L
  - 4.5.3 *Sterile pipette tips.* – capable of 20 $\mu$ L
  - 4.5.4 *Stomacher®.* – Seward or equivalent
  - 4.5.5 *Filter Stomacher® bag.* – Seward or equivalent
  - 4.5.6 *Thermometer.* – calibrated range to include 100  $\pm$  1 $^{\circ}$ C
  - 4.5.7 *Incubators.* – capable of maintaining 37  $\pm$  1 $^{\circ}$ C
  - 4.5.8 *Dry double block heater unit.* – capable of maintaining 100  $\pm$  1 $^{\circ}$ C;  
OR a *water bath* capable of maintaining 100  $\pm$  1 $^{\circ}$ C
  - 4.5.9 *Freezer.* – capable of maintaining -10 to -20 $^{\circ}$ C, for storing the 3M Molecular Detection Chill Block Tray
  - 4.5.10 *Refrigerator.* – capable of maintaining 2-8 $^{\circ}$ C, for storing the 3M MDA
  - 4.5.11 *Computer.* – compatible with the 3M Molecular Detection System (instrument)
  
- 4.6 *Determination*
  - 4.6.1 *Enrichment*
    - 4.6.1.1 **Deli turkey** - aseptically add the 125g test portion to 1125 mL Demi Fraser broth.
      - 4.6.1.1.1 Homogenize thoroughly in a filter bag for 2 minutes  $\pm$  30 seconds
      - 4.6.1.1.2 Incubate 24-30 hr at 37  $\pm$  1 $^{\circ}$ C.

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- 4.6.1.2 **Raw chicken breast** - aseptically add the 25g test portion to 475 mL Demi Fraser broth.
- 4.6.1.2.1 Homogenize thoroughly in a filter bag for  $2 \pm 0.2$  min
- 4.6.1.2.2 Incubate 28-32 hr at  $37 \pm 1^\circ\text{C}$ .
- 4.6.2 Prepare the 3M Molecular Detection speed loader tray, chill block insert, heat block insert and instrument. Equilibrate the LS tubes to room temperature ( $20\text{-}25^\circ\text{C}$ ) by setting LS tube overnight 16-18 hours. Or on the laboratory bench for at least 2 hours.
- 4.6.3 Invert the capped LS tubes to mix, up to 4 hours before use.
- 4.6.4 Transfer 20  $\mu\text{L}$  enriched sample into a LS tube.
- 4.6.5 Transfer 20  $\mu\text{L}$  Negative Control [NC], sterile enrichment medium (e.g. Demi-Fraser Broth) into a Lysis Solution [LS] tube after all enriched samples have been completed. Do not use water as a NC.
- 4.6.6 Place uncovered LS tubes in 3M Molecular detection Heat block, heat  $15\pm 1$  min at  $100\pm 1^\circ\text{C}$ . LS Solution will change from pink (cool) to yellow (hot).
- 4.6.7 Place LS tubes (without rack lid) in chill block insert (at ambient temperature) for 5-10 min. Lysis solution in LS tube will revert to pink color.
- 4.6.8 Transfer 20  $\mu\text{L}$  sample lysate from the upper portion of fluid in the LS tube into reagent tube. Mix gently by pipetting up and down 5 times.
- 4.6.9 Transfer 20  $\mu\text{L}$  of NC lysate into a reagent tube.
- 4.6.10 Transfer 20  $\mu\text{L}$  of NC lysate into a Reagent Control (RC) tube.
- 4.6.11 When all samples have been transferred, load capped tubes into speed loader tray and close lid.
- 4.6.12 Start assay.
- 4.6.13 Results and Interpretation - An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analyzed automatically by the software and are color-coded based on the result. A Positive or Negative result is determined by analysis of a number of unique curve parameters. Presumptive positive results are reported in real-time while Negative and Inspect results will be displayed after the run is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations.

**NOTE:** Even a negative sample will not give a zero reading as the 3M MDS and 3M MDA amplification reagents have a “background” relative light unit (RLU).

In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M recommends the user to repeat the assay for any Inspect samples. If the result continues to be Inspect, proceed to confirmation test using your preferred method or as specified by local regulations.

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4.6.14 *Confirmation*

All positive 3M MDA2 -*Listeria* results must be confirmed. Confirmation should be performed using the primary enrichment, carried through as described in the reference method starting with the secondary transfer and selective isolation. Confirmation should be initiated within 72 hrs. Isolate on a selective agar and identify 1 to 5 typical colonies according to the conventional tests described in the AOAC method.

5.0 3M MDA 2 -*Listeria monocytogenes*

5.1 *Applicability*

For the detection of *Listeria monocytogenes* from:

- 5.1.1 **Beef hot dogs** (25 g and 125 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 5.1.2 **Cold smoked salmon** (25 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 5.1.3 **Deli turkey** (25 g and 125 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 5.1.4 **Full fat cottage cheese** (25 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 5.1.5 **Queso fresco** (25 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 5.1.6 **Chocolate milk** (25 mL): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 5.1.7 **Vanilla ice cream** (25 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 5.1.8 **Romaine lettuce** (25 g): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 5.1.9 **Cantaloupe** (whole melon): 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 5.1.10 **Raw chicken leg pieces** (25 g): 28-32 hours of enrichment in Demi Fraser broth at 37°C
- 5.1.11 **Stainless steel**: sponge in 225 mL with 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 5.1.12 **Concrete**: sponge in 100 mL and 225 mL and sponge in 225 mL with 24-30 hours of enrichment in Demi Fraser broth at 37°C
- 5.1.13 **Plastic**: Enviroswab in 10 mL 24-30 hours of enrichment in Demi Fraser broth at 37°C

**Caution:** The 3M MDA 2 -*Listeria monocytogenes* is intended for use in a laboratory environment by professionals trained in laboratory techniques. The user should read, understand and follow all safety information in the instructions for the 3M MDS and the 3M MDA 2 -*Listeria monocytogenes* and retain the safety instructions for future reference. Follow all instructions carefully. Failure to do so may lead to inaccurate results.

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The 3M MDA2 - *Listeria monocytogenes* method may generate *Listeria monocytogenes* to levels sufficient to cause stillbirths and fatalities in pregnant women and the immuno compromised, if exposed. The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices, ISO 17025, or ISO 7218.

Follow all instructions carefully. Failure to do so may lead to inaccurate results. The 3M Molecular Detection Instrument is intended for use with samples that have undergone heat treatment during the assay lysis step, which is designed to destroy organisms present in the sample. Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M MDS (instrument).

Store the 3M MDA2 -*Listeria monocytogenes* at 2-8°C. Do not freeze. Keep kit away from light during storage. After opening the kit, check that the foil pouch is undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes should always be stored in the resealable pouch with the desiccant inside to maintain stability of the lyophilized reagents. Store resealed pouches at 2-8°C for no longer than 1 month. Do not use 3M MDA 2 -*Listeria monocytogenes* past the expiration date. Expiration date and lot number are noted on the outside label of the box.

After use, the enrichment medium and the 3M MDA 2 -*Listeria monocytogenes* tubes can potentially contain pathogenic materials. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult local regulations for disposal.

- 5.2 *Test Kit Reagents - 3M MDA 2 -Listeria monocytogenes- 96 tests*
  - 5.2.1 *Lysis Solution (LS) tubes.* – 96 (12 strips of 8 tubes)
  - 5.2.2 *Listeria Reagent tubes.* – 96 (12 strips of 8 tubes)
  - 5.2.3 *Extra caps.* – 96 (12 strips of 8 caps)
  - 5.2.4 *Reagent Control (RC).* – 16 (2 pouches of 8)
  - 5.2.5 *Quick Start Guide*
- 5.3 *Additional Supplies and Reagents*
  - 5.3.1 3M Molecular Detection System (instrument), power supply, cord, USB cable and software
  - 5.3.2 3M Molecular Detection Speed Loader Tray
  - 5.3.3 3M Molecular Detection Chill Block Tray and Chill block insert
  - 5.3.4 3M Molecular Detection Heat Block Insert
  - 5.3.5 3M Molecular Detection Cap/Decap Tool [Reagent]
  - 5.3.6 3M Molecular Detection Cap/Decap Tool [Lysis]
  - 5.3.7 Empty lysis tube rack
  - 5.3.8 Empty reagent tube rack



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- 5.3.9 Dey-Engley (D/E) Neutralizing Broth
- 5.3.10 Demi-Fraser Broth

5.4 *Additional media*

- 5.4.1 Fraser broth
- 5.4.2 Oxford Agar (OX)
- 5.4.3 Modified oxford agar (MOX)
- 5.4.4 Horse blood overlay agar (HL)
- 5.4.5 Sheep blood agar (SBA)

5.5 *Apparatus*

- 5.5.1 *Pipettes.* – capable of 20µL
- 5.5.2 *Multi-channel pipette.* – capable of 20µL
- 5.5.3 *Sterile pipette tips.* – capable of 20µL
- 5.5.4 *Stomacher®.* – Seward or equivalent
- 5.5.5 *Filter Stomacher® bags.* - Seward or equivalent
- 5.5.6 *Thermometer.* – calibrated range to include  $100 \pm 1^\circ\text{C}$  range
- 5.5.7 *Incubators.* – capable of maintaining  $37 \pm 1^\circ\text{C}$
- 5.5.8 *Dry double block heater unit.* – capable of maintaining  $100 \pm 1^\circ\text{C}$ ;  
OR a *water bath* capable of maintaining  $100 \pm 1^\circ\text{C}$
- 5.5.9 *Freezer.* – capable of maintaining -10 to -20°C, for storing the 3M  
Molecular Detection Chill Block Tray
- 5.5.10 *Refrigerator.* – capable of maintaining 2-8°C, for storing the 3M  
MDA
- 5.5.11 *Computer.* – compatible with the 3M Molecular Detection System  
(instrument)

5.6 *Determination*

- 5.6.1 *Enrichment*
  - 5.6.1.1 **Del turkey** - aseptically add the 125g test portion to 1125 mL  
Demi Fraser broth.
    - 5.6.1.1.1 Homogenize thoroughly in a filter bag for  $2 \pm 0.2$  min
    - 5.6.1.1.2 Incubate 24-30 hr at  $37 \pm 1^\circ\text{C}$ .
  - 5.6.1.2 **Raw chicken breast** - aseptically add the 25g test portion to 475  
mL Demi Fraser broth.
    - 5.6.1.2.1 Homogenize thoroughly in a filter bag for 2 minutes  $\pm$   
30 seconds
    - 5.6.1.2.2 Incubate 28-32 hr at  $37 \pm 1^\circ\text{C}$ .
- 5.6.2 Prepare the 3M Molecular Detection speed loader tray, chill block insert,  
heat block insert and instrument.
- 5.6.3 Equilibrate the LS tubes to room temperature (20-25 °C) by setting LS  
tube overnight 16-18 hours. Or on the laboratory bench for at least 2  
hours.
- 5.6.4 Invert the capped LS tubes to mix, up to 4 hours before use.
- 5.6.5 Transfer 20 µL enriched sample into a LS tube.

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- 5.6.6 Transfer 20 µL Negative Control [NC], sterile enrichment medium (e.g. Demi-Fraser Broth) into a Lysis Solution [LS] tube after all enriched samples have been completed. Do not use water as a NC.
- 5.6.7 Place uncovered LS tubes in 3M Molecular detection Heat block, heat 15±1 min at 100±1°C. LS Solution will change from pink (cool) to yellow (hot).
- 5.6.8 Place LS tubes (without rack lid) in chill block insert (at ambient temperature) for 5-10 min. Lysis solution in LS tube will revert to pink color.
- 5.6.9 Transfer 20 µL sample lysate from the upper portion of fluid in the LS tube into reagent tube. Mix gently by pipetting up and down 5 times.
- 5.6.10 Transfer 20 µL of NC lysate into a reagent tube.
- 5.6.11 Transfer 20 µL of NC lysate into a Reagent Control (RC) tube.
- 5.6.12 When all samples have been transferred, load capped tubes into speed loader tray and close lid.
- 5.6.13 Start assay.
- 5.6.14 Results and Interpretation - An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analyzed automatically by the software and are color-coded based on the result. A Positive or Negative result is determined by analysis of a number of unique curve parameters. Presumptive positive results are reported in real-time while Negative and Inspect results will be displayed after the run is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations.
- 5.6.15

**NOTE:** Even a negative sample will not give a zero reading as the 3M MDS and 3M MDA amplification reagents have a “background” relative light unit (RLU).

In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M recommends the user to repeat the assay for any Inspect samples. If the result continues to be Inspect, proceed to confirmation test using your preferred method or as specified by local regulations.

- 5.6.16 *Confirmation*  
All positive 3M MDA 2 -*Listeria monocytogenes* results must be confirmed. Confirmation should be performed using the primary enrichment, carried through as described in the reference method starting with the secondary transfer and selective isolation for deli turkey and selective isolation for raw chicken breast. Confirmation should be initiated within 72 hrs. Isolate on a selective agar and identify 1 to 5 typical colonies according to the conventional tests described in the AOAC or USDA/FSIS MLG methods.



## 6.0 Reporting Raw Data

- 6.1 Report data using the data report form in Appendix 9.2.
- 6.2 Upon completion of each food type, the laboratory will fax or email the completed data form to the Study Director.
- 6.3 Copies of all related test results (data sheets, printouts and confirmation results) should be retained by the collaborating labs for a minimum of one year.

## 7.0 Analyzing Raw Data

The resulting data will be analyzed by probability of detection (POD).

- 7.1 **Probability of Detection (POD)** - POD is the proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent. Please see the revised microbiology guidelines<sup>2</sup> for complete analysis information on POD calculations.
  - 7.1.1 Calculate:
    - 7.1.1.1 Estimate of repeatability - standard deviation ( $s_r$ )
    - 7.1.1.2 Determine POD values for each matrix and concentration - the number of positive outcomes divided by the total number of trials.
    - 7.1.1.3 Estimate of reproducibility - standard deviation of the laboratory POD values ( $s_{POD}$ ) and confidence intervals.
    - 7.1.1.4 Cross laboratory probability of detection (LPOD) - LPODs by matrix and concentration with 95% confidence intervals for candidate method (presumptive and confirmed) results.
    - 7.1.1.5 Difference of cross-laboratory probability of detection (dLPOD) - difference probability of detection is the difference between any two LPOD values.
      - 7.1.1.5.1 Estimate dLPOD<sub>C</sub> as the difference between the candidate and reference LPOD values, include CIs.
      - 7.1.1.5.2 Estimate dLPOD<sub>CP</sub> as the difference between the presumptive and confirmed LPOD values, include CIs.
      - 7.1.1.5.3 **If the CI of a dLPOD does not contain zero then the difference is statistically significant.**
    - 7.1.1.6 For each matrix, graph POD<sub>R</sub>, LPOD<sub>C</sub> and dLPOD<sub>C</sub> by level with 95% confidence intervals.

---

<sup>2</sup>AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (pre-publication document, 2012) Sharon Brunelle, Robert LaBudde, Maria Nelson, and Paul Wehling

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**8.0 Appendices**

- 8.1 Instructions to Collaborators
- 8.2 Data Report Forms
- 8.3 Study Flow Diagrams
- 8.4 Study Materials
- 8.5 Collaborator Information Sheet
- 8.6 Collaborator Comment Form
- 8.7 3M MDA2 – *Listeria* IFU
- 8.8 3M MDA2 – *Listeria monocytogenes* IFU

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## Appendix 8.1

### Instructions to Collaborators

#### General Instructions about Collaborative Studies

##### Introduction

The purpose of this document is to provide detailed instructions for performing the collaborative study for the 3M MDA (MDA) 2 -*Listeria* and the 3M MDA 2 -*Listeria monocytogenes* methods.

The trial will be conducted by Study Directors: Lisa Monteroso from 3M and Patrick Bird and Erin Crowley from Q Laboratories. The Study Directors are responsible for providing the test portions, clarifying procedures, collating the results, and submitting a final report to AOAC INTERNATIONAL. Should any questions arise before or during the course of the study, please direct them immediately to the attention of one of the Study Directors:

Patrick Bird & Erin Crowley

Co-Study Directors

Q Laboratories, Inc.

1400 Harrison Avenue

Cincinnati, Ohio 45214

Phone : 513-471-1300

Fax : 513-471-5600

Email: [pbird@qlaboratories.com](mailto:pbird@qlaboratories.com)

[ecrowley@qlaboratories.com](mailto:ecrowley@qlaboratories.com)

##### Important Information

1  
2  
3  
4  
5 1. Read the methods and Safety Instructions carefully. If you have any questions, contact one of  
6 the Study Directors. The 3M MDA2 -*Listeria* and 3M MDA2 -*Listeria monocytogenes* Assays  
7 are intended for use in a laboratory environment by professionals trained in laboratory  
8 techniques. The user should read, understand and follow all safety information in the instructions  
9 for the 3M Molecular Detection System and the 3M MDA2 -*Listeria* and 3M MDA2 -*Listeria*  
10 *monocytogenes*; retain the safety instructions for future reference. The 3M MDA2 -*Listeria* and  
11 3M MDA2 -*Listeria monocytogenes* method may generate *Listeria monocytogenes* to levels  
12 sufficient to cause stillbirths and fatalities in pregnant women and the immuno compromised, if  
13 exposed. The user must train its personnel in current proper testing techniques: for example,  
14 Good Laboratory Practices, ISO 17025, or ISO 7218. Follow all instructions carefully. Failure to  
15 do so may lead to inaccurate results. The 3M Molecular Detection Instrument is intended for use  
16 with samples that have undergone heat treatment during the assay lysis step, which is designed to  
17 destroy organisms present in the sample. Samples that have not been properly heat treated during  
18 the assay lysis step may be considered a potential biohazard and should NOT be inserted into the  
19 3M Molecular Detection System (instrument). Store the 3M MDA2 Assays at 2-8°C. Do not

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1 freeze. Keep kit away from light during storage. After opening the kit, check that the foil pouch  
2 is undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes should  
3 always be stored in the resealable pouch with the desiccant inside to maintain stability of the  
4 lyophilized reagents. Store resealed pouches at 2-8°C for no longer than 1 month. Do not use  
5 3M MDA2 Assays past the expiration date. Expiration date and lot number are noted on the  
6 outside label of the box. After use, the enrichment medium and the 3M MDA2 tubes can  
7 potentially contain pathogenic materials. When testing is complete, follow current industry  
8 standards for the disposal of contaminated waste. Consult local regulations for disposal.

9  
10 2. It is advised to make at least one practice run before the trial using your own materials so that  
11 you can minimize errors in manipulations. Check that all pipettes, equipment, and 3M MDA2  
12 supplies are on hand.

13  
14 3. Make the determination on the specified date. Store the test portions according to the  
15 instructions. It is essential for the validity of the trial that all collaborators commence the  
16 analysis of each test portion on the designated day. Immediately upon receiving each shipment,  
17 confirm the contents with the Study Director by faxing the form provided in the shipment.

18  
19 4. THIS IS A STUDY OF THE METHOD, NOT OF THE LABORATORY. THE METHOD  
20 MUST BE FOLLOWED AS CLOSELY AS PRACTICABLE, AND ANY DEVIATIONS  
21 FROM THE METHOD AS DESCRIBED, NO MATTER HOW TRIVIAL THEY MAY SEEM,  
22 MUST BE NOTED ON THE REPORT FORM.

23  
24 5. Report all of your results as soon as analyses are completed. Do not do more or less than  
25 indicated in the instructions. For example, do not do duplicate analysis and report the best or  
26 average result. More or fewer results complicate the statistical analyses and may invalidate your  
27 results. Data sheets are provided with these instructions and indicate which results are to be  
28 reported. Please include any criticisms, suggested improvements, or general comments about the  
29 3M MDAs on the Collaborators' Comments Form provided. Any results that were derived from  
30 modified protocols should be included but must be separated from the main report. Results and  
31 comments should be returned to the Study Director immediately upon completion of each  
32 portion of the study.

### 33 34 **Information about this Collaborative Study**

#### 35 36 **Shipment Schedule**

37  
38 Test portions will be shipped by overnight courier to the collaborators to arrive on a Friday. Test  
39 portion processing and analysis will begin on Monday. If the test portions do not arrive by the  
40 normal delivery time one day prior to analysis, please contact a Study Director. All test portions  
41 will be shipped refrigerated. **Analysis of test portions must be initiated on the day scheduled**  
42 **by the Study Director. Test kits and enrichment medium will be sent directly from 3M.**

#### 43 44 **Test Portion Receipt**

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Note condition of package on the data sheets and notify the Study Director if any packages appear to have been compromised or if the food product does not arrive in the appropriate condition. All refrigerated products should arrive cold to the touch and should be stored at 2-8°C (refrigerated) until the day of analysis.

**Test Portion Analysis**

Collaborators will be provided with enough sample to test each matrix using the 3M MDA2 methods and the appropriate reference method. For raw chicken breast, collaborators will receive approximately 60g. One test portion (25g) will be weighed and tested using the 3M MDA2 -*Listeria* and 3M MDA2 -*Listeria monocytogenes* and the other test portion (25g) will be weighed and tested using the reference method. For deli turkey they will receive two sets of test portions: 36 x 125g (3M MDA 2 -*Listeria monocytogenes* and 3M MDA 2 - *Listeria*) and 36 x 125g (USDA/FSIS MLG), for a total of 72 test portions. Please review the reference methods carefully before initiating analysis.

One additional 60 g control (negative) test portion will be provided to each collaborator for each matrix to determine the total plate count on the day of analysis using the 3M™ Petrifilm™ Aerobic Count Plate method (OMA 990.12). One temperature control sample will also be included in the shipment to document the temperature of the sample upon receipt. Record all results on the data sheets provided.

Food Type	Reference	Reference Pre-enrichment	3M MDA2 Enrichment	3M MDA2 Test Kit
Deli turkey	USDA/FSIS MLG 8.09	UVM	Demi Fraser Broth with FAC	<i>Listeria</i> & <i>L. monocytogenes</i>
Raw chicken breast	USDA/FSIS MLG 8.09	UVM	Demi Fraser Broth with FAC	<i>Listeria</i> & <i>L. monocytogenes</i>

\*Tryptone Soya Broth with yeast plus acriflavine (9 mg/L), nalidixic acid (36 mg/L) and cycloheximide (46 mg/L)

**Deli turkey** – use stomacher bag with filter for all 3M test portions.

**1.0 3M MDA2 -*Listeria monocytogenes* and MDA2 -*Listeria***

- 1.1 To each 125g test portion, add 1125 mL Demi Fraser broth without ferric ammonium citrate (FAC). Homogenize each sample for two minutes ± 30 seconds and incubate 24-30 h at 37 ± 1°C.
- 1.2 Prepare the 3M MDS speed loader tray, chill block insert, heat block insert and instrument following the 3M MDA 2 - *Listeria monocytogenes* Instructions for Use [IFU] and 3M Molecular Detection Instrument manual.
- 1.3 Equilibrate the LS tubes to room temperature (20-25 °C) by setting LS tube overnight 16-18 hours. Or on the laboratory bench for at least 2 hours.
- 1.4 Invert the capped LS tubes to mix, up to 4 hours before use.
- 1.5 Transfer 20 µL enriched sample into a LS tube.

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- 1 1.6 Transfer 20  $\mu$ L Negative Control [NC], sterile enrichment medium (e.g. Demi-  
2 Fraser Broth) into a Lysis Solution [LS] tube after all enriched samples have been  
3 completed. Do not use water as a NC.
- 4 1.7 Place uncovered LS tubes in 3M MDS Heat block, heat  $15 \pm 1$  min at  $100 \pm 1^\circ\text{C}$ .  
5 LS Solution will change from pink (cool) to yellow (hot).
- 6 1.8 Place LS tubes (without rack lid) in chill block insert (at ambient temperature) for  
7 5-10 min. Lysis solution in LS tube will revert to pink color.
- 8 1.9 Transfer 20  $\mu$ L sample lysate from the upper portion of fluid in the LS tube into  
9 reagent tube. Mix gently by pipetting up and down 5 times.
- 10 1.10 Transfer 20  $\mu$ L of NC lysate into a reagent tube.
- 11 1.11 Transfer 20  $\mu$ L of NC lysate into a Reagent Control (RC) tube.
- 12 1.12 Load capped tubes into speed loader tray and close lid.
- 13 1.13 Start assay.
- 14 1.14 **Validation Study Confirmation**
  - 15 1.14.1 Confirm each test portion, regardless of presumptive result.
  - 16 1.14.2 Transfer  $0.1 \pm 0.02$  ml of the enriched sample to  $10 \pm 0.5$  ml of Fraser  
17 Broth (FB). As per media preparation instructions, be sure that  
18 appropriate supplements have been added to the FB prior to inoculation.  
19 Incubate inoculated FB tubes at  $35 \pm 2^\circ\text{C}$  for  $26 \pm 2$  h.
  - 20 1.14.3 Streak a MOX plate. Streak a loopful or a drop approximating 0.1 ml of  
21 the enriched sample over the surface of the plate. Incubate the MOX at  $35$   
22  $\pm 2^\circ\text{C}$  for  $26 \pm 2$  h.
  - 23 1.14.4 Examine the MOX plates for colonies with morphology typical of *Listeria*  
24 spp. At  $26 \pm 2$  h, suspect colonies are typically small (ca. 1 mm) and are  
25 surrounded by a zone of darkening due to esculin hydrolysis.
  - 26 1.14.5 If suspect colonies are present on MOX, transfer suspect colonies to HL  
27 agar.
  - 28 1.14.6 If no suspect colonies are evident, re-incubate the MOX plate for an  
29 additional  $26 \pm 2$  hour.
  - 30 1.14.7 After  $26 \pm 2$  h of incubation, examine the FB for the potential presence of  
31 *Listeria* spp., by visual examination of the broth for darkening due to  
32 esculin hydrolysis.
  - 33 1.14.8 If any degree of FB darkening is evident, aseptically dispense a drop  
34 approximating  $0.1 \pm 0.02$  ml of FB onto a MOX plate. Swab or streak 25-  
35 40% of the surface of the MOX plate with the FB inoculum. Use a loop to  
36 streak for isolation from the initial swab/streak quadrant onto the  
37 remainder of the plate. Incubate the MOX plate at  $35 \pm 2^\circ\text{C}$  for  $26 \pm 2$  h.
  - 38 1.14.9 If no FB darkening is evident, re-incubate the FB at  $35 \pm 2^\circ\text{C}$  until a total  
39 incubation time of  $48 \pm 2$  h has been achieved.
  - 40 1.14.10 Re-examine the FB for evidence of darkening after  $48 \pm 2$  h of total  
41 incubation. If any degree of darkening is evident, swab, streak and  
42 incubate a MOX plate.
  - 43 1.14.11 If no darkening of FB is evident and no suspect MOX and/or HL colonies  
44 have been demonstrated, the sample is considered negative for *Listeria*  
45 spp.



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- 1 1.14.12 If suspect colonies are present on MOX from any source, streak for  
2 isolation on one or more HL agar plates. Incubate the streaked HL at  $35 \pm$   
3  $2^\circ\text{C}$  for  $22 \pm 4$  h.
- 4 1.14.13 After incubation, examine the HL plate(s) against backlight for translucent  
5 colonies surrounded by a small zone of  $\beta$ -hemolysis.
- 6 1.14.14 If at least one suspect colony is clearly isolated, proceed to confirmatory  
7 testing. Hold all HL plates containing suspect colonies (room temperature  
8 or refrigeration) until confirmatory testing is complete.
- 9 1.14.15 If suspect colonies or  $\beta$ -hemolytic growth are present on HL but not  
10 clearly isolated, re-streak representative suspect colonies/growth onto one  
11 or more fresh HL plates and incubate at  $35 \pm 2^\circ\text{C}$  for  $22 \pm 4$  h.
- 12 1.14.16 Confirm ALL test portions according to the USDA FSIS MLG 8.09  
13 biochemical/serological procedures. Biochemical tests will be performed  
14 using API or VITEK 2 GP. Please follow manufacturer's instructions  
15 when using any of these rapid methods.
- 16

17 **USDA FSIS Reference Method**

- 18 1.1 Add 125g test portion to 1125 mL UVM broth. Stomach (Seward 400 or  
19 equivalent) for  $2 \pm 0.2$  minutes and incubate for 23-26h at  $30 \pm 2^\circ\text{C}$ .
- 20 1.2 Transfer  $0.1 \pm 0.02$  ml of the enriched sample to  $10 \pm 0.5$  ml of Fraser Broth (FB).  
21 As per media preparation instructions, be sure that appropriate supplements have  
22 been added to the FB prior to inoculation. Incubate inoculated FB tubes at  $35 \pm$   
23  $2^\circ\text{C}$  for  $26 \pm 2$  h.
- 24 1.3 Streak a MOX plate. Streak a loopful or a drop approximating 0.1 ml of the  
25 enriched sample over the surface of the plate. Incubate the MOX at  $35 \pm 2^\circ\text{C}$  for  
26  $26 \pm 2$  h.
- 27 1.4 Examine the MOX plates for colonies with morphology typical of *Listeria* spp. At  
28  $26 \pm 2$  h, suspect colonies are typically small (ca. 1 mm) and are surrounded by a  
29 zone of darkening due to esculin hydrolysis.
- 30 1.5 If suspect colonies are present on MOX, transfer suspect colonies to HL agar.
- 31 1.6 If no suspect colonies are evident, re-incubate the MOX plate for an additional  $26$   
32  $\pm 2$  hour.
- 33 1.7 After  $26 \pm 2$  h of incubation, examine the FB for the potential presence of *Listeria*  
34 spp., by visual examination of the broth for darkening due to esculin hydrolysis.
- 35 1.8 If any degree of FB darkening is evident, aseptically dispense a drop  
36 approximating  $0.1 \pm 0.02$  ml of FB onto a MOX plate. Swab or streak 25-40% of  
37 the surface of the MOX plate with the FB inoculum. Use a loop to streak for  
38 isolation from the initial swab/streak quadrant onto the remainder of the plate.  
39 Incubate the MOX plate at  $35 \pm 2^\circ\text{C}$  for  $26 \pm 2$  h.
- 40 1.9 If no FB darkening is evident, re-incubate the FB at  $35 \pm 2^\circ\text{C}$  until a total  
41 incubation time of  $48 \pm 2$  h has been achieved.
- 42 1.10 Re-examine the FB for evidence of darkening after  $48 \pm 2$  h of total incubation.  
43 If any degree of darkening is evident, swab, streak and incubate a MOX plate.
- 44 1.11 If no darkening of FB is evident and no suspect MOX and/or HL colonies have  
45 been demonstrated, the sample is considered negative for *Listeria* spp.

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- 1 1.12 If suspect colonies are present on MOX from any source, streak for isolation on  
2 one or more HL agar plates. Incubate the streaked HL at  $35 \pm 2^\circ\text{C}$  for  $22 \pm 4$  h.  
3 1.13 After incubation, examine the HL plate(s) against backlight for translucent  
4 colonies surrounded by a small zone of  $\beta$ -hemolysis.  
5 1.14 If at least one suspect colony is clearly isolated, proceed to confirmatory testing.  
6 Hold all HL plates containing suspect colonies (room temperature or  
7 refrigeration) until confirmatory testing is complete.  
8 1.15 If suspect colonies or  $\beta$ -hemolytic growth are present on HL but not clearly  
9 isolated, re-streak representative suspect colonies/growth onto one or more fresh  
10 HL plates and incubate at  $35 \pm 2^\circ\text{C}$  for  $22 \pm 4$  h.  
11 1.16 Confirm ALL test portions according to the USDA FSIS biochemical/serological  
12 procedures. Biochemical tests will be performed using API or VITEK 2 GP.  
13 Please follow manufacturer's instructions when using any of these rapid methods.  
14

15 **Raw chicken breast** – use stomacher bag with filter for all 3M test portions.  
16

17 2.0 **3M MDA2 -*Listeria monocytogenes* and MDA2 -*Listeria***

- 18 2.1 To each 25g test portion, add 475 mL Demi Fraser broth with ferric ammonium  
19 citrate (FAC). Homogenize each sample for two minutes  $\pm$  30 seconds and  
20 incubate 28-32 h at  $37 \pm 1^\circ\text{C}$ .  
21 2.2 Prepare the 3M MDS speed loader tray, chill block insert, heat block insert and  
22 instrument following the 3M MDA 2 - *Listeria monocytogenes* Instructions for  
23 Use [IFU] and 3M Molecular Detection Instrument manual.  
24 2.3 Equilibrate the LS tubes to room temperature ( $20\text{-}25^\circ\text{C}$ ) by setting LS tube  
25 overnight 16-18 hours. Or on the laboratory bench for at least 2 hours.  
26 2.4 Invert the capped LS tubes to mix up to 4 hours before use.  
27 2.5 Transfer 20  $\mu\text{L}$  enriched sample into a LS tube.  
28 2.6 Transfer 20  $\mu\text{L}$  Negative Control [NC], sterile enrichment medium (e.g. Demi-  
29 Fraser Broth) into a Lysis Solution [LS] tube after all enriched samples have been  
30 completed. Do not use water as a NC.  
31 2.7 Place uncovered LS tubes in 3M Molecular detection Heat block, heat  $15\pm 1$  min  
32 at  $100\pm 1^\circ\text{C}$ . LS Solution will change from pink (cool) to yellow (hot).  
33 2.8 Place LS tubes (without rack lid) in chill block insert (at ambient temperature) for  
34 5-10 min. Lysis solution in LS tube will revert to pink color.  
35 2.9 Transfer 20  $\mu\text{L}$  sample lysate from the upper portion of fluid in the LS tube into  
36 reagent tube. Mix gently by pipetting up and down 5 times.  
37 2.10 Transfer 20  $\mu\text{L}$  of NC lysate into a reagent tube.  
38 2.11 Transfer 20  $\mu\text{L}$  of NC lysate into a Reagent Control (RC) tube.  
39 2.12 Load capped tubes into speed loader tray and close lid.  
40 2.13 Start assay.  
41 2.14 **Validation Study Confirmation**  
42 2.14.1 Confirm each test portion, regardless of presumptive result.  
43 2.14.2 Transfer  $0.1 \pm 0.02$  ml of the enriched sample to  $10 \pm 0.5$  ml of Fraser  
44 Broth (FB). As per media preparation instructions, be sure that  
45 appropriate supplements have been added to the FB prior to inoculation.  
46 Incubate inoculated FB tubes at  $35 \pm 2^\circ\text{C}$  for  $26 \pm 2$  h.



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- 1 2.14.3 Streak a MOX plate. Streak a loopful or a drop approximating 0.1 ml of  
2 the enriched sample over the surface of the plate. Incubate the MOX at 35  
3  $\pm 2^{\circ}\text{C}$  for  $26 \pm 2$  h.
- 4 2.14.4 Examine the MOX plates for colonies with morphology typical of *Listeria*  
5 spp. At  $26 \pm 2$  h, suspect colonies are typically small (ca. 1 mm) and are  
6 surrounded by a zone of darkening due to esculin hydrolysis.
- 7 2.14.5 If suspect colonies are present on MOX, transfer suspect colonies to HL  
8 agar.
- 9 2.14.6 If no suspect colonies are evident, re-incubate the MOX plate for an  
10 additional  $26 \pm 2$  hour.
- 11 2.14.7 After  $26 \pm 2$  h of incubation, examine the FB for the potential presence of  
12 *Listeria spp.*, by visual examination of the broth for darkening due to  
13 esculin hydrolysis.
- 14 2.14.8 If any degree of FB darkening is evident, aseptically dispense a drop  
15 approximating  $0.1 \pm 0.02$  ml of FB onto a MOX plate. Swab or streak 25-  
16 40% of the surface of the MOX plate with the FB inoculum. Use a loop to  
17 streak for isolation from the initial swab/streak quadrant onto the  
18 remainder of the plate. Incubate the MOX plate at  $35 \pm 2^{\circ}\text{C}$  for  $26 \pm 2$  h.
- 19 2.14.9 If no FB darkening is evident, re-incubate the FB at  $35 \pm 2^{\circ}\text{C}$  until a total  
20 incubation time of  $48 \pm 2$  h has been achieved.
- 21 2.14.10 Re-examine the FB for evidence of darkening after  $48 \pm 2$  h of total  
22 incubation. If any degree of darkening is evident, swab, streak and  
23 incubate a MOX plate.
- 24 2.14.11 If no darkening of FB is evident and no suspect MOX and/or HL colonies  
25 have been demonstrated, the sample is considered negative for *Listeria*  
26 spp.
- 27 2.14.12 If suspect colonies are present on MOX from any source, streak for  
28 isolation on one or more HL agar plates. Incubate the streaked HL at  $35 \pm$   
29  $2^{\circ}\text{C}$  for  $22 \pm 4$  h.
- 30 2.14.13 After incubation, examine the HL plate(s) against backlight for translucent  
31 colonies surrounded by a small zone of  $\beta$ -hemolysis.
- 32 2.14.14 If at least one suspect colony is clearly isolated, proceed to confirmatory  
33 testing. Hold all HL plates containing suspect colonies (room temperature  
34 or refrigeration) until confirmatory testing is complete.
- 35 2.14.15 If suspect colonies or  $\beta$ -hemolytic growth are present on HL but not  
36 clearly isolated, re-streak representative suspect colonies/growth onto one  
37 or more fresh HL plates and incubate at  $35 \pm 2^{\circ}\text{C}$  for  $22 \pm 4$  h.
- 38 2.14.16 Confirm ALL test portions according to the USDA FSIS MLG 8.09  
39 biochemical/serological procedures. Biochemical tests will be performed  
40 using API or VITEK 2 GP. Please follow manufacturer's instructions  
41 when using any of these rapid methods.
- 42

43 **USDA FSIS Reference Method**

- 44 1.17 Add 25g test portion to 225 mL UVM broth. Stomach (Seward 400 or equivalent)  
45 for  $2 \pm 0.2$  minutes and incubate for 20-26h at  $30 \pm 2^{\circ}\text{C}$ .

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- 1 1.18 Transfer  $0.1 \pm 0.02$  ml of the enriched sample to  $10 \pm 0.5$  ml of Fraser Broth (FB).  
2 As per media preparation instructions, be sure that appropriate supplements have  
3 been added to the FB prior to inoculation. Incubate inoculated FB tubes at  $35 \pm$   
4  $2^{\circ}\text{C}$  for  $26 \pm 2$  h.
- 5 1.19 Streak a MOX plate. Streak a loopful or a drop approximating 0.1 ml of the  
6 enriched sample over the surface of the plate. Incubate the MOX at  $35 \pm 2^{\circ}\text{C}$  for  
7  $26 \pm 2$  h.
- 8 1.20 Examine the MOX plates for colonies with morphology typical of *Listeria* spp. At  
9  $26 \pm 2$  h, suspect colonies are typically small (ca. 1 mm) and are surrounded by a  
10 zone of darkening due to esculin hydrolysis.
- 11 1.21 If suspect colonies are present on MOX, transfer suspect colonies to HL agar.  
12 1.22 If no suspect colonies are evident, re-incubate the MOX plate for an additional  $26$   
13  $\pm 2$  hour.
- 14 1.23 After  $26 \pm 2$  h of incubation, examine the FB for the potential presence of *Listeria*  
15 spp., by visual examination of the broth for darkening due to esculin hydrolysis.
- 16 1.24 If any degree of FB darkening is evident, aseptically dispense a drop  
17 approximating  $0.1 \pm 0.02$  ml of FB onto a MOX plate. Swab or streak 25-40% of  
18 the surface of the MOX plate with the FB inoculum. Use a loop to streak for  
19 isolation from the initial swab/streak quadrant onto the remainder of the plate.  
20 Incubate the MOX plate at  $35 \pm 2^{\circ}\text{C}$  for  $26 \pm 2$  h.
- 21 1.25 If no FB darkening is evident, re-incubate the FB at  $35 \pm 2^{\circ}\text{C}$  until a total  
22 incubation time of  $48 \pm 2$  h has been achieved.
- 23 1.26 Re-examine the FB for evidence of darkening after  $48 \pm 2$  h of total incubation.  
24 If any degree of darkening is evident, swab, streak and incubate a MOX plate.
- 25 1.27 If no darkening of FB is evident and no suspect MOX and/or HL colonies have  
26 been demonstrated, the sample is considered negative for *Listeria* spp.
- 27 1.28 If suspect colonies are present on MOX from any source, streak for isolation on  
28 one or more HL agar plates. Incubate the streaked HL at  $35 \pm 2^{\circ}\text{C}$  for  $22 \pm 4$  h.
- 29 1.29 After incubation, examine the HL plate(s) against backlight for translucent  
30 colonies surrounded by a small zone of  $\beta$ -hemolysis.
- 31 1.30 If at least one suspect colony is clearly isolated, proceed to confirmatory testing.  
32 Hold all HL plates containing suspect colonies (room temperature or  
33 refrigeration) until confirmatory testing is complete.
- 34 1.31 If suspect colonies or  $\beta$ -hemolytic growth are present on HL but not clearly  
35 isolated, re-streak representative suspect colonies/growth onto one or more fresh  
36 HL plates and incubate at  $35 \pm 2^{\circ}\text{C}$  for  $22 \pm 4$  h.
- 37 1.32 Confirm ALL test portions according to the USDA FSIS biochemical/serological  
38 procedures. Biochemical tests will be performed using API or VITEK 2 GP.  
39 Please follow manufacturer's instructions when using any of these rapid methods.  
40  
41

## 42 Final Results

43  
44 All results are to be recorded on the data sheets provided. For each matrix test portion, record  
45 the results reported by the 3M MDA 2 -*Listeria monocytogenes* and the 3M MDA 2-*Listeria*  
46 method along with the confirmation results for the 3M MDAs and reference methods.

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- 1 Immediately upon completion of each food type, the data sheet should be faxed or emailed to
- 2 TBD, Study Director.

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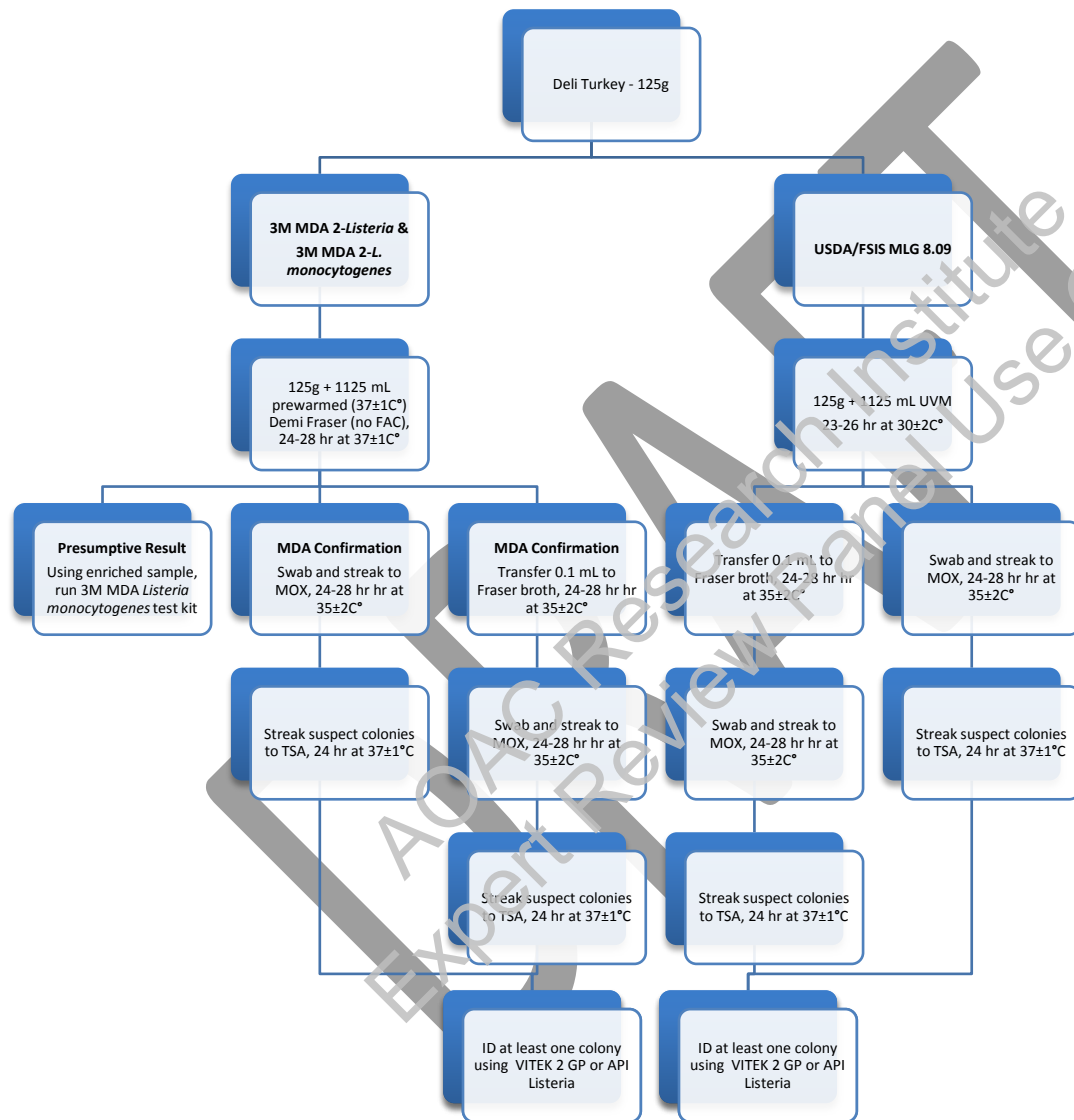




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1 Appendix 8.3a – Study Flow Diagram: Deli Turkey

2



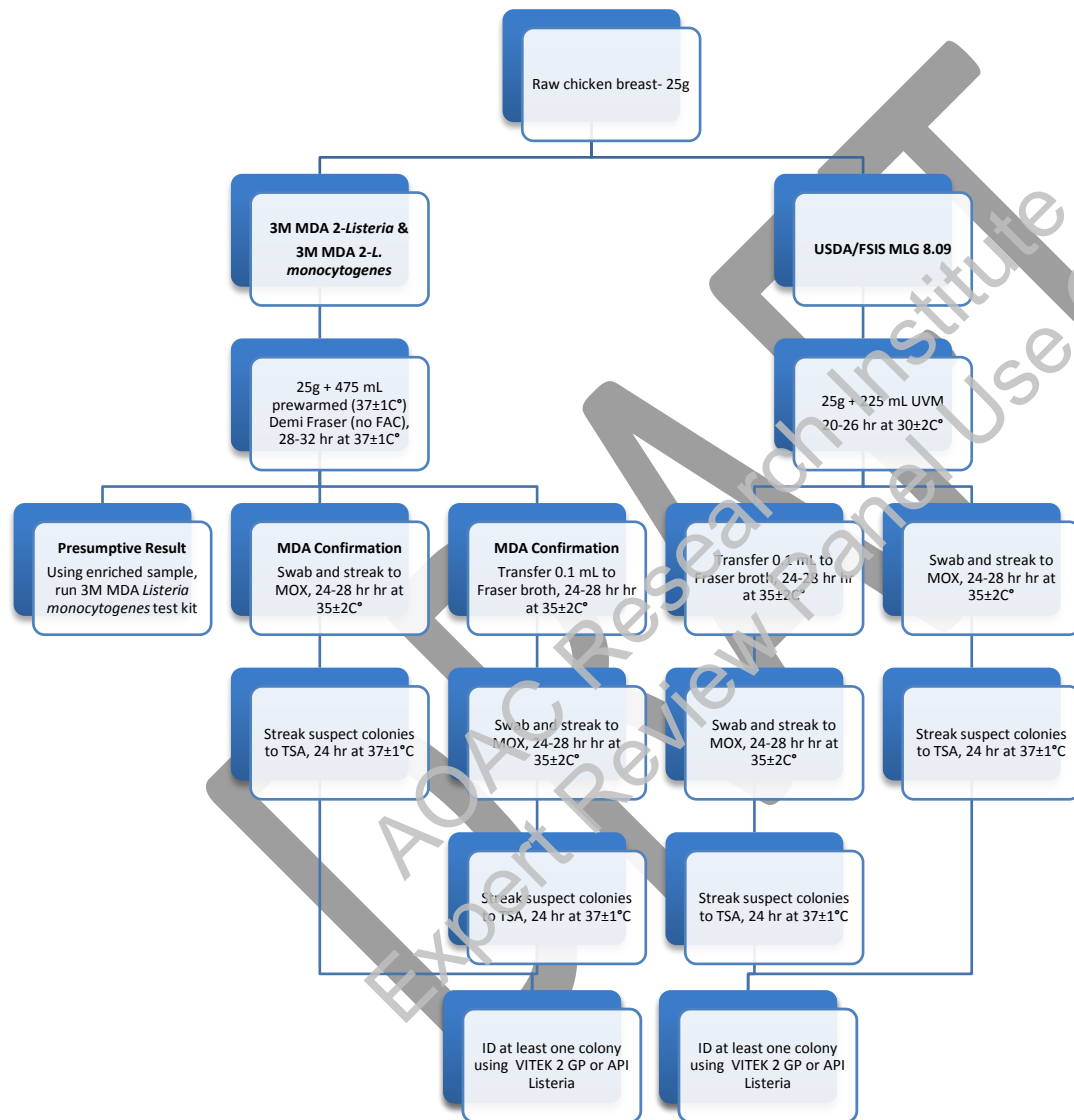
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1 **Appendix 8.3b – Study Flow Diagram: Raw Chicken Breast**

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3M MDA 2 *Listeria* species and *L. monocytogenes* Collaborative Study  
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1 **Appendix 8.4**

2 **Study Materials**

3  
4 Materials and reagents provided by the study sponsor

- 5  
6 1. 3M Molecular Detection 2 kits  
7 2. 3M Molecular Detection System (instrument), power supply, cord, USB cable and  
8 software  
9 3. 3M Molecular Detection Speed Loader Tray  
10 4. 3M Molecular Detection Chill Block Insert  
11 5. 3M Molecular Detection Heat Block Insert  
12 6. 3M Molecular Detection Cap/Decap Tool [Reagent]  
13 7. 3M Molecular Detection Cap/Decap Tool [Lysis]  
14 8. Empty lysis tube rack  
15 9. Empty reagent tube rack  
16 10. Sterile filter-tip pipette tips – capable of 20 $\mu$ L  
17 11. Filter Stomacher® bags – Seward or equivalent

18  
19 **Enrichment**

- 20 12. UVM  
21 13. Demi Fraser broth with Ferric Ammonium Citrate (FAC)  
22 14. Fraser broth with FAC

23  
24 **Selective agar**

- 25 15. MOX

26  
27 **Confirmation**

- 28 16. Blood agar (HL and SBA)  
29 17. Catalase  
30 18. Oxidase  
31 19. Motility supplies  
32 20. Tryptic Soy Agar with yeast extract  
33 21. VITEK2 GP or API *Listeria*

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- 1
- 2 **Additional Supplies and Reagents required but not provided**
- 3 1. Pipettes – capable of 20µL
- 4 2. Multi-channel (8-channel) pipette – capable of 20µL
- 5 3. Stomacher® – Seward or equivalent
- 6 4. Thermometer – calibrated range to include 100±1°C range
- 7 5. Dry double block heater unit – capable of maintaining 100 + 1°C; OR a water bath -
- 8 capable of maintaining 100 + 1°C
- 9 6. Incubators – capable of maintaining 37 ± 1°C and 35 ± 1°C
- 10 7. Refrigerator – capable of maintaining 2-8°C, for storing the 3M Molecular Detection 2
- 11 Assay
- 12 8. Computer – compatible with the 3M Molecular Detection System (instrument)
- 13
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3M MDA 2 *Listeria* species and *L. monocytogenes* Collaborative Study  
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1 **Appendix 8.5**

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**3M MDA 2 -*Listeria* and  
3M MDA 2 -*Listeria monocytogenes***

**Collaborative Study  
Collaborator Information Sheet**

---

10

11 Please complete the information below and return to TBD.

12

13 **Contact name:**

14

15 **Laboratory name:**

16

17 **Fax number:**

18

19 **Phone number:**

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21 **Email address:**

22

23 **Mailing address:**

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29 **Shipping address:**

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1 **Appendix 8.6**

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5 **3M MDA 2- *Listeria* and**  
6 **3M MDA2 - *Listeria monocytogenes***

7

8 **Collaborative Study**  
9 **Collaborator Comments Form**

10

11

12 **Collaborating Laboratory:**

13

14 **Date:**

15

16 **General Comments about Method:**

17

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## Appendix 8.7

### 3M™ Molecular Detection Assay 2 - *Listeria*

MDA2LIS96

#### PRODUCT DESCRIPTION AND INTENDED USE

3M™ Molecular Detection Assay 2 - *Listeria* is used with the 3M™ Molecular Detection System for the rapid and specific detection of *Listeria* species in enriched food and environmental samples.

The 3M Molecular Detection Assays use loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations (1, 2, 3).

The 3M Molecular Detection Assay 2 - *Listeria* is intended for use in a laboratory environment by professionals trained in laboratory techniques. 3M has not documented the use of this product in industries other than food or beverage. For example, 3M has not documented this product for testing water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M Molecular Detection Assay 2 - *Listeria* has not been evaluated with all possible testing protocols or with all possible strains of bacteria.

**As with all test methods, the source, formulation and quality of enrichment medium can influence the results.** Factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may also influence results. 3M recommends evaluation of the method including enrichment medium, in the user's environment using a sufficient number of samples with particular foods and microbial challenges to ensure that the method meets the user's criteria.

3M has evaluated the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* with Demi-Fraser Broth containing Ferric Ammonium Citrate. A typical formulation of this medium follows below.

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**Demi-Fraser Broth Base Typical Formula (g/L)**

Sodium Chloride	20 g
Sodium Phosphate, dibasic, anhydrous	9.6 g
*	
Beef Extract	5.0 g
Pancreatic Digest of Casein	5.0 g
Peptic Digest of Animal Tissue	5.0 g
Yeast Extract	5.0 g
Lithium Chloride	3.0 g
Potassium Phosphate, monobasic	1.35 g
Esculin	1.0 g
Acriflavin HCl	0.0125 g
Nalidixic Acid	0.01 g

1

2 \* Substitute: Sodium Phosphate, dibasic, dihydrate 12.0 g

3

4 **Fraser Broth Supplement**

5 (Ingredients per 10 mL vial. One vial is added to one liter of basal medium.)

6 Ferric Ammonium Citrate 0.5g/10mL

7 Final pH 7.2 ± 0.2 at 25°C

8

9 The 3M™ Molecular Detection Instrument is intended for use with samples that have undergone  
 10 heat treatment during the assay lysis step, which is designed to destroy organisms present in  
 11 the sample. Samples that have not been properly heat treated during the assay lysis step may  
 12 be considered a potential biohazard and should NOT be inserted into the 3M Molecular  
 13 Detection Instrument.

14

15 3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for  
 16 design and manufacturing.

17

18 The 3M Molecular Detection Assay 2 - *Listeria* test kit contains 96 tests, described in Table 1.

19 **Table 1. Kit Components**

Item	Identification	Quantity	Contents	Comments
------	----------------	----------	----------	----------




Lysis Solution (LS) tubes	Pink solution in clear tubes	96 (12 strips of 8 tubes)	580 µL of LS per tube	Racked and ready to use
<i>Listeria</i> Reagent tubes	Blue tubes	96 (12 strips of 8 tubes)	Lyophilized specific amplification and detection mix	Ready to use
Extra caps	Blue caps	96 (12 strips of 8 caps)		Ready to use
Reagent Control (RC)	Clear flip-top tubes	16 (2 pouches of 8 individual tubes)	Lyophilized control DNA, amplification and detection mix	Ready to use
Quick Start Guide		1		


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The Negative Control, not provided in the kit, is sterile enrichment medium, e.g., Demi-Fraser Broth. Do not use water as a Negative Control.

**SAFETY**

The user should read, understand and follow all safety information in the instructions for the 3M Molecular Detection System and the 3M Molecular Detection Assay 2 - *Listeria*. Retain the safety instructions for future reference.

 **WARNING:** Indicates a hazardous situation, which, if not avoided, could result in death or serious injury and/or property damage.

 **CAUTION:** Indicates a hazardous situation, which, if not avoided, could result in minor or moderate injury and/or property damage.

**NOTICE:** Indicates a potentially hazardous situation which, if not avoided, could result in property damage.

10

 **WARNING**

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**Do not use the 3M Molecular Detection Assay 2 - *Listeria* in the diagnosis of conditions in humans or animals.**

**The 3M Molecular Detection Assay 2 - *Listeria* method may generate *Listeria monocytogenes* levels sufficient to cause stillbirths and fatalities in pregnant women and the immuno compromised, if exposed.**

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**The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices, ISO 17025<sup>(4)</sup>, or ISO 7218<sup>(5)</sup>.**

**To reduce the risks associated with a false-negative result leading to the release of contaminated product:**

- Follow the protocol and perform the tests exactly as stated in the product instructions.
- Store the 3M Molecular Detection Assay 2 - *Listeria* as indicated on the package and in the product instructions.
- Always use the 3M Molecular Detection Assay 2 - *Listeria* by the expiration date.
- Use the 3M Molecular Detection Assay 2 - *Listeria* for food and environmental samples that have been validated internally or by a third party.
- Use the 3M Molecular Detection Assay 2 - *Listeria* only for surfaces, sanitizers, protocols and bacterial strains that have been validated internally or by a third party.
- For an environmental sample containing Neutralizing Buffer with aryl sulfonate complex, perform a 1:2 dilution before testing (1 part sample into 1 part sterile enrichment broth).  
3M™ sample handling products which include neutralizing buffer: BPPFV10NB, RS96010NB, RS9604NB, SSL10NB, XSLSSL10NB, HS10NB and HS119510NB.

**To reduce the risks associated with exposure to chemicals and biohazards:**


- It is strongly recommended that female laboratory staff be informed of the risk to a developing fetus resulting from infection of the mother through exposure to *Listeria monocytogenes*.
- Perform pathogen testing in a properly equipped laboratory under the control of trained personnel.
- Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples.
- Avoid contact with the contents of the enrichment media and reagent tubes after amplification.
- Dispose of enriched samples according to current industry standards.

**To reduce the risks associated with cross-contamination while preparing the assay:**

- Always wear gloves (to protect the user and prevent introduction of nucleases).

**To reduce the risks associated with environmental contamination:**

- Follow current industry standards for disposal of contaminated waste.

 **CAUTION**

- 1 • Do not exceed the recommended temperature setting on heater.
- 2 • Do not exceed the recommended heating time.
- 3 • Use an appropriate, calibrated thermometer to verify the 3M™ Molecular Detection Heat
- 4 Block Insert temperature (e.g., a partial immersion thermometer or digital thermocouple
- 5 thermometer, not a total immersion thermometer.) The thermometer must be placed in the
- 6 designated location in the 3M Molecular Detection Heat Block Insert.
- 7

**NOTICE**

**To reduce the risks associated with cross-contamination while preparing the assay:**

- 8 • Use of sterile, aerosol barrier (filtered), molecular biology grade pipette tips is
- 9 recommended.
- 10
- 11 • Use a new pipette tip for each sample transfer.
- 12 • Use Good Laboratory Practices to transfer the sample from the enrichment to the lysis tube.
- 13 To avoid pipettor contamination, the user may choose to add an intermediate transfer step.
- 14 For example, the user can transfer each enriched sample into a sterile tube.
- 15 • Use a molecular biology workstation containing germicidal lamp where available.
- 16

**To reduce the risks associated with a false-positive result:**

- 17 • Never open tubes post amplification.
- 18 • Always dispose of the contaminated tubes by soaking in a 1-5% (v:v in water) household
- 19 bleach solution for 1 hour and away from the assay preparation area.
- 20
- 21

22 Consult the Safety Data Sheet for additional information and local regulations for disposal.

23  
24 If you have questions about specific applications or procedures, please visit our website at  
25 [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

26  
27 **LIMITATION OF WARRANTIES / LIMITED REMEDY**

1 EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL  
2 PRODUCT PACKAGING, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES,  
3 INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR  
4 FITNESS FOR A PARTICULAR USE. If any 3M Food Safety Product is defective, 3M or its  
5 authorized distributor will, at its option, replace or refund the purchase price of the product.  
6 These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery  
7 of any suspected defects in a product and return it to 3M. Please call Customer Service (1-800-  
8 328-1671 in the U.S.) or your official 3M Food Safety representative for a Returned Goods  
9 Authorization.

### 11 **LIMITATION OF 3M LIABILITY**

12 3M WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT,  
13 SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED  
14 TO LOST PROFITS. In no event shall 3M's liability under any legal theory exceed the purchase  
15 price of the product alleged to be defective.

### 17 **USER RESPONSIBILITY**

18 Users are responsible for familiarizing themselves with product instructions and information.  
19 Visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety), or contact your local 3M representative or  
20 distributor for more information.

21 When selecting a test method, it is important to recognize that external factors such as sampling  
22 methods, testing protocols, sample preparation, handling, and laboratory technique may  
23 influence results.

24 It is the user's responsibility in selecting any test method or product to evaluate a sufficient  
25 number of samples with the appropriate matrices and microbial challenges to satisfy the user  
26 that the chosen test method meets the user's criteria.

27 It is also the user's responsibility to determine that any test methods and results meet its  
28 customers' and suppliers' requirements.

29 As with any test method, results obtained from use of any 3M Food Safety product do not  
30 constitute a guarantee of the quality of the matrices or processes tested.

31 To help customers evaluate the method for various food matrices, 3M has developed the 3M™  
32 Molecular Detection Matrix Control kit. When needed, use the Matrix Control (MC) to determine  
33 if the matrix has the ability to impact the 3M Molecular Detection Assay 2 - *Listeria* results. Test

1 several samples representative of the matrix, i.e. samples obtained from different origin, during  
2 any validation period when adopting the 3M method or when testing new or unknown matrices  
3 or matrices that have undergone raw material or process changes.

4 A matrix can be defined as a type of product with intrinsic properties such as composition and  
5 process. Differences between matrices may be as simple as the effects caused by differences  
6 in their processing or presentation for example, raw vs. pasteurized; fresh vs. dried, etc.

## 8 **STORAGE AND DISPOSAL**

9 Store the 3M Molecular Detection Assay 2 - *Listeria* at 2-8°C. Do not freeze. Keep kit away from  
10 light during storage. After opening the kit, check that the foil pouch is undamaged. If the pouch  
11 is damaged, do not use. After opening, unused reagent tubes should always be stored in the re-  
12 sealable pouch with the desiccant inside to maintain stability of the lyophilized reagents. Store  
13 resealed pouches at 2-8°C for no longer than 60days.

14  
15 Do not use 3M Molecular Detection Assay 2 - *Listeria* past the expiration date. Expiration date  
16 and lot number are noted on the outside label of the box. After use, the enrichment medium and  
17 the 3M Molecular Detection Assay 2 - *Listeria* tubes can potentially contain pathogenic  
18 materials. When testing is complete, follow current industry standards for the disposal of  
19 contaminated waste. Consult the Safety Data Sheet for additional information and local  
20 regulations for disposal.

## 22 **INSTRUCTIONS FOR USE**

23 Follow all instructions carefully. Failure to do so may lead to inaccurate results.

24 Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.)  
25 with a 1- 5% (v:v in water) household bleach solution or DNA removal solution.

## 27 **SAMPLE ENRICHMENT**

28 Table 2 presents guidance for the enrichment of food and environmental samples. It is the  
29 user's responsibility to validate alternate sampling protocols or dilution ratios to ensure this test  
30 method meets the user's criteria.

### 32 **Foods**

- 33 1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to  
34 equilibrate to ambient laboratory temperature.

- 1 2. Aseptically combine the enrichment medium and sample according to Table 2. For all meat  
2 and highly particulate samples, the use of filter bags is recommended.
- 3 3. Homogenize thoroughly by blending, stomaching, or hand mixing for  $2 \pm 0.2$  minutes.  
4 Incubate at  $37 \pm 1^\circ\text{C}$  according to Table 2.
- 5 4. For raw dairy products, transfer 0.1 mL of the primary enrichment into 10 mL of Fraser  
6 Broth. Incubate at  $37 \pm 1^\circ\text{C}$  for 20-24 hours.

### 8 **Environmental samples**

9 Sample collection devices can be a sponge hydrated with a neutralizing solution to inactivate  
10 the effects of the sanitizers. 3M recommends the use of a biocide-free cellulose sponge.  
11 Neutralizing solution can be Dey-Engley (D/E) Neutralizing Broth or Lethen broth. It is  
12 recommended to sanitize the area after sampling.

13  
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15 **WARNING:** Should you select to use Neutralizing Buffer (NB) that contains aryl sulfonate  
16 complex as the hydrating solution for the sponge, it is required to perform a 1:2 dilution (1 part  
17 sample into 1 part sterile enrichment broth) of the enriched environmental sample before testing  
18 in order to reduce the risks associated with a false-negative result leading to the release of  
19 contaminated product.

20

21 The recommended size of the sampling area to verify the presence or absence of the pathogen  
22 on the surface is at least  $100 \text{ cm}^2$  ( $10 \text{ cm} \times 10 \text{ cm}$  or  $4'' \times 4''$ ). When sampling with a sponge,  
23 cover the entire area going in two directions (left to right then up and down) or collect  
24 environmental samples following your current sampling protocol or according to the FDA BAM  
25 <sup>(1)</sup>, USDA FSIS MLG <sup>(2)</sup> or ISO 18593 <sup>(6)</sup> guidelines.

26

- 27 1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to  
28 equilibrate to ambient laboratory temperature.
- 29 2. Aseptically combine the enrichment medium and sample according to Table 2.
- 30 3. Homogenize thoroughly by blending, stomaching, or hand mixing for  $2 \pm 0.2$  minutes.  
31 Incubate at  $37 \pm 1^\circ\text{C}$  for 24-30 hours.

32

33

1 **Table 2:** Enrichment protocols using Demi-Fraser Broth Enrichment

2

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature (°C)	Enrichment Time (hr)				
Heat-processed, cooked, cured meats, poultry, seafood and fish	25 g	225	37	24-30				
Heat-processed / pasteurized dairy products								
Produce and vegetables								
Multi-component foods								
Environmental samples	1 sponge	100 or 225	37	24-30				
	1 swab	10	37	24-30				
Raw meat, poultry, seafood, fish	25 g	475	37	28-32				
Sample Matrix	Primary Enrichment (Demi-Fraser Broth)				Secondary Enrichment (Fraser Broth)			Sample Analysis Volume <sup>(a)</sup>
	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature (°C)	Enrichment Time (hr)	Sample Size	Enrichment Temperature (°C)	Enrichment Time (hr)	
Raw dairy products	25 g	225	37	20-24	Transfer 0.1 mL into 10 mL Fraser Broth	37	20-24	10 µL

3 (a) Volume of sample transferred to Lysis Solution tubes. Refer to Step 4.6 of Lysis section.

4

5 **Specific Instructions for Validated Methods**

6 **AOAC® Performance Tested Method<sup>sm</sup> # XXXXX**

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10 In AOAC PTM studies, the 3M Molecular Detection Assay 2 – *Listeria monocytogenes* was  
 11 found to be an effective method for the detection of *Listeria monocytogenes*. The matrices  
 12 tested in the study are shown in Table 3. The limit of detection of the 3M Molecular

1 Detection Assay 2 – *Listeria monocytogenes* method is 1-5 colony forming units per  
 2 validated test portion size (in Table 3).

3  
 4  
 5 **Table 3.** Enrichment protocols using Demi-Fraser Broth at  $37 \pm 1$  °C  
 6 according to AOAC Performance Tested<sup>SM</sup> Certificate #XXXXXX  
 7

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)	
Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon	25 g	225	24-30	
Raw chicken	25 g	475	26-32	
Deli turkey	125 g	1125	24-30	
Cantaloupe	Whole melon	Enough volume to allow melon to float	26-30	
Environmental samples:	Stainless steel	1 sponge	225	24-30
	Sealed concrete	1 sponge	100	
	Plastic	1 swab	10	24-30

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 9

10 **PREPARATION OF THE 3M™ MOLECULAR DETECTION SPEED LOADER TRAY**

- 11 1. Wet a cloth with a 1-5% (v:v in water) household bleach solution and wipe the 3M™  
 12 Molecular Detection Speed Loader Tray.  
 13 2. Rinse the 3M Molecular Detection Speed Loader Tray with water.  
 14 3. Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.  
 15 4. Ensure the 3M Molecular Detection Speed Loader Tray is dry before use.



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### PREPARATION OF THE 3M™ MOLECULAR DETECTION CHILL BLOCK INSERT

Place the 3M™ Molecular Detection Chill Block directly on the laboratory bench; (the 3M™ Molecular Detection Chill Block Tray is not used). Use the chill block at ambient laboratory temperature (20-25°C).

### PREPARATION OF THE 3M™ MOLECULAR DETECTION HEAT BLOCK INSERT

Place the 3M™ Molecular Detection Heat Block Insert in a dry double block heater unit. Turn on the dry block heater unit and set the temperature to allow the 3M Molecular Detection Heat Block Insert to reach and maintain a temperature of  $100 \pm 1^\circ\text{C}$ .

**NOTE:** Depending on the heater unit, allow approximately 30 minutes for the 3M Molecular Detection Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (e.g., a partial immersion thermometer or digital thermocouple thermometer, not a total immersion thermometer) placed in the designated location, verify that the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^\circ\text{C}$ .

### PREPARATION OF THE 3M™ MOLECULAR DETECTION INSTRUMENT

1. Launch the 3M™ Molecular Detection Software and log in.
2. Turn on the 3M Molecular Detection Instrument.
3. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection System User Manual for details.

**NOTE:** The 3M Molecular Detection Instrument must reach and maintain temperature of  $60^\circ\text{C}$  before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 min and is indicated by an ORANGE light on the instrument's status bar. When the instrument is ready to start a run, the status bar will turn GREEN.

### LYSIS

1. Allow the lysis solution (LS) tubes to warm up by setting the rack at room temperature (20-25 °C) overnight (16-18 hours). Alternatives to equilibrate the LS tubes to room

1 temperature are to set the LS tubes on the laboratory bench for at least 2 hours, incubate  
2 the LS tubes in a  $37 \pm 1^{\circ}\text{C}$  incubator for 1 hour or place them in a dry double block heater  
3 for 30 seconds at  $100^{\circ}\text{C}$ .

4 2. Invert the capped tubes to mix. Proceed to next step within 4 hrs.

5 3. Remove the enrichment broth from the incubator.

6 4. One LS tube is required for each sample and the Negative Control (NC) (sterile  
7 enrichment medium) sample.

8 4.1 LS tube strips can be cut to desired LS tube number. Select the number of individual LS  
9 tubes or 8-tube strips needed. Place the LS tubes in an empty rack.

10 4.2 To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipette  
11 tip for each transfer step.

12 4.3 Transfer enriched sample to LS tubes as described below:

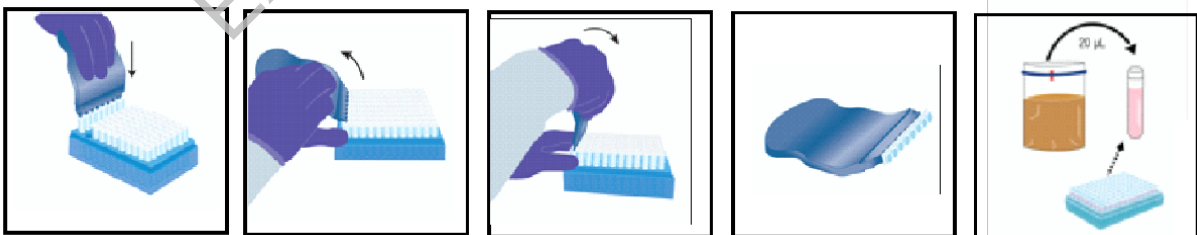
13  
14 **Transfer each enriched sample into an individual LS tube first. Transfer the NC last.**

15  
16 4.4 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip -  
17 one strip at a time.

18 4.5 Discard the LS tube cap – if lysate will be retained for retest, place the caps into a  
19 clean container for re-application after lysis. For processing of retained lysate, see  
20 Appendix A.

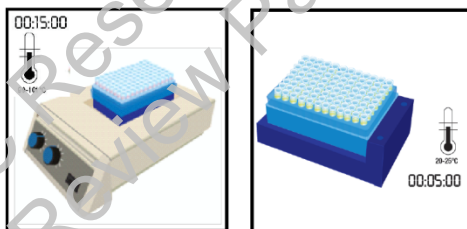
21 4.6 Transfer  $20 \mu\text{L}$  of sample into a LS tube unless otherwise indicated in Protocol Table  
22 2. e.g. Raw dairy products use  $10 \mu\text{L}$ .

23 5. Repeat step 4.2 until each individual sample has been added to a corresponding LS tube  
24 in the strip.



26  
27  
28 6. Repeat steps 4.1 to 4.6 as needed, for the number of samples to be tested.

- 1 7. When all samples have been transferred, transfer 20  $\mu$ L of NC (sterile enrichment medium,  
2 e.g., Demi-Fraser Broth) into a LS tube. Do not use water as a NC.
- 3 8. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^{\circ}\text{C}$ .
- 4 9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and  
5 heat for  $15 \pm 1$  minutes. During heating, the LS solution will change from pink (cool) to yellow  
6 (hot).
- 7 Samples that have not been properly heat treated during the assay lysis step may be  
8 considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection  
9 Instrument.
- 10 10. Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M  
11 Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes. The  
12 3M Molecular Chill Block Insert, used at ambient temperature without the Molecular Detection  
13 Chill Block Tray should sit directly on the laboratory bench. When cool, the lysis solution will  
14 revert to a pink color.
- 15
- 16 11. Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.
- 17



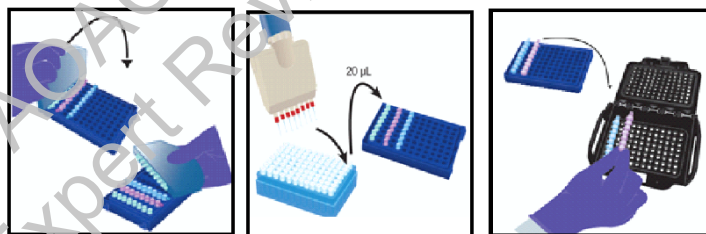
18  
19

## 20 **AMPLIFICATION**

- 21
- 22 1. One Reagent tube is required for each sample and the NC.
  - 23 1.1 Reagent tube strips can be cut to desired tube number. Select the number of individual  
24 Reagent tubes or 8-tube strips needed.
  - 25 1.2 Place Reagent tubes in an empty rack.
  - 26 1.3 Avoid disturbing the reagent pellets from the bottom of the tubes.
- 27 2. Select 1 Reagent Control (RC) tube and place in rack.
- 28 3. To avoid cross-contamination, decap one Reagent tube strip at a time and use a new  
29 pipette tip for each transfer step.
- 30 4. Transfer lysate to Reagent tubes and RC tube as described below:

1 Transfer each sample lysate into individual Reagent tubes **first** followed by the NC. Hydrate  
2 the RC tube **last**.

- 3 5. Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent tubes –  
4 one Reagent tube strip at a time. Discard cap.
- 5 5.1 Transfer 20 µL of Sample lysate from the upper ½ of the liquid (avoid precipitate) in the  
6 LS tube into corresponding Reagent tube. Dispense at an angle to avoid disturbing the  
7 pellets. Mix by gently pipetting up and down 5 times.
- 8 5.2 Repeat step 5.1 until individual Sample lysate has been added to a corresponding  
9 Reagent tube in the strip.
- 10 5.3 Cover the Reagent tubes with the provided extra caps and use the rounded side of the  
11 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and  
12 forth motion ensuring that the cap is tightly applied.
- 13 5.4 Repeat step 5.1 as needed, for the number of samples to be tested.
- 14 5.5 When all sample lysates have been transferred, repeat 4.1 to transfer 20 µL of NC  
15 lysate into a Reagent tube.
- 16 5.6 Transfer **20 µL of NC lysate into a RC tube**. Dispense at an angle to avoid disturbing  
17 the pellets. Mix by gently pipetting up and down 5 times.
- 18 6. Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader  
19 Tray. Close and latch the 3M Molecular Detection Speed Loader Tray lid.
- 20



- 21
- 22
- 23 7. Review and confirm the configured run in the 3M Molecular Detection Software.
- 24 8. Click the Start button in the software and select instrument for use. The selected  
25 instrument's lid automatically opens.
- 26 9. Place the 3M Molecular Detection Speed Loader Tray into the 3M Molecular Detection  
27 Instrument and close the lid to start the assay. Results are provided within 75 minutes,  
28 although positives may be detected sooner.

1 10. After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from  
2 the 3M Molecular Detection Instrument and dispose of the tubes by soaking in a 1-5% (v:v in  
3 water) household bleach solution for 1 hour and away from the assay preparation area.  
4

5 **NOTICE:** To minimize the risk of false positives due to cross-contamination, never open reagent  
6 tubes containing amplified DNA. This includes Reagent Control, Reagent, and Matrix Control  
7 tubes. Always dispose of sealed reagent tubes by soaking in a 1-5% (v:v in water) household  
8 bleach solution for 1 hour and away from the assay preparation area.  
9

## 10 **RESULTS AND INTERPRETATION**

11 An algorithm interprets the light output curve resulting from the detection of the nucleic acid  
12 amplification. Results are analyzed automatically by the software and are color-coded based on  
13 the result. A Positive or Negative result is determined by analysis of a number of unique curve  
14 parameters. Presumptive positive results are reported in real-time while Negative and Inspect  
15 results will be displayed after the run is completed.  
16

17 Presumptive positive samples should be confirmed as per the laboratory standard operating  
18 procedures or by following the appropriate reference method confirmation <sup>(1, 2, 3)</sup>, beginning with  
19 transfer from the primary enrichment to secondary enrichment broth (if applicable), followed by  
20 subsequent plating and confirmation of isolates using appropriate biochemical and serological  
21 methods.  
22

23 **NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular  
24 Detection Assay 2 - *Listeria* amplification reagents have a “background” relative light unit (RLU)  
25 reading.  
26

27 In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M  
28 recommends the user to repeat the assay for any Inspect samples. If the result continues to be  
29 Inspect, proceed to confirmation test using your preferred method or as specified by local  
30 regulations.  
31

32 If you have questions about specific applications or procedures, please visit our website at  
33 [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

1

2 **REFERENCES:**

- 3 1. US Food and Drug Administration Bacteriological Analysis Manual. Chapter 10: Detection  
4 and Enumeration of *Listeria monocytogenes* in Foods. Section C-6. April 2011 Version.  
5 2. US Department of Agriculture (USDA) FSIS Microbiology Laboratory Guidebook 8.08.  
6 Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry and Egg  
7 Products, and Environmental Samples. Effective Date: 6 Nov 2012.  
8 3. ISO 11290-1. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the  
9 Detection and Enumeration of *Listeria monocytogenes*. Amendment 1, 2004-10-15.  
10 4. ISO/IEC 17025. General requirements for the competence of testing and calibration  
11 laboratories.  
12 5. ISO 7218. Microbiology of food and animal feeding stuffs – General rules for microbiological  
13 examination.  
14 6. ISO 18593. Microbiology of food and animal feeding stuffs – Horizontal methods for  
15 sampling techniques from surfaces using contact plates and swabs.

16

17 **Appendix A. Protocol Interruption: Storage and re-testing of heat-treated lysates**

- 18 1. To store a heat-treated lysate, re-cap the lysis tube with a clean cap (see “LYSIS”, 4.5)  
19 2. Store at 4 to 8°C for up to 72 hours.  
20 3. Prepare a stored sample for amplification by inverting 2-3 times to mix.  
21 4. Decap the tubes.  
22 5. Place the mixed lysate tubes on 3M Molecular Detection Heat Block Insert and heat at 100  
23  $\pm 1^\circ\text{C}$  for  $5 \pm 1$  minutes.  
24 6. Remove the rack of LS tubes from the heating block and allow to cool in the 3M Molecular  
25 Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes.  
26 7. Continue the protocol at the ‘Amplification’ section detailed above.


27

28 **EXPLANATION OF PRODUCT LABEL SYMBOLS**

29

30  Caution or Warning, see product instructions

31

32  Consult product instructions

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12



The lot in a box represents the lot number



The hourglass is followed by a month and year which represent the expiration date



Storage temperature limitations

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1 **Appendix 8.8**

2

3 **3M™ Molecular Detection Assay 2 - *Listeria monocytogenes***

4 **MDA2LMO96**

5

6 **PRODUCT DESCRIPTION AND INTENDED USE**

7 3M™ Molecular Detection Assay 2 - *Listeria monocytogenes* is used with the 3M™ Molecular  
8 Detection System for the rapid and specific detection of *Listeria monocytogenes* in enriched  
9 food and environmental samples.

10

11 The 3M Molecular Detection Assays use loop-mediated isothermal amplification to rapidly  
12 amplify nucleic acid sequences with high specificity and sensitivity, combined with  
13 bioluminescence to detect the amplification. Presumptive positive results are reported in real-  
14 time while negative results are displayed after the assay is completed. Presumptive positive  
15 results should be confirmed using your preferred method or as specified by local regulations <sup>(1, 2,</sup>  
16 <sup>3)</sup>.

17

18 The 3M Molecular Detection Assay 2 - *Listeria monocytogenes* is intended for use in a  
19 laboratory environment by professionals trained in laboratory techniques. 3M has not  
20 documented the use of this product in industries other than food or beverage. For example, 3M  
21 has not documented this product for testing water, pharmaceutical, cosmetics, clinical or  
22 veterinary samples. The 3M Molecular Detection Assay - 2 *Listeria monocytogenes* has not  
23 been evaluated with all possible testing protocols or with all possible strains of bacteria.

24

25 **As with all test methods, the source, formulation and quality of enrichment medium can**  
26 **influence the results.** Factors such as sampling methods, testing protocols, sample preparation,  
27 handling, and laboratory technique may also influence results. 3M recommends evaluation of  
28 the method including enrichment medium, in the user's environment using a sufficient number of  
29 samples with particular foods and microbial challenges to ensure that the method meets the  
30 user's criteria.

31

32 3M has evaluated the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* with Demi-  
33 Fraser Broth containing Ferric Ammonium Citrate. A typical formulation of this medium follows  
34 below.

34



**Demi-Fraser Broth Base Typical Formula (g/L)**

Sodium Chloride	20 g
Sodium Phosphate, dibasic, anhydrous*	9.6 g
Beef Extract	5.0 g
Pancreatic Digest of Casein	5.0 g
Peptic Digest of Animal Tissue	5.0 g
Yeast Extract	5.0 g
Lithium Chloride	3.0 g
Potassium Phosphate, monobasic	1.35 g
Esculin	1.0 g
Acriflavin HCl	0.0125 g
Nalidixic Acid	0.01 g

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20

\* Substitute: Sodium Phosphate, dibasic, dihydrate 12.0 g

**Fraser Broth Supplement**

(Ingredients per 10 mL vial. One vial is added to one liter of basal medium.)

Ferric Ammonium Citrate 0.5g/10mL

Final pH 7.2 ± 0.2 at 25°C

The 3M™ Molecular Detection Instrument is intended for use with samples that have undergone heat treatment during the assay lysis step, which is designed to destroy organisms present in the sample. Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.

3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

The 3M Molecular Detection Assay 2 - *Listeria monocytogenes* test kit contains 96 tests, described in Table 1.

1 **Table 1. Kit Components**

2

Item	Identification	Quantity	Contents	Comments
Lysis Solution (LS) tubes	Pink solution in clear tubes	96 (12 strips of 8 tubes)	580 µL of LS per tube	Racked and ready to use
<i>Listeria monocytogenes</i> Reagent tubes	Yellow tubes	96 (12 strips of 8 tubes)	Lyophilized specific amplification and detection mix	Ready to use
Extra caps	Yellow caps	96 (12 strips of 8 caps)		Ready to use
Reagent Control (RC)	Clear flip-top tubes	16 (2 pouches of 8 individual tubes)	Lyophilized control DNA, amplification and detection mix	Ready to use
Quick Start Guide		1		

3

4


5 The Negative Control, not provided in the kit, is sterile enrichment medium, e.g., Demi-Fraser  
 6 Broth. Do not use water as a Negative Control.


7

8 **SAFETY**

9 The user should read, understand and follow all safety information in the instructions for the 3M  
 10 Molecular Detection System and the 3M Molecular Detection Assay 2 - *Listeria monocytogenes*.  
 11 Retain the safety instructions for future reference.

12

 **WARNING:** Indicates a hazardous situation, which, if not avoided, could result in death or serious injury and/or property damage.

 **CAUTION:** Indicates a hazardous situation, which, if not avoided, could result in minor or moderate injury and/or property damage.

**NOTICE:** Indicates a potentially hazardous situation which, if not avoided, could result in property damage.

13

 **WARNING**

14

15 **Do not use the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* in the diagnosis of**  
 16 **conditions in humans or animals.**

1  
2 **The 3M Molecular Detection Assay 2 - *Listeria monocytogenes* method may generate *Listeria***  
3 ***monocytogenes* to levels sufficient to cause stillbirths and fatalities in pregnant women and the**  
4 **immuno compromised, if exposed.**

5  
6 **The user must train its personnel in current proper testing techniques: for example, Good**  
7 **Laboratory Practices, ISO 17025<sup>(4)</sup>, or ISO 7218<sup>(5)</sup>.**

8  
9 **To reduce the risks associated with a false-negative result leading to the release of**  
10 **contaminated product:**

- 11 • Follow the protocol and perform the tests exactly as stated in the product instructions.
- 12 • Store the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* as indicated on the
- 13 package and in the product instructions.
- 14 • Always use the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* by the expiration
- 15 date.
- 16 • Use the 3M Molecular Detection Assay *Listeria monocytogenes* for food and environmental
- 17 samples that have been validated internally or by a third party.
- 18 • Use the 3M Molecular Detection Assay *Listeria monocytogenes* only for surfaces, sanitizers,
- 19 protocols and bacterial strains that have been validated internally or by a third party.
- 20 • For an environmental sample containing Neutralizing Buffer with aryl sulfonate complex,
- 21 perform a 1:2 dilution before testing (1 part sample into 1 part sterile enrichment broth).
- 22 3M™ sample handling products which include neutralizing buffer: BPPFV10NB,
- 23 RS96010NB, RS9604NB, SSL10NB, XSLSSL10NB, HS10NB and HS119510NB.

24

**To reduce the risks associated with exposure to chemicals and biohazards:**

- It is strongly recommended that female laboratory staff be informed of the risk to a developing fetus resulting from infection of the mother through exposure to *Listeria monocytogenes*.
- Perform pathogen testing in a properly equipped laboratory under the control of trained personnel.
- Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples.
- Avoid contact with the contents of the enrichment media and reagent tubes after

amplification.

- Dispose of enriched samples according to current industry standards.

**To reduce the risks associated with cross-contamination while preparing the assay:**

- Always wear gloves (to protect the user and prevent introduction of nucleases).

**To reduce the risks associated with environmental contamination:**

- Follow current industry standards for disposal of contaminated waste.

 **CAUTION**

- 1 • Do not exceed the recommended temperature setting on heater.
- 2 • Do not exceed the recommended heating time.
- 3 • Use an appropriate, calibrated thermometer to verify the 3M™ Molecular Detection Heat
- 4 Block Insert temperature (e.g., a partial immersion thermometer or digital thermocouple
- 5 thermometer, not a total immersion thermometer.) The thermometer must be placed in the
- 6 designated location in the 3M Molecular Detection Heat Block Insert.
- 7

**NOTICE**

- 8 **To reduce the risks associated with cross-contamination while preparing the assay:**
- 9 • Use of sterile, aerosol barrier (filtered), molecular biology grade pipette tips is
- 10 recommended.
- 11 • Use a new pipette tip for each sample transfer.
- 12 • Use Good Laboratory Practices to transfer the sample from the enrichment to the lysis tube.
- 13 To avoid pipettor contamination, the user may choose to add an intermediate transfer step.
- 14 For example, the user can transfer each enriched sample into a sterile tube.
- 15 • Use a molecular biology workstation containing germicidal lamp where available.
- 16
- 17 **To reduce the risks associated with a false-positive result:**

- 1 • Never open tubes post amplification.
- 2 • Always dispose of the contaminated tubes by soaking in a 1-5% (v:v in water) household
- 3 bleach solution for 1 hour and away from the assay preparation area.

4  
5 Consult the Safety Data Sheet for additional information and local regulations for disposal.

6  
7 If you have questions about specific applications or procedures, please visit our website at  
8 [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

#### 9 10 **LIMITATION OF WARRANTIES / LIMITED REMEDY**

11 EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL  
12 PRODUCT PACKAGING, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES,  
13 INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR  
14 FITNESS FOR A PARTICULAR USE. If any 3M Food Safety Product is defective, 3M or its  
15 authorized distributor will, at its option, replace or refund the purchase price of the product.  
16 These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery  
17 of any suspected defects in a product and return it to 3M. Please call Customer Service (1-800-  
18 328-1671 in the U.S.) or your official 3M Food Safety representative for a Returned Goods  
19 Authorization.

#### 20 21 **LIMITATION OF 3M LIABILITY**

22 3M WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT,  
23 SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED  
24 TO LOST PROFITS. In no event shall 3M's liability under any legal theory exceed the purchase  
25 price of the product alleged to be defective.

#### 26 27 **USER RESPONSIBILITY**

28 Users are responsible for familiarizing themselves with product instructions and information.  
29 Visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety), or contact your local 3M representative or  
30 distributor for more information.

31 When selecting a test method, it is important to recognize that external factors such as sampling  
32 methods, testing protocols, sample preparation, handling, and laboratory technique may  
33 influence results.

1 It is the user's responsibility in selecting any test method or product to evaluate a sufficient  
2 number of samples with the appropriate matrices and microbial challenges to satisfy the user  
3 that the chosen test method meets the user's criteria.

4 It is also the user's responsibility to determine that any test methods and results meet its  
5 customers' and suppliers' requirements.

6 As with any test method, results obtained from use of any 3M Food Safety product do not  
7 constitute a guarantee of the quality of the matrices or processes tested.

8 To help customers evaluate the method for various food matrices, 3M has developed the 3M™  
9 Molecular Detection Matrix Control kit. When needed, use the Matrix Control (MC) to determine  
10 if the matrix has the ability to impact the 3M Molecular Detection Assay 2 - *Listeria*  
11 *monocytogenes* results. Test several samples representative of the matrix, i.e. samples  
12 obtained from different origin, during any validation period when adopting the 3M method or  
13 when testing new or unknown matrices or matrices that have undergone raw material or  
14 process changes.

15 A matrix can be defined as a type of product with intrinsic properties such as composition and  
16 process. Differences between matrices may be as simple as the effects caused by differences  
17 in their processing or presentation for example, raw vs. pasteurized; fresh vs. dried, etc.

18

### 19 **STORAGE AND DISPOSAL**

20 Store the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* at 2-8°C. Do not freeze.  
21 Keep kit away from light during storage. After opening the kit, check that the foil pouch is  
22 undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes should  
23 always be stored in the re-sealable pouch with the desiccant inside to maintain stability of the  
24 lyophilized reagents. Store resealed pouches at 2-8°C for no longer than 60days.

25

26 Do not use 3M Molecular Detection Assay 2 - *Listeria monocytogenes* past the expiration date.  
27 Expiration date and lot number are noted on the outside label of the box. After use, the  
28 enrichment medium and the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* tubes  
29 can potentially contain pathogenic materials. When testing is complete, follow current industry  
30 standards for the disposal of contaminated waste. Consult the Safety Data Sheet for additional  
31 information and local regulations for disposal.

32

### 33 **INSTRUCTIONS FOR USE**

34 Follow all instructions carefully. Failure to do so may lead to inaccurate results.

1 Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.)  
2 with a 1- 5% (v:v in water) household bleach solution or DNA removal solution.

3

#### 4 **SAMPLE ENRICHMENT**

5 Table 2 presents guidance for the enrichment of food and environmental samples. It is the  
6 user's responsibility to validate alternate sampling protocols or dilution ratios to ensure this test  
7 method meets the user's criteria.

8

#### 9 **Foods**

- 10 5. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to  
11 equilibrate to ambient laboratory temperature.
- 12 6. Aseptically combine the enrichment medium and sample according to Table 2. For all meat  
13 and highly particulate samples, the use of filter bags is recommended.
- 14 7. Homogenize thoroughly by blending, stomaching, or hand mixing for  $2 \pm 0.2$  minutes.  
15 Incubate at  $37 \pm 1^\circ\text{C}$  according to Table 2.
- 16 8. For raw dairy products, transfer 0.1 mL of the primary enrichment into 10 mL of Fraser  
17 Broth. Incubate at  $37 \pm 1^\circ\text{C}$  for 20-24 hours.

18

#### 19 **Environmental samples**

20 Sample collection devices can be a sponge hydrated with a neutralizing solution to inactivate  
21 the effects of the sanitizers. 3M recommends the use of a biocide-free cellulose sponge.  
22 Neutralizing solution can be Dey-Engley (D/E) Neutralizing Broth or Lethen broth. It is  
23 recommended to sanitize the area after sampling.

24

25

26 **WARNING:** Should you select to use Neutralizing Buffer (NB) that contains aryl sulfonate  
27 complex as the hydrating solution for the sponge, it is required to perform a 1:2 dilution (1 part  
28 sample into 1 part sterile enrichment broth) of the enriched environmental sample before testing  
29 in order to reduce the risks associated with a false-negative result leading to the release of  
30 contaminated product

31

32 The recommended size of the sampling area to verify the presence or absence of the pathogen  
33 on the surface is at least  $100 \text{ cm}^2$  (10 cm x 10 cm or 4"x4"). When sampling with a sponge,  
34 cover the entire area going in two directions (left to right then up and down) or collect  
35 environmental samples following your current sampling protocol or according to the FDA BAM

- 1 (1), USDA FSIS MLG (2) or ISO 18593 (6) guidelines.  
2  
3 4. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to  
4 equilibrate to ambient laboratory temperature.  
5 5. Aseptically combine the enrichment medium and sample according to Table 2.  
6 6. Homogenize thoroughly by blending, stomaching, or hand mixing for  $2 \pm 0.2$  minutes.  
7 Incubate at  $37 \pm 1^\circ\text{C}$  for 24-30 hours.  
8  
9

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1 **Table 2:** General Enrichment protocols using Demi-Fraser Broth Enrichment at 37±1°C

2

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)				
Heat-processed, cooked, cured meats, poultry, seafood and fish	25 g	225	24-30				
Heat-processed / pasteurized dairy products							
Produce and vegetables							
Multi-component foods							
Environmental samples	1 sponge	100 or 225	24-30				
	1 swab	10	24-30				
Raw meat, poultry, seafood, fish	25 g	475	28-32				
Sample Matrix	Secondary Enrichment (Fraser Broth)						
	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)	Sample Size	Enrichment Temperature (°C)	Enrichment Time (hr)	Sample Analysis Volume(a)
Raw dairy products	25 g	225	20-24	Transfer 0.1 mL into 10 mL Fraser Broth	37	20-24	10 µL

3 (b) Volume of sample transferred to Lysis Solution tubes. Refer to Step 4.6 of Lysis section.

4

5 **Table 3.** Enrichment Protocols according to AOAC RI Cert. No. XXXXXX at 37±1°C

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)
Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold	25 g	225	24-30

smoked salmon				
Deli Turkey		125 g	1175	24-30
Environmental samples:	stainless steel, sealed concrete	1 sponge	100 or 225	24-30
	Plastic,	1 swab	10	24-30
Raw Chicken		25 g	475	28-32

1

2 **PREPARATION OF THE 3M™ MOLECULAR DETECTION SPEED LOADER TRAY**

- 3 5. Wet a cloth with a 1-5% (v:v in water) household bleach solution and wipe the 3M™  
 4 Molecular Detection Speed Loader Tray.  
 5 6. Rinse the 3M Molecular Detection Speed Loader Tray with water.  
 6 7. Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.  
 7 8. Ensure the 3M Molecular Detection Speed Loader Tray is dry before use.

8 **PREPARATION OF THE 3M™ MOLECULAR DETECTION CHILL BLOCK INSERT**

9 Place the 3M™ Molecular Detection Chill Block directly on the laboratory bench; (the 3M™  
 10 Molecular Detection Chill Block Tray is not used). Use the chill block at ambient laboratory  
 11 temperature (20-25°C).

12

13 **PREPARATION OF THE 3M™ MOLECULAR DETECTION HEAT BLOCK INSERT**

14 Place the 3M™ Molecular Detection Heat Block Insert in a dry double block heater unit. Turn on  
 15 the dry block heater unit and set the temperature to allow the 3M Molecular Detection Heat  
 16 Block Insert to reach and maintain a temperature of 100 ±1°C.

17

18 **NOTE:** Depending on the heater unit, allow approximately 30 minutes for the 3M Molecular  
 19 Detection Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer  
 20 (e.g., a partial immersion thermometer or digital thermocouple thermometer, **not** a total  
 21 immersion thermometer) placed in the designated location, verify that the 3M Molecular  
 22 Detection Heat Block Insert is at 100 ±1°C.

23

## 1 PREPARATION OF THE 3M™ MOLECULAR DETECTION INSTRUMENT

- 2 4. Launch the 3M™ Molecular Detection Software and log in.
- 3 5. Turn on the 3M Molecular Detection Instrument.
- 4 6. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection System
- 5 User Manual for details.

6

7 **NOTE:** The 3M Molecular Detection Instrument must reach and maintain temperature of 60°C

8 before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This

9 heating step takes approximately 20 minutes and is indicated by an ORANGE light on the

10 instrument's status bar. When the instrument is ready to start a run, the status bar will turn

11 GREEN.

## 12

## 13 LYSIS

- 14 5. Allow the lysis solution (LS) tubes to warm up by setting the rack at room temperature (20-
- 15 25 °C) overnight (16-18 hours). Alternatives to equilibrate the LS tubes to room
- 16 temperature are to set the LS tubes on the laboratory bench for at least 2 hours, incubate
- 17 the LS tubes in a 37 ±1°C incubator for 1 hour or place them in a dry double block heater
- 18 for 30 seconds at 100°C.
- 19 6. Invert the capped tubes to mix. Proceed to next step within 4 hrs.
- 20 7. Remove the enrichment broth from the incubator.
- 21 8. One LS tube is required for each sample and the Negative Control (NC) (sterile
- 22 enrichment medium) sample.
- 23 8.1 LS tube strips can be cut to desired LS tube number. Select the number of individual LS
- 24 tubes or 8-tube strips needed. Place the LS tubes in an empty rack.
- 25 8.2 To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipette
- 26 tip for each transfer step.
- 27 4.3 Transfer enriched sample to LS tubes as described below:

28

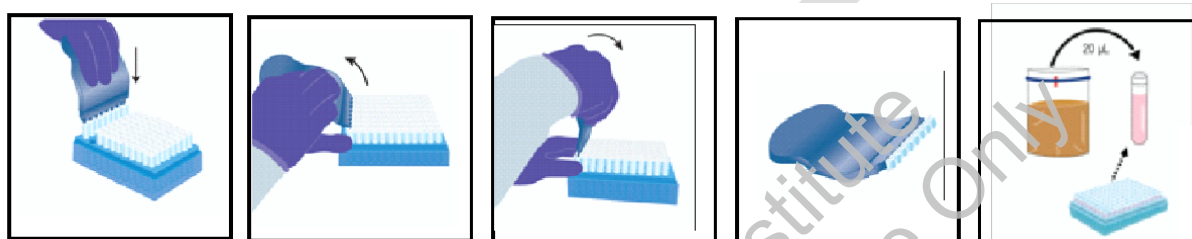
29 **Transfer each enriched sample into an individual LS tube first. Transfer the NC last.**

- 30
- 31 4.4 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip -
- 32 one strip at a time.

1 4.5 Discard the LS tube cap – if lysate will be retained for retest, place the caps into a  
2 clean container for re-application after lysis. For processing of retained lysate, see  
3 Appendix A.

4 4.6 Transfer 20  $\mu$ L of sample into a LS tube unless otherwise indicated in Protocol Table  
5 2. e.g. Raw dairy products use 10uL.

6 5. Repeat step 4.2 until each individual sample has been added to a corresponding LS tube  
7 in the strip.



9  
10  
11 6. Repeat steps 4.1 to 4.6 as needed, for the number of samples to be tested.

12 7. When all samples have been transferred, transfer 20  $\mu$ L or NC (sterile enrichment medium,  
13 e.g., Demi-Fraser Broth) into a LS tube. Do not use water as a NC.

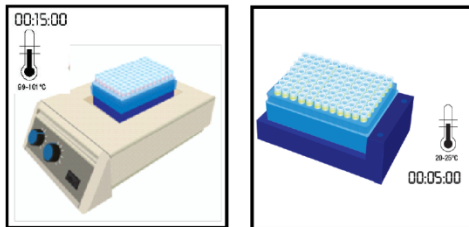
14 8. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^{\circ}\text{C}$ .

15 9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and  
16 heat for  $15 \pm 2$  minutes. During heating, the LS solution will change from pink (cool) to yellow  
17 (hot).

18 Samples that have not been properly heat treated during the assay lysis step may be  
19 considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection  
20 Instrument.

21 10. Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M  
22 Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes. The  
23 3M Molecular Chill Block Insert, used at ambient temperature without the Molecular Detection  
24 Chill Block Tray should sit directly on the laboratory bench. When cool, the lysis solution will  
25 revert to a pink color.

26  
27 11. Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.  
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## AMPLIFICATION

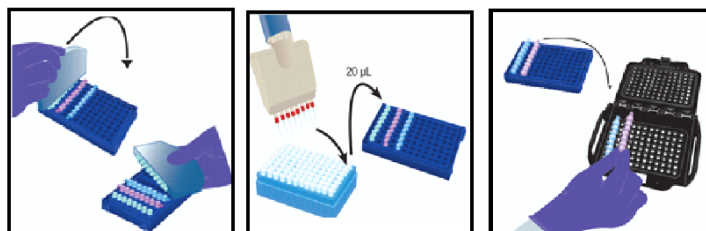
11. One Reagent tube is required for each sample and the NC.
  - 11.1 Reagent tube strips can be cut to desired tube number. Select the number of individual Reagent tubes or 8-tube strips needed.
  - 11.2 Place Reagent tubes in an empty rack.
  - 11.3 Avoid disturbing the reagent pellets from the bottom of the tubes.

12. Select 1 Reagent Control (RC) tube and place in rack.
13. To avoid cross-contamination, decap one Reagent tube strip at a time and use a new pipette tip for each transfer step.
14. Transfer lysate to Reagent tubes and RC tube as described below:

Transfer each sample lysate into individual Reagent tubes **first** followed by the NC. Hydrate the RC tube **last**.

15. Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent tubes – one Reagent tube strip at a time. Discard cap.
  - 15.1 Transfer 20 µL of Sample lysate from the upper ½ of the liquid (avoid precipitate) in the LS tube into corresponding Reagent tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
  - 15.2 Repeat step 5.1 until individual Sample lysate has been added to a corresponding Reagent tube in the strip.
  - 15.3 Cover the Reagent tubes with the provided extra caps and use the rounded side of the 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.
  - 15.4 Repeat step 5.1 as needed, for the number of samples to be tested.
  - 15.5 When all sample lysates have been transferred, repeat 4.1 to transfer 20 µL of NC lysate into a Reagent tube.
  - 15.6 Transfer **20 µL of NC lysate into a RC tube**. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.

- 1  
2  
3 16. Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader  
4 Tray. Close and latch the 3M Molecular Detection Speed Loader Tray lid.  
5



- 6  
7  
8 17. Review and confirm the configured run in the 3M Molecular Detection Software.  
9 18. Click the Start button in the software and select instrument for use. The selected  
10 instrument's lid automatically opens.  
11 19. Place the 3M Molecular Detection Speed Loader Tray into the 3M Molecular Detection  
12 Instrument and close the lid to start the assay. Results are provided within 75 minutes,  
13 although positives may be detected sooner.  
14 20. After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from  
15 the 3M Molecular Detection Instrument and dispose of the tubes by soaking in a 1-5% (v:v in  
16 water) household bleach solution for 1 hour and away from the assay preparation area.

17 **NOTICE:** To minimize the risk of false positives due to cross-contamination, never open reagent  
18 tubes containing amplified DNA. This includes Reagent Control, Reagent, and Matrix Control  
19 tubes. Always dispose of sealed reagent tubes by soaking in a 1-5% (v:v in water) household  
20 bleach solution for 1 hour and away from the assay preparation area.

21  
22 **RESULTS AND INTERPRETATION**

23 An algorithm interprets the light output curve resulting from the detection of the nucleic acid  
24 amplification. Results are analyzed automatically by the software and are color-coded based on  
25 the result. A Positive or Negative result is determined by analysis of a number of unique curve  
26 parameters. Presumptive positive results are reported in real-time while Negative and Inspect  
27 results will be displayed after the run is completed.

1 Presumptive positive samples should be confirmed as per the laboratory standard operating  
2 procedures or by following the appropriate reference method confirmation <sup>(1, 2, 3)</sup>, beginning with  
3 transfer from the primary enrichment to secondary enrichment broth (if applicable), followed by  
4 subsequent plating and confirmation of isolates using appropriate biochemical and serological  
5 methods.

6 **NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular  
7 Detection Assay 2 - *Listeria monocytogenes* amplification reagents have a “background” relative  
8 light unit (RLU).  
9

10 In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M  
11 recommends the user to repeat the assay for any Inspect samples. If the result continues to be  
12 Inspect, proceed to confirmation test using your preferred method or as specified by local  
13 regulations  
14

15 If you have questions about specific applications or procedures, please visit our website at  
16 [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.  
17

#### 18 REFERENCES:

- 19 7. US Food and Drug Administration Bacteriological Analysis Manual. Chapter 10: Detection  
20 and Enumeration of *Listeria monocytogenes* in Foods. Section C-6. April 2011 Version.
- 21 8. US Department of Agriculture (USDA) FSIS Microbiology Laboratory Guidebook 8.08.  
22 Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry and Egg  
23 Products, and Environmental Samples. Effective Date: 6 Nov 2012.
- 24 9. ISO 11290-1. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the  
25 Detection and Enumeration of *Listeria monocytogenes*. Amendment 1, 2004-10-15.
- 26 10. ISO/IEC 17025. General requirements for the competence of testing and calibration  
27 laboratories.
- 28 11. ISO 7218. Microbiology of food and animal feeding stuffs – General rules for microbiological  
29 examination.
- 30 12. ISO 18593. Microbiology of food and animal feeding stuffs – Horizontal methods for  
31 sampling techniques from surfaces using contact plates and swabs.  
32

#### 33 Appendix A. Protocol Interruption: Storage and re-testing of heat-treated lysates

- 34 8. To store a heat-treated lysate, re-cap the lysis tube with a clean cap (see “LYSIS”, 4.5)

- 1 9. Store at 4 to 8°C for up to 72 hours.
- 2 10. Prepare a stored sample for amplification by inverting 2-3 times to mix.
- 3 11. Decap the tubes.
- 4 12. Place the mixed lysate tubes on 3M Molecular Detection Heat Block Insert and heat at 100
- 5  $\pm 1^\circ\text{C}$  for  $5 \pm 1$  minutes.
- 6 13. Remove the rack of LS tubes from the heating block and allow to cool in the 3M Molecular
- 7 Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes.
- 8 14. Continue the protocol at the 'Amplification' section detailed above.

#### 11 EXPLANATION OF PRODUCT LABEL SAMPLES



13 Caution or Warning, see product instructions.



15 Consult product instructions.



17 The lot in a box represents the lot number.



18 The hourglass is followed by a month and year which represent the expiration date.



20 Storage temperature limitations.



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# Evaluation of the 3M™ Molecular Detection Assay(MDA) 2 –*Listeria* for the Detection of *Listeria* species in Select Foods and Environmental Surfaces: Collaborative Study

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**The 3M™ Molecular Detection Assay (MDA) 2 –*Listeria* uses loop-mediated isothermal amplification and bioluminescence detection to rapidly detect *Listeria* species in a broad range of food types and environmental surfaces. Using an unpaired study design, the MDA 2 -*Listeria* was compared to the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) Microbiology Laboratory Guidebook (MLG) Chapter 8.09/Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry and Egg Products, and Environmental Samples reference method for the detection of *Listeria* in deli turkey and raw chicken breast fillet. Technicians from 13 laboratories located within the continental United States and Canada participated in the collaborative study. Each matrix was evaluated at three levels of contamination: an un-inoculated control level (0 colony forming units (CFU)/test portion), a low inoculum level (0.2-2 CFU/test portion) and a high inoculum level (2-5 CFU/test portion). Statistical analysis was conducted according to the Probability of Detection (POD) statistical model. Results obtained for the low inoculum level test portions produced a dLPOD value with 95% confidence intervals of 0.04, (-0.08, 0.17) for deli turkey indicating no statistically significant difference between the candidate and reference methods. For raw chicken breast fillet a dLPOD value with 95% confidence interval of 0.16, (0.04, 0.28) indicating a statistically significant difference between the candidate and reference methods with a positive correlation in data indicating more recovery of the target analyte by the candidate method.**

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*Listeria*, particularly *L. monocytogenes*, is a pathogenic bacterium that contaminates food and is often found in food manufacturing facilities [1]. Due to the ubiquitous nature of *Listeria*, this organism is used as a hygiene indicator in all stages of the food processing chain. Detection of *Listeria* in the production and process facilities may be an indicator of unsanitary conditions [2]. The organism's ability to survive in extreme conditions make its presence in food a serious issue. In the past year, *Listeria* has been identified as the source of several high profile outbreaks involving bagged leafy greens and ice cream [3]. The 3M™ Molecular Detection Assay(MDA) 2 - *Listeria* method, using a combination of bioluminescence and isothermal amplification of nucleic

1 acid sequences, allows for the rapid and specific detection of *Listeria* species in a broad range of  
2 food types and environmental surfaces after 24 to 28 hours of pre-enrichment. After enrichment,  
3 samples are evaluated using the 3M MDA 2 - *Listeria* on the 3M™ Molecular Detection System  
4 (MDS). Presumptive positive results are reported in real-time while negative results are  
5 displayed after completion of the assay in approximately 75 minutes.

6 Prior to the collaborative study, the 3M MDA 2 - *Listeria* method was validated according to  
7 AOAC Guidelines[4] in a harmonized AOAC® Performance Tested Method<sup>SM</sup> (PTM) study.  
8 The objective of the PTM study was to demonstrate that the 3M MDA 2-*Listeria* method could  
9 detect *Listeria* in a broad range of food matrices and environmental surfaces as claimed by the  
10 manufacturer. For the 3M MDA 2- *Listeria* PTM evaluation, 13 matrices were evaluated: hot  
11 dogs (25g & 125g), salmon (25g), deli turkey (25g & 125g), cottage cheese (25g), vanilla ice  
12 cream (25g), queso fresco (25g), spinach (25g), melon (whole), raw chicken leg pieces (25g),  
13 raw chicken fillet (25g); concrete (sponge, 225 mL & 100 mL), stainless steel (sponge, 225 mL),  
14 and plastic (Enviroswab, 10 mL) environmental samples.

15 Additional PTM parameters (inclusivity, exclusivity, ruggedness, stability and lot-to-lot  
16 variability) tested in the PTM studies satisfied the performance requirements for PTM approval.  
17 The method was awarded PTM certification number 111501 on November 3<sup>rd</sup>, 2015.

18 The purpose of this collaborative study was to compare the reproducibility of the 3M MDA 2 -  
19 *Listeria* method to the United States Department of Agriculture (USDA) Food Safety Inspection  
20 Service (FSIS) -Microbiology Laboratory Guidebook (MLG) Chapter 8.09 *Isolation and*  
21 *Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and*  
22 *Environmental Samples*[5] for deli turkey (125 g) and raw chicken breast fillet.

## 24 Collaborative Study

### 26 Study Design

28 In this collaborative study, two matrices, deli turkey and raw chicken breast fillet, were evaluated.  
29 The matrices were obtained from a local retailer and screened for the presence of *Listeria* by the  
30 USDA/FSIS MLG 8.09 reference method. The raw chicken breast fillet was artificially  
31 contaminated with fresh unstressed cells of *Listeria monocytogenes*, American Type Culture  
32 Collection (ATCC) 7644, and the deli turkey was artificially contaminated with heat stressed  
33 cells of *Listeria monocytogenes*, ATCC 19115, at two inoculation levels: a high inoculation level  
34 of approximately 2-5 colony-forming units (CFU)/test portion and a low inoculation level of  
35 approximately 0.2-2 CFU/test portion. A set of un-inoculated control test portions (0 CFU/test  
36 portion) were also included.

37 Twelve replicate samples from each of the three inoculation levels were analyzed by each  
38 method. Two sets of samples (72 total) were sent to each laboratory for analysis by 3M MDA 2 -  
39 *Listeria* and the USDA/FSIS MLG Chapter 8.09 reference method due to the different sample  
40 enrichment procedures for each method. Additionally, collaborators were sent a 60 g test portion  
41 and instructed to conduct a total aerobic plate count (APC) using 3M™ Petrifilm™ Rapid Aerobic  
42 Count Plate (AOAC Official Method 2015.13) [6] on the day samples were received for the  
43 purpose of determining the total aerobic microbial load.

44 A detailed collaborative study packet outlining all necessary information related to the study  
45 including media preparation, test portion preparation and documentation of results was sent to  
46 each collaborating laboratory prior to the initiation of the study. A conference call was then  
47 conducted to discuss the details of the collaborative study packet and answer any questions from  
48 the participating laboratories.

1 *Preparation of Inocula and Test Portions*

2  
 3 The *Listeria* cultures used in this evaluation were propagated onto Tryptic Soy Agar with 5%  
 4 Sheep Blood (SBA) from a Q Laboratories frozen stock culture stored at -70°C. Each organism  
 5 was incubated for 24 ± 2 hours at 35 ± 1°C. Isolated colonies were picked to 10 mL of Brain  
 6 Heart Infusion (BHI) broth and incubated for 18 ± 0.5 hours at 35 ± 1°C. Raw chicken breast  
 7 fillet was inoculated in bulk using the fresh, broth culture. Prior to inoculation of the deli turkey,  
 8 the culture suspension was heat stressed at 55 ± 1°C in a water bath for 15 ± 0.5 minutes to obtain  
 9 a percent injury of 50-80% (as determined by plating onto selective Modified Oxford agar  
 10 (MOX) and non-selective tryptic soy agar with yeast (TSA/ye). The degree of injury was  
 11 estimated as:

$$(1 - \frac{n_{select}}{n_{nonselect}}) \times 100$$

12  
 13 where  $n_{select}$  = number of colonies on selective agar and  $n_{nonselect}$  = number of colonies on non-  
 14 selective agar. Appropriate dilutions of each culture were prepared in Butterfield's Phosphate  
 15 Diluent (BPD) based on previously established growth curves for both low and high inoculation  
 16 levels. Bulk portions of each matrix were inoculated with the diluted liquid inoculum and mixed  
 17 thoroughly to ensure an even distribution of microorganisms. The inoculated raw chicken  
 18 breastfillet was packaged into separate 30 g test portions in sterile Whirl-Pak® bags and shipped  
 19 to the collaborators. For the analysis of the deli turkey, 25 g of inoculated test product was  
 20 mixed with 100 g of un-inoculated test product to prepare 125 g test portions which were  
 21 packaged in sterile Whirl-Pak® bags and shipped to collaborators.

22 To determine the level of *Listeria* in the matrices a 5-tube most probable number (MPN) was  
 23 conducted by the coordinating laboratory on the day of the initiation of analysis using the  
 24 USDA/FSIS-MLG 8.09 reference method. For deli turkey, the MPN was determined by  
 25 analyzing 5 x 250 g test portions, the reference method test portions from the collaborating  
 26 laboratories and 5 x 65 g test portions. For the raw chicken breast fillet, the MPN of the high and  
 27 low inoculated levels was determined by analyzing 5 x 50 g test portions, the reference method  
 28 test portions from the collaborating laboratories and 5 x 10 g test portions. The MPN and 95%  
 29 confidence intervals were calculated using the LCF MPN Calculator, Version 1.6,  
 30 ([www.lcftld.com/customer/LCFMPNCalculator.exe](http://www.lcftld.com/customer/LCFMPNCalculator.exe)), provided by AOAC Research Institute  
 31 (RI) [7].

32  
 33 *Test Portion Distribution*

34  
 35 All samples were labeled with a randomized, blind-coded 3-digit number affixed to the sample  
 36 container. Test portions were shipped on a Thursday via overnight delivery according to the  
 37 Category B Dangerous Goods shipment regulations set forth by the International Air  
 38 Transportations Association (IATA). The two matrices were shipped consecutively, with  
 39 collaborating laboratories performing analysis on one matrix at a time. Upon receipt, samples  
 40 were held by the collaborating laboratory at refrigeration temperature (2-8 °C) until the  
 41 following Monday when analysis was initiated after a total equilibration time of 96 hours. All  
 42 samples were packed with cold packs to target a temperature of < 7°C during shipment.  
 43 In addition to each of the test portions and a separate APC sample, collaborators received a test  
 44 portion for each matrix labeled as 'temperature control'. Participants were instructed to obtain  
 45 the temperature of this portion upon receipt of the package, document the results on the Sample  
 46 Receipt Confirmation form provided and fax or email it back to the study director. The shipment  
 47 and hold times of the inoculated test material had been verified as a quality control measure prior  
 48 to study initiation.

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## Test Portion Analysis

Collaborators were instructed to follow the appropriate preparation and analysis as outlined in the study protocol for each matrix for both the 3M MDA 2 - *Listeria* method and reference method. For both matrices, each collaborator received 72 test portions (12 high, 12 low and 12 un-inoculated controls for each method to be performed). For the analysis of the deli turkey test portions by the 3M MDA 2 - *Listeria* method, a 125 g portion was enriched with 975 mL of Demi-Fraser (DF) broth, homogenized for 2 minutes and incubated for 24-28 hours at  $37 \pm 1^\circ\text{C}$ . For the raw chicken breast fillet test portions analyzed by the 3M MDA 2 - *Listeria* method, a 25 g portion was enriched with 475 mL of DF, homogenized for 2 minutes and incubated for 28-32 hours at  $37 \pm 1^\circ\text{C}$ .

Following enrichment, samples were assayed by the 3M MDA 2 - *Listeria* method and, regardless of presumptive result, confirmed following the USDA/FSIS MLG 8.09 reference method. Both matrices evaluated by the 3M MDA 2 - *Listeria* method were compared to samples analyzed using the USDA/FSIS MLG 8.09 reference method in an unpaired study design. All positive test portions were biochemically confirmed by the API *Listeria* biochemical test or by the VITEK 2 GPbiochemical identification test, AOAC Official Method 2012.02 [8].

## Statistical Analysis

Each collaborating laboratory recorded results for the reference method and the 3M MDA 2 - *Listeria* method on the data sheets provided. The data sheets were submitted to the study director at the end of each week of testing for statistical analysis. Data for each matrix was analyzed using the probability of detection (POD) statistical model [9]. The probability of detection (POD) was calculated as the number of positive outcomes divided by the total number of trials. The POD was calculated for the candidate presumptive results,  $\text{POD}_{\text{CP}}$ , the candidate confirmatory results (including false negative results),  $\text{POD}_{\text{CC}}$ , the difference in the candidate presumptive and confirmatory results,  $\text{dLPOD}_{\text{CP}}$ , presumptive candidate results that confirmed positive (excluding false negative results),  $\text{POD}_{\text{C}}$  the reference method,  $\text{POD}_{\text{R}}$ , and the difference in the confirmed candidate and reference methods,  $\text{dLPOD}_{\text{C}}$ . A  $\text{dLPOD}_{\text{C}}$  confidence interval not containing the point zero would indicate a statistically significant difference between the 3M MDA 2 - *Listeria* and the reference methods at the 5 % probability level. In addition to POD, the repeatability standard deviation ( $s_{\text{r}}$ ), the among laboratory repeatability standard deviation ( $s_{\text{L}}$ ), the reproducibility standard deviation ( $s_{\text{R}}$ ) and the  $P_{\text{T}}$  value were calculated. The  $s_{\text{r}}$  provides the variance of data within one laboratory, the  $s_{\text{L}}$  provides the difference in standard deviation between laboratories and the  $s_{\text{R}}$  provides the variance in data between different laboratories. The  $P_{\text{T}}$  value provides information on the homogeneity test of laboratory PODs [10].





- 1 (n) *Demi Fraser Broth* - Available from 3M Food Safety (St. Paul, MN 55144-1000,  
2 USA).
- 3 (o) *Ferric Ammonium Citrate (FAC)*, ACS grade, 5% sterilized -Available from MP  
4 Biomedicals™ or equivalent.
- 5 (p) *Disposable pipette* – capable of 20 µL
- 6 (q) *Multi-channel (8-channel) pipette* - capable of 20 µL
- 7 (r) *Sterile filter tip pipette tips* - capable of 20 µL
- 8 (s) *Filter Stomacher® bags* – Seward or equivalent.
- 9 (t) *Stomacher®*– Seward or equivalent.
- 10 (u) *Thermometer* – calibrated range to include  $100 \pm 1^\circ\text{C}$
- 11 (v) *Dry block heater unit*– capable of maintaining  $100 \pm 1^\circ\text{C}$
- 12 (w) *Incubators*. – Capable of maintaining  $37 \pm 1^\circ\text{C}$  or  $41.5 \pm 1^\circ\text{C}$ .
- 13 (x) *Freezer* – capable of maintaining -10 to -20°C, for storing the 3M Molecular  
14 Detection Chill Block Tray
- 15 (y) *Refrigerator* – capable of maintaining 2-8°C, for storing the 3M Molecular Detection  
16 Assay components
- 17 (z) *Computer* – compatible with the 3M™ Molecular Detection Instrument
- 18 (aa) *3M™ Enviroswab*, -- (hydrated with Lethen) Available from 3M Food  
19 Safety(Australia)
- 20 (bb) *3M™ Hydrated Sponge Stick with 10 mL of D/E* – Available from 3M Food  
21 Safety(St. Paul, MN 55144-1000, USA)
- 22

### 23 C. General Instructions

24

- 25 (a) Store the 3M Molecular Detection Assay 2 - *Listeria* at 2-8°C. Do not freeze. Keep  
26 kit away from light during storage. After opening the kit, check that the foil pouch is  
27 undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes  
28 should always be stored in the re-sealable pouch with the desiccant inside to maintain  
29 stability of the lyophilized reagents. Store resealed pouches at 2-8°C for no longer  
30 than 60 days. Do not use 3M Molecular Detection Assay 2 -*Listeria* past the  
31 expiration date.
- 32 (b) Follow all instructions carefully. Failure to do so may lead to inaccurate results.

#### 33 *Safety Precautions*

34 The 3M MDA2 – *Listeria* is intended for use in a laboratory environment by  
35 professionals trained in laboratory techniques. 3M has not documented the use of this  
36 product in industries other than the food and beverage industries. For example, 3M  
37 has not documented this product for testing drinking water, pharmaceutical,  
38 cosmetics, clinical or veterinary samples. The 3M MDA2 – *Listeria* has not been  
39 evaluated with all possible food products, food processes, testing protocols or with all  
40 possible strains of bacteria.

41 As with all test methods, the source of enrichment medium can influence the results.  
42 The 3M MDA 2 – *Listeria* has only been evaluated for use with the enrichment media  
43 specified in the Instructions for Use section.

1 The 3M™ Molecular Detection instrument is intended for use with samples that have  
2 undergone heat treatment during the assay lysis step, which is designed to destroy  
3 organisms present in the sample. Samples that have not been properly heat treated  
4 during the assay lysis step may be considered a potential biohazard and should NOT  
5 be inserted into the 3M MDS instrument.

6 The user should read, understand and follow all safety information in the instructions  
7 for the 3M MDS and the 3M MDA 2 – *Listeria*. Retain the safety instructions for  
8 future reference.

9 To reduce the risks associated with exposure to chemicals and biohazards:  
10 Perform pathogen testing in a properly equipped laboratory under the control of  
11 trained personnel. Always follow standard laboratory safety practices, including  
12 wearing appropriate protective apparel and eye protection while handling reagents  
13 and contaminated samples. Avoid contact with the contents of the enrichment media  
14 and reagent tubes after amplification. Dispose of enriched samples according to  
15 current industry standards.

16 *Listeria monocytogenes* is of particular concern for pregnant women, the aged and the  
17 infirmed. It is recommended that these concerned groups avoid handling this  
18 organism. After use, the enrichment media and the 3M MDA 2 - *Listeria* tubes can  
19 potentially contain pathogenic materials. Periodically decontaminate laboratory  
20 benches and equipment (pipettes, cap/decap tools, etc.) with a 1- 5% (v:v in water)  
21 household bleach solution or DNA removal solution. When testing is complete,  
22 follow current industry standards for the disposal of contaminated waste. Consult the  
23 Safety Data Sheet for additional information and local regulations for disposal.

24 To reduce the risks associated with environmental contamination: Follow current  
25 industry standards for disposal of contaminated waste.

#### 26 **D. Sample Enrichment**

##### 27 *Foods*

- 28
- 29 (a) Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate)  
30 to equilibrate to ambient laboratory temperature (20-25°C).
- 31 (b) Aseptically combine the enrichment medium and sample according to Table 2. For all  
32 meat and highly particulate samples, the use of filter bags is recommended.
- 33 (c) Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ±0.2 minutes.  
34 Incubate at 37 ±1°C according to Table A.
- 35

##### 36 *Environmental samples*

- 37 (d) Sample collection devices can be a sponge hydrated with a neutralizing solution to  
38 inactivate the effects of the sanitizers. 3M recommends the use of a biocide-free  
39 cellulose sponge. Neutralizing solution can be Dey-Engley (D/E) Neutralizing Broth  
40 or Lethen broth. It is recommended to sanitize the area after sampling.

1  
2 WARNING: Should you select to use Neutralizing Buffer (NB) that contains aryl  
3 sulfonate complex as the hydrating solution for the sponge, it is required to perform a  
4 1:2 dilution (1-part sample into 1-part sterile enrichment broth) of the enriched  
5 environmental sample before testing in order to reduce the risks associated with a  
6 false-negative result leading to the release of contaminated product. Another option is  
7 to transfer 10 µL of the NB enrichment in the LS tubes.  
8

9 (e) The recommended size of the sampling area to verify the presence or absence of the  
10 pathogen on the surface is at least 100 cm<sup>2</sup> (10 cm x 10 cm or 4"x4"). When sampling  
11 with a sponge, cover the entire area going in two directions (left to right then up and  
12 down) or collect environmental samples following your current sampling protocol or  
13 according to the FDA BAM, USDA FSIS MLG or ISO 18593 [11] guidelines.

- 14 1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium  
15 citrate) to equilibrate to ambient laboratory temperature (20-25°C).
- 16 2. Aseptically combine the enrichment medium and sample according to Table 2.
- 17 3. Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ±0.2  
18 minutes. Incubate at 37 ±1°C for 24-30 hours.

#### 19 20 E. PREPARATION OF THE 3M™ MOLECULAR DETECTION SPEED LOADER TRAY

- 21 (a) Wet a cloth or paper towel with a 1-5% (v:v in water) household bleach solution and  
22 wipe the 3M™ Molecular Detection Speed Loader Tray.
- 23 (b) Rinse the 3M Molecular Detection Speed Loader Tray with water.
- 24 (c) Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.
- 25 (d) Ensure the 3M Molecular Detection Speed Loader Tray is dry before use  
26

#### 27 Specific Instructions for Validated Methods

28 AOAC® Performance Tested Method<sup>SM</sup>#111501



31

32 In AOAC PTM studies, the 3M Molecular Detection Assay 2 – *Listeria* was found to be an  
33 effective method for the detection of *Listeria* species. The matrices tested in the study are  
34 shown in Table 2. The limit of detection of the 3M Molecular Detection Assay 2 – *Listeria*  
35 method is 1-5 colony forming units per validated test portion size (in Table 2).



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4  
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**Table 2:** Enrichment protocols using Demi-Fraser Broth at  $37 \pm 1$  °C according to AOAC Performance Tested <sup>SM</sup> Certificate #111501

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)	
Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon	25 g	225	24-30	
Raw chicken	25 g	475	28-32	
Deli turkey	125 g	1125	24-30	
Cantaloupe	Whole melon	Enough volume to allow melon to float	26-30	
Environmental samples:	Stainless steel	1 sponge	225	24-30
	Sealed concrete	1 sponge	100	24-30
	Plastic	1 swab	10	24-30

6  
7

**F. PREPARATION OF THE 3M™ MOLECULAR DETECTION HEAT BLOCK INSERT**

Place the 3M™ Molecular Detection Heat Block Insert in a dry double block heater unit. Turn on the dry block heater unit and set the temperature to allow the 3M Molecular Detection Heat Block Insert to reach and maintain a temperature of  $100 \pm 1$  °C.

**NOTE:** Depending on the heater unit, allow approximately 30minutes for the 3M Molecular Detection Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (e.g., a partial

12  
13

immersion thermometer, digital thermocouple thermometer, not a total immersion thermometer) placed in the designated location, verify that the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^\circ\text{C}$ .

### G. PREPARATION OF THE 3M MOLECULAR DETECTION INSTRUMENT

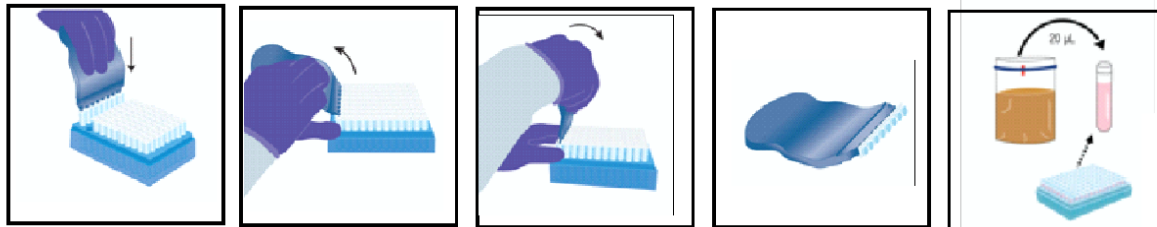
1. Launch the 3M™ Molecular Detection Software and log in.
2. Turn on the 3M Molecular Detection Instrument.
3. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection System User Manual for details.

**NOTE:** The 3M Molecular Detection Instrument must reach and maintain temperature of  $60^\circ\text{C}$  before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 minutes and is indicated by an ORANGE light on the instrument's status bar. When the instrument is ready to start a run, the status bar will turn GREEN.

### H. LYSIS

1. Allow the lysis solution (LS) tubes to warm up by setting the rack at room temperature ( $20\text{--}25^\circ\text{C}$ ) overnight (16-18 hours). Alternatives to equilibrate the LS tubes to room temperature are to set the LS tubes on the laboratory bench for at least 2 hours, incubate the LS tubes in a  $37 \pm 1^\circ\text{C}$  incubator for 1 hour or place them in a dry double block heater for 30 seconds at  $100^\circ\text{C}$ .
2. Invert the capped tubes to mix. Proceed to next step within 4 hrs.
3. Remove the enrichment broth from the incubator.
4. One LS tube is required for each sample and the Negative Control (NC) (sterile enrichment medium) sample.
  - 4.1 LS tube strips can be cut to desired LS tube number. Select the number of individual LS tubes or 8-tube strips needed. Place the LS tubes in an empty rack.
  - 4.2 To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipette tip for each transfer step.
  - 4.3 Transfer enriched sample to LS tubes as described below:

Transfer each enriched sample into individual LS tube **first**. Transfer the NC **last**.
  - 4.4 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip - one strip at a time.
  - 4.5 Discard the LS tube cap – if lysate will be retained for retest, place the caps into a clean container for re-application after lysis
  - 4.6 Transfer  $20\ \mu\text{L}$  of sample into a LS tube.
5. Repeat step 4.2 until each individual sample has been added to a corresponding LS tube in the strip as illustrated below.



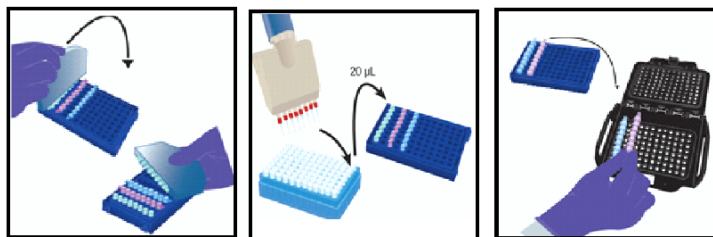
6. Repeat steps 4.1 to 4.6 as needed, for the number of samples to be tested. When all samples have been transferred, then transfer **20  $\mu$ L of NC into a LS tube**. Do not recap tubes.
7. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^\circ\text{C}$ . Place the rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for  $15 \pm 1$  minutes. During heating, the LS solution will change from pink (cool) to yellow (hot).
8. Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes. The 3M Molecular Chill block Insert, used at ambient temperature ( $20\text{-}25^\circ\text{C}$ ) without the Molecular Detection Chill Block Tray, should sit directly on the laboratory bench. When cool, the lysis solution will revert to a pink color.
6. Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.

## I. AMPLIFICATION

1. One Reagent tube is required for each sample and the NC.
  - 1.1 Reagent tubes strips can be cut to desired tube number. Select the number of individual Reagent tubes or 8-tube strips needed.
  - 1.2 Place Reagent tubes in an empty rack.
  - 1.3 Avoid disturbing the reagent pellets from the bottom of the tubes.
2. Select 1 Reagent Control (RC) tube and place in rack.
3. To avoid cross-contamination, decap one Reagent tubes strip at a time and use a new pipette tip for each transfer step.
4. Transfer lysate to Reagent tubes and RC tube as described below:

Transfer each sample lysate into individual Reagent tubes **first** followed by the NC. Hydrate the RC tube **last**.

- 4.1 Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent tubes –one Reagent tubes strip at a time. Discard cap.
- 4.2 Transfer  $20 \mu\text{L}$  of Sample lysate from the upper  $\frac{1}{2}$  of the liquid (avoid precipitate) in the LS tube into corresponding Reagent tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
- 4.3 Repeat step 4.2 until individual Sample lysate has been added to a corresponding Reagent tube in the strip.
- 4.4 Cover the Reagent tubes with the provided extra cap and use the rounded side of the 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.
- 4.5 Repeat steps 4.1 to 4.4 as needed, for the number of samples to be tested.
- 4.6 When all sample lysates have been transferred, repeat 4.1 to 4.4 to transfer  $20 \mu\text{L}$  of NC lysate into a Reagent tube.
- 4.7 Transfer  **$20 \mu\text{L}$  of NC lysate into a RC tube**. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
5. Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader Tray. See illustration below. Close and latch the 3M Molecular Detection Speed Loader Tray lid.



6. Review and confirm the configured run in the 3M Molecular Detection Software.
7. Click the Start button in the software and select instrument for use. The selected instrument's lid automatically opens.
8. Place the 3M Molecular Detection Speed Loader Tray into the 3M MDS Instrument and close the lid to start the assay. Results are provided within 75minutes, although positives may be detected sooner.
9. After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from the 3M Molecular Detection Instrument and dispose of the tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

**NOTICE:** To minimize the risk of false positives due to cross-contamination, never open reagent tubes containing amplified DNA. This includes Reagent Control, Reagent and Matrix Control tubes. Always dispose of sealed reagent tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

#### RESULTS AND INTERPRETATION

An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analyzed automatically by the software and are color-coded based on the result. A Positive or Negative result is determined by analysis of a number of unique curve parameters. Presumptive positive results are reported in real-time while Negative and Inspect results will be displayed after the run is completed.

Presumptive positive samples should be confirmed as per the laboratory standard operating procedures or by following the current version of the appropriate reference method confirmation ([FDA/BAM](#), the [USDA/FSIS-MLG](#)), beginning with transfer from the primary enrichment to secondary enrichment broth (if applicable), followed by subsequent plating and confirmation of isolates using appropriate biochemical and serological methods.

**NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular Detection Assay 2 - *Listeria* amplification reagents have a “background” relative light unit (RLU) reading.

In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M recommends the user to repeat the assay for any Inspect samples. If the result continues to be Inspect, proceed to confirmation test using your preferred method or as specified by local regulations.

1 *Results of Collaborative Study*

2  
 3 For this collaborative study, the 3M Molecular Detection Assay (MDA) 2 - *Listeria* method was  
 4 compared to the USDA FSIS MLG 8.09 reference method for deli turkey and raw chicken breast  
 5 fillet. A total of 13 laboratories throughout the United States and Canada participated in this  
 6 study, with 11 laboratories submitting data for the deli turkey and 12 laboratories submitting data  
 7 for the raw chicken breast fillet. See Table 1 for a summary of laboratory participation for each  
 8 matrix. Each laboratory analyzed 36 test portions for each method per matrix: 12 inoculated  
 9 with a high level of *Listeria*, 12 inoculated with a low level of *Listeria*, and 12 un-inoculated  
 10 controls.

11 A background screen of the matrix indicated an absence of indigenous *Listeria* species in both  
 12 matrices. Ten (10) replicate test portions (randomly sampled from 50% of the total packages used  
 13 in the analysis) were screened for the presence of *Listeria* species. All test portions produced  
 14 negative results for the target analyte.

15 Results for the heat stress analysis of the inoculum for the deli turkey are presented in Table 2.  
 16 Table 2016.1A and 2016.1B summarize the inter-laboratory results for all foods tested, including  
 17 POD statistical analysis. As per criteria outlined in Appendix J of the AOAC Validation  
 18 Guidelines, fractional positive results were obtained. Detailed results for each laboratory are  
 19 presented in Tables 2016.2A and 2016.2B. For each matrix, the level of *Listeria* was determined  
 20 by MPN on the day of initiation of analysis by the coordinating laboratory. MPN results are  
 21 presented in Tables 2016.2A and 2016.2B. The individual laboratory and sample results are  
 22 presented in Tables 1-2 of the Supplementary Materials. The APC results for each collaborating  
 23 are presented in Table 3 of the Supplementary Materials.

24  
 25 **Deli Turkey (125 g Test Portions)**

26  
 27 Deli turkey test portions were inoculated at a low and high level and were analyzed for the  
 28 detection of *Listeria* spp. Un-inoculated controls were included in each analysis. Laboratories 8  
 29 and 10 received test portions but were unable to conduct the analysis and therefore no data was  
 30 submitted. All other laboratories submitted data for both methods evaluated. The MPN levels  
 31 obtained for this matrix, with 95% confidence intervals, were 0.63 CFU/test portion (0.49, 0.80)  
 32 for the low inoculum level and 4.52 CFU/test portion (3.19, 6.42) for the high inoculum level.

33 For the low inoculum level, 68 out of 132 test portions (POD<sub>CP</sub> of 0.52) were reported as  
 34 presumptive positive by the 3M MDA 2 – *Listeria* method with 66 out of 132 test portions  
 35 (POD<sub>CC</sub> of 0.50) confirming positive. For samples that produced presumptive positive results on  
 36 the 3M MDA 2 – *Listeria* method, 66 out of 132 samples confirmed positive (POD<sub>C</sub> of 0.50). For  
 37 test portions evaluated by the USDA/FSIS MLG reference method, 60 out of 132 test portions  
 38 produced positive results. A dLPOD<sub>C</sub> value of 0.04 with 95% confidence intervals of  
 39 (-0.08, 0.17) was obtained between the candidate and reference method, indicating no statistical  
 40 significant difference between the two methods. A dLPOD<sub>CP</sub> value of 0.02 with 95% confidence  
 41 intervals of (-0.11, 0.14) was obtained between presumptive and confirmed results indicating no  
 42 statistically significant difference between the presumptive and confirmed results.

1 For the high inoculum level, 132 out of 132 test portions ( $POD_{CP}$  of 1.00) were reported as  
 2 presumptive positive by the 3M MDA 2 – *Listeria* method with 132 out of 132 test portions  
 3 ( $POD_{CC}$  of 1.00) confirming positive. For samples that produced presumptive positive results on  
 4 the 3M MDA 2 – *Listeria* method, 132 out of 132 samples confirmed positive ( $POD_C$  of 1.00). For  
 5 test portions evaluated by the USDA/FSIS MLG reference method, 132 out of 132 test portions  
 6 produced positive results. A  $dLPOD_C$  value of 0.00 with 95% confidence intervals of (-0.03,  
 7 0.03) was obtained between the candidate and reference method, indicating no statistical  
 8 significant difference between the two methods. A  $dLPOD_{CP}$  value of 0.00 with 95% confidence  
 9 intervals of (-0.03, 0.03) was obtained between presumptive and confirmed results indicating no  
 10 statistically significant difference between the presumptive and confirmed results.

11 For the un-inoculated controls, 0 out of 132 samples ( $POD_{CP}$  of 0.00) produced a presumptive  
 12 positive result by the 3M MDA 2 - *Listeria* method with 0 out of 132 test portions ( $POD_{CC}$  of  
 13 0.00) confirming positive. For samples that produced presumptive positive results on the 3M  
 14 MDA 2 – *Listeria* method, 0 out of 132 samples confirmed positive ( $POD_C$  of 0.00). For test  
 15 portions evaluated by the USDA/FSIS MLG reference method, 0 out of 132 test portions  
 16 produced positive results. A  $dLPOD_C$  value of 0.00 with 95% confidence intervals of (-0.03,  
 17 0.03) was obtained between the candidate and reference method, indicating no statistical  
 18 significant difference between the two methods. A  $dLPOD_{CP}$  value of 0.00 with 95% confidence  
 19 intervals of (-0.03, 0.00) was obtained between presumptive and confirmed results indicating no  
 20 statistically significant difference between the presumptive and confirmed results.

21  
 22 Detailed results of the POD statistical analysis are presented in Table 2016.2A and Figures 1A-  
 23 1B.

24

### 25 **Raw Chicken Breast Fillet (25 g Test Portions)**

26

27 Raw chicken breast fillet test portions were inoculated at a low and high inoculum level and were  
 28 analyzed for the detection of *Listeria* spp. Un-inoculated controls were included in each  
 29 analysis. Laboratory 11 did not participate in the evaluation of this matrix. Laboratory 10  
 30 submitted data that indicated cross contamination of the inoculating organism in the un-  
 31 inoculated control samples. Further analysis at the coordinating laboratory confirmed the cross  
 32 contamination of the un-inoculated controls. Due to this issue, the data submitted from  
 33 Laboratory 10 was not used in the statistical analysis. All other laboratories submitted data for  
 34 both methods evaluated. The MPN levels obtained for this matrix, with 95% confidence  
 35 intervals, were 0.66 CFU/test portion (0.51, 0.83) for the low level and 6.24 CFU/test portion  
 36 (3.58, 10.88) for the high level.

37 For the low inoculum level, 88 out of 132 test portions ( $POD_{CP}$  of 0.67) were reported as  
 38 presumptive positive by the 3M MDA 2 – *Listeria* method with 86 out of 132 test portions  
 39 ( $POD_{CC}$  of 0.65) confirming positive. For samples that produced presumptive positive results on  
 40 the 3M MDA 2 – *Listeria* method, 85 out of 132 test portions confirmed positive ( $POD_C$  of 0.64).  
 41 For test portions evaluated by the USDA/FSIS MLG reference method, 64 out of 132 test  
 42 portions produced positive results. A  $dLPOD_C$  value of 0.16 with 95% confidence intervals of  
 43 (0.04, 0.28) was obtained between the candidate and reference method, indicating a statistically



1 significant difference between the two methods, with a positive with a positive correlation in  
 2 data indicating more recovery of the target analyte by the candidate method. A  $dLPOD_{CP}$  value  
 3 of 0.02 with 95% confidence intervals of (-0.10, 0.13) was obtained between presumptive and  
 4 confirmed results indicating no statistically significant difference between the presumptive and  
 5 confirmed results.

6 For the high inoculum level, 131 out of 132 test portions ( $POD_{CP}$  of 0.99) were reported as  
 7 presumptive positive by the 3M MDA 2 – *Listeria* method with 132 out of 132 test portions  
 8 ( $POD_{CC}$  of 1.00) confirming positive. For samples that produced presumptive positive results on  
 9 the 3M MDA 2 – *Listeria* method, 131 out of 132 samples confirmed positive ( $POD_C$  of 0.99).  
 10 For test portions evaluated by the USDA/FSIS-MLG reference method, 132 out of 132 test  
 11 portions produced positive results. A  $dLPOD_C$  value of -0.01 with 95% confidence intervals of  
 12 (-0.04, 0.02) was obtained between the candidate and reference method, indicating no  
 13 statistically significant difference between the two methods. A  $dLPOD_{CP}$  value of -0.01 with  
 14 95% confidence intervals of (-0.04, 0.02) was obtained between presumptive and confirmed  
 15 results indicating no statistically significant difference between the presumptive and confirmed  
 16 results.

17 For the un-inoculated controls, 2 out of 132 samples ( $POD_{CP}$  of 0.02) produced a presumptive  
 18 positive result by the 3M MDA 2 - *Listeria* method with 1 out of a 132 test portions ( $POD_{CC}$  of  
 19 0.01) confirming positive. For samples that produced presumptive positive results on the 3M  
 20 MDA 2 – *Listeria* method, 1 out of 132 samples confirmed positive ( $POD_C$  of 0.01). For test  
 21 portions evaluated by the USDA/FSIS-MLG reference method, 0 out of 132 test portions  
 22 produced positive results. A  $dLPOD_C$  value of 0.01 with 95% confidence intervals of (-0.02,  
 23 0.04) was obtained between the candidate and reference method, indicating no statistical  
 24 significant difference between the two methods. A  $dLPOD_{CP}$  value of 0.01 with 95% confidence  
 25 intervals of (-0.03, 0.05) was obtained between presumptive and confirmed results indicating no  
 26 statistically significant difference between the presumptive and confirmed results.

27  
 28 Detailed results of the POD statistical analysis are presented in Table 2016.2B and Figures 1C-  
 29 1D.

### 31 Discussion

32  
 33 No negative feedback was provided by the collaborating laboratories in regard to the  
 34 performance of the 3M MDA 2- *Listeria* method. During the evaluation of the raw chicken breast  
 35 fillet, Laboratory 2 isolated *Listeria innocua* from an un-inoculated control sample. Since the  
 36 organism recovered was different from the inoculating organism, *Listeria monocytogenes*, no  
 37 just cause for removal of the data was determined and the data was included in the manuscript.  
 38 For the raw chicken breast fillet, Laboratory 10 reported isolating *Listeria monocytogenes* from  
 39 two un-inoculated control samples. The isolates were sent for further identification and it was  
 40 determined that they were the same strain as the inoculating organism, indicating that cross  
 41 contamination of the sample occurred. Due to the fact that cross contamination occurred, just  
 42 cause removal of the data was established and the data generated by Laboratory 10 was therefore  
 43 not included in the statistical analysis.

1 Overall, the data generated during this evaluation demonstrates the reproducibility of this new  
2 method. For the deli turkey analysis, the POD statistical analysis indicated that no statistically  
3 significant difference between the candidate method and the reference method or between the  
4 presumptive and confirmed results of the candidate method was obtained. For raw chicken breast  
5 fillet, a statistically significant difference was observed between the reference and the alternative  
6 method. The dLPOD data indicated a positive correlation in data indicating more recovery of the  
7 target analyte by the candidate method. One possible contribution to the higher level of recovery  
8 observed with the 3M MDA 2 – *Listeria* method was the use of Demi-Fraser Broth for the  
9 candidate method. This enrichment media formulation is less selective than the modified  
10 University of Vermont Medium used in the USDA reference method and may have contributed  
11 to the higher level of recovery observed during the evaluation. A second possible contribution to  
12 the higher level of recovery was the length of the primary enrichment. Test portions evaluated  
13 by the 3M MDA 2 – *Listeria* method were incubated for a minimum of 28 hours in the primary  
14 enrichment, while the USDA reference method had a maximum primary enrichment time of 26  
15 hours. No statistically significant difference was observed between the candidate method  
16 presumptive and confirmed results for this matrix.

## 17 **Recommendations**

18  
19  
20 It is recommended that the 3M Molecular Detection Assay 2 – *Listeria* method be adopted as  
21 Official First Action status for the detection of *Listeria* in selected foods: hot dogs (25g & 125g),  
22 salmon (25g), deli turkey (25g & 125g), cottage cheese (25g), vanilla ice cream (25g), queso  
23 fresco (25g), spinach (25g), melon (whole), raw chicken leg pieces (25g), raw chicken fillet  
24 (25g); concrete, stainless steel and plastic environmental samples.

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6  
7  
8

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**Table 1: Participation of each Collaborating Laboratory<sup>a</sup>**

Lab	Deli Turkey <sup>a,b</sup>	Raw Chicken Breast Fillet <sup>a,b</sup>
1	Y	Y
2	Y	Y
3	Y	Y
4	Y	Y
5	Y	Y
6	Y	Y
7	Y	Y
8	N	Y
9	Y	Y
10	N	Y
11	Y	N
12	Y	Y
13	Y	Y

<sup>a</sup> Y= Collaborator analyzed the food type; <sup>a</sup>N= Collaborator did not analyze food type

**Table 2: Heat-Stress Injury Results**

Matrix	Test Organism <sup>a</sup>	CFU/MOX (Selective Agar)	CFU/ TSA (Non-Selective Agar)	Degree Injury <sup>b</sup>
Deli Turkey	<i>Listeriamonocytogenes</i> ATCC 19115	9.1 x 10 <sup>8</sup>	2.5 x 10 <sup>9</sup>	60.8%

<sup>a</sup> ATCC- American Type Culture Collection

<sup>b</sup> Cultures were heat stressed for 10 minutes at 55°C in a recirculating water bath.

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**Table 2016.1A:** Summary of Results for the Detection of *Listeria* in Deli Turkey (125g)

Method <sup>a</sup>	3M™ MDA™2 - <i>Listeria</i>		
Inoculation Level	Un-inoculated	Low	High
Candidate Presumptive Positive/ Total # of Samples Analyzed	0/132	68/132	132/132
Candidate Presumptive POD (CP)	0.00 (0.00, 0.03)	0.52 (0.43, 0.60)	1.00 (0.97, 1.00)
$s_r^b$	0.00 (0.00, 0.16)	0.51 (0.45, 0.52)	0.00 (0.00, 0.16)
$s_L^c$	0.00 (0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
$s_R^d$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value <sup>e</sup>	1.0000	0.8762	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/132	66/132	132/132
Candidate Confirmed POD (CC)	0.00 (0.00, 0.03)	0.50 (0.41, 0.59)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9123	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/132	66/132	132/132
Candidate Presumptive Positive that Confirmed POD (C)	0.00 (0.00, 0.03)	0.50 (0.41, 0.59)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9123	1.0000
Positive Reference Samples/ Total # of Samples Analyzed	0/132	60/132	132/132
Reference POD	0.00 (0.00, 0.03)	0.45 (0.37, 0.54)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.11)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9829	1.0000
dLPOD (Candidate vs. Reference) <sup>f</sup>	0.00 (-0.03, 0.03)	0.04 (-0.08, 0.17)	0.00 (-0.03, 0.03)
dLPOD (Candidate Presumptive vs. Candidate Confirmed) <sup>f</sup>	0.00 (-0.03, 0.03)	0.02 (-0.11, 0.14)	0.00 (-0.03, 0.03)

<sup>a</sup> Results include 95% Confidence Intervals, <sup>r</sup> Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation

<sup>e</sup> P Value = Homogeneity test of laboratory PODs; <sup>f</sup> A confidence interval for dLPOD that does not contain the value 0 indicates a statistical significant difference between the two methods

**Table 2016.1B:** Summary of Results for the Detection of *Listeria* in Raw Chicken Breast Fillet (25g)

Method <sup>a</sup>	3M™ MDA™ 2 - <i>Listeria</i>		
Inoculation Level	Un-inoculated	Low	High
Candidate Presumptive Positive/ Total # of Samples Analyzed	2/132	88/132	131/132
Candidate Presumptive POD (CP)	0.02 (0.00, 0.06)	0.67 (0.58, 0.75)	0.99 (0.96, 1.00)
$s_r^b$	0.12 (0.11, 0.16)	0.48 (0.42, 0.52)	0.09 (0.08, 0.16)
$s_L^c$	0.00 (0.00, 0.05)	0.00 (0.00, 0.18)	0.00 (0.00, 0.04)
$s_R^d$	0.12 (0.11, 0.14)	0.48 (0.43, 0.52)	0.09 (0.08, 0.10)
P Value <sup>e</sup>	0.5190	0.6044	0.4338
Candidate Confirmed Positive/ Total # of Samples Analyzed	1/132	86/132	132/132
Candidate Confirmed POD (CC)	0.01 (0.00, 0.04)	0.65 (0.57, 0.73)	1.00 (0.97, 1.00)
$s_r$	0.09 (0.08, 0.16)	0.48 (0.43, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.04)	0.00 (0.00, 0.18)	0.00 (0.00, 0.16)
$s_R$	0.09 (0.08, 0.10)	0.48 (0.43, 0.52)	0.00 (0.00, 0.23)
P Value	0.4338	0.5632	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	1/132	85/132	131/132
Candidate Presumptive Positive that Confirmed POD (C)	0.01 (0.00, 0.04)	0.64 (0.56, 0.73)	0.99 (0.96, 1.00)
$s_r$	0.09 (0.08, 0.16)	0.48 (0.43, 0.52)	0.09 (0.08, 0.16)
$s_L$	0.00 (0.00, 0.04)	0.00 (0.00, 0.18)	0.00 (0.00, 0.04)
$s_R$	0.09 (0.08, 0.10)	0.49 (0.43, 0.52)	0.09 (0.08, 0.10)
P Value	0.4338	0.6228	0.4338
Positive Reference Samples/ Total # of Samples Analyzed	0/132	64/132	132/132
Reference POD	0.00 (0.00, 0.03)	0.48 (0.40, 0.57)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9192	1.0000
dLPOD (Candidate vs. Reference) <sup>f</sup>	0.01 (-0.02, 0.04)	0.16 (0.04, 0.28)	-0.01 (-0.04, 0.02)
dLPOD (Candidate Presumptive vs. Candidate Confirmed) <sup>f</sup>	0.01 (-0.03, 0.05)	0.02 (-0.10, 0.13)	-0.01 (-0.04, 0.02)

<sup>a</sup> Results include 95% Confidence Intervals, <sup>r</sup> Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation  
<sup>e</sup> P Value = Homogeneity test of laboratory PODs; <sup>f</sup> A confidence interval for dLPOD that does not contain the value 0 indicates a statistical significant difference between the two methods

1 **Table 2016.2A:** Comparative Results for the Detection of *Listeria* in 125 g Deli Turkey Test Portions by the 3M™ MDA 2 - *Listeria* Method vs. USDA/FSIS  
 2 MLG Chapter. 8.09 in a Collaborative Study (Un-inoculated Control)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Deli Turkey	1	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		2	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		3	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		4	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		5	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		6	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		7	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		8 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		9	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		10 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		11	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		12	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		13	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00

Estimate	All	132	0	0.00	132	0	0.00	132	0	0.00	132	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.03	-0.03
UCL				0.03			0.03			0.03			0.03	0.03	0.03
$s_r^b$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_L^c$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_R^d$				0.00			0.00			0.00			0.00		
UCL				0.00			0.00			0.00			0.00		
LCL				0.23			0.23			0.23			0.23		
$P_T^e$				1.0000			1.0000			1.0000			1.0000		

<sup>a</sup>N/A – Laboratory did not participate or submit data for this matrix

<sup>b</sup>Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup>Reproducibility Standard Deviation, <sup>e</sup> P<sub>T</sub> Value = Homogeneity test of laboratory PODs

LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

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1 **Table 2016.2A (cont'd.):** Comparative Results for the Detection of *Listeria* in 125 g Deli Turkey Test Portions by the 3M™ MDA 2 - *Listeria* Method vs.  
 2 USDA/FSIS MLG Chapter. 8.09 in a Collaborative Study (Low Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R		
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)	
	Deli Turkey	1	12	7	0.58	12	7	0.58	12	7	0.58	12	6	0.50	0.08	0.00	
		2	12	7	0.58	12	6	0.50	12	6	0.50	12	5	0.42	0.08	0.08	
		3	12	4	0.33	12	4	0.33	12	4	0.33	12	5	0.42	-0.08	0.00	
		4	12	5	0.42	12	5	0.42	12	5	0.42	12	4	0.33	0.08	0.00	
		5	12	6	0.50	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.00	
		6	12	7	0.58	12	7	0.58	12	7	0.58	12	6	0.50	0.08	0.00	
		7	12	7	0.58	12	7	0.58	12	7	0.58	12	6	0.50	0.08	0.00	
		8 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		9	12	8	0.66	12	8	0.66	12	8	0.66	12	6	0.50	0.16	0.00	
		10 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MPN/ Test Portion	11	12	7	0.58	12	6	0.50	12	6	0.50	12	7	0.58	-0.08	0.08	
		12	12	5	0.42	12	5	0.42	12	5	0.42	12	4	0.33	0.08	0.00	
		13	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00	
Estimate	0.63	All	132	68	0.52	132	66	0.50	132	66	0.50	132	60	0.45	0.04	0.02	
LCL	0.49				0.43			0.41			0.41			0.37	-0.08	-0.11	
UCL	0.80				0.60			0.59			0.59			0.54	0.17	0.14	
$s_r^b$					0.51			0.51			0.51			0.51			
LCL					0.45			0.46			0.46			0.46			
UCL					0.52			0.52			0.52			0.52			
$s_L^c$					0.00			0.00			0.00			0.00			
LCL					0.00			0.00			0.00			0.00			
UCL					0.15			0.14			0.14			0.11			
$s_R^d$					0.51			0.51			0.51			0.51			
UCL					0.46			0.46			0.46			0.46			
LCL					0.52			0.52			0.52			0.52			
$P_T^e$					0.8762			0.9123			0.9123			0.9829			

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix  
<sup>b</sup> Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation, <sup>e</sup>  $P_T$  Value = Homogeneity test of laboratory PODs  
 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

1 **Table 2016.2A (cont'd.):** Comparative Results for the Detection of *Listeria* in 125 g Deli Turkey Test Portions by the 3M™ MDA 2 - *Listeria* Method vs.  
 2 USDA/FSIS MLG Chapter. 8.09 in a Collaborative Study (High Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R		
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)	
	Deli Turkey	1	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		2	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		3	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		4	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		5	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		6	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		7	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		8 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		9	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		10 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MPN/ Test Portion	11	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		12	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		13	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
Estimate	4.52	All	132	132	1.00	132	132	1.00	132	132	1.00	132	132	1.00	0.00	0.00	
LCL	3.19				0.97			0.97			0.97			0.97	-0.03	-0.03	
UCL	6.42				1.00			1.00			1.00			1.00	0.03	0.03	
$s_r^b$					0.00			0.00			0.00			0.00			
LCL					0.00			0.00			0.00			0.00			
UCL					0.16			0.16			0.16			0.16			
$s_L^c$					0.00			0.00			0.00			0.00			
LCL					0.00			0.00			0.00			0.00			
UCL					0.16			0.16			0.16			0.16			
$s_R^d$					0.00			0.00			0.00			0.00			
UCL					0.00			0.00			0.00			0.00			
LCL					0.23			0.23			0.23			0.23			
$P_T^e$					1.000			1.000			1.000			1.000			

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix  
<sup>b</sup> Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation, <sup>e</sup>  $P_T$  Value = Homogeneity test of laboratory PODs  
 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit



1 **Table 2016.2B:** Comparative Results for the Detection of *Listeria* in 25 g Raw Chicken Breast Fillet Test Portions by the 3M™ MDA 2 - *Listeria* Method vs.  
 2 USDA/FSIS MLG Chapter. 8.09 in a Collaborative Study (Un-inoculated Control)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Raw Chicken Breast Fillet	1	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		2	12	1	0.08	12	1	0.08	12	1	0.08	12	0	0.00	0.08	0.00
		3	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		4	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		5	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		6	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		7	12	1	0.08	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.08
		8	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		9	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		10 <sup>b</sup>	12	0	0.00	12	2	0.17	12	0	0.00	12	0	0.00	0.00	-0.17
		11 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		12	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		13	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00

Estimate	All	132	2	0.02	132	1	0.01	132	1	0.01	132	0	0.00	0.01	0.01
LCL				0.00			0.00			0.00			0.00	-0.02	-0.03
UCL				0.06			0.04			0.04			0.03	0.04	0.05
$s_r^c$				0.12			0.09			0.09			0.00		
LCL				0.11			0.08			0.08			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_L^d$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.05			0.04			0.04			0.16		
$s_R^e$				0.12			0.09			0.09			0.00		
UCL				0.11			0.08			0.08			0.00		
LCL				0.14			0.10			0.10			0.23		
$P_T^f$				0.5190			0.4338			0.4338			1.0000		

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix; <sup>b</sup> Results were not used in statistical analysis due to deviation from testing protocol.  
<sup>c</sup> Repeatability Standard Deviation, <sup>d</sup> Among-Laboratory Standard Deviation, <sup>e</sup> Reproducibility Standard Deviation, <sup>f</sup>  $P_T$  Value = Homogeneity test of laboratory PODs  
 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

1 **Table 2016.2B (cont'd.):** Comparative Results for the Detection of *Listeria* in 25 g Raw Chicken Breast Fillet Test Portions by the 3M™ MDA 2 - *Listeria*  
 2 Method vs. USDA/FSIS MLG Chapter. 8.09 in a Collaborative Study (Low Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Raw Chicken Breast Fillet	1	12	7	0.58	12	7	0.58	12	7	0.58	12	7	0.58	0.00	0.00
		2	12	9	0.75	12	9	0.75	12	9	0.75	12	7	0.58	0.17	0.00
		3	12	10	0.83	12	10	0.83	12	10	0.83	12	5	0.42	0.41	0.00
		4	12	9	0.75	12	9	0.75	12	9	0.75	12	4	0.33	0.42	0.00
		5	12	8	0.66	12	8	0.66	12	8	0.66	12	5	0.42	0.22	0.00
		6	12	10	0.83	12	9	0.75	12	9	0.75	12	6	0.50	0.15	0.08
		7	12	8	0.66	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.16
		8	12	7	0.58	12	7	0.58	12	7	0.58	12	7	0.58	0.00	0.00
		9	12	8	0.66	12	9	0.75	12	8	0.66	12	7	0.58	0.08	-0.08
		10 <sup>b</sup>	12	6	0.50	12	8	0.66	12	6	0.50	12	5	0.42	0.08	-0.16
	MPN/ Test Portion	11 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		12	12	7	0.58	12	7	0.58	12	7	0.58	12	4	0.42	0.16	0.00
		13	12	5	0.42	12	5	0.42	12	5	0.42	12	6	0.50	-0.08	0.00
Estimate	0.66	All	132	88	0.67	132	86	0.65	132	85	0.64	132	64	0.48	0.16	0.02
LCL	0.51				0.58			0.57			0.56			0.40	0.04	-0.10
UCL	0.83				0.75			0.73			0.73			0.57	0.28	0.13
$s_r^c$					0.48			0.48			0.48			0.51		
LCL					0.42			0.43			0.43			0.46		
UCL					0.52			0.52			0.52			0.52		
$s_L^d$					0.00			0.00			0.00			0.00		
LCL					0.00			0.00			0.00			0.00		
UCL					0.18			0.18			0.18			0.14		
$s_R^e$					0.48			0.48			0.49			0.51		
UCL					0.43			0.43			0.43			0.46		
LCL					0.52			0.52			0.52			0.52		
$P_T^f$					0.6044			0.5632			0.6228			0.9192		

3 <sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix; <sup>b</sup> Results were not used in statistical analysis due to deviation from testing protocol.  
 4 <sup>c</sup> Repeatability Standard Deviation, <sup>d</sup> Among-Laboratory Standard Deviation, <sup>e</sup> Reproducibility Standard Deviation, <sup>f</sup>  $P_T$  Value = Homogeneity test of laboratory PODs  
 5 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit  
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1 **Table 2016.2B (cont'd.):** Comparative Results for the Detection of *Listeria* in 25 g Raw Chicken Breast Fillet Test Portions by the 3M™ MDA 2 - *Listeria*  
 2 Method vs. USDA/FSIS MLG Chapter. 8.09 in a Collaborative Study (High Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R		
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)	
	Raw Chicken Breast Fillet	1	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		2	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		3	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		4	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		5	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		6	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		7	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		8	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		9	12	11	0.92	12	12	1.00	12	11	0.92	12	12	1.00	-0.08	-0.08	
		10 <sup>b</sup>	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		MPN/ Test Portion	11 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
			13	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
Estimate	6.24	All	132	131	0.99	132	132	1.00	132	131	0.99	132	132	1.00	-0.01	-0.01	
LCL	3.58				0.96			0.97			0.96			0.97	-0.04	-0.04	
UCL	10.88				1.00			1.00			1.00			1.00	0.02	0.02	
$s_r^c$					0.09			0.00			0.09			0.00			
LCL					0.08			0.00			0.08			0.00			
UCL					0.16			0.16			0.16			0.16			
$s_L^d$					0.00			0.00			0.00			0.00			
LCL					0.00			0.00			0.00			0.00			
UCL					0.04			0.16			0.04			0.16			
$s_R^e$					0.09			0.00			0.09			0.00			
UCL					0.08			0.00			0.08			0.00			
LCL					0.10			0.23			0.10			0.23			
$P_T^f$					0.4233			1.0000			0.4338			1.0000			

<sup>a</sup>N/A – Laboratory did not participate or submit data for this matrix; <sup>b</sup>Results were not used in statistical analysis due to deviation from testing protocol.  
<sup>c</sup>Repeatability Standard Deviation, <sup>d</sup>Among-Laboratory Standard Deviation, <sup>e</sup>Reproducibility Standard Deviation, <sup>f</sup> $P_T$  Value = Homogeneity test of laboratory PODs  
 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

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**Supplementary Materials**

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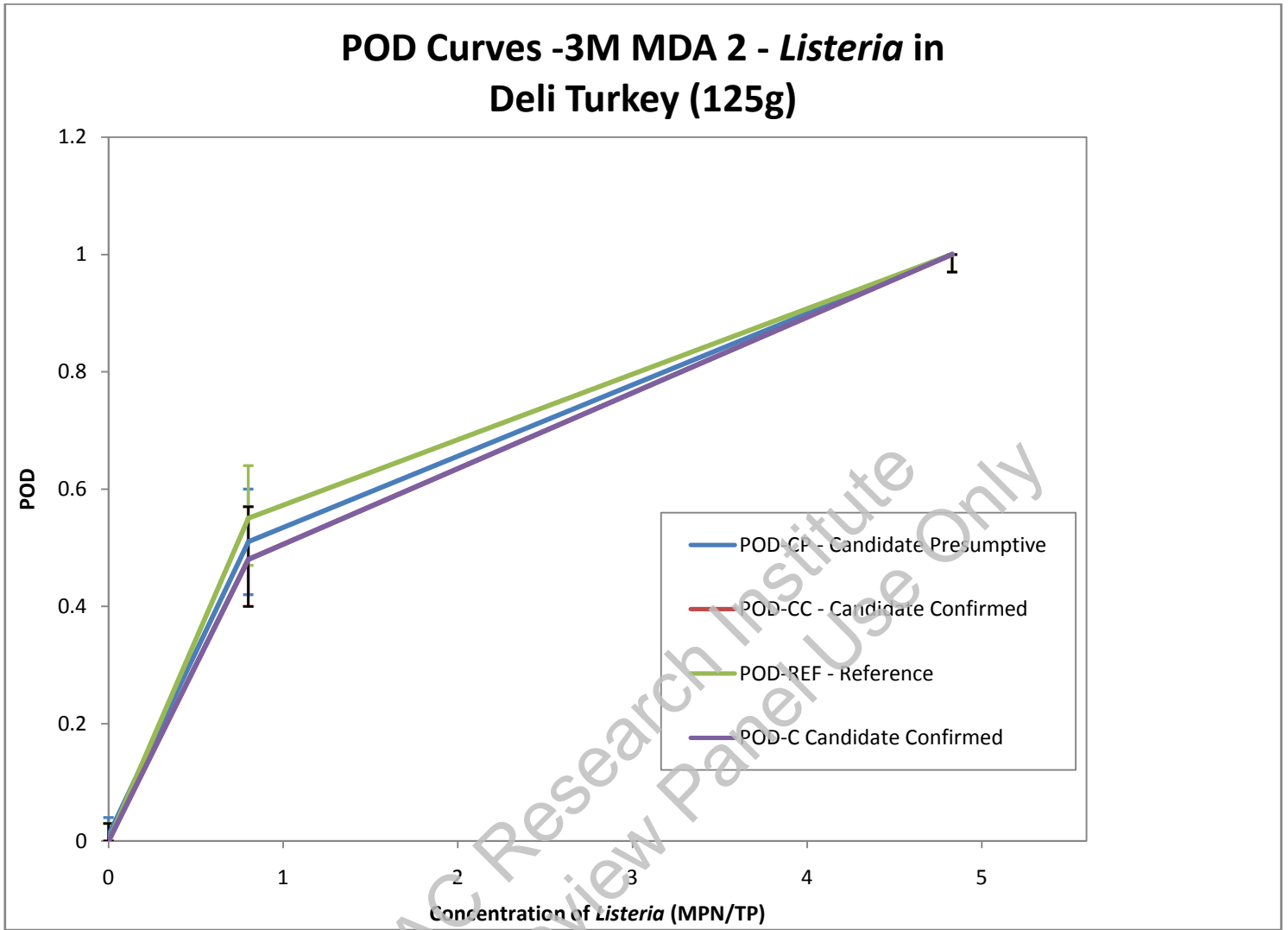
**Table 3: Results of Aerobic Plate Count for Collaborating Laboratories**

Lab	Deli Turkey (CFU/g) <sup>a</sup>	Raw Chicken Breast Fillet (CFU/g) <sup>a</sup>
1	2.0 x 10 <sup>1</sup>	2.3 x 10 <sup>4</sup>
2	< 10	3.4 x 10 <sup>4</sup>
3	< 10	4.1 x 10 <sup>3</sup>
4	3.0 x 10 <sup>1</sup>	8.3 x 10 <sup>6</sup>
5	1.0 x 10 <sup>1</sup>	5.4 x 10 <sup>3</sup>
6	< 10	6.3 x 10 <sup>4</sup>
7	6.0 x 10 <sup>1</sup>	3.6 x 10 <sup>2</sup>
8	N/A	2.9 x 10 <sup>3</sup>
9	< 10	4.6 x 10 <sup>2</sup>
10	N/A	3.7 x 10 <sup>4</sup>
11	2.0 x 10 <sup>1</sup>	N/A
12	< 10	7.4 x 10 <sup>3</sup>
13	< 10	2.7 x 10 <sup>4</sup>

<sup>a</sup> Samples analyzed by the 3M Petrifilm Rapid Aerobic Count Plate, AOAC OMA 2015.13

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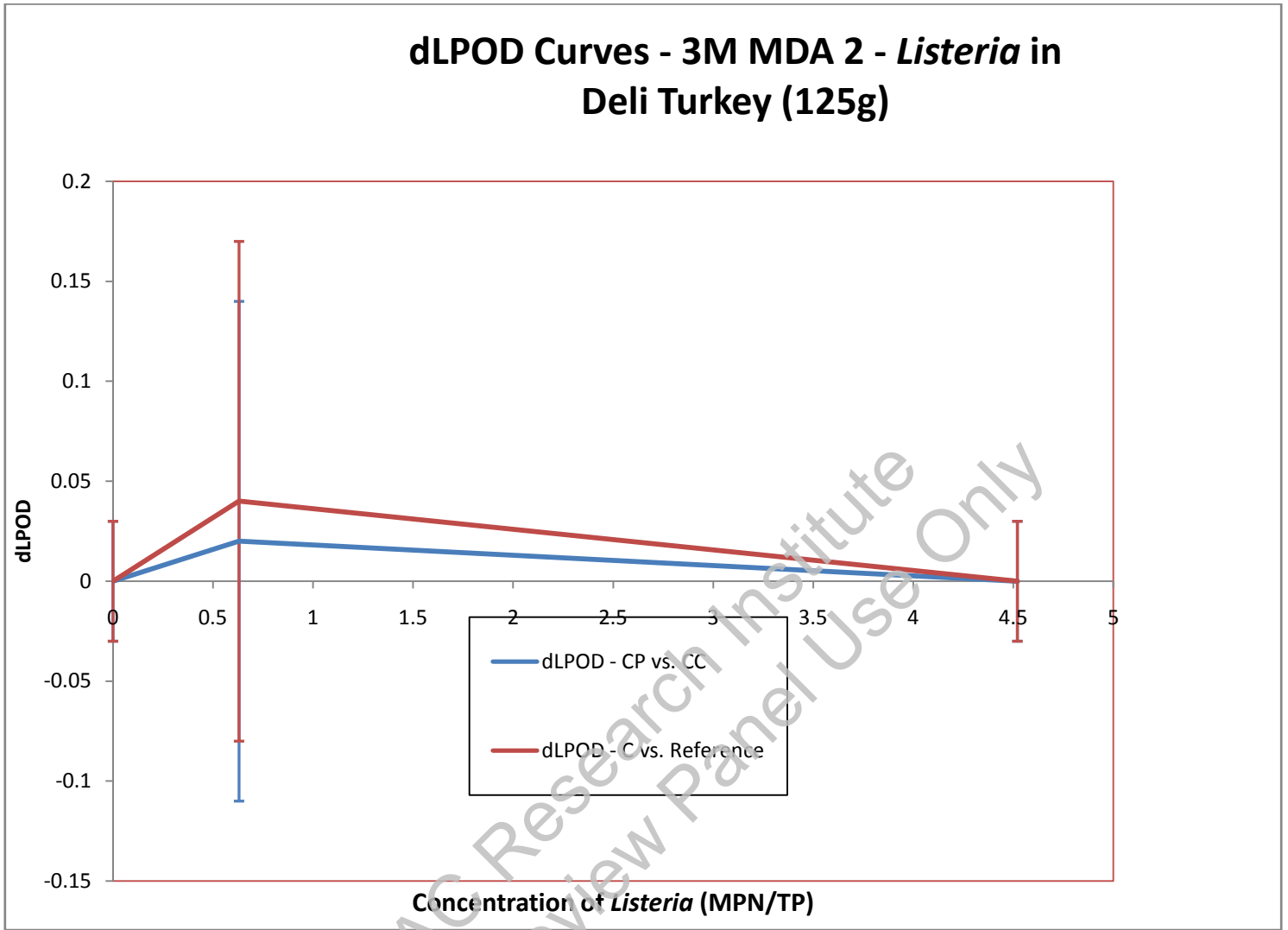
Figure 1A: POD Values of Candidate Method vs. Reference Method for 125 g Deli Turkey



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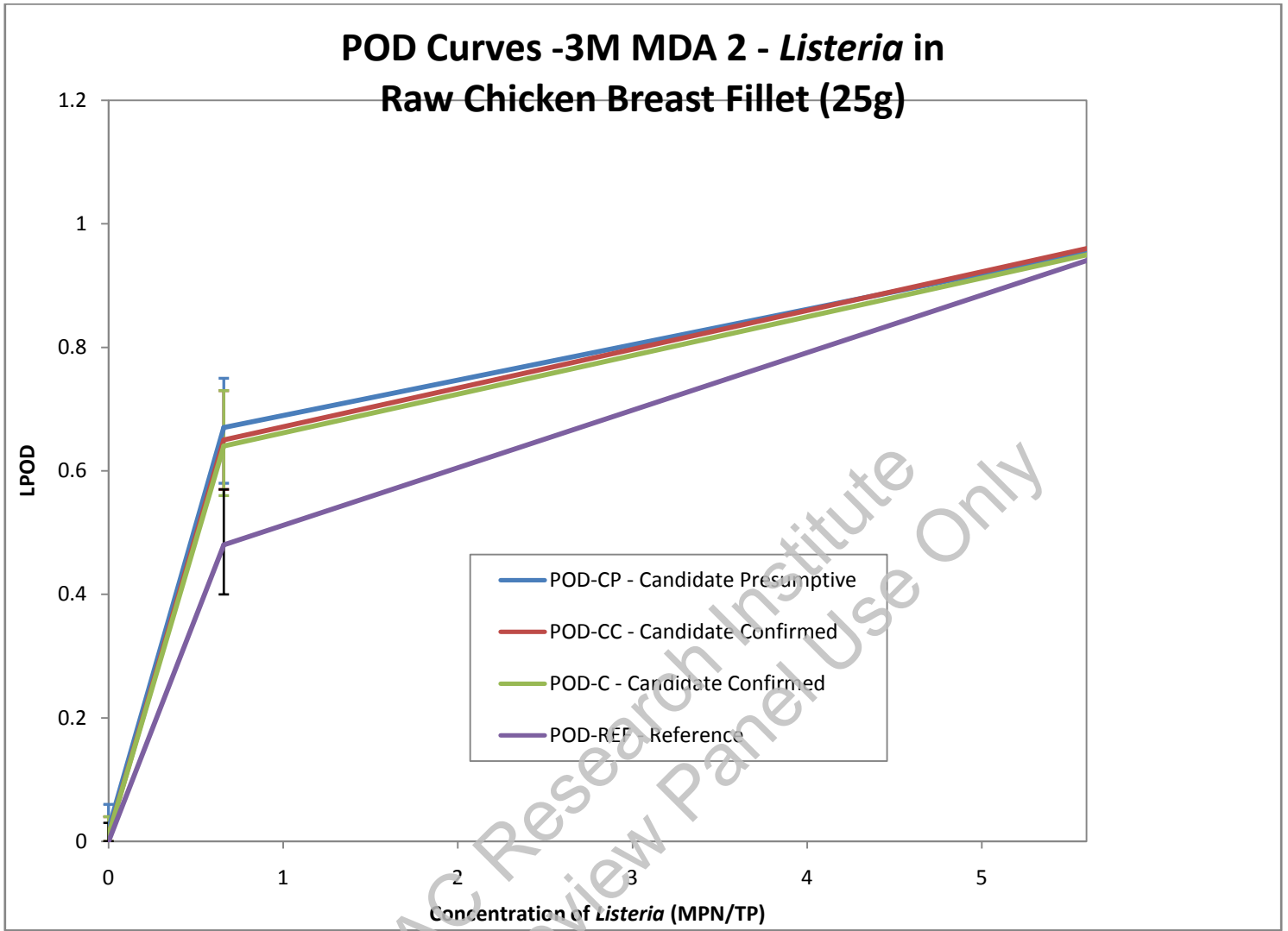
1 **Figure 1B: dLPOD Values of Candidate Method vs. Reference Method for 125 g Deli Turkey**



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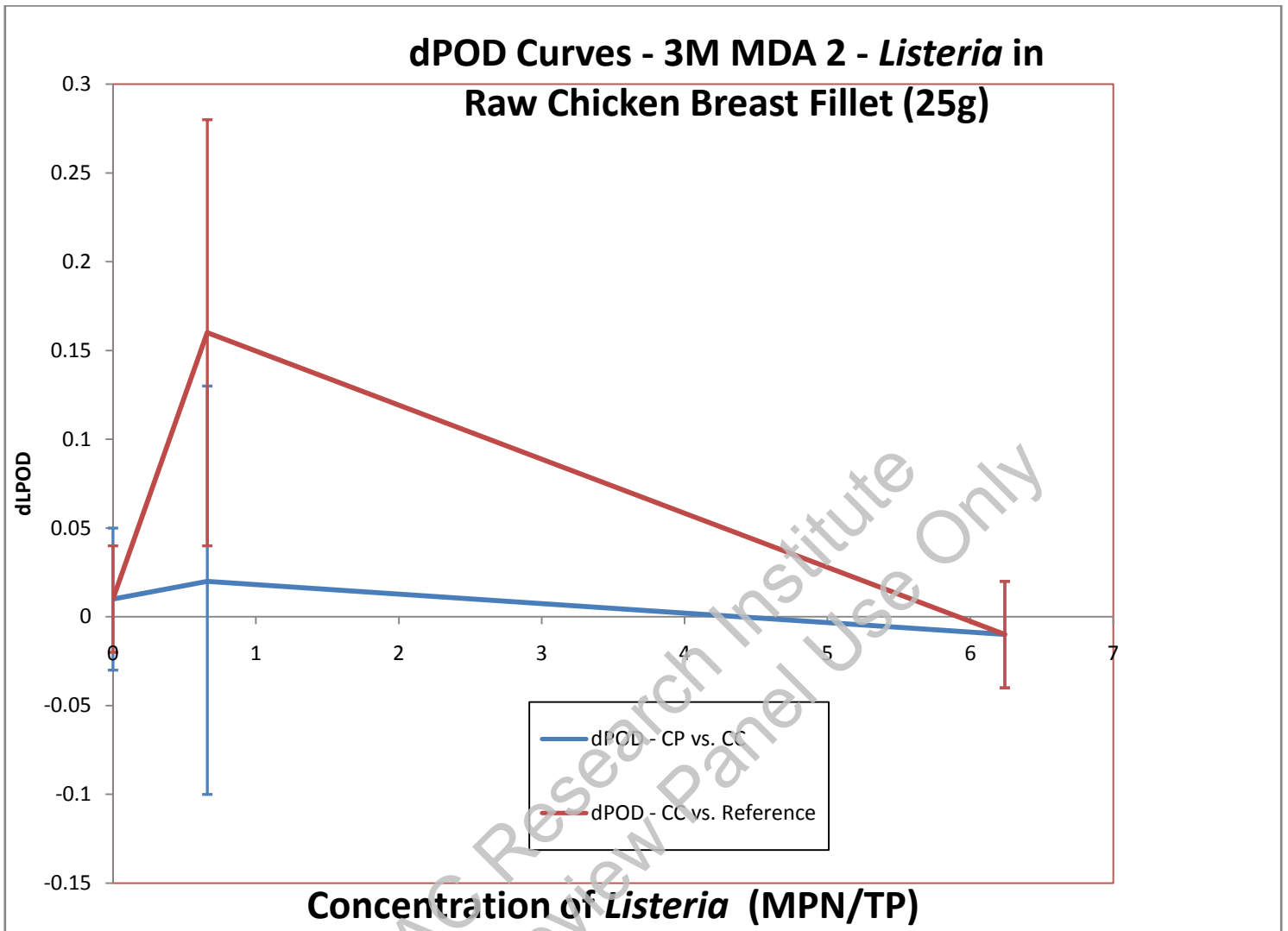
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Figure 2A: POD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet



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Figure 2B: dPOD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet



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## 3M™ Molecular Detection Assay 2 - *Listeria*

MDA2LIS96

### PRODUCT DESCRIPTION AND INTENDED USE

3M™ Molecular Detection Assay 2 - *Listeria* is used with the 3M™ Molecular Detection System for the rapid and specific detection of *Listeria* species in enriched food and environmental samples.

The 3M Molecular Detection Assays use loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations <sup>(1, 2, 3)</sup>.

The 3M Molecular Detection Assay 2 - *Listeria* is intended for use in a laboratory environment by professionals trained in laboratory techniques. 3M has not documented the use of this product in industries other than food or beverage. For example, 3M has not documented this product for testing water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M Molecular Detection Assay 2 - *Listeria* has not been evaluated with all possible testing protocols or with all possible strains of bacteria.

**As with all test methods, the source, formulation and quality of enrichment medium can influence the results.** Factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may also influence results. 3M recommends evaluation of the method including enrichment medium, in the user's environment using a sufficient number of samples with particular foods and microbial challenges to ensure that the method meets the user's criteria.

3M has evaluated the 3M Molecular Detection Assay 2 - *Listeria* with Demi-Fraser Broth containing Ferric Ammonium Citrate and Fraser Broth. A typical formulation of this medium follows below.

#### Demi-Fraser Broth Base Typical Formula (g/L)

Sodium Chloride	20 g
Sodium Phosphate, dibasic, anhydrous *	9.6 g
Beef Extract	5.0 g
Pancreatic Digest of Casein	5.0 g
Peptic Digest of Animal Tissue	5.0 g
Yeast Extract	5.0 g

Lithium Chloride	3.0 g
Potassium Phosphate, monobasic	1.35 g
Esculin	1.0 g
AcriflavinHCl	0.0125 g
Nalidixic Acid	0.01 g

\* Substitute: Sodium Phosphate, dibasic, dihydrate 12.0 g

### Fraser Broth Supplement

(Ingredients per 10 mL vial. One vial is added to one liter of basal medium.)

Ferric Ammonium Citrate 0.5g/10mL

Final pH 7.2 ± 0.2 at 25°C

The 3M™ Molecular Detection Instrument is intended for use with samples that have undergone heat treatment during the assay lysis step, which is designed to destroy organisms present in the sample. Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.

3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

The 3M Molecular Detection Assay 2 - *Listeria* test kit contains 96 tests, described in Table 1.

**Table 1.** Kit Components

Item	Identification	Quantity	Contents	Comments
Lysis Solution (LS) tubes	Pink solution in clear tubes	96 (12 strips of 8 tubes)	580 µL of LS per tube	Racked and ready to use
<i>Listeria</i> Reagent tubes	Blue tubes	96 (12 strips of 8 tubes)	Lyophilized specific amplification and detection mix	Ready to use
Extra caps	Blue caps	96 (12 strips of 8 caps)		Ready to use
Reagent Control (RC)	Clear flip-top tubes	16 (2 pouches of 8 individual tubes)	Lyophilized control DNA, amplification and detection mix	Ready to use
Quick Start Guide		1		

The Negative Control, not provided in the kit, is sterile enrichment medium, e.g., Demi-Fraser Broth. Do not use water as a Negative Control.

## SAFETY

The user should read, understand and follow all safety information in the instructions for the 3M Molecular Detection System and the 3M Molecular Detection Assay 2 - *Listeria*. Retain the safety instructions for future reference.



### WARNING:

Indicates a hazardous situation, which, if not avoided, could result in death or serious injury and/or property damage.



### CAUTION:

Indicates a hazardous situation, which, if not avoided, could result in minor or moderate injury and/or property damage.

### NOTICE:

Indicates a potentially hazardous situation which, if not avoided, could result in property damage.



### WARNING

Do not use the 3M Molecular Detection Assay 2 - *Listeria* in the diagnosis of conditions in humans or animals.

The 3M Molecular Detection Assay 2 - *Listeria* method may generate *Listeria monocytogenes* to levels sufficient to cause stillbirths and fatalities in pregnant women and the immunocompromised, if exposed.

The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices, ISO/IEC17025<sup>(4)</sup>, or ISO 17218<sup>(5)</sup>.

To reduce the risks associated with a false-negative result leading to the release of contaminated product:

- Follow the protocol and perform the tests exactly as stated in the product instructions.
- Store the 3M Molecular Detection Assay 2 - *Listeria* as indicated on the package and in the product instructions.
- Always use the 3M Molecular Detection Assay 2 - *Listeria* by the expiration date.
- Use the 3M Molecular Detection Assay 2 - *Listeria* for food and environmental samples that have been validated internally or by a third party.
- Use the 3M Molecular Detection Assay 2 - *Listeria* only for surfaces, sanitizers, protocols and bacterial strains that have been validated internally or by a third party.
- For an environmental sample containing Neutralizing Buffer with aryl sulfonate complex, perform a 1:2 dilution before testing (1 part sample into 1 part sterile enrichment broth). Another option is to

transfer 10 uL of the NB enrichment into the LS tubes. 3M™ sample handling products which include Neutralizing Buffer with aryl sulfonate complex: BPPFV10NB, RS96010NB, RS9604NB, SSL10NB, XSLSSL10NB, HS10NB and HS119510NB.

**To reduce the risks associated with exposure to chemicals and biohazards:**

- It is strongly recommended that female laboratory staff be informed of the risk to a developing fetus resulting from infection of the mother through exposure to *Listeria monocytogenes*.
- Perform pathogentesting in a properly equipped laboratory under the control of trained personnel.
- Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples.
- Avoid contact with the contents of the enrichment media and reagent tubes after amplification.
- Dispose of enriched samples according to current industry standards.
- Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.

**To reduce the risks associated with cross-contamination while preparing the assay:**

- Always wear gloves (to protect the user and prevent introduction of nucleases).

**To reduce the risks associated with environmental contamination:**

- Follow current industry standards for disposal of contaminated waste.



**CAUTION**

- Do not exceed the recommended temperature setting on heater.
- Do not exceed the recommended heating time.
- Use an appropriate, calibrated thermometer to verify the 3M™ Molecular Detection Heat Block Insert temperature (e.g., a partial immersion thermometer or digital thermocouple thermometer, not a total immersion thermometer.) The thermometer must be placed in the designated location in the 3M Molecular Detection Heat Block Insert.

**NOTICE**

**To reduce the risks associated with cross-contamination while preparing the assay:**

- Use of sterile, aerosol barrier (filtered), molecular biology grade pipette tips is recommended.
- Use a new pipette tip for each sample transfer.
- Use Good Laboratory Practices to transfer the sample from the enrichment to the lysis tube. To avoid pipettor contamination, the user may choose to add an intermediate transfer step. For example, the user can transfer each enriched sample into a sterile tube.

- Use a molecular biology workstation containing germicidal lamp where available.
- Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with a 1- 5% (v:v in water) household bleach solution or DNA removal solution.

**To reduce the risks associated with a false-positive result:**

- Never open tubes post amplification.
- Always dispose of the contaminated tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

Consult the Safety Data Sheet for additional information and local regulations for disposal.

If you have questions about specific applications or procedures, please visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

**LIMITATION OF WARRANTIES / LIMITED REMEDY**

EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL PRODUCT PACKAGING, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any 3M Food Safety Product is defective, 3M or its authorized distributor will, at its option, replace or refund the purchase price of the product. These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery of any suspected defects in a product and return it to 3M. Please call Customer Service (1-800-328-1671 in the U.S.) or your official 3M Food Safety representative for a Returned Goods Authorization.

**LIMITATION OF 3M LIABILITY**

3M WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO LOST PROFITS. In no event shall 3M's liability under any legal theory exceed the purchase price of the product alleged to be defective.

**USER RESPONSIBILITY**

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety), or contact your local 3M representative or distributor for more information.



When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may influence results.

It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and microbial challenges to satisfy the user that the chosen test method meets the user's criteria.

It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.

As with any test method, results obtained from use of any 3M Food Safety product do not constitute a guarantee of the quality of the matrices or processes tested.

To help customers evaluate the method for various food matrices, 3M has developed the 3M™ Molecular Detection Matrix Control kit. When needed, use the Matrix Control (MC) to determine if the matrix has the ability to impact the 3M Molecular Detection Assay 2 - *Listeria* results. Test several samples representative of the matrix, i.e. samples obtained from different origin, during any validation period when adopting the 3M method or when testing new or unknown matrices or matrices that have undergone raw material or process changes.

A matrix can be defined as a type of product with intrinsic properties such as composition and process. Differences between matrices may be as simple as the effects caused by differences in their processing or presentation for example, raw vs. pasteurized; fresh vs. dried, etc.

### **STORAGE AND DISPOSAL**

Store the 3M Molecular Detection Assay 2 - *Listeria* at 2-8°C. Do not freeze. Keep kit away from light during storage. After opening the kit, check that the foil pouch is undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes should always be stored in the re-sealable pouch with the desiccant inside to maintain stability of the lyophilized reagents. Store resealed pouches at 2-8°C for no longer than 60days.

Do not use 3M Molecular Detection Assay 2 - *Listeria* past the expiration date. Expiration date and lot number are noted on the outside label of the box. After use, the enrichment medium and the 3M Molecular Detection Assay 2 - *Listeria* tubes can potentially contain pathogenic materials. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Safety Data Sheet for additional information and local regulations for disposal.

### **INSTRUCTIONS FOR USE**

Follow all instructions carefully. Failure to do so may lead to inaccurate results.

Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with a 1- 5% (v:v in water) household bleach solution or DNA removal solution.

## SAMPLE ENRICHMENT

Table 2 presents guidance for the enrichment of food and environmental samples. It is the user's responsibility to validate alternate sampling protocols or dilution ratios to ensure this test method meets the user's criteria.

### Foods

1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature.
2. Aseptically combine the enrichment medium and sample according to Table 2. For all meat and highly particulate samples, the use of filter bags is recommended.
3. Homogenize thoroughly by blending, stomaching, or hand mixing for  $2 \pm 0.2$  minutes. Incubate at  $37 \pm 1^\circ\text{C}$  according to Table 2.
4. For raw dairy products, transfer 0.1mL of the primary enrichment into 10 mL of Fraser Broth. Incubate at  $37 \pm 1^\circ\text{C}$  for 20-24 hours.

### Environmental samples

Sample collection devices can be a sponge hydrated with a neutralizing solution to inactivate the effects of the sanitizers. 3M recommends the use of a biocide-free cellulose sponge. Neutralizing solution can be Dey-Engley (D/E) Neutralizing Broth or Lethen broth. It is recommended to sanitize the area after sampling.

**WARNING:** Should you select to use Neutralizing Buffer (NB) that contains aryl sulfonate complex as the hydrating solution for the sponge, it is required to perform a 1:2 dilution (1 part sample into 1 part sterile enrichment broth) of the enriched environmental sample before testing in order to reduce the risks associated with a false-negative result leading to the release of contaminated product.

The recommended size of the sampling area to verify the presence or absence of the pathogen on the surface is at least  $100 \text{ cm}^2$  (10 cm x 10 cm or 4"x4"). When sampling with a sponge, cover the entire area going in two directions (left to right then up and down) or collect environmental samples following your current sampling protocol or according to the FDA BAM <sup>(1)</sup>, USDA FSIS MLG <sup>(2)</sup> or ISO 18593 <sup>(6)</sup> guidelines.

1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature.
2. Aseptically combine the enrichment medium and sample according to Tables 2 or 3.

3. Homogenize thoroughly by blending, stomaching, or hand mixing for  $2 \pm 0.2$  minutes. Incubate at  $37 \pm 1^\circ\text{C}$  for 24-30 hours.

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**Table 2:** General enrichment protocols using Demi-Fraser Broth Enrichment at 37C+/- 1C. And Fraser Broth enrichment at 37C+/- 1C.

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature (°C)	Enrichment Time (hr)				
Heat-processed, cooked, cured meats, poultry, seafood and fish	25 g	225	37	24-30				
Heat-processed / pasteurized dairy products								
Produce and vegetables								
Multi-component foods								
Environmental samples	1 sponge	100 or 225	37	24-30				
	1 swab	10	37	24-30				
Raw meat, poultry, seafood, fish	25 g	475	37	28-32				
Sample Matrix	Primary Enrichment (Demi-Fraser Broth)				Secondary Enrichment (Fraser Broth)			Sample Analysis Volume <sup>(a)</sup>
	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature (°C)	Enrichment Time (hr)	Sample Size	Enrichment Temperature (°C)	Enrichment Time (hr)	
Raw dairy products	25 g	225	37	20-24	Transfer 0.1 mL into 10 mL Fraser Broth	37	20-24	10 µL

(a) Volume of sample transferred to Lysis Solution tubes. Refer to Step 4.6 of Lysis section.

**Specific Instructions for Validated Methods**

**AOAC® Performance Tested Method<sup>SM</sup>#111501**



In AOAC PTM studies, the 3M Molecular Detection Assay 2 – *Listeria* was found to be an effective method for the detection of *Listeria* species. The matrices tested in the study are shown in Table 3. The limit of detection of the 3M Molecular Detection Assay 2 – *Listeria* method is 1-5 colony forming units per validated test portion size (in Table 3).

**Table 3.** Enrichment protocols using Demi-Fraser Broth at  $37 \pm 1 \text{ }^\circ\text{C}$  according to AOAC Performance Tested<sup>SM</sup> Certificate #111501 [and AOAC Official Method<sup>SM</sup> 2016.xxx](#)

Sample Matrix		Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)
Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon		25 g	225	24-30
Raw chicken		25 g	475	28-32
Deli turkey		125 g	1125	24-30
Cantaloupe		Whole melon	Enough volume to allow melon to float	26-30
Environmental samples:	Stainless steel	1 sponge	225	24-30
	Sealed concrete	1 sponge	100	24-30
	Plastic	1 swab	10	24-30

#### **PREPARATION OF THE 3M™ MOLECULAR DETECTION SPEED LOADER TRAY**

1. Wet a cloth with a 1-5% (v:v in water) household bleach solution and wipe the 3M™ Molecular Detection Speed Loader Tray.
2. Rinse the 3M Molecular Detection Speed Loader Tray with water.
3. Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.
4. Ensure the 3M Molecular Detection Speed Loader Tray is dry before use.

#### **PREPARATION OF THE 3M™ MOLECULAR DETECTION CHILL BLOCK INSERT**

Place the 3M™ Molecular Detection Chill Block directly on the laboratory bench; (the 3M™ Molecular Detection Chill Block Tray is not used). Use the chill block at ambient laboratory temperature (20-25°C).

#### **PREPARATION OF THE 3M™ MOLECULAR DETECTION HEAT BLOCK INSERT**

Place the 3M™ Molecular Detection Heat Block insert in a dry double block heater unit. Turn on the dry block heater unit and set the temperature to allow the 3M Molecular Detection Heat Block Insert to reach and maintain a temperature of  $100 \pm 1^\circ\text{C}$ .

**NOTE:** Depending on the heater unit, allow approximately 30 minutes for the 3M Molecular Detection Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (e.g., a partial immersion thermometer or digital thermocouple thermometer, not a total immersion thermometer) placed in the designated location, verify that the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^\circ\text{C}$ .

#### **PREPARATION OF THE 3M™ MOLECULAR DETECTION INSTRUMENT**

1. Launch the 3M™ Molecular Detection Software and log in.
2. Turn on the 3M Molecular Detection Instrument.
3. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection System User Manual for details.

**NOTE:** The 3M Molecular Detection Instrument must reach and maintain temperature of  $60^\circ\text{C}$  before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes

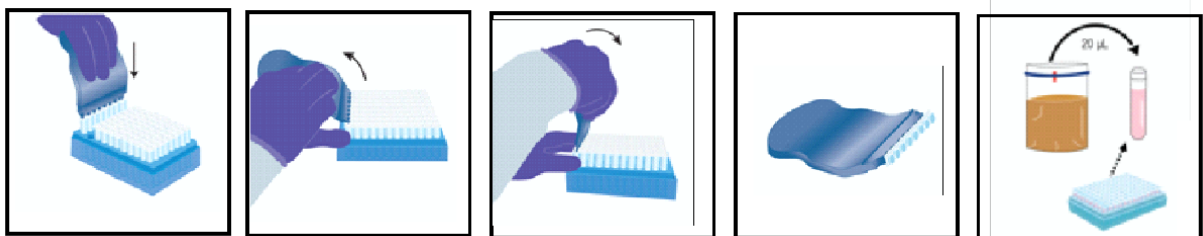
approximately 20 min and is indicated by an ORANGE light on the instrument's status bar. When the instrument is ready to start a run, the status bar will turn GREEN.

## LYSIS

1. Allow the lysis solution (LS) tubes to warm up by setting the rack at room temperature (20-25 °C) overnight (16-18 hours). Alternatives to equilibrate the LS tubes to room temperature are to set the LS tubes on the laboratory bench for at least 2 hours, incubate the LS tubes in a 37 ±1°C incubator for 1 hour or place them in a dry double block heater for 30 seconds at 100°C.
2. Invert the capped tubes to mix. Proceed to next step within 4 hrs.
3. Remove the enrichment broth from the incubator.
4. One LS tube is required for each sample and the Negative Control (NC) (sterile enrichment medium) sample.
  - 4.1 LS tube strips can be cut to desired LS tube number. Select the number of individual LS tubes or 8-tube strips needed. Place the LS tubes in an empty rack.
  - 4.2 To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipette tip foreach transfer step.
  - 4.3 Transfer enriched sample to LS tubes as described below:

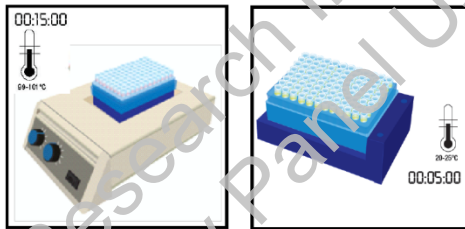
Transfer each enriched sample into an individual LS tube **first**. Transfer the NC **last**.

- 4.4 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip - one strip at a time.
  - 4.5 Discard the LS tube cap – if lysate will be retained for retest, place the caps into a clean container for re-application after lysis. For processing of retained lysate, see Appendix A.
  - 4.6 Transfer 20 µL of sample into a LS tube unless otherwise indicated in Protocol Table 2 or in Specific instructions for validated methods table 3.e.g. Raw dairy products use 10µL.
5. Repeat step 4.2 until each individual sample has been added to a corresponding LS tube in the strip.



6. Repeat steps 4.1 to 4.6 as needed, for the number of samples to be tested.

7. When all samples have been transferred, transfer 20 µL of NC (sterile enrichment medium, e.g., Demi-Fraser Broth) into a LS tube. Do not recap tubes. Do not use water as a NC.
8. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^\circ\text{C}$ .
9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for  $15 \pm 1$  minutes. During heating, the LS solution will change from pink (cool) to yellow (hot). Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.
10. Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes. The 3M Molecular Chill Block Insert, used at ambient temperature without the Molecular Detection Chill Block Tray should sit directly on the laboratory bench. When cool, the lysis solution will revert to a pink color.
11. Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.



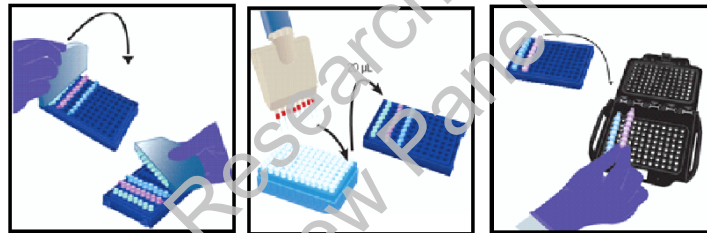
## AMPLIFICATION

1. One Reagent tube is required for each sample and the NC.
  - 1.1 Reagent tube strips can be cut to desired tube number. Select the number of individual Reagent tubes or 8-tube strips needed.
  - 1.2 Place Reagent tubes in an empty rack.
  - 1.3 Avoid disturbing the reagent pellets from the bottom of the tubes.
2. Select 1 Reagent Control (RC) tube and place in rack.
3. To avoid cross-contamination, decap one Reagent tube strip at a time and use a new pipette tip for each transfer step.
4. Transfer lysate to Reagent tubes and RC tube as described below:

Transfer each sample lysate into individual Reagent tubes **first** followed by the NC. Hydrate the RC tube **last**.
5. Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent tubes –one Reagent tube strip at a time. Discard cap.



- 5.1 Transfer 20  $\mu$ L of Sample lysate from the upper  $\frac{1}{2}$  of the liquid (avoid precipitate) in the LS tube into corresponding Reagent tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
- 5.2 Repeat step 5.1 until individual Sample lysate has been added to a corresponding Reagent tube in the strip.
- 5.3 Cover the Reagent tubes with the provided extra caps and use the rounded side of the 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.
- 5.4 Repeat step 5.1 as needed, for the number of samples to be tested.
- 5.5 When all sample lysates have been transferred, repeat 4.1 to transfer 20  $\mu$ L of NC lysate into a Reagent tube.
- 5.6 Transfer **20  $\mu$ L of NC lysate into a RC tube**. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
6. Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader Tray. Close and latch the 3M Molecular Detection Speed Loader Tray lid.



7. Review and confirm the configured run in the 3M Molecular Detection Software.
8. Click the Start button in the software and select instrument for use. The selected instrument's lid automatically opens.
9. Place the 3M Molecular Detection Speed Loader Tray into the 3M Molecular Detection Instrument and close the lid to start the assay. Results are provided within 75 minutes, although positives may be detected sooner.
10. After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from the 3M Molecular Detection Instrument and dispose of the tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

**NOTICE:** To minimize the risk of false positives due to cross-contamination, never open reagent tubes containing amplified DNA. This includes Reagent Control, Reagent, and Matrix Control tubes. Always dispose of sealed reagent tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

## RESULTS AND INTERPRETATION

An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analyzed automatically by the software and are color-coded based on the result. A Positive or Negative result is determined by analysis of a number of unique curve parameters. Presumptive positive results are reported in real-time while Negative and Inspect results will be displayed after the run is completed.

Presumptive positive samples should be confirmed as per the laboratory standard operating procedures or by following the appropriate reference method confirmation<sup>(1, 2, 3)</sup>, beginning with transfer from the primary enrichment to secondary enrichment broth (if applicable), followed by subsequent plating and confirmation of isolates using appropriate biochemical and serological methods.

**NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular Detection Assay 2 - *Listeria* amplification reagents have a “background” relative light unit (RLU) reading.

In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M recommends the user to repeat the assay for any Inspect samples. If the result continues to be Inspect, proceed to confirmation test using your preferred method or as specified by local regulations.

If you have questions about specific applications or procedures, please visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

### Appendix A. Protocol Interruption: Storage and re-testing of heat-treated lysates

1. To store a heat-treated lysate, re-cap the lysis tube with a clean cap (see “LYSIS”, 4.5)
2. Store at 4 to 8°C for up to 72 hours.
3. Prepare a stored sample for amplification by inverting 2-3 times to mix.
4. Decap the tubes.
5. Place the mixed lysate tubes on 3M Molecular Detection Heat Block Insert and heat at 100 ±1°C for 5 ±1 minutes.
6. Remove the rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes.
7. Continue the protocol at the ‘Amplification’ section detailed above.

#### REFERENCES:

1. US Food and Drug Administration Bacteriological Analysis Manual. Chapter 10: Detection and Enumeration of *Listeria monocytogenes* in Foods. January 2016 Version.
2. US Department of Agriculture (USDA) FSIS Microbiology Laboratory Guidebook 8.089. Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry and Egg Products, and Environmental Samples. Effective Date: May 1, 2013.
3. ISO 11290-1. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes*. Amendment 1, 2004-10-15.
4. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
5. ISO 7218. Microbiology of food and animal feeding stuffs – General rules for microbiological examination.
6. ISO 18593. Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.
7. ISO 6887. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.

#### EXPLANATION OF PRODUCT LABEL SYMBOLS



Caution or Warning, see product instructions



Consult product instructions



The lot in a box represents the lot number



The hourglass is followed by a month and year which represent the expiration date



Storage temperature limitations

## AOAC OMA Method Safety Checklist

<b>Submission Date</b>	2016-05-04 12:34:55
<b>First and Last Name</b>	Lisa Monteroso
<b>Organization</b>	3M
<b>E-mail</b>	LPhillips@aoac.org
<b>METHOD TITLE</b>	OMAMAN-29 C/Method Safety Checklist for MDA 2 Listeria Spp
<b>Are any materials used or compounds formed that are explosive or flammable?</b>	NO
<b>Are there any side reactions that could occur that might produce flammable or explosive products or conditions?</b>	NO
<b>Are there any hazards created from electric or mechanical equipment?</b>	NO
<b>Are pressure differentials created that could result in an explosion or implosion?</b>	NO
<b>Would there be increased hazards if the reaction temperature were increased even modestly?</b>	NO
<b>Are special procedures required if a spill of the reaction mixture occurs?</b>	NO
<b>Is there a risk in producing a dangerous aerosol?</b>	NO
<b>Are special procedures required for the disposal of reagents or reaction products?</b>	NO
<b>Are there any potential hazards in handling or storage of reagents, test samples, or standards?</b>	YES
<b>Are there any other hazards that should be addressed regarding the method?</b>	NO
<b>Does your method use chlorinated solvents?</b>	NO

**If your method uses chlorinated solvents, have non-chlorinated solvents equivalent to chlorinated solvents been investigated?**

NO

**Please note if there are any organisms and/or their products that are: (please check all that apply.)**

pathogenic

**Please note any substances used or formed that are any of the following: (please check all that apply.)**

no known associated health or safety risk

1 **A Comparative Evaluation of the 3M™ Molecular Detection Assay 2 (MDA 2) - *Listeria* for**  
2 **the Detection of *Listeria* species in a Variety of Foods and Environmental Surfaces**

3  
4 **AOAC Performance Tested Methods<sup>SM</sup> 111501**

5  
6 **Abstract**

7  
8 The 3M™ Molecular Detection Assay 2 (MDA2) - *Listeria* for the detection of *Listeria* species  
9 (*L. monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. innocua*, *L. grayi*, *L. welshimeri*, *L. fleishmanii*  
10 subsp. *coloradensis*, *L. marthii*, *L. grandensis*, *L. cornellensis* and *L. riparia*) was evaluated  
11 following the 2012 AOAC Research Institute *Performance Tested Methods<sup>SM</sup>* program  
12 guidelines. The evaluation included inclusivity/exclusivity, lot-to-lot/stability, robustness and  
13 matrix studies. The 3M MDA 2 - *Listeria* was compared to various reference methods for raw  
14 chicken, bagged raw spinach, cold smoked salmon, deli turkey, whole melons, vanilla ice cream,  
15 queso fresco, 4% milk fat cottage cheese, beef hot dogs, stainless steel, sealed concrete and  
16 plastic in the matrix study. Twenty replicates of each food matrix were analyzed at a low  
17 inoculum level of 0.2-2 colony forming units (CFU)/standard test portion, five replicates were  
18 analyzed at a high level of 2-5 CFU/test portion, and five control replicates were analyzed at 0  
19 CFU/standard test portion with the exception of naturally contaminated raw chicken (leg pieces)  
20 analyzed at the independent laboratory. Naturally contaminated raw chicken (leg pieces),  
21 analyzed at the independent laboratory, were evaluated using two separate lots consisting of  
22 twenty replicates for each lot. All environmental surfaces were analyzed using twenty replicates  
23 at a low inoculum level of ~ 50 CFU/standard area, five replicates were analyzed at a high level  
24 of ~ 100 CFU/standard area, and five control replicates were analyzed at 0 CFU/standard test  
25 area. Based on the probability of detection statistical model, there were no statistically significant  
26 differences between the number of presumptive and confirmed positive samples or between the  
27 candidate and reference method. There were no unexpected results in the lot-to-lot/stability,  
28 robustness studies. In the inclusivity/exclusivity study, 55 target organisms and 30 non-target  
29 organisms were tested following the 3M MDA 2 - *Listeria* method with all target organisms  
30 detected and none of the non-target organisms detected. The results of this evaluation  
31 demonstrate the specificity and sensitivity of the 3M MDA 2 - *Listeria* and its ability to  
32 accurately detect *Listeria* species in select food matrixes (beef hot dogs, queso fresco, vanilla ice  
33 cream, 4% milk fat cottage cheese, 3% whole milk, bagged raw spinach, cold smoked salmon,  
34 raw chicken parts, deli turkey, whole cantaloupe) and environmental surfaces (stainless steel,  
35 sealed concrete, plastic), 24-30 hours post enrichment.

36  
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43 3M Company  
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17

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21 Rockville, MD  
22

23 **Scope of Method**

- 24
- 25 a) *Target organism.*— *Listeria* species (*L. monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. innocua*,  
26 *L. grayi*, *L. welshimeri*, *L. fleishmanii* subsp. *coloradensis*, *L. cornellensis*, *L. grandensis* *L.*  
27 *marthii*, and *L. riparia*)  
28
- 29 b) *Matrixes.*— Raw chicken (leg pieces and fillets) (25 g), bagged raw spinach (25 g), cold  
30 smoked salmon (25 g), deli turkey (125 g), whole melon, vanilla ice cream (25 g), queso  
31 fresco (25 g), 4% milk fat cottage cheese (25 g), beef hot dogs (25 g), stainless steel (4" x 4",  
32 sponge enriched in 225 mL), sealed concrete (4" x 4", sponge enriched in 225 mL), plastic  
33 (1" x 1", 3M™ Tecra™ Enviroswab [3M Australia Pty Ltd] enriched in 10 mL).  
34
- 35 c) *Summary of Validated Performance Claims.*— Performance equivalent to that of the U.S.  
36 Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM) Chapter 10  
37 [1] for whole melon, cold smoked salmon, bagged raw spinach; the U.S. Department of  
38 Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook*  
39 (USDA-FSIS/MLG) 8.09 [2] for beef hot dogs, deli turkey, raw chicken, sealed concrete,  
40 plastic and stainless steel and AOAC 993.12 [3] for queso fresco, vanilla ice cream, 4% milk  
41 fat cottage cheese and for ISO 11290-1/A1 [4] for cold smoked salmon and bagged raw  
42 spinach.  
43

44 **Definitions**  
45

- 1 a) *Probability of Detection (POD)*.—The proportion of positive analytical outcomes for a  
2 qualitative method for a given matrix at a given analyte level or concentration. POD is  
3 concentration dependent. Several POD measures can be calculated;  $POD_R$  (reference method  
4 POD),  $POD_C$  (confirmed candidate method POD),  $POD_{CP}$  (candidate method presumptive  
5 result POD) and  $POD_{CC}$  (candidate method confirmation result POD).  
6 b) *Difference of Probabilities of Detection (dPOD)*.—Difference of probabilities of detection is  
7 the difference between any two POD values. If the confidence interval of a dPOD does not  
8 contain zero, then the difference is statistically significant at the 5% level.  
9

## 10 Principle of the Method

11  
12 3M™ Molecular Detection Assay 2 - *Listeria* is used with the 3M™ Molecular Detection  
13 System for the rapid and specific detection of *Listeria* species in enriched food and  
14 environmental samples.  
15

16 The 3M Molecular Detection Assays use loop-mediated isothermal amplification to rapidly  
17 amplify nucleic acid sequences with high specificity and sensitivity, combined with  
18 bioluminescence to detect the amplification. Presumptive positive results are reported in real-  
19 time while negative results are displayed after the assay is completed. Presumptive positive  
20 results should be confirmed using the laboratory's preferred method or as specified by local  
21 regulations.  
22

## 23 General Information

24  
25 *Listeria* is a small, Gram-positive rod that can be found in soil or carried by animals. Many  
26 foods have been associated with *Listeria* contamination: uncooked meats, uncooked  
27 vegetables, cantaloupe, pasteurized and unpasteurized milk, and other dairy and processed  
28 foods. [5] The resulting illness, listeriosis, from *Listeria monocytogenes* ingestion is rare but  
29 can be fatal. The disease may appear as meningitis in adults and can affect unborn children  
30 due to its ability to cross through the placenta. *Listeria* contamination often occurs post-  
31 cooking, before packaging as *Listeria* can survive longer under adverse environmental  
32 conditions that can most other vegetative bacteria that present a concern for the food safety  
33 industry. [6] Manufacturing facilities implement sanitation practices and regular surface  
34 screening for the presence of *Listeria* on food contact surfaces to monitor their food  
35 production environments. The regular surface screening for non - *Listeria monocytogenes*  
36 species is used as an indicator to monitor for the risk of *Listeria monocytogenes*  
37 contamination and is typically part of a food manufacturing facility's Hazard Analysis  
38 Critical Control Point (HACCP) plan. The 3M MDA 2 - *Listeria* was developed to provide  
39 rapid and specific detection of *Listeria* in enriched food samples and on food processing  
40 surfaces.  
41

## 42 Materials and Methods

### 43 Test Kit Information

- 44  
45 a) *Kit Name*.— Molecular Detection Assay 2 – *Listeria*  
46



1 b) Catalog Number.— MDA2LIS96  
2

3 *Test Kit Reagents - 3M MDA 2 - Listeria (96 tests)*

- 4 a) *Lysis Solution (LS) tubes.* – 96 (12 strips of 8 tubes)  
5 b) *Listeria Reagent tubes.* – 96 (12 strips of 8 tubes)  
6 c) *Extra caps.* – 96 (12 strips of 8 caps)  
7 d) *Reagent Control (RC).* – 16 (2 pouches of 8)  
8 e) *Quick Start Guide*  
9

10 *Additional Supplies and Reagents*

- 11 a) 3M Molecular Detection System (instrument), power supply, cord, USB cable and  
12 software  
13 b) 3M Molecular Detection Speed Loader Tray  
14 c) 3M Molecular Detection Chill Block insert  
15 d) 3M Molecular Detection Heat Block Insert  
16 e) 3M Molecular Detection Cap/Decap Tool [Reagent]  
17 f) 3M Molecular Detection Cap/Decap Tool [Lysis]  
18 g) Empty lysis tube rack  
19 h) Empty reagent tube rack  
20 i) Dey-Engley (D/E) Neutralizing Broth  
21 j) Demi Fraser Broth (with the addition of Ferric Ammonium Citrate [FAC])  
22 k) 3M™ Hydrated Sponge Stick with 10 mL of D/E (3M Center St. Paul, MN)  
23 l) 3M™ Molecular Detection Matrix Control Kit (3M Center St. Paul, MN)  
24

25 *Additional media*

- 26 b) Oxford Agar (OX)  
27 c)  
28 d) Fraser Broth (FB)  
29 e) Sheep Blood Agar (SBA)  
30 f) PALCAM Agar  
31 g) Ottaviani Agosti Agar (OAA)  
32 h) Trypticase Soy Agar (TSA)  
33 i) Trypticase Soy Agar with 0.6% Yeast Extract (TSA/YE)  
34 j) De Man, Rogosa and Sharpe (MRS)  
35 k) Modified Oxford Agar (MOX)  
36 l) Brain Heart Infusion (BHI) broth  
37 m) Horse Blood Overlay (HBO)  
38 n) Buffered Listeria Enrichment Broth (BLEB)  
39

40 *Apparatus*

- 41 a) *Pipettes.* – capable of 20µL  
42 b) *Multi-channel pipette.* – capable of 20µL  
43 c) *Sterile pipette tips.* – capable of 20µL  
44 d) *Stomacher®.* – Seward or equivalent  
45 e) *Filter Stomacher® bags.* - Seward or equivalent  
46 f) *Thermometer.* – calibrated range to include 100 ± 1°C range  
47 g) *Incubators.* – capable of maintaining 37 ± 1°C

- 1 h) *Dry double block heater unit.* – capable of maintaining  $100 \pm 1^{\circ}\text{C}$ ; or *a water bath*  
2 capable of maintaining  $100 \pm 1^{\circ}\text{C}$   
3 i) *Refrigerator.* – capable of maintaining  $2-8^{\circ}\text{C}$ , for storing the 3M MDA2  
4 j) *Computer.* – compatible with the 3M Molecular Detection System (instrument)  
5

## 6 **Safety Precautions**

7  
8 The user should read, understand and follow all safety information in the instructions for the 3M  
9 Molecular Detection System and the 3M Molecular Detection Assay 2 - *Listeria*. Retain the  
10 safety instructions for future reference.  
11

12 **As with all test methods, the source, formulation and quality of enrichment medium can**  
13 **influence the results.** Factors such as sampling methods, testing protocols, sample preparation,  
14 handling, and laboratory technique may also influence results. 3M recommends evaluation of  
15 the method including enrichment medium, in the user's environment using a sufficient number of  
16 samples with particular foods and microbial challenges to ensure that the method meets the  
17 user's criteria.

18 The 3M Molecular Detection Assay 2 - *Listeria* method may generate *Listeria monocytogenes* to  
19 levels sufficient to cause stillbirths and fatalities in pregnant women and the  
20 immunocompromised, if exposed.  
21

To reduce the risks associated with exposure to chemicals and biohazards:

- It is strongly recommended that female laboratory staff be informed of the risk to a developing fetus resulting from infection of the mother through exposure to *Listeria monocytogenes*
- Perform pathogen testing in a properly equipped laboratory under the control of trained personnel
- Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples
- Avoid contact with the contents of the enrichment media and reagent tubes after amplification
- Dispose of enriched samples according to current industry standards

## 22 **Sample Preparation**

23 3M recommends the use of Demi-Fraser Broth with FAC for the enrichment of food and  
24 environmental samples. Table 1 presents guidance for the enrichment of food and environmental  
25 samples. It is the user's responsibility to validate alternate sampling protocols or dilution ratios  
26 to ensure this test method meets the user's criteria.  
27

### 28 *Foods*

- 29
- 30 a) Allow the Demi-Fraser Broth enrichment medium (includes FAC) to equilibrate to ambient  
31 laboratory temperature.
  - 32 b) Aseptically combine the enrichment medium and sample according to Table 1. For all meat  
33 and highly particulate samples, the use of filter bags is recommended.
  - 34 c) Homogenize thoroughly by blending, stomaching, or hand mixing for  $2 \pm 0.2$  minutes.  
35 Incubate at  $37 \pm 1^{\circ}\text{C}$  according to Table 1.  
36

### Environmental samples

Sample collection devices can be a sponge hydrated with a neutralizing solution to inactivate the effects of the sanitizers. 3M recommends the use of a biocide-free cellulose sponge. Neutralizing solution can be Dey-Engley (D/E) Neutralizing Broth or Lethen Broth. It is recommended to sanitize the area after sampling.

The recommended size of the sampling area for verifying the presence or absence of the pathogen on the surface is at least 100 cm<sup>2</sup> (10 cm x 10 cm or 4"x4"). When sampling with a sponge, cover the entire area going in two directions (left to right then up and down) or collect environmental samples following your current sampling protocol or according to the FDA BAM, USDA FSIS MLG or ISO 18593 [7] guidelines.

1. Allow the Demi-Fraser Broth enrichment medium (includes FAC) to equilibrate to ambient laboratory temperature.
2. Aseptically combine the enrichment medium and sample according to Table 1.
3. Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ± 0.2 minutes. Incubate at 37 ± 1 °C for 24-30 hours.
4. Invert room temperature (20-25 °C) lysis tubes to mix. Proceed to next step within 4 hours. 20 µL aliquot of each enriched sample are transferred to separate lysis tubes using a new pipette tip after each sample transfer. Place uncovered samples on a dry double block heater for 15 ± 1 minutes at 100 ± 1 °C. Following the heat lysis transfer samples into the chill block insert and placed onto a sterilized lab bench and allow to cool at 18-28 °C for 5-10 minutes.
5. Add 20 µL of each lysed sample and control to separate reagent tubes, and mix by pipetting up and down five times. Analyze a matrix control tube with the samples for each matrix to verify that no interference with the assay was caused by the matrix. Use sterile Demi Fraser Broth with FAC for the Negative Control (NC).
6. Transfer a 20 µL aliquot to the NC and the Reagent Control (RC) tubes. Add a 20 µL aliquot of a randomly picked sample to the matrix control tube, mixed, and recapped. Using the 3M™ software, follow prompts to identify samples and controls. Load all samples into the Speed Loader Tray (SLT), place into the Molecular Detection System, and initiate the 3M™ MDA 2 - *Listeria* assay. Results are obtained within 75 minutes.

### Interpretation and Test Result Report

An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analyzed automatically by the software and are color-coded based on the result. A Positive or Negative result is determined by analysis of a number of unique curve parameters. Presumptive positive results are reported in real-time while Negative and Inspect results will be displayed after the run is completed.

**NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular Detection Assay 2 - *Listeria* amplification reagents have a “background” relative light unit (RLU) reading.

In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M recommends the user to repeat the assay for any Inspect samples. If the result continues to be

1 Inspect, proceed to confirmation test using your preferred method or as specified by local  
2 regulations.

### 3 4 **Confirmation**

5  
6 Presumptive positive primary enrichment samples were confirmed by following the appropriate  
7 reference method confirmation, beginning with transfer from the primary enrichment to  
8 secondary enrichment broth (if applicable), followed by subsequent plating and confirmation of  
9 isolates using appropriate biochemical and serological methods.

### 10 11 **Validation Study**

12  
13 Testing was conducted following the procedures outlined in the AOAC Research Institute  
14 *Performance Tested Methods<sup>SM</sup> Program* validation outline protocol: *Comparative Evaluation of*  
15 *the 3M<sup>TM</sup> Molecular Detection Assay 2 - Listeria monocytogenes and of the 3M<sup>TM</sup> Molecular*  
16 *Detection Assay 2 - Listeria* (February, 2015) [8]. Three brands of Demi Fraser Broth with FAC  
17 were used for this validation study; X, Y, and Z. The independent study involved a matrix study  
18 (10 matrixes), a lot-to-lot/stability evaluation, and a robustness evaluation using brand Y. The  
19 internal study involved a matrix study (4 matrixes) and an inclusivity/exclusivity study using  
20 either brand X or Z.

### 21 22 **Internal Study**

#### 23 24 *Inclusivity and Exclusivity*

#### 25 26 *Methodology*

27  
28 Fifty frozen *Listeria* strain suspensions were thawed and sub-cultured in Brain Heart Infusion  
29 (BHI) broth overnight at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The cultures were diluted in peptone salt solution in order  
30 to inoculate between 10 to 100 cells per 225 mL of Demi Fraser with FAC. The enrichment  
31 broths were then incubated for 24 hours at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , and the 3M MDA 2 - *Listeria* method  
32 was then performed and confirmed according to ISO 11290-1/A1. See Table 2a.

33  
34 Five additional *Listeria* strains were tested at 3M (St. Paul, MN). The five frozen *Listeria* strain  
35 suspensions were thawed and streaked onto Sheep Blood Agar (SBA). The SBA plates were  
36 incubated at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$  overnight. A single isolated colony from the SBA plate was sub-  
37 cultured in 10 mL of Demi Fraser Broth with FAC overnight at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The cultures were  
38 diluted using Demi Fraser broth with FAC, and the 3M MDA 2 - *Listeria* method was then  
39 performed. See Table 2b.

40  
41 Thirty frozen non-*Listeria* strain suspensions were thawed and sub-cultured in BHI broth  
42 overnight at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The cultures were diluted in Buffered Peptone Water (BPW) or de  
43 Man, Rogosa, and Sharpe (MRS) in order to inoculate  $10^5$  cells/mL. The broths were then  
44 incubated for 24 hours at the appropriate incubation temperature in order to have culture to test  
45 with the 3M MDA 2 - *Listeria* method. See Table 3.

46

1 *Results*

2  
 3 All 55 *Listeria* strains were detected by the 3M MDA 2 - *Listeria* method. *L. grayi* recovery was  
 4 enhanced when a food sample (Ultra-high-temperature [UHT] milk as described in ISO 16140  
 5 [9]) was added to the medium in the standard 1:10 dilution scheme. None of the 30 non- *Listeria*  
 6 strains were detected. See Tables 2 and 3 for study details and results.

7  
 8  
 9 **Matrix Study**

10  
 11 The method comparison study consisted of evaluating a total of 30 un-paired sample replicates  
 12 for 10 matrixes, along with naturally contaminated raw chicken (leg pieces), tested at the  
 13 independent laboratory, evaluating 20 sample replicates using two separate lots, see Table 4a.  
 14 The raw chicken (leg pieces and fillets) analyzed at the internal lab was inoculated according to  
 15 AOAC guidelines which are described below, see Table 4b. Within each sample set, there were  
 16 5 uninoculated samples (0 CFU/test portion), 20 low level inoculated samples (0.2-2 CFU/test  
 17 portion), and 5 high level inoculated samples (2-5 CFU/test portion), except for the naturally  
 18 contaminated raw chicken (leg pieces). The inoculum was prepared by transferring a single  
 19 *Listeria* colony from Trypticase Soy Agar with 5% Sheep Blood (SBA) into BHI broth and  
 20 incubating the culture at  $35 \pm 2^\circ\text{C}$  for  $24 \pm 2$  hours. Tables 4a and b presents the sample  
 21 preparation guidelines for the matrix.

22  
 23 All matrixes were screened for the presence of the target organism following the appropriate  
 24 reference method. Additionally, an aerobic plate count (APC) was conducted following the  
 25 FDA/BAM Chapter 3 reference [10] method to determine the level of background flora in each  
 26 test matrix prior to inoculation.

27  
 28 Prior to inoculation of beef hot dogs, deli turkey, and queso fresco the broth culture inoculum  
 29 was heat stressed for  $10 \pm 1$  minute at  $50 \pm 1^\circ\text{C}$  in a water bath. The degree of injury of the  
 30 culture was estimated by plating an aliquot of diluted culture onto Modified Oxford Agar (MOX)  
 31 and Tryptic Soy Agar (TSA). The agars were incubated at  $35 \pm 1^\circ\text{C}$  for  $24 \pm 2$  hours and the  
 32 colonies were counted. The degree of injury was estimated as:

33  
 34  
 35

$$\left(1 - \frac{n_{select}}{n_{nonselect}}\right) \times 100$$

36 Where  $n_{select}$  = number of colonies on selective agar and  $n_{nonselect}$  = number of colonies on non-  
 37 selective agar. Using BHI broth as the diluent, the culture was diluted to a low level expected to  
 38 yield fractional positive results (5-15 positive results) and a high level expected to yield all  
 39 positive results. Following inoculation, a bulk lot of the matrix was homogenized by hand and  
 40 held for 48-72 hours at refrigerated temperature ( $2-8^\circ\text{C}$ ) prior to analysis to allow time for the  
 41 organism to equilibrate within the sample.

42  
 43 For the inoculation of the whole melons, a single whole melon was placed into a large sterile bag  
 44 and the blossom end of the melon was inoculated with 100  $\mu\text{L}$  of the diluted *Listeria*  
 45 *monocytogenes* culture. The liquid culture was then allowed to soak into the melon. The melon

1 was inverted so that the inoculated end was on the bottom of the sterile bag. The bag was tied  
 2 closed and held for 48-72 hours at 2-8 °C.

3  
 4 For environmental surfaces, inocula were prepared by transferring a pure isolated colony of the  
 5 specified organism from SBA into BHI broth and incubated at 35 ± 2°C for 24 ± 2 hours.  
 6 Following incubation, serial dilutions were performed in BHI broth to achieve the target level  
 7 inoculum.

8  
 9 For stainless steel and sealed concrete surfaces, 4" x 4" areas were inoculated with 0.25 mL of  
 10 diluted *Listeria monocytogenes* culture. Stainless steel was also inoculated with competitor  
 11 organism *Enterococcus faecium* ATCC 19434 at 10x the level of the target organism. For plastic  
 12 surfaces, 1" x 1" areas were inoculated with 0.10 mL of diluted *Listeria seeligeri* culture. Plastic  
 13 was also inoculated with a competitor organism, *Enterococcus faecalis* ATCC 29212, at 10x the  
 14 level of the target organism. For the uninoculated test portions, sterile BHI broth was applied to  
 15 the test area. Each surface was allowed to dry for 16-24 hours at room temperature (24 ± 2°C).

16  
 17 The 3M™ Hydrated Sponge Stick (pre-moistened with Dey-Engley) (stainless steel and sealed  
 18 concrete) and 3M Tecra Enviroswabs (pre-wetted with Letheen) (plastic) were sampled by using  
 19 horizontal and vertical sweeping motions. The sponges and the swabs were held at room  
 20 temperature for 2 hours prior to analysis. To determine the inoculation level for the  
 21 environmental surfaces, aliquots of each inoculum were plated on TSA in triplicate.

22  
 23 The level of *Listeria monocytogenes* in the low level inoculum was determined by Most  
 24 Probable Number (MPN) on the day of analysis by evaluating 5 x 50 g, 20 x 25 g (reference  
 25 method test portions), and 5 x 10 g inoculated test samples. For the high inoculation level, the  
 26 MPN was determined by examining 5 x 50 g, 5 x 25 g (reference method test portions) and 5 x  
 27 10 g . The level of *Listeria* in the low level inoculum for all 125 g test portions was determined  
 28 by MPN by evaluating 5 x 250 g, 20 or 5 x 125 g (reference method test portions), and 5 x 50 g  
 29 inoculated test samples. For the high inoculation level, the MPN was determined by examining 5  
 30 x 250 g, 5 x 125 g (reference method test portions), and 5 x 50 g inoculated test samples. Each  
 31 test portion was enriched with the reference method enrichment broth at the reference method  
 32 dilution scheme and analyzed by the reference method procedure. See Table A for details. The  
 33 number of positives from the 3 test levels was used to calculate the MPN using the LCF MPN  
 34 calculator (version 1.6) provided by AOAC RI. [11]

35 (<http://www.lcfltd.com/customer/LCFMPNCalculator.exe>)

36  
 37 **Table A: MPN Test Portion Sizes**

Reference Method Test Portion	Inoculation Level	MPN Test Portions		
25 g	Low	5 x 50 g	20 x 25 g*	5 x 10 g
	High	5 x 50 g	5 x 25 g*	5 x 10 g
125 g	Low	5 x 250 g	20 x 125 g*	5 x 50 g
	High	5 x 250 g	5 x 125 g*	5 x 50 g

38 \*Test portions from reference method



1 USDA/FSIS MLG 8.09 Reference Method  
2

3 For the USDA/FSIS MLG 8.09 reference method, 25 g test portions were enriched with  $225 \pm 5$   
4 mL of modified University of Vermont Medium broth (UVM) and 125 g test portions were  
5 enriched with  $1125 \pm 25$  mL of UVM. All test portions were mechanically stomached for two  
6 minutes. The 25 g test portions were incubated at  $30 \pm 2$  °C for 20-26 hours and the 125 g test  
7 portions were incubated at  $30 \pm 2$  °C for 23-26 hours. For environmental samples, sponges were  
8 enriched with 225 mL of UVM and homogenized by hand, while swabs were enriched with 10  
9 mL of UVM and mixed by vortex. Sponges and swabs were incubated for 20-26 hours at  $30 \pm$   
10  $2^{\circ}\text{C}$ . After incubation of all test portions,  $0.1 \pm 0.02$  mL of the sample enrichment was  
11 transferred to  $10 \pm 0.5$  mL of Fraser Broth (FB) containing 0.1 mL of 5% ferric ammonium  
12 citrate and incubated at  $35 \pm 2^{\circ}\text{C}$  for  $26 \pm 2$  hours. A loopful of the sample enrichment was also  
13 streaked to MOX and incubated at  $35 \pm 2^{\circ}\text{C}$  for  $26 \pm 2$  hours.  
14

15 After  $26 \pm 2$  hours, FB was examined for any degree of darkening due to esculin hydrolysis. Any  
16 FB that displayed darkening was streaked to a MOX plate. If no darkening occurred, FB was re-  
17 incubated at  $35 \pm 2^{\circ}\text{C}$  for a total of  $48 \pm 2$  hours and re-examined for evidence of darkening. If  
18 darkening occurred, the FB was streaked to a MOX plate, if no darkening occurred, samples  
19 were considered negative. All FB streaked MOX plates were incubated at  $35 \pm 2^{\circ}\text{C}$  for  $26 \pm 2$   
20 hours.  
21

22 MOX agar plates streaked from the primary enrichment or the FB secondary enrichment were  
23 examined after  $26 \pm 2$  hours and if no suspect colonies were present, the MOX agar plate was re-  
24 incubated for an additional  $26 \pm 2$  hours at  $35 \pm 2^{\circ}\text{C}$  for a total of  $48 \pm 2$  hours. If suspect  
25 colonies were present on the MOX agar plates, these suspect colonies were streaked to Horse  
26 Blood Overlay agar (HBO) and incubated at  $35 \pm 2^{\circ}\text{C}$  for  $22 \pm 4$  hours. HBO plates were  
27 examined for hemolysis reactions and well isolated colonies were transferred to BHI broth and  
28 incubated at  $25^{\circ}\text{C}$  for  $24 \pm 2$  hours. Sample isolates from BHI broth were analyzed for tumbling  
29 motility by preparing a wet mount, analyzed by a catalase test and examined for morphology by  
30 preparing a Gram stain. Additionally, purified HBO isolates were identified using the VITEK<sup>®</sup>  
31 GP Biochemical Identification following AOAC OMA 2013.02. [12]  
32

33 FDA/BAM Chapter 10 Reference Method  
34

35 Twenty-five gram test portions were enriched in  $225 \text{ mL} \pm 5 \text{ mL}$  of Buffered Listeria  
36 Enrichment Broth (BLEB) homogenized for 2 minutes and incubated at  $30 \pm 1^{\circ}\text{C}$  for 4 hours.  
37 For whole melons, a single whole melon was enriched using BLEB with approximately 1.5 times  
38 the weight of the melon, and soaked for 1 hour at room temperature. After an hour at room  
39 temperature, the test portions were incubated at  $30 \pm 1^{\circ}\text{C}$  for 4 hours. Following 4 hours of  
40 incubation, selective supplements acriflavine (10mg/L), sodium nalidixate (40mg/L) and  
41 cycloheximide (50mg/L) were added to each test portion and incubated for an additional 20  
42 hours. After 24 hours of total incubation, the enriched samples were streaked to MOX agar plates  
43 and incubated at  $35 \pm 1^{\circ}\text{C}$  for 24-48 hours. The enriched samples were re-incubated for an  
44 additional 24 hours at  $30 \pm 1^{\circ}\text{C}$  and then streaked to a second MOX agar plate which was  
45 incubated for 24-48 hours at  $35 \pm 1^{\circ}\text{C}$ . MOX agar plates were examined for suspect colonies, and  
46 if present, at least 5 colonies were streaked to TSA containing 0.6% yeast extract (TSA/YE). The

1 TSA/YE plates were incubated at  $35 \pm 1^\circ\text{C}$  for 24-48 hours and then examined for purity. Pure  
2 colonies were tested for catalase reactivity and a Gram Stain was conducted. A pure *Listeria*  
3 colony was transferred to Trypticase Soy Broth containing 0.6% yeast extract (TSB/YE). The  
4 TSB/YE cultures were incubated at  $25 \pm 1^\circ\text{C}$  overnight, or until the broth was turbid, indicating  
5 sufficient growth. Catalase-positive organisms were stabbed into plates of 5% Sheep Blood Agar  
6 (SBA) and incubated at  $35 \pm 1^\circ\text{C}$  for 24-48 hours. The TSB/YE tubes incubated at  $25 \pm 1^\circ\text{C}$  were  
7 used to prepare a wet mount slide to determine motility pattern. After incubation, the SBA plates  
8 were examined for hemolysis. Final confirmation was conducted using the VITEK<sup>®</sup> GP  
9 Biochemical Identification card following AOAC OMA 2013.02.

#### 10 11 AOAC 993.12 Listeria Reference Method

12  
13 Twenty-five gram test portions were enriched in 225 mL  $\pm$  5 mL of selective enrichment  
14 medium, containing yeast extract (1.35 g/225 mL), acriflavine mono hydrochloride (1.125 g/225  
15 mL), nalidixic acid (sodium salt) solution (1.125 g/225 mL), and cycloheximide (2.25 g/225  
16 mL), and incubated at  $30 \pm 1^\circ\text{C}$  for 48 hours. The enriched samples were then streaked to  
17 Oxford Agar (OXA) and incubated at  $37 \pm 1^\circ\text{C}$  for 48 hours. At 48 hours, the OXA plates were  
18 examined for suspect colonies. If present, up to 5 suspect colonies were streaked to TSA/YE to  
19 obtain well isolated, pure colonies. Pure colonies on TSA/YE were analyzed for Gram-stain  
20 reaction and for catalase activity. Catalase positive, Gram positive rods colonies were then  
21 stabbed to SBA and incubated at  $37 \pm 1^\circ\text{C}$  for 48 hours. After 48 hours, the SBA plates were  
22 examined for typical hemolytic reactions. The same colony picked to SBA was also transferred  
23 to TSB/YE. The TSB/YE cultures were incubated at  $25 \pm 1^\circ\text{C}$  overnight, or until the broth was  
24 turbid, indicating sufficient growth. Another TSB/YE tube was inoculated with the same colony  
25 and incubated at  $37 \pm 1^\circ\text{C}$  for 24 hours to be used for carbohydrate utilization testing. The  
26 TSB/YE tube incubated at  $25 \pm 1^\circ\text{C}$  was used to prepare a wet mount slide to determine motility  
27 pattern. From the TSB/YE tube incubated at  $37 \pm 1^\circ\text{C}$ , Motility Test Medium (MTM) was  
28 stabbed and incubated at  $25 \pm 1^\circ\text{C}$ . After 2 days, and up to 7 days, the MTM tubes were observed  
29 for umbrella-like growth. Additionally from the TSB/YE tube, a loopful (0.1 mL) was  
30 transferred into carbohydrate fermentation broth (purple broth) containing 1.0 mL of either 5%  
31 rhamnose or 5% xylose. The purple broth tubes were incubated at  $37 \pm 1^\circ\text{C}$  and examined for up  
32 to 7 days.

#### 33 34 ISO 11290-1/A1 Reference Method

35  
36 For the ISO 11290-1/A1 reference method, 25 g test portions were enriched with 225 half Fraser  
37 broth. All test portions were mechanically stomached for two minutes. The test portions were  
38 incubated at  $30 \pm 1^\circ\text{C}$  for  $24 \pm 3$  hours. After incubation, 0.1 mL of the sample enrichment was  
39 transferred to 10 mL FB containing 0.1 mL of 5% ferric ammonium citrate and incubated at  $37 \pm$   
40  $1^\circ\text{C}$  for  $48 \pm 3$  hours. After  $48 \pm 3$  hours, a loopful of the sample secondary FB enrichment was  
41 streaked to PALCAM and Ottovani-Agosti Agar (OAA) and incubated at  $37 \pm 1^\circ\text{C}$  for  $24 \pm 3$   
42 hours. A loopful of the sample primary enrichment was also streaked to PALCAM and OAA and  
43 incubated at  $37 \pm 1^\circ\text{C}$  for  $24 \pm 3$  hours. PALCAM and OAA agar plates were examined for the  
44 presence of suspect colonies. If no suspect colonies were present, the agar plate was re-incubated  
45 for an additional 18-24 hours at  $37 \pm 1^\circ\text{C}$ . If no suspect colonies present the sample was  
46 determined to be negative for *Listeria*. If suspect colonies were present on the PALCAM or



1 OAA agar plates, these suspect colonies were streaked to HBO and incubated at  $37 \pm 1^\circ\text{C}$  for 18-  
2 24 hours. HBO plates were examined for hemolysis reactions and well-isolated colonies were  
3 transferred to BHI broth and incubated at  $25^\circ\text{C}$  for  $24 \pm 2$  hours. Sample isolates from BHI broth  
4 were analyzed for tumbling motility by preparing a wet mount, analyzed by a catalase test and  
5 examined for morphology by preparing a Gram stain. Additionally, purified HBO isolates were  
6 identified using the VITEK<sup>®</sup> GP Biochemical Identification following AOAC OMA 2013.02.  
7  
8

### 9 3M<sup>™</sup> Molecular Detection Assay 2 - Listeria

10  
11 All 25 g samples were analyzed by the 3M<sup>™</sup> MDA 2 - Listeria were enriched with 225 mL of  
12 Demi-Fraser Broth with the addition of ferric ammonium citrate; all test portions were then  
13 homogenized by stomaching thoroughly for  $2 \pm 0.2$  minutes and incubated at  $37 \pm 1^\circ\text{C}$  for 24-28  
14 hours. For the 125 g deli turkey test portions, samples were enriched with 1125 mL of Demi-  
15 Fraser Broth with the addition of ferric ammonium citrate; test portions were then homogenized  
16 by stomaching thoroughly for  $2 \pm 0.2$  minutes and incubated at  $37 \pm 1^\circ\text{C}$  for 24 hours. For  
17 whole melons, test portions were enriched with approximately 1.5 times the weight of the whole  
18 melon in Demi Fraser with ferric ammonium citrate and incubated at  $37 \pm 1^\circ\text{C}$  for 26 hours.  
19 Stainless steel sponge samples were enriched with 225 mL of Demi-Fraser Broth with the  
20 addition of ferric ammonium citrate; samples were homogenized by hand thoroughly for  $2 \pm 0.2$   
21 minutes and incubated at  $37 \pm 1^\circ\text{C}$  for 24 and 26 hours. Sealed concrete environmental surface  
22 sponge samples were enriched with 100 mL of Demi-Fraser Broth with the addition of ferric  
23 ammonium citrate; samples were thoroughly homogenized by hand for  $2 \pm 0.2$  minutes and  
24 incubated at  $37^\circ\text{C}$  for 24 and 26 hours. Plastic environmental surface swab samples were  
25 enriched with 10 mL of Demi-Fraser Broth with the addition of ferric ammonium citrate;  
26 samples were homogenized by vortexing for  $2 \pm 0.2$  minutes and incubated at  $37 \pm 1^\circ\text{C}$  for 24  
27 and 26 hours.  
28

29 Prior to analysis, lysis tubes were brought to room temperature ( $20\text{-}25^\circ\text{C}$ ) by placing the tubes on  
30 a sanitized bench top for 16-18 hours. Lysis tubes were inverted to mix up to four hours before  
31 use. The lysis tubes were then de-capped and the rubber cap was discarded. A  $20\ \mu\text{L}$  aliquot of  
32 each sample was transferred to separate lysis tubes; a new pipette tip was used after each sample  
33 transfer. Uncovered samples were placed in the 3M Molecular Detection Heat Block Insert and  
34 heated for  $15 \pm 1$  minutes at  $100 \pm 1^\circ\text{C}$ . Following the heat lysis, samples were transferred into the  
35 Chill Block Insert and placed on a sanitized laboratory bench and were allowed to cool at  $18\text{-}$   
36  $28^\circ\text{C}$  for 5-10 minutes.  
37

38 A  $20\ \mu\text{L}$  aliquot of each lysed sample and control was added to separate reagent tubes, and  
39 samples were mixed by pipetting up and down five times. A Matrix Control tube was analyzed  
40 with the samples for each matrix to verify that no interference with the assay was caused by the  
41 matrix. Also lysed was a sterile Demi Fraser Broth with FAC tube for the kit Negative Control  
42 (NC). A  $20\ \mu\text{L}$  aliquot was transferred to the NC and the Reagent Control (RC) tubes. A  $20\ \mu\text{L}$   
43 aliquot of a randomly picked sample was added to the Matrix Control tube, mixed, and recapped.  
44 Using the 3M<sup>™</sup> software, prompts were followed to identify samples and controls. All samples  
45 were loaded into the Speed Loader Tray, placed into the Molecular Detection System, and the  
46 3M<sup>™</sup> MDA 2 - Listeria assay was initiated and results were obtained within 75 minutes.

## Matrix Study Results

For each method, the probability of detection (POD) was calculated as the number of positive outcomes divided by the total number of trials. [13] The following were calculated: the candidate presumptive results,  $POD_{CP}$ ; the candidate confirmatory results,  $POD_{CC}$ ; the difference in the candidate presumptive and confirmatory results,  $dPOD_{CP}$ ; the presumptive candidate results that confirmed positive,  $POD_C (=POD_{CC})$ ; the reference method,  $POD_R$ , and the difference in the confirmed candidate and reference methods,  $dPOD_C$ . POD analyses were conducted for the MDA 2 - *Listeria* test points and compared to the appropriate reference method results. The pre-validation target analyte background screen results and APC results are presented in Table 5. The heat stress data for selected matrixes are presented in Table 6. The inoculum levels for each environmental surface are presented in Table 7. A summary of POD analyses [14] are presented in Tables 8-15. As per criteria outlined in Appendix J of the Official Methods of Analysis Manual [15], fractional positive results were obtained for all matrixes.

### Internal Study

#### *Raw chicken leg pieces (25 g)*

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 4 presumptive positives and 9 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure resulting in 5 false negative samples. There were 5 observed positives for the reference method. For the high inoculation level, there were 3 presumptive positives and 3 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There was 1 confirmed positive following the reference method. Even though 5 false negatives were confirmed, analysis show there to be no statistical difference when compared to the reference method. Detailed results of the POD analyses are presented in Tables 8 and 12. This matrix analysis was conducted using Demi Fraser with FAC brand X.

#### *Raw chicken fillet (25 g)*

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 4 presumptive positives and 4 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 5 observed positives for the reference method. For the high inoculation level, there were 2 presumptive positives and 2 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 5 confirmed positives following the reference method. Detailed results of the POD analyses are presented in Tables 8 and 12. This matrix analysis was conducted using Demi Fraser with FAC brand Z.

#### *Bagged raw spinach*

#### *Compared to FDA-BAM*

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 6 presumptive positives and 6 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation

1 procedure. There were 3 observed positives for the reference method. For the high inoculation  
2 level, there were 3 presumptive positives and 3 confirmed positives following the FDA/BAM  
3 Chapter 10 reference method confirmation procedure. There were 4 confirmed positives  
4 following the reference method. Detailed results of the POD analyses are presented in Tables 8  
5 and 12. This matrix analysis was conducted using Demi Fraser with FAC brand X.  
6  
7

8 *Compared to ISO 11290-1/A1*

9 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 6 presumptive positives  
10 and 6 confirmed positives following the ISO 11290-1/A1 reference method confirmation  
11 procedure. There were 9 observed positives for the reference method. For the high inoculation  
12 level, there were 3 presumptive positives and 3 confirmed positives following the ISO 11290-  
13 1/A1 reference method confirmation procedure. There were 4 confirmed positives following the  
14 reference method. Detailed results of the POD analyses are presented in Tables 8 and 12  
15 This matrix analysis was conducted using Demi Fraser with FAC brand X.  
16

17 *Cold smoked salmon*

18 *Compared to FDA-BAM*

19 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 10 presumptive positives  
20 and 10 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation  
21 procedure. There were 7 observed positives for the reference method. For the high inoculation  
22 level, there were 5 presumptive positives and 5 confirmed positives following the FDA/BAM  
23 Chapter 10 reference method confirmation procedure. There were 4 confirmed positives  
24 following the reference method. Detailed results of the POD analyses are presented in Tables 8  
25 and 12. This matrix analysis was conducted using Demi Fraser with FAC brand X.  
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30 *Compared to ISO 11290-1/A1*

31 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 10 presumptive positives  
32 and 10 confirmed positives following the ISO 11290-1/A1 reference method confirmation  
33 procedure. There were 8 observed positives for the reference method. For the high inoculation  
34 level, there were 5 presumptive positives and 5 confirmed positives following the ISO 11290-  
35 1/A1 reference method confirmation procedure. There were 4 confirmed positives following the  
36 reference method. Detailed results of the POD analyses are presented in Tables 8 and 12.  
37 This matrix analysis was conducted using Demi Fraser with FAC brand X.  
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41 *Independent Study using Demi Fraser with FAC brand Y.*

42 *Deli turkey (125 g)*

43  
44 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 8 presumptive positives  
45 and 8 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation  
46

1 procedure. There were 7 observed positives for the reference method. For the high inoculation  
2 level, there were 5 presumptive positives and 5 confirmed positives following the USDA/FSIS  
3 MLG 8.09 reference method confirmation procedure. There were 5 confirmed positives  
4 following the reference method. Detailed results of the POD analyses are presented in Tables 9  
5 and 13.

6  
7 *Naturally Contaminated raw chicken leg pieces (25 g)*

8  
9 *Lot 1*

10 For lot 1 of the raw chicken leg pieces of the MDA 2 - *Listeria* assay, there were 18 presumptive  
11 positives and 18 confirmed positives following the USDA/FSIS MLG 8.09 reference method  
12 confirmation procedure. There were 16 observed positives for the reference method. Detailed  
13 results of the POD analyses are presented in Tables 9 and 13.

14  
15 *Lot 2*

16 For lot 2 of the raw chicken leg pieces of the MDA *Listeria* - 2 assay, there were 12 presumptive  
17 positives and 12 confirmed positives following the USDA/FSIS MLG 8.09 reference method  
18 confirmation procedure. There were 14 observed positives for the reference method. Detailed  
19 results of the POD analyses are presented in Tables 9 and 13.

20  
21 *Whole melon*

22  
23 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 12 presumptive positives  
24 and 10 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation  
25 procedure, resulting in 2 false positives. There were 8 observed positives for the reference  
26 method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed  
27 positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There  
28 were 5 confirmed positives following the reference method. Detailed results of the POD analyses  
29 are presented in Tables 9 and 13.

30  
31 *Vanilla ice cream (25 g)*

32  
33 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 7 presumptive positives  
34 and 7 confirmed positives following the AOAC 993.12 reference method confirmation  
35 procedure. There were 7 observed positives for the reference method. For the high inoculation  
36 level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12  
37 reference method confirmation procedure. There were 5 confirmed positives following the  
38 reference method. Detailed results of the POD analyses are presented in Tables 9 and 13.

39  
40 *Queso fresco (25 g)*

41  
42 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 11 presumptive positives  
43 and 11 confirmed positives following the AOAC 993.12 reference method confirmation  
44 procedure. There were 9 observed positives for the reference method. For the high inoculation  
45 level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12

1 reference method confirmation procedure. There were 5 confirmed positives following the  
2 reference method. Detailed results of the POD analyses are presented in Tables 9 and 13.

3  
4 *4% Milk fat cottage cheese (25 g)*

5  
6 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 12 presumptive positives  
7 and 12 confirmed positives following the AOAC 993.12 reference method confirmation  
8 procedure. There were 9 observed positives for the reference method. For the high inoculation  
9 level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12  
10 reference method confirmation procedure. There were 5 confirmed positives following the  
11 reference method. Detailed results of the POD analyses are presented in Tables 9 and 13.

12  
13  
14 *Beef hot dog (25 g)*

15  
16 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 4 presumptive positives  
17 and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation  
18 procedure, resulting in 1 false negative. There were 6 observed positives for the reference  
19 method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed  
20 positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There  
21 were 5 confirmed positives following the reference method. Detailed results of the POD analyses  
22 are presented in Tables 9 and 13.

23  
24  
25 *Stainless steel (sponge enriched in 225 mL)*

26  
27 Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level  
28 of the MDA 2 - *Listeria* assay, there were 5 presumptive positives and 5 confirmed positives  
29 following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 8  
30 observed positives for the reference method. For the high inoculation level, there were 5  
31 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference  
32 method confirmation procedure. There were 5 confirmed positives following the reference  
33 method. Detailed results of the POD analyses are presented in Tables 9 and 13.

34  
35  
36 *Sealed concrete (sponge enriched in 100 mL)*

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38 Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level  
39 of the MDA 2 - *Listeria* assay, there were 15 presumptive positives and 15 confirmed positives  
40 following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 11  
41 observed positives for the reference method. For the high inoculation level, there were 5  
42 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference  
43 method confirmation procedure. There were 5 confirmed positives following the reference  
44 method. Detailed results of the POD analyses are presented in Tables 9 and 13.

45  
46 *Plastic (Enviroswab enriched in 10 mL)*



Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level of the MDA 2 - *Listeria* assay, there were 15 presumptive positives and 15 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 11 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 5 confirmed positives following the reference method. Detailed results of the POD analyses are presented in Tables 9 and 13.

## Product Consistency (Lot-to-Lot) and Stability Studies

### Methodology

Three lots of MDA 2 - *Listeria* test kits, 1 newly manufactured, 1 at the middle of its expiration, and 1 at or slightly beyond expiration were analyzed to determine the stability of the assay. For the MDA 2 - *Listeria* test kit, one *Listeria monocytogenes* ATCC 7644 isolate was analyzed at a fractional positive level (2-8 positives), and testing 10 replicates. One non-*Listeria* isolate, *Enterococcus faecalis* ATCC 29212, was analyzed at the growth level achieved in a non-selective broth, testing 5 replicates.

### Results

For the lot-to-lot/stability evaluation of the MDA 2 - *Listeria* assay, there were 6 presumptive positives out of 10 replicates for all three lots at the low inoculation level. For the 5 uninoculated control test portions, there were 0 presumptive positives out of 5 replicates for all three lots. The five control test portions were negative. See Table 16 for lot-to-lot/stability results.

## Robustness Study

### Methodology

The robustness evaluated the ability of the method to remain unaffected by minor variations in method parameters that might be expected to occur when the method is performed by an end user. Three different parameters were evaluated, which are presented in Table 18. For each parameter, one *Listeria monocytogenes* ATCC 7644 isolate was analyzed at a fractional positive level (2-8 positives), testing 10 replicates. One non-*Listeria* isolate, *Enterococcus faecalis* ATCC 29212, was analyzed at the growth level achieved in a non-selective broth, testing 5 replicates.

**Table B: Robustness Parameters**

Parameter	Low Value	Nominal Value	High Value
Lysis Time	13 minutes	15 minutes	17 minutes
Enrichment Volume	18 µL	20 µL	22 µL
Lysate Volume	18 µL	20 µL	22 µL

**Table C: Robustness Parameter Variations**

Treatment	Lysis Time	Volume	Volume	# of Replicates
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Combination		(Enrichment to lysis)	(Lysis to assay)	
1	13 minutes	18 µL	18 µL	10
2	13 minutes	18 µL	22 µL	10
3	13 minutes	22 µL	18 µL	10
4	13 minutes	22 µL	22 µL	10
5	17 minutes	18 µL	18 µL	10
6	17 minutes	18 µL	22 µL	10
7	17 minutes	22 µL	18 µL	10
8	17 minutes	22 µL	22 µL	10

*Results*

*Treatment combinations 1 and 2*

For the 13 minute lysis and the 18 µL aliquot of enrichment to lysis robustness analysis of the MDA 2 - *Listeria* assay, there were 6 presumptive positives out of 10 replicates at the low level inoculation. For the 5 uninoculated control test portions, there were 0 presumptive positives out of 5 replicates. All 5 uninoculated control test portions were negative.

For the low inoculation level, a POD value of 0.60 was obtained with a 95% confidence interval of (0.31, 0.83). All 5 non-target test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00,0.43).

*Treatment combinations 3 and 4*

For the 13 minute lysis and the 22 µL aliquot of enrichment to lysis robustness analysis of the MDA 2 - *Listeria* assay, there were 7 presumptive positives out of 10 replicates at the low level inoculation. For the 5 uninoculated control test portions, there were 0 presumptive positives out of five replicates.

For the low invocation level, a POD value of 0.70 was obtained with a 95% confidence interval of (0.40, 0.89). All 5 non-target test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.43).

*Treatment combinations 5, 6, 7 and 8*

For both 17 minutes lysis procedures robustness analysis of the MDA 2 - *Listeria* assay, there were 6 presumptive positives out of 10 replicates at the low level inoculation. For the 5 uninoculated control test portions, there were 0 presumptive positives out of 5 replicates. See Table 17 for robustness results.

For the low invocation level, a POD value of 0.50 was obtained with a 95% confidence interval of (0.24, 0.76). All 5 non-target test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.43).

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## Independent Laboratory Observations

The results of this study demonstrate the ability of the MDA 2 - *Listeria* to detect the presence of *Listeria* in various food matrixes and select environmental surfaces after 24-28 hours of a primary enrichment. The MDA 2 - *Listeria* offers the benefits of extremely high sensitivity and high specificity for the detection of *Listeria* while reducing the overall time to presumptive results. The MDA 2 - *Listeria* also reduces the total analyst time required to obtain final results by implementing a single heat lysis step and cool step, followed up by a single transfer to molecular reaction tubes. The small footprint of the Molecular Detection System requires only minimal lab and bench space, making it easy to move the instrument or pair up additional MDS units to perform multiple MDA runs concurrently. The updated software is user friendly with easy to interpret results. The ability to examine Relative Light Unit (RLU) curves makes troubleshooting more streamlined if any problems occur while testing is being conducted.

## Discussion

All matrixes evaluated in this validation study, including the raw chicken, resulted in no statistical differences when compared to the reference methods. Three different brands of Demi Fraser with FAC were used in this study, referred to as X, Y, and Z.

There were no differences between the 24 and 26 hour primary enrichment time points for the environmental surfaces tested, therefore a 24 hour minimum enrichment time will be recommended for these matrixes: stainless steel, sealed concrete and plastic.

The five false negative results with raw chicken (leg pieces) evaluated in the internal study resulted in an investigation of protocol. It was determined that the contract laboratory conducting the study used Demi Fraser with FAC brand X, which demonstrated low productivity. Brand X had also been used to analyze the inclusive list, spinach, and cold smoked salmon.

When the protocol for raw chicken was performed at the independent laboratory, using a different brand Y, two lots of naturally contaminated raw chicken (leg pieces) analyzed showed no statistical difference when compared to the reference method. Lot 1 of the naturally contaminated raw chicken (leg pieces) had zero false negatives, while Lot 2 had two false negatives.

To expand the robustness of the method to include multiple raw chicken matrixes, raw chicken fillets were chosen and brand Z was used. The raw chicken (fillet) testing resulted in no statistical difference when compared to the reference method, which highlighted the importance of internally validating any method as a system within one's own laboratory. This is why 3M has always emphasized this advisory within our package inserts;

*“As with all test methods, the source, formulation and quality of enrichment medium can influence the results. Factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may also influence results. 3M*



1 recommends evaluation of the method including enrichment medium, in the user's  
2 environment using a sufficient number of samples with particular foods and microbial  
3 challenges to ensure that the method meets the user's criteria."  
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5

## 6 Conclusion

7  
8 The 3M MDA 2 - *Listeria* AOAC PTM validation study included inclusivity/exclusivity, lot-to-  
9 lot/stability, robustness and matrix studies. The 3M MDA 2 - *Listeria* was compared to the  
10 USDA/FSIS-MLG 8.09, the FDA-BAM Chapter 10, the AOAC 993.12 or the ISO 11290-1/A1  
11 reference methods for the detection of *Listeria*. There were no significant differences between  
12 the 3M MDA 2 - *Listeria* method and the corresponding reference method for any of the  
13 matrixes evaluated. The 3M MDA 2 - *Listeria* demonstrated reliability as a rapid and sensitive  
14 method for the detection of *Listeria* species for the following new matrixes: beef hot dogs (25 g),  
15 deli turkey (125 g), queso fresco (25 g), vanilla ice cream (25 g), 4% milk fat cottage cheese (25  
16 g), whole melon, bagged raw spinach (25 g), cold smoked salmon (25 g), raw chicken (25 g),  
17 sealed concrete (sponge enriched in 225 mL), plastic (Enviroswab enriched in 10 mL), stainless  
18 steel (sponge enriched in 225 mL).  
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**Table 1. Enrichment Protocols Using Demi-Fraser Broth Enrichment at 37 ± 1°C**

Sample Matrix		Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)
Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon		25 g	225	24-30
Raw chicken		25 g	475	28-32
Deli turkey		125 g	1125	24-30
Cantaloupe		Whole melon	Enough volume to allow melon to float	26-30
Environmental samples:	Stainless steel	1 sponge	225	24-30
	Sealed concrete	1 sponge	100	24-30
	Plastic	1 swab	10	24-30

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**Table 2a. Inclusivity Study Results**

Genus	Species	Reference	Origin	Inoculation level (cfu/225ml)	3M Molecular Detection Assay 2 Half Fraser broth 24h at 37°C			
					MDA 2 - <i>Listeria</i> spp	Confirmation 100µl		
						ALOA	PALCAM	
1	<i>Listeria</i>	<i>monocytogenes</i>	1011/1410	Frozen broccoli	28	+	H+	+
2	<i>Listeria</i>	<i>monocytogenes</i>	153	Soft cheese (Munster)	39	+	H+	+
3	<i>Listeria</i>	<i>monocytogenes</i>	1973/2400	Egg and ham pastry (Quiche Lorraine)	38	+	H+	+
4	<i>Listeria</i>	<i>monocytogenes</i>	38/181	Toulouse sausages	33	+	H+	+
5	<i>Listeria</i>	<i>monocytogenes</i>	7111/7516	Pâté (Rillettes)	64	+	H+	+
6	<i>Listeria</i>	<i>monocytogenes</i>	913/1048	Black pudding	39	+	H+	+
7	<i>Listeria</i>	<i>monocytogenes</i>	A00C036	Poultry (guinea)	41	+	H+	+
8	<i>Listeria</i>	<i>monocytogenes</i>	A00C041	Sausage	38	+	H+	+
9	<i>Listeria</i>	<i>monocytogenes</i>	A00C044	Poultry (Duck)	34	+	H+	+
10	<i>Listeria</i>	<i>monocytogenes</i>	A00L097	Milk	60	+	H+	+
11	<i>Listeria</i>	<i>monocytogenes</i>	A00M009	Smoked salmon	40	+	H+	+
12	<i>Listeria</i>	<i>monocytogenes</i>	Ad 253	Semi-hard cheese	58	+	H+	+
13	<i>Listeria</i>	<i>monocytogenes</i>	Ad 266	Poultry	26	+	H+	+
14	<i>Listeria</i>	<i>monocytogenes</i>	Ad 270	Fermented sausage	42	+	H+	+
15	<i>Listeria</i>	<i>monocytogenes</i>	Ad 273	Cured delicatessen	27	+	H+	+
16	<i>Listeria</i>	<i>monocytogenes</i>	Ad 274	Ready-to-eat food (Asiatic meal)	40	+	H+	+
17	<i>Listeria</i>	<i>monocytogenes</i>	Ad 534	Fruits	54	+	H+	+
18	<i>Listeria</i>	<i>monocytogenes</i>	Ad 548	Environment (Seafood)	37	+	H+	+
19	<i>Listeria</i>	<i>monocytogenes</i>	Ad 623	Bread crumbs	50	+	H+	+
20	<i>Listeria</i>	<i>monocytogenes</i>	Ad 665	Raw milk	33	+	H+	+
21	<i>Listeria</i>	<i>grayi</i>	Ad 1198	Smoked salmon	158 (+25ml UHT milk)	+	H-	st
					1300	+	H-(2)	-
22					1300 (+25ml UHT milk)	+	H-	-
	<i>Listeria</i>	<i>grayi</i>	Ad 1443	Pork meat sausages	12(+25ml UHT milk)	+	H-	-
23	<i>Listeria</i>	<i>innocua</i>	1	Smoked salmon	40	+	H-	+
24	<i>Listeria</i>	<i>innocua</i>	Ad 658	Gorgonzola	28	+	H-	+
25	<i>Listeria</i>	<i>innocua</i>	Ad 655	Brine	19	+	H-	+
26	<i>Listeria</i>	<i>innocua</i>	Ad 660	Bread crumbs	35	+	H-	+
27	<i>Listeria</i>	<i>innocua</i>	Ad 663	Environment (dairy industry)	25	+	H-	+
28	<i>Listeria</i>	<i>innocua</i>	Ad 671	Smocked bacon	10	+	H-	+
29	<i>Listeria</i>	<i>innocua</i>	Ad 661	Soft cheese (Pont L'Evêque)	15	+	H-	+
30	<i>Listeria</i>	<i>innocua</i>	Ad 659	Environment (dairy industry)	14	+	H-	+
31	<i>Listeria</i>	<i>ivanovii</i>	Ad 466	Raw veal meat	11	+	H+	+

Genus	Species	Reference	Origin	Inoculation level (cfu/225ml)	3M Molecular Detection Assay 2 Half Fraser broth 24h at 37°C			
					MDA 2 - <i>Listeria</i> spp	Confirmation 100µl		
					ALO	PALCAM		
32	<i>Listeria</i>	<i>ivanovii</i>	Ad 662	Environment (dairy industry)	20	+	H+	+
33	<i>Listeria</i>	<i>ivanovii</i>	BR11	Environment (fish)	29	+	H+	+
34	<i>Listeria</i>	<i>ivanovii</i>	Ad 1289	Raw milk cheese	30	+	H+	+
35	<i>Listeria</i>	<i>ivanovii</i>	Ad 1290	Milk powder	17	+	H+	+
36	<i>Listeria</i>	<i>ivanovii</i>	Ad 1291	Poultry	18	+	H+	+
37	<i>Listeria</i>	<i>ivanovii</i>	Ad 1288	Sheep milk	80	+	H+	+
38	<i>Listeria</i>	<i>ivanovii</i> subsp. <i>londoniensis</i>	CIP103466	Unknown	14	+	H-	+
39	<i>Listeria</i>	<i>seeligeri</i>	Ad 649	Cheese	29	+	H-	+
40	<i>Listeria</i>	<i>seeligeri</i>	Ad 651	Environment	36	+	H-	+
41	<i>Listeria</i>	<i>seeligeri</i>	Ad 652	Environment (dairy industry)	28	+	H-	+
42	<i>Listeria</i>	<i>seeligeri</i>	Ad 674	Soft cheese (Munster)	240	+	H-	H-
43	<i>Listeria</i>	<i>seeligeri</i>	BR1	Trout	28	+	H-	+
44	<i>Listeria</i>	<i>seeligeri</i>	BR18	Environment (fish)	36	+	H-	+
45	<i>Listeria</i>	<i>seeligeri</i>	CIP100100	Unknown	8	+	st	1col
46	<i>Listeria</i>	<i>welshimeri</i>	Ad1276	Environment (Slaughterhouse)	44	+	H-	+
47	<i>Listeria</i>	<i>welshimeri</i>	Ad1235	Beef meat	26	+	H-	+
48	<i>Listeria</i>	<i>welshimeri</i>	191424	Poultry	24	+	H-	+
49	<i>Listeria</i>	<i>welshimeri</i>	Ad 1175	Ready-to-eat-food	46	+	H-	+
50	<i>Listeria</i>	<i>welshimeri</i>	Ad 650	Poultry	33	+	H-	+

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All the strains are wild strains isolated in Adria Developpement, Quimper, France.

Ad = Adria

A= Adria

BR = ANSES Lab strain

CIP = collection of Pasteur Institute

H+/- = Presence/absence Phospho-Lipolysis halo

St = plate without colonies

ANSES = French Agency for Food, Environmental, and Occupational Health and Safety, Cedex, France

*Listeria* strains #21 and #22 utilized UHT to assist with growth as ISO 16140 directs.

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Table 2b. Inclusivity Study Results

#	<i>Listeria</i> species	Reference	Origin	Level tested	MDA2LIS
51	<i>fleischmannii</i> subsp. <i>coloradensis</i>	FSL S10-1203	Running water, FL	1.8e6 CFU/mL	+
52	<i>marthii</i>	FSL S4-120	Pristine environment, forest, NY	2.65e5 CFU/mL	+
53	<i>cornellensis</i>	FSL F6-969	Water, CO	3.5e7 CFU/mL	+
54	<i>grandensis</i>	FSL F6-971	Water, CO	1.95e7 CFU/mL	+
55	<i>riparia</i>	FSL S10-1204	Running water, FL	McFarland 1 *	+

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- Table 2b are *Listeria* strains analyzed at 3M St. Paul, MN laboratory
- Cultures 51-54 were obtained from the laboratory of Dr. Martin Wiedmann at Cornell University, NY. Frozen suspensions were thawed and streaked to sheep blood agar. Single isolated colonies were picked and sub-cultured in 10 mL Demi Fraser broth with FAC overnight at 37 ± 1°C. The cultures were diluted with fresh Demi Fraser broth with FAC before testing using the 3M MDA2LIS kit.
- Culture 55 was also obtained from Cornell. The frozen suspension was thawed and streaked to sheep blood agar. This organism did not grow in broth at 37°C or at lower temperatures, therefore a McFarland 1\* suspension (approximately 3e8 CFU/mL) was prepared from a colonial isolate prior to MDA2LIS testing.
- FSL = Food Safety Laboratory Cornell University, NY

Table 3. Exclusivity Study Results

	Genus	Species	Reference	Origin	Inoculation level (cfu/ml)	3M Molecular Detection Assay 2 – <i>Listeria</i>
1	<i>Bacillus</i>	<i>cereus</i>	Ad 465	Salmon Terme	7,2x10 <sup>4</sup>	-
2	<i>Bacillus</i>	<i>circulans</i>	Ad 760	Vegetables	1,0 x 10 <sup>4</sup> (opacity +)	-
3	<i>Bacillus</i>	<i>coagulans</i>	Ad 731	Dairy product	<2,0x10 <sup>3</sup> (opacity +)	-
4	<i>Bacillus</i>	<i>licheniformis</i>	Ad 978	Dairy product	<2,0x10 <sup>3</sup> (opacity+)	-
5	<i>Bacillus</i>	<i>mycoïdes</i>	Ad 762	Milk	5,6x10 <sup>4</sup>	-
6	<i>Bacillus</i>	<i>pseudomycoïdes</i>	Ad 765	Vegetables	2,0x10 <sup>4</sup>	-
7	<i>Bacillus</i>	<i>pumilus</i>	Ad 284	Ready-to-eat	1,7x10 <sup>5</sup>	-
8	<i>Bacillus</i>	<i>weihenstephanensis</i>	Ad 726	Egg product	6,8x10 <sup>4</sup>	-
9	<i>Brochothrix</i>	<i>thermosphacta</i>	FN 15129	Trout	8,5x10 <sup>5</sup>	-
10	<i>Brochothrix</i>	<i>campestris</i>	CIP 102920T	Environment	<2,0x10 <sup>3</sup> (opacity +)	-
11	<i>Carnobacterium</i>	<i>divergens</i>	CIP 101029 <sup>1</sup>	Unknown	>2,0x10 <sup>3</sup> (opacity +)	-
12	<i>Carnobacterium</i>	<i>piscicola</i>	Ad 369	Raw milk	<2,0x10 <sup>3</sup> (opacity +)	-
13	<i>Enterococcus</i>	<i>durans</i>	Ad 149	Ham	<2,0x10 <sup>3</sup> (opacity +)	-
14	<i>Enterococcus</i>	<i>faecalis</i>	89L326	Soft cheese (Vacherin)	1,6x10 <sup>5</sup>	-
15	<i>Lactobacillus</i>	<i>brevis</i>	86L126	Ham	1,4x10 <sup>5</sup>	-
16	<i>Lactobacillus</i>	<i>curvatus</i>	Ad 380	Delicatessen	1,7x10 <sup>5</sup>	-
17	<i>Lactobacillus</i>	<i>fermentum</i>	Ad 482	Tomatoes juice	1,2x10 <sup>5</sup>	-
18	<i>Lactobacillus</i>	<i>sakei</i>	Ad 473	Ham	4,9x10 <sup>5</sup>	-
19	<i>Lactococcus</i>	<i>lactis</i> subsp <i>cremoris</i>	Ad 137	Dairy product	>2,0x10 <sup>3</sup> (opacity +)	-

20	<i>Leuconostoc</i>	<i>carosum</i>	Ad 411	Ham	$6,8 \times 10^5$	-
21	<i>Leuconostoc</i>	<i>citreum</i>	Ad 396	Ham	$4,9 \times 10^5$	-
22	<i>Micrococcus</i>	<i>luteus</i>	Ad 432	Cocktail	$<2,0 \times 10^3$ (opacity+)	-
23	<i>Pediococcus</i>	<i>pentosaceus</i>	ATCC 33316	Unknown	$1,0 \times 10^5$	-
24	<i>Propionibacterium</i>	<i>freundenreichii</i>	CNRZ 725	Dairy product	$<2,0 \times 10^3$ (opacity +)	-
25	<i>Staphylococcus</i>	<i>aureus</i>	Ad 165	Smoked delicatessen	$1,3 \times 10^5$	-
26	<i>Staphylococcus</i>	<i>aureus</i>	Ad 902	Nems	$8,2 \times 10^4$	-
27	<i>Staphylococcus</i>	<i>epidermidis</i>	Ad 931	Fruits	$2,0 \times 10^3$	-
28	<i>Staphylococcus</i>	<i>haemolyticus</i>	Ad 989	Dairy product	$2,8 \times 10^4$	-
29	<i>Streptococcus</i>	<i>bovis</i>	92L622	Dairy product	$2,0 \times 10^3$	-
30	<i>Streptococcus</i>	<i>salivarius</i>	Ad 441	Dairy product	$<2,0 \times 10^3$ (opacity +)	-

1 All the strains are wild strains isolated in Adria Developpement, Quimper, France.

2 Ad = Adria

3 A= Adria

4 BR = ANSES Lab strain

5 CIP = collection of Pasteur Institute

6 ANSES = French Agency for Food, Environmental, and Occupational Health and Safety. Cedex, France

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8 CNRZ = National Centre for Zootechnical Research, Jouy-en-Josas, France

9 ATCC = American Type Culture Collection

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**Table 4a: Independent Study - Matrixes and Inoculating Organisms**

Matrix	Inoculating Organism (Culture Condition)	Target Inoculum Level	# of Replicates	MDA 2 Testing Time Points	Reference Method (Enrichment)
Deli Turkey	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19116 (heat-stressed)	0 CFU/Test Portion	5	24 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Raw Chicken Leg Pieces	Naturally Contaminated	Lot 1 & Lot 2	20 & 20	28 hours	USDA/FSIS MLG 8.09 (UVM)
Whole Melon	<i>L. monocytogenes</i> FSL <sup>3</sup> J1-049	0 CFU/Test Portion	5	26 hours	FDA/EAM Chapter 10 (BLEB)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Vanilla Ice Cream	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19114	0 CFU/Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Queso Fresco	<i>L. monocytogenes</i> CWD <sup>2</sup> 1554 (heat-stressed)	0 CFU/Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
4% Milk Fat Cottage Cheese	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19112 & <i>L. welshimeri</i> ATCC <sup>1</sup> 35897	0 CFU/Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Beef Hot Dogs	<i>L. monocytogenes</i> ATCC <sup>1</sup> 7644 (heat-stressed)	0 CFU/Test Portion	5	24 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Stainless Steel	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19118 & <i>10x Enterococcus faecium</i> ATCC 19434	0 CFU/1" x 1" (5 cm <sup>2</sup> )	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/1" x 1" (5 cm <sup>2</sup> )	20		
		0 CFU/4" x 4" (100 cm <sup>2</sup> )	5		
Sealed Concrete	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19117	0 CFU/4" x 4" (100 cm <sup>2</sup> )	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/4" x 4" (100 cm <sup>2</sup> )	20		
		~100 CFU/4" x 4" (100 cm <sup>2</sup> )	5		
Plastic	<i>L. monocytogenes</i> ATCC <sup>1</sup> 51782 & <i>L. seeligeri</i> ATCC <sup>1</sup> 35967 &	0 CFU/1" x 1" (5 cm <sup>2</sup> )	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/1" x 1" (5 cm <sup>2</sup> )	20		
		~100 CFU/1" x 1" (5 cm <sup>2</sup> )	5		



	<i>10x Enterococcus faecalis</i> ATCC1 29212				

1 <sup>1</sup> ATCC - American Type Culture Collection

2 <sup>2</sup> CWD - University of Vermont

3 <sup>3</sup> FSL - Cornell University

4 \* Natural contamination was present during the background screen: 2 separate lots evaluated (20 replicates each), *Listeria monocytogenes*  
5 and *Listeria innocua* were isolated.

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**Table 4b: Internal Study - Matrixes and Inoculating Organisms**

Matrix	Inoculating Organism (Culture Condition)	Target Inoculum Level	# of Replicates	MDA 2 Testing Time Points	Reference Method (Enrichment)
Raw chicken <sup>2</sup> leg pieces	<i>L. ivanovii</i> Ad <sup>1</sup> 1291	0 CFU/Test Portion	5	28 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Raw chicken fillet <sup>3</sup>	<i>L. ivanovii</i> Ad 1291	0 CFU/Test Portion	5	28 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Bagged raw <sup>2</sup> spinach	<i>Listeria seeligeri</i> Ad 1754	0 CFU/Test Portion	5	24 hours	FDA/BAM Chapter 10 (BLEB)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Bagged raw <sup>2</sup> spinach	<i>Listeria seeligeri</i> Ad 1754	0 CFU/Test Portion	5	24 hours	ISO 11290-1/A1 (Half Fraser/Demi Fraser)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Cold smoked <sup>2</sup> salmon	<i>Listeria innocua</i> Ad 1674	0 CFU/Test Portion	5	24 hours	FDA/BAM Chapter 10 (BLEB)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Cold smoked <sup>2</sup> salmon	<i>Listeria innocua</i> Ad 1674	0 CFU/Test Portion	5	24 hours	ISO 11290-1/A1 (Half Fraser/Demi Fraser)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		

<sup>1</sup> Ad – Adria Developpement Culture Collection

<sup>2</sup> Tested using Demi Fraser brand (with FAC) X

<sup>3</sup> Tested using Demi Fraser brand (with FAC) Z

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**Table 5a: Independent Study - Aerobic Plate Count and Background Results (Prior to Inoculation)**

Matrix (Test Portion)	APC <sup>1</sup> (CFU/g)	Background <i>Listeria</i> (# positive/total tested)
Deli Turkey (125 g)	1.5 x 10 <sup>3</sup>	0/5 <sup>(2)</sup>
Naturally Contaminated Raw Chicken Leg Pieces (25 g)	2.8 x 10 <sup>5</sup>	4/5 <sup>(2)</sup>
Whole Melons	1.0 x 10 <sup>2</sup>	0/5 <sup>(3)</sup>
Vanilla Ice Cream (25 g)	8.0 x 10 <sup>1</sup>	0/5 <sup>(4)</sup>
Queso Fresco (25 g)	3.8 x 10 <sup>5</sup>	0/5 <sup>(4)</sup>
4% Milk Fat Cottage Cheese (25 g)	1.7 x 10 <sup>4</sup>	0/5 <sup>(4)</sup>
Beef Hot Dogs (25 g)	1.6 x 10 <sup>2</sup>	0/5 <sup>(2)</sup>
Beef Hot Dogs <sup>5</sup> (25 g)	6.0 x 10 <sup>3</sup>	0/5 <sup>(2)</sup>

<sup>1</sup> APC conducted in accordance with FDA/BAM Chapter 3

<sup>2</sup> *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines

<sup>3</sup> *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines

<sup>4</sup> *Listeria species* screen conducted following the AOAC 993.12 guidelines

<sup>5</sup> Matrix used to conduct Robustness and Lot-to-Lot testing

**Table 5b: Internal Study - Aerobic Plate Count and Background Results (Prior to Inoculation)**

Matrix (Test Portion)	APC <sup>1</sup> (CFU/g)	Background <i>Listeria</i> (# positive/total tested)
Raw Chicken Leg Pieces (25 g)	4.0 x 10 <sup>5</sup>	0/5 <sup>(2)</sup>
Raw chicken fillet (25 g)	1.4 x 10 <sup>5</sup>	0/5 <sup>(2)</sup>
Bagged Raw Spinach (25 g)	9.3 x 10 <sup>6</sup>	0/5 <sup>(3,4)</sup>
Cold Smoked Salmon (25 g)	2.0 x 10 <sup>2</sup>	0/5 <sup>(3,4)</sup>

<sup>1</sup> APC conducted in accordance with FDA/BAM Chapter 3

<sup>2</sup> *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines

<sup>3</sup> *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines

<sup>4</sup> *Listeria species* screen conducted following the ISO 11290-1/1A guidelines

**Table 6: Inoculum Heat Stress Results**

Matrix	Test Portion Size	Inoculating Organism	Agar	CFU/ g	Percent Injury
Beef Hot Dogs <sup>1</sup>	25 g	<i>Listeria monocytogenes</i> ATCC 7644	TSA	1.8 x 10 <sup>8</sup>	58.3 %
			MOX	7.5 x 10 <sup>7</sup>	
Deli Turkey	125 g	<i>Listeria monocytogenes</i> ATCC 19116	TSA	2.6 x 10 <sup>9</sup>	68.5 %
			MOX	8.2 x 10 <sup>8</sup>	
Queso Fresco	25 g	<i>Listeria monocytogenes</i> ATCC CWD 1554	TSA	2.6 x 10 <sup>9</sup>	72.7%
			MOX	7.1 x 10 <sup>8</sup>	
Beef Hot Dogs <sup>2</sup>	25 g	<i>Listeria monocytogenes</i> ATCC 7644	TSA	2.6 x 10 <sup>9</sup>	73.8%
			MOX	6.8 x 10 <sup>8</sup>	

<sup>1</sup>- Testing for Robustness and Lot to Lot

<sup>2</sup>- Matrix Study

TSA: Trypticase soy agar

MOX: modified Oxford agar

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**Table 7: Inoculum Summary Table for Whole Melons and Environmental Surfaces**

<b>Matrix</b>	<b>Whole Melons</b>		
<b>Inoculating Organism</b>	<i>Listeria monocytogenes</i> FSL J1-049		
<b>Low-Inoculum Level CFU<sup>a</sup>/Melon<sup>b</sup></b>	7		
<b>High-Inoculum Level CFU<sup>a</sup>/Test Melon<sup>b</sup></b>	55		
<b>Matrix</b>	<b>Stainless Steel</b>		
<b>Inoculating Organism</b>	<i>Listeria monocytogenes</i> ATCC 19118	<i>Enterococcus faecium</i> ATCC 19434	
<b>Low-Inoculum Level CFU<sup>a</sup>/Test Area<sup>c</sup></b>	42	530	
<b>High-Inoculum Level CFU<sup>a</sup>/Test Area<sup>c</sup></b>	400	4000	
<b>Matrix</b>	<b>Sealed Concrete</b>		
<b>Inoculating Organism</b>	<i>Listeria monocytogenes</i> ATCC 19117		
<b>Low-Inoculum Level CFU<sup>a</sup>/Test Area<sup>c</sup></b>	35		
<b>High-Inoculum Level CFU<sup>a</sup>/Test Area<sup>c</sup></b>	360		
<b>Matrix</b>	<b>Plastic</b>		
<b>Inoculating Organism</b>	<i>Listeria monocytogenes</i> ATCC 51782	<i>Listeria seeligeri</i> ATCC 35967	<i>Enterococcus faecalis</i> ATCC 29212
<b>Low-Inoculum Level CFU<sup>a</sup>/Test Area<sup>d</sup></b>	42	64	540
<b>High-Inoculum Level CFU<sup>a</sup>/Test Area<sup>d</sup></b>	420	590	3600

<sup>a</sup>CFU: aliquots of the inocula were plated in triplicate onto TSA and averaged

<sup>b</sup>Test Area: Whole Melon

<sup>c</sup>Test Area: 4" x 4" Surface Area

<sup>d</sup>Test Area: 1" x 1" Surface Area

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**Table 8: 3M™ MDA 2 - *Listeria* Assay, Candidate vs. Reference – POD Results (INTERNAL STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			USDA/FSIS MLG			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces <sup>h</sup> (25 g)	<i>L. ivanovii</i> Ad 1291	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.31 (0.14, 0.57)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
			0.5	5	3	0.60	0.23, 0.88	1	0.20	0.04, 0.62	0.40	-0.16, 0.73
Raw Chicken Fillet <sup>i</sup> (25 g)	<i>L. ivanovii</i> Ad 1291	28Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.3 (0.10, 0.5)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
			0.5	5	2	0.40	0.12, 0.77	4	0.80	0.38, 0.96	-0.40	-0.73, 0.16
Bagged Raw Spinach <sup>h</sup> (25 g)	<i>L. seeligeri</i> Ad 1754	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.26 (0.10, 0.47)	20	6	0.30	0.15, 0.52	3	0.15	0.05, 0.36	0.15	-0.11, 0.39
			1.7	5	3	0.60	0.23, 0.88	4	0.80	0.38, 0.96	-0.20	-0.60, 0.31
Bagged Raw Spinach <sup>h</sup> (25 g)	<i>L. seeligeri</i> Ad 1754	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.26 (0.10, 0.47)	20	6	0.30	0.15, 0.52	9	0.45	0.26, 0.66	-0.15	-0.41, 0.14
			1.7	5	3	0.60	0.23, 0.88	4	0.80	0.38, 0.96	-0.20	-0.60, 0.31
Cold Smoked Salmon <sup>h</sup> (25 g)	<i>L. innocua</i> Ad 1674	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.27 (0.10, 0.49)	20	10	0.50	0.30, 0.70	7	0.35	0.18, 0.57	0.15	-0.15, 0.41
			1.7	5	5	1.00	0.57, 1.00	4	0.80	0.38, 0.96	0.20	-0.26, 0.62
Cold Smoked Salmon <sup>h</sup> (25 g)	<i>L. innocua</i> Ad 1674	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.27 (0.10, 0.49)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			1.7	5	5	1.00	0.57, 1.00	4	0.80	0.38, 0.96	0.20	-0.26, 0.62

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

<sup>h</sup>Tested using Demi Fraser (with FAC) brand X

<sup>i</sup>Tested using Demi Fraser (with FAC)brand Z

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**Table 9: 3M™ MDA 2 - *Listeria* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Deli Turkey (125 g)	<i>L. monocytogenes</i> ATCC 19116	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.85)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
Raw Chicken Leg Pieces (25 g): Lot 1	Naturally Contaminated	28 Hour	1.91 (1.23, 3.80)	20	18	0.90	0.70, 0.97	15	0.80	0.58, 0.92	0.10	-0.13, 0.33
			1.12 (0.68, 1.91)	20	12	0.60	0.39, 0.78	14	0.70	0.48, 0.85	-0.10	-0.36, 0.18
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
Whole Melon	<i>L. monocytogenes</i> FSL J1-049	26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			7	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			55	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
Vanilla Ice Cream (25 g)	<i>L. monocytogenes</i> ATCC 19114	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.59 (0.33, 0.97)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes were in Table 9 were tested with Demi Fraser brand Y.



**Table 10: 3M™ MDA 2 - Listeria Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Queso Fresco (25 g)	<i>L. monocytogenes</i> CWD 1554	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	11	0.55	0.34, 0.74	9	0.45	0.26, 0.66	0.10	-0.19, 0.37
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
4% Milk Fat Cottage Cheese (25 g)	<i>L. welshimeri</i> ATCC 35897	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.69 (0.40, 1.14)	20	12	0.60	0.39, 0.78	9	0.45	0.26, 0.66	0.15	-0.15, 0.41
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Beef Hot Dogs (25 g)	<i>L. monocytogenes</i> ATCC 7644	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	5	0.25	0.11, 0.47	6	0.30	0.15, 0.52	-0.05	-0.31, 0.22
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes tested in Table 10 used Demi Fraser brand Y.

**Table 11: 3M™ MDA 2 - *Listeria* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Stainless Steel (225 mL)	<i>L. monocytogenes</i> ATCC 19118 & <i>Enterococcus faecium</i> ATCC 19434	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	8	0.40	0.22, 0.61	-0.10	-0.40, 0.13
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	8	0.40	0.22, 0.61	-0.10	-0.40, 0.13
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
Sealed Concrete (100 mL)	<i>L. monocytogenes</i> ATCC 19117	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
Plastic (10 mL)	<i>L. monocytogenes</i> ATCC 51782 & <i>Enterococcus faecalis</i> ATCC 29212	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			64 & 540	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			64 & 540	20	15	0.75	0.53, 0.89	11	0.55	0.22, 0.74	0.20	-0.09, 0.45
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes tested in Table 11 used Demi Fraser brand Y.

**Table 12: 3M™ MDA 2 - *Listeria* Assay, Presumptive vs. Confirmed – POD Results (INTERNAL STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces <sup>h</sup> (25 g)	<i>L. ivanovii</i> Ad 1291	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.31 (0.14, 0.57)	20	4	0.20	0.08, 0.42	9	0.45	0.26, 0.66	-0.25	-0.49, 0.05
			0.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Chicken Fillet <sup>i</sup> (25 g)	<i>L. ivanovii</i> Ad 1291	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.3 (0.1, 0.5)	20	4	0.80	0.38, 0.96	4	0.80	0.38, 0.96	0.00	-0.25, 0.25
			0.5	5	2	0.40	0.12, 0.77	2	0.40	0.12, 0.77	0.00	-0.46, 0.46
Bagged Raw Spinach <sup>h</sup> (25 g)	<i>L. seeligeri</i> Ad 1754	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.26 (0.10, 0.47)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.31
			1.7	5	3	1.00	0.57, 1.00	3	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cold Smoked Salmon <sup>h</sup> (25 g)	<i>L. innocua</i> Ad 1674	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.27 (0.1, 0.49)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.20	-0.26, 0.62
			1.7	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

<sup>h</sup>Tested using Demi Fraser brand (with FAC) brand X.

<sup>i</sup>Tested using Demi Fraser brand (with FAC) brand Z.

**Table 13: 3M™ MDA 2 - *Listeria* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Deli Turkey (125 g)	<i>L. monocytogenes</i> ATCC 19116	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.85)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Chicken Leg Pieces (25 g): Lot 1	Naturally Contaminated	28 Hours	1.91 (1.23, 3.80)	20	18	0.90	0.70, 0.97	18	0.90	0.70, 0.97	0.00	-0.21, 0.21
Raw Chicken Leg Pieces (25 g): Lot 2	Naturally Contaminated	28 Hours	1.12 (0.68, 1.91)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
Whole Melon	<i>L. monocytogenes</i> FSL J1-049	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
7			20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37	
55			5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43	
Vanilla Ice Cream (25 g)	<i>L. monocytogenes</i> ATCC 19114	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
0.59 (0.33, 0.97)			20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28	
4.38 (1.72, 11.15)			5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43	

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes tested in Table 13 used Demi Fraser (with FAC) brand Y.

**Table 14: 3M™ MDA 2 - *Listeria* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Queso Fresco (25 g)	<i>L. monocytogenes</i> CWD 1554	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
4% Milk Fat Cottage Cheese (25 g)	<i>L. welshimeri</i> ATCC 35897	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.69 (0.40, 01.14)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef Hot Dogs (25 g)	<i>L. monocytogenes</i> ATCC 7644	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes tested in Table 13 used Demi Fraser (with FAC) brand Y.

**Table 15: 3M™ MDA 2 - *Listeria* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Stainless Steel (225 mL)	<i>L. monocytogenes</i> ATCC 19118 & <i>Enterococcus faecium</i> ATCC 19434	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Sealed Concrete (100 mL)	<i>L. monocytogenes</i> ATCC 19117	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Plastic (10 mL)	<i>L. monocytogenes</i> ATCC 51782 & <i>Enterococcus faecalis</i> ATCC 29212	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			64 & 540	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			64 & 540	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the statistically significant at the 5% level

All matrixes tested in Table 13 used Demi Fraser (with FAC) brand Y.

**Table 16: Lot-to-Lot/Stability Study Results**

Expiration Date		Expiration Date		Expiration Date	
Lot #: 012715		Lot #: 081914		Lot #: 050114	
Sample #	Result	Sample #	Result	Sample #	Result
<i>Listeria monocytogenes</i> ATCC <sup>2</sup> 7644					
Low Level	6/10	Low Level	6/10	Low Level	6/10
Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5	Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5	Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5

<sup>1</sup>All samples were analyzed from a common lysate

<sup>2</sup>ATCC- American Type Culture Collection

**Table 17: Robustness Results**

Robustness <sup>1</sup>			Robustness <sup>1</sup>		
13 Minute Lysis			13 Minute Lysis		
Sample #	18 µL <sup>2</sup> , 18 µL <sup>3</sup>	18 µL <sup>2</sup> , 22 µL <sup>3</sup>	Sample #	22 µL <sup>2</sup> , 18 µL <sup>3</sup>	22 µL <sup>2</sup> , 22 µL <sup>3</sup>
<i>Listeria monocytogenes</i> ATCC <sup>4</sup> 7644					
Low Level	6/10	6/10	Low Level	7/10	7/10
Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5	0/5	Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5	0/5
Robustness <sup>1</sup>			Robustness <sup>1</sup>		
17 Minute Lysis			17 Minute Lysis		
Sample #	18 µL <sup>2</sup> , 18 µL <sup>3</sup>	18 µL <sup>2</sup> , 22 µL <sup>3</sup>	Sample #	22 µL <sup>2</sup> , 18 µL <sup>3</sup>	22 µL <sup>2</sup> , 22 µL <sup>3</sup>
<i>Listeria monocytogenes</i> ATCC <sup>4</sup> 7644					
Low Level	6/10	6/10	Low Level	6/10	6/10
Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5	0/5	Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5	0/5

<sup>1</sup>All samples were analyzed from a common lysate

<sup>2</sup>Volume of enrichment to lysis

<sup>3</sup>Volume of lysis to assay

<sup>4</sup>ATCC- American Type Culture Collection



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## **Evaluation of the 3M™ Molecular Detection Assay (MDA) 2 -*Listeria monocytogenes* for the Detection of *Listeria monocytogenes* in Select Foods and Environmental Surfaces: Collaborative Study**

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**The 3M™ Molecular Detection Assay (MDA) 2 –*Listeria monocytogenes* uses loop-mediated isothermal amplification of unique DNA target sequences combined with bioluminescence to rapidly detect *Listeria monocytogenes* in a broad range of food types and environmental surfaces. Using an unpaired study design, technicians from 13 laboratories located in the United States and Canada, compared the 3M MDA 2 -*Listeria monocytogenes* to the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) Microbiology Laboratory Guidebook (MLG) Chapter 8.09 *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and Environmental Samples* reference method for the detection of *Listeria monocytogenes* in deli turkey and raw chicken breast fillet. Each matrix was evaluated at three levels of contamination: an un-inoculated control level (0 colony forming units (CFU)/test portion), a low inoculum level (0.2-2 CFU/test portion) and a high inoculum level (2-5 CFU/test portion). Statistical analysis was conducted according to the Probability of Detection (POD) statistical model. Results obtained for the low inoculum level test portions produced a dLPOD value with 95% confidence intervals of 0.04, (-0.08, 0.17) for deli turkey indicating no statistically significant difference between the candidate and reference methods. For raw chicken breast fillet a dLPOD value with 95% confidence interval of 0.16, (0.04, 0.28) indicating a statistically significant difference between the candidate and reference methods with a positive correlation in data indicating more recovery of the target analyte by the candidate method.**

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*Listeria monocytogenes* is a highly pathogenic microorganism that contaminates food and can cause non-invasive gastroenteritis, or severe life-threatening illness, referred to as listeriosis [1]. Although rare, listeriosis is associated with high hospitalization and mortality rates [2]. *Listeria monocytogenes* is ubiquitous in the environment, often found in soil, water, sewage and damp environments, making it difficult to control in the food processing environment [1]. The organism's ability to survive in processing facilities has led to some highly publicized recent outbreaks, specifically in ice cream and packaged salads [3]. The 3M™ Molecular Detection



1 Assay(MDA) 2 - *Listeria monocytogenes* method, using a combination of bioluminescence and  
2 isothermal amplification of nucleic acid sequences, allows for the rapid and specific detection of  
3 *Listeria monocytogenes* in a broad range of food types and environmental surfaces after 24 to 28  
4 hours of pre-enrichment. After enrichment, samples are evaluated using the 3M MDA 2 -  
5 *Listeria monocytogenes* on the 3M™ Molecular Detection System (MDS). Presumptive positive  
6 results are reported in real-time while negative results are displayed after completion of the assay  
7 in approximately 75 minutes.

8 Prior to the collaborative study, the 3M MDA 2 - *Listeria monocytogenes* method was validated  
9 according to AOAC Guidelines[4] in a harmonized AOAC® Performance Tested Method<sup>SM</sup>  
10 (PTM) study. The objective of the PTM study was to demonstrate that the 3M MDA 2-*Listeria*  
11 *monocytogenes* method could detect *Listeria monocytogenes* in a broad range of food matrices  
12 and environmental surfaces as claimed by the manufacturer. For the 3M MDA 2- *Listeria*  
13 *monocytogenes* PTM evaluation, 13 matrices were evaluated: hot dogs (25g & 125g), salmon  
14 (25g), deli turkey (25g & 125g), cottage cheese (25g), chocolate milk (25 mL), vanilla ice cream  
15 (25g), queso fresco (25g), romaine lettuce (25g), melon (whole), raw chicken leg pieces (25g);  
16 concrete (sponge, 225 mL & 100 mL), stainless steel (sponge, 225 mL), and plastic (EnviroSwab,  
17 10 mL) environmental samples.

18 Additional PTM parameters (inclusivity, exclusivity, ruggedness, stability and lot-to-lot  
19 variability) tested in the PTM studies satisfied the performance requirements for PTM approval.  
20 The method was awarded PTM certification number 081501 on August 18<sup>th</sup>, 2015.

21 The purpose of this collaborative study was to compare the reproducibility of the 3M MDA 2 -  
22 *Listeria monocytogenes* method to the United States Department of Agriculture (USDA) Food  
23 Safety Inspection Service (FSIS) -Microbiology Laboratory Guidebook (MLG) Chapter 8.09  
24 *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg*  
25 *Products, and Environmental Samples*[5] for deli turkey (125 g) and raw chicken breast fillet.

## 26 27 **Collaborative Study**

### 28 29 *Study Design*

30  
31 In this collaborative study, two matrices, deli turkey and raw chicken breast fillet, were evaluated.  
32 The matrices were obtained from a local retailer and screened for the presence of *Listeria*  
33 *monocytogenes* by the USDA/FSIS MLG 8.09 reference method. The raw chicken breast fillet  
34 was artificially contaminated with fresh unstressed cells of *Listeria monocytogenes*, American  
35 Type Culture Collection (ATCC) 7644, and the deli turkey was artificially contaminated with  
36 heat stressed cells of *Listeria monocytogenes*, ATCC 19115, at two inoculation levels: a high  
37 inoculation level of approximately 2-5 colony-forming units (CFU)/test portion and a low  
38 inoculation level of approximately 0.2-2 CFU/test portion. A set of un-inoculated control test  
39 portions (0 CFU/test portion) were also included.

40 Twelve replicate samples from each of the three inoculation levels were analyzed by each  
41 method. Two sets of samples (72 total) were sent to each laboratory for analysis by 3M MDA 2 -  
42 *Listeria monocytogenes* and the USDA/FSIS MLG Chapter 8.09 reference method due to the  
43 different sample enrichment procedures for each method. Additionally, collaborators were sent a  
44 60 g test portion and instructed to conduct a total aerobic plate count (APC) using 3M™  
45 Petrifilm™ Rapid Aerobic Count Plate (AOAC Official Method 2015.13) [6] on the day samples  
46 were received for the purpose of determining the total aerobic microbial load.

47 A detailed collaborative study packet outlining all necessary information related to the study  
48 including media preparation, test portion preparation and documentation of results was sent to  
49 each collaborating laboratory prior to the initiation of the study. A conference call was then

1 conducted to discuss the details of the collaborative study packet and answer any questions from  
 2 the participating laboratories.

3  
 4 *Preparation of Inocula and Test Portions*

5  
 6 The *Listeria monocytogenes* cultures used in this evaluation were propagated onto Tryptic Soy  
 7 Agar with 5% Sheep Blood (SBA) from a Q Laboratories frozen stock culture stored at -70°C.  
 8 Each organism was incubated for 24 ± 2 hours at 35 ± 1°C. Isolated colonies were picked to 10  
 9 mL of Brain Heart Infusion (BHI) broth and incubated for 18 ± 0.5 hours at 35 ± 1°C. Raw  
 10 chicken breast fillet was inoculated in bulk using the fresh, broth culture. Prior to inoculation of  
 11 the deli turkey, the culture suspension was heat stressed at 55 ± 1°C in a water bath for 15  
 12 ± 0.5 minutes to obtain a percent injury of 50-80% (as determined by plating onto selective  
 13 Modified Oxford agar (MOX) and non-selective tryptic soy agar with yeast (TSA/ye). The  
 14 degree of injury was estimated as:

$$\left(1 - \frac{n_{select}}{n_{nonselect}}\right) \times 100$$

15  
 16 where  $n_{select}$  = number of colonies on selective agar and  $n_{nonselect}$  = number of colonies on non-  
 17 selective agar. Appropriate dilutions of each culture were prepared in Butterfield's Phosphate  
 18 Diluent (BPD) based on previously established growth curves for both low and high inoculation  
 19 levels. Bulk portions of each matrix were inoculated with the diluted liquid inoculum and mixed  
 20 thoroughly to ensure an even distribution of microorganisms. The inoculated raw chicken  
 21 breastfillet was packaged into separate 30 g test portions in sterile Whirl-Pak® bags and shipped  
 22 to the collaborators. For the analysis of the deli turkey, 25 g of inoculated test product was  
 23 mixed with 100 g of un-inoculated test product to prepare 125 g test portions which were  
 24 packaged in sterile Whirl-Pak® bags and shipped to collaborators.

25 To determine the level of *Listeria monocytogenes* in the matrices, a 5-tube most probable  
 26 number (MPN) was conducted by the coordinating laboratory on the day of the initiation of  
 27 analysis using the USDA/FSIS-MLG 3.09 reference method. For deli turkey, the MPN was  
 28 determined by analyzing 5 x 250 g test portions, reference method test portions from the  
 29 collaborating laboratories and 5 x 65 g test portions. For the raw chicken breast fillet, the MPN  
 30 of the high and low inoculated levels was determined by analyzing 5 x 50 g test portions, the  
 31 reference method test portions from the collaborating laboratories and 5 x 10 g test portions. The  
 32 MPN and 95% confidence intervals were calculated using the LCF MPN Calculator, Version 1.6,  
 33 ([www.lcftld.com/customer/LCFMPNCalculator.exe](http://www.lcftld.com/customer/LCFMPNCalculator.exe)), provided by AOAC Research Institute  
 34 (RI) [7].

35  
 36 *Test Portion Distribution*

37  
 38 All samples were labeled with a randomized, blind-coded 3-digit number affixed to the sample  
 39 container. Test portions were shipped on a Thursday via overnight delivery according to the  
 40 Category B Dangerous Goods shipment regulations set forth by the International Air  
 41 Transportations Association (IATA). The two matrices were shipped consecutively, with  
 42 collaborating laboratories performing analysis on one matrix at a time. Upon receipt, samples  
 43 were held by the collaborating laboratory at refrigeration temperature (2-8 °C) until the  
 44 following Monday when analysis was initiated after a total equilibration time of 96 hours. All  
 45 samples were packed with cold packs to target a temperature of < 7°C during shipment.  
 46 In addition to each of the test portions and a separate APC sample, collaborators received a test  
 47 portion for each matrix labeled as 'temperature control'. Participants were instructed to obtain  
 48 the temperature of this portion upon receipt of the package, document the results on the Sample

1 Receipt Confirmation form provided and fax or email it back to the study director. The shipment  
 2 and hold times of the inoculated test material had been verified as a quality control measure prior  
 3 to study initiation.

4  
 5 *Test Portion Analysis*

6  
 7 Collaborators were instructed to follow the appropriate preparation and analysis as outlined in  
 8 the study protocol for each matrix for both the 3M MDA 2 - *Listeria monocytogenes* method and  
 9 reference method. For both matrices, each collaborator received 72 test portions (12 high, 12 low  
 10 and 12 un-inoculated controls for each method to be performed). For the analysis of the deli  
 11 turkey test portions by the 3M MDA 2 - *Listeria monocytogenes* method, a 125 g portion was  
 12 enriched with 975 mL of Demi-Fraser (DF) with ferric ammonium citrate (FAC) broth,  
 13 homogenized for 2 minutes and incubated for 24-28 hours at  $37 \pm 1^\circ\text{C}$ . For the raw chicken  
 14 breast fillet test portions analyzed by the 3M MDA 2 - *Listeria monocytogenes* method, a 25 g  
 15 portion was enriched with 475 mL of DF, homogenized for 2 minutes and incubated for 28-32  
 16 hours at  $37 \pm 1^\circ\text{C}$ .

17 Following enrichment, samples were assayed by the 3M MDA 2 - *Listeria monocytogenes*  
 18 method and, regardless of presumptive result, confirmed following the USDA/FSIS MLG 8.09  
 19 reference method. Both matrices evaluated by the 3M MDA 2 - *Listeria monocytogenes* method  
 20 were compared to samples analyzed using the USDA/FSIS MLG 8.09 reference method in an  
 21 unpaired study design. All positive test portions were biochemically confirmed by the API  
 22 *Listeria monocytogenes* biochemical test or by the VITEK 2 GP biochemical identification test,  
 23 AOAC Official Method 2012.02 [8].

24  
 25 *Statistical Analysis*

26  
 27 Each collaborating laboratory recorded results for the reference method and the 3M MDA 2 -  
 28 *Listeria monocytogenes* method on the data sheets provided. The data sheets were submitted to  
 29 the study director at the end of each week of testing for statistical analysis. Data for each matrix  
 30 was analyzed using the probability of detection (POD) statistical model [9]. The probability of  
 31 detection (POD) was calculated as the number of positive outcomes divided by the total number  
 32 of trials. The POD was calculated for the candidate presumptive results,  $\text{POD}_{\text{CP}}$ , the candidate  
 33 confirmatory results (including false negative results),  $\text{POD}_{\text{CC}}$ , the difference in the candidate  
 34 presumptive and confirmatory results,  $\text{dLPOD}_{\text{CP}}$ , presumptive candidate results that confirmed  
 35 positive (excluding false negative results),  $\text{POD}_{\text{C}}$ , the reference method,  $\text{POD}_{\text{R}}$ , and the  
 36 difference in the confirmed candidate and reference methods,  $\text{dLPOD}_{\text{C}}$ . A  $\text{dLPOD}_{\text{C}}$  confidence  
 37 interval not containing the point zero would indicate a statistically significant difference between  
 38 the 3M MDA 2 - *Listeria monocytogenes* and the reference methods at the 5 % probability level.  
 39 In addition to POD, the repeatability standard deviation ( $s_{\text{r}}$ ), the among laboratory repeatability  
 40 standard deviation ( $s_{\text{L}}$ ), the reproducibility standard deviation ( $s_{\text{R}}$ ) and the  $P_{\text{T}}$  value were  
 41 calculated. The  $s_{\text{r}}$  provides the variance of data within one laboratory, the  $s_{\text{L}}$  provides the  
 42 difference in standard deviation between laboratories and the  $s_{\text{R}}$  provides the variance in data  
 43 between different laboratories. The  $P_{\text{T}}$  value provides information on the homogeneity test of  
 44 laboratory PODs [10].

1 **AOAC Official Method 2016.xxx**  
2 ***Listeria monocytogenes* SelectFoods and Environmental Surfaces**  
3 **3M™ Molecular Detection Assay(MDA) 2- *Listeria monocytogenes* Method**  
4 **First Action 2016**  
5

6 (Applicable to detection of *Listeria monocytogenes* hot dogs (25g & 125g), salmon (25g), deli  
7 turkey (25g & 125g), cottage cheese (25g), chocolate milk (25 mL), vanilla ice cream (25g),  
8 queso fresco (25g), romaine lettuce (25g), melon (whole), raw chicken leg pieces (25g); raw  
9 chicken breast fillet (25g), concrete (3M™ Hydrated Sponge Stick with Dey-Engley [D/E] , 225  
10 mL & 100 mL), stainless steel (3M Hydrated Sponge Stick with D/E, 225 mL), and plastic  
11 (3M™ Enviroswab with Lethen, 10 mL) environmental samples.

12  
13 *See Tables 2016.1A and 2016.1B for a summary of results of the inter-laboratory study.*

14 *See Tables 2016.2A and 2016.2B for detailed results of the inter-laboratory study*  
15

16 **A. Principle**  
17

18 The 3M™ Molecular Detection Assay (MDA) 2 - *Listeria monocytogenes* method is used with  
19 the 3M™ Molecular Detection System (MDS) for the rapid and specific detection of *Listeria*  
20 *monocytogenes* in enriched food and food process environmental samples. The 3M MDA2 -  
21 *Listeria monocytogenes* uses loop-mediated isothermal amplification of unique DNA target  
22 sequences with high specificity and sensitivity, combined with bioluminescence to detect the  
23 amplification. Presumptive positive results are reported in real-time while negative results are  
24 displayed after the assay is completed. Samples are pre-enriched in Demi Fraser with ferric  
25 ammonium citrate broth.  
26

27 **B. Apparatus and Reagents**

28 **Items (b)-(g) are available as the 3M™ Molecular Detection Assay (MDA) 2 - *Listeria***  
29 ***monocytogenes* kit from 3M Food Safety (St. Paul, MN 55144-1000, USA).**

- 30 (a) *3M Molecular Detection System (MDS100)* – Available from 3M Food Safety (St.  
31 Paul, MN 55144-1000, USA).  
32 (b) *3M Molecular Detection Assay 2 – Listeria monocytogenes reagent tubes*- 12 strips of  
33 8 tubes. Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).  
34 (c) *Lysis Solution (LS) tubes* – 12 strips of 8 tubes.  
35 (d) *Extra caps* – 12 strips of 8 caps  
36 (e) *Reagent Control* – 8 reagent tubes  
37 (f) *Quick Start Guide*  
38 (g) *3M™ Molecular Detection Speed Loader Tray* - Available from 3M Food Safety  
39 (St. Paul, MN 55144-1000, USA).  
40 (h) *3M™ Molecular Detection Chill Block Insert* - Available from 3M™ Food Safety (St.  
41 Paul, MN 55144-1000, USA).  
42 (i) *3M™ Molecular Detection Heat Block Insert* - Available from 3M Food Safety  
43 (St. Paul, MN 55144-1000, USA).  
44 (j) *3M™ Molecular Detection Cap/Decap Tool for Reagent tubes* - Available from 3M  
45 Food Safety (St. Paul, MN 55144-1000, USA).  
46 (k) *3M™ Molecular Detection Cap/Decap Tool for Lysis tubes* – Available from 3M  
47 Food Safety (St. Paul, MN 55144-1000, USA).  
48 (l) *Empty Lysis Tube Rack* - Available from 3M Food Safety (St. Paul, MN 55144-1000,  
49 USA).



- 1 (m) *Empty Reagent Tube Rack* - Available from 3M Food Safety (St. Paul, MN 55144-  
2 1000, USA).
- 3 (n) *Demi Fraser Broth* - Available from 3M Food Safety (St. Paul, MN 55144-1000,  
4 USA).
- 5 (o) *Ferric Ammonium Citrate (FAC)*, ACS grade, 5% sterilized -Available from MP  
6 Biomedicals™ or equivalent.
- 7 (p) *Disposable pipette* – capable of 20 µL
- 8 (q) *Multi-channel (8-channel) pipette* - capable of 20 µL
- 9 (r) *Sterile filter tip pipette tips* - capable of 20 µL
- 10 (s) *Filter Stomacher® bags* – Seward or equivalent.
- 11 (t) *Stomacher®*– Seward or equivalent.
- 12 (u) *Thermometer* – calibrated range to include  $100 \pm 1^{\circ}\text{C}$
- 13 (v) *Dry block heater unit*– capable of maintaining  $100 \pm 1^{\circ}\text{C}$
- 14 (w) *Incubators.* – Capable of maintaining  $37 \pm 1^{\circ}\text{C}$  or  $41.5 \pm 1^{\circ}\text{C}$ .
- 15 (x) *Freezer* – capable of maintaining -10 to -20°C, for storing the 3M Molecular  
16 Detection Chill Block Tray
- 17 (y) *Refrigerator* – capable of maintaining 2-8°C, for storing the 3M Molecular Detection  
18 Assay components
- 19 (z) *Computer* – compatible with the 3M™ Molecular Detection Instrument
- 20 (aa) *3M™ Enviroswab*, -- (hydrated with Lethen) Available from 3M Food  
21 Safety(Australia)
- 22 (bb) *3M™ Hydrated Sponge Stick with 10 mL of D/E* – Available from 3M Food  
23 Safety(St. Paul, MN 55144-1000, USA)
- 24

### 25 C. General Instructions

- 26
- 27 (a) Store the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* at 2-8°C. Do not  
28 freeze. Keep kit away from light during storage. After opening the kit, check that the  
29 foil pouch is undamaged. If the pouch is damaged, do not use. After opening, unused  
30 reagent tubes should always be stored in the re-sealable pouch with the desiccant  
31 inside to maintain stability of the lyophilized reagents. Store resealed pouches at 2-  
32 8°C for no longer than 60 days. Do not use 3M Molecular Detection Assay 2 -*Listeria*  
33 *monocytogenes* past the expiration date.
- 34 (b) Follow all instructions carefully. Failure to do so may lead to inaccurate results.

#### 35 *Safety Precautions*

36 The 3M MDA2 – *Listeria monocytogenes* is intended for use in a laboratory  
37 environment by professionals trained in laboratory techniques. 3M has not  
38 documented the use of this product in industries other than the food and beverage  
39 industries. For example, 3M has not documented this product for testing drinking  
40 water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M MDA2 –  
41 *Listeria monocytogenes* has not been evaluated with all possible food products, food  
42 processes, testing protocols or with all possible strains of bacteria.

1 As with all test methods, the source of enrichment medium can influence the results.  
2 The 3M MDA 2 – *Listeria monocytogenes* has only been evaluated for use with the  
3 enrichment media specified in the Instructions for Use section.

4 The 3M™ Molecular Detection instrument is intended for use with samples that have  
5 undergone heat treatment during the assay lysis step, which is designed to destroy  
6 organisms present in the sample. Samples that have not been properly heat treated  
7 during the assay lysis step may be considered a potential biohazard and should NOT  
8 be inserted into the 3M MDS instrument.

9 The user should read, understand and follow all safety information in the instructions  
10 for the 3M MDS and the 3M MDA 2 – *Listeria monocytogenes*. Retain the safety  
11 instructions for future reference.

12 To reduce the risks associated with exposure to chemicals and biohazards:  
13 Perform pathogen testing in a properly equipped laboratory under the control of  
14 trained personnel. Always follow standard laboratory safety practices, including  
15 wearing appropriate protective apparel and eye protection while handling reagents  
16 and contaminated samples. Avoid contact with the contents of the enrichment media  
17 and reagent tubes after amplification. Dispose of enriched samples according to  
18 current industry standards.

19 *Listeria monocytogenes* of particular concern for pregnant women, the aged and the  
20 infirmed. It is recommended that these concerned groups avoid handling this  
21 organism. After use, the enrichment medium and the 3M MDA 2 - *Listeria*  
22 *monocytogenes* tubes can potentially contain pathogenic materials. Periodically  
23 decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with  
24 a 1- 5% (v:v in water) household bleach solution or DNA removal solution. When  
25 testing is complete, follow current industry standards for the disposal of contaminated  
26 waste. Consult the Safety Data Sheet for additional information and local regulations  
27 for disposal.

28 To reduce the risks associated with environmental contamination: Follow current  
29 industry standards for disposal of contaminated waste.

#### 30 **D. Sample Enrichment**

##### 31 ***Foods***

- 32
- 33 (a) Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate)  
34 to equilibrate to ambient laboratory temperature (20-25°C).
- 35 (b) Aseptically combine the enrichment medium and sample according to Table 2. For all  
36 meat and highly particulate samples, the use of filter bags is recommended.
- 37 (c) Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ±0.2 minutes.  
38 Incubate at 37 ±1°C according to Table A.  
39

1 **Environmental samples**

2 (d) Sample collection devices can be a sponge hydrated with a neutralizing solution to  
3 inactivate the effects of the sanitizers. 3M recommends the use of a biocide-free  
4 cellulose sponge. Neutralizing solution can be Dey-Engley (D/E) Neutralizing Broth  
5 or Letheen broth. It is recommended to sanitize the area after sampling.

6  
7 WARNING: Should you select to use Neutralizing Buffer (NB) that contains aryl  
8 sulfonate complex as the hydrating solution for the sponge, it is required to perform a  
9 1:2 dilution (1-part sample into 1-part sterile enrichment broth) of the enriched  
10 environmental sample before testing in order to reduce the risks associated with a  
11 false-negative result leading to the release of contaminated product. Another option is  
12 to transfer 10 µL of the NB enrichment in the LS tubes.  
13

14 (e) The recommended size of the sampling area to verify the presence or absence of the  
15 pathogen on the surface is at least 100 cm<sup>2</sup> (10 cm x 10 cm or 4"x4"). When sampling  
16 with a sponge, cover the entire area going in two directions (left to right then up and  
17 down) or collect environmental samples following your current sampling protocol or  
18 according to the FDA BAM, USDA FSIS MLG or ISO 18593 [11] guidelines.

- 19 1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium  
20 citrate) to equilibrate to ambient laboratory temperature (20-25°C).  
21 2. Aseptically combine the enrichment medium and sample according to Table 2.  
22 3. Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ±0.2  
23 minutes. Incubate at 37 ±1°C for 24-30 hours.  
24

25 **E. PREPARATION OF THE 3M™ MOLECULAR DETECTION SPEED LOADER TRAY**

- 26 (a) Wet a cloth or paper towel with a 1-5% (v:v in water) household bleach solution and  
27 wipe the 3M™ Molecular Detection Speed Loader Tray.  
28 (b) Rinse the 3M Molecular Detection Speed Loader Tray with water.  
29 (c) Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.  
30 (d) Ensure the 3M Molecular Detection Speed Loader Tray is dry before use  
31

32 **Specific Instructions for Validated Methods**

33 **AOAC® Performance Tested Method<sup>sm</sup> # 081501**



37 In AOAC PTM studies, the 3M Molecular Detection Assay 2 – *Listeria monocytogenes*  
38 was found to be an effective method for the detection of *Listeria monocytogenes*. The

1 matrices tested in the study are shown in Table 2. The limit of detection of the 3M  
 2 Molecular Detection Assay 2 – *Listeria monocytogenes* method is 1-5 colony forming  
 3 units per validated test portion size (in Table 2).

4  
 5 **Table 2.** Enrichment protocols using Demi-Fraser Broth at  $37 \pm 1$  °C according to AOAC  
 6 Performance Tested <sup>SM</sup> Certificate #081501.

Sample Matrix		Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)
Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon		25 g	225	24-30
Raw chicken		25 g	475	28-32
Deli turkey		125 g	1125	24-30
Cantaloupe		Whole melon	Enough volume to allow melon to float	26-30
Environmental samples:	Stainless steel	1 sponge	225	24-30
	Sealed concrete	1 sponge	100	24-30
	Plastic	1 swab	10	24-30

8  
 9 **F. PREPARATION OF THE 3M™ MOLECULAR DETECTION HEAT BLOCK INSERT**

10 Place the 3M™ Molecular Detection Heat Block Insert in a dry double block heater unit.  
 11 Turn on the dry block heater unit and set the temperature to allow the 3M Molecular  
 12 Detection Heat Block Insert to reach and maintain a temperature of  $100 \pm 1$  °C.

13 **NOTE:** Depending on the heater unit, allow approximately 30minutes for the 3M Molecular Detection  
 14 Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (e.g., a partial  
 15 immersion thermometer, digital thermocouple thermometer, not a total immersion thermometer) placed in  
 16 the designated location, verify that the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1$  °C.

17 **G. PREPARATION OF THE 3M MOLECULAR DETECTION INSTRUMENT**

- 18 1. Launch the 3M™ Molecular Detection Software and log in.  
 19 2. Turn on the 3M Molecular Detection Instrument.



3. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection System User Manual for details.

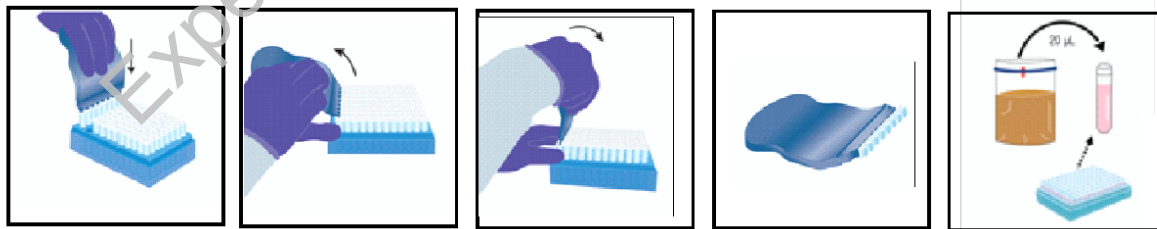
**NOTE:** The 3M Molecular Detection Instrument must reach and maintain temperature of 60°C before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 minutes and is indicated by an ORANGE light on the instrument's status bar. When the instrument is ready to start a run, the status bar will turn GREEN.

## H. LYSIS

1. Allow the lysis solution (LS) tubes to warm up by setting the rack at room temperature (20-25 °C) overnight (16-18 hours). Alternatives to equilibrate the LS tubes to room temperature are to set the LS tubes on the laboratory bench for at least 2 hours, incubate the LS tubes in a 37 ±1°C incubator for 1 hour or place them in a dry double block heater for 30 seconds at 100°C.
2. Invert the capped tubes to mix. Proceed to next step within 4 hrs.
3. Remove the enrichment broth from the incubator.
4. One LS tube is required for each sample and the Negative Control (NC) (sterile enrichment medium) sample.
  - 4.1 LS tube strips can be cut to desired LS tube number. Select the number of individual LS tubes or 8-tube strips needed. Place the LS tubes in an empty rack.
  - 4.2 To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipette tip for each transfer step.
  - 4.3 Transfer enriched sample to LS tubes as described below:

Transfer each enriched sample into individual LS tube **first**. Transfer the NC **last**.

- 4.3 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip - one strip at a time.
- 4.4 Discard the LS tube cap – if lysate will be retained for retest, place the caps into a clean container for re-application after lysis
- 4.5 Transfer 20 µL of sample into a LS tube.
- 4.6 Repeat step 4.2 until each individual sample has been added to a corresponding LS tube in the strip as illustrated below.



- 4.7 Repeat steps 4.1 to 4.4 as needed, for the number of samples to be tested. When all samples have been transferred, then transfer **20 µL of NC into a LS tube**. Do not recap tubes.
- 4.8 Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100 ±1°C. Place the rack of LS tubes in the 3M Molecular Detection Heat Block Insert and

1 heat for  $15 \pm 1$  minutes. During heating, the LS solution will change from pink (cool) to  
2 yellow (hot).

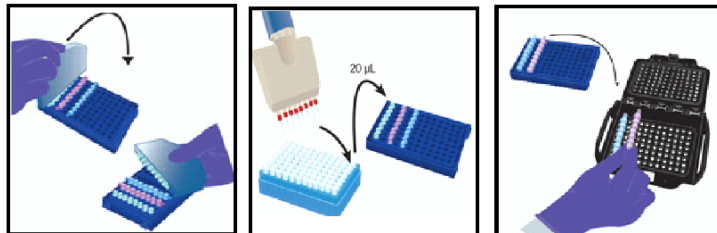
- 3 5. Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M  
4 Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes.  
5 The 3M Molecular Detection Chill Block Insert, used at ambient temperature ( $20-25^{\circ}\text{C}$ ) without the  
6 Molecular Detection Chill Block Tray, should sit directly on the laboratory bench. When  
7 cool, the lysis solution will revert to a pink color.  
8 6. Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.  
9

## 10 I. AMPLIFICATION

- 11 1. One Reagent tube is required for each sample and the NC.  
12 1.1 Reagent tubes strips can be cut to desired tube number. Select the number of individual  
13 Reagent tubes or 8-tube strips needed.  
14 1.2 Place Reagent tubes in an empty rack.  
15 1.3 Avoid disturbing the reagent pellets from the bottom of the tubes.  
16 2. Select 1 Reagent Control (RC) tube and place in rack.  
17 3. To avoid cross-contamination, decap one Reagent tubes strip at a time and use a new pipette  
18 tip for each transfer step.  
19 4. Transfer lysate to Reagent tubes and RC tube as described below:  
20

21 Transfer each sample lysate into individual Reagent tubes **first** followed by the NC. Hydrate the RC  
22 tube **last**.

- 23 4.1 Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent  
24 tubes –one Reagent tubes strip at a time. Discard cap.  
25 4.2 Transfer  $20\ \mu\text{L}$  of Sample lysate from the upper  $\frac{1}{2}$  of the liquid (avoid precipitate) in  
26 the LS tube into corresponding Reagent tube. Dispense at an angle to avoid disturbing  
27 the pellets. Mix by gently pipetting up and down 5 times.  
28 4.3 Repeat step 4.2 until individual Sample lysate has been added to a corresponding  
29 Reagent tube in the strip.  
30 4.4 Cover the Reagent tubes with the provided extra cap and use the rounded side of the  
31 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth  
32 motion ensuring that the cap is tightly applied.  
33 4.5 Repeat steps 4.1 to 4.4 as needed, for the number of samples to be tested.  
34 4.6 When all sample lysates have been transferred, repeat 4.1 to 4.4 to transfer  $20\ \mu\text{L}$  of  
35 NC lysate into a Reagent tube.  
36 4.7 Transfer  **$20\ \mu\text{L}$  of NC lysate into a RC tube**. Dispense at an angle to avoid disturbing  
37 the pellets. Mix by gently pipetting up and down 5 times.  
38 5. Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader  
39 Tray. See illustration below. Close and latch the 3M Molecular Detection Speed Loader  
40 lid.



41  
42  
43

- 1 6. Review and confirm the configured run in the 3M Molecular Detection Software.
- 2 7. Click the Start button in the software and select instrument for use. The selected instrument's
- 3 lid automatically opens.
- 4 8. Place the 3M Molecular Detection Speed Loader Tray into the 3M MDS Instrument and
- 5 close the lid to start the assay. Results are provided within 75minutes, although positives may
- 6 be detected sooner.
- 7 9. After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from
- 8 the 3M Molecular Detection Instrument and dispose of the tubes by soaking in a 1-5% (v:v in
- 9 water) household bleach solution for 1 hour and away from the assay preparation area.

10  
11 **NOTICE:** To minimize the risk of false positives due to cross-contamination, never open reagent tubes  
12 containing amplified DNA. This includes Reagent Control, Reagent and Matrix Control tubes. Always  
13 dispose of sealed reagent tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour  
14 and away from the assay preparation area.

## 15 **RESULTS AND INTERPRETATION**

16  
17 An algorithm interprets the light output curve resulting from the detection of the nucleic acid  
18 amplification. Results are analyzed automatically by the software and are color-coded based on  
19 the result. A Positive or Negative result is determined by analysis of a number of unique curve  
20 parameters. Presumptive positive results are reported in real time while Negative and Inspect  
21 results will be displayed after the run is completed.

22  
23 Presumptive positive samples should be confirmed as per the laboratory standard operating  
24 procedures or by following the current version of the appropriate reference method  
25 confirmation ([FDA/BAM](#), the [USDA/FSIS MLG](#)), beginning with transfer from the primary  
26 enrichment to secondary enrichment broth (if applicable), followed by subsequent plating and  
27 confirmation of isolates using appropriate biochemical and serological methods.

28  
29 **NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular  
30 Detection Assay 2 - *Listeria monocytogenes* amplification reagents have a "background" relative  
31 light unit (RLU) reading.

32  
33 In the rare event of any unusual light output, the algorithm labels this as "Inspect." 3M  
34 recommends the user to repeat the assay for any Inspect samples. If the result continues to be  
35 Inspect, proceed to confirmation test using your preferred method or as specified by local  
36 regulations.

### 37 *Results of Collaborative Study*

38  
39 For this collaborative study, the 3M Molecular Detection Assay (MDA) 2 - *Listeria*  
40 *monocytogenes* method was compared to the USDA FSIS MLG 8.09 reference method for deli  
41 turkey and raw chicken breast fillet. A total of 13 laboratories throughout the United States and  
42 Canada participated in this study, with 11 laboratories submitting data for the deli turkey and 12  
43 laboratories submitting data for the raw chicken breast fillet. See Table 1 for a summary of  
44 laboratory participation for each matrix. Each laboratory analyzed 36 test portions for each  
45

1 method per matrix: 12 inoculated with a high level of *Listeria monocytogenes*, 12 inoculated  
 2 with a low level of *Listeria monocytogenes*, and 12 un-inoculated controls.  
 3 A background screen of the matrix indicated an absence of indigenous *Listeria monocytogenes* in  
 4 both matrices. Ten (10) replicate test portions (randomly sampled from 50% of the total packages  
 5 used in the analysis) were screened for the presence of *Listeria monocytogenes*. All test portions  
 6 produced negative results for the target analyte.

7 Results for the heat stress analysis of the inoculum for the deli turkey are presented in Table 2.  
 8 Table 2016.1A and 2016.1B summarize the inter-laboratory results for all foods tested, including  
 9 POD statistical analysis. As per criteria outlined in Appendix J of the AOAC Validation  
 10 Guidelines, fractional positive results were obtained. Detailed results for each laboratory are  
 11 presented in Tables 2016.2A and 2016.2B. For each matrix, the level of *Listeria monocytogenes*  
 12 was determined by MPN on the day of initiation of analysis by the coordinating laboratory. MPN  
 13 results are presented in Tables 2016.2A and 2016.2B. The individual laboratory and sample  
 14 results are presented in Tables 1-2 of the Supplementary Materials. The APC results for each  
 15 collaborating are presented in Table 3 of the Supplementary Materials.  
 16

### 17 **Deli Turkey (125 g Test Portions)**

18  
 19 Deli turkey test portions were inoculated at a low and high level and were analyzed for the  
 20 detection of *Listeria monocytogenes*. Un-inoculated controls were included in each analysis.  
 21 Laboratories 8 and 10 received test portions but were unable to conduct the analysis and  
 22 therefore no data was submitted. All other laboratories submitted data for both methods  
 23 evaluated. The MPN levels obtained for this matrix, with 95% confidence intervals, were 0.63  
 24 CFU/test portion (0.49, 0.80) for the low inoculum level and 4.52 CFU/test portion (3.19, 6.42)  
 25 for the high inoculum level.

26 For the low inoculum level, 69 out of 132 test portions (POD<sub>CP</sub> of 0.52) were reported as  
 27 presumptive positive by the 3M MDA 2 – *Listeria monocytogenes* method with 66 out of 132 test  
 28 portions (POD<sub>CC</sub> of 0.50) confirming positive. For samples that produced presumptive positive  
 29 results on the 3M MDA 2 – *Listeria monocytogenes* method, 66 out of 132 samples confirmed  
 30 positive (POD<sub>C</sub> of 0.50). For test portions evaluated by the USDA/FSIS MLG reference method,  
 31 60 out of 132 test portions produced positive results. A dLPOD<sub>C</sub> value of 0.04 with 95%  
 32 confidence intervals of (-0.08, 0.17) was obtained between the candidate and reference method,  
 33 indicating no statistically significant difference between the two methods. A dLPOD<sub>CP</sub> value of  
 34 0.02 with 95% confidence intervals of (-0.10, 0.15) was obtained between presumptive and  
 35 confirmed results indicating no statistically significant difference between the presumptive and  
 36 confirmed results.

37 For the high inoculum level, 132 out of 132 test portions (POD<sub>CP</sub> of 1.00) were reported as  
 38 presumptive positive by the 3M MDA 2 – *Listeria monocytogenes* method with 132 out of 132  
 39 test portions (POD<sub>CC</sub> of 1.00) confirming positive. For samples that produced presumptive  
 40 positive results on the 3M MDA 2 – *Listeria monocytogenes* method, 132 out of 132 samples  
 41 confirmed positive (POD<sub>C</sub> of 1.00). For test portions evaluated by the USDA/FSIS MLG  
 42 reference method, 132 out of 132 test portions produced positive results. A dLPOD<sub>C</sub> value of

1 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between the candidate and  
 2 reference method, indicating no statistically significant difference between the two methods.  
 3 A dLPOD<sub>CP</sub> value of 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between  
 4 presumptive and confirmed results indicating no statistically significant difference between the  
 5 presumptive and confirmed results.

6 For the un-inoculated controls, 0 out of 132 samples (POD<sub>CP</sub> of 0.00) produced a presumptive  
 7 positive result by the 3M MDA 2 - *Listeria monocytogenes* method with 0 out of 132 test portions  
 8 (POD<sub>CC</sub> of 0.00) confirming positive. For samples that produced presumptive positive results on  
 9 the 3M MDA 2 - *Listeria monocytogenes* method, 0 out of 132 samples confirmed positive  
 10 (POD<sub>C</sub> of 0.00). For test portions evaluated by the USDA/FSIS MLG reference method, 0 out of  
 11 132 test portions produced positive results. A dLPOD<sub>C</sub> value of 0.00 with 95% confidence  
 12 intervals of (-0.03, 0.03) was obtained between the candidate and reference method, indicating  
 13 no statistically significant difference between the two methods. A dLPOD<sub>CP</sub> value of 0.00 with  
 14 95% confidence intervals of (-0.03, 0.00) was obtained between presumptive and confirmed  
 15 results indicating no statistically significant difference between the presumptive and confirmed  
 16 results.

17  
 18 Detailed results of the POD statistical analysis are presented in Table 2016.2A and Figures 1A-  
 19 1B.

### 21 **Raw Chicken Breast Fillet (25 g Test Portions)**

22  
 23 Raw chicken breast fillet test portions were inoculated at a low and high inoculum level and were  
 24 analyzed for the detection of *Listeria monocytogenes*. Un-inoculated controls were included in  
 25 each analysis. Laboratory 11 did not participate in the evaluation of this matrix. Laboratory 10  
 26 submitted data that indicated cross contamination of the inoculating organism in the un-  
 27 inoculated control samples. Further analysis at the coordinating laboratory confirmed the cross  
 28 contamination of the un-inoculated controls. Due to this issue, the data submitted from  
 29 Laboratory 10 was not used in the statistical analysis. All other laboratories submitted data for  
 30 both methods evaluated. The MPN levels obtained for this matrix, with 95% confidence  
 31 intervals, were 0.66 CFU/test portion (0.51, 0.83) for the low level and 6.24 CFU/test portion  
 32 (3.58, 10.88) for the high level.

33 For the low inoculum level, 86 out of 132 test portions (POD<sub>CP</sub> of 0.65) were reported as  
 34 presumptive positive by the 3M MDA 2 - *Listeria monocytogenes* method with 86 out of 132 test  
 35 portions (POD<sub>CC</sub> of 0.65) confirming positive. For samples that produced presumptive positive  
 36 results on the 3M MDA 2 - *Listeria monocytogenes* method, 85 out of 132 test portions confirmed  
 37 positive (POD<sub>C</sub> of 0.64). For test portions evaluated by the USDA/FSIS MLG reference method,  
 38 64 out of 132 test portions produced positive results. A dLPOD<sub>C</sub> value of 0.16 with 95%  
 39 confidence intervals of (0.04, 0.28) was obtained between the candidate and reference method,  
 40 indicating a statistically significant difference between the two methods, with a positive  
 41 correlation in data indicating more recovery of the target analyte by the candidate method. A  
 42 dLPOD<sub>CP</sub> value of 0.00 with 95% confidence intervals of (-0.12, 0.12) was obtained between



1 presumptive and confirmed results indicating no statistically significant difference between the  
 2 presumptive and confirmed results.

3 For the high inoculum level, 129 out of 132 test portions ( $POD_{CP}$  of 0.98) were reported as  
 4 presumptive positive by the 3M MDA 2 – *Listeria monocytogenes* method with 132 out of 132  
 5 test portions ( $POD_{CC}$  of 1.00) confirming positive. For samples that produced presumptive  
 6 positive results on the 3M MDA 2 – *Listeria monocytogenes* method, 129 out of 132 samples  
 7 confirmed positive ( $POD_C$  of 0.98). For test portions evaluated by the USDA/FSIS-MLG  
 8 reference method, 132 out of 132 test portions produced positive results. A  $dLPOD_C$  value of  
 9 -0.02 with 95% confidence intervals of (-0.06, 0.01) was obtained between the candidate and  
 10 reference method, indicating no statistically significant difference between the two methods. A  
 11  $dLPOD_{CP}$  value of -0.02 with 95% confidence intervals of (-0.06, 0.01) was obtained between  
 12 presumptive and confirmed results indicating no statistically significant difference between the  
 13 presumptive and confirmed results.

14 For the un-inoculated controls, 0 out of 132 samples ( $POD_{CP}$  of 0.00) produced a presumptive  
 15 positive result by the 3M MDA 2 - *Listeria monocytogenes* method with 0 out of 132 test  
 16 portions ( $POD_{CC}$  of 0.00) confirming positive. For samples that produced presumptive positive  
 17 results on the 3M MDA 2 – *Listeria monocytogenes* method, 0 out of 132 samples confirmed  
 18 positive ( $POD_C$  of 0.00). For test portions evaluated by the USDA/FSIS MLG reference method,  
 19 0 out of 132 test portions produced positive results. A  $dLPOD_C$  value of 0.00 with 95%  
 20 confidence intervals of (-0.03, 0.03) was obtained between the candidate and reference method,  
 21 indicating no statistical significant difference between the two methods. A  $dLPOD_{CP}$  value of  
 22 0.00 with 95% confidence intervals of (-0.03, 0.00) was obtained between presumptive and  
 23 confirmed results indicating no statistically significant difference between the presumptive and  
 24 confirmed results.

25  
 26 Detailed results of the POD statistical analysis are presented in Table 2016.2B and Figures 1C-  
 27 1D.

## 30 Discussion

31  
 32 No negative feedback was provided by the collaborating laboratories in regard to the  
 33 performance of the 3M MDA 2- *Listeria monocytogenes* method. For the raw chicken breast  
 34 fillet, Laboratory 10 reported isolating *Listeria monocytogenes* from two un-inoculated control  
 35 samples. The isolates were sent for further identification and it was determined that they were  
 36 the same strain as the inoculating organism, indicating that cross contamination of the sample  
 37 occurred. Due to the fact that cross contamination occurred, just cause for removal of the data  
 38 was established and the data generated by Laboratory 10 was therefore not included in the  
 39 statistical analysis.

40 Overall, the data generated during this evaluation demonstrates the reproducibility of this new  
 41 method. For the deli turkey analysis, the POD statistical analysis indicated that no statistically  
 42 significant difference between the candidate method and the reference method or between the  
 43 presumptive and confirmed results of the candidate method was obtained. For raw chicken

1 breastfillet, a statistically significant difference was observed between the reference and the  
2 alternative method. The dLPOD data indicated a positive correlation in data indicating more  
3 recovery of the target analyte by the candidate method. One possible contribution to the higher  
4 level of recovery observed with the 3M MDA 2 – *Listeria monocytogenes* method was the use of  
5 Demi-Fraser Broth for the candidate method. This enrichment media formulation is less  
6 selective than the modified University of Vermont Medium used in the USDA reference method  
7 and may have contributed to the higher level of recovery observed during the evaluation.  
8 A second possible contribution to the higher level of recovery was the length of the primary  
9 enrichment. Test portions evaluated by the 3M MDA 2 – *Listeria monocytogenes* method were  
10 incubated for a minimum of 28 hours in the primary enrichment, while the USDA reference  
11 method had a maximum primary enrichment time of 26 hours. No statistically significant  
12 difference was observed between the candidate method presumptive and confirmed results for  
13 this matrix.

## 14 **Recommendations**

15  
16  
17 It is recommended that the 3M Molecular Detection Assay 2 – *Listeria*  
18 *monocytogenes* method be adopted as Official First Action status for the detection of *Listeria*  
19 *monocytogenes* in selected foods: hot dogs (25g & 125g), salmon (25g), deli turkey (25g &  
20 125g), cottage cheese (25g), chocolate milk (25 mL), vanilla ice cream (25g), queso fresco (25g),  
21 romaine lettuce (25g), melon (whole), raw chicken leg pieces (25g), raw chicken breast fillet,  
22 concrete (sponge, 225 mL & 100 mL), stainless steel (sponge, 225 mL), and plastic (Enviroswab,  
23 10 mL) environmental samples.

## 24 **Acknowledgements**

25  
26  
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- 1 (10) Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook  
2 (2013), <http://lcf ltd.com/aoac/aoac-binary-v2-3.xls> (Accessed March, 2016)  
3  
4 (11) ISO 18593:2004 -*Microbiology of food and animal feeding stuffs – Horizontal methods*  
5 *for sampling techniques from surfaces using contact plates and swabs.*

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**Table 1: Participation of each Collaborating Laboratory**

Lab	Deli Turkey <sup>a,b</sup>	Raw Chicken Breast Fillet <sup>a,b</sup>
1	Y	Y
2	Y	Y
3	Y	Y
4	Y	Y
5	Y	Y
6	Y	Y
7	Y	Y
8	N	Y
9	Y	Y
10	N	Y
11	Y	N
12	Y	Y
13	Y	Y

<sup>a</sup> Y= Collaborator analyzed the food type; <sup>a</sup>N= Collaborator did not analyze food type

**Table 2: Heat-Stress Injury Results**

Matrix	Test Organism <sup>a</sup>	CFU/MOX (Selective Agar)	CFU/ TSA (Non-Selective Agar)	Degree Injury <sup>b</sup>
Deli Turkey	<i>Listeria monocytogenes</i> ATCC 19115	9.1 x 10 <sup>8</sup>	2.5 x 10 <sup>9</sup>	60.8%

<sup>a</sup> ATCC- American Type Culture Collection

<sup>b</sup> Cultures were heat stressed for 10 minutes at 55°C in a recirculating water bath.

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**Table 2016.1A:** Summary of Results for the Detection of *Listeria monocytogenes* in Deli Turkey (125g)

Method <sup>a</sup>	3M™ MDA™ 2 - <i>Listeria monocytogenes</i>		
Inoculation Level	Un-inoculated	Low	High
Candidate Presumptive Positive/ Total # of Samples Analyzed	0/132	69/132	132/132
Candidate Presumptive POD (CP)	0.00 (0.00, 0.03)	0.52 (0.43, 0.61)	1.00 (0.97, 1.00)
$s_r^b$	0.00 (0.00, 0.16)	0.51 (0.45, 0.52)	0.00 (0.00, 0.16)
$s_L^c$	0.00 (0.00, 0.16)	0.00 (0.00, 0.16)	0.00 (0.00, 0.16)
$s_R^d$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value <sup>e</sup>	1.0000	0.8091	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/132	66/132	132/132
Candidate Confirmed POD (CC)	0.00 (0.00, 0.03)	0.50 (0.41, 0.59)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9123	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/132	66/132	132/132
Candidate Presumptive Positive that Confirmed POD (C)	0.00 (0.00, 0.03)	0.50 (0.41, 0.59)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9123	1.0000
Positive Reference Samples/ Total # of Samples Analyzed	0/132	60/132	132/132
Reference POD	0.00 (0.00, 0.03)	0.45 (0.37, 0.54)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.11)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9829	1.0000
dLPOD (Candidate vs. Reference) <sup>f</sup>	0.00 (-0.03, 0.03)	0.04 (-0.08, 0.17)	0.00 (-0.03, 0.03)
dLPOD (Candidate Presumptive vs. Candidate Confirmed) <sup>f</sup>	0.00 (-0.03, 0.03)	0.02 (-0.10, 0.15)	0.00 (-0.03, 0.03)

<sup>a</sup> Results include 95% Confidence Intervals, <sup>r</sup> Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation

<sup>e</sup> P Value = Homogeneity test of laboratory PODs; <sup>f</sup> A confidence interval for dLPOD that does not contain the value 0 indicates a statistical significant difference between the two methods

**Table 2016.1B:** Summary of Results for the Detection of *Listeria monocytogenes* in Raw Chicken Breast Fillet (25g)

Method <sup>a</sup>	3M™ MDA™ 2 - <i>Listeria monocytogenes</i>		
Inoculation Level	Un-inoculated	Low	High
Candidate Presumptive Positive/ Total # of Samples Analyzed	0/132	86/132	129/132
Candidate Presumptive POD (CP)	0.00 (0.00, 0.03)	0.65 (0.57, 0.73)	0.98 (0.93, 0.99)
$s_r^b$	0.00 (0.00, 0.16)	0.48 (0.43, 0.52)	0.15 (0.13, 0.17)
$s_L^c$	0.00 (0.00, 0.16)	0.00 (0.00, 0.17)	0.03 (0.00, 0.08)
$s_R^d$	0.00 (0.00, 0.23)	0.48 (0.43, 0.52)	0.15 (0.13, 0.18)
P Value <sup>e</sup>	1.0000	0.7057	0.1089
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/132	86/132	132/132
Candidate Confirmed POD (CC)	0.00 (0.00, 0.03)	0.65 (0.57, 0.73)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.48 (0.43, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.18)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.48 (0.43, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.5632	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/132	85/132	129/132
Candidate Presumptive Positive that Confirmed POD (C)	0.00 (0.00, 0.03)	0.64 (0.56, 0.73)	0.98 (0.93, 0.99)
$s_r$	0.00 (0.00, 0.16)	0.48 (0.43, 0.52)	0.15 (0.13, 0.17)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.18)	0.03 (0.00, 0.08)
$s_R$	0.00 (0.00, 0.23)	0.49 (0.43, 0.52)	0.15 (0.13, 0.18)
P Value	1.0000	0.6228	0.1089
Positive Reference Samples/ Total # of Samples Analyzed	0/132	64/132	132/132
Reference POD	0.00 (0.00, 0.03)	0.48 (0.40, 0.57)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9192	1.0000
dLPOD (Candidate vs. Reference) <sup>f</sup>	0.00 (-0.03, 0.03)	0.16 (0.04, 0.28)	-0.02 (-0.06, 0.01)
dLPOD (Candidate Presumptive vs. Candidate Confirmed) <sup>f</sup>	0.00 (-0.03, 0.03)	0.00 (-0.12, 0.12)	-0.02 (-0.06, 0.01)

<sup>a</sup> Results include 95% Confidence Intervals, <sup>r</sup> Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation

<sup>e</sup> P Value = Homogeneity test of laboratory PODs; <sup>f</sup> A confidence interval for dLPOD that does not contain the value 0 indicates a statistical significant difference between the two methods

1 **Table 2016.2A:** Comparative Results for the Detection of *Listeria monocytogenes* in 125 g Deli Turkey Test Portions by the 3M™ MDA 2 - *Listeria*  
 2 *monocytogenes* Method vs. USDA/FSIS MLG Chapter 8.09 in a Collaborative Study (Un-inoculated Control)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Deli Turkey	1	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		2	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		3	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		4	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		5	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		6	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		7	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		8 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		9	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		10 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		11	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		12	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		13	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00

Estimate	All	132	0	0.00	132	0	0.00	132	0	0.00	132	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.03	-0.03
UCL				0.03			0.03			0.03			0.03	0.03	0.03
$s_r^b$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_L^c$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_R^d$				0.00			0.00			0.00			0.00		
UCL				0.00			0.00			0.00			0.00		
LCL				0.23			0.23			0.23			0.23		
$P_T^e$				1.0000			1.0000			1.0000			1.0000		

<sup>a</sup>N/A – Laboratory did not participate or submit data for this matrix  
<sup>b</sup>Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup>Reproducibility Standard Deviation, <sup>e</sup> P<sub>T</sub> Value = Homogeneity test of laboratory PODs  
 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

1 **Table 2016.2A (cont'd.):** Comparative Results for the Detection of *Listeria monocytogenes* in 125 g Deli Turkey Test Portions by the 3M™ MDA 2 - *Listeria*  
 2 *monocytogenes* Method vs. USDA/FSIS MLG Chapter 8.09 in a Collaborative Study (Low Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R		
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)	
	Deli Turkey	1	12	7	0.58	12	7	0.58	12	7	0.58	12	6	0.50	0.08	0.00	
		2	12	7	0.58	12	6	0.50	12	6	0.50	12	5	0.42	0.08	0.08	
		3	12	4	0.33	12	4	0.33	12	4	0.33	12	5	0.42	-0.08	0.00	
		4	12	5	0.42	12	5	0.42	12	5	0.42	12	4	0.33	0.08	0.00	
		5	12	6	0.50	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.00	
		6	12	8	0.66	12	7	0.58	12	7	0.58	12	6	0.50	0.08	0.00	
		7	12	7	0.58	12	7	0.58	12	7	0.58	12	6	0.50	0.08	0.00	
		8 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		9	12	8	0.66	12	8	0.66	12	8	0.66	12	6	0.50	0.16	0.00	
		10 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MPN/ Test Portion	11	12	7	0.58	12	6	0.50	12	6	0.50	12	7	0.58	-0.08	0.08	
		12	12	5	0.42	12	5	0.42	12	5	0.42	12	4	0.33	0.08	0.00	
		13	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00	
Estimate	0.63	All	132	69	0.52	132	66	0.50	132	66	0.50	132	60	0.45	0.04	0.02	
LCL	0.49				0.43			0.41			0.41			0.37	-0.08	-0.10	
UCL	0.80				0.61			0.59			0.59			0.54	0.17	0.15	
$s_r^b$					0.51			0.51			0.51			0.51			
LCL					0.45			0.46			0.46			0.46			
UCL					0.52			0.52			0.52			0.52			
$s_L^c$					0.00			0.00			0.00			0.00			
LCL					0.00			0.00			0.00			0.00			
UCL					0.16			0.14			0.14			0.11			
$s_R^d$					0.51			0.51			0.51			0.51			
UCL					0.46			0.46			0.46			0.46			
LCL					0.52			0.52			0.52			0.52			
$P_T^e$					0.8091			0.9123			0.9123			0.9829			

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix  
<sup>b</sup> Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation, <sup>e</sup>  $P_T$  Value = Homogeneity test of laboratory PODs  
 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

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1 **Table 2016.2A (cont'd.):** Comparative Results for the Detection of *Listeria monocytogenes* in 125 g Deli Turkey Test Portions by the 3M™ MDA 2 - *Listeria*  
 2 *monocytogenes* Method vs. USDA/FSIS MLG Chapter 8.09 in a Collaborative Study (High Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Deli Turkey	1	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		2	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		3	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		4	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		5	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		6	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		7	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		8 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		9	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		10 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MPN/ Test Portion	11	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		12	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		13	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
Estimate	4.52	All	132	132	1.00	132	132	1.00	132	132	1.00	132	132	1.00	0.00	0.00
LCL	3.19				0.97			0.97			0.97			0.97	-0.03	-0.03
UCL	6.42				1.00			1.00			1.00			1.00	0.03	0.03
s <sub>r</sub> <sup>b</sup>					0.00			0.00			0.00			0.00		
LCL					0.00			0.00			0.00			0.00		
UCL					0.16			0.16			0.16			0.16		
s <sub>L</sub> <sup>c</sup>					0.00			0.00			0.00			0.00		
LCL					0.00			0.00			0.00			0.00		
UCL					0.16			0.16			0.16			0.16		
s <sub>R</sub> <sup>d</sup>					0.00			0.00			0.00			0.00		
UCL					0.00			0.00			0.00			0.00		
LCL					0.23			0.23			0.23			0.23		
P <sub>T</sub> <sup>e</sup>					1.000			1.000			1.000			1.000		

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix  
<sup>b</sup> Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation, <sup>e</sup> P<sub>T</sub> Value = Homogeneity test of laboratory PODs  
 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

1 **Table 2016.2B:** Comparative Results for the Detection of *Listeria monocytogenes* in 25 g Raw Chicken Breast Fillet Test Portions by the 3M™ MDA 2 - *Listeria*  
 2 *monocytogenes* Method vs. USDA/FSIS MLG Chapter 8.09 in a Collaborative Study (Un-inoculated Control)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Raw Chicken Breast Fillet	1	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		2	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		3	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		4	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		5	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		6	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		7	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.08
		8	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		9	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		10 <sup>b</sup>	12	0	0.00	12	2	0.17	12	0	0.00	12	0	0.00	0.00	-0.17
		11 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		12	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		13	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00

Estimate	All	132	0	0.00	132	0	0.00	132	0	0.00	132	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.03	-0.03
UCL				0.03			0.03			0.03			0.03	0.03	0.03
$s_r^c$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_L^d$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_R^e$				0.00			0.00			0.00			0.00		
UCL				0.00			0.00			0.00			0.00		
LCL				0.23			0.23			0.23			0.23		
$P_T^f$				1.0000			1.0000			1.0000			1.0000		

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix; <sup>b</sup> Results were not used in statistical analysis due to deviation from testing protocol.

<sup>c</sup> Repeatability Standard Deviation, <sup>d</sup> Among-Laboratory Standard Deviation, <sup>e</sup> Reproducibility Standard Deviation, <sup>f</sup>  $P_T$  Value = Homogeneity test of laboratory PODs

LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

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1 **Table 2016.2B (cont'd.):** Comparative Results for the Detection of *Listeria monocytogenes* in 25 g Raw Chicken Breast Fillet Test Portions by the 3M™ MDA 2 -  
 2 *Listeria monocytogenes* Method vs. USDA/FSIS MLG Chapter 8.09 in a Collaborative Study (Low Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Raw Chicken Breast Fillet	1	12	7	0.58	12	7	0.58	12	7	0.58	12	7	0.58	0.00	0.00
		2	12	9	0.75	12	9	0.75	12	9	0.75	12	7	0.58	0.17	0.00
		3	12	10	0.83	12	10	0.83	12	10	0.83	12	5	0.42	0.41	0.00
		4	12	9	0.75	12	9	0.75	12	9	0.75	12	4	0.33	0.42	0.00
		5	12	8	0.66	12	8	0.66	12	8	0.66	12	5	0.42	0.22	0.00
		6	12	9	0.75	12	9	0.75	12	9	0.75	12	6	0.50	0.15	0.08
		7	12	7	0.58	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.16
		8	12	7	0.58	12	7	0.58	12	7	0.58	12	7	0.58	0.00	0.00
		9	12	8	0.66	12	9	0.75	12	8	0.66	12	7	0.58	0.08	-0.08
		10 <sup>b</sup>	12	6	0.50	12	8	0.66	12	6	0.50	12	5	0.42	0.08	-0.16
	MPN/ Test Portion	11 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		12	12	7	0.58	12	7	0.58	12	7	0.58	12	4	0.42	0.16	0.00
		13	12	5	0.42	12	5	0.42	12	5	0.42	12	6	0.50	-0.08	0.00
Estimate	0.66	All	132	86	0.65	132	86	0.65	132	85	0.64	132	64	0.48	0.16	0.00
LCL	0.51				0.57			0.57			0.56			0.40	0.04	-0.12
UCL	0.83				0.73			0.73			0.73			0.57	0.28	0.12
$s_r^c$					0.48			0.48			0.48			0.51		
LCL					0.43			0.43			0.43			0.46		
UCL					0.52			0.52			0.52			0.52		
$s_L^d$					0.00			0.00			0.00			0.00		
LCL					0.00			0.00			0.00			0.00		
UCL					0.17			0.18			0.18			0.14		
$s_R^e$					0.48			0.48			0.49			0.51		
UCL					0.43			0.43			0.43			0.46		
LCL					0.52			0.52			0.52			0.52		
$P_T^f$					0.7057			0.5632			0.6228			0.9192		

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix; <sup>b</sup> Results were not used in statistical analysis due to deviation from testing protocol.

<sup>c</sup> Repeatability Standard Deviation, <sup>d</sup> Among-Laboratory Standard Deviation, <sup>e</sup> Reproducibility Standard Deviation, <sup>f</sup>  $P_T$  Value = Homogeneity test of laboratory PODs

LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

1 **Table 2016.2B (cont'd.):** Comparative Results for the Detection of *Listeria monocytogenes* in 25 g Raw Chicken Breast Fillet Test Portions by the 3M™ MDA 2 -  
 2 *Listeria monocytogenes* Method vs. USDA/FSIS MLG Chapter 8.09 in a Collaborative Study (High Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R		
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)	
	Raw Chicken Breast Fillet	1	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		2	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		3	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		4	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		5	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		6	12	10	0.83	12	12	1.00	12	10	0.83	12	12	1.00	0.00	0.00	
		7	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		8	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		9	12	11	0.92	12	12	1.00	12	11	0.92	12	12	1.00	-0.08	-0.08	
		10 <sup>b</sup>	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		MPN/ Test Portion	11 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
			13	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
Estimate	6.24	All	132	129	0.98	132	132	1.00	132	129	0.98	132	132	1.00	-0.02	-0.02	
LCL	3.58				0.93			0.97			0.93			0.97	-0.06	-0.06	
UCL	10.88				0.99			1.00			0.99			1.00	0.01	0.01	
$s_r^c$					0.15			0.00			0.15			0.00			
LCL					0.13			0.00			0.13			0.00			
UCL					0.17			0.16			0.17			0.16			
$s_L^d$					0.03			0.00			0.03			0.00			
LCL					0.00			0.00			0.00			0.00			
UCL					0.08			0.16			0.08			0.16			
$s_R^e$					0.15			0.00			0.15			0.00			
UCL					0.13			0.00			0.13			0.00			
LCL					0.18			0.23			0.18			0.23			
$P_T^f$					0.1089			1.0000			0.1089			1.0000			

<sup>a</sup>N/A – Laboratory did not participate or submit data for this matrix; <sup>b</sup>Results were not used in statistical analysis due to deviation from testing protocol.

<sup>c</sup>Repeatability Standard Deviation, <sup>d</sup>Among-Laboratory Standard Deviation, <sup>e</sup>Reproducibility Standard Deviation, <sup>f</sup> $P_T$  Value = Homogeneity test of laboratory PODs

LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

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**Supplementary Materials**

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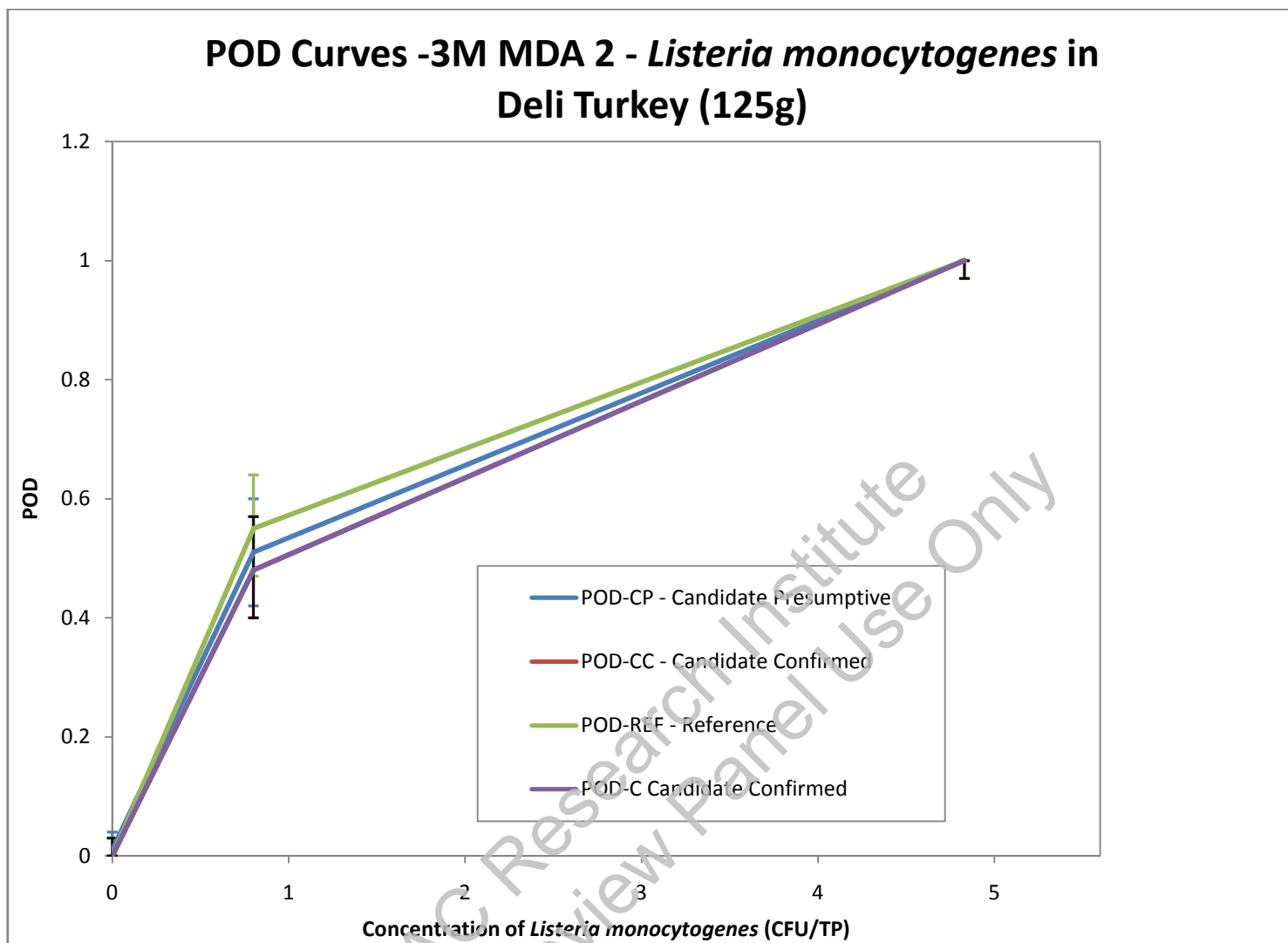
**Table 3: Results of Aerobic Plate Count for Collaborating Laboratories**

Lab	Deli Turkey (CFU/g) <sup>a</sup>	Raw Chicken Breast Fillet (CFU/g) <sup>a</sup>
1	2.0 x 10 <sup>1</sup>	2.3 x 10 <sup>4</sup>
2	< 10	3.4 x 10 <sup>4</sup>
3	< 10	4.1 x 10 <sup>3</sup>
4	3.0 x 10 <sup>1</sup>	8.3 x 10 <sup>6</sup>
5	1.0 x 10 <sup>1</sup>	5.4 x 10 <sup>3</sup>
6	< 10	6.3 x 10 <sup>4</sup>
7	6.0 x 10 <sup>1</sup>	3.6 x 10 <sup>2</sup>
8	N/A	2.9 x 10 <sup>3</sup>
9	< 10	4.6 x 10 <sup>2</sup>
10	N/A	3.7 x 10 <sup>4</sup>
11	2.0 x 10 <sup>1</sup>	N/A
12	< 10	7.4 x 10 <sup>3</sup>
13	< 10	2.7 x 10 <sup>4</sup>

<sup>a</sup> Samples analyzed by the 3M Petrifilm Rapid Aerobic Count Plate, AOAC OMA 2015.13

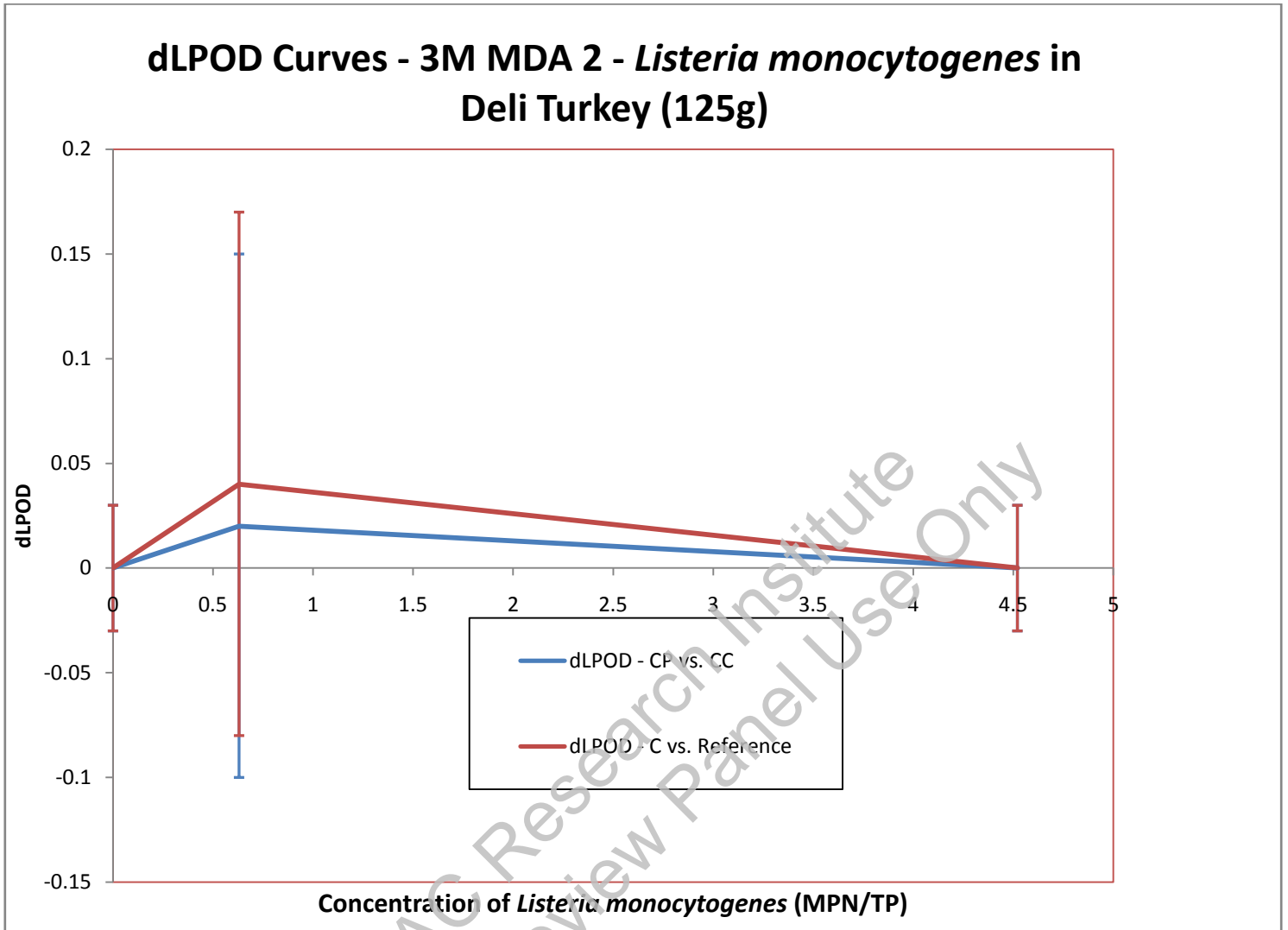
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Figure 1A: POD Values of Candidate Method vs. Reference Method for 125 g Deli Turkey



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Figure 1B: dLPOD Values of Candidate Method vs. Reference Method for 125 g Deli Turkey

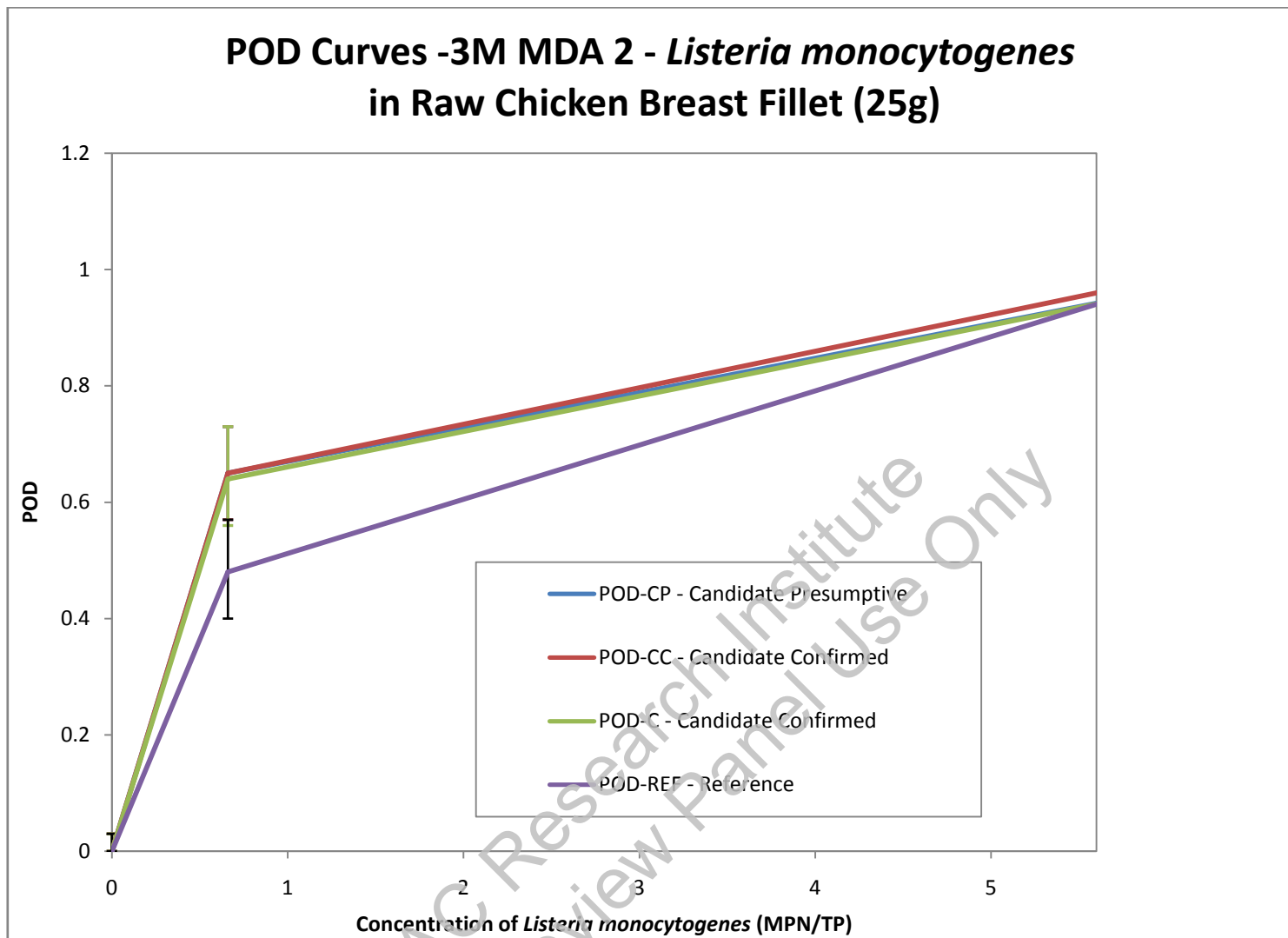


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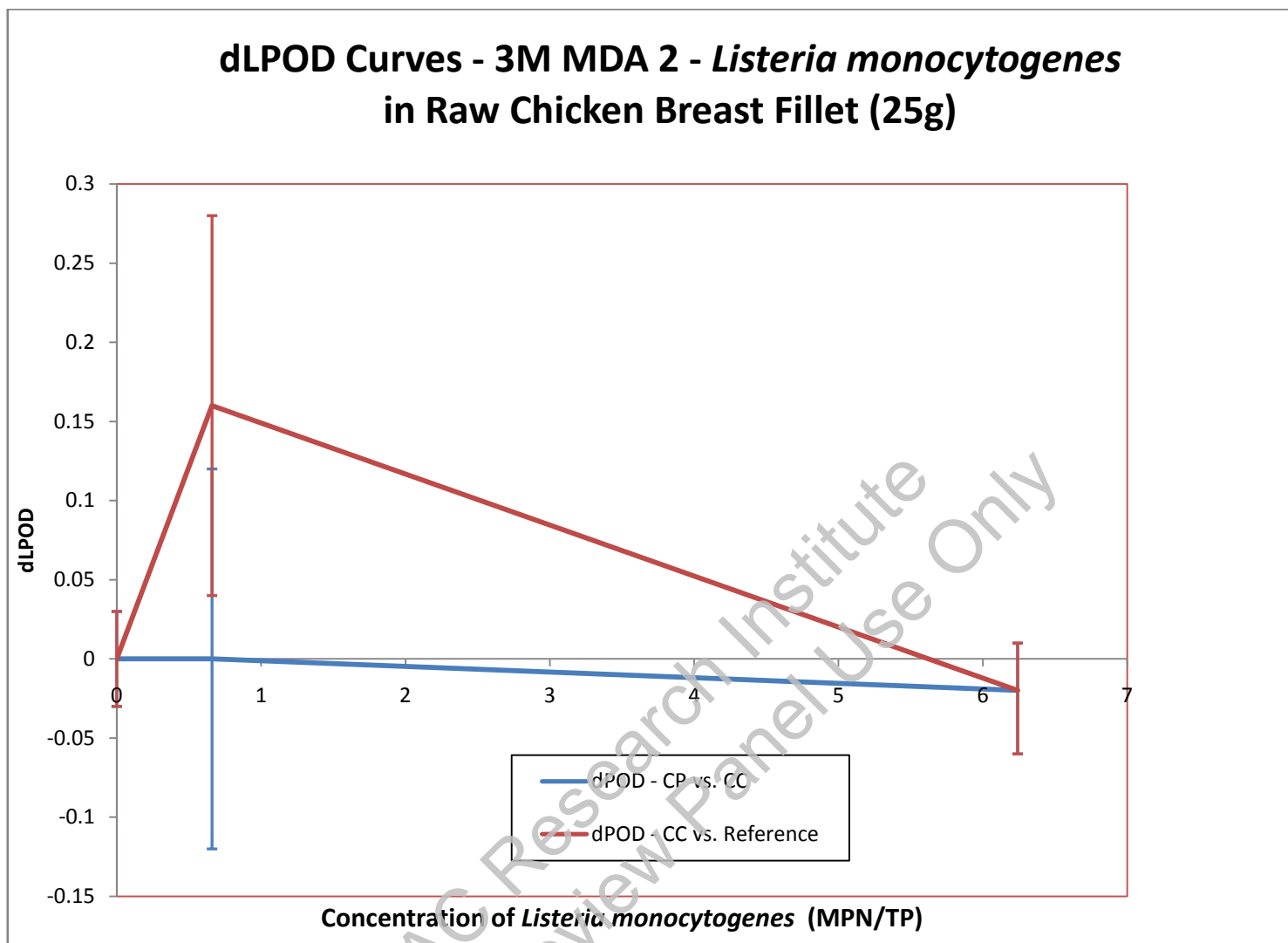


Figure 2A: POD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet



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Figure 2B: dLPOD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet



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## 3M™ Molecular Detection Assay 2 - *Listeria monocytogenes*

MDA2LMO96

### PRODUCT DESCRIPTION AND INTENDED USE

3M™ Molecular Detection Assay 2 - *Listeria monocytogenes* is used with the 3M™ Molecular Detection System for the rapid and specific detection of *Listeria monocytogenes* in enriched food and environmental samples.

The 3M Molecular Detection Assays use loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations <sup>(1, 2, 3)</sup>.

The 3M Molecular Detection Assay 2 - *Listeria monocytogenes* is intended for use in a laboratory environment by professionals trained in laboratory techniques. 3M has not documented the use of this product in industries other than food or beverage. For example, 3M has not documented this product for testing water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M Molecular Detection Assay - 2 *Listeria monocytogenes* has not been evaluated with all possible testing protocols or with all possible strains of bacteria.

**As with all test methods, the source, formulation, and quality of enrichment medium can influence the results.** Factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may also influence results. 3M recommends evaluation of the method including enrichment medium, in the user's environment using a sufficient number of samples with particular foods and microbial challenges to ensure that the method meets the user's criteria.

3M has evaluated the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* with Demi-Fraser Broth containing Ferric Ammonium Citrate and Fraser Broth. A typical formulation of this medium follows below.

#### Demi-Fraser Broth Base Typical Formula (g/L)

Sodium Chloride	20 g
Sodium Phosphate, dibasic, anhydrous*	9.6 g
Beef Extract	5.0 g
Pancreatic Digest of Casein	5.0 g
Peptic Digest of Animal Tissue	5.0 g
Yeast Extract	5.0 g

Lithium Chloride	3.0 g
Potassium Phosphate, monobasic	1.35 g
Esculin	1.0 g
Acridine HCl	0.0125 g
Nalidixic Acid	0.01 g

\* Substitute: Sodium Phosphate, dibasic, dihydrate 12.0 g

### Fraser Broth Supplement

(Ingredients per 10 mL vial. One vial is added to one liter of basal medium.)

Ferric Ammonium Citrate 0.5g/10mL

Final pH 7.2 ± 0.2 at 25°C

The 3M™ Molecular Detection Instrument is intended for use with samples that have undergone heat treatment during the assay lysis step, which is designed to destroy organisms present in the sample. Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.

3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

The 3M Molecular Detection Assay 2 - *Listeria monocytogenes* test kit contains 96 tests, described in Table 1.

**Table 1.** Kit Components

Item	Identification	Quantity	Contents	Comments
Lysis Solution (LS) tubes	Pink solution in clear tubes	96 (12 strips of 8 tubes)	580 µL of LS per tube	Racked and ready to use
<i>Listeria monocytogenes</i> Reagent tubes	Yellow tubes	96 (12 strips of 8 tubes)	Lyophilized specific amplification and detection mix	Ready to use
Extra caps	Yellow caps	96 (12 strips of 8 caps)		Ready to use
Reagent Control (RC)	Clear flip-top tubes	16 (2 pouches of 8 individual tubes)	Lyophilized control DNA, amplification and detection mix	Ready to use
Quick Start Guide		1		

The Negative Control, not provided in the kit, is sterile enrichment medium, e.g., Demi-Fraser Broth. Do not use water as a Negative Control.

## SAFETY

The user should read, understand and follow all safety information in the instructions for the 3M Molecular Detection System and the 3M Molecular Detection Assay 2 - *Listeria monocytogenes*. Retain the safety instructions for future reference.



### WARNING:

Indicates a hazardous situation, which, if not avoided, could result in death or serious injury and/or property damage.



### CAUTION:

Indicates a hazardous situation, which, if not avoided, could result in minor or moderate injury and/or property damage.

### NOTICE:

Indicates a potentially hazardous situation which, if not avoided, could result in property damage.



## WARNING

Do not use the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* in the diagnosis of conditions in humans or animals.

The 3M Molecular Detection Assay 2 - *Listeria monocytogenes* method may generate *Listeria monocytogenes* to levels sufficient to cause stillbirths and fatalities in pregnant women and the immunocompromised, if exposed.

The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices, ISO/IEC17025<sup>(4)</sup>, or ISO 7218<sup>(5)</sup>.

To reduce the risks associated with a false-negative result leading to the release of contaminated product:

- Follow the protocol and perform the tests exactly as stated in the product instructions.
- Store the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* as indicated on the package and in the product instructions.
- Always use the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* by the expiration date.
- Use the 3M Molecular Detection Assay *Listeria monocytogenes* for food and environmental samples that have been validated internally or by a third party.

- Use the 3M Molecular Detection Assay 2 -*Listeria monocytogenes* only for surfaces, sanitizers, protocols and bacterial strains that have been validated internally or by a third party.
- For an environmental sample containing Neutralizing Buffer with aryl sulfonate complex, perform a 1:2 dilution before testing (1 part sample into 1 part sterile enrichment broth). Another option is to transfer 10 uL of the NB enrichment into the LS tubes. 3M™ sample handling products which include Neutralizing Buffer with aryl sulfonate complex: BPPFV10NB, RS96010NB, RS9604NB, SSL10NB, XSLSSL10NB, HS10NB and HS119510NB.

**To reduce the risks associated with exposure to chemicals and biohazards:**

- It is strongly recommended that female laboratory staff be informed of the risk to a developing fetus resulting from infection of the mother through exposure to *Listeria monocytogenes*.
- Perform pathogentesting in a properly equipped laboratory under the control of trained personnel.
- Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples.
- Avoid contact with the contents of the enrichment media and reagent tubes after amplification.
- Dispose of enriched samples according to current industry standards.
- Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.

**To reduce the risks associated with cross-contamination while preparing the assay:**

- Always wear gloves (to protect the user and prevent introduction of nucleases).

**To reduce the risks associated with environmental contamination:**

- Follow current industry standards for disposal of contaminated waste.



**CAUTION**

- Do not exceed the recommended temperature setting on heater.
- Do not exceed the recommended heating time.
- Use an appropriate, calibrated thermometer to verify the 3M™ Molecular Detection Heat Block Insert temperature (e.g., a partial immersion thermometer or digital thermocouple thermometer, not a total immersion thermometer.) The thermometer must be placed in the designated location in the 3M Molecular Detection Heat Block Insert.

**NOTICE**

**To reduce the risks associated with cross-contamination while preparing the assay:**

- Use of sterile, aerosol barrier (filtered), molecular biology grade pipette tips is recommended.
- Use a new pipette tip for each sample transfer.
- Use Good Laboratory Practices to transfer the sample from the enrichment to the lysis tube. To avoid pipettor contamination, the user may choose to add an intermediate transfer step. For example, the user can transfer each enriched sample into a sterile tube.
- Use a molecular biology workstation containing germicidal lamp where available.
- Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with a 1- 5% (v:v in water) household bleach solution or DNA removal solution.

**To reduce the risks associated with a false-positive result:**

- Never open tubes post amplification.
- Always dispose of the contaminated tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

Consult the Safety Data Sheet for additional information and local regulations for disposal.

If you have questions about specific applications or procedures, please visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

**LIMITATION OF WARRANTIES / LIMITED REMEDY**

EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL PRODUCT PACKAGING, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any 3M Food Safety Product is defective, 3M or its authorized distributor will, at its option, replace or refund the purchase price of the product. These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery of any suspected defects in a product and return it to 3M. Please call Customer Service (1-800-328-1671 in the U.S.) or your official 3M Food Safety representative for a Returned Goods Authorization.

**LIMITATION OF 3M LIABILITY**

3M WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO LOST PROFITS. In no event shall 3M's liability under any legal theory exceed the purchase price of the product alleged to be defective.

**USER RESPONSIBILITY**

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety), or contact your local 3M representative or distributor for more information.

When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may influence results.

It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and microbial challenges to satisfy the user that the chosen test method meets the user's criteria.

It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.

As with any test method, results obtained from use of any 3M Food Safety product do not constitute a guarantee of the quality of the matrices or processes tested.

To help customers evaluate the method for various food matrices, 3M has developed the 3M™ Molecular Detection Matrix Control kit. When needed, use the Matrix Control (MC) to determine if the matrix has the ability to impact the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* results. Test several samples representative of the matrix, i.e. samples obtained from different origin, during any validation period when adopting the 3M method or when testing new or unknown matrices or matrices that have undergone raw material or process changes.

A matrix can be defined as a type of product with intrinsic properties such as composition and process. Differences between matrices may be as simple as the effects caused by differences in their processing or presentation for example, raw vs. pasteurized; fresh vs. dried, etc.

## **STORAGE AND DISPOSAL**

Store the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* at 2-8°C. Do not freeze. Keep kit away from light during storage. After opening the kit, check that the foil pouch is undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes should always be stored in the re-sealable pouch with the desiccant inside to maintain stability of the lyophilized reagents. Store resealed pouches at 2-8°C for no longer than 60days.

Do not use 3M Molecular Detection Assay 2 - *Listeria monocytogenes* past the expiration date. Expiration date and lot number are noted on the outside label of the box. After use, the enrichment medium and the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* tubes can potentially contain pathogenic materials. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Safety Data Sheet for additional information and local regulations for disposal.

## **INSTRUCTIONS FOR USE**



Follow all instructions carefully. Failure to do so may lead to inaccurate results.

Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with a 1-5% (v:v in water) household bleach solution or DNA removal solution.

## **SAMPLE ENRICHMENT**

Table 2 presents guidance for the enrichment of food and environmental samples. It is the user's responsibility to validate alternate sampling protocols or dilution ratios to ensure this test method meets the user's criteria.

### **Foods**

1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature.
2. Aseptically combine the enrichment medium and sample according to Table 2. For all meat and highly particulate samples, the use of filter bags is recommended.
3. Homogenize thoroughly by blending, stomaching, or hand mixing for  $2 \pm 0.2$  minutes. Incubate at  $37 \pm 1^\circ\text{C}$  according to Table 2.
4. For raw dairy products, transfer 0.1mL of the primary enrichment into 10 mL of Fraser Broth. Incubate at  $37 \pm 1^\circ\text{C}$  for 20-24 hours.

### **Environmental samples**

Sample collection devices can be a sponge hydrated with a neutralizing solution to inactivate the effects of the sanitizers. 3M recommends the use of a biocide-free cellulose sponge. Neutralizing solution can be Dey-Engley (D/E) Neutralizing Broth or Lethen broth. It is recommended to sanitize the area after sampling.

**WARNING:** Should you select to use Neutralizing Buffer (NB) that contains aryl sulfonate complex as the hydrating solution for the sponge, it is required to perform a 1:2 dilution (1 part sample into 1 part sterile enrichment broth) of the enriched environmental sample before testing in order to reduce the risks associated with a false-negative result leading to the release of contaminated product

The recommended size of the sampling area to verify the presence or absence of the pathogen on the surface is at least  $100 \text{ cm}^2$  (10 cm x 10 cm or 4"x4"). When sampling with a sponge, cover the entire area going in two directions (left to right then up and down) or collect environmental samples following your current sampling protocol or according to the FDA BAM <sup>(1)</sup>, USDA FSIS MLG <sup>(2)</sup> or ISO 18593 <sup>(6)</sup> guidelines.

1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature.
2. Aseptically combine the enrichment medium and sample according to Table 2 or 3.
3. Homogenize thoroughly by blending, stomaching, or hand mixing for  $2 \pm 0.2$  minutes. Incubate at  $37 \pm 1^\circ\text{C}$  for 24-30 hours.

**Table 2:** General enrichment protocols using Demi-Fraser Broth at  $37 \pm 1^\circ\text{C}$  and Fraser Broth as needed

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)			
Heat-processed, cooked, cured meats, poultry, seafood and fish	25 g	225	24-30			
Heat-processed / pasteurized dairy products						
Produce and vegetables						
Multi-component foods						
Environmental samples (a)	1 sponge	100 or 225	24-30			
	1 swab	10	24-30			
Raw meat, poultry, seafood, fish (b)	25 g	475	23-32			
Sample Matrix	Primary Enrichment (Demi-Fraser Broth)			Secondary Enrichment (Fraser Broth)	Sample Analysis Volume(a)	
	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)	Sample Size		Enrichment Time (hr)
Raw dairy products	25 g	225	20-24	Transfer 0.1 mL into 10 mL Fraser Broth	20-24	10 $\mu\text{L}$

(a) Volume of sample transferred to Lysis Solution tubes. Refer to Step 4.6 of Lysis section.

**Specific Instructions for Validated Methods**

**AOAC® Performance Tested Method<sup>sm</sup># 081501**



In AOAC PTM studies, the 3M Molecular Detection Assay 2 – *Listeria monocytogenes* was found to be an effective method for the detection of *Listeria monocytogenes*. The matrices tested in the study are shown in Table 3. The limit of detection of the 3M Molecular Detection Assay 2 – *Listeria monocytogenes* method is 1-5 colony forming units per validated test portion size (in Table 3).

**Table 3.** Enrichment protocols using Demi-Fraser Broth at  $37 \pm 1 \text{ }^\circ\text{C}$  according to AOAC Performance Tested <sup>SM</sup> Certificate #081501 [and Official Method<sup>SM</sup>2016.xxx-](#)

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)	
Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon	25 g	225	24-30	
Raw chicken	25 g	475	28-32	
Deli turkey	125 g	1125	24-30	
Cantaloupe	Whole melon	Enough volume to allow melon to float	26-30	
Environmental samples:	Stainless steel	1 sponge	225	24-30
	Sealed concrete	1 sponge	100	24-30

	Plastic	1 swab	10	24-30
--	---------	--------	----	-------

#### PREPARATION OF THE 3M™ MOLECULAR DETECTION SPEED LOADER TRAY

1. Wet a cloth with a 1-5% (v:v in water) household bleach solution and wipe the 3M™ Molecular Detection Speed Loader Tray.
2. Rinse the 3M Molecular Detection Speed Loader Tray with water.
3. Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.
4. Ensure the 3M Molecular Detection Speed Loader Tray is dry before use.

#### PREPARATION OF THE 3M™ MOLECULAR DETECTION CHILL BLOCK INSERT

Place the 3M™ Molecular Detection Chill Block directly on the laboratory bench; (the 3M™ Molecular Detection Chill Block Tray is not used). Use the chill block at a ambient laboratory temperature (20-25°C).

#### PREPARATION OF THE 3M™ MOLECULAR DETECTION HEAT BLOCK INSERT

Place the 3M™ Molecular Detection Heat Block Insert in a dry doubleblock heater unit. Turn on the dry block heater unit and set the temperature to allow the 3M Molecular Detection Heat Block Insert to reach and maintain a temperature of  $100 \pm 1^\circ\text{C}$ .

**NOTE:** Depending on the heater unit, allow approximately 30 minutes for the 3M Molecular Detection Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (e.g., a partial immersion thermometer or digital thermocouple thermometer, **not** a total immersion thermometer) placed in the designated location, verify that the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^\circ\text{C}$ .

#### PREPARATION OF THE 3M™ MOLECULAR DETECTION INSTRUMENT

1. Launch the 3M™ Molecular Detection Software and log in.
2. Turn on the 3M Molecular Detection Instrument.
3. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection System User Manual for details.

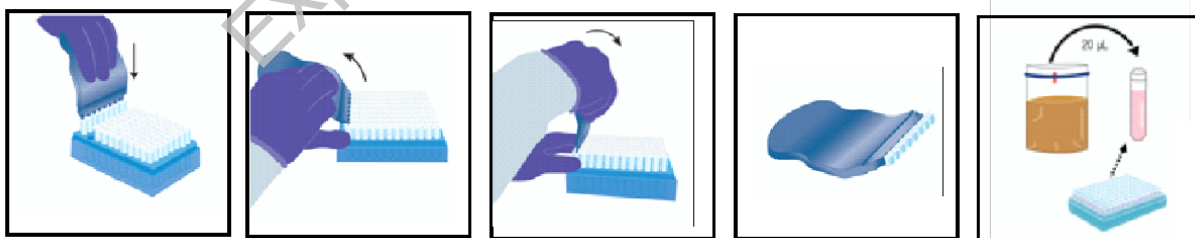
**NOTE:** The 3M Molecular Detection Instrument must reach and maintain temperature of  $60^\circ\text{C}$  before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 minutes and is indicated by an ORANGE light on the instrument's status bar. When the instrument is ready to start a run, the status bar will turn GREEN.

## LYSIS

1. Allow the lysis solution (LS) tubes to warm up by setting the rack at room temperature (20-25 °C) overnight (16-18 hours). Alternatives to equilibrate the LS tubes to room temperature are to set the LS tubes on the laboratory bench for at least 2 hours, incubate the LS tubes in a 37 ±1°C incubator for 1 hour or place them in a dry double block heater for 30 seconds at 100°C.
2. Invert the capped tubes to mix. Proceed to next step within 4 hrs.
3. Remove the enrichment broth from the incubator.
4. One LS tube is required for each sample and the Negative Control (NC) (sterile enrichment medium) sample.
  - 4.1 LS tube strips can be cut to desired LS tube number. Select the number of individual LS tubes or 8-tube strips needed. Place the LS tubes in an empty rack.
  - 4.2 To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipette tip for each transfer step.
  - 4.3 Transfer enriched sample to LS tubes as described below:

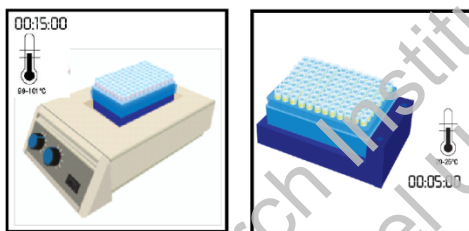
Transfer each enriched sample into an individual LS tube **first**. Transfer the NC **last**.

- 4.4 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip - one strip at a time.
  - 4.5 Discard the LS tube cap – if lysate will be retained for retest, place the caps into a clean container for re-application after lysis. For processing of retained lysate, see Appendix A.
  - 4.6 Transfer 20 µL of sample into a LS tube unless otherwise indicated in Protocol Table 2 or in Specific instructions for validated methods table 3.e.g. Raw dairy products use 10µL.
5. Repeat step 4.2 until each individual sample has been added to a corresponding LS tube in the strip.



6. Repeat steps 4.1 to 4.6 as needed, for the number of samples to be tested.
7. When all samples have been transferred, transfer 20 µL of NC (sterile enrichment medium, e.g., Demi-Fraser Broth) into a LS tube. Do not use water as a NC.

8. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^{\circ}\text{C}$ .
9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for  $15 \pm 1$  minutes. During heating, the LS solution will change from pink (cool) to yellow (hot).  
Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.
10. Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes. The 3M Molecular Chill Block Insert, used at ambient temperature without the Molecular Detection Chill Block Tray should sit directly on the laboratory bench. When cool, the lysis solution will revert to a pink color.
11. Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.



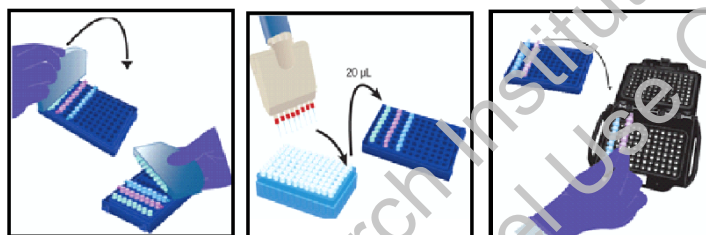
#### AMPLIFICATION

1. One Reagent tube is required for each sample and the NC.
  - 1.1 Reagent tube strips can be cut to desired tube number. Select the number of individual Reagent tubes or 8-tube strips needed.
  - 1.2 Place Reagent tubes in an empty rack.
  - 1.3 Avoid disturbing the reagent pellets from the bottom of the tubes.
2. Select 1 Reagent Control (RC) tube and place in rack.
3. To avoid cross-contamination, decap one Reagent tube strip at a time and use a new pipette tip for each transfer step.
4. Transfer lysate to Reagent tubes and RC tube as described below:

Transfer each sample lysate into individual Reagent tubes **first** followed by the NC. Hydrate the RC tube **last**.
5. Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent tubes –one Reagent tube strip at a time. Discard cap.
  - 5.1 Transfer 20  $\mu\text{L}$  of Sample lysate from the upper  $\frac{1}{2}$  of the liquid (avoid precipitate) in the LS tube into corresponding Reagent tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
  - 5.2 Repeat step 5.1 until individual Sample lysate has been added to a corresponding Reagent tube in the strip.

- 5.3 Cover the Reagent tubes with the provided extra caps and use the rounded side of the 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.
- 5.4 Repeat step 5.1 as needed, for the number of samples to be tested.
- 5.5 When all sample lysates have been transferred, repeat 4.1 to transfer 20  $\mu\text{L}$  of NC lysate into a Reagent tube.
- 5.6 Transfer **20  $\mu\text{L}$  of NC lysate into a RC tube**. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.

6. Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader Tray. Close and latch the 3M Molecular Detection Speed Loader Tray lid.



7. Review and confirm the configured run in the 3M Molecular Detection Software.
8. Click the Start button in the software and select instrument for use. The selected instrument's lid automatically opens.
9. Place the 3M Molecular Detection Speed Loader Tray into the 3M Molecular Detection Instrument and close the lid to start the assay. Results are provided within 75 minutes, although positives may be detected sooner.
10. After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from the 3M Molecular Detection Instrument and dispose of the tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

**NOTICE:** To minimize the risk of false positives due to cross-contamination, never open reagent tubes containing amplified DNA. This includes Reagent Control, Reagent, and Matrix Control tubes. Always dispose of sealed reagent tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

## RESULTS AND INTERPRETATION

An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analyzed automatically by the software and are color-coded based on the result. A Positive or Negative result is determined by analysis of a number of unique curve parameters. Presumptive positive

results are reported in real-time while Negative and Inspect results will be displayed after the run is completed.

Presumptive positive samples should be confirmed as per the laboratory standard operating procedures or by following the appropriate reference method confirmation<sup>(1, 2, 3)</sup>, beginning with transfer from the primary enrichment to secondary enrichment broth (if applicable), followed by subsequent plating and confirmation of isolates using appropriate biochemical and serological methods.

**NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular Detection Assay 2 - *Listeria monocytogenes* amplification reagents have a “background” relative light unit (RLU) reading.

In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M recommends the user to repeat the assay for any Inspect samples. If the result continues to be Inspect, proceed to confirmation test using your preferred method or as specified by local regulations.

If you have questions about specific applications or procedures, please visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

#### **Appendix A. Protocol Interruption: Storage and re-testing of heat-treated lysates**

1. To store a heat-treated lysate, recap the lysis tube with a clean cap (see “LYSIS”, 4.5)
2. Store at 4 to 8°C for up to 72 hours.
3. Prepare a stored sample for amplification by inverting 2-3 times to mix.
4. Decap the tubes.
5. Place the mixed lysate tubes on 3M Molecular Detection Heat Block Insert and heat at 100 ±1°C for 5 ±1 minutes.
6. Remove the rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes.
7. Continue the protocol at the ‘Amplification’ section detailed above.

#### **REFERENCES:**

1. US Food and Drug Administration Bacteriological Analysis Manual. Chapter 10: Detection and Enumeration of *Listeria monocytogenes* in Foods. January 2016 Version.



2. US Department of Agriculture (USDA) FSIS Microbiology Laboratory Guidebook 8.09. Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry and Egg Products, and Environmental Samples. Effective Date: May 1, 2013.
3. ISO 11290-1. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes*. Amendment 1, 2004-10-15.
4. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
5. ISO 7218. Microbiology of food and animal feeding stuffs – General rules for microbiological examination.
6. ISO 18593. Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.
7. ISO 6887. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.

#### EXPLANATION OF PRODUCT LABEL SAMPLES



Caution or Warning, see product instructions.



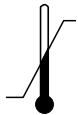
Consult product instructions.



The lot in a box represents the lot number.



The hourglass is followed by a month and year which represent the expiration date.



Storage temperature limitations.



## AOAC OMA Method Safety Checklist

<b>Submission Date</b>	2016-05-04 12:36:15
<b>First and Last Name</b>	Lisa Monteroso
<b>Organization</b>	3M
<b>E-mail</b>	LPhillips@aoac.org
<b>METHOD TITLE</b>	OMAMAN-30 C/Method Safety Checklist for MDA 2 ListeriaM
<b>Are any materials used or compounds formed that are explosive or flammable?</b>	NO
<b>Are there any side reactions that could occur that might produce flammable or explosive products or conditions?</b>	NO
<b>Are there any hazards created from electric or mechanical equipment?</b>	NO
<b>Are pressure differentials created that could result in an explosion or implosion?</b>	NO
<b>Would there be increased hazards if the reaction temperature were increased even modestly?</b>	NO
<b>Are special procedures required if a spill of the reaction mixture occurs?</b>	NO
<b>Is there a risk in producing a dangerous aerosol?</b>	NO
<b>Are special procedures required for the disposal of reagents or reaction products?</b>	NO
<b>Are there any potential hazards in handling or storage of reagents, test samples, or standards?</b>	YES
<b>Are there any other hazards that should be addressed regarding the method?</b>	NO
<b>Does your method use chlorinated solvents?</b>	NO

**If your method uses chlorinated solvents, have non-chlorinated solvents equivalent to chlorinated solvents been investigated?**

NO

**Please note if there are any organisms and/or their products that are: (please check all that apply.)**

pathogenic

**Please note any substances used or formed that are any of the following: (please check all that apply.)**

no known associated health or safety risk

1 **A Comparative Evaluation of the 3M™ Molecular Detection Assay 2 (MDA 2) –*Listeria***  
2 ***monocytogenes* for the Detection of *Listeria monocytogenes* in a Variety of Foods and**  
3 **Environmental Surfaces**

4  
5 **AOAC Performance Tested Methods<sup>SM</sup>XXXXXX**

6  
7 **Abstract**

8  
9 The 3M™ Molecular Detection Assay 2 (MDA2)–*Listeria monocytogenes* for the detection of  
10 *Listeria monocytogenes* was evaluated following the AOAC Research Institute *Performance*  
11 *Tested Methods<sup>SM</sup>* program guidelines. The evaluation included inclusivity/exclusivity, lot-to-  
12 lot/stability, robustness and matrix studies. The 3M MDA2 –*Listeria monocytogenes* was  
13 compared to various reference methods for beef hot dogs, deli turkey, raw chicken leg pieces,  
14 sealed concrete, plastic, stainless steel, whole melons, bagged raw spinach, romaine lettuce, cold  
15 smoked salmon, queso fresco, 4% milk fat cottage cheese, 3% chocolate whole milk and vanilla  
16 ice cream in the matrix study. Twenty replicates of each food matrix were analyzed at a low  
17 inoculum level of 0.2-2 colony forming units (CFU)/test portion, five replicates were analyzed at  
18 a high level of 2-5 CFU/test portion, and five control replicates were analyzed at 0 CFU/test  
19 portion with the exception of raw chicken leg pieces. Naturally contaminated raw chicken leg  
20 pieces were evaluated using two separate lots consisting of twenty replicates for each lot. All  
21 environmental surfaces were analyzed using twenty replicates at a low inoculum level of ~50  
22 CFU/standard test area, five replicates were analyzed at a high level of ~100 CFU/standard test  
23 area, and five control replicates were analyzed at 0 CFU/standard test area. Based on the  
24 probability of detection statistical model, there were no statistically significant differences  
25 between the number of presumptive and confirmed positive samples or between the candidate  
26 and reference method. There were no unexpected results in the lot-to-lot/stability, robustness  
27 studies. In the inclusivity/exclusivity study, 50 target organisms and 30 non-target organisms  
28 were tested following the 3M MDA2 – *Listeria monocytogenes* method with all target organisms  
29 detected and none of the non-target organisms detected. The results of this evaluation  
30 demonstrate the specificity and sensitivity of the 3M MDA2 –*Listeria monocytogenes* and its  
31 ability to accurately detect *Listeria monocytogenes* in select food matrices and environmental  
32 surfaces, 24-30 hours post enrichment.

33  
34 **Authors**

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36 Ben Bastin, Jonathan Flannery, Erin Crowley, Patrick Bird, M. Joseph Benzinger, Jr., James  
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38  
39 **Submitting Company**

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43 St. Paul, MN 55144-1000

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45 **Independent Laboratory**

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14

15 Reviewer  
16 Tony Hitchins  
17 FDA-Retired  
18 Rockville, MD  
19

#### 20 **Scope of Method**

- 21
- 22 a) *Target organism.*— *Listeria monocytogenes*  
23
- 24 b) *Matrices.*— Beef hot dogs (25 g), deli turkey (125 g), queso fresco (25 g), vanilla ice cream  
25 (25 g), 4% milk fat cottage cheese (25 g), 3% chocolate whole milk (25 mL), whole melon,  
26 romaine lettuce (25 g), bagged raw spinach (25 g), cold smoked salmon (25 g), raw chicken  
27 leg pieces (25 g), sealed concrete (sponge enriched in 100 mL, 4" x 4"), plastic (Enviroswab  
28 enriched in 10 mL, 1" x 1"), stainless steel (sponge enriched in 225 mL, 4" x 4").  
29
- 30 c) *Summary of Validated Performance Claims.*— Performance equivalent to that of the U.S.  
31 Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM) Chapter 10  
32 [1] for whole melon, cold smoked salmon, romaine lettuce; the U.S. Department of  
33 Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook*  
34 (USDA-FSIS/MLG) 8.09 [2] for beef hot dogs, deli turkey, raw chicken leg pieces, sealed  
35 concrete, plastic and stainless steel and AOAC 993.12 [3] for queso fresco, vanilla ice cream,  
36 4% milk fat cottage cheese, 3% chocolate whole milk and the ISO 11290-1/A1 [4] for cold  
37 smoked salmon and bagged raw spinach.  
38

#### 39 **Definitions**

- 40
- 41 a) *Probability of Detection (POD).*—The proportion of positive analytical outcomes for a  
42 qualitative method for a given matrix at a given analyte level or concentration. POD is  
43 concentration dependent. Several POD measures can be calculated; POD<sub>R</sub> (reference method  
44 POD), POD<sub>C</sub> (confirmed candidate method POD), POD<sub>CP</sub> (candidate method presumptive  
45 result POD) and POD<sub>CC</sub> (candidate method confirmation result POD).

- 1 b) *Difference of Probabilities of Detection (dPOD)*.—Difference of probabilities of detection is  
2 the difference between any two POD values. If the confidence interval of a dPOD does not  
3 contain zero, then the difference is statistically significant at the 5% level.  
4

## 5 **Principle of the Method**

6  
7 3M™ Molecular Detection Assay 2 –*Listeria monocytogenes* used with the 3M™ Molecular  
8 Detection System for the rapid and specific detection of *Listeria* species in enriched food and  
9 environmental samples.

10 The 3M Molecular Detection Assays use loop-mediated isothermal amplification to rapidly  
11 amplify nucleic acid sequences with high specificity and sensitivity, combined with  
12 bioluminescence to detect the amplification. Presumptive positive results are reported in real-  
13 time while negative results are displayed after the assay is completed. Presumptive positive  
14 results should be confirmed using the laboratory's preferred method or as specified by local  
15 regulations.  
16

## 17 **General Information**

18  
19 *Listeria* is a small, Gram-positive rod that can be found in soil or carried by animals. Many  
20 foods have been associated with *Listeria* contamination: uncooked meats, uncooked  
21 vegetables, cantaloupe, pasteurized and unpasteurized milk, and other dairy and processed  
22 foods. [5] The resulting illness, listeriosis, from *Listeria monocytogenes* ingestion is rare but  
23 can be fatal. The disease may appear as meningitis in adults and can affect unborn children  
24 due to its ability to cross through the placenta. *Listeria* contamination often occurs post-  
25 cooking, before packaging as *Listeria* can survive longer under adverse environmental  
26 conditions than most other vegetative bacteria that present a concern for the food safety  
27 industry. [6] Manufacturing facilities implement sanitation practices and regular surface  
28 screening for the presence of *Listeria* on food contact surfaces to monitor their food  
29 production environments. The 3M MDA2 –*Listeria monocytogenes* was developed to provide  
30 rapid and specific detection of *Listeria monocytogenes* in enriched food samples and on food  
31 processing surfaces.  
32

## 33 **Materials and Methods**

### 34 **Test Kit Information**

- 35  
36  
37 a) *Kit Name*.—Molecular Detection Assay 2 – *Listeria monocytogenes*  
38 b) *Catalog Number*.—MDA2LM96  
39

### 40 *Test Kit Reagents - 3M MDA2 – Listeria monocytogenes*(96 tests)

- 41 a) *Lysis Solution (LS) tubes*. – 96 (12 strips of 8 tubes)  
42 b) *Listeria monocytogenes Reagent tubes*. – 96 (12 strips of 8 tubes)  
43 c) *Extra caps*. – 96 (12 strips of 8 caps)  
44 d) *Reagent Control (RC)*. – 16 (2 pouches of 8)  
45 e) *Quick Start Guide*  
46

### 47 *Additional Supplies and Reagents*

- 1 a) 3M Molecular Detection System (instrument), power supply, cord, USB cable and
- 2 software
- 3 b) 3M Molecular Detection Speed Loader Tray
- 4 c) 3M Molecular Detection Chill Block Tray and Chill block insert
- 5 d) 3M Molecular Detection Heat Block Insert
- 6 e) 3M Molecular Detection Cap/Decap Tool [Reagent]
- 7 f) 3M Molecular Detection Cap/Decap Tool [Lysis]
- 8 g) Empty lysis tube rack
- 9 h) Empty reagent tube rack
- 10 i) Dey-Engley (D/E) Neutralizing Broth
- 11 j) Demi-Fraser broth (with additional of ferric ammonium citrate)
- 12

#### 13 *Additional media*

- 14 a) Oxford Agar (OX)b) Sheep blood agar (SBA)
- 15 b) Fraser broth (FB)
- 16 c) Sheep blood agar (SBA)
- 17 d) PALCAM agar
- 18 e) Ottaviani Agosti Agar (OAA)
- 19 f) Trypticase soy agar (TSA)
- 20 g) Trypticase soy agar with 0.6% Yeast extract (TSA/YE)
- 21 h) De Man, Rogosa and Sharpe (MRS)
- 22 i) Modified Oxford agar (MOX)
- 23 j) Brain heart infusion (BHI) broth
- 24 k) Horse blood overlay (HBO)
- 25 l) Buffered Listeria enrichment broth (BULEB)
- 26

#### 27 *Apparatus*

- 28 a) *Pipettes.* – capable of 20µL
- 29 b) *Multi-channel pipette.* – capable of 20µL
- 30 c) *Sterile pipette tips.* – capable of 20µL
- 31 d) *Stomacher®.* – Seward or equivalent
- 32 e) *Filter Stomacher® bags.* – Seward or equivalent
- 33 f) *Thermometer.* – calibrated range to include 100 ± 1°C range
- 34 g) *Incubators.* – capable of maintaining 37 ± 1°C
- 35 h) *Dry double block heater unit.* – capable of maintaining 100 ± 1°C; *ora water bath*
- 36 *capable of maintaining 100 ± 1°C*
- 37 i) *Freezer.* – capable of maintaining -10 to -20°C, for storing the 3M Molecular Detection
- 38 *Chill Block Tray*
- 39 j) *Refrigerator.* – capable of maintaining 2-8°C, for storing the 3M MDA2
- 40 k) *Computer.* – compatible with the 3M Molecular Detection System (instrument)
- 41

#### 42 **Safety Precautions**

43  
44 The user should read, understand and follow all safety information in the instructions for the 3M  
45 Molecular Detection System and the 3M Molecular Detection Assay 2 –*Listeriamonocytogenes*.  
46 Retain the safety instructions for future reference.



1  
2 The 3M Molecular Detection Assay 2 –*Listeria monocytogenes* method may generate *Listeria*  
3 *monocytogenes* to levels sufficient to cause stillbirths and fatalities in pregnant women and the  
4 immunocompromised, if exposed.  
5

To reduce the risks associated with exposure to chemicals and biohazards:

- It is strongly recommended that female laboratory staff be informed of the risk to a developing fetus resulting from infection of the mother through exposure to *Listeria monocytogenes*
- Perform pathogen testing in a properly equipped laboratory under the control of trained personnel
- Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples
- Avoid contact with the contents of the enrichment media and reagent tubes after amplification
- Dispose of enriched samples according to current industry standards

### 6 **Enrichment Sample Preparation**

7 3M recommends the use of Demi-Fraser Broth (with addition of ferric ammonium citrate) for the  
8 enrichment of food and environmental samples. Table 1 presents guidance for the enrichment of  
9 food and environmental samples. It is the user's responsibility to validate alternate sampling  
10 protocols or dilution ratios to ensure this test method meets the user's criteria.  
11  
12

#### 13 *Foods*

- 14 a) Allow the Demi-Fraser Broth enrichment medium to equilibrate to ambient laboratory  
15 temperature.
- 16 b) Aseptically combine the enrichment medium and sample according to Table 1. For all meat  
17 and highly particulate samples, the use of filter bags is recommended.
- 18 c) Homogenize thoroughly by blending, stomaching, or hand mixing for  $2 \pm 0.2$  minutes.  
19 Incubate at  $37 \pm 1^\circ\text{C}$  according to Table 1.  
20

#### 21 *Environmental samples*

22 Sample collection devices can be a sponge hydrated with a neutralizing solution to inactivate the  
23 effects of the sanitizers. 3M recommends the use of a biocide-free cellulose sponge. Neutralizing  
24 solution can be Dey-Engley (D/E) Neutralizing Broth or Lethen broth. It is recommended to  
25 sanitize the area after sampling.  
26

27 The recommended size of the sampling area for verifying the presence or absence of the pathogen  
28 on the surface is at least  $100 \text{ cm}^2$  (10 cm x 10 cm or 4"x4"). When sampling with a sponge,  
29 cover the entire area going in two directions (left to right then up and down) or collect  
30 environmental samples following your current sampling protocol or according to the FDA BAM,  
31 USDA FSIS MLG or ISO 18593 (7) guidelines.  
32

- 33 1. Allow the Demi-Fraser Broth enrichment medium to equilibrate to ambient laboratory  
34 temperature.
- 35 2. Aseptically combine the enrichment medium and sample according to Table 1.
- 36 3. Homogenize thoroughly by blending, stomaching, or hand mixing for  $2 \pm 0.2$  minutes.

1 Incubate at  $37 \pm 1^\circ\text{C}$  for 24-30 hours.

- 2 4. Invert room temperature ( $20\text{-}25^\circ\text{C}$ ) lysis tubes to mix. Proceed to next step within 4 hours. 20  
3  $\mu\text{L}$  aliquot of each enriched sample are is transferred to separate lysis tubes using a new  
4 pipette tip after each sample transfer. Place uncovered samples on a dry bath incubator for 15  
5  $\pm 2$  minutes at  $100 \pm 1^\circ\text{C}$ . Following the heat lysis transfer samples to the sterilized lab bench  
6 and allow to cool at  $18\text{-}28^\circ\text{C}$  for 5-10 minutes.
- 7 5. Add 20  $\mu\text{L}$  of each lysed sample and control to separate reagent tubes, and mix by pipetting  
8 up and down five times. Analyze a matrix control tube with the samples for each matrix to  
9 verify that no interference with the assay was caused by the matrix. Use sterile Demi Fraser  
10 Broth for the Negative Control (NC). Transfer a 20  $\mu\text{L}$  aliquot to the NC and the Reagent  
11 Control (RC) tubes. Add a 20  $\mu\text{L}$  aliquot of a randomly picked sample to the matrix control  
12 tube, mixed, and recapped. Using the 3M<sup>TM</sup> software, follow prompts to identify samples and  
13 controls. Load all samples into the Speed Loader Tray (SLT), place into the Molecular  
14 Detection System, and initiate the 3M<sup>TM</sup> MDA2 *Listeria monocytogenes* assay. Results are  
15 obtained within 75 minutes.

### 17 Interpretation and Test Result Report

18 An algorithm interprets the light output curve resulting from the detection of the nucleic acid  
19 amplification. Results are analyzed automatically by the software and are color-coded based on  
20 the result. A Positive or Negative result is determined by analysis of a number of unique curve  
21 parameters. Presumptive positive results are reported in real-time while Negative and Inspect  
22 results will be displayed after the run is completed.

23  
24 **NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular  
25 Detection Assay 2 –*Listeria monocytogenes* amplification reagents have a “background” relative  
26 light unit (RLU).

27  
28 In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M  
29 recommends the user to repeat the assay for any Inspect samples. If the result continues to be  
30 Inspect, proceed to confirmation test using your preferred method or as specified by local  
31 regulations.

### 32 Confirmation

33  
34 Presumptive positive samples of primary enrichments were confirmed by following the  
35 appropriate reference method confirmation, beginning with transfer from the primary enrichment  
36 to secondary enrichment broth (if applicable), followed by subsequent plating and confirmation  
37 of isolates using appropriate biochemical and serological methods.

### 38 Validation Study

39  
40 Testing was conducted following the procedures outlined in the AOAC Research Institute  
41 *Performance Tested Methods<sup>SM</sup> Program* validation outline protocol: *Comparative Evaluation of*  
42 *the 3M<sup>TM</sup> Molecular Detection Assay 2-Listeria monocytogenes and of the 3M<sup>TM</sup> Molecular*  
43 *Detection Assay 2 Listeria* (February, 2015) [8]. The independent study involved a matrix study  
44 (9 matrices), a lot-to-lot/stability evaluation, and a robustness evaluation. The internal study  
45 involved a matrix study (3 matrices) and an inclusivity/exclusivity study.

## Internal Study

### *Inclusivity and Exclusivity*

#### *Methodology*

Fifty frozen *Listeria monocytogenes* strain suspensions were thawed and sub-cultured in brain heart infusion (BHI) broth overnight at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The cultures were diluted in peptone salt solution in order to inoculate between 10 to 100 cells per 225 mL of Demi Fraser. The enrichment broths were incubated for 24 hours at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , and the 3M MDA2 - *Listeria monocytogenes* method was then performed.

Thirty frozen non-*Listeria monocytogenes* strain suspensions were thawed and sub-cultured grown in BHI broth overnight at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The cultures were diluted in buffered peptone water (BPW) or de Man, Rogosa, and Sharpe (MRS) in order to inoculate  $10^5$  cells/mL. The broths were then incubated for 24 hours at the appropriate incubation temperature in order to have culture to test with the 3M MDA2 - *Listeria monocytogenes* method.

#### *Results*

All 50 *Listeria monocytogenes* strains were detected by the 3M MDA2 - *Listeria monocytogenes* method. None of the 30 non-*Listeria monocytogenes* strains were detected. See Tables 2 and 3 for study details and results.

## Matrix Study

The matrix study consisted of evaluating a total of 30 un-paired sample replicates for 11 matrices, along with a raw chicken leg pieces evaluating 20 sample replicates using two separate lots. Within each sample set, there were 5 uninoculated samples (0 CFU/test portion), 20 low level inoculated samples (0.2-2 CFU/test portion), and 5 high level inoculated samples (2-5 CFU/test portion), except for raw chicken leg pieces that were naturally contaminated with the target analyte. The inoculum was prepared by transferring a single *Listeria monocytogenes* colony from Trypticase soy agar with 5% sheep blood (SBA) into BHI broth and incubating the culture at  $35 \pm 2^{\circ}\text{C}$  for  $24 \pm 2$  hours. Table 4 presents the sample preparation guidelines for the matrix. All matrices were screened for the presence of the target organism following the appropriate reference method. Additionally, an aerobic plate count (APC) was conducted following the FDA/BAM Chapter 3 reference [9] method to determine the level of background flora in each test matrix prior to inoculation.

Prior to inoculation of beef hot dogs, deli turkey, and queso fresco the broth culture inoculum was heat stressed for  $10 \pm 1$  minute at  $50 \pm 1^{\circ}\text{C}$  in a water bath. The degree of injury of the culture was estimated by plating an aliquot of diluted culture onto modified Oxford agar (MOX) and Tryptic Soy agar (TSA). The agars were incubated at  $35 \pm 1^{\circ}\text{C}$  for  $24 \pm 2$  hours and the colonies were counted. The degree of injury was estimated as:

$$\left(1 - \frac{n_{select}}{n_{nonselect}}\right) \times 100$$

1 Where  $n_{select}$  = number of colonies on selective agar and  $n_{nonselect}$  = number of colonies on non-  
2 selective agar. Using BHI broth as the diluent, the culture was diluted to a low level expected to  
3 yield fractional positive results (5-15 positive results) and a high level expected to yield all  
4 positive results. Following inoculation, a bulk lot of the matrix was homogenized by hand and  
5 held for 48-72 hours at refrigerated temperature (2-8 °C) prior to analysis to allow time for the  
6 organism to equilibrate within the sample.

7 For melons, a single whole melon was placed into a large sterile bag and the blossom end of  
8 the melon was inoculated with 100 µL of the diluted *Listeria monocytogenes* culture. The liquid  
9 culture was then allowed to soak into the melon. The melon was then inverted so that the  
10 inoculated end was on the bottom of the sterile bag. The bag was tied closed and held for 48-72  
11 hours at 2-8 °C.

12 For environmental surfaces, inocula were prepared by transferring a pure isolated colony of  
13 the specified organism from SBA into BHI broth and incubated at  $35 \pm 2^\circ\text{C}$  for  $24 \pm 2$  hours.  
14 Following incubation, serial dilutions were performed in BHI broth to achieve the target level  
15 inoculum.

16 For stainless steel and sealed concrete surfaces, 4" x 4" areas were inoculated with 0.25 mL  
17 of diluted *Listeria monocytogenes* culture. Stainless steel was also inoculated with competitor  
18 organism *Enterococcus faecium* ATCC 19434 at 10x the level of the target organism. For plastic  
19 surfaces, 1" x 1" areas were inoculated with 0.10 mL of diluted *Listeria monocytogenes* culture.  
20 Plastic was also inoculated with a competitor organism, *Enterococcus faecalis* ATCC 29212, at  
21 10x the level of the target organism. For the uninoculated test portions, sterile BHI broth was  
22 applied to the test area. Each surface was allowed to dry for 16-24 hours at room temperature ( $24$   
23  $\pm 2^\circ\text{C}$ ).

24 The 3M hydrated sampling sponges (pre-moistened with Dey-Engley) (stainless steel and  
25 sealed concrete) and 3M™ Tecra™ Enviro Swabs (pre-wetted with Letheen) (plastic) were  
26 sampled by using horizontal and vertical sweeping motions. The sponges and the swabs were  
27 held at room temperature for 2 hours prior to analysis. To determine the inoculation level for the  
28 environmental surfaces, aliquots of each inoculum were plated on TSA in triplicate.

29 The level of *Listeria monocytogenes* in the low level inoculum and high level inoculum was  
30 determined by Most Probable Number (MPN) on the day of analysis by evaluating 5 x 50 g, 20 x  
31 25 g (reference method test portions), and 5 x 10 g inoculated test samples for the low  
32 inoculation level and by examining 5 x 25 g, 5 x 5 g and 5 x 1 g for the high inoculation level.  
33 The level of *Listeria monocytogenes* in the low level inoculum and high level inoculum for all 125  
34 g test portions was determined by MPN by evaluating 5 x 250 g, 20 or 5 x 125 g (reference  
35 method test portions), and 5 x 50 g inoculated test samples. Each test portion was enriched with  
36 the reference method enrichment broth at the reference method dilution scheme and analyzed by  
37 the reference method procedure. The number of positives from the 3 test levels was used to  
38 calculate the MPN using the LCF MPN calculator (version 1.6) provided by AOAC RI. [10]  
39 (<http://www.lcfltd.com/customer/LCFMPNCalculator.exe>)  
40

#### 41 USDA/FSIS MLG 8.09 Reference Method

42  
43  
44 For the USDA/FSIS MLG 8.09 reference method, 25 g test portions were enriched with  $225 \pm 5$   
45 mL of modified University of Vermont Medium broth (UVM) and 125 g test portions were  
46 enriched with  $1125 \pm 25$  mL of UVM. All test portions were mechanically stomached for two  
47 minutes. The 25 g test portions were incubated at  $30 \pm 2^\circ\text{C}$  for 20-26 hours and the 125 g test

1 portions were incubated at  $30 \pm 2$  °C for 23-26 hours. For environmental samples, sponges were  
2 enriched with 225 mL of UVM and homogenized by hand while swabs were enriched with 10  
3 mL of UVM and mixed by vortex. Sponges and swabs were incubated for 20-26 hours at  $30 \pm$   
4  $2^{\circ}\text{C}$ . After incubation of all test portions,  $0.1 \pm 0.02$  mL of the sample enrichment was  
5 transferred to  $10 \pm 0.5$  mL of Fraser Broth (FB) containing 0.1 mL of 5% ferric ammonium  
6 citrate and incubated at  $35 \pm 2^{\circ}\text{C}$  for  $26 \pm 2$  hours. A loopful of the sample enrichment was also  
7 streaked to MOX and incubated at  $35 \pm 2^{\circ}\text{C}$  for  $26 \pm 2$  hours.

8 After  $26 \pm 2$  hours, FB was examined for any degree of darkening due to esculin hydrolysis.  
9 Any FB that displayed darkening was streaked to a MOX plate. If no darkening occurred, FB  
10 was re-incubated at  $35 \pm 2^{\circ}\text{C}$  for a total of  $48 \pm 2$  hours and re-examined for evidence of  
11 darkening. If darkening occurred, the FB was streaked to a MOX plate, if no darkening  
12 occurred, samples were considered negative. All FB streaked MOX plates were incubated at  $35$   
13  $\pm 2^{\circ}\text{C}$  for  $26 \pm 2$  hours.

14 MOX agar plates streaked from the primary enrichment or the FB secondary enrichment were  
15 examined after  $26 \pm 2$  hours and if no suspect colonies were present, the MOX agar plate was re-  
16 incubated for an additional  $26 \pm 2$  hours at  $35 \pm 2^{\circ}\text{C}$  for a total of  $48 \pm 2$  hours. If suspect  
17 colonies were present on the MOX agar plates, these suspect colonies were streaked to Horse  
18 Blood Overlay agar (HBO) and incubated at  $35 \pm 2^{\circ}\text{C}$  for  $22 \pm 4$  hours. HBO plates were  
19 examined for hemolysis reactions and well isolated colonies were transferred to BHI broth and  
20 incubated at  $25^{\circ}\text{C}$  for  $24 \pm 2$  hours. Sample isolates from BHI broth were analyzed for tumbling  
21 motility by preparing a wet mount, analyzed by a catalase test and examined for morphology by  
22 preparing a Gram stain. Additionally, purified HBC isolates were identified using the VITEK<sup>®</sup>  
23 GP Biochemical Identification following AOAC OMA 2013.02. [11]

#### 24 FDA/BAM Chapter 10 Reference Method

25  
26  
27 Twenty-five gram test portions were enriched in  $225 \text{ mL} \pm 5 \text{ mL}$  of Buffered Listeria  
28 Enrichment Broth (BLEB) homogenized for 2 minutes and incubated at  $30 \pm 1^{\circ}\text{C}$  for 4 hours.  
29 For whole melons, a single whole melon was enriched using BLEB with approximately 1.5 times  
30 the weight of the melon, and soaked for 1 hour at room temperature. After an hour at room  
31 temperature, the test portions were incubated at  $30 \pm 1^{\circ}\text{C}$  for 4 hours. Following 4 hours of  
32 incubation, selective supplements acriflavine (10mg/L), sodium nalidixate (40mg/L) and  
33 cycloheximide (50mg/L) were added to each test portion and incubated for an additional 20  
34 hours. After 24 hours of total incubation, the enriched samples were streaked to MOX agar plates  
35 and incubated at  $35 \pm 1^{\circ}\text{C}$  for 24-48 hours. The enriched samples were re-incubated for an  
36 additional 24 hours at  $30 \pm 1^{\circ}\text{C}$  and then streaked to a second MOX agar plate which was  
37 incubated for 24-48 hours at  $35 \pm 1^{\circ}\text{C}$ . MOX agar plates were examined for suspect colonies, and  
38 if present, at least 5 colonies were streaked to TSA containing 0.6% yeast extract (TSA/YE). The  
39 TSA/YE plates were incubated at  $35 \pm 1^{\circ}\text{C}$  for 24-48 hours and then examined for purity. Pure  
40 colonies were tested for catalase reactivity and a Gram Stain was conducted. A pure *Listeria*  
41 colony was transferred to Trypticase Soy Broth containing 0.6% yeast extract (TSB/YE). The  
42 TSB/YE cultures were incubated at  $25 \pm 1^{\circ}\text{C}$  overnight, or until the broth was turbid, indicating  
43 sufficient growth. Catalase-positive organisms were stabbed into plates of 5% Sheep Blood Agar  
44 (SBA) and incubated at  $35 \pm 1^{\circ}\text{C}$  for 24-48 hours. The TSB/YE tubes incubated at  $25 \pm 1^{\circ}\text{C}$   
45 were used to prepare a wet mount slide to determine motility pattern. After incubation, the SBA



1 plates were examined for hemolysis. Final confirmation was conducted using the VITEK<sup>®</sup> GP  
2 Biochemical Identification card following AOAC OMA 2013.02.  
3

#### 4 AOAC 993.12 Listeria Reference Method 5

6 Twenty-five gram test portions were enriched in 225 mL ± 5 mL of selective enrichment  
7 medium, containing yeast extract (1.35 g/225 mL), acriflavine mono hydrochloride (1.125 g/225  
8 mL), nalidixic acid (sodium salt) solution (1.125 g/225 mL), and cycloheximide (2.25 g/225  
9 mL), and incubated at 30 ± 1 °C for 48 hours. The enriched samples were then streaked to  
10 Oxford Agar (OXA) and incubated at 37 ± 1°C for 48 hours. At 48 hours, the OXA plates were  
11 examined for suspect colonies. If present, up to 5 suspect colonies were streaked to TSA/YE to  
12 obtain well isolated, pure colonies. Pure colonies on TSA/YE were analyzed for Gram-stain  
13 reaction and for catalase activity. Catalase positive, Gram positive rods colonies were then  
14 stabbed to SBA and incubated at 37 ± 1 °C for 48 hours. After 48 hours, the SBA plates were  
15 examined for typical hemolytic reactions. The same colony picked to SBA was also transferred  
16 to TSB/YE. The TSB/YE cultures were incubated at 25 ± 1 °C overnight, or until the broth was  
17 turbid, indicating sufficient growth. Another TSB/YE tube was inoculated with the same colony  
18 and incubated at 37 ± 1 °C for 24 hours to be used for carbohydrate utilization testing. The  
19 TSB/YE tube incubated at 25 ± 1 °C were used to prepare a wet mount slide to determine motility  
20 pattern. From the TSB/YE tube incubated at 37 ± 1 °C, Motility Test Medium (MTM) was  
21 stabbed and incubated at 25 ± 1 °C. After 2 days, and up to 7 days, the MTM tubes were observed  
22 for umbrella-like growth. Additionally from the TSB/YE tube, a loopful (0.1 mL) was  
23 transferred into carbohydrate fermentation broth (purple broth) containing 1.0 mL of either 5%  
24 rhamnose or 5% xylose. The purple broth tubes were incubated at 37 ± 1 °C and examined for up  
25 to 7 days.  
26

#### 27 ISO 11290-1/A1 Reference Method 28

29 For the ISO 11290-1/A1 reference method, 25 g test portions were enriched with 225 half Fraser  
30 broth. All test portions were mechanically stomached for two minutes. The test portions were  
31 incubated at 30 ± 1°C for 24 ± 3 hours. After incubation, 0.1 mL of the sample enrichment was  
32 transferred to 10 mL FB containing 0.1 mL of 5% ferric ammonium citrate and incubated at 37 ±  
33 1°C for 48 ± 3 hours. After 48 ± 3 hours, a loopful of the sample secondary FB enrichment was  
34 streaked to PALCAM and OAA(Ottovani-Agosti Agar) and incubated at 37 ± 1°C for 24 ± 3  
35 hours. A loopful of the sample primary enrichment was also streaked to PALCAM and OAA and  
36 incubated at 37 ± 1°C for 24 ± 3 hours. PALCAM and OAA agar plates were examined for the  
37 presence of suspect colonies. If no suspect colonies were present, the agar plate was re-incubated  
38 for an additional 18-24 hours at 37 ± 1°C. If no suspect colonies present the sample was  
39 determined to be negative for *Listeria*. If suspect colonies were present on the PALCAM or  
40 OAA agar plates, these suspect colonies were streaked to HBO and incubated at 37 ± 1°C for 18-  
41 24 hours. HBO plates were examined for hemolysis reactions and well-isolated colonies were  
42 transferred to BHI broth and incubated at 25°C for 24 ± 2 hours. Sample isolates from BHI broth  
43 were analyzed for tumbling motility by preparing a wet mount, analyzed by a catalase test and  
44 examined for morphology by preparing a Gram stain. Additionally, purified HBO isolates were  
45 identified using the VITEK<sup>®</sup> GP Biochemical Identification following AOAC OMA 2013.02.  
46

### 3M™ Molecular Detection Assay 2 –*Listeriamonocytogenes*

All 25 g samples were analyzed by the 3M™ MDA2 - *Listeriamonocytogenes* were enriched with 225 mL of Demi-Fraser Broth containing ferric ammonium citrate; all test portions were then homogenized by stomaching thoroughly for  $2 \pm 0.2$  minutes and incubated at  $37 \pm 1$  °C for 24-28 hours. For the 125 g deli turkey test portions, samples were enriched with 1125 mL of Demi-Fraser Broth; test portions were then homogenized by stomaching thoroughly for  $2 \pm 0.2$  minutes and incubated at  $37 \pm 1$  °C for 24 hours. For whole melons, test portions were enriched with approximately 1.5 times the weight of the whole melon in Demi Fraser and incubated at  $37 \pm 1$  °C for 26 hours. For stainless steel, sponge samples were enriched with 225 mL of Demi-Fraser Broth; samples were homogenized by hand thoroughly for  $2 \pm 0.2$  minutes and incubated at  $37 \pm 1$  °C for 24-26 hours. Sealed concrete environmental surface sponge samples were enriched with 100 mL of Demi-Fraser Broth; samples were homogenized by hand thoroughly for  $2 \pm 0.2$  minutes and incubated at 37°C for 24-26 hours. Plastic environmental surface swab samples were enriched with 10 mL of Demi-Fraser Broth; samples were homogenized by vortexing thoroughly for  $2 \pm 0.2$  minutes and incubated at  $37 \pm 1$  °C for 24-26 hours.

Prior to analysis, lysis tubes were brought to room temperature (20-25 °C) by placing the tubes on a sanitized bench top for 16-18 hours. Lysis tubes were inverted to mix up to four hours before use. The lysis tubes were then de-capped and the rubber cap was discarded. A 20 µL aliquot of each sample was transferred to separate lysis tubes; a new pipette tip was used after each sample transfer. Uncovered samples were placed on a dry bath incubator for  $15 \pm 2$  minutes at  $100 \pm 1$  °C. Following the heat lysis, samples were transferred to the sanitized laboratory bench and were allowed to cool at 18-28 °C for 5-10 minutes.

A 20 µL aliquot of each lysed sample and control was added to separate reagent tubes, and samples were mixed by pipetting up and down five times. A matrix control tube was analyzed with the samples for each matrix to verify that no interference with the assay was caused by the matrix. A sample of sterile Demi Fraser Broth was lysed for the kit Negative Control (NC). A 20 µL aliquot was transferred to the NC and the Reagent Control (RC) tubes. A 20 µL aliquot of a randomly picked sample was added to the matrix control tube, mixed, and recapped. Using the 3M™ software, prompts were followed to identify samples and controls. All samples were loaded into the Speed Loader Tray, placed into the Molecular Detection System, and the 3M™ MDA2 *Listeria monocytogenes* assay was initiated and results were obtained within 75 minutes.

#### **Matrix Study Results**

For each method, the probability of detection (POD) was calculated as the number of positive outcomes divided by the total number of trials. [12] The following were calculated: ~~for~~ the candidate presumptive results,  $POD_{CP}$ ; the candidate confirmatory results,  $POD_{CC}$ ; the difference in the candidate presumptive and confirmatory results,  $dPOD_{CP}$ ; the presumptive candidate results that confirmed positive,  $POD_C (= POD_{CC})$ ; the reference method,  $POD_R$ , and the difference in the confirmed candidate and reference methods,  $dPOD_C$ . POD analyses were conducted for the MDA2 - *Listeriamonocytogenes* test points and compared to the appropriate reference method results. The pre-validation target analyte background screen results and APC results are presented in Table 5. The heat stress data for selected matrices are presented in Table 6. The

1 inoculum levels for each environmental surface are presented in Table 7. A summary of POD  
2 analyses [13] are presented in Tables 8-15. As per criteria outlined in Appendix J of the Official  
3 Methods of Analysis Manual [13], fractional positive results were obtained for all matrices.  
4

#### 5 Internal Study

##### 7 *Raw Chicken Leg Pieces (25 g) – 28 hour Primary Enrichment*

9 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 10  
10 presumptive positives and 10 confirmed positives following the USDA/FSIS MLG 8.09  
11 reference method confirmation procedure. There were 12 observed positives for the reference  
12 method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed  
13 positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There  
14 were 5 confirmed positive following the reference method. Detailed results of the POD analyses  
15 are presented in Tables 8 and 12.  
16

##### 17 *Bagged raw spinach*

19 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 9  
20 presumptive positives and 10 confirmed positives following the ISO 11290-1/A1 reference  
21 method confirmation procedure resulting in 1 false negative. There were 10 observed positives  
22 for the reference method. For the high inoculation level, there were 5 presumptive positives and  
23 5 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure.  
24 There were 4 confirmed positives following the reference method. Detailed results of the POD  
25 analyses are presented in Tables 8 and 12.  
26  
27

##### 28 *Cold smoked salmon*

##### 30 *Compared to FDA-BAM*

31 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 10  
32 presumptive positives, and 10 confirmed positives following the FDA/BAM Chapter 10 reference  
33 method confirmation procedure. There were 7 observed positives for the reference method. For  
34 the high inoculation level, there were 3 presumptive positives and 3 confirmed positives  
35 following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 5  
36 confirmed positives following the reference method. Detailed results of the POD analyses are  
37 presented in Tables 8 and 12.  
38

##### 39 *Compared to ISO 11290-1/A1*

40 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 10  
41 presumptive positives and 10 confirmed positives following the ISO 11290-1/A1 reference  
42 method confirmation procedure. There were 9 observed positives for the reference method. For  
43 the high inoculation level, there were 3 presumptive positives and 3 confirmed positives  
44 following the ISO 11290-1/A1 reference method confirmation procedure. There were 4  
45 confirmed positives following the reference method. Detailed results of the POD analyses are  
46 presented in Tables 8 and 12.



1  
2  
3 Independent Study

4  
5 *Deli Turkey (125 g)*

6  
7 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 8  
8 presumptive positives and 8 confirmed positives following the USDA/FSIS MLG 8.09 reference  
9 method confirmation procedure. There were 7 observed positives for the reference method. For  
10 the high inoculation level, there were 5 presumptive positives and 5 confirmed positives  
11 following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 5  
12 confirmed positives following the reference method. Detailed results of the POD analyses are  
13 presented in Tables 9 and 13.

14  
15 *Raw Chicken Leg Pieces (25 g)*

16  
17 *Lot 1*

18 For the lot 1 of the raw chicken leg pieces of the MDA2 - *Listeria monocytogenes* assay, there  
19 were 17 presumptive positives and 17 confirmed positives following the USDA/FSIS MLG 8.09  
20 reference method confirmation procedure. There were 16 observed positives for the reference  
21 method. Detailed results of the POD analyses are presented in Tables 9 and 13.

22  
23 *Lot 2*

24 For the lot 2 of the raw chicken leg pieces of the MDA2 - *Listeria monocytogenes* assay, there  
25 were 10 presumptive positives and 10 confirmed positives following the USDA/FSIS MLG 8.09  
26 reference method confirmation procedure. There were 14 observed positives for the reference  
27 method. Detailed results of the POD analyses are presented in Tables 9 and 13.

28  
29 *Whole Melon*

30  
31 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 10  
32 presumptive positives, and 10 confirmed positives following the FDA/BAM Chapter 10 reference  
33 method confirmation procedure. There were 8 observed positives for the reference method. For  
34 the high inoculation level, there were 5 presumptive positives and 5 confirmed positives  
35 following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 5  
36 confirmed positives following the reference method. Detailed results of the POD analyses are  
37 presented in Tables 9 and 13.

38  
39  
40 *Vanilla Ice Cream (25 g)*

41  
42 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 7  
43 presumptive positives and 7 confirmed positives following the AOAC 993.12 reference method  
44 confirmation procedure. There were 7 observed positives for the reference method. For the high  
45 inoculation level, there were 5 presumptive positives and 5 confirmed positives following the  
46 AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives

1 following the reference method. Detailed results of the POD analyses are presented in Tables 9  
2 and 13.

3  
4 *Romaine lettuce (25 g)*

5  
6 For the low inoculation level of the MDA2 -*Listeria monocytogenes* assay, there were 7  
7 presumptive positives and 7 confirmed positives following the FDA/BAM Chapter 10 reference  
8 method confirmation procedure. There were 6 observed positives for the reference method. For  
9 the high inoculation level, there were 5 presumptive positives and 5 confirmed positives  
10 following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 5  
11 confirmed positives following the reference method. Detailed results of the POD analyses are  
12 presented in Tables 10 and 14.

13  
14 *Queso Fresco (25 g)*

15  
16 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 11  
17 presumptive positives and 11 confirmed positives following the AOAC 993.12 reference method  
18 confirmation procedure. There were 9 observed positives for the reference method. For the high  
19 inoculation level, there were 5 presumptive positives and 5 confirmed positives following the  
20 AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives  
21 following the reference method. Detailed results of the POD analyses are presented in Tables 10  
22 and 14.

23  
24 *4% Milk Fat Cottage Cheese (25 g)*

25  
26 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 10  
27 presumptive positives and 10 confirmed positives following the AOAC 993.12 reference method  
28 confirmation procedure. There were 8 observed positives for the reference method. For the high  
29 inoculation level, there were 5 presumptive positives and 5 confirmed positives following the  
30 AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives  
31 following the reference method. Detailed results of the POD analyses are presented in Tables 10  
32 and 14.

33  
34 *3% Chocolate Whole Milk (25 mL)*

35  
36 For the low inoculation level of the MDA2 -*Listeria monocytogenes* assay, there were 9  
37 presumptive positives and 9 confirmed positives following the AOAC 993.12 reference method  
38 confirmation procedure. There were 6 observed positives for the reference method. For the high  
39 inoculation level, there were 5 presumptive positives and 5 confirmed positives following the  
40 AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives  
41 following the reference method. Detailed results of the POD analyses are presented in Tables 10  
42 and 14.

43  
44 *Beef Hot Dog (25 g)*

45

1 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 4  
2 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference  
3 method confirmation procedure, resulting in 1 false negative. There were 6 observed positives  
4 for the reference method. For the high inoculation level, there were 5 presumptive positives and  
5 5 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation  
6 procedure. There were 5 confirmed positives following the reference method. Detailed results of  
7 the POD analyses are presented in Tables 10 and 14.  
8

9 *Stainless Steel (enriched in 225 mL)*

10  
11 Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level  
12 of the MDA2 - *Listeria monocytogenes* assay, there were 5 presumptive positives and 5  
13 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation  
14 procedure. There were 8 observed positives for the reference method. For the high inoculation  
15 level, there were 5 presumptive positives and 5 confirmed positives following the USDA/FSIS  
16 MLG 8.09 reference method confirmation procedure. There were 5 confirmed positives  
17 following the reference method. Detailed results of the POD analyses are presented in Tables 11  
18 and 15.  
19

20 *Sealed Concrete (enriched in 100 mL)*

21  
22 *24-hour primary enrichment* - For the low inoculation level of the MDA2 –  
23 *Listeria monocytogenes* assay, there were 15 presumptive positives and 15 confirmed positives  
24 following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 11  
25 observed positives for the reference method. For the high inoculation level, there were 5  
26 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference  
27 method confirmation procedure. There were 5 confirmed positives following the reference  
28 method. Detailed results of the PCD analyses are presented in Tables 11 and 15.  
29

30 *26-hour primary enrichment* - For the low inoculation level of the MDA2 –  
31 *Listeria monocytogenes* assay, there were 16 presumptive positives and 15 confirmed positives  
32 following the USDA/FSIS MLG 8.09 reference method confirmation procedure, resulting in 1  
33 false positive. There were 11 observed positives for the reference method. For the high  
34 inoculation level, there were 5 presumptive positives and 5 confirmed positives following the  
35 USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 5 confirmed  
36 positives following the reference method. Detailed results of the POD analyses are presented in  
37 Tables 11 and 15.  
38

39 *Plastic (enriched in 10 mL)*

40  
41 Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level  
42 of the MDA2 - *Listeria monocytogenes* assay, there were 12 presumptive positives and 12  
43 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation  
44 procedure. There were 10 observed positives for the reference method. For the high inoculation  
45 level, there were 5 presumptive positives and 5 confirmed positives following the USDA/FSIS  
46 MLG 8.09 reference method confirmation procedure. There were 5 confirmed positives

1 following the reference method. Detailed results of the POD analyses are presented in Tables 11  
2 and 15.

3  
4 All five uninoculated control test portions were negative by both the MDA2 - *Listeria*  
5 *monocytogenes* assay and the corresponding reference method for each matrix above.  
6

## 7 8 **Product Consistency (Lot-to-Lot) and Stability Studies**

### 9 10 *Methodology*

11 Three lots of MDA2 - *Listeria monocytogenes* test kits, 1 newly manufactured, 1 at the middle of  
12 its expiration, and 1 at or slightly beyond expiration was analyzed to determine the stability of  
13 the assay. For the MDA2 - *Listeria monocytogenes* test kit, one *Listeria monocytogenes* ATCC  
14 7644 isolate was analyzed at a fractional positive level (2-8 positives), and testing 10 replicates.  
15 One non-*Listeria monocytogenes* isolate, *Enterococcus faecalis* ATCC 29212, was analyzed at the  
16 growth level achieved in a non-selective broth, testing 5 replicates.  
17

### 18 *Results*

19 For the lot-to-lot/stability evaluation of the MDA2 - *Listeria monocytogenes* assay, there were 6  
20 presumptive positives out of 10 replicates for all three lots at the low inoculation level. For the 5  
21 uninoculated control test portions, there were 0 presumptive positives out of 5 replicates for all  
22 three lots. The five control test portions were negative. See Tables 16 for lot-to-lot/stability  
23 results.  
24

## 25 **Robustness Study**

### 26 27 *Methodology*

28 The robustness evaluated the ability of the method to remain unaffected by minor variations in  
29 method parameters that might be expected to occur when the method is performed by an end  
30 user. Three different parameters were evaluated, which are presented in Table 17. For each  
31 parameter, one *Listeria monocytogenes* ATCC 7644 isolate was analyzed at a fractional positive  
32 level (2-8 positives), testing 10 replicates. One non-*Listeria monocytogenes* isolate, *Enterococcus*  
33 *faecalis* ATCC 29212, was analyzed at the growth level achieved in a non-selective broth, testing  
34 5 replicates.  
35

### 36 37 *Results*

38 For the robustness analysis of the MDA2 - *Listeria monocytogenes* assay, there were 6  
39 presumptive positives out of 10 replicates for each variation evaluated. For the 5 control test  
40 portions, there were 0 presumptive positives out of 5 replicates. See Table 17 for robustness  
41 results.  
42

## 43 **Independent Laboratory Observations**

44  
45 The results of this study demonstrate the ability of the MDA2 *Listeria monocytogenes* to detect  
46 the presence of *Listeria monocytogenes* in various food matrices and select environmental

1 surfaces after 24-28 hours of a primary enrichment. The MDA2 *Listeria monocytogenes* offers  
2 the benefits of extremely high sensitivity and high specificity for the detection of *Listeria*  
3 *monocytogenes* while reducing the overall time to presumptive results. The MDA2 *Listeria*  
4 *monocytogenes* also reduces the total analyst time required to obtain final results by  
5 implementing a single heat lysis step and cool step, followed up by a single transfer to molecular  
6 reaction tubes. The small foot print of the Molecular Detection System requires only minimal lab  
7 and bench space, making it easy to move the instrument or pair up additional MDS units to  
8 perform multiple MDA runs concurrently. The updated software is user friendly with easy to  
9 interpret results. The ability to examine Relative Light Unit (RLU) curves makes trouble  
10 shooting more streamlined if any problems occur while testing is being conducted.

## 11 Discussion

12 There were no differences between the 24- and 26-hour enrichment time points for the  
13 environmental surfaces tested, therefore a 24-hour minimum enrichment will be recommended  
14 for these matrices.

## 15 Conclusion

16 The 3M MDA2 -*Listeria monocytogenes* AOAC PTM validation study included an  
17 inclusivity/exclusivity, lot-to-lot/stability, robustness and matrix studies. The 3M MDA2 –  
18 *Listeria monocytogenes* was compared to the USDA/FSIS-MLG 8.09, the FDA-BAM  
19 Chapter 10, the AOAC 993.12 or the ISO 11290-1/A1 reference methods for the detection  
20 of *Listeria monocytogenes*. There were no significant differences between the 3M MDA2 -  
21 *Listeria monocytogenes* method and the corresponding reference method for any of the  
22 matrices evaluated. The 3M MDA2 -*Listeria monocytogenes* demonstrated reliability as a  
23 rapid and sensitive method for the detection of *Listeria monocytogenes* for the following  
24 matrices: beef hot dogs (25 g), deli turkey (125 g), queso fresco (25 g), vanilla ice cream (25  
25 g), 4% milk fat cottage cheese (25 g), 3% chocolate whole milk (25 mL), whole melon,  
26 romaine lettuce (25 g), bagged raw spinach (25 g), cold smoked salmon (25 g), raw chicken  
27 leg pieces (25 g), sealed concrete (sponge enriched in 100mL), plastic (Enviroswab enriched  
28 in 10 mL), stainless steel (sponge enriched in 225 mL).

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**Table 1. Enrichment Protocols Using Demi-Fraser Broth Enrichment at  $37 \pm 1 \text{ }^\circ\text{C}$**

Sample Matrix		Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)
Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon		25 g	225	24-30
Deli Turkey		125 g	1175	24-30
Environmental samples:	stainless steel, sealed concrete	1 sponge	100 or 225	24-30
	Plastic,	1 swab	10	24-30
Raw Chicken		25 g	475	28-32

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**Table 2. Inclusivity Study Results**

INCLUSIVITY								
Strain	Species	Reference	Origin	Inoculation level (cfu/225ml)	3M MDA 2 Demi Fraser broth 24h at 37°C			
					MDA2 - <i>Listeria monocytogenes</i>	Confirmation 100µl		
						ALOA	Palcam	
1	<i>Listeria</i>	<i>monocytogenes</i>	153	Soft cheese (Munster)	39	+	H+	+
2	<i>Listeria</i>	<i>monocytogenes</i>	1011/1410	Frozen broccoli	28	+	H+	+
3	<i>Listeria</i>	<i>monocytogenes</i>	1972/2399	Puff pastry with mushrooms	38	+	H+	+
4	<i>Listeria</i>	<i>monocytogenes</i>	1973/2400	Puff pastry egg and ham (Quiche-lorraine)	36	+	H+	+
5	<i>Listeria</i>	<i>monocytogenes</i>	2407/3139	Tripes with tomatoes	31	+	H+	+
6	<i>Listeria</i>	<i>monocytogenes</i>	2760/3145	Raw bacon	44	+	H+	+
7	<i>Listeria</i>	<i>monocytogenes</i>	32.183	Croque-Monsieur	31	+	H+	+
8	<i>Listeria</i>	<i>monocytogenes</i>	38/181	Toulouse sausages	33	+	H+	+
9	<i>Listeria</i>	<i>monocytogenes</i>	5721/6179	Smoked bacon	49	+	H+	+
10	<i>Listeria</i>	<i>monocytogenes</i>	7111/7516	Pâté (Rillettes)	64	+	H+	+
11	<i>Listeria</i>	<i>monocytogenes</i>	850/109	Ready-To-Eat (RTE) food (deli salad with seafood)	34	+	H+	+
12	<i>Listeria</i>	<i>monocytogenes</i>	877/113	Environmental sample (pastry)	28	+	H+	+
13	<i>Listeria</i>	<i>monocytogenes</i>	913/1048	Black pudding	39	+	H+	+
14	<i>Listeria</i>	<i>monocytogenes</i>	A00C014	Sausage	37	+	H+	+
15	<i>Listeria</i>	<i>monocytogenes</i>	A00C022	Merguez	33	+	H+	+
16	<i>Listeria</i>	<i>monocytogenes</i>	A00C024	Sausage	21	+	H+	+
17	<i>Listeria</i>	<i>monocytogenes</i>	A00C036	Poultry (guinea)	41	+	H+	+
18	<i>Listeria</i>	<i>monocytogenes</i>	A00C039	Sausages	20	+	H+	+
19	<i>Listeria</i>	<i>monocytogenes</i>	A00C040	Cooked delicatessen (Museau)	36	+	H+	+
20	<i>Listeria</i>	<i>monocytogenes</i>	A00C041	Sausage	38	+	H+	+
21	<i>Listeria</i>	<i>monocytogenes</i>	A00C042	Raw sausage	32	+	H+	+



INCLUSIVITY								
Strain	Species	Reference	Origin	Inoculation level (cfu/225ml)	3M MDA 2 Demi Fraser broth 24h at 37°C			
					MDA2 - <i>Listeria monocytogenes</i>	Confirmation 100µl		
						ALOA	Palcam	
22	<i>Listeria</i>	<i>monocytogenes</i>	A00C043	Smoked Bacon	50	+	H+	+
23	<i>Listeria</i>	<i>monocytogenes</i>	A00C044	Poultry (duck)	34	+	H+	+
24	<i>Listeria</i>	<i>monocytogenes</i>	A00C052	RTE food (Ossobucco with turkey)	34	+	H+	+
25	<i>Listeria</i>	<i>monocytogenes</i>	A00C053	Gizzards	30	+	H+	+
26	<i>Listeria</i>	<i>monocytogenes</i>	A00C054	Beef heart	30	+	H+	+
27	<i>Listeria</i>	<i>monocytogenes</i>	A00C055	Raw sausages	43	+	H+	+
28	<i>Listeria</i>	<i>monocytogenes</i>	A00E008	Environmental sample	27	+	H+	+
29	<i>Listeria</i>	<i>monocytogenes</i>	A00E049	Environmental sample (smoked salmon)	41	+	H+	+
30	<i>Listeria</i>	<i>monocytogenes</i>	A00E082	Environmental sample (smoked salmon)	33	+	H+	+
31	<i>Listeria</i>	<i>monocytogenes</i>	A00L097	Milk	60	+	H+	+
32	<i>Listeria</i>	<i>monocytogenes</i>	A00M009	Smoked salmon	40	+	H+	+
33	<i>Listeria</i>	<i>monocytogenes</i>	A00M032	Smoked salmon	11	+	H+	+
34	<i>Listeria</i>	<i>monocytogenes</i>	A00M045	Smoked salmon	62	+	H+	+
35	<i>Listeria</i>	<i>monocytogenes</i>	A00M083	Smoked salmon	79	+	H+	+
36	<i>Listeria</i>	<i>monocytogenes</i>	Ad235	Poultry	45	+	H+	+
37	<i>Listeria</i>	<i>monocytogenes</i>	Ad253	Hard cheese	58	+	H+	+
38	<i>Listeria</i>	<i>monocytogenes</i>	Ad260	Semi hard cheese	27	+	H+	+
39	<i>Listeria</i>	<i>monocytogenes</i>	Ad265	Tongue	62	+	H+	+
40	<i>Listeria</i>	<i>monocytogenes</i>	Ad266	Poultry	26	+	H+	+
41	<i>Listeria</i>	<i>monocytogenes</i>	Ad267	Dry sausage	26	+	H+	+
42	<i>Listeria</i>	<i>monocytogenes</i>	Ad268	Cured ham	35	+	H+	+

INCLUSIVITY								
Strain	Species	Reference	Origin	Inoculation level (cfu/225ml)	3M MDA 2 Demi Fraser broth 24h at 37°C			
					MDA2 - <i>Listeria monocytogenes</i>	Confirmation 100µl		
						ALOA	Palcam	
43	<i>Listeria</i>	<i>monocytogenes</i>	Ad270	Fermented sausage	42	+	H+	+
44	<i>Listeria</i>	<i>monocytogenes</i>	Ad272	Fermented sausage	56	+	H+	+
45	<i>Listeria</i>	<i>monocytogenes</i>	Ad273	Cured delicatessen	27	+	H+	+
46	<i>Listeria</i>	<i>monocytogenes</i>	Ad274	Ready-to-eat food (Asiatic meal)	40	+	H+	+
47	<i>Listeria</i>	<i>monocytogenes</i>	Ad534	Fruits	54	+	H+	+
48	<i>Listeria</i>	<i>monocytogenes</i>	Ad544	Onion	43	+	H+	+
49	<i>Listeria</i>	<i>monocytogenes</i>	Ad546	Flour	83	+	H+	+
50	<i>Listeria</i>	<i>monocytogenes</i>	Ad623	Bread crumbs	50	+	H+	+

1 All the strains are wild strains isolated in Adria Developpement, Quimper, France.

2 Ad = Adria

3 A= Adria

4 C= Origin from Meat Meat products

5 M = Origin from Fish and Seafood

6 L = Origin from Milk and Dairy products

7 E = Origin from Productionenvironment

8 H+ = Phosphy-Lipolysis halo

9

10

11

12

**Table 3. Exclusivity Study Results**

EXCLUSIVITY						
Strain	Species	Reference	Origin	Inoculation level (cfu/ml)	3M MDA2 – <i>Listeria monocytogenes</i>	
1	<i>Listeria</i>	<i>grayi</i>	Ad 1198	Smoked salmon	3.2 x 10 <sup>5</sup>	-

EXCLUSIVITY						
Strain	Species	Reference	Origin	Inoculation level (cfu/ml)	3M MDA2 – <i>Listeria monocytogenes</i>	
2	<i>Listeria</i>	<i>grayi</i>	Ad 1443	Pork meat sausages	4.9 x 10 <sup>5</sup>	-
3	<i>Listeria</i>	<i>innocua</i>	1	Smoked salmon	4.9 x 10 <sup>5</sup>	-
4	<i>Listeria</i>	<i>innocua</i>	Ad 658	Gorgonzola	5.9 x 10 <sup>5</sup>	-
5	<i>Listeria</i>	<i>ivanovii</i>	Ad 466	Raw veal meat	3.3 x 10 <sup>5</sup>	-
6	<i>Listeria</i>	<i>ivanovii</i>	Ad 662	Environment (dairy industry)	3.6 x 10 <sup>5</sup>	-
7	<i>Listeria</i>	<i>seeligeri</i>	Ad 649	Cheese	5.4 x 10 <sup>5</sup>	-
8	<i>Listeria</i>	<i>seeligeri</i>	BR1	Trout	5.1 x 10 <sup>5</sup>	-
9	<i>Listeria</i>	<i>welshimeri</i>	Ad1276	Environment (Slaughterhouse)	5.2 x 10 <sup>5</sup>	-
10	<i>Listeria</i>	<i>welshimeri</i>	Ad1175	Ready-to-eat-food	6.4 x 10 <sup>5</sup>	-
11	<i>Bacillus</i>	<i>cereus</i>	Ad 465	Salmon Terine	7.2 x 10 <sup>4</sup>	-
12	<i>Bacillus</i>	<i>circulans</i>	Ad 760	Vegetables	2.0 x 10 <sup>4</sup>	-
13	<i>Bacillus</i>	<i>coagulans</i>	Ad 731	Dairy product	<2.0 x 10 <sup>3</sup> (opacity +)	-
14	<i>Bacillus</i>	<i>licheniformis</i>	Ad 978	Dairy product	1.6 x 10 <sup>4</sup>	-
15	<i>Bacillus</i>	<i>pumilus</i>	Ad 284	Ready-to-eat	1.7 x 10 <sup>5</sup>	-
16	<i>Brochotrix</i>	<i>campestris</i>	CIP 102920T	Environment	<2.0 x 10 <sup>3</sup> (opacity +)	-
17	<i>Carnobacterium</i>	<i>piscicola</i>	Ad 369	Raw milk	<2.0 x 10 <sup>3</sup> (opacity +)	-
18	<i>Enterococcus</i>	<i>durans</i>	Ad 149	Ham	<2.0 x 10 <sup>3</sup> (opacity +)	-
19	<i>Enterococcus</i>	<i>faecalis</i>	89L326	Soft cheese (Vacherin)	1.6 x 10 <sup>5</sup>	-
20	<i>Lactobacillus</i>	<i>brevis</i>	86L126	Ham	1.4 x 10 <sup>5</sup>	-
21	<i>Lactobacillus</i>	<i>curvatus</i>	Ad 380	Delicatessen	<2.0 x 10 <sup>3</sup> (opacity +)	-
22	<i>Lactobacillus</i>	<i>sakei</i>	Ad 473	Ham	<2.0 x 10 <sup>3</sup> (opacity	-

EXCLUSIVITY						
Strain	Species	Reference	Origin	Inoculation level (cfu/ml)	3M MDA2 – <i>Listeria monocytogenes</i>	
				+) )		
23	<i>Leuconostoc</i>	<i>carosum</i>	Ad 411	Ham	<2.0 x 10 <sup>3</sup> (opacity +)	-
24	<i>Leuconostoc</i>	<i>citreum</i>	Ad 396	Ham	4.9 x 10 <sup>5</sup>	-
25	<i>Micrococcus</i>	<i>luteus</i>	Ad 432	Cocktail	<2.0 x 10 <sup>3</sup> (opacity +)	-
26	<i>Staphylococcus</i>	<i>aureus</i>	Ad 165	Smoked delicatessen	<2.0 x 10 <sup>3</sup> (opacity +)	-
27	<i>Staphylococcus</i>	<i>epidermidis</i>	Ad 931	Fruits	2.0 x 10 <sup>3</sup>	-
28	<i>Staphylococcus</i>	<i>haemolyticus</i>	Ad 989	Dairy product	2.8 x 10 <sup>4</sup>	-
29	<i>Streptococcus</i>	<i>bovis</i>	92L622	Cheese	2.0 x 10 <sup>3</sup>	-
30	<i>Streptococcus</i>	<i>salivarius</i>	Ad 441	Dairy product	<2.0 x 10 <sup>3</sup> (opacity +)	-

All the strains are wild strains isolated in Adria Developpement, Quimper, France.

Ad = Adria

A= Adria

C= Origin from Meat Meat products

M = Origin from Fish and Seafood

L = Origin from Milk and Dairy products

E = Origin from Productionenvironment

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**Table 4a: Independent Study - Matrices and Inoculating Organisms**

Matrix	Inoculating Organism (Culture Condition)	Target Inoculum Level	# of Replicates	MDA2 Testing Time Points	Reference Method (Enrichment)
Beef Hot Dogs	<i>L. monocytogenes</i> ATCC <sup>1</sup> 7644 (heat-stressed)	0 CFU/Test Portion	5	24 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Deli Turkey	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19116 (heat-stressed)	0 CFU/Test Portion	5	24 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Queso Fresco	<i>L. monocytogenes</i> CWD <sup>2</sup> 1554 (heat-stressed)	0 CFU/Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Vanilla Ice Cream	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19114	0 CFU/Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
4% Milk Fat Cottage Cheese	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19112 & <i>L. welshimeri</i> ATCC <sup>1</sup> 35897	0 CFU/Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Whole Melon	<i>L. monocytogenes</i> FSL <sup>3</sup> J1-049	0 CFU/Test Portion	5	26 hours	FDA/BAM Chapter 10 (BLEB)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Raw Chicken Leg Pieces	Naturally Contaminated	Lot 1 & Lot 2	20 & 20	28 hours	USDA/FSIS MLG 8.09 (UVM)
Romaine Lettuce	<i>L. monocytogenes</i> ATCC <sup>1</sup> 49594	0 CFU/ Test Portion	5	24 hours	FDA/BAM Chapter 10 (BLEB)
		0.2-2 CFU/ Test Portion	20		
		2-5 CFU/ Test Portion	5		
3% Chocolate Whole Milk	<i>L. monocytogenes</i> ATCC <sup>1</sup> BAA-751	0 CFU/ Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/ Test Portion	20		
		2-5 CFU/ Test Portion	5		
Sealed Concrete	<i>L. monocytogenes</i> ATCC1 19117	0 CFU/4" x 4" (100 cm <sup>2</sup> )	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/4" x 4" (100 cm <sup>2</sup> )	20		
		~100 CFU/4" x 4" (100 cm <sup>2</sup> )	5		
Plastic	<i>L. monocytogenes</i> ATCC1 51782 & <i>L. seeligeri</i> ATCC1 35967 & <i>10x Enterococcus faecalis</i> ATCC1 29212	0 CFU/1" x 1" (5 cm <sup>2</sup> )	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/1" x 1" (5 cm <sup>2</sup> )	20		
		~100 CFU/1" x 1" (5 cm <sup>2</sup> )	5		

1

2

**Table 4a: Independent Study - Matrices and Inoculating Organisms (Continued)**

Matrix	Inoculating Organism (Culture Condition)	Target Inoculum Level	# of Replicates	MDA2 Testing Time Points	Reference Method (Enrichment)
Stainless Steel	<i>L. monocytogenes</i> ATCC 19118 & <i>10x Enterococcus faecium</i> ATCC 19434	0 CFU/1" x 1" (5 cm <sup>2</sup> )	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/1" x 1" (5 cm <sup>2</sup> )	20		
		0 CFU/4" x 4" (100 cm <sup>2</sup> )	5		

<sup>1</sup> ATCC - American Type Culture Collection

<sup>2</sup> CWD - University of Vermont

<sup>3</sup> FSL - Cornell University

\* Natural contamination was present during the background screen: 2 separate lots evaluated (20 replicates each)

**Table 4b: Internal Study - Matrices and Inoculating Organisms**

Matrix	Inoculating Organism (Culture Condition)	Target Inoculum Level	# of Replicates	MDA2 Testing Time Points	Reference Method (Enrichment)
Raw chicken leg pieces	<i>L. monocytogenes</i> Ad <sup>1</sup> 668	0 CFU/Test Portion	5	28 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Bagged raw spinach	<i>L. monocytogenes</i> Ad 543	0 CFU/Test Portion	5	24 hours	ISO 11290-1/A1 (Half/Demi Fraser)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Cold smoked salmon	<i>L. monocytogenes</i> Ad 670	0 CFU/Test Portion	5	24 hours	FDA/BAM Chapter 10 (BLEB)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Cold smoked salmon	<i>L. monocytogenes</i> Ad 670	0 CFU/Test Portion	5	24 hours	ISO 11290-1/A1 (Half/Demi Fraser)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		

<sup>1</sup> Ad - Adria Development Culture Collection

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**Table 5a: Independent Study - Aerobic Plate Count and Background Results (Prior to Inoculation)**

Matrix (Test Portion)	APC <sup>1</sup> (CFU/g)	<i>Listeria</i> Pathogen Screen
Beef Hot Dogs <sup>5</sup> (25 g)	6.0 x 10 <sup>3</sup>	0/5 Detected <sup>2</sup>
Deli Turkey (125 g)	1.5 x 10 <sup>3</sup>	0/5 Detected <sup>2</sup>
Raw Chicken Leg Pieces (25 g)	2.8 x 10 <sup>5</sup>	4/5 Detected <sup>2</sup>
Whole Melons	1.0 x 10 <sup>2</sup>	0/5 Detected <sup>3</sup>
Vanilla Ice Cream (25 g)	8.0 x 10 <sup>1</sup>	0/5 Detected <sup>4</sup>
Queso Fresco (25 g)	3.8 x 10 <sup>5</sup>	0/5 Detected <sup>4</sup>
4% Milk Fat Cottage Cheese (25 g)	1.7 x 10 <sup>4</sup>	0/5 Detected <sup>4</sup>
Beef Hot Dogs (25 g)	1.6 x 10 <sup>2</sup>	0/5 Detected <sup>2</sup>

<sup>1</sup> APC conducted in accordance with FDA/BAM Chapter 3

<sup>2</sup> *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines

<sup>3</sup> *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines

<sup>4</sup> *Listeria species* screen conducted following the AOAC 993.12 guidelines

<sup>5</sup> Matrix used to conduct Robustness and Lot-to-Lot testing

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**Table 5b: Internal Study - Aerobic Plate Count and Background Results (Prior to Inoculation)**

Matrix (Test Portion)	APC <sup>1</sup> (CFU/g)	Background <i>Listeria</i>
Raw Chicken Leg Pieces (25 g)	5.4 x 10 <sup>5</sup>	None Detected
Bagged Raw Spinach (25 g)	8.0 x 10 <sup>6</sup>	None Detected
Cold Smoked Salmon (25 g)	4.0 x 10 <sup>2</sup>	None Detected

<sup>1</sup> APC conducted in accordance with FDA/BAM Chapter 3

<sup>2</sup> *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines

<sup>3</sup> *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines

<sup>4</sup> *Listeria species* screen conducted following the ISO 11290-1/1A guidelines

**Table 6: Inoculum Heat Stress Results**

Matrix	Test Portion Size	Inoculating Organism	Agar	CFU/ g	Percent Injury
Beef Hot Dogs <sup>1</sup>	25 g	<i>Listeria monocytogenes</i> ATCC 7644	TSA	1.8 x 10 <sup>8</sup>	58.3 %
			MOX	7.5 x 10 <sup>7</sup>	
Deli Turkey	125 g	<i>Listeria monocytogenes</i> ATCC 19116	TSA	2.6 x 10 <sup>9</sup>	68.5 %
			MOX	8.2 x 10 <sup>8</sup>	
Queso Fresco	25 g	<i>Listeria monocytogenes</i> ATCC CWD 1554	TSA	2.6 x 10 <sup>9</sup>	72.7%
			MOX	7.1 x 10 <sup>8</sup>	
Beef Hot Dogs <sup>2</sup>	25 g	<i>Listeria monocytogenes</i> ATCC 7644	TSA	2.6 x 10 <sup>9</sup>	73.8%
			MOX	6.8 x 10 <sup>8</sup>	

<sup>1</sup> - Testing for Robustness and Lot to Lot

<sup>2</sup> - Matrix Study

TSA: Trypticase soy agar

MOX: modified Oxford agar



**Table 7: Inoculum Summary Table for Whole Melons and Environmental Surfaces**

<b>Matrix</b>	<b>Whole Melons</b>	
<b>Inoculating Organism</b>	<i>Listeria monocytogenes</i> FSL J1-049	
<b>Low-Inoculum Level CFU<sup>a</sup>/Melon<sup>b</sup></b>	7	
<b>High-Inoculum Level CFU<sup>a</sup>/Test Melon<sup>b</sup></b>	55	
<b>Matrix</b>	<b>Stainless Steel</b>	
<b>Inoculating Organism</b>	<i>Listeria monocytogenes</i> ATCC 19118	<i>Enterococcus faecium</i> ATCC 19434
<b>Low-Inoculum Level CFU<sup>a</sup>/Test Area<sup>c</sup></b>	42	530
<b>High-Inoculum Level CFU<sup>a</sup>/Test Area<sup>c</sup></b>	400	4000
<b>Matrix</b>	<b>Sealed Concrete</b>	
<b>Inoculating Organism</b>	<i>Listeria monocytogenes</i> ATCC 19117	
<b>Low-Inoculum Level CFU<sup>a</sup>/Test Area<sup>c</sup></b>	35	
<b>High-Inoculum Level CFU<sup>a</sup>/Test Area<sup>c</sup></b>	360	
<b>Matrix</b>	<b>Plastic</b>	
<b>Inoculating Organism</b>	<i>Listeria monocytogenes</i> ATCC 51782	<i>Enterococcus faecalis</i> ATCC 29212
<b>Low-Inoculum Level CFU<sup>a</sup>/Test Area<sup>d</sup></b>	42	540
<b>High-Inoculum Level CFU<sup>a</sup>/Test Area<sup>d</sup></b>	420	3600

<sup>a</sup>CFU: aliquots of the inocula were plated in triplicate onto TSA and averaged

<sup>b</sup>Test Area: Whole Melon

<sup>c</sup>Test Area: 4" x 4" Surface Area

<sup>d</sup>Test Area: 1" x 1" Surface Area

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**Table 8: 3M™ MDA 2 *Listeria monocytogenes* Assay, Candidate vs. Reference – POD Results (INTERNAL STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			USDA/FSIS MLG			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces (25 g)	<i>L. monocytogenes</i> Ad 668	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.91 (0.6, 1.39)	20	10	0.50	0.30, 0.70	12	0.60	0.39, 0.78	-0.10	-0.37, 0.19
			2.2	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			ISO 11290-1			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI							
Bagged Raw Spinach (25 g)	<i>L. monocytogenes</i> Ad 543	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.17 (0.74, 1.84)	20	9	0.45	0.26, 0.66	10	0.50	0.30, 0.70	-0.05	-0.34, 0.25
			2.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			FDA-BAM			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI							
Cold Smoked Salmon (25 g)	<i>L. monocytogenes</i> Ad 670	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.4 (0.18, 0.72)	20	10	0.50	0.30, 0.70	7	0.35	0.15, 0.60	0.15	-0.20, 0.47
			2.7	5	3	0.60	0.23, 0.88	5	1.00	0.57, 1.00	-0.40	-0.77, 0.12
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			ISO 11290-1			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI							
Cold Smoked Salmon (25 g)	<i>L. monocytogenes</i> Ad 670	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.4 (0.18, 0.72)	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.27, 0.36
			2.7	5	3	0.60	0.23, 0.88	4	0.80	0.38, 0.96	-0.20	-0.60, 0.31

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

**Table 9: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Deli Turkey (125 g)	<i>L. monocytogenes</i> ATCC 19116	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.85)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI							
Raw Chicken Leg Pieces (25 g): Lot 1	Naturally Contaminated	28 Hour	1.91 (1.23, 3.80)	20	17	0.85	0.64, 0.95	15	0.80	0.58, 0.92	0.05	-0.19, 0.29
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI							
Raw Chicken Leg Pieces (25 g): Lot 2	Naturally Contaminated	28 Hour	1.12 (0.68, 1.91)	20	10	0.50	0.30, 0.70	14	0.70	0.48, 0.85	-0.20	-0.45, 0.10
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI							
Whole Melon	<i>L. monocytogenes</i> FSL J1-049	26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			7	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			55	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI							
Vanilla Ice Cream (25 g)	<i>L. monocytogenes</i> ATCC 19114	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.59 (0.33, 0.97)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

**Table 10: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Romaine Lettuce (25 g)	<i>L. monocytogenes</i> ATCC 49594	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.40 (0.18, 0.70)	20	7	0.35	0.18, 0.57	6	0.30	0.15, 0.52	0.05	-0.23, 0.32
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
Queso Fresco (25 g)	<i>L. monocytogenes</i> CWD 1554	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	11	0.55	0.34, 0.74	9	0.45	0.26, 0.66	0.10	-0.19, 0.37
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
4% Milk Fat Cottage Cheese (25 g)	<i>L. monocytogenes</i> ATCC 19112	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.55 (0.40, 0.92)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			1.64 (0.84, 3.20)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
3% Chocolate Whole Milk (25 g)	<i>L. monocytogenes</i> ATCC BAA-751	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	9	0.45	0.26, 0.66	6	0.30	0.15, 0.52	0.15	-0.14, 0.41
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
Beef Hot Dogs (25 g)	<i>L. monocytogenes</i> ATCC 7644	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	5	0.25	0.11, 0.47	6	0.30	0.15, 0.52	-0.05	-0.31, 0.22
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

**Table 11: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Stainless Steel (225 mL)	<i>L. monocytogenes</i> ATCC 19118 & <i>Enterococcus faecium</i> ATCC 19434	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	8	0.40	0.22, 0.61	-0.10	-0.40, 0.13
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	8	0.40	0.22, 0.61	-0.10	-0.40, 0.13
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Sealed Concrete (100 mL)	<i>L. monocytogenes</i> ATCC 19117	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Plastic (10 mL)	<i>L. monocytogenes</i> ATCC 51782 & <i>Enterococcus faecalis</i> ATCC 29212	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 540	20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 540	20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

**Table 12: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INTERNAL STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>d</sub> <sup>CP</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces (25 g)	<i>L. monocytogenes</i> Ad 668	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.91 (0.6, 1.39)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
			2.2	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Bagged Raw Spinach (25 g)	<i>L. monocytogenes</i> Ad 543	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.17 (0.74, 1.84)	20	9	0.45	0.26, 0.66	10	0.50	0.30, 0.70	-0.05	-0.33, 0.24
			2.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cold Smoked Salmon (25 g)	<i>L. monocytogenes</i> Ad 670	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.4 (0.18, 0.72)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
			2.7	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AGAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

**Table 13: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Deli Turkey (125 g)	<i>L. monocytogenes</i> ATCC 19116	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.85)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Chicken Leg Pieces (25 g): Lot 1	Naturally Contaminated	28 Hours	1.91 (1.23, 3.80)	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.23, 0.23
Raw Chicken Leg Pieces (25 g): Lot 2	Naturally Contaminated	28 Hours	1.12 (0.68, 1.91)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
Whole Melon	<i>L. monocytogenes</i> FSL J1-049	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
7			20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28	
55			5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43	
Vanilla Ice Cream (25 g)	<i>L. monocytogenes</i> ATCC 19114	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
0.59 (0.33, 0.97)			20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28	
4.38 (1.72, 11.15)			5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43	

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level



**Table 14: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Romaine Lettuce (25 g)	<i>L. monocytogenes</i> ATCC 49594	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.40 (0.18, 0.70)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Queso Fresco (25 g)	<i>L. monocytogenes</i> CWD 1554	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
4% Milk Fat Cottage Cheese (25 g)	<i>L. monocytogenes</i> ATCC 19112	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.55 (0.40, 0.92)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
			1.64 (0.84, 3.20)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
3% Chocolate Whole Milk (25 g)	<i>L. monocytogenes</i> ATCC BAA-751	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Beef Hot Dogs (25 g)	<i>L. monocytogenes</i> ATCC 7644	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level



**Table 15: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Stainless Steel (225 mL)	<i>L. monocytogenes</i> ATCC 19118 & <i>Enterococcus faecium</i> ATCC 19434	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Sealed Concrete (100 mL)	<i>L. monocytogenes</i> ATCC 19117	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	16	0.80	0.58, 0.92	15	0.75	0.53, 0.89	0.05	-0.21, 0.30
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Plastic (10 mL)	<i>L. monocytogenes</i> ATCC 51782 & <i>Enterococcus faecalis</i> ATCC 29212	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the diistically significant at the 5% level

**Table 16: Lot-to-Lot/Stability Study Results**

Expiration Date		Expiration Date		Expiration Date	
Lot #: 012715		Lot #: 081914		Lot #: 050114	
Sample #	Result	Sample #	Result	Sample #	Result
<i>Listeria monocytogenes</i> ATCC <sup>2</sup> 7644					
Low Level	6/10	Low Level	6/10	Low Level	6/10
Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5	Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5	Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5

<sup>1</sup>- All samples were analyzed from a common lysate

<sup>2</sup>ATCC- American Type Culture Collection

**Table 17: Robustness Results**

Robustness <sup>1</sup>			Robustness <sup>1</sup>		
13 Minute Lysis			13 Minute Lysis		
Sample #	18 $\mu$ L <sup>2</sup> , 18 $\mu$ L <sup>3</sup>	18 $\mu$ L <sup>2</sup> , 22 $\mu$ L <sup>3</sup>	Sample #	22 $\mu$ L <sup>2</sup> , 18 $\mu$ L <sup>3</sup>	22 $\mu$ L <sup>2</sup> , 22 $\mu$ L <sup>3</sup>
<i>Listeria monocytogenes</i> ATCC <sup>4</sup> 7644					
Low Level	6/10	6/10	Low Level	6/10	6/10
Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5	0/5	Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5	0/5
Robustness <sup>1</sup>			Robustness <sup>1</sup>		
17 Minute Lysis			17 Minute Lysis		
Sample #	18 $\mu$ L <sup>2</sup> , 18 $\mu$ L <sup>3</sup>	18 $\mu$ L <sup>2</sup> , 22 $\mu$ L <sup>3</sup>	Sample #	22 $\mu$ L <sup>2</sup> , 18 $\mu$ L <sup>3</sup>	22 $\mu$ L <sup>2</sup> , 22 $\mu$ L <sup>3</sup>
<i>Listeria monocytogenes</i> ATCC <sup>4</sup> 7644					
Low Level	6/10	6/10	Low Level	6/10	6/10
Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5	0/5	Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5	0/5

<sup>1</sup>- All samples were analyzed from a common lysate

<sup>2</sup>-Volume of enrichment to lysis

<sup>3</sup>-Volume of lysis to assay

<sup>4</sup>ATCC- American Type Culture Collection

Is the Test Kit Method scientifically and technically sound?		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	Yes	Thank You for your comments and review
ER5	Yes	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review
Have sufficient Controls been used, including those required to calculate the rate of false-positive and		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	Yes	Thank You for your comments and review
ER5	Yes	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review
Is sufficient information included for system suitability determination and product performance or acceptance testing?		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	Yes	Thank You for your comments and review
ER5	Yes	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review
Are the conclusions statements valid based upon data presented?		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	Yes	Thank You for your comments and review
ER5	Yes	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review
Do you agree that the evidence or data from this and previous studies support the proposed		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	Yes	Thank You for your comments and review
ER5	Yes	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review
Are there sufficient data points per product evaluated in accordance with AOAC requirements?		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	Yes	Thank You for your comments and review
ER5	Yes	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review
General Comments about the method scope/applicability:		

ER1	OMAMAN-30: EVALUATION OF 3M™ MOLECULAR DETECTION ASSAY 2 - LISTERIA FOR THE DETECTION OF LISTERIA IN SELECTED FOODS AND ENVIRONMENTAL SURFACES: COLLABORATIVE STUDY is a simple, rapid and easy to perform method.	Agreed! Thank you
ER2	Well-defined	Thank you
ER3	The method has a broad scope and applicability.	Agreed! Thank you
ER4	Between the PTM and OMA study, the method scope was study appropriately	Agreed! Thank you
ER5	The scope and applicability seem appropriate.	Thank you
ER6	Appropriately written	Thank you
Pros/Strengths of the Manuscript:		
ER1	The method is described in detail and in a friendly format.	Thank you
ER2	Well-written and clearly explained	Thank you
ER3	Manuscript is generally well written	Thank you
ER4	Manuscript was clear and thorough	Thank you
ER5	Generally well written	Thank you
ER6	The manuscript describes the study as required.	Thank you
Cons/Weakness of the Manuscript:		
ER1	NO	Thank you
ER2	None	Thank you
ER3	None	Thank you
ER4	The official method was written incorrectly	Revised the method and tables to reflect the homogenization process
ER5	Three minor points:	
	1. Page 97, line 39. This sentence needs rewriting	Unable to find this page in the "flip book" .
	2. Page 109, Lines 11 and 13. Spaces missing between some words. This happens throughout Ms.	
	3. Page 136, Table 3 (Supplementary). Lab 4, Raw chicken count seems to be a high outlier. Is there a typo?	~130 lbs of raw chicken breast was processed for the study. Varying degrees of APC results would be expected, although the variability observed is higher than typically seen.
ER6	Amended Submission:	
	1. Page 4, line 9 says the deli turkey was incubated for 24-28 hours; Table 3 of package insert says 24-30 hours, as does the collaborative study protocol and the Table 2 on Page 9 of the manuscript. Please clarify.	Corrected to 24-30 hours.

	2. Page 9, Table 2 - There is no table before this table in the manuscript. Label this as Table A to differentiate from the tables in the back of the manuscript and per page 7, line 34.	Revised to "Table A" in five instances. Page 9 Table header, page 9 line 3, Page 8 line 21.
	3. Please number Tables 1-3 of the Supplementary Material and include it with the other table of the manuscript. There are duplicate numbers of tables which make it a little confusing.	Supplementary tables are now numbered 3, 4, and 5.
	4. Tables 2016.2A - 2016.2B please define in footnote to table: N,X.	Added footnote explanation, N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)
Supporting Data and information: Does data from collaborative study support the method as written?		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	Yes	Thank You for your comments and review
ER5	Yes	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review
Supporting Data and information: Does data collected support the criteria given in the collaborative study protocol?		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	Yes	Thank You for your comments and review
ER5	Yes	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review
Are there any concerns regarding the safety of the method?		
ER1	No	Thank You for your comments and review
ER2	No	Thank You for your comments and review
ER3	Safety Review not available	Thank You for your comments and review
ER4	no	Thank You for your comments and review
ER5	no	Thank You for your comments and review
ER6	none, please also see review provided by Safety Advisor	Thank You for your comments and review
Are there any concerns regarding the data manipulation, data tables, or statistical analysis?		
ER1	No	Thank You for your comments and review
ER2	No	Thank You for your comments and review
ER3	Table 2. Heat injury, raw chicken product is not heat treated so not necessary to injure the cells	Addressed this in both the table and body of the manuscript

	Table 3. APC for Deli Turkey was not determined to be >1 log to meet the background flora criteria.	Per Appendix J, background flora at 10 x the limit of the competitor organism is only required for one food type, which would have been achieved by the raw chicken breast.
	Table3. Raw Chicken APC was variable - 1000s to 1,000,000s. It's not clear why so much variability between laboratories	~130 lbs of raw chicken breast was processed for the study. Varying degrees of APC results would be expected, although the variability observed is higher than typically seen.
ER4	no	Thank You for your comments and review
ER5	Statistical review not available. Flip book page 100, lin 35. Change 'variance' to 'variability' (two instances). S is standard deviation (variability) and s^2 is variance. The relevant footnotes in Tables 2016.1A and .1B are correct.	Revised variance to variability.
ER6	Please also see refer by statistical advisor	Thank you
Is the Validation Study Manuscript in a format acceptable to AOAC?		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	Yes	Thank You for your comments and review
ER5	Yes	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review
Is the method described in sufficient detail so that it is relatively easy to understand, including equations and procedures for calculation of results (are all terms explained)?		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	NO, there are problems with the official method write up (see below). The method will not be acceptable until the method is corrected.	
	1. Page 5, Section B. Were any samples homogenized by blending? Section D© refers to blending, but a blender does not appear in section B. If blenders were used in either the PTM or OMA validation studies, the "blender" should appear in the Apparatus and Reagents section.	Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.
	2. Page 6, line 32 (section C(b)). Change "...inaccurate results." To "false negative or false positive results."	Covered in the Safety sections of the Package inserts.

	<p>3. Page 7, line 33. Blending, stomaching and hand mixing can give significantly different results with certain commodities. This is particularly the case with leafy greens and seeds. If different types of homogenization were used for different commodities then they should specifically be listed in the method. Only sample preparation procedures that were used in the PTM and OMA validation studies can be specified in the instructions, since that is what was validated. "Table A" would be the appropriate location for this information.</p>	<p>Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.</p>
	<p>PTM 111501 indicates (Page 11, line 12-16) that "all" 25 and 125g food samples were homogenized by stomaching, so stomaching should be indicated for these products. It is not clear how the cantaloupes were prepared.</p>	<p>Currently revising the PTMs to reflect the method of homognization. See above response.</p>
	<p>4. Page 9, Table 2. There is no Table 1, so how can there be a Table 2? Moreover, Page7/line 34 refers to "Table A" which appears to be in the Table found on page 9. Please make sure that the instructions on page 7 refer the reader to the correct table.</p>	<p>Revised Table 2 to Table A. (five instances)</p>
	<p>5. Page 9, Table 2. Please be sure to add a sample preparation column that specifies homogenization procedures for the different commodities if different sample prep procedures were used in the validation studies.</p>	<p>Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.</p>
<p><b>ER4 Package Insert comments:</b></p>		
	<p>1. Page 9, line 3. It appears that the method was validated with a sample preparation procedure that only included stomaching, except cantaloupes, so "blending" should be removed from the instructions. I believe that hand mixing is appropriate for cantaloupes, but this must be spedified in the method.</p>	<p>Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.</p>

	2. There should be an applicability statement, specific to AOAC, In the package insert.	Per AOAC website: <a href="http://www.aoac.org/imis15_prod/AOAC_Docs/SPDS/Formats_AOAC_collab.pdf">http://www.aoac.org/imis15_prod/AOAC_Docs/SPDS/Formats_AOAC_collab.pdf</a> In the Package Insert the applicability statement is prior to the table. In AOAC PTM studies, the 3M Molecular Detection Assay 2 – Listeria was found to be an effective method for the detection of Listeria species. The matrices tested in the study are shown in Table 3. The limit of detection of the 3M Molecular Detection Assay 2 – Listeria method is 1-5 colony forming units per validated test portion size (in Table 3).
	3. There should be an apparatus/media section that details all of the equipment needed to perform the test that is not supplied by 3 in the package insert.	The Package insert lists ALL components that come with the kit.
ER5	Yes	
ER6	Yes	
Are the figures and tables sufficiently explanatory without the need to refer to the text?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
Are all the figures and tables pertinent?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
Could some be omitted and covered by a simple statement?		
ER1	NO	<a href="#">Thank You for your comments and review</a>
ER2	NO	<a href="#">Thank You for your comments and review</a>
ER3	NO	<a href="#">Thank You for your comments and review</a>
ER4	NO	<a href="#">Thank You for your comments and review</a>
ER5	NO	<a href="#">Thank You for your comments and review</a>
ER6	NO	<a href="#">Thank You for your comments and review</a>



Are the references complete and correctly annotated?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
Does the method contain adequate safety precaution reference and/or statements?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
Recommendation: Do you recommend that the ERP adopt this method as an AOAC Official Method of Analysis (First Action status)?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	No, Both the official method and the package insert need to be revised, as stated earlier in this review, since they both contain sample preparation procedures (specifically blending) that were not validated in either the PTM study or the OMA study. Once the methods instructions have been corrected and are aligned with the validation studies, then I would recommend approval of the method for First Action Status	
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>



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1 **Evaluation of the 3M™ Molecular Detection Assay(MDA) 2 –**  
2 ***Listeria* for the Detection of *Listeria* species in Select Foods and**  
3 **Environmental Surfaces: Collaborative Study**  
4

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7  
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14 Repeck, C. Zook, C. Barnes, B. Bastin, D. Isfort

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15  
16 **The 3M™ Molecular Detection Assay (MDA) 2 –*Listeria* uses loop-mediated**  
17 **isothermal amplification and bioluminescence detection to rapidly detect *Listeria***  
18 **species in a broad range of food types and environmental surfaces. Using an**  
19 **unpaired study design, the MDA 2 -*Listeria* was compared to the United States**  
20 **Department of Agriculture (USDA) Food Safety Inspection Service (FSIS)**  
21 **Microbiology Laboratory Guidebook (MLG) Chapter 8.09 *Isolation and***  
22 ***Identification of Listeria monocytogenes from Red Meat, Poultry and Egg***  
23 ***Products, and Environmental Samples* reference method for the detection of**  
24 ***Listeria* in deli turkey and raw chicken breast fillet. Technicians from 13 laboratories**  
25 **located within the continental United States and Canada participated in the**  
26 **collaborative study. Each matrix was evaluated at three levels of contamination:**  
27 **an un-inoculated control level (0 colony forming units (CFU)/test portion), a low**  
28 **inoculum level (0.2-2 CFU/test portion) and a high inoculum level (2-5 CFU/test**  
29 **portion). Statistical analysis was conducted according to the Probability of**  
30 **Detection (POD) statistical model. Results obtained for the low inoculum level test**  
31 **portions produced a dLPOD value with 95% confidence intervals of 0.04, (-0.08,**  
32 **0.17) for deli turkey indicating [the difference between methods was not](#)**  
33 **[statistically significant at the 0.05 probability level. no statistically significant](#)**  
34 **[difference between the candidate and reference methods.](#) For raw chicken breast**  
35 **fillet a dLPOD value with 95% confidence interval of 0.16, (0.04, 0.28) indicating**  
36 **a statistically significant difference [between the candidate and reference methods](#)**  
37 **[an observed higher proportion of positive results by the candidate method than](#)**  
38 **[the reference method. with a positive correlation in data indicating more recovery](#)**  
39 **[of the target analyte by the candidate method.](#)**  
40

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41 *Listeria*, particularly *L. monocytogenes*, is a pathogenic bacterium that contaminates food and is  
42 often found in food manufacturing facilities [1]. Due to the ubiquitous nature of *Listeria*, this  
43 organism is used as a hygiene indicator in all stages of the food processing chain. Detection of  
44 *Listeria* in the production and process facilities may be an indicator of unsanitary conditions [2].  
45 The organism's ability to survive in extreme conditions make its presence in food a serious issue.  
46 In the past year, *Listeria* has been identified as the source of several high profile outbreaks

1 involving bagged leafy greens and ice cream [3]. The 3M™ Molecular Detection Assay (MDA) 2 -  
2 *Listeria* method, using a combination of bioluminescence and isothermal amplification of nucleic  
3 acid sequences, allows for the rapid and specific detection of *Listeria* species in a broad range of  
4 food types and environmental surfaces after 24 to ~~28~~32 hours of pre-enrichment. After  
5 enrichment, samples are evaluated using the 3M MDA 2 - *Listeria* on the 3M™ Molecular  
6 Detection System (MDS). Presumptive positive results are reported in real-time while negative  
7 results are displayed after completion of the assay in approximately 75 minutes.  
8 Prior to the collaborative study, the 3M MDA 2 - *Listeria* method was validated according to  
9 AOAC Guidelines [4] in a harmonized AOAC® Performance Tested Method<sup>SM</sup> (PTM) study.  
10 The objective of the PTM study was to demonstrate that the 3M MDA 2-*Listeria* method could  
11 detect *Listeria* in a broad range of food matrices and environmental surfaces as claimed by the  
12 manufacturer. For the 3M MDA 2- *Listeria* PTM evaluation, 13 matrices were evaluated: hot  
13 dogs (25g & 125g), salmon (25g), deli turkey (25g & 125g), cottage cheese (25g), vanilla ice  
14 cream (25g), queso fresco (25g), spinach (25g), melon (whole), raw chicken leg pieces (25g),  
15 raw chicken fillet (25g); concrete (sponge, 225 mL & 100 mL), stainless steel (sponge, 225 mL),  
16 and plastic (EnviroSwab, 10 mL) environmental samples.  
17 Additional PTM parameters (inclusivity, exclusivity, ruggedness, stability and lot-to-lot  
18 variability) tested in the PTM studies satisfied the performance requirements for PTM approval.  
19 The method was awarded PTM certification number 111501 on November 3<sup>rd</sup>, 2015.  
20 The purpose of this collaborative study was to compare the reproducibility of the 3M MDA 2 -  
21 *Listeria* method to the United States Department of Agriculture (USDA) Food Safety Inspection  
22 Service (FSIS) -Microbiology Laboratory Guidebook (MLG) Chapter 8.09 *Isolation and*  
23 *Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and*  
24 *Environmental Samples* [5] for deli turkey (125 g) and raw chicken breast fillet.

## 26 Collaborative Study

### 27 Study Design

28  
29  
30 In this collaborative study, two matrices, deli turkey and raw chicken breast fillet, were evaluated.  
31 The matrices were obtained from a local retailer and screened for the presence of *Listeria* by the  
32 USDA/FSIS MLG 8.09 reference method. The raw chicken breast fillet was artificially  
33 contaminated with fresh unstressed cells of *Listeria monocytogenes*, American Type Culture  
34 Collection (ATCC) 7644, and the deli turkey was artificially contaminated with heat stressed  
35 cells (Table 2) of *Listeria monocytogenes*, ATCC 19115, at two inoculation levels: a high  
36 inoculation level of approximately 2-5 colony-forming units (CFU)/test portion and a low  
37 inoculation level of approximately 0.2-2 CFU/test portion. A set of un-inoculated control test  
38 portions (0 CFU/test portion) were also included.  
39 Twelve replicate samples from each of the three inoculation levels were analyzed by each  
40 method. Two sets of samples (72 total) were sent to each laboratory for analysis by 3M MDA 2 -  
41 *Listeria* and the USDA/FSIS MLG Chapter 8.09 reference method due to the different sample  
42 enrichment procedures for each method. Additionally, collaborators were sent a 60 g test portion  
43 and instructed to conduct a total aerobic plate count (APC) using 3M™ Petrifilm™ Rapid Aerobic  
44 Count Plate (AOAC Official Method 2015.13) [6] on the day samples were received for the  
45 purpose of determining the total aerobic microbial load.  
46 A detailed collaborative study packet outlining all necessary information related to the study  
47 including media preparation, test portion preparation and documentation of results was sent to  
48 each collaborating laboratory prior to the initiation of the study. A conference call was then

1 conducted to discuss the details of the collaborative study packet and answer any questions from  
2 the participating laboratories.

### 3 4 *Preparation of Inocula and Test Portions*

5  
6 The *Listeria* cultures used in this evaluation were propagated onto Tryptic Soy Agar with 5%  
7 Sheep Blood (SBA) from a Q Laboratories frozen stock culture stored at -70°C. Each organism  
8 was incubated for 24 ± 2 hours at 35 ± 1°C. Isolated colonies were picked to 10 mL of Brain  
9 Heart Infusion (BHI) broth and incubated for 18 ± 0.5 hours at 35 ± 1°C. Raw chicken breast  
10 fillet was inoculated in bulk using the fresh, broth culture. Prior to inoculation of the deli turkey,  
11 the culture suspension was heat stressed at 55 ± 1°C in a water bath for 15 ± 0.5 minutes to obtain  
12 a percent injury of 50-80% (as determined by plating onto selective Modified Oxford agar  
13 (MOX) and non-selective tryptic soy agar with yeast (TSA/ye). The degree of injury was  
14 estimated as:

$$(1 - \frac{n_{select}}{n_{nonselect}}) \times 100$$

15  
16 where  $n_{select}$  = number of colonies on selective agar and  $n_{nonselect}$  = number of colonies on non-  
17 selective agar. Appropriate dilutions of each culture were prepared in Butterfield's Phosphate  
18 Diluent (BPD) based on previously established growth curves for both low and high inoculation  
19 levels. Bulk portions of each matrix were inoculated with the diluted liquid inoculum and mixed  
20 thoroughly to ensure an even distribution of microorganisms. The inoculated raw chicken  
21 breast fillet was packaged into separate 30 g test portions in sterile Whirl-Pak® bags and shipped  
22 to the collaborators. For the analysis of the deli turkey, 25 g of inoculated test product was  
23 mixed with 100 g of un-inoculated test product to prepare 125 g test portions which were  
24 packaged in sterile Whirl-Pak® bags and shipped to collaborators.

25 To determine the level of *Listeria* in the matrices, a 5-tube most probable number (MPN) was  
26 conducted by the coordinating laboratory on the day of the initiation of analysis using the  
27 USDA/FSIS-MLG 8.09 reference method. For deli turkey, the MPN was determined by  
28 analyzing 5 x 250 g test portions, the reference method test portions from the collaborating  
29 laboratories and 5 x 65 g test portions. For the raw chicken breast fillet, the MPN of the high and  
30 low inoculated levels was determined by analyzing 5 x 50 g test portions, the reference method  
31 test portions from the collaborating laboratories and 5 x 10 g test portions. The MPN and 95%  
32 confidence intervals were calculated using the LCF MPN Calculator, Version 1.6,  
33 ([www.lcftld.com/customer/LCFMPNCalculator.exe](http://www.lcftld.com/customer/LCFMPNCalculator.exe)), provided by AOAC Research Institute  
34 (RI) [7].

### 35 36 *Test Portion Distribution*

37  
38 All samples were labeled with a randomized, blind-coded 3-digit number affixed to the sample  
39 container. Test portions were shipped on a Thursday via overnight delivery according to the  
40 Category B Dangerous Goods shipment regulations set forth by the International Air  
41 Transportations Association (IATA). The two matrices were shipped consecutively, with  
42 collaborating laboratories performing analysis on one matrix at a time. Upon receipt, samples  
43 were held by the collaborating laboratory at refrigeration temperature (2-8 °C) until the  
44 following Monday when analysis was initiated after a total equilibration time of 96 hours. All  
45 samples were packed with cold packs to target a temperature of < 7°C during shipment.  
46 In addition to each of the test portions and a separate APC sample, collaborators received a test  
47 portion for each matrix labeled as 'temperature control'. Participants were instructed to obtain the  
48 temperature of this portion upon receipt of the package, document the results on the Sample

1 Receipt Confirmation form provided and fax or email it back to the study director. The shipment  
2 and hold times of the inoculated test material had been verified as a quality control measure prior  
3 to study initiation.

#### 4 5 *Test Portion Analysis*

6  
7 Collaborators were instructed to follow the appropriate preparation and analysis as outlined in  
8 the study protocol for each matrix for both the 3M MDA 2 - *Listeria* method and reference  
9 method. For both matrices, each collaborator received 72 test portions (12 high, 12 low and 12  
10 un-inoculated controls for each method to be performed). For the analysis of the deli turkey test  
11 portions by the 3M MDA 2 - *Listeria* method, a 125 g portion was enriched with 975 mL of  
12 Demi-Fraser (DF) broth, homogenized for 2 minutes and incubated for 24-28 hours at  $37 \pm 1^\circ\text{C}$ .  
13 For the raw chicken breast fillet test portions analyzed by the 3M MDA 2 - *Listeria* method, a 25  
14 g portion was enriched with 475 mL of DF, homogenized for 2 minutes and incubated for 28-32  
15 hours at  $37 \pm 1^\circ\text{C}$ .

16 Following enrichment, samples were assayed by the 3M MDA 2 - *Listeria* method and,  
17 regardless of presumptive result, confirmed following the USDA/FSIS MLG 8.09 reference  
18 method. Both matrices evaluated by the 3M MDA 2 - *Listeria* method were compared to  
19 samples analyzed using the USDA/FSIS MLG 8.09 reference method in an unpaired study  
20 design. All positive test portions were biochemically confirmed by the API *Listeria* biochemical  
21 test or by the VITEK 2 GP biochemical identification test, AOAC Official Method 2012.02 [8].  
22

#### 23 *Statistical Analysis*

24  
25 Each collaborating laboratory recorded results for the reference method and the 3M MDA 2 -  
26 *Listeria* method on the data sheets provided. The data sheets were submitted to the study director  
27 at the end of each week of testing for statistical analysis. Data for each matrix was analyzed  
28 using the probability of detection (POD) statistical model [9]. POD statistical analysis was  
29 conducted using AOAC Binary Data Interlaboratory Study Workbook, Version 2.3 [10]. The  
30 probability of detection (POD) was calculated as the number of positive outcomes divided by the  
31 total number of trials. The POD was calculated for the candidate presumptive results,  $\text{POD}_{\text{CP}}$ , the  
32 candidate confirmatory results (excluding those with presumptive negative results including false  
33 negative results),  $\text{POD}_{\text{CC}}$ , the difference in the candidate presumptive and confirmatory results,  
34  $\text{dLPOD}_{\text{CP}}$ , presumptive candidate results that confirmed positive (including those with  
35 presumptive negative results excluding false negative results),  $\text{POD}_{\text{C}}$ , the reference method,  
36  $\text{POD}_{\text{R}}$ , and the difference in the confirmed candidate and reference methods,  $\text{dLPOD}_{\text{C}}$ . A  
37  $\text{dLPOD}_{\text{C}}$  confidence interval not containing the point zero would indicate a statistically  
38 significant difference between the 3M MDA 2 - *Listeria* and the reference methods at the 5 %  
39 probability level. In addition to POD, the repeatability standard deviation ( $s_{\text{r}}$ ), the among  
40 laboratory repeatability standard deviation ( $s_{\text{L}}$ ), the reproducibility standard deviation ( $s_{\text{R}}$ ) and the  
41  $P_{\text{T}}$  value were calculated. The  $s_{\text{r}}$  provides the variance-variability of data within one laboratory,  
42 the  $s_{\text{L}}$  provides the difference in standard deviation between laboratories and the  $s_{\text{R}}$  provides the  
43 variance-variability in data between different laboratories. The  $P_{\text{T}}$  value provides information on  
44 the homogeneity test of laboratory PODs [10].  
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8 **AOAC Official Method 2016.xxx**  
9 **Listeria species in Select Foods and Environmental Surfaces**  
10 **3M™ Molecular Detection Assay (MDA) 2- Listeria Method**  
11 **First Action 2016**

12 (Applicable to detection of *Listeria* species in hot dogs (25g & 125g), salmon (25g), deli turkey  
13 (25g & 125g), cottage cheese (25g), vanilla ice cream (25g), queso fresco (25g), spinach (25g),  
14 melon (whole), raw chicken leg pieces (25g), raw chicken fillet (25g); concrete (3M™ Hydrated  
15 Sponge Stick with D/E, 225 mL & 100 mL), stainless steel (3M™ Hydrated Sponge Stick with  
16 D/E, 225 mL), and plastic (3M™ EnviroSwab with Lethen, 10 mL) environmental samples.

17  
18 *See Tables 2016.1A and 2016.1B for a summary of results of the inter-laboratory study.*

19 *See Tables 2016.2A and 2016.2B for detailed results of the inter-laboratory study*

20  
21 **A. Principle**

22  
23 The 3M™ Molecular Detection Assay (MDA) 2 - *Listeria* method is used with the 3M™  
24 Molecular Detection System (MDS) for the rapid and specific detection of *Listeria* in enriched  
25 food and food process environmental samples. The 3M MDA2 - *Listeria* uses loop-mediated  
26 isothermal amplification of unique DNA target sequences with high specificity and sensitivity,  
27 combined with bioluminescence to detect the amplification. Presumptive positive results are  
28 reported in real-time while negative results are displayed after the assay is completed. Samples  
29 are pre-enriched in Demi Fraser broth with ferric ammonium citrate (FAC).

30  
31 **B. Apparatus and Reagents**

32 **Items (b)-(g) are available as the 3M™ Molecular Detection Assay (MDA) 2 - *Listeria* kit**  
33 **from 3M Food Safety (St. Paul, MN 55144-1000, USA).**

34 (a) *3M Molecular Detection System (MDS100)* – Available from 3M Food Safety (St.  
35 Paul, MN 55144-1000, USA).

36 (b) *3M Molecular Detection Assay 2 – Listeria reagent tubes*- 12 strips of 8 tubes.  
37 Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).

38 (c) *Lysis Solution (LS) tubes* – 12 strips of 8 tubes.

39 (d) *Extra caps* – 12 strips of 8 caps

40 (e) *Reagent Control* – 8 reagent tubes

41 (f) *Quick Start Guide*

42 (g) *3M™ Molecular Detection Speed Loader Tray* - Available from 3M Food Safety  
43 (St. Paul, MN 55144-1000, USA).

44 (h) *3M™ Molecular Detection Chill Block Insert* - Available from 3M™ Food Safety (St.  
45 Paul, MN 55144-1000, USA).

46 (i) *3M™ Molecular Detection Heat Block Insert* - Available from 3M Food Safety  
47 (St. Paul, MN 55144-1000, USA).

48 (j) *3M™ Molecular Detection Cap/Decap Tool for Reagent tubes* - Available from 3M  
49 Food Safety (St. Paul, MN 55144-1000, USA).

- 1 (k) 3M™ Molecular Detection Cap/Decap Tool for Lysis tubes – Available from 3M  
 2 Food Safety (St. Paul, MN 55144-1000, USA).  
 3 (l) Empty Lysis Tube Rack - Available from 3M Food Safety (St. Paul, MN 55144-1000,  
 4 USA).  
 5 (m) Empty Reagent Tube Rack - Available from 3M Food Safety (St. Paul, MN 55144-  
 6 1000, USA).  
 7 (n) Demi Fraser Broth - Available from 3M Food Safety (St. Paul, MN 55144-1000,  
 8 USA).  
 9 (o) Ferric Ammonium Citrate (FAC), ACS grade, 5% sterilized -Available from MP  
 10 Biomedicals™ or equivalent.  
 11 (p) Disposable pipette – capable of 20 µL  
 12 (q) Multi-channel (8-channel) pipette - capable of 20 µL  
 13 (r) Sterile filter tip pipette tips - capable of 20 µL  
 14 (s) Filter Stomacher® bags – Seward or equivalent.  
 15 (t) Stomacher® – Seward or equivalent.  
 16 (u) Thermometer – calibrated range to include 100 ± 1°C  
 17 (v) Dry block heater unit – capable of maintaining 100 ± 1°C  
 18 (w) Incubators. – Capable of maintaining 37 ± 1°C or 41.5 ± 1°C.  
 19 ~~(x) Freezer – capable of maintaining 10 to 20°C, for storing the 3M Molecular~~  
 20 ~~Detection Chill Block Tray~~  
 21 ~~(y)(x) Refrigerator – capable of maintaining 2-8°C, for storing the 3M Molecular~~  
 22 ~~Detection Assay components~~  
 23 ~~(z)(y) Computer – compatible with the 3M™ Molecular Detection Instrument~~  
 24 ~~(aa)(z) 3M™ EnviroSwab, -- (hydrated with Letheen) Available from 3M Food~~  
 25 ~~Safety(Australia)~~  
 26 ~~(bb)(aa) 3M™ Hydrated Sponge Stick with 10 mL of D/E – Available from 3M~~  
 27 ~~Food Safety(St. Paul, MN 55144-1000, USA)~~

### 29 C. General Instructions

31 (a) Store the 3M Molecular Detection Assay 2 - *Listeria* at 2-8°C. Do not freeze. Keep  
 32 kit away from light during storage. After opening the kit, check that the foil pouch is  
 33 undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes  
 34 should always be stored in the re-sealable pouch with the desiccant inside to maintain  
 35 stability of the lyophilized reagents. Store resealed pouches at 2-8°C for no longer  
 36 than 60 days. Do not use 3M Molecular Detection Assay 2 -*Listeria* past the  
 37 expiration date.

38 (b) Follow all instructions carefully. Failure to do so may lead to inaccurate results.

#### 39 Safety Precautions

40 The 3M MDA2 – *Listeria* is intended for use in a laboratory environment by  
 41 professionals trained in laboratory techniques. 3M has not documented the use of this  
 42 product in industries other than the food and beverage industries. For example, 3M  
 43 has not documented this product for testing drinking water, pharmaceutical,  
 44 cosmetics, clinical or veterinary samples. The 3M MDA2 – *Listeria* has not been



1 evaluated with all possible food products, food processes, testing protocols or with all  
2 possible strains of bacteria.

3 As with all test methods, the source of enrichment medium can influence the results.  
4 The 3M MDA 2 – *Listeria* has only been evaluated for use with the enrichment media  
5 specified in the Instructions for Use section.

6 The 3M™ Molecular Detection instrument is intended for use with samples that have  
7 undergone heat treatment during the assay lysis step, which is designed to destroy  
8 organisms present in the sample. Samples that have not been properly heat treated  
9 during the assay lysis step may be considered a potential biohazard and should NOT  
10 be inserted into the 3M MDS instrument.

11 The user should read, understand and follow all safety information in the instructions  
12 for the 3M MDS and the 3M MDA 2 – *Listeria*. Retain the safety instructions for  
13 future reference.

14 To reduce the risks associated with exposure to chemicals and biohazards:  
15 Perform pathogen testing in a properly equipped laboratory under the control of  
16 trained personnel. Always follow standard laboratory safety practices, including  
17 wearing appropriate protective apparel and eye protection while handling reagents  
18 and contaminated samples. Avoid contact with the contents of the enrichment media  
19 and reagent tubes after amplification. Dispose of enriched samples according to  
20 current industry standards.

21 *Listeria monocytogenes* is of particular concern for pregnant women, the aged and the  
22 infirmed. It is recommended that these concerned groups avoid handling this  
23 organism. After use, the enrichment medium and the 3M MDA 2 - *Listeria* tubes can  
24 potentially contain pathogenic materials. Periodically decontaminate laboratory  
25 benches and equipment (pipettes, cap/decap tools, etc.) with a 1- 5% (v:v in water)  
26 household bleach solution or DNA removal solution. When testing is complete,  
27 follow current industry standards for the disposal of contaminated waste. Consult the  
28 Safety Data Sheet for additional information and local regulations for disposal.

29 To reduce the risks associated with environmental contamination: Follow current  
30 industry standards for disposal of contaminated waste.

#### 31 **D. Sample Enrichment**

##### 32 ***Foods***

- 33
- 34 (a) Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate)  
35 to equilibrate to ambient laboratory temperature (20-25°C).
- 36 (b) Aseptically combine the enrichment medium and sample according to Table 2A. For  
37 all meat and highly particulate samples, the use of filter bags is recommended.
- 38 (c) Homogenize thoroughly by ~~blending~~-stomaching, or hand mixing for 2 ±0.2 minutes.  
39 Incubate at 37 ±1°C according to Table A.

1  
2 **Environmental samples**

3 (d) Sample collection devices can be a sponge hydrated with a neutralizing solution to  
4 inactivate the effects of the sanitizers. 3M recommends the use of a biocide-free  
5 cellulose sponge. Neutralizing solution can be Dey-Engley (D/E) Neutralizing Broth  
6 or Lethen broth. It is recommended to sanitize the area after sampling.

7  
8 WARNING: Should you select to use Neutralizing Buffer (NB) that contains aryl  
9 sulfonate complex as the hydrating solution for the sponge, it is required to perform a  
10 1:2 dilution (1-part sample into 1-part sterile enrichment broth) of the enriched  
11 environmental sample before testing in order to reduce the risks associated with a  
12 false-negative result leading to the release of contaminated product. Another option is  
13 to transfer 10 µL of the NB enrichment in the LS tubes.

14  
15 (e) The recommended size of the sampling area to verify the presence or absence of the  
16 pathogen on the surface is at least 100 cm<sup>2</sup> (10 cm x 10 cm or 4"x4"). When sampling  
17 with a sponge, cover the entire area going in two directions (left to right then up and  
18 down) or collect environmental samples following your current sampling protocol or  
19 according to the FDA BAM, USDA FSIS MLG or ISO 18593 [11] guidelines.

- 20 1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium  
21 citrate) to equilibrate to ambient laboratory temperature (20-25°C).  
22 2. Aseptically combine the enrichment medium and sample according to Table 2A.  
23 3. Homogenize thoroughly by ~~blending~~~~vortexing~~ or ~~stomaching~~~~or hand mixing~~  
24 for 2 ±0.2 minutes. Incubate at 37 ±1°C for 24-30 hours.

25  
26 **E. PREPARATION OF THE 3M™ MOLECULAR DETECTION SPEED LOADER TRAY**

- 27 (a) Wet a cloth or paper towel with a 1-5% (v:v in water) household bleach solution and  
28 wipe the 3M™ Molecular Detection Speed Loader Tray.  
29 (b) Rinse the 3M Molecular Detection Speed Loader Tray with water.  
30 (c) Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.  
31 (d) Ensure the 3M Molecular Detection Speed Loader Tray is dry before use  
32

33 **Specific Instructions for Validated Methods**

34 **AOAC® Performance Tested Method<sup>SM</sup>#111501**



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1 In AOAC PTM studies, the 3M Molecular Detection Assay 2 – *Listeria* was found to be an  
 2 effective method for the detection of *Listeria* species. The matrices tested in the study are  
 3 shown in Table 2A. The limit of detection of the 3M Molecular Detection Assay 2 – *Listeria*  
 4 method is 1-5 colony forming units per validated test portion size (in Table 2A).

5  
6

7 **Table 2A:** Enrichment protocols using Demi-Fraser Broth at  $37 \pm 1$  °C  
 8 according to AOAC Performance Tested <sup>SM</sup> Certificate #111501  
 9

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)	
Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon	25 g	225	24-30	
Raw chicken	25 g	475	28-32	
Deli turkey	125 g	1125	24-30	
Cantaloupe <sup>a</sup>	Whole melon	Enough volume to allow melon to float	26-30	
Environmental samples:	Stainless steel	1 sponge	225	24-30
	Sealed concrete	1 sponge	100	24-30
	Plastic <sup>b</sup>	1 swab	10	24-30

10 All samples for the AOAC validation were homogenized by stomaching unless otherwise noted.

11 a = Homogenize sample by hand mixing

12 b = Homogenize sample by vortexing

13  
14 **F. PREPARATION OF THE 3M™ MOLECULAR DETECTION HEAT BLOCK INSERT**

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- 1 Place the 3M™ Molecular Detection Heat Block Insert in a dry double block heater unit.
- 2 Turn on the dry block heater unit and set the temperature to allow the 3M Molecular
- 3 Detection Heat Block Insert to reach and maintain a temperature of 100 ±1°C.

4 **NOTE:** Depending on the heater unit, allow approximately 30minutes for the 3M Molecular Detection  
5 Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (e.g., a partial  
6 immersion thermometer, digital thermocouple thermometer, not a total immersion thermometer) placed in  
7 the designated location, verify that the 3M Molecular Detection Heat Block Insert is at 100 ±1°C.

#### 8 **G. PREPARATION OF THE 3M MOLECULAR DETECTION INSTRUMENT**

- 9 1. Launch the 3M™ Molecular Detection Software and log in.
- 10 2. Turn on the 3M Molecular Detection Instrument.
- 11 3. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection
- 12 System User Manual for details.

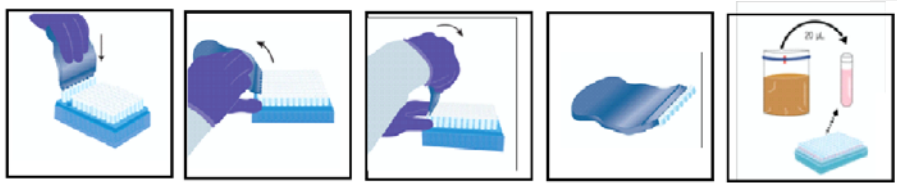
13  
14 **NOTE:** The 3M Molecular Detection Instrument must reach and maintain temperature of 60°C before  
15 inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes  
16 approximately 20 minutes and is indicated by an ORANGE light on the instrument's status bar. When the  
17 instrument is ready to start a run, the status bar will turn GREEN.

#### 18 **H. LYSIS**

- 19 1. Allow the lysis solution (LS) tubes to warm up by setting the rack at room temperature (20-  
20 25 °C) overnight (16-18 hours). Alternatives to equilibrate the LS tubes to room  
21 temperature are to set the LS tubes on the laboratory bench for at least 2 hours, incubate the  
22 LS tubes in a 37 ±1°C incubator for 1 hour or place them in a dry double block heater for  
23 30 seconds at 100±1°C.
- 24 2. Invert the capped tubes to mix. Proceed to next step within 4 hrs.
- 25 3. Remove the enrichment broth from the incubator.
- 26 4. One LS tube is required for each sample and the Negative Control (NC) (sterile enrichment  
27 medium) sample.
  - 28 4.1 LS tube strips can be cut to desired LS tube number. Select the number of individual LS  
29 tubes or 8-tube strips needed. Place the LS tubes in an empty rack.
  - 30 4.2 To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipette  
31 tip for each transfer step.
  - 32 4.3 Transfer enriched sample to LS tubes as described below:

33  
34 **Transfer each enriched sample into individual LS tube **first**. Transfer the NC **last**.**

- 35  
36 4.4 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip -  
37 one strip at a time.
- 38 4.5 Discard the LS tube cap – if lysate will be retained for retest, place the caps into a clean  
39 container for re-application after lysis
- 40 4.6 Transfer 20 µL of sample into a LS tube.
- 41 5. Repeat step 4.2 until each individual sample has been added to a corresponding LS tube in  
42 the strip as illustrated below.



1  
2

- 3 6. Repeat steps 4.1 to 4.6 as needed, for the number of samples to be tested. When all samples  
 4 have been transferred, then transfer **20 µL of NC into a LS tube**. Do not recap tubes.  
 5 7. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100  
 6 ±1°C. Place the rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat  
 7 for 15 ±1 minutes. During heating, the LS solution will change from pink (cool) to yellow  
 8 (hot).  
 9 8. Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M  
 10 Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes.  
 11 The 3M Molecular Chill block Insert, used at ambient temperature (20-25°C) without the  
 12 Molecular Detection Chill Block Tray, should sit directly on the laboratory bench. When  
 13 cool, the lysis solution will revert to a pink color.

14 9.6- Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.

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16 **I. AMPLIFICATION**

- 17 1. One Reagent tube is required for each sample and the NC.  
 18 1.1 Reagent tubes strips can be cut to desired tube number. Select the number of individual  
 19 Reagent tubes or 8-tube strips needed.  
 20 1.2 Place Reagent tubes in an empty rack.  
 21 1.3 Avoid disturbing the reagent pellets from the bottom of the tubes.  
 22 2. Select 1 Reagent Control (RC) tube and place in rack.  
 23 3. To avoid cross-contamination, decap one Reagent tubes strip at a time and use a new pipette  
 24 tip for each transfer step.  
 25 4. Transfer lysate to Reagent tubes and RC tube as described below:

27 Transfer each sample lysate into individual Reagent tubes **first** followed by the NC. Hydrate the RC  
 28 tube **last**.

- 29 4.1 Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent  
 30 tubes –one Reagent tubes strip at a time. Discard cap.  
 31 4.2 Transfer 20 µL of Sample lysate from the upper ½ of the liquid (avoid precipitate) in  
 32 the LS tube into corresponding Reagent tube. Dispense at an angle to avoid disturbing  
 33 the pellets. Mix by gently pipetting up and down 5 times.  
 34 4.3 Repeat step 4.2 until individual Sample lysate has been added to a corresponding  
 35 Reagent tube in the strip.  
 36 4.4 Cover the Reagent tubes with the provided extra cap and use the rounded side of the  
 37 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth  
 38 motion ensuring that the cap is tightly applied.  
 39 4.5 Repeat steps 4.1 to 4.4 as needed, for the number of samples to be tested.  
 40 4.6 When all sample lysates have been transferred, repeat 4.1 to 4.4 to transfer 20 µL of  
 41 NC lysate into a Reagent tube.

- 1 4.7 Transfer **20 µL of NC lysate into a RC tube**. Dispense at an angle to avoid disturbing  
2 the pellets. Mix by gently pipetting up and down 5 times.
- 3 5. Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader  
4 Tray. See illustration below. Close and latch the 3M Molecular Detection Speed Loader Tray  
5 lid.



- 6
- 7
- 8
- 9 6. Review and confirm the configured run in the 3M Molecular Detection Software.
- 10 7. Click the Start button in the software and select instrument for use. The selected instrument's  
11 lid automatically opens.
- 12 8. Place the 3M Molecular Detection Speed Loader Tray into the 3M MDS Instrument and  
13 close the lid to start the assay. Results are provided within 75minutes, although positives may  
14 be detected sooner.
- 15 9. After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from  
16 the 3M Molecular Detection Instrument and dispose of the tubes by soaking in a 1-5% (v:v in  
17 water) household bleach solution for 1 hour and away from the assay preparation area.
- 18

19 **NOTICE:** To minimize the risk of false positives due to cross-contamination, never open reagent tubes  
20 containing amplified DNA. This includes Reagent Control, Reagent and Matrix Control tubes. Always  
21 dispose of sealed reagent tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour  
22 and away from the assay preparation area.

#### 24 **RESULTS AND INTERPRETATION**

25 An algorithm interprets the light output curve resulting from the detection of the nucleic acid  
26 amplification. Results are analyzed automatically by the software and are color-coded based on  
27 the result. A Positive or Negative result is determined by analysis of a number of unique curve  
28 parameters. Presumptive positive results are reported in real-time while Negative and Inspect  
29 results will be displayed after the run is completed.

30

31 Presumptive positive samples should be confirmed as per the laboratory standard operating  
32 procedures or by following the current version of the appropriate reference method  
33 confirmation ([FDA/BAM](#), the [USDA/FSIS-MLG](#)), beginning with transfer from the primary  
34 enrichment to secondary enrichment broth (if applicable), followed by subsequent plating and  
35 confirmation of isolates using appropriate biochemical and serological methods.

36

37 **NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular  
38 Detection Assay 2 - *Listeria* amplification reagents have a "background" relative light unit (RLU)  
39 reading.

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1 In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M  
2 recommends the user to repeat the assay for any Inspect samples. If the result continues to be  
3 Inspect, proceed to confirmation test using your preferred method or as specified by local  
4 regulations.

#### 5 6 *Results of Collaborative Study*

7  
8 For this collaborative study, the 3M Molecular Detection Assay (MDA) 2 - *Listeria* method was  
9 compared to the USDA FSIS MLG 8.09 reference method for deli turkey and raw chicken breast  
10 fillet. A total of 13 laboratories throughout the United States and Canada participated in this  
11 study, with 11 laboratories submitting data for the deli turkey and 12 laboratories submitting data  
12 for the raw chicken breast fillet. See Table 1 for a summary of laboratory participation for each  
13 matrix. Each laboratory analyzed 36 test portions for each method per matrix: 12 inoculated  
14 with a high level of *Listeria*, 12 inoculated with a low level of *Listeria*, and 12 un-inoculated  
15 controls.

16 A background screen of the matrix indicated an absence of indigenous *Listeria* species in both  
17 matrices. Ten (10) replicate test portions (randomly sampled from 50% of the total packages used  
18 in the analysis) were screened for the presence of *Listeria* species. All test portions produced  
19 negative results for the target analyte.

20 Results for the heat stress analysis of the inoculum for the deli turkey are presented in Table 2.

21 [The raw chicken breast fillet is not heat treated, therefore it was not necessary to injure the](#)  
22 [cells.](#) Table 2016.1A and 2016.1B summarize the inter-laboratory results for all foods tested,  
23 including POD statistical analysis. As per criteria outlined in Appendix J of the AOAC Validation  
24 Guidelines, fractional positive results were obtained. Detailed results for each laboratory are  
25 presented in Tables 2016.2A and 2016.2B. For each matrix, the level of *Listeria* was determined  
26 by MPN on the day of initiation of analysis by the coordinating laboratory. MPN results are  
27 presented in Tables 2016.2A and 2016.2B. The individual laboratory and sample results are  
28 presented in Tables 1-2 of the Supplementary Materials. The APC results for each collaborating  
29 are presented in Table 53 of the Supplementary Materials.

#### 30 31 **Deli Turkey (125 g Test Portions)**

32  
33 Deli turkey test portions were inoculated at a low and high level and were analyzed for the  
34 detection of *Listeria* spp. Un-inoculated controls were included in each analysis. Laboratories 8  
35 and 10 received test portions but were unable to conduct the analysis and therefore no data was  
36 submitted. All other laboratories submitted data for both methods evaluated. The MPN levels  
37 obtained for this matrix, with 95% confidence intervals, were 0.63 CFU/test portion (0.49, 0.80)  
38 for the low inoculum level and 4.52 CFU/test portion (3.19, 6.42) for the high inoculum level.

39 For the low inoculum level, 68 out of 132 test portions (POD<sub>CP</sub> of 0.52) were reported as  
40 presumptive positive by the 3M MDA 2 – *Listeria* method with 66 out of 132 test portions  
41 (POD<sub>CC</sub> of 0.50) confirming positive. For samples that produced presumptive positive results on  
42 the 3M MDA 2 – *Listeria* method, 66 out of 132 samples confirmed positive (POD<sub>C</sub> of 0.50). For

1 test portions evaluated by the USDA/FSIS MLG reference method, 60 out of 132 test portions  
2 produced positive results. A dLPOD<sub>C</sub> value of 0.04 with 95% confidence intervals of  
3 (-0.08, 0.17) was obtained between the candidate and reference method, indicating [the difference](#)  
4 [between methods was not statistically significant at the 0.05 probability level.](#)~~no statistical~~  
5 ~~significant difference between the two methods.~~ A dLPOD<sub>CP</sub> value of 0.02 with 95% confidence  
6 intervals of (-0.11, 0.14) was obtained between presumptive and confirmed results indicating [the](#)  
7 [difference between presumptive and confirmed results was not statistically significant at the 0.05](#)  
8 [probability level.](#)~~no statistically significant difference between the presumptive and confirmed~~  
9 ~~results.~~

10 For the high inoculum level, 132 out of 132 test portions (POD<sub>CP</sub> of 1.00) were reported as  
11 presumptive positive by the 3M MDA 2 – *Listeria* method with 132 out of 132 test portions  
12 (POD<sub>CC</sub> of 1.00) confirming positive. For samples that produced presumptive positive results on  
13 the 3M MDA 2 – *Listeria* method, 132 out of 132 samples confirmed positive (POD<sub>C</sub> of 1.00). For  
14 test portions evaluated by the USDA/FSIS MLG reference method, 132 out of 132 test portions  
15 produced positive results. A dLPOD<sub>C</sub> value of 0.00 with 95% confidence intervals of (-0.03,  
16 0.03) was obtained between the candidate and reference method, indicating [the difference](#)  
17 [between methods was not statistically significant at the 0.05 probability level.](#)~~no statistical~~  
18 ~~significant difference between the two methods.~~ A dLPOD<sub>CP</sub> value of 0.00 with 95% confidence  
19 intervals of (-0.03, 0.03) was obtained between presumptive and confirmed results indicating [the](#)  
20 [difference between presumptive and confirmed results was not statistically significant at the 0.05](#)  
21 [probability level.](#)~~no statistically significant difference between the presumptive and confirmed~~  
22 ~~results.~~

23 For the un-inoculated controls, 0 out of 132 samples (POD<sub>CP</sub> of 0.00) produced a presumptive  
24 positive result by the 3M MDA 2 – *Listeria* method with 0 out of 132 test portions (POD<sub>CC</sub> of  
25 0.00) confirming positive. For samples that produced presumptive positive results on the 3M  
26 MDA 2 – *Listeria* method, 0 out of 132 samples confirmed positive (POD<sub>C</sub> of 0.00). For test  
27 portions evaluated by the USDA/FSIS MLG reference method, 0 out of 132 test portions  
28 produced positive results. A dLPOD<sub>C</sub> value of 0.00 with 95% confidence intervals of (-0.03,  
29 0.03) was obtained between the candidate and reference method, indicating [the difference](#)  
30 [between methods was not statistically significant at the 0.05 probability level.](#)~~no statistical~~  
31 ~~significant difference between the two methods.~~ A dLPOD<sub>CP</sub> value of 0.00 with 95% confidence  
32 intervals of (-0.03, 0.00) was obtained between presumptive and confirmed results indicating [the](#)  
33 [difference between presumptive and confirmed results was not statistically significant at the 0.05](#)  
34 [probability level.](#)~~no statistically significant difference between the presumptive and confirmed~~  
35 ~~results.~~

36  
37 Detailed results of the POD statistical analysis are presented in Table 2016.2A and Figures 1A-  
38 1B.

#### 39 40 **Raw Chicken Breast Fillet (25 g Test Portions)**

41  
42 Raw chicken breast fillet test portions were inoculated at a low and high inoculum level and were  
43 analyzed for the detection of *Listeria* spp. Un-inoculated controls were included in each



1 analysis. Laboratory 11 did not participate in the evaluation of this matrix. Laboratory 10  
2 submitted data that indicated cross contamination of the inoculating organism in the un-  
3 inoculated control samples. Further analysis at the coordinating laboratory confirmed the cross  
4 contamination of the un-inoculated controls. Due to this issue, the data submitted from  
5 Laboratory 10 was not used in the statistical analysis. All other laboratories submitted data for  
6 both methods evaluated. The MPN levels obtained for this matrix, with 95% confidence intervals,  
7 were 0.66 CFU/test portion (0.51, 0.83) for the low level and 6.24 CFU/test portion (3.58, 10.88)  
8 for the high level.

9 For the low inoculum level, 88 out of 132 test portions ( $POD_{CP}$  of 0.67) were reported as  
10 presumptive positive by the 3M MDA 2 – *Listeria* method with 86 out of 132 test portions  
11 ( $POD_{CC}$  of 0.65) confirming positive. For samples that produced presumptive positive results on  
12 the 3M MDA 2 – *Listeria* method, 85 out of 132 test portions confirmed positive ( $POD_C$  of 0.64).  
13 For test portions evaluated by the USDA/FSIS MLG reference method, 64 out of 132 test  
14 portions produced positive results. A  $dLPOD_C$  value of 0.16 with 95% confidence intervals of  
15 (0.04, 0.28) was obtained between the candidate and reference method, indicating a statistically  
16 significant difference between the two methods, with [an observed higher proportion of positive](#)  
17 [results by the candidate method than the reference method, positive with a positive correlation in](#)  
18 [data indicating more recovery of the target analyte by the candidate method.](#) A  $dLPOD_{CP}$  value of  
19 0.02 with 95% confidence intervals of (-0.10, 0.13) was obtained between presumptive and  
20 confirmed results indicating [the difference between presumptive and confirmed results was not](#)  
21 [statistically significant at the 0.05 probability level, no statistically significant difference between](#)  
22 [the presumptive and confirmed results.](#)

23 For the high inoculum level, 131 out of 132 test portions ( $POD_{CP}$  of 0.99) were reported as  
24 presumptive positive by the 3M MDA 2 – *Listeria* method with 132 out of 132 test portions  
25 ( $POD_{CC}$  of 1.00) confirming positive. For samples that produced presumptive positive results on  
26 the 3M MDA 2 – *Listeria* method, 131 out of 132 samples confirmed positive ( $POD_C$  of 0.99).  
27 For test portions evaluated by the USDA/FSIS-MLG reference method, 132 out of 132 test  
28 portions produced positive results. A  $dLPOD_C$  value of -0.01 with 95% confidence intervals of  
29 (-0.04, 0.02) was obtained between the candidate and reference method, indicating [the difference](#)  
30 [between methods was not statistically significant at the 0.05 probability level, no statistically](#)  
31 [significant difference between the two methods.](#) A  $dLPOD_{CP}$  value of -0.01 with 95%  
32 confidence intervals of (-0.04, 0.02) was obtained between presumptive and confirmed results  
33 indicating [the difference between presumptive and confirmed results was not statistically](#)  
34 [significant at the 0.05 probability level, no statistically significant difference between the](#)  
35 [presumptive and confirmed results.](#)

36 For the un-inoculated controls, 2 out of 132 samples ( $POD_{CP}$  of 0.02) produced a presumptive  
37 positive result by the 3M MDA 2 - *Listeria* method with 1 out of a 132 test portions ( $POD_{CC}$  of  
38 0.01) confirming positive. For samples that produced presumptive positive results on the 3M  
39 MDA 2 – *Listeria* method, 1 out of 132 samples confirmed positive ( $POD_C$  of 0.01). For test  
40 portions evaluated by the USDA/FSIS-MLG reference method, 0 out of 132 test portions  
41 produced positive results. A  $dLPOD_C$  value of 0.01 with 95% confidence intervals of (-0.02,  
42 0.04) was obtained between the candidate and reference method, indicating [the difference](#)  
43 [between methods was not statistically significant at the 0.05 probability level, no statistical](#)

1 ~~significant difference between the two methods.~~ A dLPOD<sub>CP</sub> value of 0.01 with 95% confidence  
2 intervals of (-0.03, 0.05) was obtained between presumptive and confirmed results indicating the  
3 difference between presumptive and confirmed results was not statistically significant at the 0.05  
4 probability level.~~no statistically significant difference between the presumptive and confirmed~~  
5 ~~results.~~

6  
7 Detailed results of the POD statistical analysis are presented in Table 2016.2B and Figures 1C-  
8 1D.

## 9 **Discussion**

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11  
12 No negative feedback was provided by the collaborating laboratories in regard to the  
13 performance of the 3M MDA 2- *Listeria* method. During the evaluation of the raw chicken breast  
14 fillet, Laboratory 2 isolated *Listeria innocua* from an un-inoculated control sample. Since the  
15 organism recovered was different from the inoculating organism, *Listeria monocytogenes*, no  
16 just cause for removal of the data was determined and the data was included in the manuscript.  
17 For the raw chicken breast fillet, Laboratory 10 reported isolating *Listeria monocytogenes* from  
18 two un-inoculated control samples. The isolates were sent for further identification and it was  
19 determined that they were the same strain as the inoculating organism, indicating that cross  
20 contamination of the sample occurred. Due to the fact that cross contamination occurred, just  
21 cause removal of the data was established and the data generated by Laboratory 10 was therefore  
22 not included in the statistical analysis.

23 Overall, the data generated during this evaluation demonstrates the reproducibility of this new  
24 method. For the deli turkey analysis, the POD statistical analysis indicated the difference  
25 between the candidate method and reference method was not statistically significant at the 0.05  
26 probability level. And that the difference between presumptive and confirmed candidate method  
27 was not statistically significant at the 0.05 probability level.~~that no statistically significant~~  
28 ~~difference between the candidate method and the reference method or between the presumptive~~  
29 ~~and confirmed results of the candidate method was obtained.~~ For raw chicken breast fillet, a  
30 statistically significant difference was observed between the reference and the alternative  
31 method. The dLPOD being significantly greater than zero showed an observed higher proportion  
32 of positive results by the candidate method than the reference method.~~The dLPOD data indicated~~  
33 ~~a positive correlation in data indicating more recovery of the target analyte by the candidate~~  
34 ~~method.~~ One possible contribution for the higher observed proportion positive results to the  
35 higher level of recovery observed with the 3M MDA 2 – *Listeria* method was the use of Demi-  
36 Fraser Broth for the candidate method. This enrichment media formulation is less selective than  
37 the modified University of Vermont Medium used in the USDA reference method and may have  
38 contributed to the higher level of recovery observed during the evaluation. A second possible  
39 contribution for the higher observed proportion positive results to the higher level of recovery  
40 was the length of the primary enrichment. Test portions evaluated by the 3M MDA 2 – *Listeria*  
41 method were incubated for a minimum of 28 hours in the primary enrichment, while the USDA  
42 reference method had a maximum primary enrichment time of 26 hours. No statistically

1 significant difference was observed between the candidate method presumptive and confirmed  
2 results for this matrix.

3

#### 4 **Recommendations**

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6 It is recommended that the 3M Molecular Detection Assay 2 – *Listeria* method be adopted as  
7 Official First Action status for the detection of *Listeria* in selected foods: hot dogs (25g & 125g),  
8 salmon (25g), deli turkey (25g & 125g), cottage cheese (25g), vanilla ice cream (25g), queso  
9 fresco (25g), spinach (25g), melon (whole), raw chicken leg pieces (25g), raw chicken fillet  
10 (25g); concrete, stainless steel and plastic environmental samples.

11

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13

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24

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39 Klass and Colleen Sweeney.

40

41

42

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41 *for sampling techniques from surfaces using contact plates and swabs*.

**Table 1: Participation of each Collaborating Laboratory<sup>a</sup>**

Lab	Deli Turkey <sup>a,b</sup>	Raw Chicken Breast Fillet <sup>a,b</sup>
1	Y	Y
2	Y	Y
3	Y	Y
4	Y	Y
5	Y	Y
6	Y	Y
7	Y	Y
8	N	Y
9	Y	Y
10	N	Y
11	Y	N
12	Y	Y
13	Y	Y

<sup>a</sup> Y= Collaborator analyzed the food type; <sup>a</sup>N= Collaborator did not analyze food type

**Table 2: Heat-Stress Injury Results**

Matrix	Test Organism <sup>a</sup>	CFU/MOX (Selective Agar)	CFU/TSA (Non-Selective Agar)	Degree Injury <sup>b</sup>
Deli Turkey	<i>Listeria monocytogenes</i> ATCC 19115	9.1 x 10 <sup>8</sup>	2.5 x 10 <sup>9</sup>	60.8%
<a href="#">Raw Chicken Breast Fillet</a>	<a href="#">Listeria monocytogenes ATCC 7644</a>	<a href="#">Not applicable</a>	<a href="#">Not applicable</a>	<a href="#">Raw chicken product is not heat treated so not necessary to injure the cells.</a>

<sup>a</sup> ATCC- American Type Culture Collection

<sup>b</sup> Cultures were heat stressed for 10 minutes at 55°C in a recirculating water bath.

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**Table 2016.1A:** Summary of Results for the Detection of *Listeria* in Deli Turkey (125g)

Method <sup>a</sup>	3M™ MDA™ 2 - <i>Listeria</i>		
	Un-inoculated	Low	High
Candidate Presumptive Positive/ Total # of Samples Analyzed	0/132	68/132	132/132
Candidate Presumptive POD (CP)	0.00 (0.00, 0.03)	0.52 (0.43, 0.60)	1.00 (0.97, 1.00)
$s_r^b$	0.00 (0.00, 0.16)	0.51 (0.45, 0.52)	0.00 (0.00, 0.16)
$s_L^c$	0.00 (0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
$s_R^d$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value <sup>e</sup>	1.0000	0.8762	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/132	66/132	132/132
Candidate Confirmed POD (CC)	0.00 (0.00, 0.03)	0.50 (0.41, 0.59)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9123	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/132	66/132	132/132
Candidate Presumptive Positive that Confirmed POD (C)	0.00 (0.00, 0.03)	0.50 (0.41, 0.59)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9123	1.0000
Positive Reference Samples/ Total # of Samples Analyzed	0/132	60/132	132/132
Reference POD	0.00 (0.00, 0.03)	0.45 (0.37, 0.54)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.11)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9829	1.0000
dLPOD (Candidate vs. Reference) <sup>f</sup>	0.00 (-0.03, 0.03)	0.04 (-0.08, 0.17)	0.00 (-0.03, 0.03)

dLPOD (Candidate Presumptive vs. Candidate Confirmed) <sup>f</sup>	0.00 (-0.03, 0.03)	0.02 (-0.11, 0.14)	0.00 (-0.03, 0.03)
--	-----------------------	-----------------------	-----------------------

<sup>a</sup>Results include 95% Confidence Intervals, <sup>b</sup>Repeatability Standard Deviation, <sup>c</sup>Among-Laboratory Standard Deviation, <sup>d</sup>Reproducibility Standard Deviation

<sup>e</sup>P Value = Homogeneity test of laboratory PODs; <sup>f</sup>A confidence interval for dLPOD that does not contain the value 0 indicates a statistical significant difference between the two methods

**Table 2016.1B:**Summary of Results for the Detection of *Listeria* in Raw Chicken Breast Fillet (25g)

Method <sup>a</sup>	3M <sup>TM</sup> MDA <sup>TM</sup> 2 - <i>Listeria</i>		
	Un-inoculated	Low	High
Inoculation Level			
Candidate Presumptive Positive/ Total # of Samples Analyzed	2/132	88/132	131/132
Candidate Presumptive POD (CP)	0.02 (0.00, 0.06)	0.67 (0.58, 0.75)	0.99 (0.96, 1.00)
s <sub>r</sub> <sup>b</sup>	0.12 (0.11, 0.16)	0.48 (0.42, 0.52)	0.09 (0.08, 0.16)
s <sub>L</sub> <sup>c</sup>	0.00 (0.00, 0.05)	0.00 (0.00, 0.18)	0.00 (0.00, 0.04)
s <sub>R</sub> <sup>d</sup>	0.12 (0.11, 0.14)	0.48 (0.43, 0.52)	0.09 (0.08, 0.10)
P Value <sup>e</sup>	0.5190	0.6044	0.4338
Candidate Confirmed Positive/ Total # of Samples Analyzed	1/132	86/132	132/132
Candidate Confirmed POD (CC)	0.01 (0.00, 0.04)	0.65 (0.57, 0.73)	1.00 (0.97, 1.00)
s <sub>r</sub>	0.09 (0.08, 0.16)	0.48 (0.43, 0.52)	0.00 (0.00, 0.16)
s <sub>L</sub>	0.00 (0.00, 0.04)	0.00 (0.00, 0.18)	0.00 (0.00, 0.16)
s <sub>R</sub>	0.09 (0.08, 0.10)	0.48 (0.43, 0.52)	0.00 (0.00, 0.23)
P Value	0.4338	0.5632	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	1/132	85/132	131/132
Candidate Presumptive Positive that Confirmed POD (C)	0.01 (0.00, 0.04)	0.64 (0.56, 0.73)	0.99 (0.96, 1.00)
s <sub>r</sub>	0.09 (0.08, 0.16)	0.48 (0.43, 0.52)	0.09 (0.08, 0.16)
s <sub>L</sub>	0.00 (0.00, 0.04)	0.00 (0.00, 0.18)	0.00 (0.00, 0.04)
s <sub>R</sub>	0.09 (0.08, 0.10)	0.49 (0.43, 0.52)	0.09 (0.08, 0.10)
P Value	0.4338	0.6228	0.4338
Positive Reference Samples/ Total # of Samples Analyzed	0/132	64/132	132/132
Reference POD	0.00 (0.00, 0.03)	0.48 (0.40, 0.57)	1.00 (0.97, 1.00)
s <sub>r</sub>	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
s <sub>L</sub>	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
s <sub>R</sub>	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9192	1.0000
dLPOD (Candidate vs. Reference) <sup>f</sup>	0.01	0.16	-0.01

	(-0.02, 0.04)	(0.04, 0.28)	(-0.04, 0.02)
dLPOD (Candidate Presumptive vs. Candidate Confirmed) <sup>f</sup>	0.01 (-0.03, 0.05)	0.02 (-0.10, 0.13)	-0.01 (-0.04, 0.02)

<sup>a</sup>Results include 95% Confidence Intervals, <sup>r</sup>Repeatability Standard Deviation, <sup>s</sup>Among-Laboratory Standard Deviation, <sup>d</sup>Reproducibility Standard Deviation

<sup>e</sup> P Value = Homogeneity test of laboratory PODs; <sup>f</sup> A confidence interval for dLPOD that does not contain the value 0 indicates a statistical significant difference between the two methods

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1 **Table 2016.2A:** Comparative Results for the Detection of *Listeria* in 125 g Deli Turkey Test Portions by the 3M™ MDA 2 - *Listeria* Method vs. USDA/FSIS  
 2 MLG Chapter. 8.09 in a Collaborative Study (Un-inoculated Control)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
		1	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		2	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		3	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		4	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		5	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		6	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		7	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		8 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		9	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		10 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		11	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		12	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		13	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00

Estimate	All	132	0	0.00	132	0	0.00	132	0	0.00	132	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.03	-0.03
UCL				0.03			0.03			0.03			0.03	0.03	0.03
$s_r^b$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_L^c$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_R^d$				0.00			0.00			0.00			0.00		
UCL				0.00			0.00			0.00			0.00		
LCL				0.23			0.23			0.23			0.23		
$P_T^e$				1.0000			1.0000			1.0000			1.0000		

<sup>a</sup>N/A – Laboratory did not participate or submit data for this matrix

<sup>b</sup>Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation, <sup>e</sup> P<sub>T</sub> Value = Homogeneity test of laboratory PODs

LCL – Lower Confidence Limit; UCL – Upper Confidence Limit, N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)

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- 1 **Table 2016.2A (cont'd.):** Comparative Results for the Detection of *Listeria* in 125 g Deli Turkey Test Portions by the 3M™ MDA 2 - *Listeria* Method vs.  
 2 USDA/FSIS MLG Chapter. 8.09 in a Collaborative Study (Low Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R		
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)	
	Deli Turkey	1	12	7	0.58	12	7	0.58	12	7	0.58	12	6	0.50	0.08	0.00	
		2	12	7	0.58	12	6	0.50	12	6	0.50	12	5	0.42	0.08	0.08	
		3	12	4	0.33	12	4	0.33	12	4	0.33	12	5	0.42	-0.08	0.00	
		4	12	5	0.42	12	5	0.42	12	5	0.42	12	4	0.33	0.08	0.00	
		5	12	6	0.50	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.00	
		6	12	7	0.58	12	7	0.58	12	7	0.58	12	6	0.50	0.08	0.00	
		7	12	7	0.58	12	7	0.58	12	7	0.58	12	6	0.50	0.08	0.00	
		8 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		9	12	8	0.66	12	8	0.66	12	8	0.66	12	6	0.50	0.16	0.00	
		10 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		MPN/ Test Portion	11	12	7	0.58	12	6	0.50	12	6	0.50	12	7	0.58	-0.08	0.08
			12	12	5	0.42	12	5	0.42	12	5	0.42	12	4	0.33	0.08	0.00
			13	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
Estimate	0.63	All	132	68	0.52	132	66	0.50	132	66	0.50	132	60	0.45	0.04	0.02	
LCL	0.49				0.43			0.41			0.41			0.37	-0.08	-0.11	
UCL	0.80				0.60			0.59			0.59			0.54	0.17	0.14	
s <sub>r</sub> <sup>b</sup>					0.51			0.51			0.51			0.51			
LCL					0.45			0.46			0.46			0.46			
UCL					0.52			0.52			0.52			0.52			
s <sub>L</sub> <sup>c</sup>					0.00			0.00			0.00			0.00			
LCL					0.00			0.00			0.00			0.00			
UCL					0.15			0.14			0.14			0.11			
s <sub>R</sub> <sup>d</sup>					0.51			0.51			0.51			0.51			
UCL					0.46			0.46			0.46			0.46			
LCL					0.52			0.52			0.52			0.52			
P <sub>T</sub> <sup>e</sup>					0.8762			0.9123			0.9123			0.9829			

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix

<sup>b</sup> Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation, <sup>e</sup> P<sub>T</sub> Value = Homogeneity test of laboratory PODs  
 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit, **N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)**

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- 1 **Table 2016.2A (cont'd.):** Comparative Results for the Detection of *Listeria* in 125 g Deli Turkey Test Portions by the 3M™ MDA 2 - *Listeria* Method vs.  
 2 USDA/FSIS MLG Chapter. 8.09 in a Collaborative Study (High Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R		
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)	
	Deli Turkey	1	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		2	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		3	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		4	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		5	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		6	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		7	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		8 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
		9	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		10 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
		MPN/ Test Portion	11	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
			12	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
			13	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
Estimate	4.52	All	132	132	1.00	132	132	1.00	132	132	1.00	132	132	1.00	0.00	0.00	
LCL	3.19				0.97			0.97			0.97			0.97	-0.03	-0.03	
UCL	6.42				1.00			1.00			1.00			1.00	0.03	0.03	
s <sub>r</sub> <sup>b</sup>					0.00			0.00			0.00			0.00			
LCL					0.00			0.00			0.00			0.00			
UCL					0.16			0.16			0.16			0.16			
s <sub>L</sub> <sup>c</sup>					0.00			0.00			0.00			0.00			
LCL					0.00			0.00			0.00			0.00			
UCL					0.16			0.16			0.16			0.16			
s <sub>R</sub> <sup>d</sup>					0.00			0.00			0.00			0.00			
UCL					0.00			0.00			0.00			0.00			
LCL					0.23			0.23			0.23			0.23			
P <sub>T</sub> <sup>e</sup>					1.000			1.000			1.000			1.000			

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix  
<sup>b</sup> Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation, <sup>e</sup> P<sub>T</sub> Value = Homogeneity test of laboratory PODs  
 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit, N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)

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1 **Table 2016.2B:** Comparative Results for the Detection of *Listeria* in 25 g Raw Chicken Breast Fillet Test Portions by the 3M™ MDA 2 - *Listeria* Method vs.  
 2 USDA/FSIS MLG Chapter. 8.09 in a Collaborative Study (Un-inoculated Control)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Raw Chicken Breast Fillet	1	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		2	12	1	0.08	12	1	0.08	12	1	0.08	12	0	0.00	0.08	0.00
		3	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		4	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		5	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		6	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		7	12	1	0.08	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.08
		8	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		9	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		10 <sup>b</sup>	12	0	0.00	12	2	0.17	12	0	0.00	12	0	0.00	0.00	-0.17
		11 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		12	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		13	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00

Estimate	All	132	2	0.02	132	1	0.01	132	1	0.01	132	0	0.00	0.01	0.01
LCL				0.00			0.00			0.00			0.00	-0.02	-0.03
UCL				0.06			0.04			0.04			0.03	0.04	0.05
$s_r^c$				0.12			0.09			0.09			0.00		
LCL				0.11			0.08			0.08			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_L^d$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.05			0.04			0.04			0.16		
$s_R^e$				0.12			0.09			0.09			0.00		
UCL				0.11			0.08			0.08			0.00		
LCL				0.14			0.10			0.10			0.23		
$P_T^f$				0.5190			0.4338			0.4338			1.0000		

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix; <sup>b</sup> Results were not used in statistical analysis due to deviation from testing protocol.  
<sup>c</sup> Repeatability Standard Deviation, <sup>d</sup> Among-Laboratory Standard Deviation, <sup>e</sup> Reproducibility Standard Deviation, <sup>f</sup> P<sub>T</sub> Value = Homogeneity test of laboratory PODs  
 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit, N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)

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1 **Table 2016.2B (cont'd.):** Comparative Results for the Detection of *Listeria* in 25 g Raw Chicken Breast Fillet Test Portions by the 3M™ MDA 2 - *Listeria*  
 2 Method vs. USDA/FSIS MLG Chapter. 8.09 in a Collaborative Study (Low Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Raw Chicken Breast Fillet	1	12	7	0.58	12	7	0.58	12	7	0.58	12	7	0.58	0.00	0.00
		2	12	9	0.75	12	9	0.75	12	9	0.75	12	7	0.58	0.17	0.00
		3	12	10	0.83	12	10	0.83	12	10	0.83	12	5	0.42	0.41	0.00
		4	12	9	0.75	12	9	0.75	12	9	0.75	12	4	0.33	0.42	0.00
		5	12	8	0.66	12	8	0.66	12	8	0.66	12	5	0.42	0.22	0.00
		6	12	10	0.83	12	9	0.75	12	9	0.75	12	6	0.50	0.15	0.08
		7	12	8	0.66	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.16
		8	12	7	0.58	12	7	0.58	12	7	0.58	12	7	0.58	0.00	0.00
		9	12	8	0.66	12	9	0.75	12	8	0.66	12	7	0.58	0.08	-0.08
		10 <sup>b</sup>	12	6	0.50	12	8	0.66	12	6	0.50	12	5	0.42	0.08	-0.16
	MPN/ Test Portion	11 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		12	12	7	0.58	12	7	0.58	12	7	0.58	12	4	0.42	0.16	0.00
		13	12	5	0.42	12	5	0.42	12	5	0.42	12	6	0.50	-0.08	0.00
Estimate	0.66	All	132	88	0.67	132	86	0.65	132	85	0.64	132	64	0.48	0.16	0.02
LCL	0.51				0.58			0.57			0.56			0.40	0.04	-0.10
UCL	0.83				0.75			0.73			0.73			0.57	0.28	0.13
$s_r^c$					0.48			0.48			0.48			0.51		
LCL					0.42			0.43			0.43			0.46		
UCL					0.52			0.52			0.52			0.52		
$s_L^d$					0.00			0.00			0.00			0.00		
LCL					0.00			0.00			0.00			0.00		
UCL					0.18			0.18			0.18			0.14		
$s_R^e$					0.48			0.48			0.49			0.51		
UCL					0.43			0.43			0.43			0.46		
LCL					0.52			0.52			0.52			0.52		
$P_T^f$					0.6044			0.5632			0.6228			0.9192		

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix; <sup>b</sup> Results were not used in statistical analysis due to deviation from testing protocol.

<sup>c</sup> Repeatability Standard Deviation, <sup>d</sup> Among-Laboratory Standard Deviation, <sup>e</sup> Reproducibility Standard Deviation, <sup>f</sup>  $P_T$  Value = Homogeneity test of laboratory PODs

LCL – Lower Confidence Limit; UCL – Upper Confidence Limit, **N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)**

1 **Table 2016.2B (cont'd.):** Comparative Results for the Detection of *Listeria* in 25 g Raw Chicken Breast Fillet Test Portions by the 3M™ MDA 2 - *Listeria*  
 2 Method vs. USDA/FSIS MLG Chapter. 8.09 in a Collaborative Study (High Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R		
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)	
Raw Chicken Breast Fillet		1	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		2	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		3	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		4	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		5	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		6	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		7	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		8	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		9	12	11	0.92	12	12	1.00	12	11	0.92	12	12	1.00	-0.08	-0.08	
		10 <sup>b</sup>	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
		MPN/ Test Portion	11 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
			13	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00	
Estimate	6.24	All	132	131	0.99	132	132	1.00	132	131	0.99	132	132	1.00	-0.01	-0.01	
LCL	3.58				0.96			0.97			0.96			0.97	-0.04	-0.04	
UCL	10.88				1.00			1.00			1.00			1.00	0.02	0.02	
$s_r^c$					0.09			0.00			0.09			0.00			
LCL					0.08			0.00			0.08			0.00			
UCL					0.16			0.16			0.16			0.16			
$s_L^d$					0.00			0.00			0.00			0.00			
LCL					0.00			0.00			0.00			0.00			
UCL					0.04			0.16			0.04			0.16			
$s_R^e$					0.09			0.00			0.09			0.00			
UCL					0.08			0.00			0.08			0.00			
LCL					0.10			0.23			0.10			0.23			
$P_T^f$					0.4338			1.0000			0.4338			1.0000			

3 <sup>a</sup>N/A – Laboratory did not participate or submit data for this matrix; <sup>b</sup>Results were not used in statistical analysis due to deviation from testing protocol.  
 4 <sup>c</sup>Repeatability Standard Deviation, <sup>d</sup>Among-Laboratory Standard Deviation, <sup>e</sup>Reproducibility Standard Deviation, <sup>f</sup> $P_T$  Value = Homogeneity test of laboratory PODs  
 5 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit, N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)  
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**Table 53: Results of Aerobic Plate Count for Collaborating Laboratories**

Lab	Deli Turkey (CFU/g) <sup>a</sup>	Raw Chicken Breast Fillet (CFU/g) <sup>a</sup>
1	2.0 x 10 <sup>1</sup>	2.3 x 10 <sup>4</sup>
2	< 10	3.4 x 10 <sup>4</sup>
3	< 10	4.1 x 10 <sup>3</sup>
4	3.0 x 10 <sup>1</sup>	8.3 x 10 <sup>6</sup>
5	1.0 x 10 <sup>1</sup>	5.4 x 10 <sup>3</sup>
6	< 10	6.3 x 10 <sup>4</sup>
7	6.0 x 10 <sup>1</sup>	3.6 x 10 <sup>2</sup>
8	N/A	2.9 x 10 <sup>3</sup>
9	< 10	4.6 x 10 <sup>2</sup>
10	N/A	3.7 x 10 <sup>4</sup>
11	2.0 x 10 <sup>1</sup>	N/A
12	< 10	7.4 x 10 <sup>3</sup>
13	< 10	2.7 x 10 <sup>4</sup>

<sup>a</sup>Samples analyzed by the 3M Petrifilm Rapid Aerobic Count Plate, AOAC OMA 2015.13

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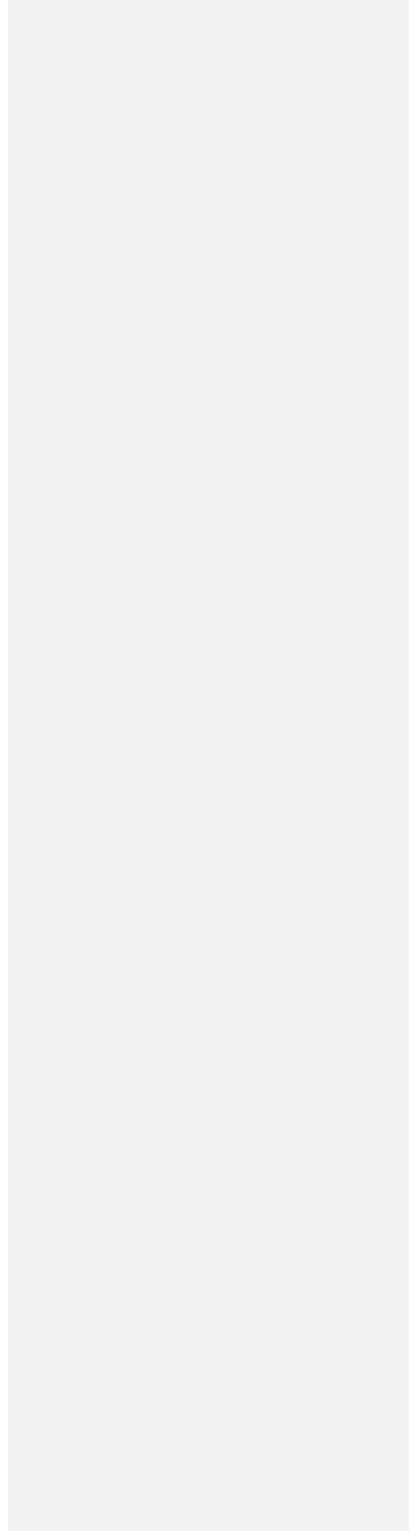
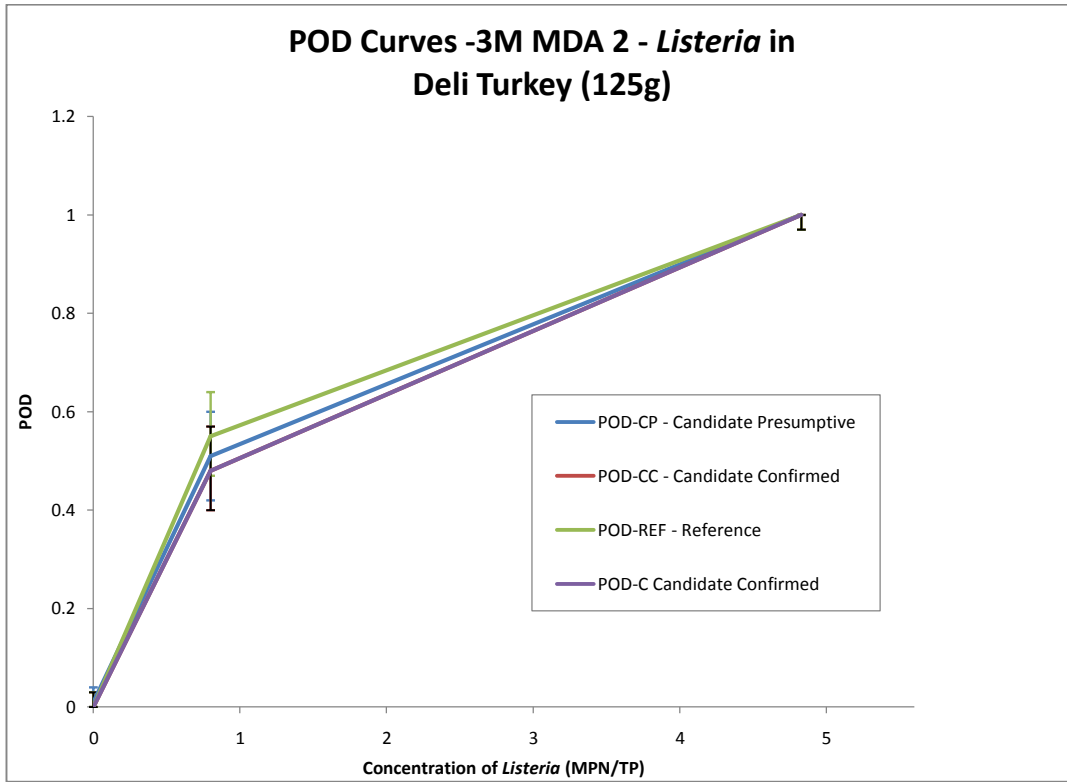


Figure 1A: POD Values of Candidate Method vs. Reference Method for 125 g Deli Turkey



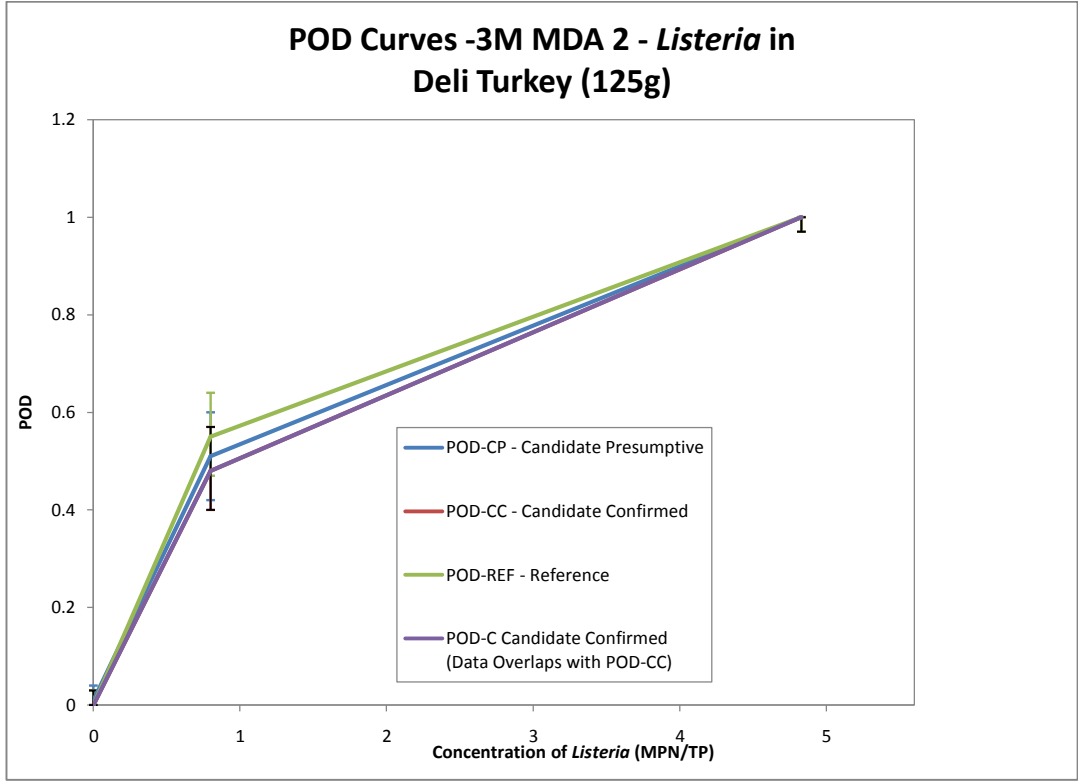


Figure 1B: dLPOD Values of Candidate Method vs. Reference Method for 125 g Deli Turkey

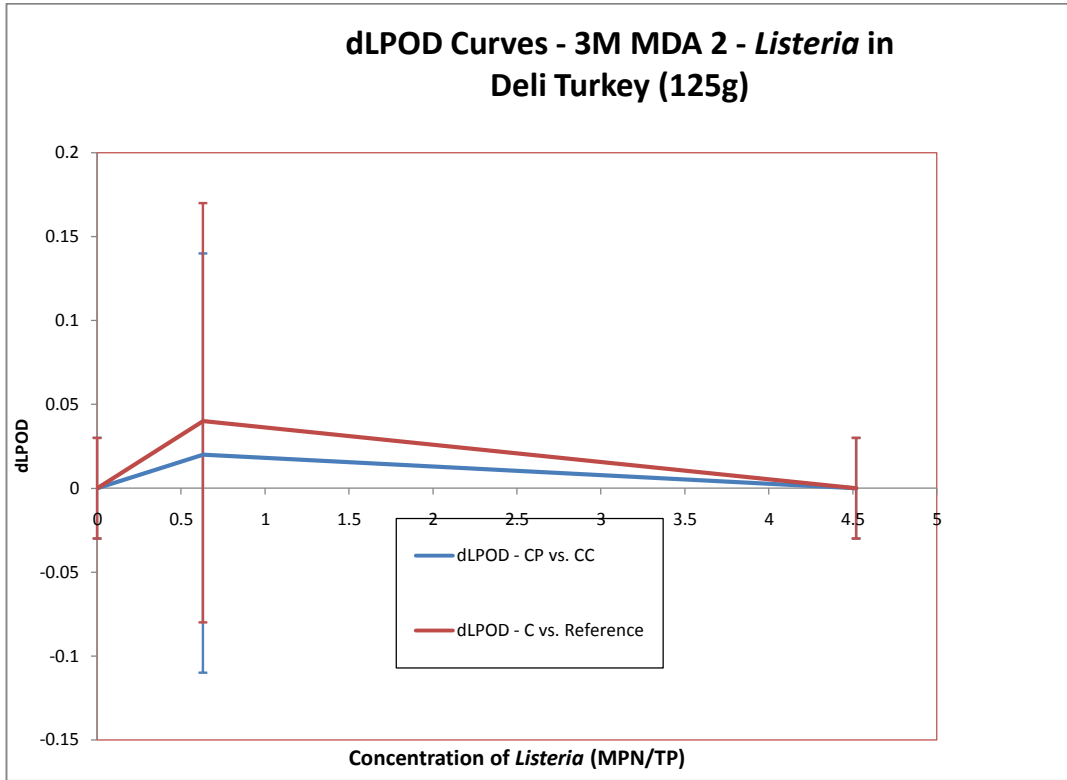
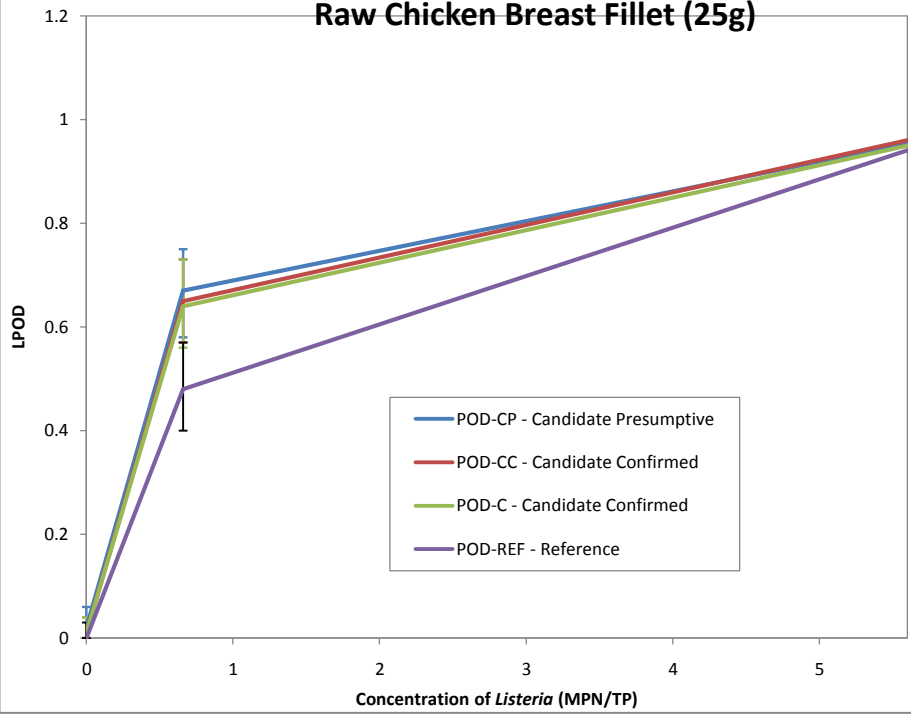


Figure 2A: POD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet

**POD Curves -3M MDA 2 - *Listeria* in Raw Chicken Breast Fillet (25g)**



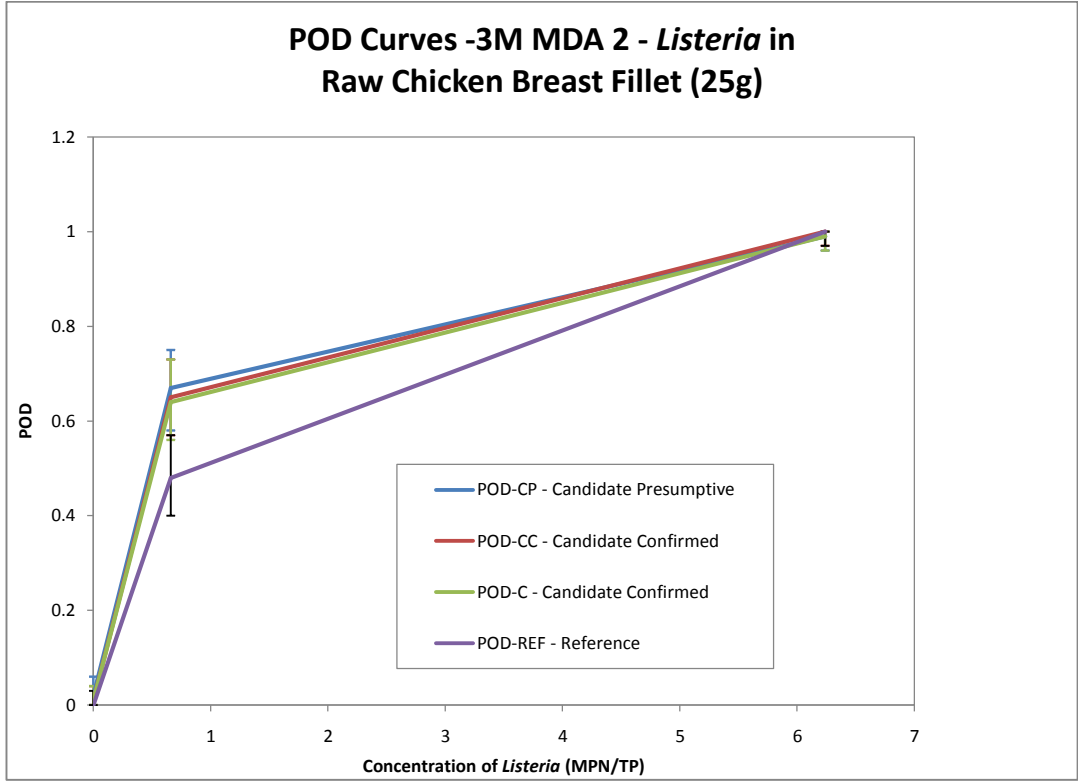
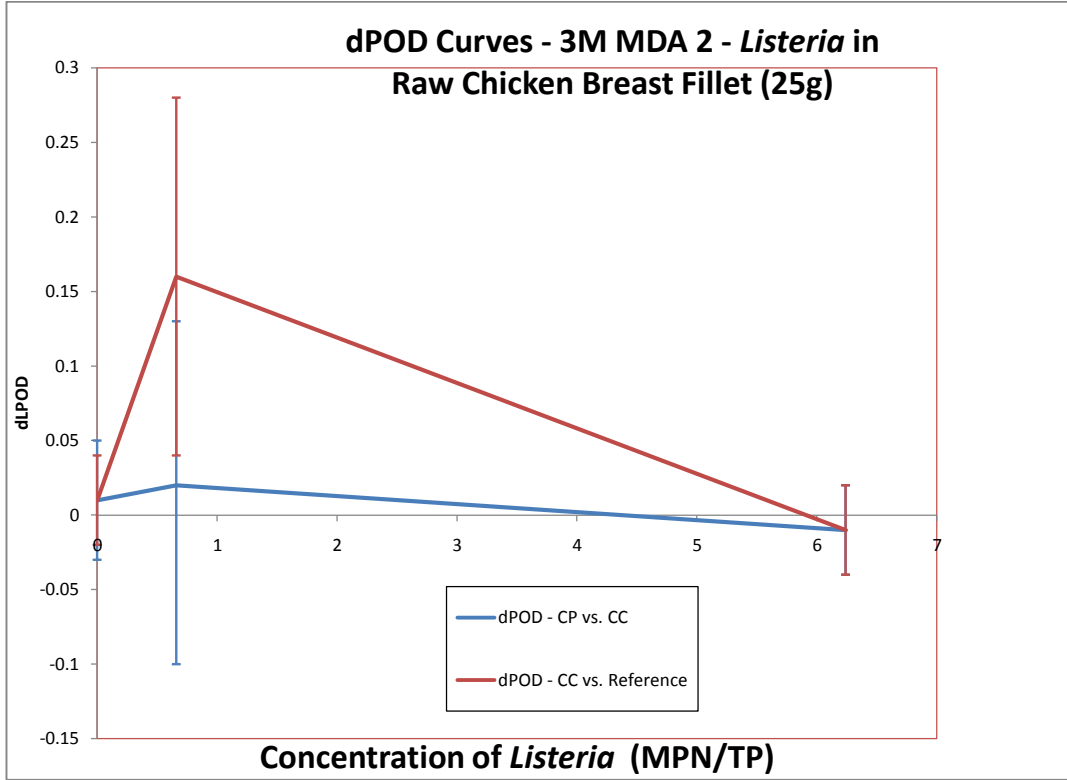


Figure 2B: dPOD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet



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# 3M™ Molecular Detection Assay 2 - *Listeria*

MDA2LIS96

## PRODUCT DESCRIPTION AND INTENDED USE

3M™ Molecular Detection Assay 2 - *Listeria* is used with the 3M™ Molecular Detection System for the rapid and specific detection of *Listeria* species in enriched food and environmental samples.

The 3M Molecular Detection Assays use loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations <sup>(1, 2, 3)</sup>.

The 3M Molecular Detection Assay 2 - *Listeria* is intended for use in a laboratory environment by professionals trained in laboratory techniques. 3M has not documented the use of this product in industries other than food or beverage. For example, 3M has not documented this product for testing water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M Molecular Detection Assay 2 - *Listeria* has not been evaluated with all possible testing protocols or with all possible strains of bacteria.

**As with all test methods, the source, formulation and quality of enrichment medium can influence the results.** Factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may also influence results. 3M recommends evaluation of the method including enrichment medium, in the user's environment using a sufficient number of samples with particular foods and microbial challenges to ensure that the method meets the user's criteria.

3M has evaluated the 3M Molecular Detection Assay 2 - *Listeria* with Demi-Fraser Broth and Fraser Broth as needed. A typical formulation of this medium follows below.

### Demi-Fraser Broth Base Typical Formula (g/L)

Sodium Chloride	20 g
Sodium Phosphate, dibasic, anhydrous *	9.6 g
Beef Extract	5.0 g
Pancreatic Digest of Casein	5.0 g
Peptic Digest of Animal Tissue	5.0 g
Yeast Extract	5.0 g



Lithium Chloride	3.0 g
Potassium Phosphate, monobasic	1.35 g
Esculin	1.0 g
Acriflavin HCl	0.0125 g
Nalidixic Acid	0.01 g

\* Substitute: Sodium Phosphate, dibasic, dihydrate 12.0 g

### Fraser Broth Supplement

(Ingredients per 10 mL vial. One vial is added to one liter of basal medium.)

Ferric Ammonium Citrate 0.5g/10mL

Final pH 7.2 ± 0.2 at 25°C

The 3M™ Molecular Detection Instrument is intended for use with samples that have undergone heat treatment during the assay lysis step, which is designed to destroy organisms present in the sample. Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.

3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

The 3M Molecular Detection Assay 2 - *Listeria* test kit contains 96 tests, described in Table 1.

**Table 1.** Kit Components

Item	Identification	Quantity	Contents	Comments
Lysis Solution (LS) tubes	Pink solution in clear tubes	96 (12 strips of 8 tubes)	580 µL of LS per tube	Racked and ready to use
<i>Listeria</i> Reagent tubes	Blue tubes	96 (12 strips of 8 tubes)	Lyophilized specific amplification and detection mix	Ready to use
Extra caps	Blue caps	96 (12 strips of 8 caps)		Ready to use
Reagent Control (RC)	Clear flip-top tubes	16 (2 pouches of 8 individual tubes)	Lyophilized control DNA, amplification and detection mix	Ready to use
Quick Start Guide		1		

The Negative Control, not provided in the kit, is sterile enrichment medium, e.g., Demi-Fraser Broth. Do not use water as a Negative Control.

## SAFETY

The user should read, understand and follow all safety information in the instructions for the 3M Molecular Detection System and the 3M Molecular Detection Assay 2 - *Listeria*. Retain the safety instructions for future reference.



### WARNING:

Indicates a hazardous situation, which, if not avoided, could result in death or serious injury and/or property damage.



### CAUTION:

Indicates a hazardous situation, which, if not avoided, could result in minor or moderate injury and/or property damage.

### NOTICE:

Indicates a potentially hazardous situation which, if not avoided, could result in property damage.



## WARNING

**Do not use the 3M Molecular Detection Assay 2 - *Listeria* in the diagnosis of conditions in humans or animals.**

**The 3M Molecular Detection Assay 2 - *Listeria* method may generate *Listeria monocytogenes* to levels sufficient to cause stillbirths and fatalities in pregnant women and the immunocompromised, if exposed.**

**The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices, ISO/IEC 17025<sup>(4)</sup>, or ISO 7218<sup>(5)</sup>. The user should complete the 3M Molecular Detection System operator qualification training, as described in the "Installation Qualification (IQ) / Operational Qualification (OQ) Protocols and Instructions for 3M Molecular Detection System" document<sup>(6)</sup>.**

**To reduce the risks associated with a false-negative result leading to the release of contaminated product:**

- Follow the protocol and perform the tests exactly as stated in the product instructions.
- Store the 3M Molecular Detection Assay 2 - *Listeria* as indicated on the package and in the product instructions.
- Always use the 3M Molecular Detection Assay 2 - *Listeria* by the expiration date.
- Use the 3M Molecular Detection Assay 2 - *Listeria* for food and environmental samples that have been validated internally or by a third party.
- Use the 3M Molecular Detection Assay 2 - *Listeria* only for surfaces, sanitizers, protocols and bacterial strains that have been validated internally or by a third party.

- For an environmental sample containing Neutralizing Buffer with aryl sulfonate complex, perform a 1:2 dilution before testing (1 part sample into 1 part sterile enrichment broth). Another option is to transfer 10 µL of the NB enrichment into the LS tubes. 3M™ sample handling products which include Neutralizing Buffer with aryl sulfonate complex: BPPFV10NB, RS96010NB, RS9604NB, SSL10NB, XSLSSL10NB, HS10NB and HS119510NB.

**To reduce the risks associated with exposure to chemicals and biohazards:**

- It is strongly recommended that female laboratory staff be informed of the risk to a developing fetus resulting from infection of the mother through exposure to *Listeria monocytogenes*.
- Perform pathogentesting in a properly equipped laboratory under the control of trained personnel.
- Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples.
- Avoid contact with the contents of the enrichment media and reagent tubes after amplification.
- Dispose of enriched samples according to current industry standards.
- Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.

**To reduce the risks associated with cross-contamination while preparing the assay:**

- Always wear gloves (to protect the user and prevent introduction of nucleases).

**To reduce the risks associated with environmental contamination:**

- Follow current industry standards for disposal of contaminated waste.



**CAUTION**

- Do not exceed the recommended temperature setting on heater.
- Do not exceed the recommended heating time.
- Use an appropriate, calibrated thermometer to verify the 3M™ Molecular Detection Heat Block Insert temperature (e.g., a partial immersion thermometer or digital thermocouple thermometer, not a total immersion thermometer.) The thermometer must be placed in the designated location in the 3M Molecular Detection Heat Block Insert.

**NOTICE**

**To reduce the risks associated with cross-contamination while preparing the assay:**

- Use of sterile, aerosol barrier (filtered), molecular biology grade pipette tips is recommended.
- Use a new pipette tip for each sample transfer.

- Use Good Laboratory Practices to transfer the sample from the enrichment to the lysis tube. To avoid pipettor contamination, the user may choose to add an intermediate transfer step. For example, the user can transfer each enriched sample into a sterile tube.
- Use a molecular biology workstation containing germicidal lamp where available.
- Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with a 1- 5% (v:v in water) household bleach solution or DNA removal solution.

**To reduce the risks associated with a false-positive result:**

- Never open tubes post amplification.
- Always dispose of the contaminated tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

Consult the Safety Data Sheet for additional information and local regulations for disposal.

If you have questions about specific applications or procedures, please visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

**LIMITATION OF WARRANTIES / LIMITED REMEDY**

EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL PRODUCT PACKAGING, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any 3M Food Safety Product is defective, 3M or its authorized distributor will, at its option, replace or refund the purchase price of the product. These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery of any suspected defects in a product and return it to 3M. Please call Customer Service (1-800-328-1671 in the U.S.) or your official 3M Food Safety representative for a Returned Goods Authorization.

**LIMITATION OF 3M LIABILITY**

3M WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO LOST PROFITS. In no event shall 3M's liability under any legal theory exceed the purchase price of the product alleged to be defective.

**USER RESPONSIBILITY**

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety), or contact your local 3M representative or distributor for more information.

When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may influence results.

It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and microbial challenges to satisfy the user that the chosen test method meets the user's criteria.

It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.

As with any test method, results obtained from use of any 3M Food Safety product do not constitute a guarantee of the quality of the matrices or processes tested.

To help customers evaluate the method for various food matrices, 3M has developed the 3M™ Molecular Detection Matrix Control kit. When needed, use the Matrix Control (MC) to determine if the matrix has the ability to impact the 3M Molecular Detection Assay 2 - *Listeria* results. Test several samples representative of the matrix, i.e. samples obtained from different origin, during any validation period when adopting the 3M method or when testing new or unknown matrices or matrices that have undergone raw material or process changes.

A matrix can be defined as a type of product with intrinsic properties such as composition and process. Differences between matrices may be as simple as the effects caused by differences in their processing or presentation for example, raw vs. pasteurized; fresh vs. dried, etc.

## **STORAGE AND DISPOSAL**

Store the 3M Molecular Detection Assay 2 - *Listeria* at 2-8°C. Do not freeze. Keep kit away from light during storage. After opening the kit, check that the foil pouch is undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes should always be stored in the re-sealable pouch with the desiccant inside to maintain stability of the lyophilized reagents. Store resealed pouches at 2-8°C for no longer than 60days.

Do not use 3M Molecular Detection Assay 2 - *Listeria* past the expiration date. Expiration date and lot number are noted on the outside label of the box. After use, the enrichment medium and the 3M Molecular Detection Assay 2 - *Listeria* tubes can potentially contain pathogenic materials. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Safety Data Sheet for additional information and local regulations for disposal.

## **INSTRUCTIONS FOR USE**

Follow all instructions carefully. Failure to do so may lead to inaccurate results.

Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with a 1- 5% (v:v in water) household bleach solution or DNA removal solution.

See Section “ Specific Instructions for validated methods “ for specific requirements:

Table 3 for enrichment protocols according to AOAC Performance Tested <sup>SM</sup>Certificate #111501

Table 4 for enrichment protocols according to NF Validation certificate 3M XXXXX

### **SAMPLE ENRICHMENT**

Table 2 presents guidance for the enrichment of food and environmental samples. It is the user’s responsibility to validate alternate sampling protocols or dilution ratios to ensure this test method meets the user’s criteria.

### **Foods**

1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature.
2. Aseptically combine the enrichment medium and sample according to Table 2. For all meat and highly particulate samples, the use of filter bags is recommended.
3. Homogenize thoroughly by blending, stomaching, or hand mixing for  $2 \pm 0.2$  minutes. Incubate at  $37 \pm 1^\circ\text{C}$  according to Table 2.
4. For raw dairy products, transfer 0.1mL of the primary enrichment into 10 mL of Fraser Broth. Incubate at  $37 \pm 1^\circ\text{C}$  for 20-24 hours.

### **Environmental samples**

Sample collection devices can be a sponge hydrated with a neutralizing solution to inactivate the effects of the sanitizers. 3M recommends the use of a biocide-free cellulose sponge. Neutralizing solution can be Dey-Engley (D/E) Neutralizing Broth or Lethen broth. It is recommended to sanitize the area after sampling.

**WARNING:** Should you select to use Neutralizing Buffer (NB) that contains aryl sulfonate complex as the hydrating solution for the sponge, it is required to perform a 1:2 dilution (1 part sample into 1 part sterile enrichment broth) of the enriched environmental sample before testing in order to reduce the risks associated with a false-negative result leading to the release of contaminated product.

The recommended size of the sampling area to verify the presence or absence of the pathogen on the surface is at least 100 cm<sup>2</sup> (10 cm x 10 cm or 4”x4”). When sampling with a sponge, cover the entire area going in two directions (left to right then up and down) or collect environmental samples following

your current sampling protocol or according to the FDA BAM<sup>(1)</sup>, USDA FSIS MLG<sup>(2)</sup> or ISO 18593<sup>(7)</sup> guidelines.

1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature.
2. Aseptically combine the enrichment medium and sample according to Tables 2,3 or 4.
3. Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ±0.2 minutes. Incubate at 37 ±1°C for 24-30 hours.

**Table 2:** General enrichment protocols using Demi-Fraser Broth Enrichment at 37 ±1°C and Fraser Broth as needed.

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature (°C)	Enrichment Time (hr)				
Heat-processed, cooked, cured meats, poultry, seafood and fish	25 g	225	37	24-30				
Heat-processed / pasteurized dairy products								
Produce and vegetables								
Multi-component foods								
Environmental samples	1 sponge	100 or 225	37	24-30				
	1 swab	10	37	24-30				
Raw meat, poultry, seafood, fish	25 g	475	37	28-32				
Sample Matrix	Primary Enrichment (Demi-Fraser Broth)				Secondary Enrichment (Fraser Broth)			Sample Analysis Volume <sup>(a)</sup>
	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature (°C)	Enrichment Time (hr)	Sample Size	Enrichment Temperature (°C)	Enrichment Time (hr)	
Raw dairy products	25 g	225	37	20-24	Transfer 0.1 mL into 10 mL Fraser Broth	37	20-24	10 µL

(a) Volume of sample transferred to Lysis Solution tubes. Refer to Step 4.6 of Lysis section.

**Specific Instructions for Validated Methods**  
**AOAC® Performance Tested Method<sup>SM</sup>#111501**



In AOAC PTM studies, the 3M Molecular Detection Assay 2 – *Listeria* was found to be an effective method for the detection of *Listeria* species. The matrices tested in the study are shown in Table 3. The limit of detection of the 3M Molecular Detection Assay 2 – *Listeria* method is 1-5 colony forming units per validated test portion size (in Table 3).

**Table 3.** Enrichment protocols using Demi-Fraser Broth at 37 ± 1 °C according to AOAC Performance Tested<sup>SM</sup> Certificate #111501

Sample Matrix		Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)
Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon		25 g	225	24-30
Raw chicken		25 g	475	28-32
Deli turkey		125 g	1125	24-30
Cantaloupe <sup>(a)</sup>		Whole melon	Enough volume to allow melon to float	26-30
Environmental samples:	Stainless steel	1 sponge	225	24-30
	Sealed concrete	1 sponge	100	24-30
	Plastic <sup>(b)</sup>	1 swab	10	24-30

All samples for the AOAC validation were homogenized by stomaching unless otherwise noted.

(a) Homogenize sample by hand mixing

(b) Homogenize sample by vortexing



NF VALIDATION by AFNOR Certification



3M 01/04-05/16

ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS

<http://nf-validation.afnor.org/en>

For more information about end of validity, please refer to NF VALIDATION certificate available on the website mentioned above.

NF VALIDATION certified method in compliance with ISO 16140-2<sup>(8)</sup> in comparison to ISO 11290-1<sup>(3)</sup>

**Scope of the validation:** All human food and environmental samples  
(excluding primary production samples)

**Sample preparation:** Samples should be prepared according to EN ISO 11290-1<sup>(3)</sup> and EN ISO 6887<sup>(9)</sup>

**Software version:** See certificate

**Table 4.** Enrichment protocols according to NF VALIDATION certified method 3M 01/04-05/16.

General Protocol	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature ( $\pm 1^{\circ}\text{C}$ )	Enrichment Time (hr)	Sample Analysis Volume <sup>(a)</sup>	Recommended Interruption Point			
All food samples(except ed raw meats, raw seafood and raw dairy products)	25 g	225	37	24-30	20 $\mu\text{L}$	<ul style="list-style-type: none"> <li>•DF broth up to 72 hr</li> <li>•lysate at <math>-20^{\circ}\text{C}</math></li> <li>•lysate at <math>4^{\circ}\text{C}</math> up to 72 hr</li> </ul>			
Environmental samples	25 g, 1 swab, or 1 wipe	225	37	24-30	20 $\mu\text{L}$	<ul style="list-style-type: none"> <li>•DF broth up to 72 hr</li> <li>•lysate at <math>-20^{\circ}\text{C}</math></li> </ul>			
Specific Protocol 1	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature ( $\pm 1^{\circ}\text{C}$ )	Enrichment Time (hr)	Sample Analysis Volume (a) ( $\mu\text{L}$ )	Recommended Interruption Point			
Raw meats and raw seafood	25 g	475	37	28-32	20 $\mu\text{L}$	<ul style="list-style-type: none"> <li>•DF broth up to 72 hr</li> <li>•lysate at <math>-20^{\circ}\text{C}</math></li> <li>•lysate at <math>4^{\circ}\text{C}</math> up to 72 hr</li> </ul>			
Specific Protocol 2	Primary Enrichment (Demi-Fraser Broth)				Secondary Enrichment (Fraser Broth)				
	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature ( $\pm 1^{\circ}\text{C}$ )	Enrichment Time (hr)	Sample Size	Enrichment Temperature ( $\pm 1^{\circ}\text{C}$ )	Enrichment Time (hr)	Sample Analysis Volume <sup>(a)</sup>	Recommended Interruption Point
Raw dairy products	25 g	225	37	20-24	Transfer 0.1 mL into 10 mL Fraser Broth	37	20-24	10 $\mu\text{L}$	<ul style="list-style-type: none"> <li>•DF broth upto 72 hr</li> <li>•lysate at <math>-20^{\circ}\text{C}</math></li> </ul>

(a) Volume of sample transferred to Lysis Solution tubes. Refer to Step 4.6 of Lysis section.

(b) Volume of sample transferred to Lysis Solution tubes. Refer to Step 4.6 of Lysis section.

**Note**

- Samples larger than 25g have not been tested in the NF validation study.

#### **PREPARATION OF THE 3M™ MOLECULAR DETECTION SPEED LOADER TRAY**

1. Wet a cloth with a 1-5% (v:v in water) household bleach solution and wipe the 3M™ Molecular Detection Speed Loader Tray.
2. Rinse the 3M Molecular Detection Speed Loader Tray with water.
3. Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.
4. Ensure the 3M Molecular Detection Speed Loader Tray is dry before use.

#### **PREPARATION OF THE 3M™ MOLECULAR DETECTION CHILL BLOCK INSERT**

Place the 3M™ Molecular Detection Chill Block directly on the laboratory bench; (the 3M™ Molecular Detection Chill Block Tray is not used). Use the chill block at ambient laboratory temperature (20-25°C).

#### **PREPARATION OF THE 3M™ MOLECULAR DETECTION HEAT BLOCK INSERT**

Place the 3M™ Molecular Detection Heat Block Insert in a dry double block heater unit. Turn on the dry block heater unit and set the temperature to allow the 3M Molecular Detection Heat Block Insert to reach and maintain a temperature of  $100 \pm 1^\circ\text{C}$ .

**NOTE:** Depending on the heater unit, allow approximately 30 minutes for the 3M Molecular Detection Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (e.g., a partial immersion thermometer or digital thermocouple thermometer, not a total immersion thermometer) placed in the designated location, verify that the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^\circ\text{C}$ .

#### **PREPARATION OF THE 3M™ MOLECULAR DETECTION INSTRUMENT**

1. Launch the 3M™ Molecular Detection Software and log in.
2. Turn on the 3M Molecular Detection Instrument.
3. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection System User Manual for details.

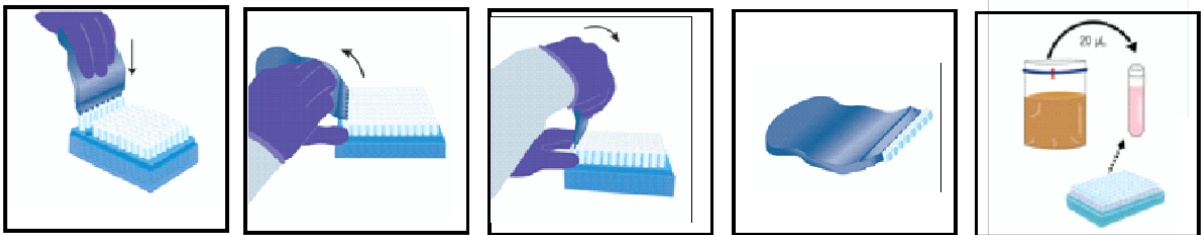
**NOTE:** The 3M Molecular Detection Instrument must reach and maintain temperature of  $60^\circ\text{C}$  before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 min and is indicated by an ORANGE light on the instrument's status bar. When the instrument is ready to start a run, the status bar will turn GREEN.

## LYSIS

1. Allow the lysis solution (LS) tubes to warm up by setting the rack at room temperature (20-25 °C) overnight (16-18 hours). Alternatives to equilibrate the LS tubes to room temperature are to set the LS tubes on the laboratory bench for at least 2 hours, incubate the LS tubes in a 37 ±1°C incubator for 1 hour or place them in a dry double block heater for 30 seconds at 100°C.
2. Invert the capped tubes to mix. Proceed to next step within 4 hrs.
3. Remove the enrichment broth from the incubator.
4. One LS tube is required for each sample and the Negative Control (NC) (sterile enrichment medium) sample.
  - 4.1 LS tube strips can be cut to desired LS tube number. Select the number of individual LS tubes or 8-tube strips needed. Place the LS tubes in an empty rack.
  - 4.2 To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipette tip for each transfer step.
  - 4.3 Transfer enriched sample to LS tubes as described below:

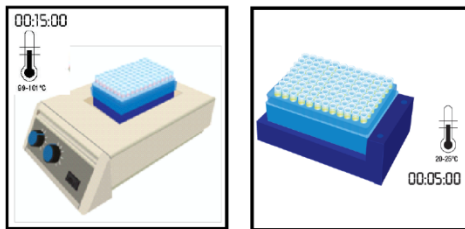
Transfer each enriched sample into an individual LS tube **first**. Transfer the NC **last**.

- 4.4 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip - one strip at a time.
  - 4.5 Discard the LS tube cap – if lysate will be retained for retest, place the caps into a clean container for re-application after lysis. For processing of retained lysate, see Appendix A.
  - 4.6 Transfer 20 µL of sample into a LS tube unless otherwise indicated in Protocol Tables 2, 3, and 4(e.g., raw dairy products use 10 µL).
5. Repeat step 4.2 until each individual sample has been added to a corresponding LS tube in the strip.



6. Repeat steps 4.1 to 4.6 as needed, for the number of samples to be tested.
7. When all samples have been transferred, transfer 20 µL of NC (sterile enrichment medium, e.g., Demi-Fraser Broth) into a LS tube. Do not use water as a NC.

8. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^\circ\text{C}$ .
9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for  $15 \pm 1$  minutes. During heating, the LS solution will change from pink (cool) to yellow (hot). Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.
10. Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes. The 3M Molecular Chill Block Insert, used at ambient temperature without the Molecular Detection Chill Block Tray should sit directly on the laboratory bench. When cool, the lysis solution will revert to a pink color.
11. Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.



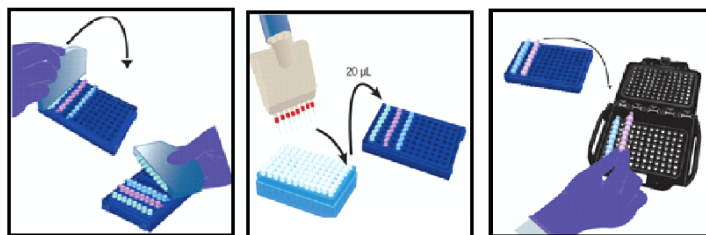
## AMPLIFICATION

1. One Reagent tube is required for each sample and the NC.
  - 1.1 Reagent tube strips can be cut to desired tube number. Select the number of individual Reagent tubes or 8-tube strips needed.
  - 1.2 Place Reagent tubes in an empty rack.
  - 1.3 Avoid disturbing the reagent pellets from the bottom of the tubes.
2. Select 1 Reagent Control (RC) tube and place in rack.
3. To avoid cross-contamination, decap one Reagent tube strip at a time and use a new pipette tip for each transfer step.
4. Transfer lysate to Reagent tubes and RC tube as described below:

Transfer each sample lysate into individual Reagent tubes **first** followed by the NC. Hydrate the RC tube **last**.

5. Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent tubes –one Reagent tube strip at a time. Discard cap.

- 5.1 **Transfer 20  $\mu$ L of Sample lysate from the upper  $\frac{1}{2}$  of the liquid (avoid precipitate) in the LS tube into corresponding Reagent tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.**
- 5.2 Repeat step 5.1 until individual Sample lysate has been added to a corresponding Reagent tube in the strip.
- 5.3 Cover the Reagent tubes with the provided extra caps and use the rounded side of the 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.
- 5.4 Repeat step 5.1 as needed, for the number of samples to be tested.
- 5.5 When all sample lysates have been transferred, repeat 4.1 to transfer 20  $\mu$ L of NC lysate into a Reagent tube.
- 5.6 **Transfer 20  $\mu$ L of NC lysate into a RC tube.** Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
6. Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader Tray. Close and latch the 3M Molecular Detection Speed Loader Tray lid.



7. Review and confirm the configured run in the 3M Molecular Detection Software.
8. Click the Start button in the software and select instrument for use. The selected instrument's lid automatically opens.
9. Place the 3M Molecular Detection Speed Loader Tray into the 3M Molecular Detection Instrument and close the lid to start the assay. Results are provided within 75 minutes, although positives may be detected sooner.
10. After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from the 3M Molecular Detection Instrument and dispose of the tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

**NOTICE:** To minimize the risk of false positives due to cross-contamination, never open reagent tubes containing amplified DNA. This includes Reagent Control, Reagent, and Matrix Control tubes. Always dispose of sealed reagent tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

## RESULTS AND INTERPRETATION

An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analyzed automatically by the software and are color-coded based on the result. A Positive or Negative result is determined by analysis of a number of unique curve parameters. Presumptive positive results are reported in real-time while Negative and Inspect results will be displayed after the run is completed.

Presumptive positive samples should be confirmed as per the laboratory standard operating procedures or by following the appropriate reference method confirmation <sup>(1, 2, 3)</sup>, beginning with transfer from the primary enrichment to secondary enrichment broth (if applicable), followed by subsequent plating and confirmation of isolates using appropriate biochemical and serological methods.

In the context of the NF VALIDATION, all samples identified as positive by the 3M Molecular Detection Assay 2 – *Listeria* must be confirmed by one of the following tests:

**Option 1:** Using the ISO 11290-1<sup>(3)</sup> standard starting from Demi-Fraser enrichment

**Option 2:** Implementing a confirmation method consisting of the following: Transfer 0.1 mL of the Demi-Fraser broth. Streak directly onto selective agar described in ISO 11290-1<sup>(3)</sup>.

**Option 3:** Using nucleic acid probes as described in EN ISO 7218<sup>(5)</sup> standard, performed on isolated colonies, from selective agar (see Options 1 or 2).

**Option 4:** Using any other method certified NF VALIDATION, the principle of which must be different from 3M Molecular Detection Assay 2 – *Listeria*. The complete protocol described for this second validated method must be used. All steps prior to the start of confirmation must be common to both methods.

In the event of discordant results (presumptive positive with the alternative method, non-confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

**NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular Detection Assay 2 - *Listeria* amplification reagents have a “background” relative light unit (RLU) reading.

In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M recommends the user to repeat the assay for any Inspect samples. If the result continues to be Inspect, proceed to confirmation test using your preferred method or as specified by local regulations.

If you have questions about specific applications or procedures, please visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

#### **Appendix A. Protocol Interruption: Storage and re-testing of heat-treated lysates**

1. To store a heat-treated lysate, re-cap the lysis tube with a clean cap (see “LYSIS”, 4.5)
2. Store at 4 to 8°C for up to 72 hours.
3. Prepare a stored sample for amplification by inverting 2-3 times to mix.
4. Decap the tubes.
5. Place the mixed lysate tubes on 3M Molecular Detection Heat Block Insert and heat at  $100 \pm 1^\circ\text{C}$  for  $5 \pm 1$  minutes.
6. Remove the rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes.
7. Continue the protocol at the ‘Amplification’ section detailed above.

#### **REFERENCES:**

1. US Food and Drug Administration Bacteriological Analysis Manual. Chapter 10: Detection and Enumeration of *Listeria monocytogenes* in Foods. January 2016 Version.
2. US Department of Agriculture (USDA) FSIS Microbiology Laboratory Guidebook 8.08. Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry and Egg Products, and Environmental Samples. Effective Date: May 1, 2013.
3. ISO 11290-1. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes*. Amendment 1, 2004-10-15.
4. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
5. ISO 7218. Microbiology of food and animal feeding stuffs – General rules for microbiological examination.
6. 3M. Installation Qualification (IQ) / Operational Qualification (OQ) Protocols and Instructions for 3M Molecular Detection System.
7. ISO 18593. Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.



8. ISO 16140-2 Microbiology of the food chain – Method validation – Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.
9. ISO 6887. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.

#### EXPLANATION OF PRODUCT LABEL SYMBOLS



Caution or Warning, see product instructions



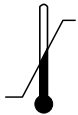
Consult product instructions



The lot in a box represents the lot number



The hourglass is followed by a month and year which represent the expiration date



Storage temperature limitations

1 **A Comparative Evaluation of the 3M™ Molecular Detection Assay 2 (MDA 2) - *Listeria* for**  
2 **the Detection of *Listeria* species in a Variety of Foods and Environmental Surfaces**

3  
4 **AOAC Performance Tested Methods<sup>SM</sup> 111501**

5  
6 **Abstract**

7  
8 The 3M™ Molecular Detection Assay 2 (MDA2) - *Listeria* for the detection of *Listeria* species  
9 (*L. monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. innocua*, *L. grayi*, *L. welshimeri*, *L. fleishmanii*  
10 subsp. *coloradensis*, *L. marthii*, *L. grandensis*, *L. cornellensis*, *L. aquatic*, *L. newyorkensis*, *L.*  
11 *weihenstephanensis*, *L. baoriae*, *L. fleishmanii* subsp. *fleishmanii*, *L. ivanovii* subsp. *londoniensis*  
12 and *L. riparia*) was evaluated following the 2012 AOAC Research Institute *Performance Tested*  
13 *Methods<sup>SM</sup>* program guidelines. The evaluation included inclusivity/exclusivity, lot-to-  
14 lot/stability, robustness and matrix studies. The 3M MDA 2 - *Listeria* was compared to various  
15 reference methods for raw chicken, bagged raw spinach, cold smoked salmon, deli turkey, whole  
16 melons, vanilla ice cream, queso fresco, 4% milk fat cottage cheese, beef hot dogs, stainless  
17 steel, sealed concrete and plastic in the matrix study. Twenty replicates of each food matrix were  
18 analyzed at a low inoculum level of 0.2-2 colony forming units (CFU)/standard test portion, five  
19 replicates were analyzed at a high level of 2-5 CFU/test portion, and five control replicates were  
20 analyzed at 0 CFU/standard test portion with the exception of naturally contaminated raw  
21 chicken (leg pieces) analyzed at the independent laboratory. Naturally contaminated raw chicken  
22 (leg pieces), analyzed at the independent laboratory, were evaluated using two separate lots  
23 consisting of twenty replicates for each lot. All environmental surfaces were analyzed using  
24 twenty replicates at a low inoculum level of ~ 50 CFU/standard area, five replicates were  
25 analyzed at a high level of ~ 100 CFU/standard area, and five control replicates were analyzed at  
26 0 CFU/standard test area. Based on the probability of detection statistical model, there were no  
27 statistically significant differences between the number of presumptive and confirmed positive  
28 samples or between the candidate and reference method. There were no unexpected results in the  
29 lot-to-lot/stability, robustness studies. In the inclusivity/exclusivity study, 55 target organisms  
30 and 30 non-target organisms were tested following the 3M MDA 2 - *Listeria* method with all  
31 target organisms detected and none of the non-target organisms detected. The results of this  
32 evaluation demonstrate the specificity and sensitivity of the 3M MDA 2 - *Listeria* and its ability  
33 to accurately detect *Listeria* species in select food matrixes (beef hot dogs, queso fresco, vanilla  
34 ice cream, 4% milk fat cottage cheese, 3% whole milk, bagged raw spinach, cold smoked  
35 salmon, raw chicken parts, deli turkey, whole cantaloupe) and environmental surfaces (stainless  
36 steel, sealed concrete, plastic), 24-30 hours post enrichment.

37  
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23  
24 **Scope of Method**

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26 a) *Target organism.*— *Listeria* species (*L. monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. innocua*,  
27 *L. grayi*, *L. welshimeri*, *L. fleishmanii* subsp. *coloradensis*, *L. marthii*, *L. grandensis*, *L.*  
28 *cornellensis*, *L. aquatic*, *L. newyorkensis*, *L. weihenstephanesis*, *L. baoriae*, *L. fleishmanii*  
29 *subsp. fleishmanii*, *L. ivanovii* subsp. *londoniensis* and *L. riparia*) (~~*L. monocytogenes*, *L.*~~  
30 ~~*seeligeri*, *L. ivanovii*, *L. innocua*, *L. grayi*, *L. welshimeri*, *L. fleishmanii* subsp. *coloradensis*,  
31 ~~*L. cornellensis*, *L. grandensis*, *L. marthii*, and *L. riparia*~~)  
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33 b) *Matrixes.*— Raw chicken (leg pieces and fillets) (25 g), bagged raw spinach (25 g), cold  
34 smoked salmon (25 g), deli turkey (125 g), whole melon, vanilla ice cream (25 g), queso  
35 fresco (25 g), 4% milk fat cottage cheese (25 g), beef hot dogs (25 g), stainless steel (4" x 4",  
36 sponge enriched in 225 mL), sealed concrete (4" x 4", sponge enriched in 225 mL), plastic  
37 (1" x 1", 3M™ Teera™ Enviro Sswab [3M Australia Pty Ltd] enriched in 10 mL).  
38  
39 c) *Summary of Validated Performance Claims.*— Performance equivalent to that of the U.S.  
40 Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM) Chapter 10  
41 [1] for whole melon, cold smoked salmon, bagged raw spinach; the U.S. Department of  
42 Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook*  
43 (USDA-FSIS/MLG) 8.09 [2] for beef hot dogs, deli turkey, raw chicken, sealed concrete,  
44 plastic and stainless steel and AOAC 993.12 [3] for queso fresco, vanilla ice cream, 4% milk  
45 fat cottage cheese and for ISO 11290-1/A1 [4] for cold smoked salmon and bagged raw  
46 spinach.~~

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## Definitions

- a) *Probability of Detection (POD)*.—The proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent. Several POD measures can be calculated;  $POD_R$  (reference method POD),  $POD_C$  (confirmed candidate method POD),  $POD_{CP}$  (candidate method presumptive result POD) and  $POD_{CC}$  (candidate method confirmation result POD).
- b) *Difference of Probabilities of Detection (dPOD)*.—Difference of probabilities of detection is the difference between any two POD values. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

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## Principle of the Method

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3M™ Molecular Detection Assay 2 - *Listeria* is used with the 3M™ Molecular Detection System for the rapid and specific detection of *Listeria* species in enriched food and environmental samples.

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The 3M Molecular Detection Assays use loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed. Presumptive positive results should be confirmed using the laboratory's preferred method or as specified by local regulations.

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## General Information

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*Listeria* is a small, Gram-positive rod that can be found in soil or carried by animals. Many foods have been associated with *Listeria* contamination: uncooked meats, uncooked vegetables, cantaloupe, pasteurized and unpasteurized milk, and other dairy and processed foods. [5] The resulting illness, listeriosis, from *Listeria monocytogenes* ingestion is rare but can be fatal. The disease may appear as meningitis in adults and can affect unborn children due to its ability to cross through the placenta. *Listeria* contamination often occurs post-cooking, before packaging as *Listeria* can survive longer under adverse environmental conditions than most other vegetative bacteria that present a concern for the food safety industry. [6] Manufacturing facilities implement sanitation practices and regular surface screening for the presence of *Listeria* on food contact surfaces to monitor their food production environments. The regular surface screening for non - *Listeria monocytogenes* species is used as an indicator to monitor for the risk of *Listeria monocytogenes* contamination and is typically part of a food manufacturing facility's Hazard Analysis Critical Control Point (HACCP) plan. The 3M MDA 2 - *Listeria* was developed to provide rapid and specific detection of *Listeria* in enriched food samples and on food processing surfaces.

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## Materials and Methods

1 Test Kit Information

- 2  
3 a) *Kit Name.*— Molecular Detection Assay 2 – *Listeria*  
4 b) *Catalog Number.*— MDA2LIS96  
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6 *Test Kit Reagents - 3M MDA 2 - Listeria (96 tests)*

- 7 a) *Lysis Solution (LS) tubes.* – 96 (12 strips of 8 tubes)  
8 b) *Listeria Reagent tubes.* – 96 (12 strips of 8 tubes)  
9 c) *Extra caps.* – 96 (12 strips of 8 caps)  
10 d) *Reagent Control (RC).* – 16 (2 pouches of 8)  
11 e) *Quick Start Guide*  
12

13 *Additional Supplies and Reagents*

- 14 a) 3M Molecular Detection System (instrument), power supply, cord, USB cable and  
15 software  
16 b) 3M Molecular Detection Speed Loader Tray  
17 c) 3M Molecular Detection Chill Block insert  
18 d) 3M Molecular Detection Heat Block Insert  
19 e) 3M Molecular Detection Cap/Decap Tool [Reagent]  
20 f) 3M Molecular Detection Cap/Decap Tool [Lysis]  
21 g) Empty lysis tube rack  
22 h) Empty reagent tube rack  
23 i) Dey-Engley (D/E) Neutralizing Broth  
24 j) Demi Fraser Broth (with the addition of Ferric Ammonium Citrate [FAC])  
25 k) 3M™ Hydrated Sponge Stick with 10 mL of D/E (3M Center St. Paul, MN)  
26 l) 3M™ Molecular Detection Matrix Control Kit (3M Center St. Paul, MN)  
27

28 *Additional media*

- 29 b) Oxford Agar (OX)  
30 c)  
31 d) Fraser Broth (FB)  
32 e) Sheep Blood Agar (SBA)  
33 f) PALCAM Agar  
34 g) Ottaviani Agosti Agar (OAA)  
35 h) Trypticase Soy Agar (TSA)  
36 i) Trypticase Soy Agar with 0.6% Yeast Extract (TSA/YE)  
37 j) De Man, Rogosa and Sharpe (MRS)  
38 k) Modified Oxford Agar (MOX)  
39 l) Brain Heart Infusion (BHI) broth  
40 m) Horse Blood Overlay (HBO)  
41 n) Buffered *Listeria* Enrichment Broth (BLEB)  
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43 *Apparatus*

- 44 a) *Pipettes.* – capable of 20µL  
45 b) *Multi-channel pipette.* – capable of 20µL  
46 c) *Sterile pipette tips.* – capable of 20µL  
47 d) *Stomacher®.* – Seward or equivalent

- 1 e) *Filter Stomacher*® bags. - Seward or equivalent  
2 f) *Thermometer*. – calibrated range to include  $100 \pm 1^\circ\text{C}$  range  
3 g) *Incubators*. – capable of maintaining  $37 \pm 1^\circ\text{C}$   
4 h) *Dry double block heater unit*. – capable of maintaining  $100 \pm 1^\circ\text{C}$ ; or a *water bath*  
5 capable of maintaining  $100 \pm 1^\circ\text{C}$   
6 i) *Refrigerator*. – capable of maintaining  $2\text{-}8^\circ\text{C}$ , for storing the 3M MDA2  
7 j) *Computer*. – compatible with the 3M Molecular Detection System (instrument)  
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### 9 **Safety Precautions**

10 The user should read, understand and follow all safety information in the instructions for the 3M  
11 Molecular Detection System and the 3M Molecular Detection Assay 2 - *Listeria*. Retain the  
12 safety instructions for future reference.  
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14  
15 **As with all test methods, the source, formulation and quality of enrichment medium can**  
16 **influence the results.** Factors such as sampling methods, testing protocols, sample preparation,  
17 handling, and laboratory technique may also influence results. 3M recommends evaluation of  
18 the method including enrichment medium, in the user's environment using a sufficient number of  
19 samples with particular foods and microbial challenges to ensure that the method meets the  
20 user's criteria.

21 The 3M Molecular Detection Assay 2 - *Listeria* method may generate *Listeria monocytogenes* to  
22 levels sufficient to cause stillbirths and fatalities in pregnant women and the  
23 immunocompromised, if exposed.  
24

To reduce the risks associated with exposure to chemicals and biohazards:

- It is strongly recommended that female laboratory staff be informed of the risk to a developing fetus resulting from infection of the mother through exposure to *Listeria monocytogenes*
- Perform pathogen testing in a properly equipped laboratory under the control of trained personnel
- Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples
- Avoid contact with the contents of the enrichment media and reagent tubes after amplification
- Dispose of enriched samples according to current industry standards

### 25 **Sample Preparation**

26 3M recommends the use of Demi-Fraser Broth with FAC for the enrichment of food and  
27 environmental samples. Table 1 presents guidance for the enrichment of food and environmental  
28 samples. It is the user's responsibility to validate alternate sampling protocols or dilution ratios  
29 to ensure this test method meets the user's criteria.  
30

#### 31 *Foods*

- 32
- 33 a) Allow the Demi-Fraser Broth enrichment medium (includes FAC) to equilibrate to ambient  
34 laboratory temperature.
  - 35 b) Aseptically combine the enrichment medium and sample according to Table 1. For all meat  
36 and highly particulate samples, the use of filter bags is recommended.

- 1 | c) Homogenize thoroughly by ~~blending, stomaching,~~ or hand mixing for 2 ±0.2 minutes.  
2 | Incubate at 37 ±1°C according to Table 1.  
3 |

#### 4 | *Environmental samples*

5 | Sample collection devices can be a sponge hydrated with a neutralizing solution to inactivate the  
6 | effects of the sanitizers. 3M recommends the use of a biocide-free cellulose sponge. Neutralizing  
7 | solution can be Dey-Engley (D/E) Neutralizing Broth or Lethen Broth. It is recommended to  
8 | sanitize the area after sampling.  
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10 | The recommended size of the sampling area for verifying the presence or absence of the  
11 | pathogen on the surface is at least 100 cm<sup>2</sup> (10 cm x 10 cm or 4"x4"). When sampling with a  
12 | sponge, cover the entire area going in two directions (left to right then up and down) or collect  
13 | environmental samples following your current sampling protocol or according to the FDA BAM,  
14 | USDA FSIS MLG or ISO 18593 [7] guidelines.  
15 |

- 16 | 1. Allow the Demi-Fraser Broth enrichment medium (includes FAC) to equilibrate to ambient  
17 | laboratory temperature.  
18 | 2. Aseptically combine the enrichment medium and sample according to Table 1.  
19 | 3. Homogenize thoroughly by ~~blending, vortexing or~~ stomaching, ~~or hand mixing~~ for 2 ±0.2  
20 | minutes. Incubate at 37 ±1°C for 24-30 hours.  
21 | 4. Invert room temperature (20-25 °C) lysis tubes to mix. Proceed to next step within 4 hours.  
22 | 20 µL aliquot of each enriched sample are is transferred to separate lysis tubes using a new  
23 | pipette tip after each sample transfer. Place uncovered samples on a dry double block heater  
24 | for 15 ± 1 minutes at 100 ± 1 °C. Following the heat lysis transfer samples into the chill  
25 | block insert and placed onto a sterilized lab bench and allow to cool at 18-28 °C for 5-10  
26 | minutes.  
27 | 5. Add 20 µL of each lysed sample and control to separate reagent tubes, and mix by pipetting  
28 | up and down five times. Analyze a matrix control tube with the samples for each matrix to  
29 | verify that no interference with the assay was caused by the matrix. Use sterile Demi Fraser  
30 | Broth with FAC for the Negative Control (NC).  
31 | 6. Transfer a 20 µL aliquot to the NC and the Reagent Control (RC) tubes. Add a 20 µL aliquot  
32 | of a randomly picked sample to the matrix control tube, mixed, and recapped. Using the 3M™  
33 | software, follow prompts to identify samples and controls. Load all samples into the Speed  
34 | Loader Tray (SLT), place into the Molecular Detection System, and initiate the 3M™ MDA 2  
35 | - *Listeria* assay. Results are obtained within 75 minutes.  
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#### 38 | **Interpretation and Test Result Report**

39 | An algorithm interprets the light output curve resulting from the detection of the nucleic acid  
40 | amplification. Results are analyzed automatically by the software and are color-coded based on  
41 | the result. A Positive or Negative result is determined by analysis of a number of unique curve  
42 | parameters. Presumptive positive results are reported in real-time while Negative and Inspect  
43 | results will be displayed after the run is completed.  
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1 **NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular  
2 Detection Assay 2 - *Listeria* amplification reagents have a “background” relative light unit  
3 (RLU) reading.  
4

5 In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M  
6 recommends the user to repeat the assay for any Inspect samples. If the result continues to be  
7 Inspect, proceed to confirmation test using your preferred method or as specified by local  
8 regulations.  
9

### 10 **Confirmation**

11 Presumptive positive primary enrichment samples were confirmed by following the appropriate  
12 reference method confirmation, beginning with transfer from the primary enrichment to  
13 secondary enrichment broth (if applicable), followed by subsequent plating and confirmation of  
14 isolates using appropriate biochemical and serological methods.  
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### 17 **Validation Study**

18 Testing was conducted following the procedures outlined in the AOAC Research Institute  
19 *Performance Tested Methods<sup>SM</sup> Program* validation outline protocol: *Comparative Evaluation of*  
20 *the 3M<sup>TM</sup> Molecular Detection Assay 2 - Listeria monocytogenes and of the 3M<sup>TM</sup> Molecular*  
21 *Detection Assay 2 - Listeria* (February, 2015) [8]. Three brands of Demi Fraser Broth with FAC  
22 were used for this validation study; X, Y, and Z. The independent study involved a matrix study  
23 (10 matrixes), a lot-to-lot/stability evaluation, and a robustness evaluation using brand Y. The  
24 internal study involved a matrix study (4 matrixes) and an inclusivity/exclusivity study using  
25 either brand X or Z.  
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### 28 **Internal Study**

#### 29 *Inclusivity and Exclusivity*

#### 30 *Methodology*

31 Fifty frozen *Listeria* strain suspensions were thawed and sub-cultured in Brain Heart Infusion  
32 (BHI) broth overnight at 37°C ± 1°C. The cultures were diluted in peptone salt solution in order  
33 to inoculate between 10 to 100 cells per 225 mL of Demi Fraser with FAC. The enrichment  
34 broths were then incubated for 24 hours at 37°C ± 1°C, and the 3M MDA 2 - *Listeria* method  
35 was then performed and confirmed according to ISO 11290-1/A1. See Table 2a.  
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40 ~~Five-Thirteen~~ additional *Listeria* strains were tested at 3M (St. Paul, MN). The ~~five-thirteen~~  
41 frozen *Listeria* strain suspensions were thawed, or lyophilized cultures were revived, and then  
42 streaked onto Sheep Blood Agar (SBA). The SBA plates were incubated at 37°C ± 1°C  
43 overnight. A single isolated colony from the SBA plate was sub-cultured in 10 mL of Demi  
44 Fraser Broth with FAC overnight at 37°C ± 1°C. The cultures were diluted using Demi Fraser  
45 broth with FAC, and the 3M MDA 2 – *Listeria* method was then performed. See Table 2b.  
46



1 Thirty frozen non-*Listeria* strain suspensions were thawed and sub-cultured in BHI broth  
2 overnight at 37°C ± 1°C. The cultures were diluted in Buffered Peptone Water (BPW) or de  
3 Man, Rogosa, and Sharpe (MRS) in order to inoculate 10<sup>5</sup> cells/mL. The broths were then  
4 incubated for 24 hours at the appropriate incubation temperature in order to have culture to test  
5 with the 3M MDA 2 - *Listeria* method. See Table 3.

## 6 7 *Results*

8  
9 All ~~55-63~~ *Listeria* strains were detected by the 3M MDA 2 - *Listeria* method. *L. grayi* recovery  
10 was enhanced when a food sample (Ultra-high-temperature [UHT] milk as described in ISO  
11 16140 [9]) was added to the medium in the standard 1:10 dilution scheme. None of the 30 non-  
12 *Listeria* strains were detected. See Tables [2a](#), [2b](#) and 3 for study details and results.

## 13 14 15 **Matrix Study**

16  
17 The method comparison study consisted of evaluating a total of 30 un-paired sample replicates  
18 for 10 matrixes, along with naturally contaminated raw chicken (leg pieces), tested at the  
19 independent laboratory, evaluating 20 sample replicates using two separate lots, see Table 4a.  
20 The raw chicken (leg pieces and fillets) analyzed at the internal lab was inoculated according to  
21 AOAC guidelines which are described below, see Table 4b. Within each sample set, there were  
22 5 uninoculated samples (0 CFU/test portion), 20 low level inoculated samples (0.2-2 CFU/test  
23 portion), and 5 high level inoculated samples (2-5 CFU/test portion), except for the naturally  
24 contaminated raw chicken (leg pieces). The inoculum was prepared by transferring a single  
25 *Listeria* colony from Trypticase Soy Agar with 5% Sheep Blood (SBA) into BHI broth and  
26 incubating the culture at 35 ± 2°C for 24 ± 2 hours. Tables 4a and b presents the sample  
27 preparation guidelines for the matrix.

28  
29 All matrixes were screened for the presence of the target organism following the appropriate  
30 reference method. Additionally, an aerobic plate count (APC) was conducted following the  
31 FDA/BAM Chapter 3 reference [10] method to determine the level of background flora in each  
32 test matrix prior to inoculation.

33  
34 Prior to inoculation of beef hot dogs, deli turkey, and queso fresco the broth culture inoculum  
35 was heat stressed for 10 ± 1 minute at 50 ± 1°C in a water bath. The degree of injury of the  
36 culture was estimated by plating an aliquot of diluted culture onto Modified Oxford Agar (MOX)  
37 and Tryptic Soy Agar (TSA). The agars were incubated at 35 ± 1°C for 24 ± 2 hours and the  
38 colonies were counted. The degree of injury was estimated as:

$$(1 - \frac{n_{select}}{n_{nonselect}}) \times 100$$

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40  
41  
42 Where  $n_{select}$  = number of colonies on selective agar and  $n_{nonselect}$  = number of colonies on non-  
43 selective agar. Using BHI broth as the diluent, the culture was diluted to a low level expected to  
44 yield fractional positive results (5-15 positive results) and a high level expected to yield all  
45 positive results. Following inoculation, a bulk lot of the matrix was homogenized by hand and

1 held for 48-72 hours at refrigerated temperature (2-8 °C) prior to analysis to allow time for the  
2 organism to equilibrate within the sample.  
3

4 For the inoculation of the whole melons, a single whole melon was placed into a large sterile bag  
5 and the blossom end of the melon was inoculated with 100 µL of the diluted *Listeria*  
6 *monocytogenes* culture. The liquid culture was then allowed to soak into the melon. The melon  
7 was inverted so that the inoculated end was on the bottom of the sterile bag. The bag was tied  
8 closed and held for 48-72 hours at 2-8 °C.  
9

10 For environmental surfaces, inocula were prepared by transferring a pure isolated colony of the  
11 specified organism from SBA into BHI broth and incubated at 35 ± 2°C for 24 ± 2 hours.  
12 Following incubation, serial dilutions were performed in BHI broth to achieve the target level  
13 inoculum.  
14

15 For stainless steel and sealed concrete surfaces, 4" x 4" areas were inoculated with 0.25 mL of  
16 diluted *Listeria monocytogenes* culture. Stainless steel was also inoculated with competitor  
17 organism *Enterococcus faecium* ATCC 19434 at 10x the level of the target organism. For plastic  
18 surfaces, 1" x 1" areas were inoculated with 0.10 mL of diluted *Listeria seeligeri* culture. Plastic  
19 was also inoculated with a competitor organism, *Enterococcus faecalis* ATCC 29212, at 10x the  
20 level of the target organism. For the uninoculated test portions, sterile BHI broth was applied to  
21 the test area. Each surface was allowed to dry for 16-24 hours at room temperature (24 ± 2°C).  
22

23 The 3M™ Hydrated Sponge Stick (pre-moistened with Dey-Engley) (stainless steel and sealed  
24 concrete) and 3M Tecra Enviroswabs (pre-wetted with Lethen) (plastic) were sampled by using  
25 horizontal and vertical sweeping motions. The sponges and the swabs were held at room  
26 temperature for 2 hours prior to analysis. To determine the inoculation level for the  
27 environmental surfaces, aliquots of each inoculum were plated on TSA in triplicate.  
28

29 The level of *Listeria monocytogenes* in the low level inoculum was determined by Most  
30 Probable Number (MPN) on the day of analysis by evaluating 5 x 50 g, 20 x 25 g (reference  
31 method test portions), and 5 x 10 g inoculated test samples. For the high inoculation level, the  
32 MPN was determined by examining 5 x 50 g, 5 x 25 g (reference method test portions) and 5 x  
33 10 g . The level of *Listeria* in the low level inoculum for all 125 g test portions was determined  
34 by MPN by evaluating 5 x 250 g, 20 or 5 x 125 g (reference method test portions), and 5 x 50 g  
35 inoculated test samples. For the high inoculation level, the MPN was determined by examining 5  
36 x 250 g, 5 x 125 g (reference method test portions), and 5 x 50 g inoculated test samples. Each  
37 test portion was enriched with the reference method enrichment broth at the reference method  
38 dilution scheme and analyzed by the reference method procedure. See Table A for details. The  
39 number of positives from the 3 test levels was used to calculate the MPN using the LCF MPN  
40 calculator (version 1.6) provided by AOAC RI. [11]  
41 (<http://www.lcfltd.com/customer/LCFMPNCalculator.exe>)  
42  
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**Table A: MPN Test Portion Sizes**

Reference Method Test Portion	Inoculation Level	MPN Test Portions		
		5 x 50 g	20 x 25 g*	5 x 10 g
25 g	Low	5 x 50 g	20 x 25 g*	5 x 10 g

	High	5 x 50 g	5 x 25 g*	5 x 10 g
125 g	Low	5 x 250 g	20 x 125 g*	5 x 50 g
	High	5 x 250 g	5 x 125 g*	5 x 50 g

\*Test portions from reference method

#### USDA/FSIS MLG 8.09 Reference Method

For the USDA/FSIS MLG 8.09 reference method, 25 g test portions were enriched with 225 ± 5 mL of modified University of Vermont Medium broth (UVM) and 125 g test portions were enriched with 1125 ± 25 mL of UVM. All test portions were mechanically stomached for two minutes. The 25 g test portions were incubated at 30 ± 2 °C for 20-26 hours and the 125 g test portions were incubated at 30 ± 2 °C for 23-26 hours. For environmental samples, sponges were enriched with 225 mL of UVM and homogenized by hand, while swabs were enriched with 10 mL of UVM and mixed by vortex. Sponges and swabs were incubated for 20-26 hours at 30 ± 2°C. After incubation of all test portions, 0.1 ± 0.02 mL of the sample enrichment was transferred to 10 ± 0.5 mL of Fraser Broth (FB) containing 0.1 mL of 5% ferric ammonium citrate and incubated at 35 ± 2°C for 26 ± 2 hours. A loopful of the sample enrichment was also streaked to MOX and incubated at 35 ± 2°C for 26 ± 2 hours.

After 26 ± 2 hours, FB was examined for any degree of darkening due to esculin hydrolysis. Any FB that displayed darkening was streaked to a MOX plate. If no darkening occurred, FB was re-incubated at 35 ± 2°C for a total of 48 ± 2 hours and re-examined for evidence of darkening. If darkening occurred, the FB was streaked to a MOX plate, if no darkening occurred, samples were considered negative. All FB streaked MOX plates were incubated at 35 ± 2°C for 26 ± 2 hours.

MOX agar plates streaked from the primary enrichment or the FB secondary enrichment were examined after 26 ± 2 hours and if no suspect colonies were present, the MOX agar plate was re-incubated for an additional 26 ± 2 hours at 35 ± 2°C for a total of 48 ± 2 hours. If suspect colonies were present on the MOX agar plates, these suspect colonies were streaked to Horse Blood Overlay agar (HBO) and incubated at 35 ± 2°C for 22 ± 4 hours. HBO plates were examined for hemolysis reactions and well isolated colonies were transferred to BHI broth and incubated at 25°C for 24 ± 2 hours. Sample isolates from BHI broth were analyzed for tumbling motility by preparing a wet mount, analyzed by a catalase test and examined for morphology by preparing a Gram stain. Additionally, purified HBO isolates were identified using the VITEK<sup>®</sup> GP Biochemical Identification following AOAC OMA 2013.02. [12]

#### FDA/BAM Chapter 10 Reference Method

Twenty-five gram test portions were enriched in 225 mL ± 5 mL of Buffered Listeria Enrichment Broth (BLEB) homogenized for 2 minutes and incubated at 30 ± 1°C for 4 hours. For whole melons, a single whole melon was enriched using BLEB with approximately 1.5 times the weight of the melon, and soaked for 1 hour at room temperature. After an hour at room temperature, the test portions were incubated at 30 ± 1°C for 4 hours. Following 4 hours of incubation, selective supplements acriflavine (10mg/L), sodium nalidixate (40mg/L) and cycloheximide (50mg/L) were added to each test portion and incubated for an additional 20

1 hours. After 24 hours of total incubation, the enriched samples were streaked to MOX agar plates  
2 and incubated at  $35 \pm 1$  °C for 24-48 hours. The enriched samples were re-incubated for an  
3 additional 24 hours at  $30 \pm 1$  °C and then streaked to a second MOX agar plate which was  
4 incubated for 24-48 hours at  $35 \pm 1$ °C. MOX agar plates were examined for suspect colonies, and  
5 if present, at least 5 colonies were streaked to TSA containing 0.6% yeast extract (TSA/YE). The  
6 TSA/YE plates were incubated at  $35 \pm 1$ °C for 24-48 hours and then examined for purity. Pure  
7 colonies were tested for catalase reactivity and a Gram Stain was conducted. A pure *Listeria*  
8 colony was transferred to Trypticase Soy Broth containing 0.6% yeast extract (TSB/YE). The  
9 TSB/YE cultures were incubated at  $25 \pm 1$ °C overnight, or until the broth was turbid, indicating  
10 sufficient growth. Catalase-positive organisms were stabbed into plates of 5% Sheep Blood Agar  
11 (SBA) and incubated at  $35 \pm 1$ °C for 24-48 hours. The TSB/YE tubes incubated at  $25 \pm 1$ °C were  
12 used to prepare a wet mount slide to determine motility pattern. After incubation, the SBA plates  
13 were examined for hemolysis. Final confirmation was conducted using the VITEK® GP  
14 Biochemical Identification card following AOAC OMA 2013.02.

#### 15 AOAC 993.12 Listeria Reference Method

16  
17  
18 Twenty-five gram test portions were enriched in 225 mL  $\pm$  5 mL of selective enrichment  
19 medium, containing yeast extract (1.35 g/225 mL), acriflavine mono hydrochloride (1.125 g/225  
20 mL), nalidixic acid (sodium salt) solution (1.125 g/225 mL), and cycloheximide (2.25 g/225  
21 mL), and incubated at  $30 \pm 1$  °C for 48 hours. The enriched samples were then streaked to  
22 Oxford Agar (OXA) and incubated at  $37 \pm 1$ °C for 48 hours. At 48 hours, the OXA plates were  
23 examined for suspect colonies. If present, up to 5 suspect colonies were streaked to TSA/YE to  
24 obtain well isolated, pure colonies. Pure colonies on TSA/YE were analyzed for Gram-stain  
25 reaction and for catalase activity. Catalase positive, Gram positive rods colonies were then  
26 stabbed to SBA and incubated at  $37 \pm 1$  °C for 48 hours. After 48 hours, the SBA plates were  
27 examined for typical hemolytic reactions. The same colony picked to SBA was also transferred  
28 to TSB/YE. The TSB/YE cultures were incubated at  $25 \pm 1$  °C overnight, or until the broth was  
29 turbid, indicating sufficient growth. Another TSB/YE tube was inoculated with the same colony  
30 and incubated at  $37 \pm 1$  °C for 24 hours to be used for carbohydrate utilization testing. The  
31 TSB/YE tube incubated at  $25 \pm 1$  °C was used to prepare a wet mount slide to determine motility  
32 pattern. From the TSB/YE tube incubated at  $37 \pm 1$  °C, Motility Test Medium (MTM) was  
33 stabbed and incubated at  $25 \pm 1$  °C. After 2 days, and up to 7 days, the MTM tubes were observed  
34 for umbrella-like growth. Additionally from the TSB/YE tube, a loopful (0.1 mL) was  
35 transferred into carbohydrate fermentation broth (purple broth) containing 1.0 mL of either 5%  
36 rhamnose or 5% xylose. The purple broth tubes were incubated at  $37 \pm 1$  °C and examined for up  
37 to 7 days.

#### 38 ISO 11290-1/A1 Reference Method

39  
40  
41 For the ISO 11290-1/A1 reference method, 25 g test portions were enriched with 225 half Fraser  
42 broth. All test portions were mechanically stomached for two minutes. The test portions were  
43 incubated at  $30 \pm 1$ °C for  $24 \pm 3$  hours. After incubation, 0.1 mL of the sample enrichment was  
44 transferred to 10 mL FB containing 0.1 mL of 5% ferric ammonium citrate and incubated at  $37 \pm$   
45  $1$ °C for  $48 \pm 3$  hours. After  $48 \pm 3$  hours, a loopful of the sample secondary FB enrichment was  
46 streaked to PALCAM and Ottovani-Agosti Agar (OAA) and incubated at  $37 \pm 1$ °C for  $24 \pm 3$

1 hours. A loopful of the sample primary enrichment was also streaked to PALCAM and OAA and  
2 incubated at  $37 \pm 1^\circ\text{C}$  for  $24 \pm 3$  hours. PALCAM and OAA agar plates were examined for the  
3 presence of suspect colonies. If no suspect colonies were present, the agar plate was re-incubated  
4 for an additional 18-24 hours at  $37 \pm 1^\circ\text{C}$ . If no suspect colonies present the sample was  
5 determined to be negative for *Listeria*. If suspect colonies were present on the PALCAM or  
6 OAA agar plates, these suspect colonies were streaked to HBO and incubated at  $37 \pm 1^\circ\text{C}$  for 18-  
7 24 hours. HBO plates were examined for hemolysis reactions and well-isolated colonies were  
8 transferred to BHI broth and incubated at  $25^\circ\text{C}$  for  $24 \pm 2$  hours. Sample isolates from BHI broth  
9 were analyzed for tumbling motility by preparing a wet mount, analyzed by a catalase test and  
10 examined for morphology by preparing a Gram stain. Additionally, purified HBO isolates were  
11 identified using the VITEK<sup>®</sup> GP Biochemical Identification following AOAC OMA 2013.02.  
12  
13

### 14 3M<sup>™</sup> Molecular Detection Assay 2 - Listeria

15  
16 All 25 g samples were analyzed by the 3M<sup>™</sup> MDA 2 - *Listeria* were enriched with 225 mL of  
17 Demi-Fraser Broth with the addition of ferric ammonium citrate; all test portions were then  
18 homogenized by stomaching thoroughly for  $2 \pm 0.2$  minutes and incubated at  $37 \pm 1^\circ\text{C}$  for 24-28  
19 hours. For the 125 g deli turkey test portions, samples were enriched with 1125 mL of Demi-  
20 Fraser Broth with the addition of ferric ammonium citrate; test portions were then homogenized  
21 by stomaching thoroughly for  $2 \pm 0.2$  minutes and incubated at  $37 \pm 1^\circ\text{C}$  for 24 hours. For  
22 whole melons, test portions were enriched with approximately 1.5 times the weight of the whole  
23 melon in Demi Fraser with ferric ammonium citrate and incubated at  $37 \pm 1^\circ\text{C}$  for 26 hours.  
24 Stainless steel sponge samples were enriched with 225 mL of Demi-Fraser Broth with the  
25 addition of ferric ammonium citrate; samples were homogenized by hand thoroughly for  $2 \pm 0.2$   
26 minutes and incubated at  $37 \pm 1^\circ\text{C}$  for 24 and 26 hours. Sealed concrete environmental surface  
27 sponge samples were enriched with 100 mL of Demi-Fraser Broth with the addition of ferric  
28 ammonium citrate; samples were thoroughly homogenized by hand for  $2 \pm 0.2$  minutes and  
29 incubated at  $37^\circ\text{C}$  for 24 and 26 hours. Plastic environmental surface swab samples were  
30 enriched with 10 mL of Demi-Fraser Broth with the addition of ferric ammonium citrate;  
31 samples were homogenized by vortexing for  $2 \pm 0.2$  minutes and incubated at  $37 \pm 1^\circ\text{C}$  for 24  
32 and 26 hours.  
33

34 Prior to analysis, lysis tubes were brought to room temperature ( $20\text{-}25^\circ\text{C}$ ) by placing the tubes on  
35 a sanitized bench top for 16-18 hours. Lysis tubes were inverted to mix up to four hours before  
36 use. The lysis tubes were then de-capped and the rubber cap was discarded. A  $20\ \mu\text{L}$  aliquot of  
37 each sample was transferred to separate lysis tubes; a new pipette tip was used after each sample  
38 transfer. Uncovered samples were placed in the 3M Molecular Detection Heat Block Insert and  
39 heated for  $15 \pm 1$  minutes at  $100 \pm 1^\circ\text{C}$ . Following the heat lysis, samples were transferred into the  
40 Chill Block Insert and placed on a sanitized laboratory bench and were allowed to cool at  $18\text{-}$   
41  $28^\circ\text{C}$  for 5-10 minutes.  
42

43 A  $20\ \mu\text{L}$  aliquot of each lysed sample and control was added to separate reagent tubes, and  
44 samples were mixed by pipetting up and down five times. A Matrix Control tube was analyzed  
45 with the samples for each matrix to verify that no interference with the assay was caused by the  
46 matrix. Also lysed was a sterile Demi Fraser Broth with FAC tube for the kit Negative Control

1 (NC). A 20 µL aliquot was transferred to the NC and the Reagent Control (RC) tubes. A 20 µL  
2 aliquot of a randomly picked sample was added to the Matrix Control tube, mixed, and recapped.  
3 Using the 3M™ software, prompts were followed to identify samples and controls. All samples  
4 were loaded into the Speed Loader Tray, placed into the Molecular Detection System, and the  
5 3M™ MDA 2 - *Listeria* assay was initiated and results were obtained within 75 minutes.  
6

## 7 **Matrix Study Results**

8  
9 For each method, the probability of detection (POD) was calculated as the number of positive  
10 outcomes divided by the total number of trials. [13] The following were calculated: the candidate  
11 presumptive results, POD<sub>CP</sub>; the candidate confirmatory results, POD<sub>CC</sub>; the difference in the  
12 candidate presumptive and confirmatory results, dPOD<sub>CP</sub>; the presumptive candidate results that  
13 confirmed positive, POD<sub>C</sub> (=POD<sub>CC</sub>); the reference method, POD<sub>R</sub>, and the difference in the  
14 confirmed candidate and reference methods, dPOD<sub>C</sub>. POD analyses were conducted for the  
15 MDA 2 - *Listeria* test points and compared to the appropriate reference method results. The pre-  
16 validation target analyte background screen results and APC results are presented in Table 5. The  
17 heat stress data for selected matrixes are presented in Table 6. The inoculum levels for each  
18 environmental surface are presented in Table 7. A summary of POD analyses [14] are presented  
19 in Tables 8-15. As per criteria outlined in Appendix J of the Official Methods of Analysis  
20 Manual [15], fractional positive results were obtained for all matrixes.  
21

### 22 Internal Study

#### 23 *Raw chicken leg pieces (25 g)*

24  
25 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 4 presumptive positives  
26 and 9 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation  
27 procedure resulting in 5 false negative samples. There were 5 observed positives for the  
28 reference method. For the high inoculation level, there were 3 presumptive positives and 3  
29 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation  
30 procedure. There was 1 confirmed positive following the reference method. Even though 5 false  
31 negatives were confirmed, analysis show there to be no statistical difference when compared to  
32 the reference method. Detailed results of the POD analyses are presented in Tables 8 and 12.  
33 This matrix analysis was conducted using Demi Fraser with FAC brand X.  
34  
35

#### 36 *Raw chicken fillet (25 g)*

37  
38 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 4 presumptive positives  
39 and 4 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation  
40 procedure. There were 5 observed positives for the reference method. For the high inoculation  
41 level, there were 2 presumptive positives and 2 confirmed positives following the USDA/FSIS  
42 MLG 8.09 reference method confirmation procedure. There were 5 confirmed positives  
43 following the reference method. Detailed results of the POD analyses are presented in Tables 8  
44 and 12. This matrix analysis was conducted using Demi Fraser with FAC brand Z.  
45  
46

1 *Bagged raw spinach*

2  
3 *Compared to FDA-BAM*

4 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 6 presumptive positives  
5 and 6 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation  
6 procedure. There were 3 observed positives for the reference method. For the high inoculation  
7 level, there were 3 presumptive positives and 3 confirmed positives following the FDA/BAM  
8 Chapter 10 reference method confirmation procedure. There were 4 confirmed positives  
9 following the reference method. Detailed results of the POD analyses are presented in Tables 8  
10 and 12. This matrix analysis was conducted using Demi Fraser with FAC brand X.

11  
12  
13 *Compared to ISO 11290-1/A1*

14 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 6 presumptive positives  
15 and 6 confirmed positives following the ISO 11290-1/A1 reference method confirmation  
16 procedure. There were 9 observed positives for the reference method. For the high inoculation  
17 level, there were 3 presumptive positives and 3 confirmed positives following the ISO 11290-  
18 1/A1 reference method confirmation procedure. There were 4 confirmed positives following the  
19 reference method. Detailed results of the POD analyses are presented in Tables 8 and 12  
20 This matrix analysis was conducted using Demi Fraser with FAC brand X.

21  
22 *Cold smoked salmon*

23  
24 *Compared to FDA-BAM*

25 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 10 presumptive positives  
26 and 10 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation  
27 procedure. There were 7 observed positives for the reference method. For the high inoculation  
28 level, there were 5 presumptive positives and 5 confirmed positives following the FDA/BAM  
29 Chapter 10 reference method confirmation procedure. There were 4 confirmed positives  
30 following the reference method. Detailed results of the POD analyses are presented in Tables 8  
31 and 12. This matrix analysis was conducted using Demi Fraser with FAC brand X.

32  
33  
34  
35 *Compared to ISO 11290-1/A1*

36 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 10 presumptive positives  
37 and 10 confirmed positives following the ISO 11290-1/A1 reference method confirmation  
38 procedure. There were 8 observed positives for the reference method. For the high inoculation  
39 level, there were 5 presumptive positives and 5 confirmed positives following the ISO 11290-  
40 1/A1 reference method confirmation procedure. There were 4 confirmed positives following the  
41 reference method. Detailed results of the POD analyses are presented in Tables 8 and 12.  
42 This matrix analysis was conducted using Demi Fraser with FAC brand X.

43  
44  
45  
46 *Independent Study using Demi Fraser with FAC brand Y.*

1  
2 *Deli turkey (125 g)*  
3

4 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 8 presumptive positives  
5 and 8 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation  
6 procedure. There were 7 observed positives for the reference method. For the high inoculation  
7 level, there were 5 presumptive positives and 5 confirmed positives following the USDA/FSIS  
8 MLG 8.09 reference method confirmation procedure. There were 5 confirmed positives  
9 following the reference method. Detailed results of the POD analyses are presented in Tables 9  
10 and 13.

11 *Naturally Contaminated raw chicken leg pieces (25 g)*  
12  
13

14 *Lot 1*

15 For lot 1 of the raw chicken leg pieces of the MDA 2 - *Listeria* assay, there were 18 presumptive  
16 positives and 18 confirmed positives following the USDA/FSIS MLG 8.09 reference method  
17 confirmation procedure. There were 16 observed positives for the reference method. Detailed  
18 results of the POD analyses are presented in Tables 9 and 13.

19 *Lot 2*

20 For lot 2 of the raw chicken leg pieces of the MDA *Listeria* - 2 assay, there were 12 presumptive  
21 positives and 12 confirmed positives following the USDA/FSIS MLG 8.09 reference method  
22 confirmation procedure. There were 14 observed positives for the reference method. Detailed  
23 results of the POD analyses are presented in Tables 9 and 13.

24 *Whole melon*  
25

26 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 12 presumptive positives  
27 and 10 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation  
28 procedure, resulting in 2 false positives. There were 8 observed positives for the reference  
29 method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed  
30 positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There  
31 were 5 confirmed positives following the reference method. Detailed results of the POD analyses  
32 are presented in Tables 9 and 13.

33 *Vanilla ice cream (25 g)*  
34  
35

36 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 7 presumptive positives  
37 and 7 confirmed positives following the AOAC 993.12 reference method confirmation  
38 procedure. There were 7 observed positives for the reference method. For the high inoculation  
39 level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12  
40 reference method confirmation procedure. There were 5 confirmed positives following the  
41 reference method. Detailed results of the POD analyses are presented in Tables 9 and 13.

42 *Queso fresco (25 g)*  
43  
44  
45  
46



1 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 11 presumptive positives  
2 and 11 confirmed positives following the AOAC 993.12 reference method confirmation  
3 procedure. There were 9 observed positives for the reference method. For the high inoculation  
4 level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12  
5 reference method confirmation procedure. There were 5 confirmed positives following the  
6 reference method. Detailed results of the POD analyses are presented in Tables 9 and 13.

7  
8 *4% Milk fat cottage cheese (25 g)*  
9

10 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 12 presumptive positives  
11 and 12 confirmed positives following the AOAC 993.12 reference method confirmation  
12 procedure. There were 9 observed positives for the reference method. For the high inoculation  
13 level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12  
14 reference method confirmation procedure. There were 5 confirmed positives following the  
15 reference method. Detailed results of the POD analyses are presented in Tables 9 and 13.

16  
17  
18 *Beef hot dog (25 g)*  
19

20 For the low inoculation level of the MDA 2 - *Listeria* assay, there were 4 presumptive positives  
21 and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation  
22 procedure, resulting in 1 false negative. There were 6 observed positives for the reference  
23 method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed  
24 positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There  
25 were 5 confirmed positives following the reference method. Detailed results of the POD analyses  
26 are presented in Tables 9 and 13.

27  
28  
29 *Stainless steel (sponge enriched in 225 mL)*  
30

31 Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level  
32 of the MDA 2 - *Listeria* assay, there were 5 presumptive positives and 5 confirmed positives  
33 following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 8  
34 observed positives for the reference method. For the high inoculation level, there were 5  
35 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference  
36 method confirmation procedure. There were 5 confirmed positives following the reference  
37 method. Detailed results of the POD analyses are presented in Tables 9 and 13.

38  
39  
40 *Sealed concrete (sponge enriched in 100 mL)*  
41

42 Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level  
43 of the MDA 2 - *Listeria* assay, there were 15 presumptive positives and 15 confirmed positives  
44 following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 11  
45 observed positives for the reference method. For the high inoculation level, there were 5  
46 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference

1 method confirmation procedure. There were 5 confirmed positives following the reference  
2 method. Detailed results of the POD analyses are presented in Tables 9 and 13.

3  
4 *Plastic (Enviroswab enriched in 10 mL)*

5  
6 Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level  
7 of the MDA 2 - *Listeria* assay, there were 15 presumptive positives and 15 confirmed positives  
8 following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 11  
9 observed positives for the reference method. For the high inoculation level, there were 5  
10 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference  
11 method confirmation procedure. There were 5 confirmed positives following the reference  
12 method. Detailed results of the POD analyses are presented in Tables 9 and 13.

13  
14  
15 **Product Consistency (Lot-to-Lot) and Stability Studies**

16  
17 *Methodology*

18 Three lots of MDA 2 - *Listeria* test kits, 1 newly manufactured, 1 at the middle of its expiration,  
19 and 1 at or slightly beyond expiration were analyzed to determine the stability of the assay. For  
20 the MDA 2 - *Listeria* test kit, one *Listeria monocytogenes* ATCC 7644 isolate was analyzed at a  
21 fractional positive level (2-8 positives), and testing 10 replicates. One non-*Listeria* isolate,  
22 *Enterococcus faecalis* ATCC 29212, was analyzed at the growth level achieved in a non-  
23 selective broth, testing 5 replicates.

24  
25 *Results*

26 For the lot-to-lot/stability evaluation of the MDA 2 - *Listeria* assay, there were 6 presumptive  
27 positives out of 10 replicates for all three lots at the low inoculation level. For the 5 uninoculated  
28 control test portions, there were 0 presumptive positives out of 5 replicates for all three lots. The  
29 five control test portions were negative. See Table 16 for lot-to-lot/stability results.

30  
31 **Robustness Study**

32  
33 *Methodology*

34 The robustness evaluated the ability of the method to remain unaffected by minor variations in  
35 method parameters that might be expected to occur when the method is performed by an end  
36 user. Three different parameters were evaluated, which are presented in Table 18. For each  
37 parameter, one *Listeria monocytogenes* ATCC 7644 isolate was analyzed at a fractional positive  
38 level (2-8 positives), testing 10 replicates. One non-*Listeria* isolate, *Enterococcus faecalis* ATCC  
39 29212, was analyzed at the growth level achieved in a non-selective broth, testing 5 replicates.

40  
41 **Table B: Robustness Parameters**

42

Parameter	Low Value	Nominal Value	High Value
Lysis Time	13 minutes	15 minutes	17 minutes
Enrichment Volume	18 µL	20 µL	22 µL
Lysate Volume	18 µL	20 µL	22 µL

1  
2  
3  
**Table C: Robustness Parameter Variations**

Treatment Combination	Lysis Time	Volume (Enrichment to lysis)	Volume (Lysis to assay)	# of Replicates
1	13 minutes	18 µL	18 µL	10
2	13 minutes	18 µL	22 µL	10
3	13 minutes	22 µL	18 µL	10
4	13 minutes	22 µL	22 µL	10
5	17 minutes	18 µL	18 µL	10
6	17 minutes	18 µL	22 µL	10
7	17 minutes	22 µL	18 µL	10
8	17 minutes	22 µL	22 µL	10

4  
5  
6  
*Results*

7  
8  
9  
*Treatment combinations 1 and 2*

10 For the 13 minute lysis and the 18 µL aliquot of enrichment to lysis robustness analysis of the  
11 MDA 2 - *Listeria* assay, there were 6 presumptive positives out of 10 replicates at the low level  
12 inoculation. For the 5 uninoculated control test portions, there were 0 presumptive positives out  
13 of 5 replicates. All 5 uninoculated control test portions were negative.

14  
15 For the low inoculation level, a POD value of 0.60 was obtained with a 95% confidence interval  
16 of (0.31, 0.83). All 5 non-target test portions were negative with a POD value of 0.00 with a 95%  
17 confidence interval of (0.00,0.43).

18  
19  
20  
*Treatment combinations 3 and 4*

21 For the 13 minute lysis and the 22 µL aliquot of enrichment to lysis robustness analysis of the  
22 MDA 2 - *Listeria* assay, there were 7 presumptive positives out of 10 replicates at the low level  
23 inoculation. For the 5 uninoculated control test portions, there were 0 presumptive positives out  
24 of five replicates.

25  
26 For the low invocation level, a POD value of 0.70 was obtained with a 95% confidence interval  
27 of (0.40, 0.89). All 5 non-target test portions were negative with a POD value of 0.00 with a 95%  
28 confidence interval of (0.00, 0.43).

29  
30  
31  
*Treatment combinations 5, 6, 7 and 8*

32 For both 17 minutes lysis procedures robustness analysis of the MDA 2 - *Listeria* assay, there  
33 were 6 presumptive positives out of 10 replicates at the low level inoculation. For the 5  
34 uninoculated control test portions, there were 0 presumptive positives out of 5 replicates. See  
35 Table 17 for robustness results.

1  
2 For the low invocation level, a POD value of 0.50 was obtained with a 95% confidence interval  
3 of (0.24, 0.76). All 5 non-target test portions were negative with a POD value of 0.00 with a 95%  
4 confidence interval of (0.00, 0.43).  
5

#### 6 **Independent Laboratory Observations**

7

8 The results of this study demonstrate the ability of the MDA 2 - *Listeria* to detect the presence of  
9 *Listeria* in various food matrixes and select environmental surfaces after 24-28 hours of a  
10 primary enrichment. The MDA 2 - *Listeria* offers the benefits of extremely high sensitivity and  
11 high specificity for the detection of *Listeria* while reducing the overall time to presumptive  
12 results. The MDA 2 - *Listeria* also reduces the total analyst time required to obtain final results  
13 by implementing a single heat lysis step and cool step, followed up by a single transfer to  
14 molecular reaction tubes. The small footprint of the Molecular Detection System requires only  
15 minimal lab and bench space, making it easy to move the instrument or pair up additional MDS  
16 units to perform multiple MDA runs concurrently. The updated software is user friendly with  
17 easy to interpret results. The ability to examine Relative Light Unit (RLU) curves makes trouble  
18 shooting more streamlined if any problems occur while testing is being conducted.  
19

#### 20 **Discussion**

21

22 All matrixes evaluated in this validation study, including the raw chicken, resulted in no  
23 statistical differences when compared to the reference methods. Three different brands of Demi  
24 Fraser with FAC were used in this study, referred to as X, Y, and Z.  
25

26 There were no differences between the 24 and 26 hour primary enrichment time points for the  
27 environmental surfaces tested, therefore a 24 hour minimum enrichment time will be  
28 recommended for these matrixes: stainless steel, sealed concrete and plastic.  
29

30 The five false negative results with raw chicken (leg pieces) evaluated in the internal study  
31 resulted in an investigation of protocol. It was determined that the contract laboratory  
32 conducting the study used Demi Fraser with FAC brand X, which demonstrated low  
33 productivity. Brand X had also been used to analyze the inclusive list, spinach, and cold smoked  
34 salmon.  
35

36 When the protocol for raw chicken was performed at the independent laboratory, using a  
37 different brand Y, two lots of naturally contaminated raw chicken (leg pieces) analyzed showed  
38 no statistical difference when compared to the reference method. Lot 1 of the naturally  
39 contaminated raw chicken (leg pieces) had zero false negatives, while Lot 2 had two false  
40 negatives.  
41

42 To expand the robustness of the method to include multiple raw chicken matrixes, raw chicken  
43 fillets were chosen and brand Z was used. The raw chicken (fillet) testing resulted in no  
44 statistical difference when compared to the reference method, which highlighted the importance  
45 of internally validating any method as a system within one's own laboratory. This is why 3M  
46 has always emphasized this advisory within our package inserts;

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3 **“As with all test methods, the source, formulation and quality of enrichment medium**  
4 **can influence the results. Factors such as sampling methods, testing protocols, sample**  
5 **preparation, handling, and laboratory technique may also influence results. 3M**  
6 **recommends evaluation of the method including enrichment medium, in the user’s**  
7 **environment using a sufficient number of samples with particular foods and microbial**  
8 **challenges to ensure that the method meets the user’s criteria.”**  
9

## 10 **Conclusion**

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12 The 3M MDA 2 - *Listeria* AOAC PTM validation study included inclusivity/exclusivity, lot-to-  
13 lot/stability, robustness and matrix studies. The 3M MDA 2 - *Listeria* was compared to the  
14 USDA/FSIS-MLG 8.09, the FDA-BAM Chapter 10, the AOAC 993.12 or the ISO 11290-1/A1  
15 reference methods for the detection of *Listeria*. There were no significant differences between  
16 the 3M MDA 2 - *Listeria* method and the corresponding reference method for any of the  
17 matrixes evaluated. The 3M MDA 2 - *Listeria* demonstrated reliability as a rapid and sensitive  
18 method for the detection of *Listeria* species for the following new matrixes: beef hot dogs (25 g),  
19 deli turkey (125 g), queso fresco (25 g), vanilla ice cream (25 g), 4% milk fat cottage cheese (25  
20 g), whole melon, bagged raw spinach (25 g), cold smoked salmon (25 g), raw chicken (25 g),  
21 sealed concrete (sponge enriched in 225 mL), plastic (Enviro\_sSwab enriched in 10 mL),  
22 stainless steel (sponge enriched in 225 mL).  
23

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1 **Table 1. Enrichment Protocols Using Demi-Fraser Broth Enrichment at 37 ± 1°C**

2 **Specific Instructions for Validated Methods**

3 **AOAC® Performance Tested Method<sup>SM</sup> #111501**



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8 In AOAC PTM studies, the 3M Molecular Detection Assay 2 – *Listeria* was found to be an effective method for the

9 detection of *Listeria* species. The matrices tested in the study are shown in Table 3. The limit of detection of the 3M

10 Molecular Detection Assay 2 – *Listeria* method is 1-5 colony forming units per validated test portion size (in Table 3).

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12

13 **Table 1. Enrichment protocols using Demi-Fraser Broth at 37 ± 1 °C**

14 **according to AOAC Performance Tested<sup>SM</sup> Certificate #111501**

15

<u>Sample Matrix</u>	<u>Sample Size</u>	<u>Enrichment Broth Volume (mL)</u>	<u>Enrichment Time (hr)</u>
<u>Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon</u>	<u>25 g</u>	<u>225</u>	<u>24-30</u>

	<u>Raw chicken</u>	<u>25 g</u>	<u>475</u>	<u>28-32</u>
	<u>Deli turkey</u>	<u>125 g</u>	<u>1125</u>	<u>24-30</u>
	<u>Cantaloupe<sup>a</sup></u>	<u>Whole melon</u>	<u>Enough volume to allow melon to float</u>	<u>26-30</u>
<u>Environmental samples:</u>	<u>Stainless steel</u>	<u>1 sponge</u>	<u>225</u>	<u>24-30</u>
	<u>Sealed concrete</u>	<u>1 sponge</u>	<u>100</u>	<u>24-30</u>
	<u>Plastic<sup>b</sup></u>	<u>1 swab</u>	<u>10</u>	<u>24-30</u>

All samples for the AOAC validation were homogenized by stomaching unless otherwise noted.

a = Homogenize sample by hand mixing

b = Homogenize sample by vortexing

<b>Sample Matrix</b>	<b>Sample Size</b>	<b>Enrichment Broth Volume (mL)</b>	<b>Enrichment Time (hr)</b>
Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon	25 g	225	24-30
Raw chicken	25 g	475	28-32
Deli turkey	125 g	1125	24-30

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	Cantaloupe	Whole melon	Enough volume to allow melon to float	26-30
Environmental samples:	Stainless steel	1 sponge	225	24-30
	Sealed concrete	1 sponge	100	24-30
	Plastic	1 swab	10	24-30

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**Table 2a. Inclusivity Study Results**

Genus	Species	Reference	Origin	Inoculation level (cfu/225ml)	3M Molecular Detection Assay 2 Half Fraser broth 24h at 37°C			
					MDA 2 - <i>Listeria</i> spp	Confirmation 100µl		
					ALOA	PALCAM		
1	<i>Listeria</i>	<i>monocytogenes</i>	1011/1410	Frozen broccoli	28	+	H+	+
2	<i>Listeria</i>	<i>monocytogenes</i>	153	Soft cheese (Munster)	39	+	H+	+
3	<i>Listeria</i>	<i>monocytogenes</i>	1973/2400	Egg and ham pastry (Quiche Lorraine)	38	+	H+	+
4	<i>Listeria</i>	<i>monocytogenes</i>	38/181	Toulouse sausages	33	+	H+	+
5	<i>Listeria</i>	<i>monocytogenes</i>	7111/7516	Pâté (Rillettes)	64	+	H+	+
6	<i>Listeria</i>	<i>monocytogenes</i>	913/1048	Black pudding	39	+	H+	+
7	<i>Listeria</i>	<i>monocytogenes</i>	A00C036	Poultry (guinea)	41	+	H+	+
8	<i>Listeria</i>	<i>monocytogenes</i>	A00C041	Sausage	38	+	H+	+
9	<i>Listeria</i>	<i>monocytogenes</i>	A00C044	Poultry (Duck)	34	+	H+	+
10	<i>Listeria</i>	<i>monocytogenes</i>	A00L097	Milk	60	+	H+	+
11	<i>Listeria</i>	<i>monocytogenes</i>	A00M009	Smoked salmon	40	+	H+	+
12	<i>Listeria</i>	<i>monocytogenes</i>	Ad 253	Semi-hard cheese	58	+	H+	+
13	<i>Listeria</i>	<i>monocytogenes</i>	Ad 266	Poultry	26	+	H+	+
14	<i>Listeria</i>	<i>monocytogenes</i>	Ad 270	Fermented sausage	42	+	H+	+
15	<i>Listeria</i>	<i>monocytogenes</i>	Ad 273	Cured delicatessen	27	+	H+	+
16	<i>Listeria</i>	<i>monocytogenes</i>	Ad 274	Ready-to-eat food (Asiatic meal)	40	+	H+	+
17	<i>Listeria</i>	<i>monocytogenes</i>	Ad 534	Fruits	54	+	H+	+
18	<i>Listeria</i>	<i>monocytogenes</i>	Ad 548	Environment (Seafood)	37	+	H+	+
19	<i>Listeria</i>	<i>monocytogenes</i>	Ad 623	Bread crumbs	50	+	H+	+
20	<i>Listeria</i>	<i>monocytogenes</i>	Ad 665	Raw milk	33	+	H+	+
21	<i>Listeria</i>	<i>grayi</i>	Ad 1198	Smoked salmon	158 (+25ml UHT milk)	+	H-	st
					1300	+	H-(2)	-
22	<i>Listeria</i>	<i>grayi</i>	Ad 1443	Pork meat sausages	1300 (+25ml UHT milk)	+	H-	-
					12(+25ml UHT milk)	+	H-	-
23	<i>Listeria</i>	<i>innocua</i>	1	Smoked salmon	40	+	H-	+
24	<i>Listeria</i>	<i>innocua</i>	Ad 658	Gorgonzola	28	+	H-	+
25	<i>Listeria</i>	<i>innocua</i>	Ad 655	Brine	19	+	H-	+
26	<i>Listeria</i>	<i>innocua</i>	Ad 660	Bread crumbs	35	+	H-	+
27	<i>Listeria</i>	<i>innocua</i>	Ad 663	Environment (dairy industry)	25	+	H-	+
28	<i>Listeria</i>	<i>innocua</i>	Ad 671	Smocked bacon	10	+	H-	+
29	<i>Listeria</i>	<i>innocua</i>	Ad 661	Soft cheese (Pont L'Evêque)	15	+	H-	+
30	<i>Listeria</i>	<i>innocua</i>	Ad 659	Environment (dairy industry)	14	+	H-	+
31	<i>Listeria</i>	<i>ivanovii</i>	Ad 466	Raw veal meat	11	+	H+	+

Genus	Species	Reference	Origin	Inoculation level (cfu/225ml)	3M Molecular Detection Assay 2 Half Fraser broth 24h at 37°C		
					MDA 2 - <i>Listeria</i> spp	Confirmation 100µl	
						ALOA	PALCAM
32	<i>Listeria ivanovii</i>	Ad 662	Environment (dairy industry)	20	+	H+	+
33	<i>Listeria ivanovii</i>	BR11	Environment (fish)	29	+	H+	+
34	<i>Listeria ivanovii</i>	Ad 1289	Raw milk cheese	30	+	H+	+
35	<i>Listeria ivanovii</i>	Ad 1290	Milk powder	17	+	H+	+
36	<i>Listeria ivanovii</i>	Ad 1291	Poultry	18	+	H+	+
37	<i>Listeria ivanovii</i>	Ad 1288	Sheep milk	80	+	H+	+
38	<i>Listeria ivanovii</i> subsp. <i>londoniensis</i>	CIP103466	Unknown	14	+	H-	+
39	<i>Listeria seeligeri</i>	Ad 649	Cheese	29	+	H-	+
40	<i>Listeria seeligeri</i>	Ad 651	Environment	36	+	H-	+
41	<i>Listeria seeligeri</i>	Ad 652	Environment (dairy industry)	28	+	H-	+
42	<i>Listeria seeligeri</i>	Ad 674	Soft cheese (Munster)	240	+	H-	H-
43	<i>Listeria seeligeri</i>	BR1	Trout	28	+	H-	+
44	<i>Listeria seeligeri</i>	BR18	Environment (fish)	36	+	H-	+
45	<i>Listeria seeligeri</i>	CIP100100	Unknown	8	+	st	1col
46	<i>Listeria welshimeri</i>	Ad1276	Environment (Slaughterhouse)	44	+	H-	+
47	<i>Listeria welshimeri</i>	Ad1235	Beef meat	26	+	H-	+
48	<i>Listeria welshimeri</i>	191424	Poultry	24	+	H-	+
49	<i>Listeria welshimeri</i>	Ad 1175	Ready-to-eat-food	46	+	H-	+
50	<i>Listeria welshimeri</i>	Ad 650	Poultry	33	+	H-	+

- 1
- 2 All the strains are wild strains isolated in Adria Developpement, Quimper, France.
- 3 Ad = Adria
- 4 A= Adria
- 5 BR = ANSES Lab strain
- 6 CIP = collection of Pasteur Institute
- 7 H+/- = Presence/absence Phospho-Lipolysis halo
- 8 St = plate without colonies
- 9 ANSES = French Agency for Food, Environmental, and Occupational Health and Safety, Cedex, France
- 10 *Listeria* strains #21 and #22 utilized UHT to assist with growth as ISO 16140 directs.
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Table 2b. Inclusivity Study Results

#	<i>Listeria</i> species	Reference	Origin	Level tested	MDA2LIS	3
51	<i>faecalis</i> subsp. <i>coloradensis</i>	FSL S10-1203	Running water, Grazing pasture USA, Florida	1.8e5 CFU/mL	±	4
52	<i>marthii</i>	FSL S10-1204	Pristine environment, forest USA, NY	2.05e5 CFU/mL	±	5
53	<i>gornetensis</i>	FSL H69706-06	Water, USA, Colorado	3.3e7 CFU/mL	±	6
54	<i>san diegoensis</i>	FSL H69706-07	Water, USA, Colorado	1.95e7 CFU/mL	±	7
55	<i>riparia</i>	FSL S10-1204	Running water, FL- Florida	3.05e5 CFU/mL	±	8
56	<i>aquatica</i>	FSL S10-1188	Running water, USA, Florida	1.37e6 CFU/mL	±	9
57	<i>booriae</i>	FSL A5-0281	Non-food-contact surface in a dairy processing plant, USA, New York	2.75e5 CFU/mL	±	10
58	<i>fleischmannii</i> subsp. <i>fleischmannii</i>	DSM 24998	Hard cheese, Switzerland, Suisse Romande	1.28e6 CFU/mL	±	11
59	<i>ivanovii</i>	ATCC 19119	Sheep, Bulgaria	4.50e5 CFU/mL	±	12
60	<i>ivanovii</i> subsp. <i>londoniensis</i>	ATCC 49954	Food, France	7.40e5 CFU/mL	±	13
61	<i>newyorkensis</i>	FSL M6-0635	Non-food-contact surface in a seafood processing plant, USA, New York	3.05e5 CFU/mL	±	14
62	<i>rocourtiae</i>	CIP 109804	Pre-cut lettuce, Austria, Siezenheim	7.30e5 CFU/mL	±	15
63	<i>weihenstephanensis</i>	LMG 26374	Water plant <i>Lemna trisulca</i> from pond, Germany, Bavaria	3.30e4 CFU/mL	±	16

- Table 2b are *Listeria* strains analyzed at 3M St. Paul, MN laboratory
- Cultures 51-54 were obtained from the laboratory of Dr. Martin Wiedmann at Cornell University, NY and from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany. Frozen suspensions were thawed and streaked to sheep blood agar. Single isolated colonies were picked and sub-cultured in 10 mL Demi Fraser broth with FAC overnight at 37 ± 1°C. The cultures were diluted with fresh Demi Fraser broth with FAC before testing using the 3M MDA2LIS kit.
- Culture 55 was tested a second time and the results are indicated in red.
  - LMG = Laboratorium voor Microbiologie, Universiteit Gent (Laboratory of Microbiology, Department of Biochemistry and Microbiology, Faculty of Sciences of Ghent University, Belgium)
  - DSM = Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures)
  - FSL = Food Safety Laboratory Cornell University, NY
- Culture 55 was also obtained from Cornell. The frozen suspension was thawed and streaked to sheep blood agar. This organism did not grow in broth at 37°C or at lower temperatures, therefore a McFarland 1<sup>+</sup> suspension (approximately 3e8 CFU/mL) was prepared from a colonial isolate prior to MDA2LIS testing.
- FSL = Food Safety Laboratory Cornell University, NY

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Table 3. Exclusivity Study Results

	Genus	Species	Reference	Origin	Inoculation level (cfu/ml)	3M Molecular Detection Assay 2 – <i>Listeria</i>
1	<i>Bacillus</i>	<i>cereus</i>	Ad 465	Salmon Terrine	7,2x10 <sup>4</sup>	-
2	<i>Bacillus</i>	<i>circulans</i>	Ad 760	Vegetables	1,0 x 10 <sup>4</sup> (opacity +)	-
3	<i>Bacillus</i>	<i>coagulans</i>	Ad 731	Dairy product	<2,0x10 <sup>3</sup> (opacity +)	-
4	<i>Bacillus</i>	<i>licheniformis</i>	Ad 978	Dairy product	<2,0x10 <sup>3</sup> (opacity+)	-
5	<i>Bacillus</i>	<i>mycoïdes</i>	Ad 762	Milk	5,6x10 <sup>4</sup>	-
6	<i>Bacillus</i>	<i>pseudomycoïdes</i>	Ad 765	Vegetables	2,0x10 <sup>4</sup>	-
7	<i>Bacillus</i>	<i>pumilus</i>	Ad 284	Ready-to-eat	1,7x10 <sup>3</sup>	-
8	<i>Bacillus</i>	<i>weihenstephanensis</i>	Ad 726	Egg product	6,8x10 <sup>4</sup>	-
9	<i>Brochothrix</i>	<i>thermosphacta</i>	EN 15129	Trout	8,5x10 <sup>5</sup>	-
10	<i>Brochrotrix</i>	<i>campestris</i>	CIP 102920T	Environment	<2,0x10 <sup>3</sup> (opacity +)	-
11	<i>Carnobacterium</i>	<i>divergens</i>	CIP 101029 <sup>1</sup>	Unknown	>2,0x10 <sup>3</sup> (opacity +)	-
12	<i>Carnobacterium</i>	<i>piscicola</i>	Ad 369	Raw milk	<2,0x10 <sup>3</sup> (opacity +)	-
13	<i>Enterococcus</i>	<i>durans</i>	Ad 149	Ham	<2,0x10 <sup>3</sup> (opacity +)	-
14	<i>Enterococcus</i>	<i>faecalis</i>	89L326	Soft cheese (Vacherin)	1,6x10 <sup>5</sup>	-
15	<i>Lactobacillus</i>	<i>brevis</i>	86L126	Ham	1,4x10 <sup>5</sup>	-
16	<i>Lactobacillus</i>	<i>curvatus</i>	Ad 380	Delicatessen	1,7x10 <sup>5</sup>	-
17	<i>Lactobacillus</i>	<i>fermentum</i>	Ad 482	Tomatoes juice	1,2x10 <sup>5</sup>	-
18	<i>Lactobacillus</i>	<i>sakei</i>	Ad 473	Ham	4,9x10 <sup>5</sup>	-
19	<i>Lactococcus</i>	<i>lactis subsp cremoris</i>	Ad 137	Dairy product	>2,0x10 <sup>3</sup> (opacity +)	-
20	<i>Leuconostoc</i>	<i>carosum</i>	Ad 411	Ham	6,8x10 <sup>5</sup>	-
21	<i>Leuconostoc</i>	<i>citreum</i>	Ad 396	Ham	4,9x10 <sup>5</sup>	-
22	<i>Micrococcus</i>	<i>luteus</i>	Ad 432	Cocktail	<2,0x10 <sup>3</sup> (opacity+)	-
23	<i>Pediococcus</i>	<i>pentosaceus</i>	ATCC 33316	Unknown	1,0x10 <sup>5</sup>	-
24	<i>Propionibacterium</i>	<i>freundenreichii</i>	CNRZ 725	Dairy product	<2,0x10 <sup>3</sup> (opacity +)	-
25	<i>Staphylococcus</i>	<i>aureus</i>	Ad 165	Smoked delicatessen	1,3x10 <sup>5</sup>	-
26	<i>Staphylococcus</i>	<i>aureus</i>	Ad 902	Nems	8,2x10 <sup>4</sup>	-
27	<i>Staphylococcus</i>	<i>epidermidis</i>	Ad 931	Fruits	2,0x10 <sup>3</sup>	-
28	<i>Staphylococcus</i>	<i>haemolyticus</i>	Ad 989	Dairy product	2,8x10 <sup>4</sup>	-
29	<i>Streptococcus</i>	<i>bovis</i>	92L622	Dairy product	2,0x10 <sup>3</sup>	-
30	<i>Streptococcus</i>	<i>salivarius</i>	Ad 441	Dairy product	<2,0x10 <sup>3</sup> (opacity +)	-

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All the strains are wild strains isolated in Adria Developpement, Quimper, France.

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Ad = Adria

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A= Adria

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BR = ANSES Lab strain

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CIP = collection of Pasteur Institute

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ANSES = French Agency for Food, Environmental, and Occupational Health and Safety, Cedex, France

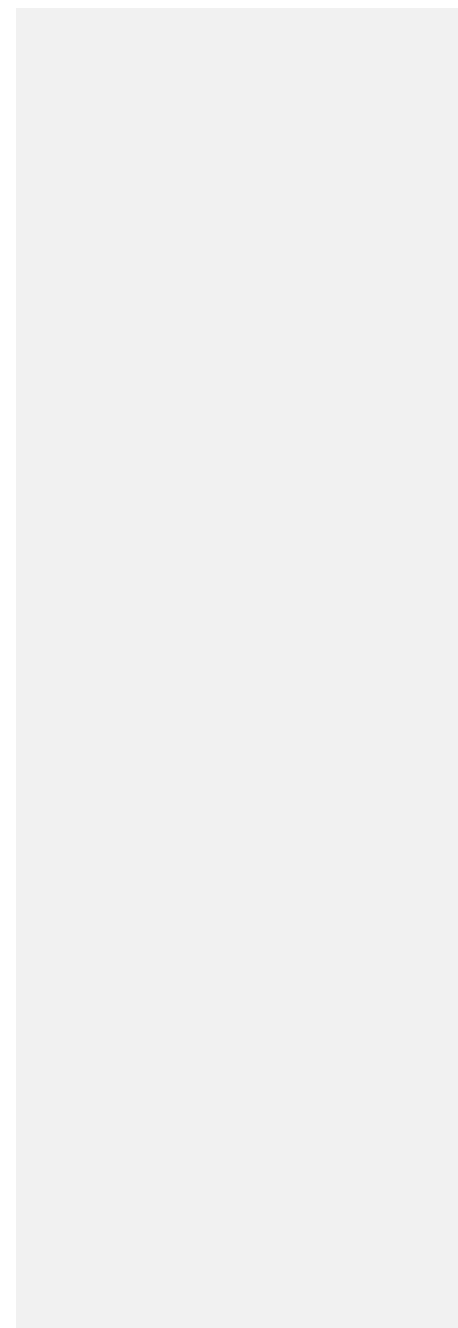
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CNRZ = National Centre for Zootechnical Research, Jouy-en-Josas, France

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1 ATCC = American Type Culture Collection

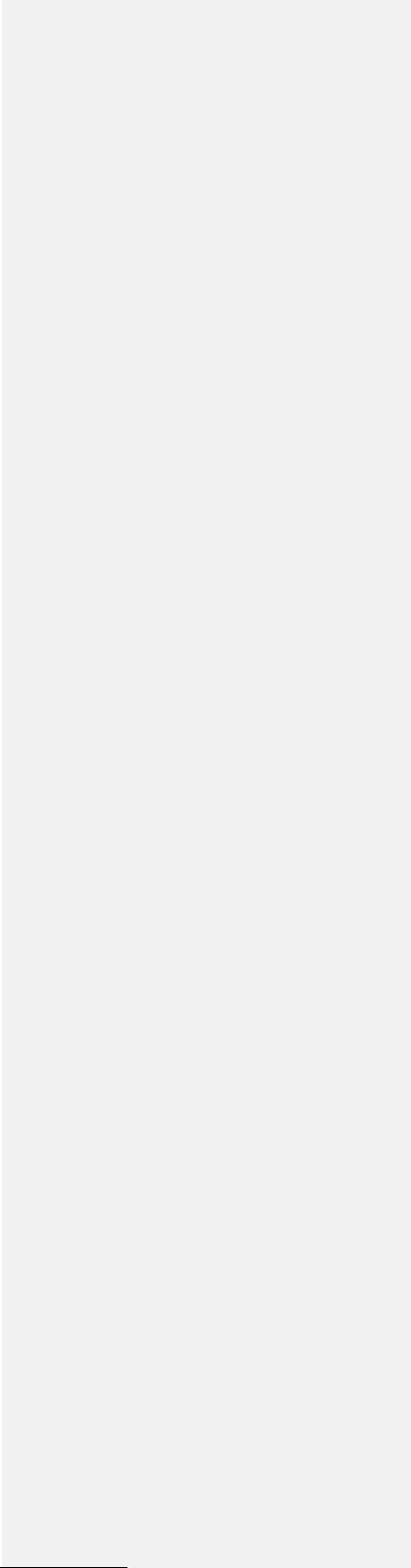


**Table 4a: Independent Study - Matrixes and Inoculating Organisms**

Matrix	Inoculating Organism (Culture Condition)	Target Inoculum Level	# of Replicates	MDA 2 Testing Time Points	Reference Method (Enrichment)
Deli Turkey	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19116 (heat-stressed)	0 CFU/Test Portion	5	24 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Raw Chicken Leg Pieces	Naturally Contaminated	Lot 1 & Lot 2	20 & 20	28 hours	USDA/FSIS MLG 8.09 (UVM)
Whole Melon	<i>L. monocytogenes</i> FSL <sup>3</sup> J1-049	0 CFU/Test Portion	5	26 hours	FDA/BAM Chapter 10 (BLEB)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Vanilla Ice Cream	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19114	0 CFU/Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Queso Fresco	<i>L. monocytogenes</i> CWD <sup>2</sup> 1554 (heat-stressed)	0 CFU/Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
4% Milk Fat Cottage Cheese	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19112 & <i>L. welshimeri</i> ATCC <sup>1</sup> 35897	0 CFU/Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Beef Hot Dogs	<i>L. monocytogenes</i> ATCC <sup>1</sup> 7644 (heat-stressed)	0 CFU/Test Portion	5	24 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Stainless Steel	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19118 & <i>10x Enterococcus faecium</i> ATCC <sup>1</sup> 19434	0 CFU/1" x 1" (5 cm <sup>2</sup> )	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/1" x 1" (5 cm <sup>2</sup> )	20		
		0 CFU/4" x 4" (100 cm <sup>2</sup> )	5		
Sealed Concrete	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19117	0 CFU/4" x 4" (100 cm <sup>2</sup> )	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/4" x 4" (100 cm <sup>2</sup> )	20		
		~100 CFU/4" x 4" (100 cm <sup>2</sup> )	5		
Plastic	<i>L. monocytogenes</i> ATCC <sup>1</sup> 51782 & <i>L. seeligeri</i> ATCC <sup>1</sup> 35967 &	0 CFU/1" x 1" (5 cm <sup>2</sup> )	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/1" x 1" (5 cm <sup>2</sup> )	20		
		~100 CFU/1" x 1" (5 cm <sup>2</sup> )	5		

	<i>10x Enterococcus faecalis ATCC1 29212</i>				
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- 1 <sup>1</sup> ATCC - American Type Culture Collection
- 2 <sup>2</sup> CWD - University of Vermont
- 3 <sup>3</sup> FSL - Cornell University
- 4 <sup>\*</sup> Natural contamination was present during the background screen: 2 separate lots evaluated (20 replicates each), *Listeria monocytogenes*
- 5 and *Listeria innocua* were isolated.





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**Table 4b: Internal Study - Matrixes and Inoculating Organisms**

Matrix	Inoculating Organism (Culture Condition)	Target Inoculum Level	# of Replicates	MDA 2 Testing Time Points	Reference Method (Enrichment)
Raw chicken <sup>2</sup> leg pieces	<i>L. ivanovii</i> Ad <sup>1</sup> 1291	0 CFU/Test Portion	5	28 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Raw chicken fillet <sup>3</sup>	<i>L. ivanovii</i> Ad 1291	0 CFU/Test Portion	5	28 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Bagged raw <sup>2</sup> spinach	<i>Listeria seeligeri</i> Ad 1754	0 CFU/Test Portion	5	24 hours	FDA/BAM Chapter 10 (BLEB)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Bagged raw <sup>2</sup> spinach	<i>Listeria seeligeri</i> Ad 1754	0 CFU/Test Portion	5	24 hours	ISO 11290-1/A1 (Half Fraser/Demi Fraser)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Cold smoked <sup>2</sup> salmon	<i>Listeria innocua</i> Ad 1674	0 CFU/Test Portion	5	24 hours	FDA/BAM Chapter 10 (BLEB)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Cold smoked <sup>2</sup> salmon	<i>Listeria innocua</i> Ad 1674	0 CFU/Test Portion	5	24 hours	ISO 11290-1/A1 (Half Fraser/Demi Fraser)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		

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<sup>1</sup> Ad – Adria Developpement Culture Collection<sup>2</sup> Tested using Demi Fraser brand (with FAC) X<sup>3</sup> Tested using Demi Fraser brand (with FAC) Z

**Table 5a: Independent Study - Aerobic Plate Count and Background Results (Prior to Inoculation)**

Matrix (Test Portion)	APC <sup>1</sup> (CFU/g)	Background <i>Listeria</i> (# positive/total tested)
Deli Turkey (125 g)	1.5 x 10 <sup>3</sup>	0/5 <sup>(2)</sup>
Naturally Contaminated Raw Chicken Leg Pieces (25 g)	2.8 x 10 <sup>5</sup>	4/5 <sup>(2)</sup>
Whole Melons	1.0 x 10 <sup>2</sup>	0/5 <sup>(3)</sup>
Vanilla Ice Cream (25 g)	8.0 x 10 <sup>1</sup>	0/5 <sup>(4)</sup>
Queso Fresco (25 g)	3.8 x 10 <sup>5</sup>	0/5 <sup>(4)</sup>
4% Milk Fat Cottage Cheese (25 g)	1.7 x 10 <sup>4</sup>	0/5 <sup>(4)</sup>
Beef Hot Dogs (25 g)	1.6 x 10 <sup>2</sup>	0/5 <sup>(2)</sup>
Beef Hot Dogs <sup>5</sup> (25 g)	6.0 x 10 <sup>3</sup>	0/5 <sup>(2)</sup>

<sup>1</sup> APC conducted in accordance with FDA/BAM Chapter 3

<sup>2</sup> *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines

<sup>3</sup> *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines

<sup>4</sup> *Listeria species* screen conducted following the AOAC 993.12 guidelines

<sup>5</sup> Matrix used to conduct Robustness and Lot-to-Lot testing

**Table 5b: Internal Study - Aerobic Plate Count and Background Results (Prior to Inoculation)**

Matrix (Test Portion)	APC <sup>1</sup> (CFU/g)	Background <i>Listeria</i> (# positive/total tested)
Raw Chicken Leg Pieces (25 g)	4.0 x 10 <sup>5</sup>	0/5 <sup>(2)</sup>
Raw chicken fillet (25 g)	1.4 x 10 <sup>5</sup>	0/5 <sup>(2)</sup>
Bagged Raw Spinach (25 g)	9.3 x 10 <sup>6</sup>	0/5 <sup>(3,4)</sup>
Cold Smoked Salmon (25 g)	2.0 x 10 <sup>2</sup>	0/5 <sup>(3,4)</sup>

<sup>1</sup> APC conducted in accordance with FDA/BAM Chapter 3

<sup>2</sup> *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines

<sup>3</sup> *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines

<sup>4</sup> *Listeria species* screen conducted following the ISO 11290-1/1A guidelines

**Table 6: Inoculum Heat Stress Results**

Matrix	Test Portion Size	Inoculating Organism	Agar	CFU/ g	Percent Injury
Beef Hot Dogs <sup>1</sup>	25 g	<i>Listeria monocytogenes</i> ATCC 7644	TSA	1.8 x 10 <sup>8</sup>	58.3 %
			MOX	7.5 x 10 <sup>7</sup>	
Deli Turkey	125 g	<i>Listeria monocytogenes</i> ATCC 19116	TSA	2.6 x 10 <sup>9</sup>	68.5 %
			MOX	8.2 x 10 <sup>8</sup>	
Queso Fresco	25 g	<i>Listeria monocytogenes</i> ATCC CWD 1554	TSA	2.6 x 10 <sup>9</sup>	72.7%
			MOX	7.1 x 10 <sup>8</sup>	
Beef Hot Dogs <sup>2</sup>	25 g	<i>Listeria monocytogenes</i> ATCC 7644	TSA	2.6 x 10 <sup>9</sup>	73.8%
			MOX	6.8 x 10 <sup>8</sup>	

<sup>1</sup>- Testing for Robustness and Lot to Lot

<sup>2</sup>- Matrix Study

TSA: Trypticase soy agar

MOX: modified Oxford agar

**Table 7: Inoculum Summary Table for Whole Melons and Environmental Surfaces**

Matrix	Whole Melons		
Inoculating Organism	<i>Listeria monocytogenes</i> FSL J1-049		
Low-Inoculum Level CFU <sup>a</sup> /Melon <sup>b</sup>	7		
High-Inoculum Level CFU <sup>a</sup> /Test Melon <sup>b</sup>	55		
Matrix	Stainless Steel		
Inoculating Organism	<i>Listeria monocytogenes</i> ATCC 19118	<i>Enterococcus faecium</i> ATCC 19434	
Low-Inoculum Level CFU <sup>a</sup> /Test Area <sup>c</sup>	42	530	
High-Inoculum Level CFU <sup>a</sup> /Test Area <sup>c</sup>	400	4000	
Matrix	Sealed Concrete		
Inoculating Organism	<i>Listeria monocytogenes</i> ATCC 19117		
Low-Inoculum Level CFU <sup>a</sup> /Test Area <sup>c</sup>	35		
High-Inoculum Level CFU <sup>a</sup> /Test Area <sup>c</sup>	360		
Matrix	Plastic		
Inoculating Organism	<i>Listeria monocytogenes</i> ATCC 51782	<i>Listeria seeligeri</i> ATCC 35967	<i>Enterococcus faecalis</i> ATCC 29212
Low-Inoculum Level CFU <sup>a</sup> /Test Area <sup>d</sup>	42	64	540
High-Inoculum Level CFU <sup>a</sup> /Test Area <sup>d</sup>	420	590	3600

<sup>a</sup>CFU: aliquots of the inocula were plated in triplicate onto TSA and averaged

<sup>b</sup>Test Area: Whole Melon

<sup>c</sup>Test Area: 4" x 4" Surface Area

<sup>d</sup>Test Area: 1" x 1" Surface Area

**Table 8: 3M™ MDA 2 - Listeria Assay, Candidate vs. Reference – POD Results (INTERNAL STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			USDA/FSIS MLG			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces <sup>h</sup> (25 g)	<i>L. ivanovii</i> Ad 1291	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.31 (0.14, 0.57)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
			0.5	5	3	0.60	0.23, 0.88	1	0.20	0.04, 0.62	0.40	-0.16, 0.73
Raw Chicken Fillet <sup>i</sup> (25 g)	<i>L. ivanovii</i> Ad 1291	28Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.3 (0.10, 0.5)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
			0.5	5	2	0.40	0.12, 0.77	4	0.80	0.38, 0.96	-0.40	-0.73, 0.16
Bagged Raw Spinach <sup>h</sup> (25 g)	<i>L. seeligeri</i> Ad 1754	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.26 (0.10, 0.47)	20	6	0.30	0.15, 0.52	3	<b>0.15</b>	<b>0.05, 0.36</b>	<b>0.15</b>	<b>-0.11, 0.39</b>
			1.7	5	3	0.60	0.23, 0.88	4	<b>0.80</b>	<b>0.38, 0.96</b>	<b>-0.20</b>	<b>-0.60, 0.31</b>
Bagged Raw Spinach <sup>h</sup> (25 g)	<i>L. seeligeri</i> Ad 1754	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.26 (0.10, 0.47)	20	6	0.30	0.15, 0.52	9	0.45	0.26, 0.66	-0.15	-0.41, 0.14
			1.7	5	3	0.60	0.23, 0.88	4	0.80	0.38, 0.96	-0.20	-0.60, 0.31
Cold Smoked Salmon <sup>h</sup> (25 g)	<i>L. innocua</i> Ad 1674	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.27 (0.10, 0.49)	20	10	0.50	0.30, 0.70	7	0.35	0.18, 0.57	0.15	-0.15, 0.41
			1.7	5	5	1.00	0.57, 1.00	4	0.80	0.38, 0.96	0.20	-0.26, 0.62
Cold Smoked Salmon <sup>h</sup> (25 g)	<i>L. innocua</i> Ad 1674	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.27 (0.10, 0.49)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			1.7	5	5	1.00	0.57, 1.00	4	0.80	0.38, 0.96	0.20	-0.26, 0.62

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

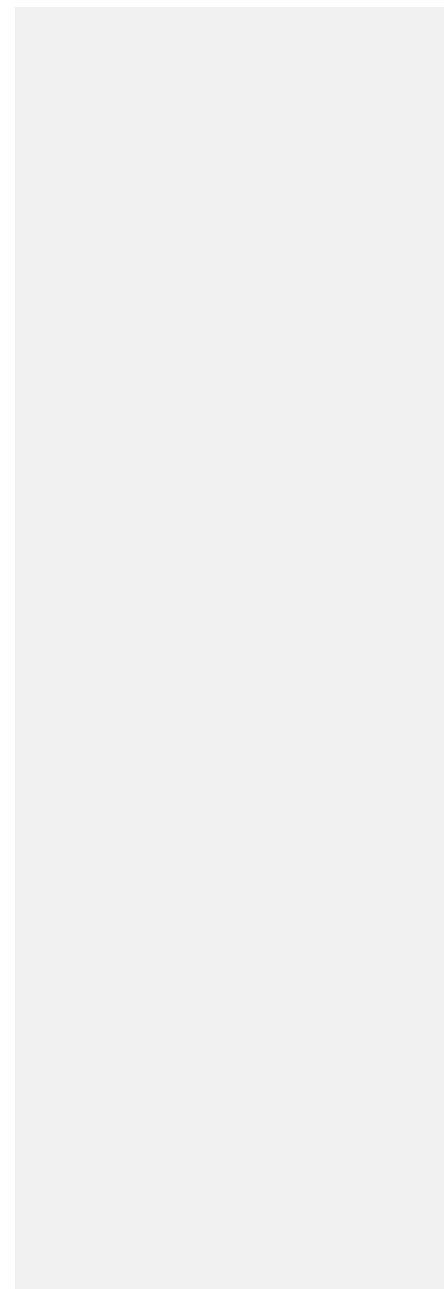
<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

<sup>h</sup>Tested using Demi Fraser (with FAC) brand X

<sup>i</sup>Tested using Demi Fraser (with FAC) brand Z



**Table 9: 3M™ MDA 2 - *Listeria* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Deli Turkey (125 g)	<i>L. monocytogenes</i> ATCC 19116	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.85)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces (25 g): Lot 1	Naturally Contaminated	28 Hour	1.91 (1.23, 3.80)	20	18	0.90	0.70, 0.97	16	0.80	0.58, 0.92	0.10	-0.13, 0.33
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces (25 g): Lot 2	Naturally Contaminated	28 Hour	1.12 (0.68, 1.91)	20	12	0.60	0.39, 0.78	14	0.70	0.48, 0.85	-0.10	-0.36, 0.18
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Whole Melon	<i>L. monocytogenes</i> FSL J1-049	26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			7	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			55	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Vanilla Ice Cream (25 g)	<i>L. monocytogenes</i> ATCC 19114	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.59 (0.33, 0.97)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes were in Table 9 were tested with Demi Fraser brand Y.

**Table 10: 3M™ MDA 2 - Listeria Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Queso Fresco (25 g)	<i>L. monocytogenes</i> CWD 1554	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	11	0.55	0.34, 0.74	9	0.45	0.26, 0.66	0.10	-0.19, 0.37
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
4% Milk Fat Cottage Cheese (25 g)	<i>L. welshimeri</i> ATCC 35897	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.69 (0.40, 1.14)	20	12	0.60	0.39, 0.78	9	0.45	0.26, 0.66	0.15	-0.15, 0.41
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Beef Hot Dogs (25 g)	<i>L. monocytogenes</i> ATCC 7644	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	5	0.25	0.11, 0.47	6	0.30	0.15, 0.52	-0.05	-0.31, 0.22
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes tested in Table 10 used Demi Fraser brand Y.



**Table 11: 3M™ MDA 2 - *Listeria* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Stainless Steel (225 mL)	<i>L. monocytogenes</i> ATCC 19118 & <i>Enterococcus faecium</i> ATCC 19434	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	8	0.40	0.22, 0.61	-0.10	-0.40, 0.13
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	8	0.40	0.22, 0.61	-0.10	-0.40, 0.13
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Sealed Concrete (100 mL)	<i>L. monocytogenes</i> ATCC 19117	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Plastic (10 mL)	<i>L. monocytogenes</i> ATCC 51782 & <i>Enterococcus faecalis</i> ATCC 29212	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			64 & 540	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			64 & 540	20	15	0.75	0.53, 0.89	11	0.55	0.22, 0.74	0.20	-0.09, 0.45
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes tested in Table 11 used Demi Fraser brand Y.

**Table 12: 3M™ MDA 2 - Listeria Assay, Presumptive vs. Confirmed – POD Results (INTERNAL STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces <sup>h</sup> (25 g)	<i>L. ivanovii</i> Ad 1291	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.31 (0.14, 0.57)	20	4	0.20	0.08, 0.42	9	0.45	0.26, 0.66	-0.25	-0.49, 0.05
			0.5	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Chicken Fillet <sup>i</sup> (25 g)	<i>L. ivanovii</i> Ad 1291	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.3 (0.1, 0.5)	20	4	0.80	0.38, 0.96	4	0.80	0.38, 0.96	0.00	-0.25, 0.25
			0.5	5	2	0.40	0.12, 0.77	2	0.40	0.12, 0.77	0.00	-0.46, 0.46
Bagged Raw Spinach <sup>h</sup> (25 g)	<i>L. seeligeri</i> Ad 1754	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.26 (0.10, 0.47)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.31
			1.7	5	3	1.00	0.57, 1.00	3	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cold Smoked Salmon <sup>h</sup> (25 g)	<i>L. innocua</i> Ad 1674	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.27 (0.1, 0.49)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.20	-0.26, 0.62
			1.7	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

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<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

<sup>h</sup>Tested using Demi Fraser brand (with FAC) brand X.

<sup>i</sup>Tested using Demi Fraser brand (with FAC) brand Z.

**Table 13: 3M™ MDA 2 - Listeria Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Deli Turkey (125 g)	<i>L. monocytogenes</i> ATCC 19116	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.85)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces (25 g): Lot 1	Naturally Contaminated	28 Hours	1.91 (1.23, 3.80)	20	18	0.90	0.70, 0.97	18	0.90	0.70, 0.97	0.00	-0.21, 0.21
Raw Chicken Leg Pieces (25 g): Lot 2	Naturally Contaminated	28 Hours	1.12 (0.68, 1.91)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Whole Melon	<i>L. monocytogenes</i> FSL J1-049	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			7	20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37
			55	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Vanilla Ice Cream (25 g)	<i>L. monocytogenes</i> ATCC 19114	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.59 (0.33, 0.97)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrices tested in Table 13 used Demi Fraser (with FAC) brand Y.

**Table 14: 3M™ MDA 2 - *Listeria* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Queso Fresco (25 g)	<i>L. monocytogenes</i> CWD 1554	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI							
4% Milk Fat Cottage Cheese (25 g)	<i>L. welshimeri</i> ATCC 35897	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.69 (0.40, 01.14)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef Hot Dogs (25 g)	<i>L. monocytogenes</i> ATCC 7644	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

All matrixes tested in Table 13 used Demi Fraser (with FAC) brand Y.

**Table 15: 3M™ MDA 2 - *Listeria* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Stainless Steel (225 mL)	<i>L. monocytogenes</i> ATCC 19118 & <i>Enterococcus faecium</i> ATCC 19434	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Sealed Concrete (100 mL)	<i>L. monocytogenes</i> ATCC 19117	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Plastic (10 mL)	<i>L. monocytogenes</i> ATCC 51782 & <i>Enterococcus faecalis</i> ATCC 29212	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			64 & 540	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			64 & 540	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the statistically significant at the 5% level

All matrixes tested in Table 13 used Demi Fraser (with FAC) brand Y.

**Table 16: Lot-to-Lot/Stability Study Results**

Expiration Date Lot #: 012715		Expiration Date Lot #: 081914		Expiration Date Lot #: 050114	
Sample #	Result	Sample #	Result	Sample #	Result
<i>Listeria monocytogenes</i> ATCC <sup>2</sup> 7644					
Low Level	6/10	Low Level	6/10	Low Level	6/10
Un-inoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5	Un-inoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5	Un-inoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5

<sup>1</sup>All samples were analyzed from a common lysate

<sup>2</sup>ATCC- American Type Culture Collection

**Table 17: Robustness Results**

Robustness <sup>1</sup> 13 Minute Lysis			Robustness <sup>1</sup> 13 Minute Lysis		
Sample #	18 µL <sup>2</sup> , 18 µL <sup>3</sup>	18 µL <sup>2</sup> , 22 µL <sup>3</sup>	Sample #	22 µL <sup>2</sup> , 18 µL <sup>3</sup>	22 µL <sup>2</sup> , 22 µL <sup>3</sup>
<i>Listeria monocytogenes</i> ATCC <sup>4</sup> 7644					
Low Level	6/10	6/10	Low Level	7/10	7/10
Un-inoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5	0/5	Un-inoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5	0/5
Robustness <sup>1</sup> 17 Minute Lysis			Robustness <sup>1</sup> 17 Minute Lysis		
Sample #	18 µL <sup>2</sup> , 18 µL <sup>3</sup>	18 µL <sup>2</sup> , 22 µL <sup>3</sup>	Sample #	22 µL <sup>2</sup> , 18 µL <sup>3</sup>	22 µL <sup>2</sup> , 22 µL <sup>3</sup>
<i>Listeria monocytogenes</i> ATCC <sup>4</sup> 7644					
Low Level	6/10	6/10	Low Level	6/10	6/10
Un-inoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5	0/5	Un-inoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC 29212)	0/5	0/5

<sup>1</sup>All samples were analyzed from a common lysate

<sup>2</sup>Volume of enrichment to lysis

<sup>3</sup>Volume of lysis to assay

<sup>4</sup>ATCC- American Type Culture Collection

Is the Test Kit Method scientifically and technically sound?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
Have sufficient Controls been used, including those required to calculate the rate of false-positive and		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
Is sufficient information included for system suitability determination and product performance or acceptance testing?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
Are the conclusions statements valid based upon data presented?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
Do you agree that the evidence or data from this and previous studies support the proposed		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
Are there sufficient data points per product evaluated in accordance with AOAC requirements?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
General Comments about the method scope/applicability:		

ER1	OMAMAN-30: EVALUATION OF 3M™ MOLECULAR DETECTION ASSAY 2 - LISTERIA FOR THE DETECTION OF LISTERIA IN SELECTED FOODS AND ENVIRONMENTAL SURFACES: COLLABORATIVE STUDY is a simple, rapid and easy to perform method.	Agreed! Thank you
ER2	Well-defined	Thank you
ER3	The method has a broad scope and applicability.	Agreed! Thank you
ER4	Between the PTM and OMA study, the method scope was study appropriately	Agreed! Thank you
ER5	The scope and applicability seem appropriate.	Thank you
ER6	Appropriately written	Thank you
Pros/Strengths of the Manuscript:		
ER1	The method is described in detail and in a friendly format.	Thank you
ER2	Well-written and clearly explained	Thank you
ER3	Manuscript is generally well written	Thank you
ER4	Manuscript was clear and thorough	Thank you
ER5	Generally well written	Thank you
ER6	The manuscript describes the study as required.	Thank you
Cons/Weakness of the Manuscript:		
ER1	NO	Thank you
ER2	None	Thank you
ER3	None	Thank you
ER4	The official method was written incorrectly	Revised the method and tables to reflect the homogenization process
ER5	Three minor points:	
	1. Page 97, line 39. This sentence needs rewriting	Unable to find this page in the "flip book" .
	2. Page 109, Lines 11 and 13. Spaces missing between some words. This happens throughout Ms.	
	3. Page 136, Table 3 (Supplementary). Lab 4, Raw chicken count seems to be a high outlier. Is there a typo?	~130 lbs of raw chicken breast was processed for the study. Varying degrees of APC results would be expected, although the variability observed is higher than typically seen.
ER6	Amended Submission:	
	1. Page 4, line 9 says the deli turkey was incubated for 24-28 hours; Table 3 of package insert says 24-30 hours, as does the collaborative study protocol and the Table 2 on Page 9 of the manuscript. Please clarify.	Corrected to 24-30 hours.



	2. Page 9, Table 2 - There is no table before this table in the manuscript. Label this as Table A to differentiate from the tables in the back of the manuscript and per page 7, line 34.	Revised to "Table A" in five instances. Page 9 Table header, page 9 line 3, Page 8 line 21.
	3. Please number Tables 1-3 of the Supplementary Material and include it with the other table of the manuscript. There are duplicate numbers of tables which make it a little confusing.	Supplementary tables are now numbered 3, 4, and 5.
	4. Tables 2016.2A - 2016.2B please define in footnote to table: N,X.	Added footnote explanation, N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)
Supporting Data and information: Does data from collaborative study support the method as written?		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	Yes	Thank You for your comments and review
ER5	Yes	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review
Supporting Data and information: Does data collected support the criteria given in the collaborative study protocol?		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	Yes	Thank You for your comments and review
ER5	Yes	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review
Are there any concerns regarding the safety of the method?		
ER1	No	Thank You for your comments and review
ER2	No	Thank You for your comments and review
ER3	Safety Review not available	Thank You for your comments and review
ER4	no	Thank You for your comments and review
ER5	no	Thank You for your comments and review
ER6	none, please also see review provided by Safety Advisor	Thank You for your comments and review
Are there any concerns regarding the data manipulation, data tables, or statistical analysis?		
ER1	No	Thank You for your comments and review
ER2	No	Thank You for your comments and review
ER3	Table 2. Heat injury, raw chicken product is not heat treated so not necessary to injure the cells	Addressed this in both the table and body of the manuscript

	Table 3. APC for Deli Turkey was not determined to be >1 log to meet the background flora criteria.	Per Appendix J, background flora at 10 x the limit of the competitor organism is only required for one food type, which would have been achieved by the raw chicken breast.
	Table3. Raw Chicken APC was variable - 1000s to 1,000,000s. It's not clear why so much variability between laboratories	~130 lbs of raw chicken breast was processed for the study. Varying degrees of APC results would be expected, although the variability observed is higher than typically seen.
ER4	no	Thank You for your comments and review
ER5	Statistical review not available. Flip book page 100, lin 35. Change 'variance' to 'variability' (two instances). S is standard deviation (variability) and s^2 is variance. The relevant footnotes in Tables 2016.1A and .1B are correct.	Revised variance to variability.
ER6	Please also see refer by statistical advisor	Thank you
Is the Validation Study Manuscript in a format acceptable to AOAC?		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	Yes	Thank You for your comments and review
ER5	Yes	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review
Is the method described in sufficient detail so that it is relatively easy to understand, including equations and procedures for calculation of results (are all terms explained)?		
ER1	Yes	Thank You for your comments and review
ER2	Yes	Thank You for your comments and review
ER3	Yes	Thank You for your comments and review
ER4	NO, there are problems with the official method write up (see below). The method will not be acceptable until the method is corrected.	
	1. Page 5, Section B. Were any samples homogenized by blending? Section D© refers to blending, but a blender does not appear in section B. If blenders were used in either the PTM or OMA validation studies, the "blender" should appear in the Apparatus and Reagents section.	Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.
	2. Page 6, line 32 (section C(b)). Change "...inaccurate results." To "false negative or false positive results."	Covered in the Safety sections of the Package inserts.

	<p>3. Page 7, line 33. Blending, stomaching and hand mixing can give significantly different results with certain commodities. This is particularly the case with leafy greens and seeds. If different types of homogenization were used for different commodities then they should specifically be listed in the method. Only sample preparation procedures that were used in the PTM and OMA validation studies can be specified in the instructions, since that is what was validated. "Table A" would be the appropriate location for this information.</p>	<p>Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.</p>
	<p>PTM 111501 indicates (Page 11, line 12-16) that "all" 25 and 125g food samples were homogenized by stomaching, so stomaching should be indicated for these products. It is not clear how the cantaloupes were prepared.</p>	<p>Currently revising the PTMs to reflect the method of homognization. See above response.</p>
	<p>4. Page 9, Table 2. There is no Table 1, so how can there be a Table 2? Moreover, Page7/line 34 refers to "Table A" which appears to be in the Table found on page 9. Please make sure that the instructions on page 7 refer the reader to the correct table.</p>	<p>Revised Table 2 to Table A. (five instances)</p>
	<p>5. Page 9, Table 2. Please be sure to add a sample preparation column that specifies homogenization procedures for the different commodities if different sample prep procedures were used in the validation studies.</p>	<p>Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.</p>
<p><b>ER4 Package Insert comments:</b></p>		
	<p>1. Page 9, line 3. It appears that the method was validated with a sample preparation procedure that only included stomaching, except cantaloupes, so "blending" should be removed from the instructions. I believe that hand mixing is appropriate for cantaloupes, but this must be spedified in the method.</p>	<p>Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.</p>

	2. There should be an applicability statement, specific to AOAC, In the package insert.	Per AOAC website: <a href="http://www.aoac.org/imis15_prod/AOAC_Docs/SPDS/Formats_AOAC_collab.pdf">http://www.aoac.org/imis15_prod/AOAC_Docs/SPDS/Formats_AOAC_collab.pdf</a> In the Package Insert the applicability statement is prior to the table. In AOAC PTM studies, the 3M Molecular Detection Assay 2 – Listeria was found to be an effective method for the detection of Listeria species. The matrices tested in the study are shown in Table 3. The limit of detection of the 3M Molecular Detection Assay 2 – Listeria method is 1-5 colony forming units per validated test portion size (in Table 3).
	3. There should be an apparatus/media section that details all of the equipment needed to perform the test that is not supplied by 3 in the package insert.	The Package insert lists ALL components that come with the kit.
ER5	Yes	
ER6	Yes	
Are the figures and tables sufficiently explanatory without the need to refer to the text?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
Are all the figures and tables pertinent?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
Could some be omitted and covered by a simple statement?		
ER1	NO	<a href="#">Thank You for your comments and review</a>
ER2	NO	<a href="#">Thank You for your comments and review</a>
ER3	NO	<a href="#">Thank You for your comments and review</a>
ER4	NO	<a href="#">Thank You for your comments and review</a>
ER5	NO	<a href="#">Thank You for your comments and review</a>
ER6	NO	<a href="#">Thank You for your comments and review</a>

Are the references complete and correctly annotated?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
Does the method contain adequate safety precaution reference and/or statements?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	Yes	<a href="#">Thank You for your comments and review</a>
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>
Recommendation: Do you recommend that the ERP adopt this method as an AOAC Official Method of Analysis (First Action status)?		
ER1	Yes	<a href="#">Thank You for your comments and review</a>
ER2	Yes	<a href="#">Thank You for your comments and review</a>
ER3	Yes	<a href="#">Thank You for your comments and review</a>
ER4	No, Both the official method and the package insert need to be revised, as stated earlier in this review, since they both contain sample preparation procedures (specifically blending) that were not validated in either the PTM study or the OMA study. Once the methods instructions have been corrected and are aligned with the validation studies, then I would recommend approval of the method for First Action Status	
ER5	Yes	<a href="#">Thank You for your comments and review</a>
ER6	Yes	<a href="#">Thank You for your comments and review</a>



1 **Evaluation of the 3M™ Molecular Detection Assay(MDA) 2 -*Listeria***  
2 ***monocytogenes*for the Detection of *Listeria monocytogenes*inSelectFoods and**  
3 **Environmental Surfaces: Collaborative Study**

4  
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7  
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10  
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12 E. Sjogren, M. Shekhawat, L. Ma, C. Timmons, C. Diaz Proano,A. Calle,Z. Metz, D. Baumler, R.  
13 Smith, D. Wood, E. Maranan, C. Chavarria,J. Miller, B. Schindler, C. Gies, E. Budge, Z. Geurin, A.  
14 Repeck, C. Zook, C. Barnes, B. Bastin, D. Isfort

15  
16 **The 3M™ Molecular Detection Assay (MDA) 2 –*Listeria monocytogenes*usesloop-**  
17 **mediated isothermal amplification of unique DNA target sequences combined**  
18 **with bioluminescence to rapidly detect *Listeria monocytogenes*in a broad range**  
19 **of food types and environmental surfaces. Using an unpaired study design,**  
20 **technicians from 13 laboratories located in the United States and Canada,**  
21 **compared the 3M MDA 2 -*Listeria monocytogenes*to the United States Department**  
22 **of Agriculture (USDA) Food Safety Inspection Service (FSIS) Microbiology**  
23 **Laboratory Guidebook (MLG) Chapter 8.09*Isolation and Identification of Listeria***  
24 ***monocytogenes from Red Meat, Poultry and Egg Products, and Environmental***  
25 ***Samples* reference method for the detection of *Listeria monocytogenes*in deli**  
26 **turkey and raw chicken breast fillet. Each matrix was evaluated at three levels of**  
27 **contamination: an un-inoculated control level**  
28 **(0 colony forming units (CFU)/test portion), a low inoculum level (0.2-2 CFU/test**  
29 **portion) and a high inoculum level (2-5 CFU/test portion).Statistical analysis was**  
30 **conducted according to the Probability of Detection (POD) statistical**  
31 **model.Results obtained for the low inoculum level test portions produced a**  
32 **dLPOD value with 95% confidence intervals of 0.04, (-0.08, 0.17) for deli turkey**  
33 **indicating the difference between methods was not statistically significant at the**  
34 **0.05 probability level.no statistically significant difference between the candidate**  
35 **and reference methods.For raw chicken breast fillet a dLPOD valewith 95%**  
36 **confidence interval of 0.16, (0.04, 0.28)indicating a statistically significant**  
37 **difference an observed higher proportion of positive results by the candidate**  
38 **method than the reference method,indicating astatisticallysignificant**  
39 **differencebetween the candidate and reference methods with a positive**  
40 **correlation in data indicating more recovery of the target analyte by the candidate**  
41 **method.**

42  
43 *Listeriamonocytogenes* is a highly pathogenic microorganism that contaminates food and can  
44 cause non-invasive gastroenteritis, or severe life-threatening illness, referred to as listeriosis [1].  
45 Although rare, listeriosis is associated with high hospitalization and mortality rates [2].

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1 *Listeria monocytogenes* is ubiquitous in the environment, often found in soil, water, sewage and  
2 damp environments, making it difficult to control in the food processing environment [1]. The  
3 organism's ability to survive in processing facilities has led to some highly publicized recent  
4 outbreaks, specifically in ice cream and packaged salads [3]. The 3M™ Molecular Detection  
5 Assay(MDA) 2 - *Listeria monocytogenes* method, using a combination of bioluminescence and  
6 isothermal amplification of nucleic acid sequences, allows for the rapid and specific detection of  
7 *Listeria monocytogenes* in a broad range of food types and environmental surfaces after 24 to  
8 | 2832 hours of pre-enrichment. After enrichment, samples are evaluated using the 3M MDA 2 -  
9 *Listeria monocytogenes* on the 3M™ Molecular Detection System (MDS). Presumptive positive  
10 results are reported in real-time while negative results are displayed after completion of the assay  
11 in approximately 75 minutes.  
12 Prior to the collaborative study, the 3M MDA 2 - *Listeria monocytogenes* method was validated  
13 according to AOAC Guidelines[4] in a harmonized AOAC® Performance Tested Method<sup>SM</sup>  
14 (PTM) study. The objective of the PTM study was to demonstrate that the 3M MDA 2-*Listeria*  
15 *monocytogenes* method could detect *Listeria monocytogenes* in a broad range of food matrices  
16 and environmental surfaces as claimed by the manufacturer. For the 3M MDA 2- *Listeria*  
17 *monocytogenes* PTM evaluation, 13 matrices were evaluated: hot dogs (25g & 125g), salmon  
18 (25g), deli turkey (25g & 125g), cottage cheese (25g), chocolate milk (25 mL), vanilla ice cream  
19 (25g), queso fresco (25g), romaine lettuce (25g), melon (whole), raw chicken leg pieces (25g);  
20 concrete (sponge, 225 mL & 100 mL), stainless steel (sponge, 225 mL), and plastic  
21 | (EnviroSwab, 10 mL) environmental samples.  
22 Additional PTM parameters (inclusivity, exclusivity, ruggedness, stability and lot-to-lot  
23 variability) tested in the PTM studies satisfied the performance requirements for PTM approval.  
24 The method was awarded PTM certification number 081501 on August 18<sup>th</sup>, 2015.  
25 The purpose of this collaborative study was to compare the reproducibility of the 3M MDA 2 -  
26 *Listeria monocytogenes* method to the United States Department of Agriculture (USDA) Food  
27 Safety Inspection Service (FSIS) -Microbiology Laboratory Guidebook (MLG) Chapter 8.09  
28 *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg*  
29 *Products, and Environmental Samples*[5] for deli turkey (125 g) and raw chicken breast fillet.  
30

## 31 Collaborative Study

### 32 Study Design

33  
34  
35 In this collaborative study, two matrices, deli turkey and raw chicken breast fillet,  
36 were evaluated. The matrices were obtained from a local retailer and screened for the presence of  
37 *Listeria monocytogenes* by the USDA/FSIS MLG 8.09 reference method. The raw chicken breast  
38 fillet was artificially contaminated with fresh unstressed cells of *Listeria monocytogenes*,  
39 American Type Culture Collection (ATCC) 7644, and the deli turkey was artificially  
40 | contaminated with heat stressed cells (Table 2) of *Listeria monocytogenes*, ATCC 19115, at two  
41 inoculation levels: a high inoculation level of approximately 2-5 colony-forming units (CFU)/test  
42 portion and a low inoculation level of approximately 0.2-2 CFU/test portion. A set of un-  
43 inoculated control test portions (0 CFU/test portion) were also included.  
44 Twelve replicate samples from each of the three inoculation levels were analyzed by each  
45 method. Two sets of samples (72 total) were sent to each laboratory for analysis by 3M MDA 2 -  
46 *Listeria monocytogenes* and the USDA/FSIS MLG Chapter 8.09 reference method due to the  
47 different sample enrichment procedures for each method. Additionally, collaborators were sent a  
48 60 g test portion and instructed to conduct a total aerobic plate count (APC) using 3M™



1 Petrifilm™ Rapid Aerobic Count Plate (AOAC Official Method 2015.13) [6] on the day samples  
2 were received for the purpose of determining the total aerobic microbiological load.  
3 A detailed collaborative study packet outlining all necessary information related to the study  
4 including media preparation, test portion preparation and documentation of results was sent to  
5 each collaborating laboratory prior to the initiation of the study. A conference call was then  
6 conducted to discuss the details of the collaborative study packet and answer any questions from  
7 the participating laboratories.

#### 8 9 *Preparation of Inocula and Test Portions*

10  
11 The *Listeria monocytogenes* cultures ([ATCC 7644](#) and [ATCC 19115](#)) used in this evaluation  
12 were propagated onto Tryptic Soy Agar with 5% Sheep Blood (SBA) from a Q Laboratories  
13 frozen stock culture stored at -70°C. Each organism was incubated for 24 ± 2 hours at 35 ± 1°C.  
14 Isolated colonies were picked to 10 mL of Brain Heart Infusion (BHI) broth and incubated for 18  
15 ± 0.5 hours at 35 ± 1°C. Raw chicken breast fillet was inoculated in bulk using the fresh, broth  
16 culture. Prior to inoculation of the deli turkey, the culture suspension was heat stressed at 55 ±  
17 1°C in a water bath for 15 ± 0.5 minutes to obtain a percent injury of 50-80% (as determined by  
18 plating onto selective Modified Oxford agar (MOX) and non-selective tryptic soy agar with yeast  
19 (TSA/ye). The degree of injury was estimated as:

$$\left(1 - \frac{n_{select}}{n_{nonselect}}\right) \times 100$$

20  
21 where  $n_{select}$  = number of colonies on selective agar and  $n_{nonselect}$  = number of colonies on non-  
22 selective agar. Appropriate dilutions of each culture were prepared in Butterfield's Phosphate  
23 Diluent (BPD) based on previously established growth curves for both low and high inoculation  
24 levels. Bulk portions of each matrix were inoculated with the diluted liquid inoculum and mixed  
25 thoroughly to ensure an even distribution of microorganisms. The inoculated raw chicken  
26 breast fillet was packaged into separate 30 g test portions in sterile Whirl-Pak® bags and shipped  
27 to the collaborators. For the analysis of the deli turkey, 25 g of inoculated test product was  
28 mixed with 100 g of un-inoculated test product to prepare 125 g test portions which were  
29 packaged in sterile Whirl-Pak® bags and shipped to collaborators.

30 To determine the level of *Listeria monocytogenes* in the matrices, a 5-tube most probable  
31 number (MPN) was conducted by the coordinating laboratory on the day of the initiation of  
32 analysis using the USDA/FSIS-MLG 8.09 reference method. For deli turkey, the MPN was  
33 determined by analyzing 5 x 250 g test portions, reference method test portions from the  
34 collaborating laboratories and 5 x 65 g test portions. For the raw chicken breast fillet, the MPN  
35 of the high and low inoculated levels was determined by analyzing 5 x 50 g test portions, the  
36 reference method test portions from the collaborating laboratories and 5 x 10 g test portions. The  
37 MPN and 95% confidence intervals were calculated using the LCF MPN Calculator, Version 1.6,  
38 ([www.lcftld.com/customer/LCFMPNCalculator.exe](http://www.lcftld.com/customer/LCFMPNCalculator.exe)), provided by AOAC Research Institute  
39 (RI) [7].

#### 40 41 *Test Portion Distribution*

42  
43 All samples were labeled with a randomized, blind-coded 3-digit number affixed to the sample  
44 container. Test portions were shipped on a Thursday via overnight delivery according to the  
45 Category B Dangerous Goods shipment regulations set forth by the International Air  
46 Transportations Association (IATA). The two matrices were shipped consecutively, with  
47 collaborating laboratories performing analysis on one matrix at a time. Upon receipt, samples  
48 were held by the collaborating laboratory at refrigeration temperature (2-8 °C) until the

1 following Monday when analysis was initiated after a total equilibration time of 96 hours. All  
2 samples were packed with cold packs to target a temperature of  $< 7^{\circ}\text{C}$  during shipment.  
3 In addition to each of the test portions and a separate APC sample, collaborators received a test  
4 portion for each matrix labeled as 'temperature control'. Participants were instructed to obtain  
5 the temperature of this portion upon receipt of the package, document the results on the Sample  
6 Receipt Confirmation form provided and fax or email it back to the study director. The shipment  
7 and hold times of the inoculated test material had been verified as a quality control measure prior  
8 to study initiation.

#### 9 10 *Test Portion Analysis*

11  
12 Collaborators were instructed to follow the appropriate preparation and analysis as outlined in  
13 the study protocol for each matrix for both the 3M MDA 2 - *Listeria monocytogenes* method and  
14 reference method. For both matrices, each collaborator received 72 test portions (12 high, 12 low  
15 and 12 un-inoculated controls for each method to be performed). For the analysis of the deli  
16 turkey test portions by the 3M MDA 2 - *Listeria monocytogenes* method, a 125 g portion was  
17 enriched with 975 mL of Demi-Fraser (DF) with ferric ammonium citrate (FAC) broth,  
18 homogenized for 2 minutes and incubated for 24-~~28~~30 hours at  $37 \pm 1^{\circ}\text{C}$ . For the raw chicken  
19 breast fillet test portions analyzed by the 3M MDA 2 - *Listeria monocytogenes* method, a 25 g  
20 portion was enriched with 475 mL of DF, homogenized for 2 minutes and incubated for 28-32  
21 hours at  $37 \pm 1^{\circ}\text{C}$ .

22 Following enrichment, samples were assayed by the 3M MDA 2 - *Listeria monocytogenes*  
23 method and, regardless of presumptive result, confirmed following the USDA/FSIS MLG 8.09  
24 reference method. Both matrices evaluated by the 3M MDA 2 - *Listeria monocytogenes* method  
25 were compared to samples analyzed using the USDA/FSIS MLG 8.09 reference method in an  
26 unpaired study design. All positive test portions were biochemically confirmed by the API  
27 *Listeria monocytogenes* biochemical test or by the VITEK 2 GP biochemical identification test,  
28 AOAC Official Method 2012.02 [8].

#### 29 30 *Statistical Analysis*

31  
32 Each collaborating laboratory recorded results for the reference method and the 3M MDA 2 -  
33 *Listeria monocytogenes* method on the data sheets provided. The data sheets were submitted to  
34 the study director at the end of each week of testing for statistical analysis. Data for each matrix  
35 was analyzed using the probability of detection (POD) statistical model [9]. [POD statistical  
36 analysis was conducted using AOAC Binary Data Interlaboratory Study Workbook, Version 2.3  
37 \[10\]](#). The probability of detection (POD) was calculated as the number of positive outcomes  
38 divided by the total number of trials. The POD was calculated for the candidate presumptive  
39 results,  $\text{POD}_{\text{CP}}$ , the candidate confirmatory results ([excluding those with presumptive negative  
40 results including false negative results](#)),  $\text{POD}_{\text{CC}}$ , the difference in the candidate presumptive and  
41 confirmatory results,  $\text{dLPOD}_{\text{CP}}$ , presumptive candidate results that confirmed positive ([including  
42 those with presumptive negative results](#)) [excluding false negative results](#)),  $\text{POD}_{\text{C}}$ , the reference  
43 method,  $\text{POD}_{\text{R}}$ , and the difference in the confirmed candidate and reference methods,  $\text{dLPOD}_{\text{C}}$ .  
44 A  $\text{dLPOD}_{\text{C}}$  confidence interval not containing the point zero would indicate a statistically  
45 significant difference between the 3M MDA 2 - *Listeria monocytogenes* and the reference  
46 methods at the 5 % probability level. In addition to POD, the repeatability standard deviation  
47 ( $s_r$ ), the among laboratory repeatability standard deviation ( $s_L$ ), the reproducibility standard  
48 deviation ( $s_R$ ) and the  $P_T$  value were calculated. The  $s_r$  provides the [variability variance](#) of data  
49 within one laboratory, the  $s_L$  provides the difference in standard deviation between laboratories

1 and the  $s_R$  provides the variability ~~variance~~ in data between different laboratories. The  $P_T$  value  
2 provides information on the homogeneity test of laboratory PODs. ~~[10]~~

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8 **AOAC Official Method 2016.xxx**  
9 ***Listeria monocytogenes* in Select Foods and Environmental Surfaces**  
10 **3M™ Molecular Detection Assay (MDA) 2 - *Listeria monocytogenes* Method**  
11 **First Action 2016**  
12

13 (Applicable to detection of *Listeria monocytogenes* hot dogs (25g & 125g), salmon (25g), deli  
14 turkey (25g & 125g), cottage cheese (25g), chocolate milk (25 mL), vanilla ice cream (25g),  
15 queso fresco (25g), romaine lettuce (25g), melon (whole), raw chicken leg pieces (25g); raw  
16 chicken breast fillet (25g), concrete (3M™ Hydrated Sponge Stick with Dey-Engley [D/E], 225  
17 mL & 100 mL), stainless steel (3M Hydrated Sponge Stick with D/E, 225 mL), and plastic  
18 (3M™ EnviroS~~Swab~~ with Lethen, 10 mL) environmental samples.

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19  
20 See Tables 2016.1A and 2016.1B for a summary of results of the inter-laboratory study.  
21 See Tables 2016.2A and 2016.2B for detailed results of the inter-laboratory study  
22

23 **A. Principle**  
24

25 The 3M™ Molecular Detection Assay (MDA) 2 - *Listeria monocytogenes* method is used with  
26 the 3M™ Molecular Detection System (MDS) for the rapid and specific detection of *Listeria*  
27 *monocytogenes* in enriched food and food process environmental samples. The 3M MDA 2 -  
28 *Listeria monocytogenes* uses loop-mediated isothermal amplification of unique DNA target  
29 sequences with high specificity and sensitivity, combined with bioluminescence to detect the  
30 amplification. Presumptive positive results are reported in real-time while negative results are  
31 displayed after the assay is completed. Samples are pre-enriched in Demi Fraser with ferric  
32 ammonium citrate broth.  
33

34 **B. Apparatus and Reagents**

35 **Items (b)-(g) are available as the 3M™ Molecular Detection Assay (MDA) 2 - *Listeria***  
36 ***monocytogenes* kit from 3M Food Safety (St. Paul, MN 55144-1000, USA).**

- 37 (a) 3M Molecular Detection System (MDS100) – Available from 3M Food Safety (St.  
38 Paul, MN 55144-1000, USA).  
39 (b) 3M Molecular Detection Assay 2 – *Listeria monocytogenes* reagent tubes- 12 strips of  
40 8 tubes. Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).  
41 (c) Lysis Solution (LS) tubes – 12 strips of 8 tubes.  
42 (d) Extra caps – 12 strips of 8 caps  
43 (e) Reagent Control – 8 reagent tubes  
44 (f) Quick Start Guide  
45 (g) 3M™ Molecular Detection Speed Loader Tray - Available from 3M Food Safety  
46 (St. Paul, MN 55144-1000, USA).  
47 (h) 3M™ Molecular Detection Chill Block Insert - Available from 3M™ Food Safety (St.  
48 Paul, MN 55144-1000, USA).  
49 (i) 3M™ Molecular Detection Heat Block Insert - Available from 3M Food Safety

- (St. Paul, MN 55144-1000, USA).
- (j) *3M™ Molecular Detection Cap/Decap Tool for Reagent tubes* - Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- (k) *3M™ Molecular Detection Cap/Decap Tool for Lysis tubes* – Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- (l) *Empty Lysis Tube Rack* - Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- (m) *Empty Reagent Tube Rack* - Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- (n) *Demi Fraser Broth* - Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- (o) *Ferric Ammonium Citrate (FAC)*, ACS grade, 5% sterilized -Available from MP Biomedicals™ or equivalent.
- (p) *Disposable pipette* – capable of 20 µL
- (q) *Multi-channel (8-channel) pipette* - capable of 20 µL
- (r) *Sterile filter tip pipette tips* - capable of 20 µL
- (s) *Filter Stomacher® bags* – Seward or equivalent.
- (t) *Stomacher®*– Seward or equivalent.
- (u) *Thermometer* – calibrated range to include  $100 \pm 1^{\circ}\text{C}$
- (v) *Dry block heater unit*– capable of maintaining  $100 \pm 1^{\circ}\text{C}$
- (w) *Incubators*. – Capable of maintaining  $37 \pm 1^{\circ}\text{C}$  ~~or  $41.5 \pm 1^{\circ}\text{C}$ .~~
- ~~(x) *Freezer* – capable of maintaining  $10$  to  $20^{\circ}\text{C}$ , for storing the 3M Molecular Detection Chill Block Tray~~
- ~~(y)(x) *Refrigerator* – capable of maintaining  $2$ - $8^{\circ}\text{C}$ , for storing the 3M Molecular Detection Assay components~~
- ~~(z)(y) *Computer* – compatible with the 3M™ Molecular Detection Instrument~~
- ~~(aa)(z) *3M™ EnviroS Swab*, -- (hydrated with Letheen) Available from 3M Food Safety(Australia)~~
- ~~(bb)(aa) *3M™ Hydrated Sponge Stick with 10 mL of D/E* – Available from 3M Food Safety(St. Paul, MN 55144-1000, USA)~~

### C. General Instructions

(a) Store the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* at  $2$ - $8^{\circ}\text{C}$ . Do not freeze. Keep kit away from light during storage. After opening the kit, check that the foil pouch is undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes should always be stored in the re-sealable pouch with the desiccant inside to maintain stability of the lyophilized reagents. Store resealed pouches at  $2$ - $8^{\circ}\text{C}$  for no longer than 60 days. Do not use 3M Molecular Detection Assay 2 -*Listeria monocytogenes* past the expiration date.

(b) Follow all instructions carefully. Failure to do so may lead to inaccurate results.

#### *Safety Precautions*

The 3M MDA2 – *Listeria monocytogenes* is intended for use in a laboratory environment by professionals trained in laboratory techniques. 3M has not documented the use of this product in industries other than the food and beverage

1 industries. For example, 3M has not documented this product for testing drinking  
2 water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M MDA2 –  
3 *Listeria monocytogenes* has not been evaluated with all possible food products, food  
4 processes, testing protocols or with all possible strains of bacteria.

5 As with all test methods, the source of enrichment medium can influence the results.  
6 The 3M MDA 2 – *Listeria monocytogenes* has only been evaluated for use with the  
7 enrichment media specified in the Instructions for Use section.

8 The 3M™ Molecular Detection instrument is intended for use with samples that have  
9 undergone heat treatment during the assay lysis step, which is designed to destroy  
10 organisms present in the sample. Samples that have not been properly heat treated  
11 during the assay lysis step may be considered a potential biohazard and should NOT  
12 be inserted into the 3M MDS instrument.

13 The user should read, understand and follow all safety information in the instructions  
14 for the 3M MDS and the 3M MDA 2 – *Listeria monocytogenes*. Retain the safety  
15 instructions for future reference.

16 To reduce the risks associated with exposure to chemicals and biohazards:  
17 Perform pathogen testing in a properly equipped laboratory under the control of  
18 trained personnel. Always follow standard laboratory safety practices, including  
19 wearing appropriate protective apparel and eye protection while handling reagents  
20 and contaminated samples. Avoid contact with the contents of the enrichment media  
21 and reagent tubes after amplification. Dispose of enriched samples according to  
22 current industry standards.

23 *Listeria monocytogenes* of particular concern for pregnant women, the aged and the  
24 infirmed. It is recommended that these concerned groups avoid handling this  
25 organism. After use, the enrichment medium and the 3M MDA 2 - *Listeria*  
26 *monocytogenes* tubes can potentially contain pathogenic materials. Periodically  
27 decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with  
28 a 1- 5% (v:v in water) household bleach solution or DNA removal solution. When  
29 testing is complete, follow current industry standards for the disposal of contaminated  
30 waste. Consult the Safety Data Sheet for additional information and local regulations  
31 for disposal.

32 To reduce the risks associated with environmental contamination: Follow current  
33 industry standards for disposal of contaminated waste.

#### 34 **D. Sample Enrichment**

##### 35 ***Foods***

- 36  
37 (a) Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate)  
38 to equilibrate to ambient laboratory temperature (20-25°C).  
39 (b) Aseptically combine the enrichment medium and sample according to Table 2A. For

1 all meat and highly particulate samples, the use of filter bags is recommended.  
2 (c) Homogenize thoroughly by ~~blending~~, stomaching, or hand mixing for 2 ±0.2 minutes.  
3 Incubate at 37 ±1°C according to Table A.  
4

5 ***Environmental samples***

6 (d) Sample collection devices can be a sponge hydrated with a neutralizing solution to  
7 inactivate the effects of the sanitizers. 3M recommends the use of a biocide-free  
8 cellulose sponge. Neutralizing solution can be Dey-Engley (D/E) Neutralizing Broth  
9 or Lethen broth. It is recommended to sanitize the area after sampling.

10 WARNING: Should you select to use Neutralizing Buffer (NB) that contains aryl  
11 sulfonate complex as the hydrating solution for the sponge, it is required to perform a  
12 1:2 dilution (1-part sample into 1-part sterile enrichment broth) of the enriched  
13 environmental sample before testing in order to reduce the risks associated with a  
14 false-negative result leading to the release of contaminated product. Another option is  
15 to transfer 10 µL of the NB enrichment in the LS tubes.  
16  
17

18 (e) The recommended size of the sampling area to verify the presence or absence of the  
19 pathogen on the surface is at least 100 cm<sup>2</sup> (10 cm x 10 cm or 4"x4"). When sampling  
20 with a sponge, cover the entire area going in two directions (left to right then up and  
21 down) or collect environmental samples following your current sampling protocol or  
22 according to the FDA BAM, USDA FSIS MLG or ISO 18593 [11] guidelines.

- 23 1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium  
24 citrate) to equilibrate to ambient laboratory temperature (20-25°C).
- 25 2. Aseptically combine the enrichment medium and sample according to Table 2A.
- 26 3. Homogenize thoroughly by ~~blending~~vortexing or, stomaching, ~~or hand mixing~~  
27 for 2 ±0.2 minutes. Incubate at 37 ±1°C for 24-30hours.

28  
29 **E. PREPARATION OF THE 3M™ MOLECULAR DETECTION SPEED LOADER TRAY**

- 30 (a) Wet a cloth or paper towel with a 1-5% (v:v in water) household bleach solution and  
31 wipe the 3M™ Molecular Detection Speed Loader Tray.
- 32 (b) Rinse the 3M Molecular Detection Speed Loader Tray with water.
- 33 (c) Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.
- 34 (d) Ensure the 3M Molecular Detection Speed Loader Tray is dry before use.  
35

36 **Specific Instructions for Validated Methods**  
37 **AOAC® Performance Tested Method<sup>sm</sup> # 081501**



In AOAC PTM studies, the 3M Molecular Detection Assay 2 – *Listeria monocytogenes* was found to be an effective method for the detection of *Listeria monocytogenes*. The matrices tested in the study are shown in Table 2A. The limit of detection of the 3M Molecular Detection Assay 2 – *Listeria monocytogenes* method is 1-5 colony forming units per validated test portion size (in Table 2A).

**Table 2A.** Enrichment protocols using Demi-Fraser Broth at  $37 \pm 1$  °C according to AOAC Performance Tested <sup>SM</sup> Certificate #081501.

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)	
Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon	25 g	225	24-30	
Raw chicken	25 g	475	28-32	
Deli turkey	125 g	1125	24-30	
Cantaloupe <sup>a</sup>	Whole melon	Enough volume to allow melon to float	26-30	
Environmental samples:	Stainless steel	1 sponge	225	24-30
	Sealed concrete	1 sponge	100	24-30
	Plastic <sup>b</sup>	1 swab	10	24-30

All samples for the AOAC validation were homogenized by stomaching unless otherwise noted.

a = Homogenize sample by hand mixing

b = Homogenize sample by vortexing

#### F. PREPARATION OF THE 3M™ MOLECULAR DETECTION HEAT BLOCK INSERT

Place the 3M™ Molecular Detection Heat Block Insert in a dry double block heater unit. Turn on the dry block heater unit and set the temperature to allow the 3M Molecular Detection Heat Block Insert to reach and maintain a temperature of  $100 \pm 1$  °C.

1 **NOTE:** Depending on the heater unit, allow approximately 30minutes for the 3M Molecular Detection  
2 Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (e.g., a partial  
3 immersion thermometer, digital thermocouple thermometer, not a total immersion thermometer) placed in  
4 the designated location, verify that the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^{\circ}\text{C}$ .

#### 5 **G. PREPARATION OF THE 3M MOLECULAR DETECTION INSTRUMENT**

- 6 1. Launch the 3M™ Molecular Detection Software and log in.
- 7 2. Turn on the 3M Molecular Detection Instrument.
- 8 3. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection  
9 System User Manual for details.

10  
11 **NOTE:** The 3M Molecular Detection Instrument must reach and maintain temperature of  $60^{\circ}\text{C}$  before  
12 inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes  
13 approximately 20 minutes and is indicated by an ORANGE light on the instrument's status bar. When the  
14 instrument is ready to start a run, the status bar will turn GREEN.

#### 15 **H. LYSIS**

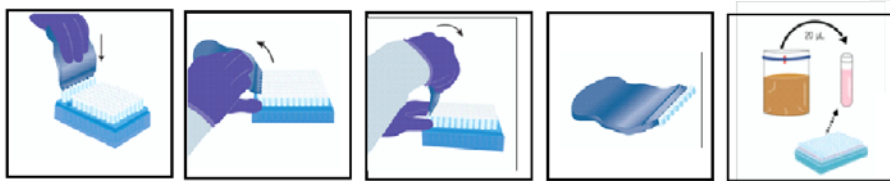
- 16 1. Allow the lysis solution (LS) tubes to warm up by setting the rack at room temperature (20-  
17  $25^{\circ}\text{C}$ ) overnight (16-18 hours). Alternatives to equilibrate the LS tubes to room  
18 temperature are to set the LS tubes on the laboratory bench for at least 2 hours, incubate the  
19 LS tubes in a  $37 \pm 1^{\circ}\text{C}$  incubator for 1 hour or place them in a dry double block heater for  
20 30 seconds at  $100^{\circ}\text{C}$ .
- 21 2. Invert the capped tubes to mix. Proceed to next step within 4 hrs.
- 22 3. Remove the enrichment broth from the incubator.
- 23 4. One LS tube is required for each sample and the Negative Control (NC) (sterile enrichment  
24 medium) sample.
  - 25 4.1 LS tube strips can be cut to desired LS tube number. Select the number of individual LS  
26 tubes or 8-tube strips needed. Place the LS tubes in an empty rack.
  - 27 4.2 To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipette  
28 tip for each transfer step.
  - 29 4.3 Transfer enriched sample to LS tubes as described below:

30  
31 **Transfer each enriched sample into individual LS tube *first*. Transfer the NC *last*.**

- 32  
33 4.3 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip -  
34 one strip at a time.
- 35 4.4 Discard the LS tube cap – if lysate will be retained for retest, place the caps into a clean  
36 container for re-application after lysis
- 37 4.5 Transfer  $20 \mu\text{L}$  of sample into a LS tube.
- 38 4.6 Repeat step 4.2 until each individual sample has been added to a corresponding LS tube  
39 in the strip as illustrated below.

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add space between paragraphs of the same  
style, Tab stops: Not at 4.45"





- 1  
2
- 3 4.7 Repeat steps 4.1 to 4.4 as needed, for the number of samples to be tested. When all  
4 samples have been transferred, then transfer **20  $\mu$ L of NC into a LS tube**. Do not recap  
5 tubes.
- 6 4.8 Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100  
7  $\pm 1^\circ\text{C}$ . Place the rack of LS tubes in the 3M Molecular Detection Heat Block Insert and  
8 heat for 15  $\pm 1$  minutes. During heating, the LS solution will change from pink (cool) to  
9 yellow (hot).
- 10 5. Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M  
11 Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes.  
12 The 3M Molecular Chill Block Insert, used at ambient temperature (20-25 $^\circ\text{C}$ ) without the  
13 Molecular Detection Chill Block Tray, should sit directly on the laboratory bench. When  
14 cool, the lysis solution will revert to a pink color.
- 15 6. Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.

16  
17 **I. AMPLIFICATION**

- 18 1. One Reagent tube is required for each sample and the NC.
- 19 1.1 Reagent tubes strips can be cut to desired tube number. Select the number of individual  
20 Reagent tubes or 8-tube strips needed.
- 21 1.2 Place Reagent tubes in an empty rack.
- 22 1.3 Avoid disturbing the reagent pellets from the bottom of the tubes.
- 23 2. Select 1 Reagent Control (RC) tube and place in rack.
- 24 3. To avoid cross-contamination, decap one Reagent tubes strip at a time and use a new pipette  
25 tip for each transfer step.
- 26 4. Transfer lysate to Reagent tubes and RC tube as described below:

27  
28 **Transfer each sample lysate into individual Reagent tubes first followed by the NC. Hydrate the RC**  
29 **tube last.**

- 30 4.1 Use the 3M<sup>TM</sup> Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent  
31 tubes –one Reagent tubes strip at a time. Discard cap.
- 32 4.2 Transfer 20  $\mu$ L of Sample lysate from the upper  $\frac{1}{2}$  of the liquid (avoid precipitate) in  
33 the LS tube into corresponding Reagent tube. Dispense at an angle to avoid disturbing  
34 the pellets. Mix by gently pipetting up and down 5 times.
- 35 4.3 Repeat step 4.2 until individual Sample lysate has been added to a corresponding  
36 Reagent tube in the strip.
- 37 4.4 Cover the Reagent tubes with the provided extra cap and use the rounded side of the  
38 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth  
39 motion ensuring that the cap is tightly applied.
- 40 4.5 Repeat steps 4.1 to 4.4 as needed, for the number of samples to be tested.
- 41 4.6 When all sample lysates have been transferred, repeat 4.1 to 4.4 to transfer 20  $\mu$ L of  
42 NC lysate into a Reagent tube.

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- 1 4.7 Transfer **20 µL of NC lysate into a RC tube**. Dispense at an angle to avoid disturbing  
2 the pellets. Mix by gently pipetting up and down 5 times.
- 3 5. Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader  
4 Tray. See illustration below. Close and latch the 3M Molecular Detection Speed Loader  
5 lid.



- 6  
7  
8
- 9 6. Review and confirm the configured run in the 3M Molecular Detection Software.
- 10 7. Click the Start button in the software and select instrument for use. The selected instrument's  
11 lid automatically opens.
- 12 8. Place the 3M Molecular Detection Speed Loader Tray into the 3M MDS Instrument and  
13 close the lid to start the assay. Results are provided within 75 minutes, although positives may  
14 be detected sooner.
- 15 9. After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from  
16 the 3M Molecular Detection Instrument and dispose of the tubes by soaking in a 1-5% (v:v in  
17 water) household bleach solution for 1 hour and away from the assay preparation area.
- 18

19 **NOTICE:** To minimize the risk of false positives due to cross-contamination, never open reagent tubes  
20 containing amplified DNA. This includes Reagent Control, Reagent and Matrix Control tubes. Always  
21 dispose of sealed reagent tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour  
22 and away from the assay preparation area.

#### 23 **RESULTS AND INTERPRETATION**

24 An algorithm interprets the light output curve resulting from the detection of the nucleic acid  
25 amplification. Results are analyzed automatically by the software and are color-coded based on  
26 the result. A Positive or Negative result is determined by analysis of a number of unique curve  
27 parameters. Presumptive positive results are reported in real-time while Negative and Inspect  
28 results will be displayed after the run is completed.

29  
30 Presumptive positive samples should be confirmed as per the laboratory standard operating  
31 procedures or by following the current version of the appropriate reference method  
32 confirmation ([FDA/BAM](#), the [USDA/FSIS-MLG](#)), beginning with transfer from the primary  
33 enrichment to secondary enrichment broth (if applicable), followed by subsequent plating and  
34 confirmation of isolates using appropriate biochemical and serological methods.

35  
36  
37 **NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular  
38 Detection Assay 2 - *Listeria monocytogenes* amplification reagents have a "background" relative  
39 light unit (RLU) reading.

40

1 In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M  
2 recommends the user to repeat the assay for any Inspect samples. If the result continues to be  
3 Inspect, proceed to confirmation test using your preferred method or as specified by local  
4 regulations.

#### 5 6 *Results of Collaborative Study*

7  
8 For this collaborative study, the 3M Molecular Detection Assay (MDA) 2 - *Listeria*  
9 *monocytogenes* method was compared to the USDA FSIS MLG 8.09 reference method for deli  
10 turkey and raw chicken breast fillet. A total of 13 laboratories throughout the United States and  
11 Canada participated in this study, with 11 laboratories submitting data for the deli turkey and 12  
12 laboratories submitting data for the raw chicken breast fillet. See Table 1 for a summary of  
13 laboratory participation for each matrix. Each laboratory analyzed 36 test portions for each  
14 method per matrix: 12 inoculated with a high level of *Listeria monocytogenes*, 12 inoculated  
15 with a low level of *Listeria monocytogenes*, and 12 un-inoculated controls.

16 A background screen of the matrix indicated an absence of indigenous *Listeria monocytogenes* in  
17 both matrices. Ten (10) replicate test portions (randomly sampled from 50% of the total packages  
18 used in the analysis) were screened for the presence of *Listeria monocytogenes*. All test portions  
19 produced negative results for the target analyte.

20 Results for the heat stress analysis of the inoculum for the deli turkey are presented in Table 2.

21 [The raw chicken breast fillet is not heat treated, therefore it was not necessary to injure the](#)  
22 [cells.](#) Table 2016.1A and 2016.1B summarize the inter-laboratory results for all foods tested,  
23 including POD statistical analysis. As per criteria outlined in Appendix J of the AOAC  
24 Validation Guidelines, fractional positive results were obtained. Detailed results for each  
25 laboratory are presented in Tables 2016.2A and 2016.2B. For each matrix, the level of *Listeria*  
26 *monocytogenes* was determined by MPN on the day of initiation of analysis by the coordinating  
27 laboratory. MPN results are presented in Tables 2016.2A and 2016.2B. The individual laboratory  
28 and sample results are presented in Tables 1-2 of the Supplementary Materials. The APC results  
29 for each collaborating are presented in Table 35 of the Supplementary Materials.

#### 30 31 **Deli Turkey (125 g Test Portions)**

32  
33 Deli turkey test portions were inoculated at a low and high level and were analyzed for the  
34 detection of *Listeria monocytogenes*. Un-inoculated controls were included in each analysis.  
35 Laboratories 8 and 10 received test portions but were unable to conduct the analysis and  
36 therefore no data was submitted. All other laboratories submitted data for both methods  
37 evaluated. The MPN levels obtained for this matrix, with 95% confidence intervals, were 0.63  
38 CFU/test portion (0.49, 0.80) for the low inoculum level and 4.52 CFU/test portion (3.19, 6.42)  
39 for the high inoculum level.

40 For the low inoculum level, 69 out of 132 test portions (POD<sub>CP</sub> of 0.52) were reported as  
41 presumptive positive by the 3M MDA 2 – *Listeria monocytogenes* method with 66 out of 132 test  
42 portions (POD<sub>CC</sub> of 0.50) confirming positive. For samples that produced presumptive positive  
43 results on the 3M MDA 2 – *Listeria monocytogenes* method, 66 out of 132 samples confirmed

1 positive (POD<sub>C</sub> of 0.50). For test portions evaluated by the USDA/FSIS MLG reference method,  
2 60 out of 132 test portions produced positive results. A dLPOD<sub>C</sub> value of 0.04 with 95%  
3 confidence intervals of (-0.08, 0.17) was obtained between the candidate and reference method,  
4 indicating ~~the difference between methods was not statistically significant at the 0.05 probability~~  
5 ~~level. no statistically significant difference between the two methods.~~ A dLPOD<sub>CP</sub> value of 0.02  
6 with 95% confidence intervals of (-0.10, 0.15) was obtained between presumptive and confirmed  
7 results indicating ~~the difference between presumptive and confirmed methods was not~~  
8 ~~statistically significant at the 0.05 probability level. no statistically significant difference between~~  
9 ~~the presumptive and confirmed results.~~

10 For the high inoculum level, 132 out of 132 test portions (POD<sub>CP</sub> of 1.00) were reported as  
11 presumptive positive by the 3M MDA 2 – *Listeria monocytogenes* method with 132 out of 132  
12 test portions (POD<sub>CC</sub> of 1.00) confirming positive. For samples that produced presumptive  
13 positive results on the 3M MDA 2 – *Listeria monocytogenes* method, 132 out of 132 samples  
14 confirmed positive (POD<sub>C</sub> of 1.00). For test portions evaluated by the USDA/FSIS MLG  
15 reference method, 132 out of 132 test portions produced positive results. A dLPOD<sub>C</sub> value of  
16 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between the candidate and  
17 reference method, indicating ~~the difference between methods was not statistically significant at~~  
18 ~~the 0.05 probability level. no statistically significant difference between the two methods.~~

19 A dLPOD<sub>CP</sub> value of 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between  
20 presumptive and confirmed results indicating ~~the difference between presumptive and confirmed~~  
21 ~~methods was not statistically significant at the 0.05 probability level. no statistically significant~~  
22 ~~difference between the presumptive and confirmed results.~~

23 For the un-inoculated controls, 0 out of 132 samples (POD<sub>CP</sub> of 0.00) produced a presumptive  
24 positive result by the 3M MDA 2 – *Listeria monocytogenes* method with 0 out of 132 test portions  
25 (POD<sub>CC</sub> of 0.00) confirming positive. For samples that produced presumptive positive results on  
26 the 3M MDA 2 – *Listeria monocytogenes* method, 0 out of 132 samples confirmed positive  
27 (POD<sub>C</sub> of 0.00). For test portions evaluated by the USDA/FSIS MLG reference method, 0 out of  
28 132 test portions produced positive results. A dLPOD<sub>C</sub> value of 0.00 with 95% confidence  
29 intervals of (-0.03, 0.03) was obtained between the candidate and reference method, indicating  
30 ~~the difference between methods was not statistically significant at the 0.05 probability level. no~~  
31 ~~statistically significant difference between the two methods.~~ A dLPOD<sub>CP</sub> value of 0.00 with 95%  
32 confidence intervals of (-0.03, 0.00) was obtained between presumptive and confirmed results  
33 indicating ~~the difference between presumptive and confirmed methods was not statistically~~  
34 ~~significant at the 0.05 probability level. no statistically significant difference between the~~  
35 ~~presumptive and confirmed results.~~

36  
37 Detailed results of the POD statistical analysis are presented in Table 2016.2A and Figures 1A-  
38 1B.

#### 40 **Raw Chicken Breast Fillet (25 g Test Portions)**

41  
42 Raw chicken breast fillet test portions were inoculated at a low and high inoculum level and were  
43 analyzed for the detection of *Listeria monocytogenes*. Un-inoculated controls were included in

1 each analysis. Laboratory 11 did not participate in the evaluation of this matrix. Laboratory 10  
2 submitted data that indicated cross contamination of the inoculating organism in the un-  
3 inoculated control samples. Further analysis at the coordinating laboratory confirmed the cross  
4 contamination of the un-inoculated controls. Due to this issue, the data submitted from  
5 Laboratory 10 was not used in the statistical analysis. All other laboratories submitted data for  
6 both methods evaluated. The MPN levels obtained for this matrix, with 95% confidence  
7 intervals, were 0.66 CFU/test portion (0.51, 0.83) for the low level and 6.24 CFU/test portion  
8 (3.58, 10.88) for the high level.

9 For the low inoculum level, 86 out of 132 test portions ( $POD_{CP}$  of 0.65) were reported as  
10 presumptive positive by the 3M MDA 2 – *Listeria monocytogenes* method with 86 out of 132 test  
11 portions ( $POD_{CC}$  of 0.65) confirming positive. For samples that produced presumptive positive  
12 results on the 3M MDA 2 – *Listeria monocytogenes* method, 85 out of 132 test portions confirmed  
13 positive ( $POD_C$  of 0.64). For test portions evaluated by the USDA/FSIS MLG reference method,  
14 64 out of 132 test portions produced positive results. A  $dLPOD_C$  value of 0.16 with 95%  
15 confidence intervals of (0.04, 0.28) was obtained between the candidate and reference method,  
16 indicating a statistically significant difference between the two methods, with a positive  
17 correlation in data indicating more recovery of the target analyte by the candidate method. A  
18  $dLPOD_{CP}$  value of 0.00 with 95% confidence intervals of (-0.12, 0.12) was obtained between  
19 presumptive and confirmed results indicating [the difference between presumptive and confirmed](#)  
20 [methods was not statistically significant at the 0.05 probability level.](#)~~no statistically significant~~  
21 ~~difference between the presumptive and confirmed results.~~

22 For the high inoculum level, 129 out of 132 test portions ( $POD_{CP}$  of 0.98) were reported as  
23 presumptive positive by the 3M MDA 2 – *Listeria monocytogenes* method with 132 out of 132  
24 test portions ( $POD_{CC}$  of 1.00) confirming positive. For samples that produced presumptive  
25 positive results on the 3M MDA 2 – *Listeria monocytogenes* method, 129 out of 132 samples  
26 confirmed positive ( $POD_C$  of 0.98). For test portions evaluated by the USDA/FSIS-MLG  
27 reference method, 132 out of 132 test portions produced positive results. A  $dLPOD_C$  value of  
28 -0.02 with 95% confidence intervals of (-0.06, 0.01) was obtained between the candidate and  
29 reference method, indicating [the difference between methods was not statistically significant at](#)  
30 [the 0.05 probability level.](#)~~no statistically significant difference between the two methods.~~ A  
31  $dLPOD_{CP}$  value of -0.02 with 95% confidence intervals of (-0.06, 0.01) was obtained between  
32 presumptive and confirmed results [indicating the difference between presumptive and confirmed](#)  
33 [methods was not statistically significant at the 0.05 probability level.](#)~~indicating no statistically~~  
34 ~~significant difference between the presumptive and confirmed results.~~

35 For the un-inoculated controls, 0 out of 132 samples ( $POD_{CP}$  of 0.00) produced a presumptive  
36 positive result by the 3M MDA 2 - *Listeria monocytogenes* method with 0 out of 132 test  
37 portions ( $POD_{CC}$  of 0.00) confirming positive. For samples that produced presumptive positive  
38 results on the 3M MDA 2 – *Listeria monocytogenes* method, 0 out of 132 samples confirmed  
39 positive ( $POD_C$  of 0.00). For test portions evaluated by the USDA/FSIS MLG reference method,  
40 0 out of 132 test portions produced positive results. A  $dLPOD_C$  value of 0.00 with 95%  
41 confidence intervals of (-0.03, 0.03) was obtained between the candidate and reference method,  
42 indicating [the difference between methods was not statistically significant at the 0.05 probability](#)  
43 [level.](#)~~no statistical significant difference between the two methods.~~ A  $dLPOD_{CP}$  value of 0.00

1 with 95% confidence intervals of (-0.03, 0.00) was obtained between presumptive and confirmed  
2 results indicating the difference between presumptive and confirmed methods was not  
3 statistically significant at the 0.05 probability level.~~indicating no statistically significant~~  
4 ~~difference between the presumptive and confirmed results.~~

5  
6 Detailed results of the POD statistical analysis are presented in Table 2016.2B and Figures 1C-  
7 1D.

## 8 9 10 **Discussion**

11  
12 No negative feedback was provided by the collaborating laboratories in regard to the  
13 performance of the 3M MDA 2- *Listeria monocytogenes* method. For the raw chicken breast fillet,  
14 Laboratory 10 reported isolating *Listeria monocytogenes* from two un-inoculated control samples.  
15 The isolates were sent for further identification and it was determined that they were the same  
16 strain as the inoculating organism, indicating that cross contamination of the sample occurred.  
17 Due to the fact that cross contamination occurred, just cause for removal of the data was  
18 established and the data generated by Laboratory 10 was therefore not included in the statistical  
19 analysis.

20 Overall, the data generated during this evaluation demonstrates the reproducibility of this new  
21 method. For the deli turkey analysis, the POD statistical analysis indicated the difference  
22 between the candidate method and reference method was not statistically significant at the 0.05  
23 probability level. ~~And that no statistically significant difference between the candidate method~~  
24 ~~and the reference method or between the presumptive and confirmed results.~~ difference between  
25 presumptive and confirmed candidate method was not statistically significant at the 0.05  
26 probability level of the candidate method was obtained. For raw chicken breast fillet, a  
27 statistically significant difference was observed between the reference and the alternative  
28 method. ~~The dLPOD data indicated.~~ The dLPOD being significantly greater than zero showed an  
29 observed higher proportion of positive results by the candidate method than the reference  
30 method, a positive correlation in data indicating more recovery of the target analyte by the  
31 candidate method. One possible contribution for the higher observed proportion positive results to  
32 the higher level of recovery observed with the 3M MDA 2 – *Listeria monocytogenes* method was  
33 the use of Demi-Fraser Broth for the candidate method. This enrichment media formulation is  
34 less selective than the modified University of Vermont Medium used in the USDA reference  
35 method and may have contributed to the higher observed proportion positive results level of  
36 recovery observed during the evaluation.

37 A second possible contribution to the higher for the higher observed proportion positive  
38 results level of recovery was is the length of the primary enrichment. Test portions evaluated by  
39 the 3M MDA 2 – *Listeria monocytogenes* method were incubated for a minimum of 28 hours in  
40 the primary enrichment, while the USDA reference method had a maximum primary enrichment  
41 time of 26 hours. No statistically significant difference was observed between the candidate  
42 method presumptive and confirmed results for this matrix.

1 **Recommendations**

2  
3 It is recommended that the 3M Molecular Detection Assay 2 – *Listeria*  
4 *monocytogenes* method be adopted as Official First Action status for the detection of *Listeria*  
5 *monocytogenes* in selected foods: hot dogs (25g & 125g), salmon (25g), deli turkey (25g &  
6 125g), cottage cheese (25g), chocolate milk (25 mL), vanilla ice cream (25g), queso fresco (25g),  
7 romaine lettuce (25g), melon (whole), raw chicken leg pieces (25g); raw chicken breast fillet,  
8 concrete (sponge, 225 mL & 100 mL), stainless steel (sponge, 225 mL), and plastic  
9 (EnviroSwab, 10 mL) environmental samples.

10  
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12  
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36 Sweeney.

37  
38  
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1 **Table 1: Participation of each Collaborating Laboratory**

Lab	Deli Turkey <sup>a,b</sup>	Raw Chicken Breast/Fillet <sup>a,b</sup>
1	Y	Y
2	Y	Y
3	Y	Y
4	Y	Y
5	Y	Y
6	Y	Y
7	Y	Y
8	N	Y
9	Y	Y
10	N	Y
11	Y	N
12	Y	Y
13	Y	Y

2 <sup>a</sup> Y= Collaborator analyzed the food type; <sup>b</sup>N= Collaborator did not analyze food type

3  
4  
5  
6 **Table 2: Heat-Stress Injury Results**

Matrix	Test Organism <sup>a</sup>	CFU/MOX (Selective Agar)	CFU/TSA (Non-Selective Agar)	Degree Injury <sup>b</sup>
Deli Turkey	<i>Listeria monocytogenes</i> ATCC 19115	9.1 x 10 <sup>8</sup>	2.5 x 10 <sup>9</sup>	60.8%
<a href="#">Raw Chicken Breast Fillet</a>	<a href="#">Listeria monocytogenes ATCC 7644</a>	Not applicable	Not applicable	<a href="#">Raw chicken product is not heat treated so not necessary to injure the cells.</a>

8 <sup>a</sup> ATCC- American Type Culture Collection

9 <sup>b</sup> Cultures were heat stressed for 10 minutes at 55°C in a recirculating water bath.

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**Table 2016.1A:** Summary of Results for the Detection of *Listeria monocytogenes* in Deli Turkey (125g)

Method <sup>a</sup>	3M™ MDA™ 2 - <i>Listeria monocytogenes</i>		
	Un-inoculated	Low	High
Candidate Presumptive Positive/ Total # of Samples Analyzed	0/132	69/132	132/132
Candidate Presumptive POD (CP)	0.00 (0.00, 0.03)	0.52 (0.43, 0.61)	1.00 (0.97, 1.00)
$s_r^b$	0.00 (0.00, 0.16)	0.51 (0.45, 0.52)	0.00 (0.00, 0.16)
$s_L^c$	0.00 (0.00, 0.16)	0.00 (0.00, 0.16)	0.00 (0.00, 0.16)
$s_R^d$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value <sup>e</sup>	1.0000	0.8091	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/132	66/132	132/132
Candidate Confirmed POD (CC)	0.00 (0.00, 0.03)	0.50 (0.41, 0.59)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9123	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/132	66/132	132/132
Candidate Presumptive Positive that Confirmed POD (C)	0.00 (0.00, 0.03)	0.50 (0.41, 0.59)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9123	1.0000
Positive Reference Samples/ Total # of Samples Analyzed	0/132	60/132	132/132
Reference POD	0.00 (0.00, 0.03)	0.45 (0.37, 0.54)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.11)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9829	1.0000
dLPOD (Candidate vs. Reference) <sup>f</sup>	0.00 (-0.03, 0.03)	0.04 (-0.08, 0.17)	0.00 (-0.03, 0.03)

dLPOD (Candidate Presumptive vs. Candidate Confirmed) <sup>f</sup>	0.00 (-0.03, 0.03)	0.02 (-0.10, 0.15)	0.00 (-0.03, 0.03)
--	-----------------------	-----------------------	-----------------------

<sup>a</sup>Results include 95% Confidence Intervals, <sup>b</sup>Repeatability Standard Deviation, <sup>c</sup>Among-Laboratory Standard Deviation, <sup>d</sup>Reproducibility Standard Deviation

<sup>e</sup> P Value = Homogeneity test of laboratory PODs; <sup>f</sup> A confidence interval for dLPOD that does not contain the value 0 indicates a statistical significant difference between the two methods

**Table 2016.1B: Summary of Results for the Detection of *Listeria monocytogenes* in Raw Chicken Breast Fillet (25g)**

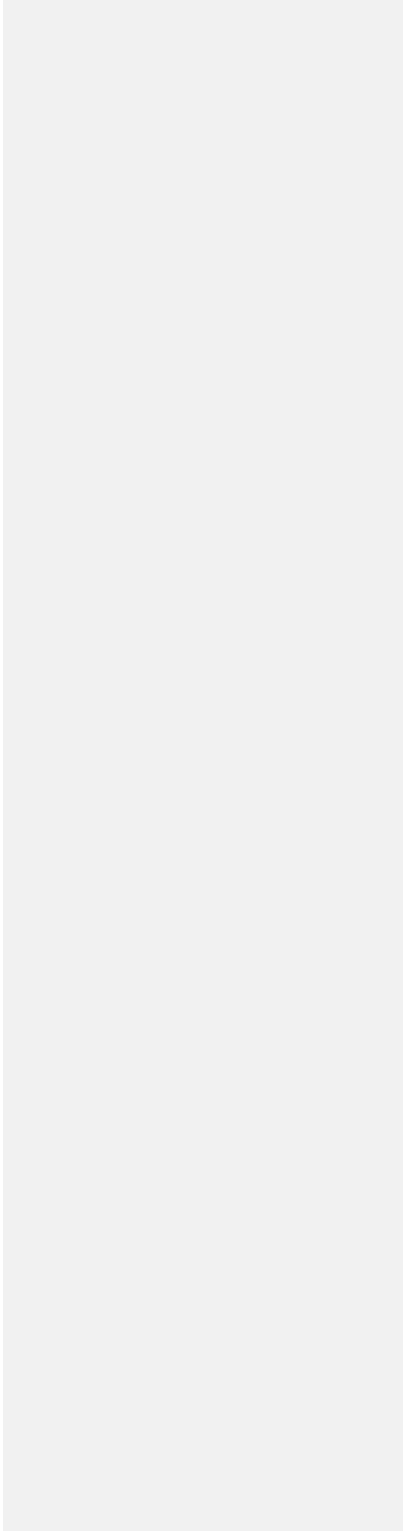
Method <sup>a</sup>	3M <sup>TM</sup> MDA <sup>TM</sup> 2 - <i>Listeria monocytogenes</i>		
	Un-inoculated	Low	High
Candidate Presumptive Positive/ Total # of Samples Analyzed	0/132	86/132	129/132
Candidate Presumptive POD (CP)	0.00 (0.00, 0.03)	0.65 (0.57, 0.73)	0.98 (0.93, 0.99)
$s_r^b$	0.00 (0.00, 0.16)	0.48 (0.43, 0.52)	0.15 (0.13, 0.17)
$s_L^c$	0.00 (0.00, 0.16)	0.00 (0.00, 0.17)	0.03 (0.00, 0.08)
$s_R^d$	0.00 (0.00, 0.23)	0.48 (0.43, 0.52)	0.15 (0.13, 0.18)
P Value <sup>e</sup>	1.0000	0.7057	0.1089
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/132	86/132	132/132
Candidate Confirmed POD (CC)	0.00 (0.00, 0.03)	0.65 (0.57, 0.73)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.48 (0.43, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.18)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.48 (0.43, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.5632	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/132	85/132	129/132
Candidate Presumptive Positive that Confirmed POD (C)	0.00 (0.00, 0.03)	0.64 (0.56, 0.73)	0.98 (0.93, 0.99)
$s_r$	0.00 (0.00, 0.16)	0.48 (0.43, 0.52)	0.15 (0.13, 0.17)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.18)	0.03 (0.00, 0.08)
$s_R$	0.00 (0.00, 0.23)	0.49 (0.43, 0.52)	0.15 (0.13, 0.18)
P Value	1.0000	0.6228	0.1089
Positive Reference Samples/ Total # of Samples Analyzed	0/132	64/132	132/132
Reference POD	0.00 (0.00, 0.03)	0.48 (0.40, 0.57)	1.00 (0.97, 1.00)
$s_r$	0.00 (0.00, 0.16)	0.51 (0.46, 0.52)	0.00 (0.00, 0.16)
$s_L$	0.00 (0.00, 0.16)	0.00 (0.00, 0.14)	0.00 (0.00, 0.16)
$s_R$	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P Value	1.0000	0.9192	1.0000
dLPOD (Candidate vs. Reference) <sup>f</sup>	0.00	0.16	-0.02

	(-0.03, 0.03)	(0.04, 0.28)	(-0.06, 0.01)
dLPOD (Candidate Presumptive vs. Candidate Confirmed) <sup>f</sup>	0.00 (-0.03, 0.03)	0.00 (-0.12, 0.12)	-0.02 (-0.06, 0.01)

<sup>a</sup>Results include 95% Confidence Intervals, <sup>f</sup> Repeatability Standard Deviation, <sup>e</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation

<sup>g</sup> P Value = Homogeneity test of laboratory PODs; <sup>f</sup> A confidence interval for dLPOD that does not contain the value 0 indicates a statistical significant difference between the two methods

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1 **Table 2016.2A:** Comparative Results for the Detection of *Listeria monocytogenes* in 125 g Deli Turkey Test Portions by the 3M™ MDA 2 - *Listeria*  
 2 *monocytogenes* Method vs. USDA/FSIS MLG Chapter 8.09 in a Collaborative Study (Un-inoculated Control)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
		1	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		2	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		3	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		4	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
	Deli Turkey	5	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		6	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		7	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		8 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		9	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		10 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		11	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		12	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		13	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00

Estimate	All	132	0	0.00	132	0	0.00	132	0	0.00	132	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.03	-0.03
UCL				0.03			0.03			0.03			0.03	0.03	0.03
$s_r^b$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_L^c$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_R^d$				0.00			0.00			0.00			0.00		
UCL				0.00			0.00			0.00			0.00		
LCL				0.23			0.23			0.23			0.23		
$P_T^e$				1.0000			1.0000			1.0000			1.0000		

<sup>a</sup>N/A – Laboratory did not participate or submit data for this matrix

<sup>b</sup>Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation, <sup>e</sup> P<sub>T</sub> Value = Homogeneity test of laboratory PODs

LCL – Lower Confidence Limit; UCL – Upper Confidence Limit, **N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)**

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1 **Table 2016.2A (cont'd.):** Comparative Results for the Detection of *Listeria monocytogenes* in 125 g Deli Turkey Test Portions by the 3M™ MDA 2 - *Listeria*  
 2 *monocytogenes* Method vs. USDA/FSIS MLG Chapter 8.09 in a Collaborative Study (Low Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R		
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)	
	Deli Turkey	1	12	7	0.58	12	7	0.58	12	7	0.58	12	6	0.50	0.08	0.00	
		2	12	7	0.58	12	6	0.50	12	6	0.50	12	5	0.42	0.08	0.08	
		3	12	4	0.33	12	4	0.33	12	4	0.33	12	5	0.42	-0.08	0.00	
		4	12	5	0.42	12	5	0.42	12	5	0.42	12	4	0.33	0.08	0.00	
		5	12	6	0.50	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.00	
		6	12	8	0.66	12	7	0.58	12	7	0.58	12	6	0.50	0.08	0.00	
		7	12	7	0.58	12	7	0.58	12	7	0.58	12	6	0.50	0.08	0.00	
		8 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		9	12	8	0.66	12	8	0.66	12	8	0.66	12	6	0.50	0.16	0.00	
		10 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		MPN/ Test Portion	11	12	7	0.58	12	6	0.50	12	6	0.50	12	7	0.58	-0.08	0.08
			12	12	5	0.42	12	5	0.42	12	5	0.42	12	4	0.33	0.08	0.00
			13	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
Estimate	0.63	All	132	69	0.52	132	66	0.50	132	66	0.50	132	60	0.45	0.04	0.02	
LCL	0.49				0.43			0.41			0.41			0.37	-0.08	-0.10	
UCL	0.80				0.61			0.59			0.59			0.54	0.17	0.15	
s <sub>r</sub> <sup>b</sup>					0.51			0.51			0.51			0.51			
LCL					0.45			0.46			0.46			0.46			
UCL					0.52			0.52			0.52			0.52			
s <sub>L</sub> <sup>c</sup>					0.00			0.00			0.00			0.00			
LCL					0.00			0.00			0.00			0.00			
UCL					0.16			0.14			0.14			0.11			
s <sub>R</sub> <sup>d</sup>					0.51			0.51			0.51			0.51			
UCL					0.46			0.46			0.46			0.46			
LCL					0.52			0.52			0.52			0.52			
P <sub>T</sub> <sup>e</sup>					0.8091			0.9123			0.9123			0.9829			

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix

<sup>b</sup> Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation, <sup>e</sup> P<sub>T</sub> Value = Homogeneity test of laboratory PODs  
 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit, N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)

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1 **Table 2016.2A (cont'd.):** Comparative Results for the Detection of *Listeria monocytogenes* in 125 g Deli Turkey Test Portions by the 3M™ MDA 2 - *Listeria*  
 2 *monocytogenes* Method vs. USDA/FSIS MLG Chapter 8.09 in a Collaborative Study (High Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
		1	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		2	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		3	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		4	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		5	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		6	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		7	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		8 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		9	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		10 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		11	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		12	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		13	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
Estimate	4.52	All	132	132	1.00	132	132	1.00	132	132	1.00	132	132	1.00	0.00	0.00
LCL	3.19				0.97			0.97			0.97			0.97	-0.03	-0.03
UCL	6.42				1.00			1.00			1.00			1.00	0.03	0.03
s <sub>r</sub> <sup>b</sup>					0.00			0.00			0.00			0.00		
LCL					0.00			0.00			0.00			0.00		
UCL					0.16			0.16			0.16			0.16		
s <sub>L</sub> <sup>c</sup>					0.00			0.00			0.00			0.00		
LCL					0.00			0.00			0.00			0.00		
UCL					0.16			0.16			0.16			0.16		
s <sub>R</sub> <sup>d</sup>					0.00			0.00			0.00			0.00		
UCL					0.00			0.00			0.00			0.00		
LCL					0.23			0.23			0.23			0.23		
P <sub>T</sub> <sup>e</sup>					1.000			1.000			1.000			1.000		

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix  
<sup>b</sup> Repeatability Standard Deviation, <sup>c</sup> Among-Laboratory Standard Deviation, <sup>d</sup> Reproducibility Standard Deviation, <sup>e</sup> P<sub>T</sub> Value = Homogeneity test of laboratory PODs  
 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit, N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)

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1 **Table 2016.2B:** Comparative Results for the Detection of *Listeria monocytogenes* in 25 g Raw Chicken Breast Fillet Test Portions by the 3M™ MDA 2 - *Listeria*  
 2 *monocytogenes* Method vs. USDA/FSIS MLG Chapter 8.09 in a Collaborative Study (Un-inoculated Control)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Raw Chicken Breast Fillet	1	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		2	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		3	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		4	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		5	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		6	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		7	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.08
		8	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		9	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		10 <sup>b</sup>	12	0	0.00	12	2	0.17	12	0	0.00	12	0	0.00	0.00	-0.17
		11 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		12	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		13	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00

Estimate	All	132	0	0.00	132	0	0.00	132	0	0.00	132	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.03	-0.03
UCL				0.03			0.03			0.03			0.03	0.03	0.03
$s_r^c$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_L^d$				0.00			0.00			0.00			0.00		
LCL				0.00			0.00			0.00			0.00		
UCL				0.16			0.16			0.16			0.16		
$s_R^e$				0.00			0.00			0.00			0.00		
UCL				0.00			0.00			0.00			0.00		
LCL				0.23			0.23			0.23			0.23		
$P_T^f$				1.0000			1.0000			1.0000			1.0000		

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix; <sup>b</sup> Results were not used in statistical analysis due to deviation from testing protocol.  
<sup>c</sup> Repeatability Standard Deviation, <sup>d</sup> Among-Laboratory Standard Deviation, <sup>e</sup> Reproducibility Standard Deviation, <sup>f</sup> P<sub>r</sub> Value = Homogeneity test of laboratory PODs  
 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit, N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)

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1 **Table 2016.2B (cont'd.):** Comparative Results for the Detection of *Listeria monocytogenes* in 25 g Raw Chicken Breast Fillet Test Portions by the 3M™ MDA 2 -  
 2 *Listeria monocytogenes* Method vs. USDA/FSIS MLG Chapter 8.09 in a Collaborative Study (Low Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Raw Chicken Breast Fillet	1	12	7	0.58	12	7	0.58	12	7	0.58	12	7	0.58	0.00	0.00
		2	12	9	0.75	12	9	0.75	12	9	0.75	12	7	0.58	0.17	0.00
		3	12	10	0.83	12	10	0.83	12	10	0.83	12	5	0.42	0.41	0.00
		4	12	9	0.75	12	9	0.75	12	9	0.75	12	4	0.33	0.42	0.00
		5	12	8	0.66	12	8	0.66	12	8	0.66	12	5	0.42	0.22	0.00
		6	12	9	0.75	12	9	0.75	12	9	0.75	12	6	0.50	0.15	0.08
		7	12	7	0.58	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.16
		8	12	7	0.58	12	7	0.58	12	7	0.58	12	7	0.58	0.00	0.00
		9	12	8	0.66	12	9	0.75	12	8	0.66	12	7	0.58	0.08	-0.08
		10 <sup>b</sup>	12	6	0.50	12	8	0.66	12	6	0.50	12	5	0.42	0.08	-0.16
	MPN/ Test Portion	11 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		12	12	7	0.58	12	7	0.58	12	7	0.58	12	4	0.42	0.16	0.00
		13	12	5	0.42	12	5	0.42	12	5	0.42	12	6	0.50	-0.08	0.00
Estimate	0.66	All	132	86	0.65	132	86	0.65	132	85	0.64	132	64	0.48	0.16	0.00
LCL	0.51				0.57			0.57			0.56			0.40	0.04	-0.12
UCL	0.83				0.73			0.73			0.73			0.57	0.28	0.12
$s_r^c$					0.48			0.48			0.48			0.51		
LCL					0.43			0.43			0.43			0.46		
UCL					0.52			0.52			0.52			0.52		
$s_L^d$					0.00			0.00			0.00			0.00		
LCL					0.00			0.00			0.00			0.00		
UCL					0.17			0.18			0.18			0.14		
$s_R^e$					0.48			0.48			0.49			0.51		
UCL					0.43			0.43			0.43			0.46		
LCL					0.52			0.52			0.52			0.52		
$P_T^f$					0.7057			0.5632			0.6228			0.9192		

<sup>a</sup> N/A – Laboratory did not participate or submit data for this matrix; <sup>b</sup> Results were not used in statistical analysis due to deviation from testing protocol.

<sup>c</sup> Repeatability Standard Deviation, <sup>d</sup> Among-Laboratory Standard Deviation, <sup>e</sup> Reproducibility Standard Deviation, <sup>f</sup>  $P_T$  Value = Homogeneity test of laboratory PODs

LCL – Lower Confidence Limit; UCL – Upper Confidence Limit, **N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)**

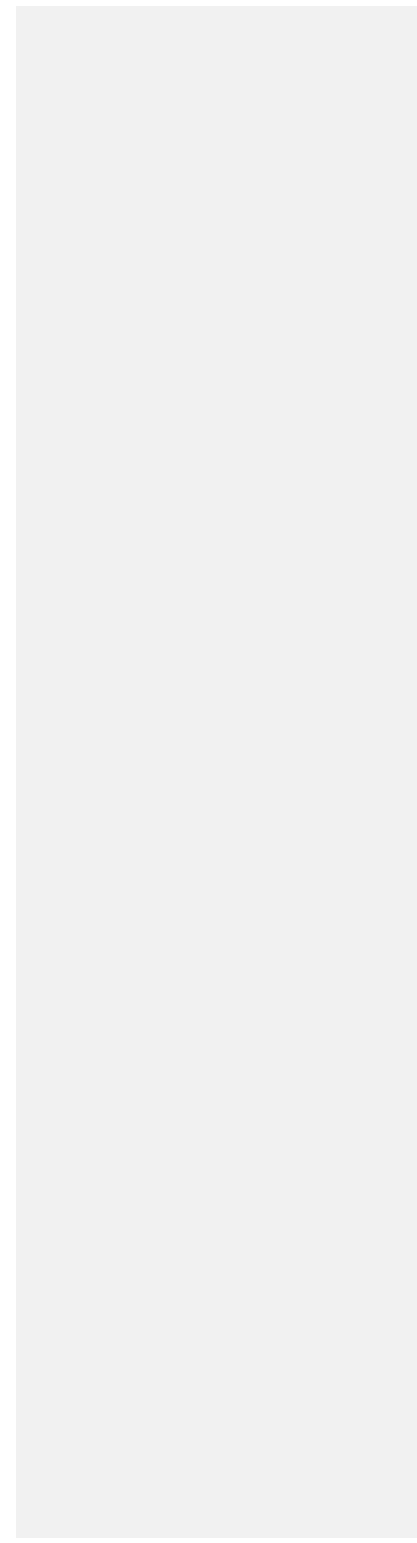
1 **Table 2016.2B (cont'd.):** Comparative Results for the Detection of *Listeria monocytogenes* in 25 g Raw Chicken Breast Fillet Test Portions by the 3M™ MDA 2 -  
 2 *Listeria monocytogenes* Method vs. USDA/FSIS MLG Chapter 8.09 in a Collaborative Study (High Inoculum Level)

Statistic	Matrix/ Inoculation Level	Laboratory	Candidate presumptive (CP)			Candidate confirmed (CC)			Candidate result (C)			Reference method (R)			C vs. R	
			N	X	POD (CP)	N	X	POD (CC)	N	X	POD (C)	N	X	POD (R)	dLPOD (C,R)	dLPOD (CP,CC)
	Raw Chicken Breast Fillet	1	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		2	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		3	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		4	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		5	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		6	12	10	0.83	12	12	1.00	12	10	0.83	12	12	1.00	0.00	0.00
		7	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		8	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		9	12	11	0.92	12	12	1.00	12	11	0.92	12	12	1.00	-0.08	-0.08
		10 <sup>b</sup>	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
	MPN/ Test Portion	11 <sup>a</sup>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		12	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		13	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
Estimate	6.24	All	132	129	0.98	132	132	1.00	132	129	0.98	132	132	1.00	-0.02	-0.02
LCL	3.58				0.93			0.97			0.93			0.97	-0.06	-0.06
UCL	10.88				0.99			1.00			0.99			1.00	0.01	0.01
$s_r^c$					0.15			0.00			0.15			0.00		
LCL					0.13			0.00			0.13			0.00		
UCL					0.17			0.16			0.17			0.16		
$s_L^d$					0.03			0.00			0.03			0.00		
LCL					0.00			0.00			0.00			0.00		
UCL					0.08			0.16			0.08			0.16		
$s_R^e$					0.15			0.00			0.15			0.00		
UCL					0.13			0.00			0.13			0.00		
LCL					0.18			0.23			0.18			0.23		
$P_T^f$					0.1089			1.0000			0.1089			1.0000		

3 <sup>a</sup>N/A – Laboratory did not participate or submit data for this matrix; <sup>b</sup>Results were not used in statistical analysis due to deviation from testing protocol.  
 4 <sup>c</sup>Repeatability Standard Deviation, <sup>d</sup>Among-Laboratory Standard Deviation, <sup>e</sup>Reproducibility Standard Deviation, <sup>f</sup> $P_T$  Value = Homogeneity test of laboratory PODs  
 5 LCL – Lower Confidence Limit; UCL – Upper Confidence Limit, N = number of samples tested, X = number of test samples with a positive result (presumptive or confirmed)  
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**Supplementary Materials**







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**Table 35: Results of Aerobic Plate Count for Collaborating Laboratories**

Lab	Deli Turkey (CFU/g) <sup>a</sup>	Raw Chicken Breast Fillet (CFU/g) <sup>a</sup>
1	2.0 x 10 <sup>1</sup>	2.3 x 10 <sup>4</sup>
2	< 10	3.4 x 10 <sup>4</sup>
3	< 10	4.1 x 10 <sup>3</sup>
4	3.0 x 10 <sup>1</sup>	8.3 x 10 <sup>6</sup>
5	1.0 x 10 <sup>1</sup>	5.4 x 10 <sup>3</sup>
6	< 10	6.3 x 10 <sup>4</sup>
7	6.0 x 10 <sup>1</sup>	3.6 x 10 <sup>2</sup>
8	N/A	2.9 x 10 <sup>3</sup>
9	< 10	4.6 x 10 <sup>2</sup>
10	N/A	3.7 x 10 <sup>4</sup>
11	2.0 x 10 <sup>1</sup>	N/A
12	< 10	7.4 x 10 <sup>3</sup>
13	< 10	2.7 x 10 <sup>4</sup>

<sup>a</sup>Samples analyzed by the 3M Petrifilm Rapid Aerobic Count Plate, AOAC OMA 2015.13

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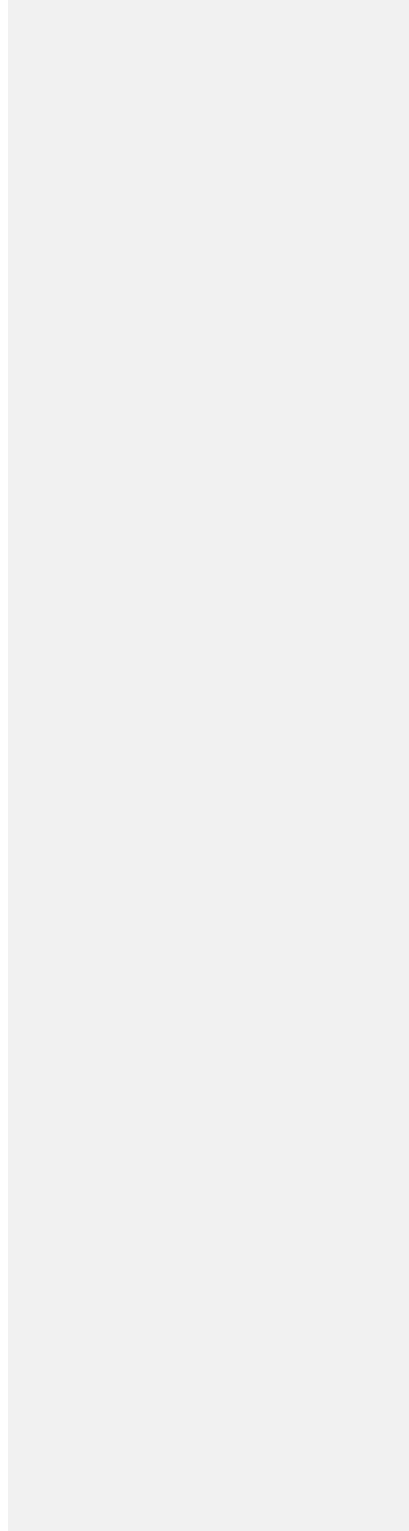
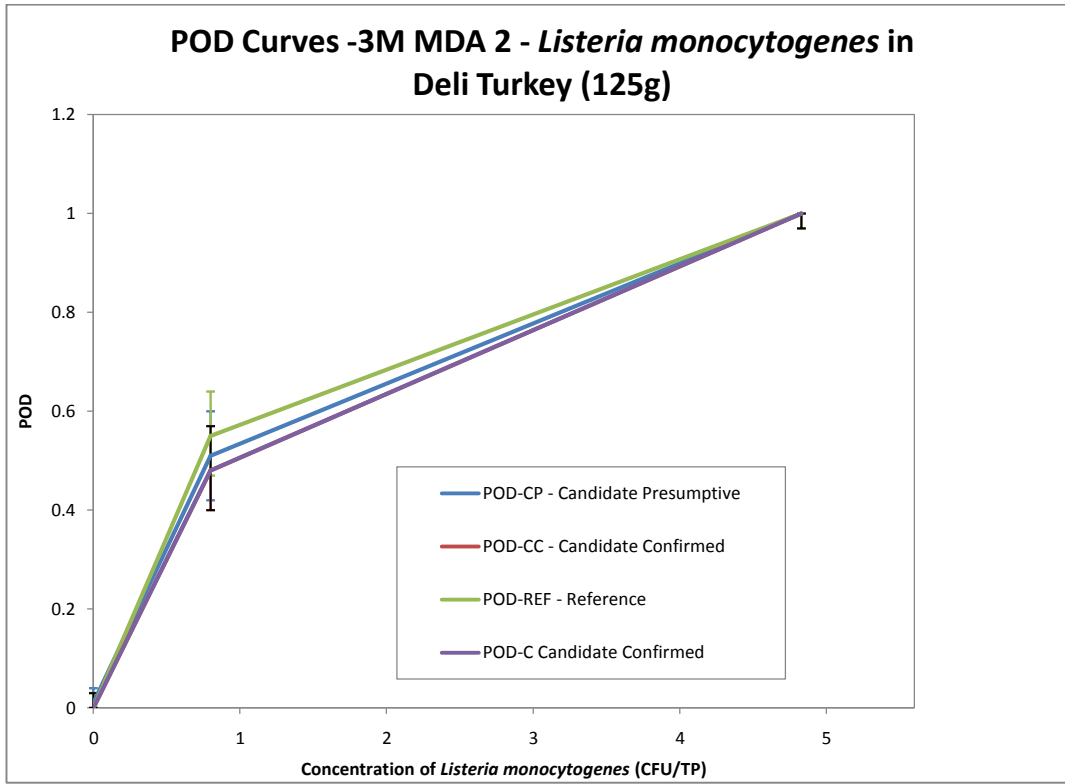


Figure 1A: POD Values of Candidate Method vs. Reference Method for 125 g Deli Turkey



### POD Curves -3M MDA 2 - *Listeria monocytogenes* in Deli Turkey (125g)

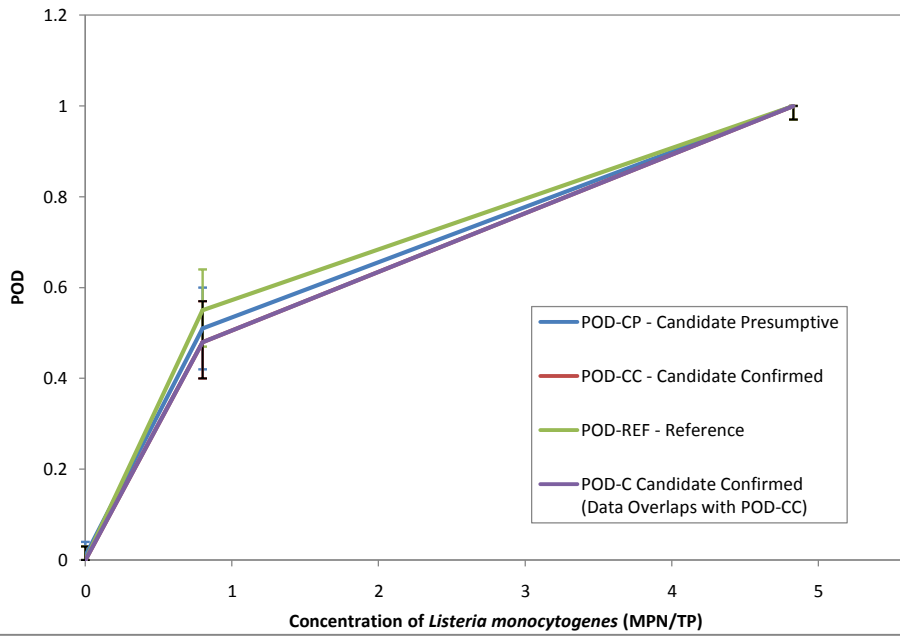


Figure 1B: dLPOD Values of Candidate Method vs. Reference Method for 125 g Deli Turkey



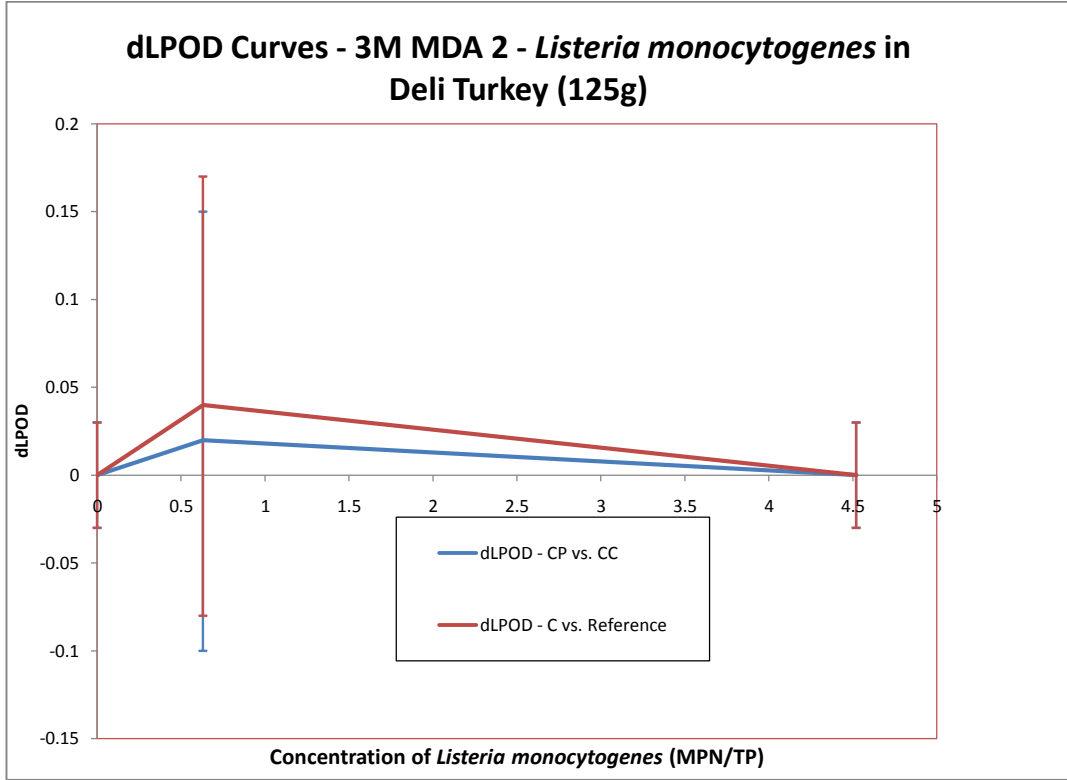
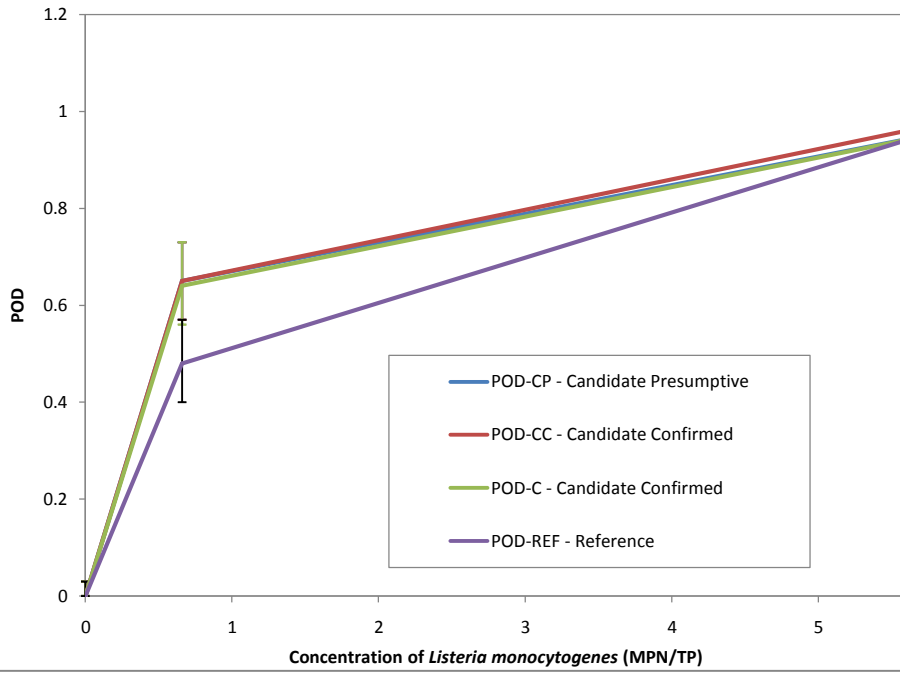


Figure 2A: POD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet

### POD Curves -3M MDA 2 - *Listeria monocytogenes* in Raw Chicken Breast Fillet (25g)



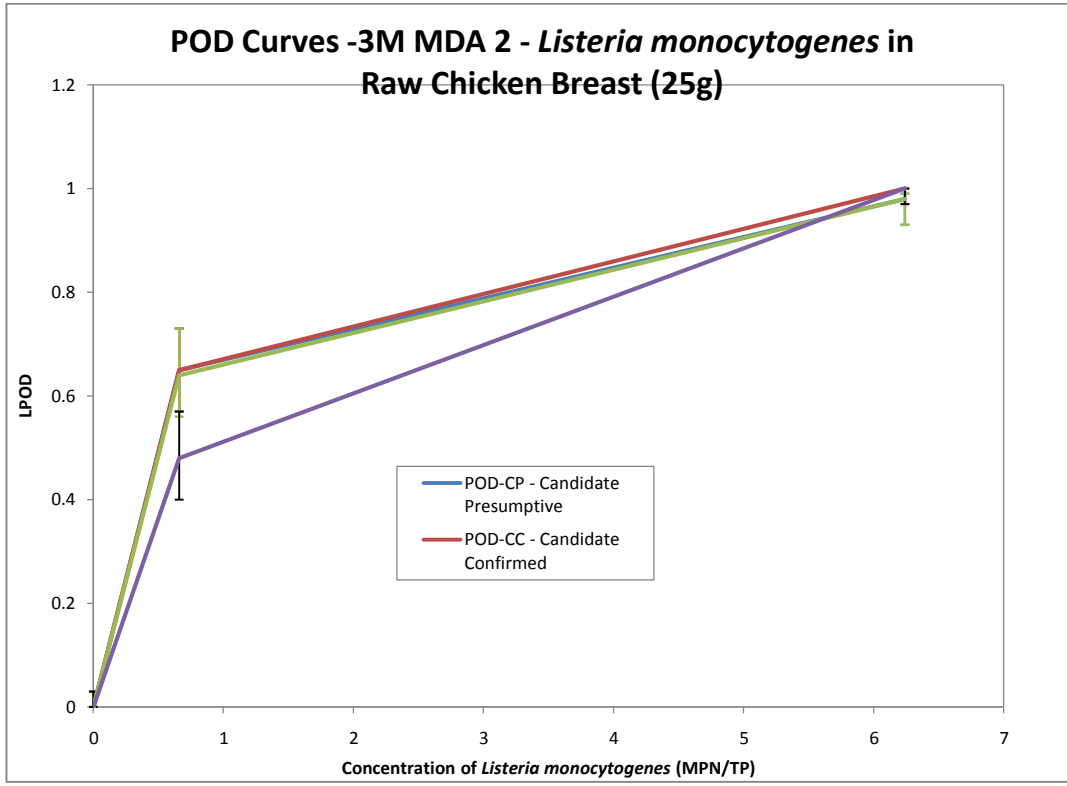
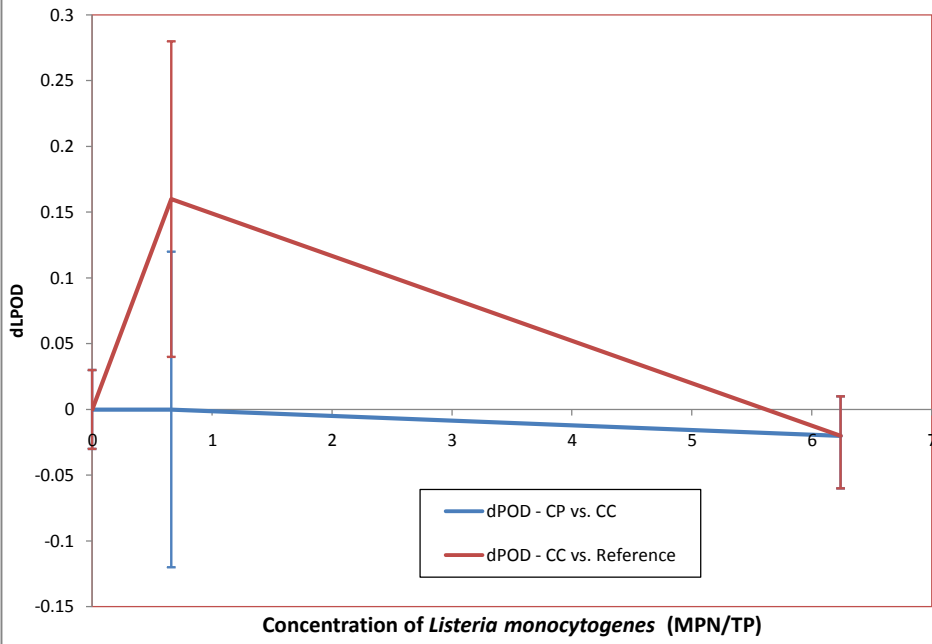


Figure 2B: dPOD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet

### dLPOD Curves - 3M MDA 2 - *Listeria monocytogenes* in Raw Chicken Breast Fillet (25g)



1  
2  
3

# 3M™ Molecular Detection Assay 2 - *Listeria monocytogenes*

MDA2LMO96

## PRODUCT DESCRIPTION AND INTENDED USE

3M™ Molecular Detection Assay 2 - *Listeria monocytogenes* is used with the 3M™ Molecular Detection System for the rapid and specific detection of *Listeria monocytogenes* in enriched food and environmental samples.

The 3M Molecular Detection Assays use loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations <sup>(1, 2, 3)</sup>.

The 3M Molecular Detection Assay 2 - *Listeria monocytogenes* is intended for use in a laboratory environment by professionals trained in laboratory techniques. 3M has not documented the use of this product in industries other than food or beverage. For example, 3M has not documented this product for testing water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M Molecular Detection Assay - 2 *Listeria monocytogenes* has not been evaluated with all possible testing protocols or with all possible strains of bacteria.

**As with all test methods, the source, formulation and quality of enrichment medium can influence the results.** Factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may also influence results. 3M recommends evaluation of the method including enrichment medium, in the user's environment using a sufficient number of samples with particular foods and microbial challenges to ensure that the method meets the user's criteria.

3M has evaluated the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* with Demi-Fraser Broth and Fraser Broth as needed. A typical formulation of this medium follows below.

### Demi-Fraser Broth Base Typical Formula (g/L)

Sodium Chloride	20 g
Sodium Phosphate, dibasic, anhydrous*	9.6 g
Beef Extract	5.0 g
Pancreatic Digest of Casein	5.0 g
Peptic Digest of Animal Tissue	5.0 g
Yeast Extract	5.0 g

Lithium Chloride	3.0 g
Potassium Phosphate, monobasic	1.35 g
Esculin	1.0 g
Acriflavin HCl	0.0125 g
Nalidixic Acid	0.01 g

\* Substitute: Sodium Phosphate, dibasic, dihydrate 12.0 g

### Fraser Broth Supplement

(Ingredients per 10 mL vial. One vial is added to one liter of basal medium.)

Ferric Ammonium Citrate 0.5g/10mL

Final pH 7.2 ± 0.2 at 25°C

The 3M™ Molecular Detection Instrument is intended for use with samples that have undergone heat treatment during the assay lysis step, which is designed to destroy organisms present in the sample. Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.

3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

The 3M Molecular Detection Assay 2 - *Listeria monocytogenes* test kit contains 96 tests, described in Table 1.

**Table 1. Kit Components**

Item	Identification	Quantity	Contents	Comments
Lysis Solution (LS) tubes	Pink solution in clear tubes	96 (12 strips of 8 tubes)	580 µL of LS per tube	Racked and ready to use
<i>Listeria monocytogenes</i> Reagent tubes	Yellow tubes	96 (12 strips of 8 tubes)	Lyophilized specific amplification and detection mix	Ready to use
Extra caps	Yellow caps	96 (12 strips of 8 caps)		Ready to use
Reagent Control (RC)	Clear flip-top tubes	16 (2 pouches of 8 individual tubes)	Lyophilized control DNA, amplification and detection mix	Ready to use
Quick Start Guide		1		

The Negative Control, not provided in the kit, is sterile enrichment medium, e.g., Demi-Fraser Broth. Do not use water as a Negative Control.

## SAFETY

The user should read, understand and follow all safety information in the instructions for the 3M Molecular Detection System and the 3M Molecular Detection Assay 2 - *Listeria monocytogenes*. Retain the safety instructions for future reference.



### WARNING:

Indicates a hazardous situation, which, if not avoided, could result in death or serious injury and/or property damage.



### CAUTION:

Indicates a hazardous situation, which, if not avoided, could result in minor or moderate injury and/or property damage.

### NOTICE:

Indicates a potentially hazardous situation which, if not avoided, could result in property damage.



## WARNING

Do not use the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* in the diagnosis of conditions in humans or animals.

The 3M Molecular Detection Assay 2 - *Listeria monocytogenes* method may generate *Listeria monocytogenes* to levels sufficient to cause stillbirths and fatalities in pregnant women and the immunocompromised, if exposed.

The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices, ISO 17025<sup>(4)</sup>, or ISO 7218<sup>(5)</sup>. The user should complete the 3M Molecular Detection System operator qualification training, as described in the "Installation Qualification (IQ) / Operational Qualification (OQ) Protocols and Instructions for 3M Molecular Detection System" document<sup>(6)</sup>.

**To reduce the risks associated with a false-negative result leading to the release of contaminated product:**

- Follow the protocol and perform the tests exactly as stated in the product instructions.
- Store the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* as indicated on the package and in the product instructions.
- Always use the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* by the expiration date.

- Use the 3M Molecular Detection Assay *Listeria monocytogenes* for food and environmental samples that have been validated internally or by a third party.
- Use the 3M Molecular Detection Assay *Listeria monocytogenes* only for surfaces, sanitizers, protocols and bacterial strains that have been validated internally or by a third party.
- For an environmental sample containing Neutralizing Buffer with aryl sulfonate complex, perform a 1:2 dilution before testing (1 part sample into 1 part sterile enrichment broth). Another option is to transfer 10 µL of the NB enrichment into the LS tubes. 3M™ sample handling products which include Neutralizing Buffer with aryl sulfonate complex: BPPFV10NB, RS96010NB, RS9604NB, SSL10NB, XLSL10NB, HS10NB and HS119510NB.

**To reduce the risks associated with exposure to chemicals and biohazards:**

- It is strongly recommended that female laboratory staff be informed of the risk to a developing fetus resulting from infection of the mother through exposure to *Listeria monocytogenes*.
- Perform pathogentesting in a properly equipped laboratory under the control of trained personnel.
- Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples.
- Avoid contact with the contents of the enrichment media and reagent tubes after amplification.
- Dispose of enriched samples according to current industry standards.
- Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.

**To reduce the risks associated with cross-contamination while preparing the assay:**

- Always wear gloves (to protect the user and prevent introduction of nucleases).

**To reduce the risks associated with environmental contamination:**

- Follow current industry standards for disposal of contaminated waste.



**CAUTION**

- Do not exceed the recommended temperature setting on heater.
- Do not exceed the recommended heating time.
- Use an appropriate, calibrated thermometer to verify the 3M™ Molecular Detection Heat Block Insert temperature (e.g., a partial immersion thermometer or digital thermocouple thermometer, not a total immersion thermometer.) The thermometer must be placed in the designated location in the 3M Molecular Detection Heat Block Insert.



## NOTICE

### To reduce the risks associated with cross-contamination while preparing the assay:

- Use of sterile, aerosol barrier (filtered), molecular biology grade pipette tips is recommended.
- Use a new pipette tip for each sample transfer.
- Use Good Laboratory Practices to transfer the sample from the enrichment to the lysis tube. To avoid pipettor contamination, the user may choose to add an intermediate transfer step. For example, the user can transfer each enriched sample into a sterile tube.
- Use a molecular biology workstation containing germicidal lamp where available.
- Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with a 1- 5% (v:v in water) household bleach solution or DNA removal solution.

### To reduce the risks associated with a false-positive result:

- Never open tubes post amplification.
- Always dispose of the contaminated tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

Consult the Safety Data Sheet for additional information and local regulations for disposal.

If you have questions about specific applications or procedures, please visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

### LIMITATION OF WARRANTIES / LIMITED REMEDY

EXCEPT AS EXPRESSLY STATED IN A LIMITED WARRANTY SECTION OF INDIVIDUAL PRODUCT PACKAGING, 3M DISCLAIMS ALL EXPRESS AND IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, ANY WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. If any 3M Food Safety Product is defective, 3M or its authorized distributor will, at its option, replace or refund the purchase price of the product. These are your exclusive remedies. You must promptly notify 3M within sixty days of discovery of any suspected defects in a product and return it to 3M. Please call Customer Service (1-800-328-1671 in the U.S.) or your official 3M Food Safety representative for a Returned Goods Authorization.

### LIMITATION OF 3M LIABILITY

3M WILL NOT BE LIABLE FOR ANY LOSS OR DAMAGES, WHETHER DIRECT, INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO

LOST PROFITS. In no event shall 3M's liability under any legal theory exceed the purchase price of the product alleged to be defective.

## **USER RESPONSIBILITY**

Users are responsible for familiarizing themselves with product instructions and information. Visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety), or contact your local 3M representative or distributor for more information.

When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, and laboratory technique may influence results.

It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and microbial challenges to satisfy the user that the chosen test method meets the user's criteria.

It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.

As with any test method, results obtained from use of any 3M Food Safety product do not constitute a guarantee of the quality of the matrices or processes tested.

To help customers evaluate the method for various food matrices, 3M has developed the 3M™ Molecular Detection Matrix Control kit. When needed, use the Matrix Control (MC) to determine if the matrix has the ability to impact the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* results. Test several samples representative of the matrix, i.e. samples obtained from different origin, during any validation period when adopting the 3M method or when testing new or unknown matrices or matrices that have undergone raw material or process changes.

A matrix can be defined as a type of product with intrinsic properties such as composition and process. Differences between matrices may be as simple as the effects caused by differences in their processing or presentation for example, raw vs. pasteurized; fresh vs. dried, etc.

## **STORAGE AND DISPOSAL**

Store the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* at 2-8°C. Do not freeze. Keep kit away from light during storage. After opening the kit, check that the foil pouch is undamaged. If the pouch is damaged, do not use. After opening, unused reagent tubes should always be stored in the re-sealable pouch with the desiccant inside to maintain stability of the lyophilized reagents. Store resealed pouches at 2-8°C for no longer than 60days.

Do not use 3M Molecular Detection Assay 2 - *Listeria monocytogenes* past the expiration date. Expiration date and lot number are noted on the outside label of the box. After use, the enrichment medium and the 3M Molecular Detection Assay 2 - *Listeria monocytogenes* tubes can potentially contain pathogenic materials. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Safety Data Sheet for additional information and local regulations for disposal.

## INSTRUCTIONS FOR USE

Follow all instructions carefully. Failure to do so may lead to inaccurate results.

Periodically decontaminate laboratory benches and equipment (pipettes, cap/decap tools, etc.) with a 1- 5% (v:v in water) household bleach solution or DNA removal solution.

## SAMPLE ENRICHMENT

Table 2 presents guidance for the enrichment of food and environmental samples. It is the user's responsibility to validate alternate sampling protocols or dilution ratios to ensure this test method meets the user's criteria.

### Foods

1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature.
2. Aseptically combine the enrichment medium and sample according to Table 2. For all meat and highly particulate samples, the use of filter bags is recommended.
3. Homogenize thoroughly by blending, stomaching, or hand mixing for  $2 \pm 0.2$  minutes. Incubate at  $37 \pm 1^\circ\text{C}$  according to Table 2.
4. For raw dairy products, transfer 0.1mL of the primary enrichment into 10 mL of Fraser Broth. Incubate at  $37 \pm 1^\circ\text{C}$  for 20-24 hours.

### Environmental samples

Sample collection devices can be a sponge hydrated with a neutralizing solution to inactivate the effects of the sanitizers. 3M recommends the use of a biocide-free cellulose sponge. Neutralizing solution can be Dey-Engley (D/E) Neutralizing Broth or Lethen broth. It is recommended to sanitize the area after sampling.

**WARNING:** Should you select to use Neutralizing Buffer (NB) that contains aryl sulfonate complex as the hydrating solution for the sponge, it is required to perform a 1:2 dilution (1 part sample into 1 part sterile enrichment broth) of the enriched environmental sample before testing in order to reduce the

risks associated with a false-negative result leading to the release of contaminated product

The recommended size of the sampling area to verify the presence or absence of the pathogen on the surface is at least 100 cm<sup>2</sup> (10 cm x 10 cm or 4"x4"). When sampling with a sponge, cover the entire area going in two directions (left to right then up and down) or collect environmental samples following your current sampling protocol or according to the FDA BAM <sup>(1)</sup>, USDA FSIS MLG <sup>(2)</sup> or ISO 18593 <sup>(7)</sup> guidelines.

1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature.
2. Aseptically combine the enrichment medium and sample according to Table 2.
3. Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ± 0.2 minutes. Incubate at 37 ± 1°C for 24-30 hours.

**Table 2:** General enrichment protocols using Demi-Fraser Broth at 37 ± 1 °C

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)
Heat-processed, cooked, cured meats, poultry, seafood and fish	25 g	225	24-30
Heat-processed / pasteurized dairy products			
Produce and vegetables			
Multi-component foods			
Environmental samples (a)	1 sponge	100 or 225	24-30
	1 swab	10	24-30
Raw meat, poultry, seafood, fish (b)	25 g	475	28-32

Sample Matrix	Primary Enrichment (Demi-Fraser Broth)			Secondary Enrichment (Fraser Broth)	Sample Analysis Volume(a)	
	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)	Sample Size		Enrichment Time (hr)
Raw dairy products	25 g	225	20-24	Transfer 0.1 mL into 10 mL	20-24	10 µL

(a) Volume of sample transferred to Lysis Solution tubes. Refer to Step 4.6 of Lysis section.

### Specific Instructions for Validated Methods

#### AOAC® Performance Tested Method<sup>sm</sup> # 081501



In AOAC PTM studies, the 3M Molecular Detection Assay 2 – *Listeria monocytogenes* was found to be an effective method for the detection of *Listeria monocytogenes*. The matrices tested in the study are shown in Table 3. The limit of detection of the 3M Molecular Detection Assay 2 – *Listeria monocytogenes* method is 1-5 colony forming units per validated test portion size (in Table 3).

**Table 3.** Enrichment protocols using Demi-Fraser Broth at  $37 \pm 1$  °C according to AOAC Performance Tested<sup>SM</sup> Certificate #081501.

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)
Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon	25 g	225	24-30
Raw chicken	25 g	475	28-32
Deli turkey	125 g	1125	24-30
Cantaloupe <sup>(a)</sup>	Whole melon	Enough volume to allow melon to float	26-30
Stainless	1 sponge	225	24-30

	steel			
	Sealed concrete	1 sponge	100	24-30
	Plastic <sup>(b)</sup>	1 swab	10	24-30

All samples for the AOAC validation were homogenized by stomaching unless otherwise noted.

(a) Homogenize sample by hand mixing

(b) Homogenize sample by vortexing

#### PREPARATION OF THE 3M™ MOLECULAR DETECTION SPEED LOADER TRAY

1. Wet a cloth with a 1-5% (v:v in water) household bleach solution and wipe the 3M™ Molecular Detection Speed Loader Tray.
2. Rinse the 3M Molecular Detection Speed Loader Tray with water.
3. Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.
4. Ensure the 3M Molecular Detection Speed Loader Tray is dry before use.

#### PREPARATION OF THE 3M™ MOLECULAR DETECTION CHILL BLOCK INSERT

Place the 3M™ Molecular Detection Chill Block directly on the laboratory bench; (the 3M™ Molecular Detection Chill Block Tray is not used). Use the chill block at ambient laboratory temperature (20-25°C).

#### PREPARATION OF THE 3M™ MOLECULAR DETECTION HEAT BLOCK INSERT

Place the 3M™ Molecular Detection Heat Block Insert in a dry doubleblock heater unit. Turn on the dry block heater unit and set the temperature to allow the 3M Molecular Detection Heat Block Insert to reach and maintain a temperature of 100 ±1°C.

**NOTE:** Depending on the heater unit, allow approximately 30 minutes for the 3M Molecular Detection Heat Block Insert to reach temperature. Using an appropriate, calibrated thermometer (e.g., a partial immersion thermometer or digital thermocouple thermometer, **not** a total immersion thermometer) placed in the designated location, verify that the 3M Molecular Detection Heat Block Insert is at 100 ±1°C.

#### PREPARATION OF THE 3M™ MOLECULAR DETECTION INSTRUMENT

1. Launch the 3M™ Molecular Detection Software and log in.
2. Turn on the 3M Molecular Detection Instrument.

3. Create or edit a run with data for each sample. Refer to the 3M Molecular Detection System User Manual for details.

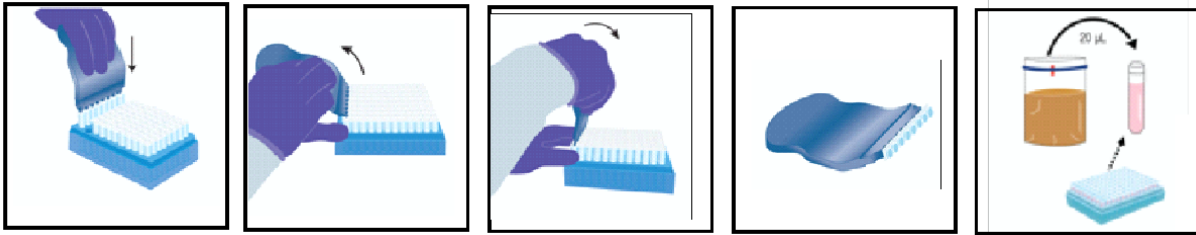
**NOTE:** The 3M Molecular Detection Instrument must reach and maintain temperature of 60°C before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 minutes and is indicated by an ORANGE light on the instrument's status bar. When the instrument is ready to start a run, the status bar will turn GREEN.

## LYSIS

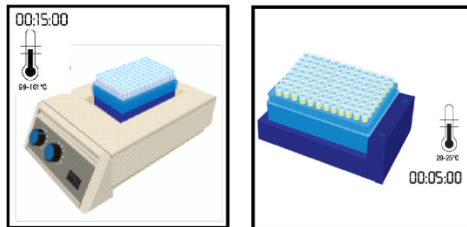
1. Allow the lysis solution (LS) tubes to warm up by setting the rack at room temperature (20-25 °C) overnight (16-18 hours). Alternatives to equilibrate the LS tubes to room temperature are to set the LS tubes on the laboratory bench for at least 2 hours, incubate the LS tubes in a 37 ±1°C incubator for 1 hour or place them in a dry double block heater for 30 seconds at 100°C.
2. Invert the capped tubes to mix. Proceed to next step within 4 hrs.
3. Remove the enrichment broth from the incubator.
4. One LS tube is required for each sample and the Negative Control (NC) (sterile enrichment medium) sample.
  - 4.1 LS tube strips can be cut to desired LS tube number. Select the number of individual LS tubes or 8-tube strips needed. Place the LS tubes in an empty rack.
  - 4.2 To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipette tip for each transfer step.
  - 4.3 Transfer enriched sample to LS tubes as described below:

Transfer each enriched sample into an individual LS tube **first**. Transfer the NC **last**.

- 4.4 Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip - one strip at a time.
  - 4.5 Discard the LS tube cap – if lysate will be retained for retest, place the caps into a clean container for re-application after lysis. For processing of retained lysate, see Appendix A.
  - 4.6 Transfer 20 µL of sample into a LS tube unless otherwise indicated in Protocol Table 2. e.g. Raw dairy products use 10µL.
5. Repeat step 4.2 until each individual sample has been added to a corresponding LS tube in the strip.



6. Repeat steps 4.1 to 4.6 as needed, for the number of samples to be tested.
7. When all samples have been transferred, transfer 20 µL of NC (sterile enrichment medium, e.g., Demi-Fraser Broth) into a LS tube. Do not use water as a NC.
8. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at  $100 \pm 1^\circ\text{C}$ .
9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for  $15 \pm 1$  minutes. During heating, the LS solution will change from pink (cool) to yellow (hot). Samples that have not been properly heat treated during the assay lysis step may be considered a potential biohazard and should NOT be inserted into the 3M Molecular Detection Instrument.
10. Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes. The 3M Molecular Detection Chill Block Insert, used at ambient temperature without the Molecular Detection Chill Block Tray should sit directly on the laboratory bench. When cool, the lysis solution will revert to a pink color.
11. Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.



#### AMPLIFICATION

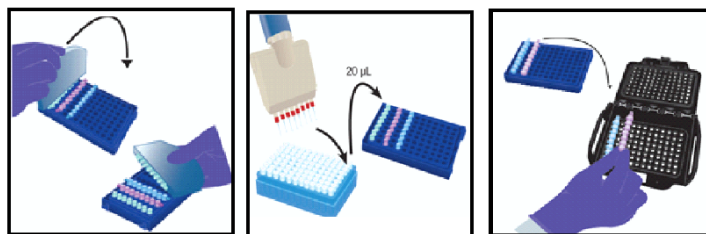
1. One Reagent tube is required for each sample and the NC.
  - 1.1 Reagent tube strips can be cut to desired tube number. Select the number of individual Reagent tubes or 8-tube strips needed.
  - 1.2 Place Reagent tubes in an empty rack.
  - 1.3 Avoid disturbing the reagent pellets from the bottom of the tubes.
2. Select 1 Reagent Control (RC) tube and place in rack.
3. To avoid cross-contamination, decap one Reagent tube strip at a time and use a new pipette tip for each transfer step.



- Transfer lysate to Reagent tubes and RC tube as described below:

Transfer each sample lysate into individual Reagent tubes **first** followed by the NC. Hydrate the RC tube **last**.

- Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent tubes –one Reagent tube strip at a time. Discard cap.
  - Transfer 20 µL of Sample lysate from the upper ½ of the liquid (avoid precipitate) in the LS tube into corresponding Reagent tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
  - Repeat step 5.1 until individual Sample lysate has been added to a corresponding Reagent tube in the strip.
  - Cover the Reagent tubes with the provided extra caps and use the rounded side of the 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.
  - Repeat step 5.1 as needed, for the number of samples to be tested.
  - When all sample lysates have been transferred, repeat 4.1 to transfer 20 µL of NC lysate into a Reagent tube.
  - Transfer **20 µL of NC lysate into a RC tube**. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.
- Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader Tray. Close and latch the 3M Molecular Detection Speed Loader Tray lid.



- Review and confirm the configured run in the 3M Molecular Detection Software.
- Click the Start button in the software and select instrument for use. The selected instrument's lid automatically opens.
- Place the 3M Molecular Detection Speed Loader Tray into the 3M Molecular Detection Instrument and close the lid to start the assay. Results are provided within 75 minutes, although positives may be detected sooner.

10. After the assay is complete, remove the 3M Molecular Detection Speed Loader Tray from the 3M Molecular Detection Instrument and dispose of the tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

**NOTICE:** To minimize the risk of false positives due to cross-contamination, never open reagent tubes containing amplified DNA. This includes Reagent Control, Reagent, and Matrix Control tubes. Always dispose of sealed reagent tubes by soaking in a 1-5% (v:v in water) household bleach solution for 1 hour and away from the assay preparation area.

## RESULTS AND INTERPRETATION

An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analyzed automatically by the software and are color-coded based on the result. A Positive or Negative result is determined by analysis of a number of unique curve parameters. Presumptive positive results are reported in real-time while Negative and Inspect results will be displayed after the run is completed.

Presumptive positive samples should be confirmed as per the laboratory standard operating procedures or by following the appropriate reference method confirmation <sup>(1, 2, 3)</sup>, beginning with transfer from the primary enrichment to secondary enrichment broth (if applicable), followed by subsequent plating and confirmation of isolates using appropriate biochemical and serological methods.

**NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular Detection Assay 2 - *Listeria monocytogenes* amplification reagents have a “background” relative light unit (RLU) reading.

In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M recommends the user to repeat the assay for any Inspect samples. If the result continues to be Inspect, proceed to confirmation test using your preferred method or as specified by local regulations

If you have questions about specific applications or procedures, please visit our website at [www.3M.com/foodsafety](http://www.3M.com/foodsafety) or contact your local 3M representative or distributor.

## REFERENCES:

1. US Food and Drug Administration Bacteriological Analysis Manual. Chapter 10: Detection and Enumeration of *Listeria monocytogenes* in Foods. Section C-6. April 2011 Version.
2. US Department of Agriculture (USDA) FSIS Microbiology Laboratory Guidebook 8.08. Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry and Egg Products, and Environmental Samples. Effective Date: 6 Nov 2012.

3. ISO 11290-1. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes*. Amendment 1, 2004-10-15.
4. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
5. ISO 7218. Microbiology of food and animal feeding stuffs – General rules for microbiological examination.
6. 3M. Installation Qualification (IQ) / Operational Qualification (OQ) Protocols and Instructions for 3M Molecular Detection System
7. ISO 18593. Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.

**Appendix A. Protocol Interruption: Storage and re-testing of heat-treated lysates**

1. To store a heat-treated lysate, re-cap the lysis tube with a clean cap (see “LYSIS”, 4.5)
2. Store at 4 to 8°C for up to 72 hours.
3. Prepare a stored sample for amplification by inverting 2-3 times to mix.
4. Decap the tubes.
5. Place the mixed lysate tubes on 3M Molecular Detection Heat Block Insert and heat at 100 ±1°C for 5 ±1 minutes.
6. Remove the rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes.
7. Continue the protocol at the ‘Amplification’ section detailed above.

**EXPLANATION OF PRODUCT LABEL SAMPLES**



Caution or Warning, see product instructions.



Consult product instructions.



The lot in a box represents the lot number.



The hourglass is followed by a month and year which represent the expiration date.



Storage temperature limitations.



1                   **A Comparative Evaluation of the 3M™ Molecular Detection Assay 2 (MDA 2) –*Listeria***  
2                   ***monocytogenes* for the Detection of *Listeria monocytogenes* in a Variety of Foods and**  
3                   **Environmental Surfaces**

4  
5                   **AOAC Performance Tested Methods<sup>SM</sup> ~~XXXXXX~~081501**

6  
7                   **Abstract**

8  
9                   The 3M™ Molecular Detection Assay 2 (MDA2)–*Listeria monocytogenes* for the detection of  
10                  *Listeria monocytogenes* was evaluated following the AOAC Research Institute *Performance*  
11                  *Tested Methods<sup>SM</sup>* program guidelines. The evaluation included inclusivity/exclusivity, lot-to-  
12                  lot/stability, robustness and matrix studies. The 3M MDA2 –*Listeria monocytogenes* was  
13                  compared to various reference methods for beef hot dogs, deli turkey, raw chicken leg pieces,  
14                  sealed concrete, plastic, stainless steel, whole melons, bagged raw spinach, romaine lettuce, cold  
15                  smoked salmon, queso fresco, 4% milk fat cottage cheese, 3% chocolate whole milk and vanilla  
16                  ice cream in the matrix study. Twenty replicates of each food matrix were analyzed at a low  
17                  inoculum level of 0.2-2 colony forming units (CFU)/test portion, five replicates were analyzed at  
18                  a high level of 2-5 CFU/test portion, and five control replicates were analyzed at 0 CFU/test  
19                  portion with the exception of raw chicken leg pieces. Naturally contaminated raw chicken leg  
20                  pieces were evaluated using two separate lots consisting of twenty replicates for each lot. All  
21                  environmental surfaces were analyzed using twenty replicates at a low inoculum level of ~50  
22                  CFU/standard test area, five replicates were analyzed at a high level of ~100 CFU/standard test  
23                  area, and five control replicates were analyzed at 0 CFU/standard test area. Based on the  
24                  probability of detection statistical model, there were no statistically significant differences  
25                  between the number of presumptive and confirmed positive samples or between the candidate  
26                  and reference method. There were no unexpected results in the lot-to-lot/stability, robustness  
27                  studies. In the inclusivity/exclusivity study, 50 target organisms and 30-43 non-target organisms  
28                  were tested following the 3M MDA2 - *Listeria monocytogenes* method with all target organisms  
29                  detected and none of the non-target organisms detected. The results of this evaluation  
30                  demonstrate the specificity and sensitivity of the 3M MDA2 - *Listeria monocytogenes* and its  
31                  ability to accurately detect *Listeria monocytogenes* in select food matrices and environmental  
32                  surfaces, 24-30 hours post enrichment.

33  
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19  
20 **Scope of Method**

21  
22 a) *Target organism.*— *Listeria monocytogenes*

23  
24 b) *Matrices.*— Beef hot dogs (25 g), deli turkey (125 g), queso fresco (25 g), vanilla ice cream  
25 (25 g), 4% milk fat cottage cheese (25 g), 3% chocolate whole milk (25 mL), whole melon,  
26 romaine lettuce (25 g), bagged raw spinach (25 g), cold smoked salmon (25 g), raw chicken  
27 leg pieces (25 g), sealed concrete (sponge enriched in 100 mL, 4" x 4"), plastic  
28 (EnviroSwab enriched in 10 mL, 1" x 1"), stainless steel (sponge enriched in 225 mL, 4" x  
29 4").

30  
31 ⇨ *Summary of Validated Performance Claims.*— Performance equivalent to that of the U.S.  
32 Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM) Chapter 10  
33 [1] for whole melon, cold smoked salmon, romaine lettuce; the U.S. Department of  
34 Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook*  
35 (USDA-FSIS/MLG) 8.09 [2] for beef hot dogs, deli turkey, raw chicken leg pieces, sealed  
36 concrete, plastic and stainless steel and AOAC 993.12 [3] for queso fresco, vanilla ice cream,  
37 4% milk fat cottage cheese, 3% chocolate whole milk and the ISO 11290-1/A1 [4] for cold  
38 smoked salmon and bagged raw spinach.

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39  
40 **Definitions**

41  
42 a) *Probability of Detection (POD).*—The proportion of positive analytical outcomes for a  
43 qualitative method for a given matrix at a given analyte level or concentration. POD is  
44 concentration dependent. Several POD measures can be calculated; POD<sub>R</sub> (reference method  
45 POD), POD<sub>C</sub> (confirmed candidate method POD), POD<sub>CP</sub> (candidate method presumptive  
46 result POD) and POD<sub>CC</sub> (candidate method confirmation result POD).

1 b) *Difference of Probabilities of Detection (dPOD)*.—Difference of probabilities of detection is  
2 the difference between any two POD values. If the confidence interval of a dPOD does not  
3 contain zero, then the difference is statistically significant at the 5% level.  
4

## 5 **Principle of the Method**

6 3M™ Molecular Detection Assay 2 –*Listeriamonocytogenes* used with the 3M™ Molecular  
7 Detection System for the rapid and specific detection of *Listeria* species in enriched food and  
8 environmental samples.  
9

10 The 3M Molecular Detection Assays use loop-mediated isothermal amplification to rapidly  
11 amplify nucleic acid sequences with high specificity and sensitivity, combined with  
12 bioluminescence to detect the amplification. Presumptive positive results are reported in real-  
13 time while negative results are displayed after the assay is completed. Presumptive positive  
14 results should be confirmed using the laboratory's preferred method or as specified by local  
15 regulations.  
16

## 17 **General Information**

18 *Listeria* is a small, Gram-positive rod that can be found in soil or carried by animals. Many  
19 foods have been associated with *Listeria* contamination: uncooked meats, uncooked  
20 vegetables, cantaloupe, pasteurized and unpasteurized milk, and other dairy and processed  
21 foods. [5] The resulting illness, listeriosis, from *Listeria monocytogenes* ingestion is rare but  
22 can be fatal. The disease may appear as meningitis in adults and can affect unborn children  
23 due to its ability to cross through the placenta. *Listeria* contamination often occurs post-  
24 cooking, before packaging as *Listeria* can survive longer under adverse environmental  
25 conditions that can most other vegetative bacteria that present a concern for the food safety  
26 industry. [6] Manufacturing facilities implement sanitation practices and regular surface  
27 screening for the presence of *Listeria* on food contact surfaces to monitor their food  
28 production environments. The 3M MDA2–*Listeria monocytogenes* was developed to  
29 provide rapid and specific detection of *Listeriamonocytogenes* in enriched food samples and  
30 on food processing surfaces.  
31

## 32 **Materials and Methods**

### 33 **Test Kit Information**

- 34  
35  
36 a) *Kit Name*.—Molecular Detection Assay 2 – *Listeriamonocytogenes*  
37 b) *Catalog Number*.—MDA2LM96  
38

### 39 *Test Kit Reagents - 3M MDA2 - Listeriamonocytogenes*(96 tests)

- 40 a) *Lysis Solution (LS) tubes*. – 96 (12 strips of 8 tubes)  
41 b) *Listeriamonocytogenes Reagent tubes*. – 96 (12 strips of 8 tubes)  
42 c) *Extra caps*. – 96 (12 strips of 8 caps)  
43 d) *Reagent Control (RC)*. – 16 (2 pouches of 8)  
44 e) *Quick Start Guide*  
45

### 46 *Additional Supplies and Reagents* 47

- 1 a) 3M Molecular Detection System (instrument), power supply, cord, USB cable and  
2 software  
3 b) 3M Molecular Detection Speed Loader Tray  
4 c) 3M Molecular Detection Chill Block Tray and Chill block insert  
5 d) 3M Molecular Detection Heat Block Insert  
6 e) 3M Molecular Detection Cap/Decap Tool [Reagent]  
7 f) 3M Molecular Detection Cap/Decap Tool [Lysis]  
8 g) Empty lysis tube rack  
9 h) Empty reagent tube rack  
10 i) Dey-Engley (D/E) Neutralizing Broth  
11 j) Demi-Fraser broth (with additional of ferric ammonium citrate)

#### 13 *Additional media*

- 14 a) Oxford Agar (OX)b) Sheep blood agar (SBA)  
15 b) Fraser broth (FB)  
16 c) Sheep blood agar (SBA)  
17 d) PALCAM agar  
18 e) Ottaviani Agosti Agar (OAA)  
19 f) Trypticase soy agar (TSA)  
20 g) Trypticase soy agar with 0.6% Yeast extract (TSA/YE)  
21 h) De Man, Rogosa and Sharpe (MRS)  
22 i) Modified Oxford agar (MOX)  
23 j) Brain heart infusion (BHI) broth  
24 k) Horse blood overlay (HBO)  
25 l) Buffered Listeria enrichment broth (BLEB)

#### 27 *Apparatus*

- 28 a) *Pipettes*. – capable of 20µL  
29 b) *Multi-channel pipette*. – capable of 20µL  
30 c) *Sterile pipette tips*. – capable of 20µL  
31 d) *Stomacher*®. – Seward or equivalent  
32 e) *Filter Stomacher*® *bags*. - Seward or equivalent  
33 f) *Thermometer*. – calibrated range to include 100 ± 1°C range  
34 g) *Incubators*. – capable of maintaining 37 ± 1°C  
35 h) *Dry double block heater unit*. – capable of maintaining 100 ± 1°C; *ora water bath*  
36 capable of maintaining 100 ± 1°C  
37 ~~i) *Freezer*. – capable of maintaining 10 to 20°C, for storing the 3M Molecular Detection~~  
38 ~~*Chill Block Tray*~~  
39 ~~†) *Refrigerator*. – capable of maintaining 2-8°C, for storing the 3M MDA2~~  
40 ~~‡) *Computer*. – compatible with the 3M Molecular Detection System (instrument)~~

#### 42 **Safety Precautions**

43  
44 The user should read, understand and follow all safety information in the instructions for the 3M  
45 Molecular Detection System and the 3M Molecular Detection Assay 2 –*Listeriamonocytogenes*.  
46 Retain the safety instructions for future reference.



1  
2 The 3M Molecular Detection Assay 2 –*Listeria monocytogenes* method may generate *Listeria*  
3 *monocytogenes* to levels sufficient to cause stillbirths and fatalities in pregnant women and the  
4 immunocompromised, if exposed.  
5

To reduce the risks associated with exposure to chemicals and biohazards:

- It is strongly recommended that female laboratory staff be informed of the risk to a developing fetus resulting from infection of the mother through exposure to *Listeria monocytogenes*
- Perform pathogentesting in a properly equipped laboratory under the control of trained personnel
- Always follow standard laboratory safety practices, including wearing appropriate protective apparel and eye protection while handling reagents and contaminated samples
- Avoid contact with the contents of the enrichment media and reagent tubes after amplification
- Dispose of enriched samples according to current industry standards

#### 6 7 **Enrichment Sample Preparation**

8 3M recommends the use of Demi-Fraser Broth (with addition of ferric ammonium citrate) for the  
9 enrichment of food and environmental samples. Table 1 presents guidance for the enrichment of  
10 food and environmental samples. It is the user's responsibility to validate alternate sampling  
11 protocols or dilution ratios to ensure this test method meets the user's criteria.  
12

#### 13 *Foods*

- 14 a) Allow the Demi-Fraser Broth enrichment medium to equilibrate to ambient laboratory  
15 temperature.
- 16 b) Aseptically combine the enrichment medium and sample according to Table 1. For all meat  
17 and highly particulate samples, the use of filter bags is recommended.
- 18 c) Homogenize thoroughly by ~~blending, stomaching, or hand mixing~~ for 2 ±0.2 minutes.  
19 Incubate at 37 ±1°C according to Table 1.  
20

#### 21 *Environmental samples*

22 Sample collection devices can be a sponge hydrated with a neutralizing solution to inactivate the  
23 effects of the sanitizers. 3M recommends the use of a biocide-free cellulose sponge. Neutralizing  
24 solution can be Dey-Engley (D/E) Neutralizing Broth or Letheen broth. It is recommended to  
25 sanitize the area after sampling.  
26

27 The recommended size of the sampling area for verifying the presence or absence of the pathogen  
28 on the surface is at least 100 cm<sup>2</sup> (10 cm x 10 cm or 4"x4"). When sampling with a sponge,  
29 cover the entire area going in two directions (left to right then up and down) or collect  
30 environmental samples following your current sampling protocol or according to the FDA BAM,  
31 USDA FSIS MLG or ISO 18593 (7) guidelines.  
32

- 33 1. Allow the Demi-Fraser Broth enrichment medium to equilibrate to ambient laboratory  
34 temperature.
- 35 2. Aseptically combine the enrichment medium and sample according to Table 1.
- 36 3. Homogenize thoroughly by ~~blending, vortexing, or stomaching, or hand mixing~~ for 2 ±0.2

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1 minutes. Incubate at  $37 \pm 1^\circ\text{C}$  for 24-30 hours.

- 2 4. Invert room temperature ( $20\text{-}25^\circ\text{C}$ ) lysis tubes to mix. Proceed to next step within 4 hours. 20  
3  $\mu\text{L}$  aliquot of each enriched sample are is transferred to separate lysis tubes using a new  
4 pipette tip after each sample transfer. Place uncovered samples on a dry bath incubator for 15  
5  $\pm 2$  minutes at  $100 \pm 1^\circ\text{C}$ . Following the heat lysis transfer samples to the sterilized lab bench  
6 and allow to cool at  $18\text{-}28^\circ\text{C}$  for 5-10 minutes.
- 7 5. Add 20  $\mu\text{L}$  of each lysed sample and control to separate reagent tubes, and mix by pipetting  
8 up and down five times. Analyze a matrix control tube with the samples for each matrix to  
9 verify that no interference with the assay was caused by the matrix. Use sterile Demi Fraser  
10 Broth for the Negative Control (NC). Transfer a 20  $\mu\text{L}$  aliquot to the NC and the Reagent  
11 Control (RC) tubes. Add a 20  $\mu\text{L}$  aliquot of a randomly picked sample to the matrix control  
12 tube, mixed, and recapped. Using the 3M™ software, follow prompts to identify samples and  
13 controls. Load all samples into the Speed Loader Tray (SLT), place into the Molecular  
14 Detection System, and initiate the 3M™ MDA2 - *Listeria monocytogenes* assay. Results are  
15 obtained within 75 minutes.

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### 17 Interpretation and Test Result Report

18 An algorithm interprets the light output curve resulting from the detection of the nucleic acid  
19 amplification. Results are analyzed automatically by the software and are color-coded based on  
20 the result. A Positive or Negative result is determined by analysis of a number of unique curve  
21 parameters. Presumptive positive results are reported in real-time while Negative and Inspect  
22 results will be displayed after the run is completed.

24 **NOTE:** Even a negative sample will not give a zero reading as the system and 3M Molecular  
25 Detection Assay 2 - *Listeria monocytogenes* amplification reagents have a “background” relative  
26 light unit (RLU) [reading](#).

28 In the rare event of any unusual light output, the algorithm labels this as “Inspect.” 3M  
29 recommends the user to repeat the assay for any Inspect samples. If the result continues to be  
30 Inspect, proceed to confirmation test using your preferred method or as specified by local  
31 regulations.

### 33 Confirmation

35 Presumptive positive samples of primary enrichments were confirmed by following the  
36 appropriate reference method confirmation, beginning with transfer from the primary enrichment  
37 to secondary enrichment broth (if applicable), followed by subsequent plating and confirmation  
38 of isolates using appropriate biochemical and serological methods.

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### 40 Validation Study

41  
42 Testing was conducted following the procedures outlined in the AOAC Research Institute  
43 *Performance Tested Methods<sup>SM</sup> Program* validation outline protocol: *Comparative Evaluation of*  
44 *the 3M™ Molecular Detection Assay 2-*Listeria monocytogenes* and of the 3M™ Molecular*  
45 *Detection Assay 2-*Listeria** (February, 2015) [8]. The independent study involved a matrix study  
46 (9 matrices), a lot-to-lot/stability evaluation, and a robustness evaluation. The internal study  
47 involved a matrix study (3 matrices) and an inclusivity/exclusivity study.

1  
2 **Internal Study**

3  
4 *Inclusivity and Exclusivity*

5  
6 *Methodology*

7 Fifty frozen *Listeria monocytogenes* strain suspensions were thawed and sub-cultured in brain heart  
8 infusion (BHI) broth overnight at 37°C ± 1°C. The cultures were diluted in peptone salt  
9 solution in order to inoculate between 10 to 100 cells per 225 mL of Demi Fraser. The  
10 enrichment broths were incubated for 24 hours at 37°C ± 1°C, and the 3M MDA2 -  
11 *Listeria monocytogenes* method was then performed.

12 Thirty frozen non-*Listeria monocytogenes* strain suspensions were thawed and sub-cultured  
13 grown in BHI broth overnight at 37°C ± 1°C. The cultures were diluted in buffered peptone water  
14 (BPW) or de Man, Rogosa, and Sharpe (MRS) in order to inoculate 10<sup>5</sup> cells/mL. The broths  
15 were then incubated for 24 hours at the appropriate incubation temperature in order to have  
16 culture to test with the 3M MDA2 -*Listeria monocytogenes* method (Table 3a).

17  
18 An additional thirteen frozen non-*Listeria monocytogenes* strain suspensions were thawed, or  
19 lyophilized cultures were revived, and then streaked to sheep blood agar (SBA). Single isolated  
20 colonies were picked and sub-cultured in 10 mL Demi-Fraser broth with FAC for 24 hours at  
21 37°C ± 1°C. The cultures were diluted with fresh Demi Fraser broth with FAC before testing  
22 using the 3M MDA2 - *Listeria monocytogenes* kit (Table 3b).

23  
24 *Results*

25 All 50 *Listeria monocytogenes* strains were detected by the 3M MDA2 -  
26 *Listeria monocytogenes* method. None of the 30-43 non-*Listeria monocytogenes* strains were  
27 detected. See Tables 2 and 3a, 3b for study details and results.  
28  
29

30 **Matrix Study**

31  
32 The matrix study consisted of evaluating a total of 30 un-paired sample replicates for 11  
33 matrices, along with a raw chicken leg pieces evaluating 20 sample replicates using two separate  
34 lots. Within each sample set, there were 5 un-inoculated samples (0 CFU/test portion), 20 low  
35 level inoculated samples (0.2-2 CFU/test portion), and 5 high level inoculated samples (2-5  
36 CFU/test portion), except for raw chicken leg pieces that were naturally contaminated with the  
37 target analyte. The inoculum was prepared by transferring a single *Listeria monocytogenes* colony  
38 from Trypticase soy agar with 5% sheep blood (SBA) into BHI broth and incubating the culture  
39 at 35 ± 2°C for 24 ± 2 hours. Table 4 presents the sample preparation guidelines for the matrix.  
40 All matrices were screened for the presence of the target organism following the appropriate  
41 reference method. Additionally, an aerobic plate count (APC) was conducted following the  
42 FDA/BAM Chapter 3 reference [9] method to determine the level of background flora in each  
43 test matrix prior to inoculation.

44 Prior to inoculation of beef hot dogs, deli turkey, and queso fresco the broth culture inoculum  
45 was heat stressed for 10 ± 1 minute at 50 ± 1°C in a water bath. The degree of injury of the  
46 culture was estimated by plating an aliquot of diluted culture onto modified Oxford agar (MOX)

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1 and Tryptic Soy agar (TSA). The agars were incubated at  $35 \pm 1^\circ\text{C}$  for  $24 \pm 2$  hours and the  
2 colonies were counted. The degree of injury was estimated as:  
3

$$(1 - \frac{n_{select}}{n_{nonselect}}) \times 100$$

4  
5  
6 Where  $n_{select}$  = number of colonies on selective agar and  $n_{nonselect}$  = number of colonies on non-  
7 selective agar. Using BHI broth as the diluent, the culture was diluted to a low level expected to  
8 yield fractional positive results (5-15 positive results) and a high level expected to yield all  
9 positive results. Following inoculation, a bulk lot of the matrix was homogenized by hand and  
10 held for 48-72 hours at refrigerated temperature ( $2-8^\circ\text{C}$ ) prior to analysis to allow time for the  
11 organism to equilibrate within the sample.

12 For melons, a single whole melon was placed into a large sterile bag and the blossom end of  
13 the melon was inoculated with  $100\ \mu\text{L}$  of the diluted *Listeria monocytogenes* culture. The liquid  
14 culture was then allowed to soak into the melon. The melon was then inverted so that the  
15 inoculated end was on the bottom of the sterile bag. The bag was tied closed and held for 48-72  
16 hours at  $2-8^\circ\text{C}$ .

17 For environmental surfaces, inocula were prepared by transferring a pure isolated colony of  
18 the specified organism from SBA into BHI broth and incubated at  $35 \pm 2^\circ\text{C}$  for  $24 \pm 2$  hours.  
19 Following incubation, serial dilutions were performed in BHI broth to achieve the target level  
20 inoculum.

21 For stainless steel and sealed concrete surfaces,  $4'' \times 4''$  areas were inoculated with  $0.25\ \text{mL}$   
22 of diluted *Listeria monocytogenes* culture. Stainless steel was also inoculated with competitor  
23 organism *Enterococcus faecium* ATCC 19434 at  $10\times$  the level of the target organism. For plastic  
24 surfaces,  $1'' \times 1''$  areas were inoculated with  $0.10\ \text{mL}$  of diluted *Listeria monocytogenes* culture.  
25 Plastic was also inoculated with a competitor organism, *Enterococcus faecalis* ATCC 29212, at  
26  $10\times$  the level of the target organism. For the un-inoculated test portions, sterile BHI broth was  
27 applied to the test area. Each surface was allowed to dry for 16-24 hours at room temperature ( $24$   
28  $\pm 2^\circ\text{C}$ ).

29 The 3M hydrated sampling sponges (pre-moistened with Dey-Engley) (stainless steel and  
30 sealed concrete) and 3M™ Tecra™ Enviro Swabs (pre-wetted with Lethen) (plastic) were  
31 sampled by using horizontal and vertical sweeping motions. The sponges and the swabs were  
32 held at room temperature for 2 hours prior to analysis. To determine the inoculation level for the  
33 environmental surfaces, aliquots of each inoculum were plated on TSA in triplicate.

34 The level of *Listeria monocytogenes* in the low level inoculum and high level inoculum was  
35 determined by Most Probable Number (MPN) on the day of analysis by evaluating  $5 \times 50\ \text{g}$ ,  $20 \times$   
36  $25\ \text{g}$  (reference method test portions), and  $5 \times 10\ \text{g}$  inoculated test samples for the low  
37 inoculation level and by examining  $5 \times 25\ \text{g}$ ,  $5 \times 5\ \text{g}$  and  $5 \times 1\ \text{g}$  for the high inoculation level.  
38 The level of *Listeria monocytogenes* in the low level inoculum and high level inoculum for all  
39  $125\ \text{g}$  test portions was determined by MPN by evaluating  $5 \times 250\ \text{g}$ ,  $20$  or  $5 \times 125\ \text{g}$  (reference  
40 method test portions), and  $5 \times 50\ \text{g}$  inoculated test samples. Each test portion was enriched with  
41 the reference method enrichment broth at the reference method dilution scheme and analyzed by  
42 the reference method procedure. The number of positives from the 3 test levels was used to  
43 calculate the MPN using the LCF MPN calculator (version 1.6) provided by AOAC RI. [10]  
44 (<http://www.lcf ltd.com/customer/LCFMPNCalculator.exe>)  
45

1  
2 USDA/FSIS MLG 8.09 Reference Method  
3

4 For the USDA/FSIS MLG 8.09 reference method, 25 g test portions were enriched with  $225 \pm 5$   
5 mL of modified University of Vermont Medium broth (UVM) and 125 g test portions were  
6 enriched with  $1125 \pm 25$  mL of UVM. All test portions were mechanically stomached for two  
7 minutes. The 25 g test portions were incubated at  $30 \pm 2$  °C for 20-26 hours and the 125 g test  
8 portions were incubated at  $30 \pm 2$  °C for 23-26 hours. For environmental samples, sponges were  
9 enriched with 225 mL of UVM and homogenized by hand while swabs were enriched with 10  
10 mL of UVM and mixed by vortex. Sponges and swabs were incubated for 20-26 hours at  $30 \pm$   
11  $2^{\circ}\text{C}$ . After incubation of all test portions,  $0.1 \pm 0.02$  mL of the sample enrichment was  
12 transferred to  $10 \pm 0.5$  mL of Fraser Broth (FB) containing 0.1 mL of 5% ferric ammonium  
13 citrate and incubated at  $35 \pm 2^{\circ}\text{C}$  for  $26 \pm 2$  hours. A loopful of the sample enrichment was also  
14 streaked to MOX and incubated at  $35 \pm 2^{\circ}\text{C}$  for  $26 \pm 2$  hours.

15 After  $26 \pm 2$  hours, FB was examined for any degree of darkening due to esculin hydrolysis.  
16 Any FB that displayed darkening was streaked to a MOX plate. If no darkening occurred, FB  
17 was re-incubated at  $35 \pm 2^{\circ}\text{C}$  for a total of  $48 \pm 2$  hours and re-examined for evidence of  
18 darkening. If darkening occurred, the FB was streaked to a MOX plate, if no darkening  
19 occurred, samples were considered negative. All FB streaked MOX plates were incubated at  $35$   
20  $\pm 2^{\circ}\text{C}$  for  $26 \pm 2$  hours.

21 MOX agar plates streaked from the primary enrichment or the FB secondary enrichment were  
22 examined after  $26 \pm 2$  hours and if no suspect colonies were present, the MOX agar plate was re-  
23 incubated for an additional  $26 \pm 2$  hours at  $35 \pm 2^{\circ}\text{C}$  for a total of  $48 \pm 2$  hours. If suspect  
24 colonies were present on the MOX agar plates, these suspect colonies were streaked to Horse  
25 Blood Overlay agar (HBO) and incubated at  $35 \pm 2^{\circ}\text{C}$  for  $22 \pm 4$  hours. HBO plates were  
26 examined for hemolysis reactions and well isolated colonies were transferred to BHI broth and  
27 incubated at  $25^{\circ}\text{C}$  for  $24 \pm 2$  hours. Sample isolates from BHI broth were analyzed for tumbling  
28 motility by preparing a wet mount, analyzed by a catalase test and examined for morphology by  
29 preparing a Gram stain. Additionally, purified HBO isolates were identified using the VITEK<sup>®</sup>  
30 GP Biochemical Identification following AOAC OMA 2013.02. [11]

31 FDA/BAM Chapter 10 Reference Method  
32  
33

34 Twenty-five gram test portions were enriched in  $225 \text{ mL} \pm 5 \text{ mL}$  of Buffered Listeria  
35 Enrichment Broth (BLEB) homogenized for 2 minutes and incubated at  $30 \pm 1^{\circ}\text{C}$  for 4 hours.  
36 For whole melons, a single whole melon was enriched using BLEB with approximately 1.5 times  
37 the weight of the melon, and soaked for 1 hour at room temperature. After an hour at room  
38 temperature, the test portions were incubated at  $30 \pm 1^{\circ}\text{C}$  for 4 hours. Following 4 hours of  
39 incubation, selective supplements acriflavine (10mg/L), sodium nalidixate (40mg/L) and  
40 cycloheximide (50mg/L) were added to each test portion and incubated for an additional 20  
41 hours. After 24 hours of total incubation, the enriched samples were streaked to MOX agar plates  
42 and incubated at  $35 \pm 1^{\circ}\text{C}$  for 24-48 hours. The enriched samples were re-incubated for an  
43 additional 24 hours at  $30 \pm 1^{\circ}\text{C}$  and then streaked to a second MOX agar plate which was  
44 incubated for 24-48 hours at  $35 \pm 1^{\circ}\text{C}$ . MOX agar plates were examined for suspect colonies, and  
45 if present, at least 5 colonies were streaked to TSA containing 0.6% yeast extract (TSA/YE). The  
46 TSA/YE plates were incubated at  $35 \pm 1^{\circ}\text{C}$  for 24-48 hours and then examined for purity. Pure

1 colonies were tested for catalase reactivity and a Gram Stain was conducted. A pure *Listeria*  
2 colony was transferred to Trypticase Soy Broth containing 0.6% yeast extract (TSB/YE). The  
3 TSB/YE cultures were incubated at  $25 \pm 1$  °C overnight, or until the broth was turbid, indicating  
4 sufficient growth. Catalase-positive organisms were stabbed into plates of 5% Sheep Blood Agar  
5 (SBA) and incubated at  $35 \pm 1$  °C for 24-48 hours. The TSB/YE tubes incubated at  $25 \pm 1$  °C  
6 were used to prepare a wet mount slide to determine motility pattern. After incubation, the SBA  
7 plates were examined for hemolysis. Final confirmation was conducted using the VITEK® GP  
8 Biochemical Identification card following AOAC OMA 2013.02.

#### 9 10 AOAC 993.12 Listeria Reference Method

11  
12 Twenty-five gram test portions were enriched in 225 mL  $\pm$  5 mL of selective enrichment  
13 medium, containing yeast extract (1.35 g/225 mL), acriflavine mono hydrochloride (1.125 g/225  
14 mL), nalidixic acid (sodium salt) solution (1.125 g/225 mL), and cycloheximide (2.25 g/225  
15 mL), and incubated at  $30 \pm 1$  °C for 48 hours. The enriched samples were then streaked to  
16 Oxford Agar (OXA) and incubated at  $37 \pm 1$  °C for 48 hours. At 48 hours, the OXA plates were  
17 examined for suspect colonies. If present, up to 5 suspect colonies were streaked to TSA/YE to  
18 obtain well isolated, pure colonies. Pure colonies on TSA/YE were analyzed for Gram-stain  
19 reaction and for catalase activity. Catalase positive, Gram positive rods colonies were then  
20 stabbed to SBA and incubated at  $37 \pm 1$  °C for 48 hours. After 48 hours, the SBA plates were  
21 examined for typical hemolytic reactions. The same colony picked to SBA was also transferred  
22 to TSB/YE. The TSB/YE cultures were incubated at  $25 \pm 1$  °C overnight, or until the broth was  
23 turbid, indicating sufficient growth. Another TSB/YE tube was inoculated with the same colony  
24 and incubated at  $37 \pm 1$  °C for 24 hours to be used for carbohydrate utilization testing. The  
25 TSB/YE tube incubated at  $25 \pm 1$  °C were used to prepare a wet mount slide to determine motility  
26 pattern. From the TSB/YE tube incubated at  $37 \pm 1$  °C, Motility Test Medium (MTM) was  
27 stabbed and incubated at  $25 \pm 1$  °C. After 2 days, and up to 7 days, the MTM tubes were observed  
28 for umbrella-like growth. Additionally from the TSB/YE tube, a loopful (0.1 mL) was  
29 transferred into carbohydrate fermentation broth (purple broth) containing 1.0 mL of either 5%  
30 rhamnose or 5% xylose. The purple broth tubes were incubated at  $37 \pm 1$  °C and examined for up  
31 to 7 days.

#### 32 33 ISO 11290-1/A1 Reference Method

34  
35 For the ISO 11290-1/A1 reference method, 25 g test portions were enriched with 225 half Fraser  
36 broth. All test portions were mechanically stomached for two minutes. The test portions were  
37 incubated at  $30 \pm 1$  °C for  $24 \pm 3$  hours. After incubation, 0.1 mL of the sample enrichment was  
38 transferred to 10 mLFB containing 0.1 mL of 5% ferric ammonium citrate and incubated at  $37 \pm$   
39  $1$  °C for  $48 \pm 3$  hours. After  $48 \pm 3$  hours, a loopful of the sample secondary FB enrichment was  
40 streaked to PALCAM and OAA (Ottovani-Agosti Agar) and incubated at  $37 \pm 1$  °C for  $24 \pm 3$   
41 hours. A loopful of the sample primary enrichment was also streaked to PALCAM and OAA and  
42 incubated at  $37 \pm 1$  °C for  $24 \pm 3$  hours. PALCAM and OAA agar plates were examined for the  
43 presence of suspect colonies. If no suspect colonies were present, the agar plate was re-incubated  
44 for an additional 18-24 hours at  $37 \pm 1$  °C. If no suspect colonies present the sample was  
45 determined to be negative for *Listeria*. If suspect colonies were present on the PALCAM or  
46 OAA agar plates, these suspect colonies were streaked to HBO and incubated at  $37 \pm 1$  °C for 18-

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1 24 hours. HBO plates were examined for hemolysis reactions and well-isolated colonies were  
2 transferred to BHI broth and incubated at 25°C for 24 ± 2 hours. Sample isolates from BHI broth  
3 were analyzed for tumbling motility by preparing a wet mount, analyzed by a catalase test and  
4 examined for morphology by preparing a Gram stain. Additionally, purified HBO isolates were  
5 identified using the VITEK® GP Biochemical Identification following AOAC OMA 2013.02.  
6  
7

### 8 3M™ Molecular Detection Assay 2 –*Listeria monocytogenes*

9

10 All 25 g samples were analyzed by the 3M™MDA2 - *Listeriamonocytogenes*were enriched  
11 with 225 mL of Demi-Fraser Broth containing ferric ammonium citrate; all test portions were  
12 then homogenized by stomaching thoroughly for 2 ± 0.2 minutes and incubated at 37 ±1 °C for  
13 24-28 hours. For the 125 g deli turkey test portions, samples were enriched with 1125 mL of  
14 Demi-Fraser Broth; test portions were then homogenized by stomaching thoroughly for 2 ± 0.2  
15 minutes and incubated at 37 ±1 °C for 24 hours. For whole melons, test portions were enriched  
16 with approximately 1.5 times the weight of the whole melon in Demi Fraser and incubated at 37  
17 ±1 °C for 26 hours. For stainless steel, sponge samples were enriched with 225 mL of Demi-  
18 Fraser Broth; samples were homogenized by hand thoroughly for 2 ± 0.2 minutes and incubated  
19 at 37 ±1 °C for 24-26 hours. Sealed concrete environmental surface sponge samples were  
20 enriched with 100 mL of Demi-Fraser Broth; samples were homogenized by hand thoroughly for  
21 2 ± 0.2 minutes and incubated at 37°C for 24-26 hours. Plastic environmental surface swab  
22 samples were enriched with 10 mL of Demi-Fraser Broth; samples were homogenized by  
23 vortexing thoroughly for 2 ± 0.2 minutes and incubated at 37 ±1 °C for 24-26 hours.

24 Prior to analysis, lysis tubes were brought to room temperature (20-25 °C) by placing the  
25 tubes on a sanitized bench top for 16-18 hours. Lysis tubes were inverted to mix up to four hours  
26 before use. The lysis tubes were then de-capped and the rubber cap was discarded. A 20 µL  
27 aliquot of each sample was transferred to separate lysis tubes; a new pipette tip was used after  
28 each sample transfer. Uncovered samples were placed on a dry bath incubator for 15 ± 2minutes  
29 at 100 ± 1 °C. Following the heat lysis, samples were transferred to the sanitized  
30 laboratorybench and were allowed to cool at 18-28 °C for 5-10 minutes.

31 A 20 µL aliquot of each lysed sample and control was added to separate reagent tubes, and  
32 samples were mixed by pipetting up and down five times. A matrix control tube was analyzed  
33 with the samples for each matrix to verify that no interference with the assay was caused by the  
34 matrix. A sample of sterile Demi Fraser Broth was lysed for the kit Negative Control (NC). A  
35 20 µL aliquot was transferred to the NC and the Reagent Control (RC) tubes. A 20 µL aliquot of  
36 a randomly picked sample was added to the matrix control tube, mixed, and recapped. Using the  
37 3M™ software, prompts were followed to identify samples and controls. All samples were  
38 loaded into the Speed Loader Tray, placed into the Molecular Detection System, and the  
39 3M™MDA2 - *Listeria monocytogenes*assay was initiated and results were obtained within 75  
40 minutes.  
41

### 42 **Matrix Study Results**

43

44 For each method, the probability of detection (POD) was calculated as the number of positive  
45 outcomes divided by the total number of trials. [12] The following were calculated:forthe  
46 candidate presumptive results, POD<sub>CP</sub>; the candidate confirmatory results, POD<sub>CC</sub>; the difference

1 in the candidate presumptive and confirmatory results,  $dPOD_{CP}$ ; the presumptive candidate results  
2 that confirmed positive,  $POD_C (= POD_{CC})$ ; the reference method,  $POD_R$ , and the difference in the  
3 confirmed candidate and reference methods,  $dPOD_C$ . POD analyses were conducted for the  
4 MDA2 - *Listeria monocytogenes* test points and compared to the appropriate reference method  
5 results. The pre-validation target analyte background screen results and APC results are  
6 presented in Table 5. The heat stress data for selected matrices are presented in Table 6. The  
7 inoculum levels for each environmental surface are presented in Table 7. A summary of POD  
8 analyses [13] are presented in Tables 8-15. As per criteria outlined in Appendix J of the Official  
9 Methods of Analysis Manual [13], fractional positive results were obtained for all matrices.

#### 10 Internal Study

##### 11 *Raw Chicken Leg Pieces (25 g) – 28 hour Primary Enrichment*

12  
13 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 10  
14 presumptive positives and 10 confirmed positives following the USDA/FSIS MLG 8.09  
15 reference method confirmation procedure. There were 12 observed positives for the reference  
16 method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed  
17 positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There  
18 were 5 confirmed positive following the reference method. Detailed results of the POD analyses  
19 are presented in Tables 8 and 12.

##### 20 *Bagged raw spinach*

21 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 9  
22 presumptive positives and 10 confirmed positives following the ISO 11290-1/A1 reference  
23 method confirmation procedure resulting in 1 false negative. There were 10 observed positives  
24 for the reference method. For the high inoculation level, there were 5 presumptive positives and  
25 5 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure.  
26 There were 4 confirmed positives following the reference method. Detailed results of the POD  
27 analyses are presented in Tables 8 and 12.

##### 28 *Cold smoked salmon*

##### 29 *Compared to FDA-BAM*

30 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 10  
31 presumptive positives and 10 confirmed positives following the FDA/BAM Chapter 10 reference  
32 method confirmation procedure. There were 7 observed positives for the reference method. For  
33 the high inoculation level, there were 3 presumptive positives and 3 confirmed positives  
34 following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 5  
35 confirmed positives following the reference method. Detailed results of the POD analyses are  
36 presented in Tables 8 and 12.

##### 37 *Compared to ISO 11290-1/A1*



1 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 10  
2 presumptive positives and 10 confirmed positives following the ISO 11290-1/A1 reference  
3 method confirmation procedure. There were 9 observed positives for the reference method. For  
4 the high inoculation level, there were 3 presumptive positives and 3 confirmed positives  
5 following the ISO 11290-1/A1 reference method confirmation procedure. There were 4  
6 confirmed positives following the reference method. Detailed results of the POD analyses are  
7 presented in Tables 8 and 12.  
8

9  
10 Independent Study

11  
12 *Deli Turkey (125 g)*

13  
14 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 8  
15 presumptive positives and 8 confirmed positives following the USDA/FSIS MLG 8.09 reference  
16 method confirmation procedure. There were 7 observed positives for the reference method. For  
17 the high inoculation level, there were 5 presumptive positives and 5 confirmed positives  
18 following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 5  
19 confirmed positives following the reference method. Detailed results of the POD analyses are  
20 presented in Tables 9 and 13.  
21

22 *Raw Chicken Leg Pieces (25 g)*

23  
24 *Lot 1*

25 For the lot 1 of the raw chicken leg pieces of the MDA2 - *Listeria monocytogenes* assay, there  
26 were 17 presumptive positives and 17 confirmed positives following the USDA/FSIS MLG 8.09  
27 reference method confirmation procedure. There were 16 observed positives for the reference  
28 method. Detailed results of the POD analyses are presented in Tables 9 and 13.  
29

30 *Lot 2*

31 For the lot 2 of the raw chicken leg pieces of the MDA2 - *Listeria monocytogenes* assay, there  
32 were 10 presumptive positives and 10 confirmed positives following the USDA/FSIS MLG 8.09  
33 reference method confirmation procedure. There were 14 observed positives for the reference  
34 method. Detailed results of the POD analyses are presented in Tables 9 and 13.  
35

36 *Whole Melon*

37  
38 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 10  
39 presumptive positives and 10 confirmed positives following the FDA/BAM Chapter 10 reference  
40 method confirmation procedure. There were 8 observed positives for the reference method. For  
41 the high inoculation level, there were 5 presumptive positives and 5 confirmed positives  
42 following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 5  
43 confirmed positives following the reference method. Detailed results of the POD analyses are  
44 presented in Tables 9 and 13.  
45  
46

1 *Vanilla Ice Cream (25 g)*

2  
3 For the low inoculation level of the MDA2 -*Listeria monocytogenes* assay, there were 7  
4 presumptive positives and 7 confirmed positives following the AOAC 993.12 reference method  
5 confirmation procedure. There were 7 observed positives for the reference method. For the high  
6 inoculation level, there were 5 presumptive positives and 5 confirmed positives following the  
7 AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives  
8 following the reference method. Detailed results of the POD analyses are presented in Tables 9  
9 and 13.

10  
11 *Romaine lettuce (25 g)*

12  
13 For the low inoculation level of the MDA2 -*Listeria monocytogenes* assay, there were 7  
14 presumptive positives and 7 confirmed positives following the FDA/BAM Chapter 10 reference  
15 method confirmation procedure. There were 6 observed positives for the reference method. For  
16 the high inoculation level, there were 5 presumptive positives and 5 confirmed positives  
17 following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 5  
18 confirmed positives following the reference method. Detailed results of the POD analyses are  
19 presented in Tables 10 and 14.

20  
21 *Queso Fresco (25 g)*

22  
23 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 11  
24 presumptive positives and 11 confirmed positives following the AOAC 993.12 reference method  
25 confirmation procedure. There were 9 observed positives for the reference method. For the high  
26 inoculation level, there were 5 presumptive positives and 5 confirmed positives following the  
27 AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives  
28 following the reference method. Detailed results of the POD analyses are presented in Tables 10  
29 and 14.

30  
31 *4% Milk Fat Cottage Cheese (25 g)*

32  
33 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 10  
34 presumptive positives and 10 confirmed positives following the AOAC 993.12 reference method  
35 confirmation procedure. There were 8 observed positives for the reference method. For the high  
36 inoculation level, there were 5 presumptive positives and 5 confirmed positives following the  
37 AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives  
38 following the reference method. Detailed results of the POD analyses are presented in Tables 10  
39 and 14.

40  
41 *3% Chocolate Whole Milk (25 mL)*

42  
43 For the low inoculation level of the MDA2 -*Listeria monocytogenes* assay, there were 9  
44 presumptive positives and 9 confirmed positives following the AOAC 993.12 reference method  
45 confirmation procedure. There were 6 observed positives for the reference method. For the high  
46 inoculation level, there were 5 presumptive positives and 5 confirmed positives following the

1 AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives  
2 following the reference method. Detailed results of the POD analyses are presented in Tables 10  
3 and 14.

4  
5 *Beef Hot Dog (25 g)*

6  
7 For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 4  
8 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference  
9 method confirmation procedure, resulting in 1 false negative. There were 6 observed positives  
10 for the reference method. For the high inoculation level, there were 5 presumptive positives and  
11 5 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation  
12 procedure. There were 5 confirmed positives following the reference method. Detailed results of  
13 the POD analyses are presented in Tables 10 and 14.

14  
15 *Stainless Steel (enriched in 225 mL)*

16  
17 Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level  
18 of the MDA2 - *Listeria monocytogenes* assay, there were 5 presumptive positives and 5  
19 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation  
20 procedure. There were 8 observed positives for the reference method. For the high inoculation  
21 level, there were 5 presumptive positives and 5 confirmed positives following the USDA/FSIS  
22 MLG 8.09 reference method confirmation procedure. There were 5 confirmed positives  
23 following the reference method. Detailed results of the POD analyses are presented in Tables 11  
24 and 15.

25  
26 *Sealed Concrete (enriched in 100 mL)*

27  
28 *24-hour primary enrichment* - For the low inoculation level of the MDA2 –  
29 *Listeria monocytogenes* assay, there were 15 presumptive positives and 15 confirmed positives  
30 following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 11  
31 observed positives for the reference method. For the high inoculation level, there were 5  
32 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference  
33 method confirmation procedure. There were 5 confirmed positives following the reference  
34 method. Detailed results of the POD analyses are presented in Tables 11 and 15.

35  
36 *26-hour primary enrichment* - For the low inoculation level of the MDA2 –  
37 *Listeria monocytogenes* assay, there were 16 presumptive positives and 15 confirmed positives  
38 following the USDA/FSIS MLG 8.09 reference method confirmation procedure, resulting in 1  
39 false positive. There were 11 observed positives for the reference method. For the high  
40 inoculation level, there were 5 presumptive positives and 5 confirmed positives following the  
41 USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 5 confirmed  
42 positives following the reference method. Detailed results of the POD analyses are presented in  
43 Tables 11 and 15.

44  
45 *Plastic (enriched in 10 mL)*

46

1 Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level  
2 of the MDA2 - *Listeria monocytogenes* assay, there were 12 presumptive positives and 12  
3 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation  
4 procedure. There were 10 observed positives for the reference method. For the high inoculation  
5 level, there were 5 presumptive positives and 5 confirmed positives following the USDA/FSIS  
6 MLG 8.09 reference method confirmation procedure. There were 5 confirmed positives  
7 following the reference method. Detailed results of the POD analyses are presented in Tables 11  
8 and 15.

9  
10 All five un-inoculated control test portions were negative by both the MDA2 - *Listeria*  
11 *monocytogenes* assay and the corresponding reference method for each matrix above.

## 12 13 14 **Product Consistency (Lot-to-Lot) and Stability Studies**

### 15 16 *Methodology*

17 Three lots of MDA2 - *Listeria monocytogenes* test kits, 1 newly manufactured, 1 at the middle of  
18 its expiration, and 1 at or slightly beyond expiration was analyzed to determine the stability of  
19 the assay. For the MDA2 - *Listeria monocytogenes* test kit, one *Listeria monocytogenes* ATCC  
20 7644 isolate was analyzed at a fractional positive level (2-8 positives), and testing 10 replicates.  
21 One non-*Listeria monocytogenes* isolate, *Enterococcus faecalis* ATCC 29212, was analyzed at  
22 the growth level achieved in a non-selective broth, testing 5 replicates.

### 23 24 *Results*

25 For the lot-to-lot/stability evaluation of the MDA2 - *Listeria monocytogenes* assay, there were 6  
26 presumptive positives out of 10 replicates for all three lots at the low inoculation level. For the 5  
27 un-inoculated control test portions, there were 0 presumptive positives out of 5 replicates for all  
28 three lots. The five control test portions were negative. See Tables 16 for lot-to-lot/stability  
29 results.

## 30 31 **Robustness Study**

### 32 33 *Methodology*

34 The robustness evaluated the ability of the method to remain unaffected by minor variations in  
35 method parameters that might be expected to occur when the method is performed by an end  
36 user. Three different parameters were evaluated, which are presented in Table 17. For each  
37 parameter, one *Listeria monocytogenes* ATCC 7644 isolate was analyzed at a fractional positive  
38 level (2-8 positives), testing 10 replicates. One non-*Listeria monocytogenes* isolate, *Enterococcus*  
39 *faecalis* ATCC 29212, was analyzed at the growth level achieved in a non-selective broth, testing  
40 5 replicates.

### 41 42 *Results*

43  
44 For the robustness analysis of the MDA2 - *Listeria monocytogenes* assay, there were 6  
45 presumptive positives out of 10 replicates for each variation evaluated. For the 5 control test

1 portions, there were 0 presumptive positives out of 5 replicates. See Table 17 for robustness  
2 results.

### 3 4 **Independent Laboratory Observations**

5  
6 The results of this study demonstrate the ability of the MDA2-*Listeria monocytogenes* to detect  
7 the presence of *Listeria monocytogenes* in various food matrices and select environmental  
8 surfaces after 24-28 hours of a primary enrichment. The MDA2-*Listeria monocytogenes* offers  
9 the benefits of extremely high sensitivity and high specificity for the detection of *Listeria*  
10 *monocytogenes* while reducing the overall time to presumptive results. The MDA2-*Listeria*  
11 *monocytogenes* also reduces the total analyst time required to obtain final results by  
12 implementing a single heat lysis step and cool step, followed up by a single transfer to molecular  
13 reaction tubes. The small foot print of the Molecular Detection System requires only minimal lab  
14 and bench space, making it easy to move the instrument or pair up additional MDS units to  
15 perform multiple MDA runs concurrently. The updated software is user friendly with easy to  
16 interpret results. The ability to examine Relative Light Unit (RLU) curves makes trouble  
17 shooting more streamlined if any problems occur while testing is being conducted.

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### 18 19 **Discussion**

20  
21 There were no differences between the 24- and 26-hour enrichment time points for the  
22 environmental surfaces tested, therefore a 24-hour minimum enrichment will be recommended  
23 for these matrices.

### 24 25 **Conclusion**

26  
27 The 3M MDA2-*Listeria monocytogenes* AOAC PTM validation study included an  
28 inclusivity/exclusivity, lot-to-lot/stability, robustness and matrix studies. The 3M MDA2-  
29 *Listeria monocytogenes* was compared to the USDA/FSIS-MLG 8.09, the FDA-BAM  
30 Chapter 10, the AOAC 993.12 or the ISO 11290-1/A1 reference methods for the detection  
31 of *Listeria monocytogenes*. There were no significant differences between the 3M MDA2-  
32 *Listeria monocytogenes* method and the corresponding reference method for any of the  
33 matrices evaluated. The 3M MDA2-*Listeria monocytogenes* demonstrated reliability as a  
34 rapid and sensitive method for the detection of *Listeria monocytogenes* for the following  
35 matrices: beef hot dogs (25 g), deli turkey (125 g), queso fresco (25 g), vanilla ice cream (25  
36 g), 4% milk fat cottage cheese (25 g), 3% chocolate whole milk (25 mL), whole melon,  
37 romaine lettuce (25 g), bagged raw spinach (25 g), cold smoked salmon (25 g), raw chicken  
38 leg pieces (25 g), sealed concrete (sponge enriched in 100mL), plastic (EnviroSwab  
39 enriched in 10 mL), stainless steel (sponge enriched in 225 mL).

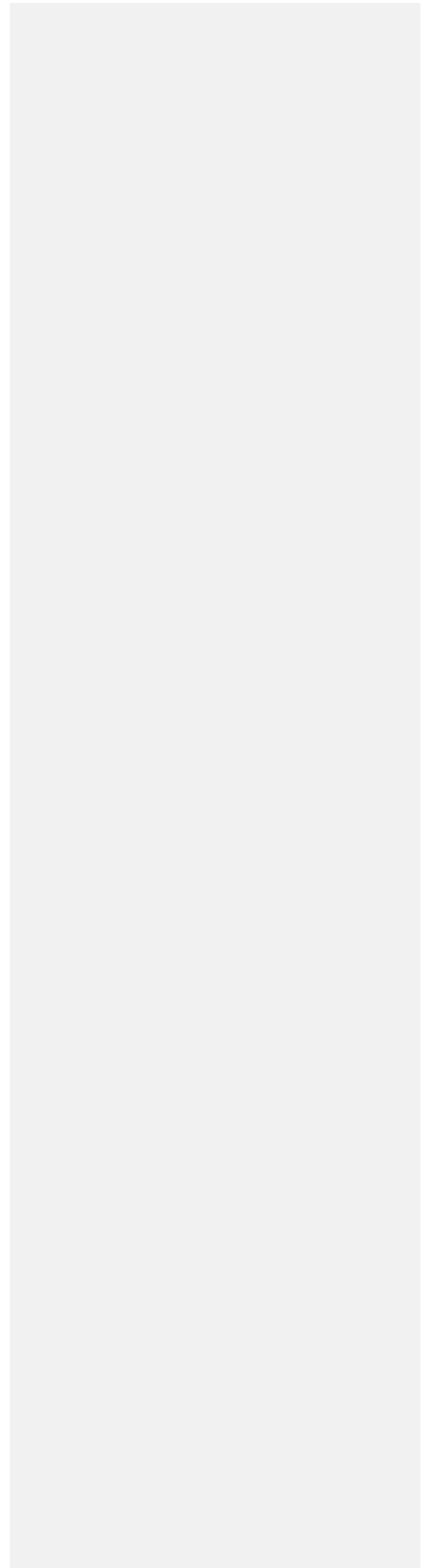
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**Table 1. Enrichment Protocols Using Demi-Fraser Broth Enrichment at 37 ± 1 °C**  
**Specific Instructions for Validated Methods**  
**AOAC® Performance Tested Method<sup>SM</sup> # 081501**



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In AOAC PTM studies, the 3M Molecular Detection Assay 2 – *Listeria monocytogenes* was found to be an effective method for the detection of *Listeria monocytogenes*. The matrices tested in the study are shown in Table 3. The limit of detection of the 3M Molecular Detection Assay 2 – *Listeria monocytogenes* method is 1-5 colony forming units per validated test portion size (in Table 3).

**Table 1. Enrichment protocols using Demi-Fraser Broth at 37 ± 1 °C according to AOAC Performance Tested<sup>SM</sup> Certificate #081501.**

<u>Sample Matrix</u>	<u>Sample Size</u>	<u>Enrichment Broth Volume (mL)</u>	<u>Enrichment Time (hr)</u>
<u>Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon</u>	<u>25 g</u>	<u>225</u>	<u>24-30</u>



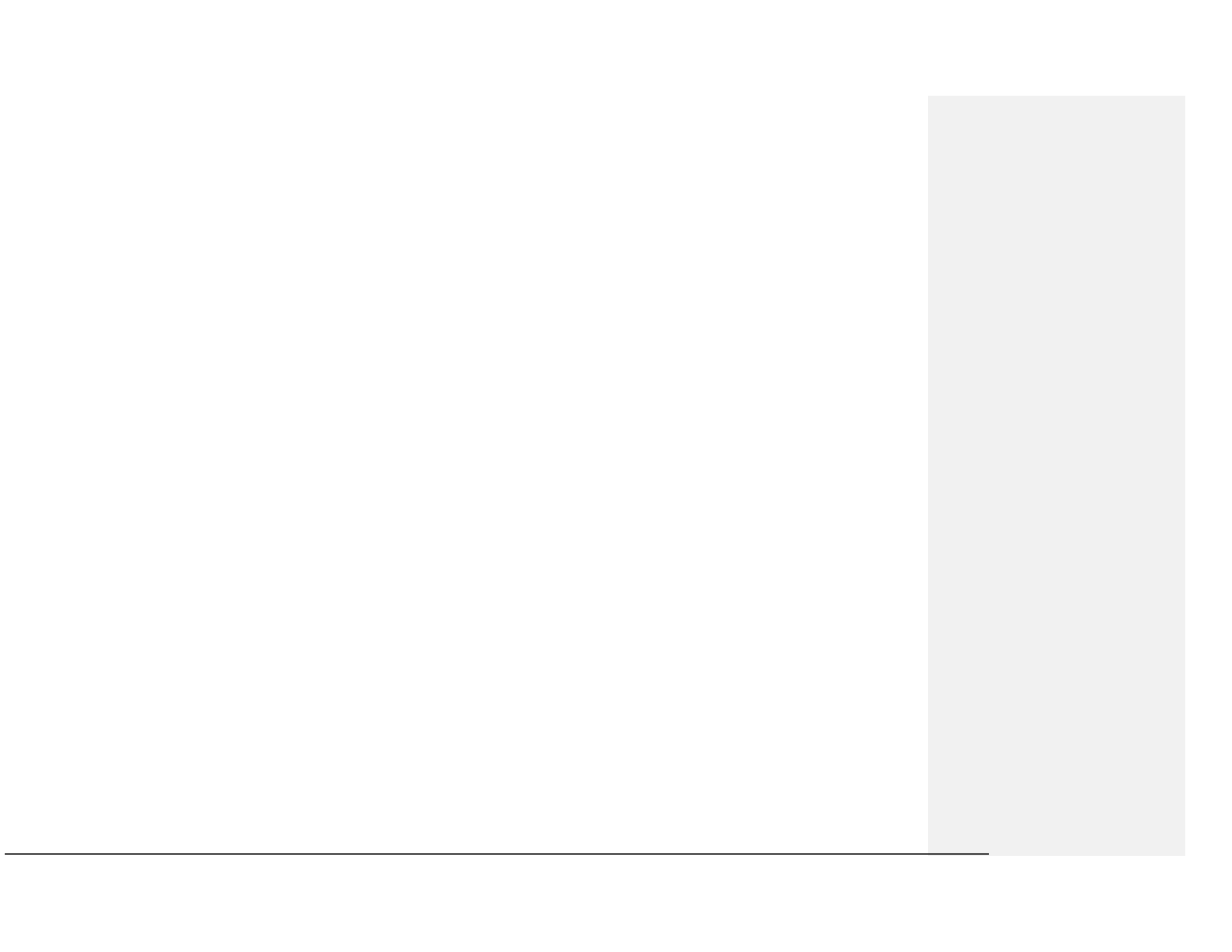
	<a href="#">Raw chicken</a>	<a href="#">25 g</a>	<a href="#">475</a>	<a href="#">28-32</a>
	<a href="#">Deli turkey</a>	<a href="#">125 g</a>	<a href="#">1125</a>	<a href="#">24-30</a>
	<a href="#">Cantaloupe<sup>a</sup></a>	<a href="#">Whole melon</a>	<a href="#">Enough volume to allow melon to float</a>	<a href="#">26-30</a>
<a href="#">Environmental samples:</a>	<a href="#">Stainless steel</a>	<a href="#">1 sponge</a>	<a href="#">225</a>	<a href="#">24-30</a>
	<a href="#">Sealed concrete</a>	<a href="#">1 sponge</a>	<a href="#">100</a>	<a href="#">24-30</a>
	<a href="#">Plastic<sup>b</sup></a>	<a href="#">1 swab</a>	<a href="#">10</a>	<a href="#">24-30</a>

[All samples for the AOAC validation were homogenized by stomaching unless otherwise noted.](#)

[a = Homogenize sample by hand mixing](#)

[b = Homogenize sample by vortexing](#)

<a href="#">Sample Matrix</a>	<a href="#">Sample Size</a>	<a href="#">Enrichment Broth Volume (mL)</a>	<a href="#">Enrichment Time (hr)</a>
<a href="#">Beef hot dogs, Queso Fresco, Vanilla Ice Cream, 4% Milk Fat Cottage Cheese, 3% chocolate whole milk, romaine lettuce, bagged raw spinach, cold smoked salmon</a>	<a href="#">25 g</a>	<a href="#">225</a>	<a href="#">24-30</a>
<a href="#">Deli Turkey</a>	<a href="#">125-g</a>	<a href="#">1175</a>	<a href="#">24-30</a>
<a href="#">Environmental samples:</a>	<a href="#">stainless steel, sealed concrete</a>	<a href="#">1-sponge</a>	<a href="#">100 or 225</a>
	<a href="#">Plastic,</a>	<a href="#">1 swab</a>	<a href="#">10</a>
<a href="#">Raw Chicken</a>	<a href="#">25 g</a>	<a href="#">475</a>	<a href="#">28-32</a>



**Table 2. Inclusivity Study Results**

INCLUSIVITY								
Strain	Species	Reference	Origin	Inoculation level (cfu/225ml)	3M MDA 2 Demi Fraser broth 24h at 37°C			
					MDA2 - <i>Listeria monocytogenes</i>	Confirmation 100µl		
						ALOA	Palcam	
1	<i>Listeria</i>	<i>monocytogenes</i>	153	Soft cheese (Munster)	39	+	H+	+
2	<i>Listeria</i>	<i>monocytogenes</i>	1011/1410	Frozen broccoli	28	+	H+	+
3	<i>Listeria</i>	<i>monocytogenes</i>	1972/2399	Puff pastry with mushrooms	38	+	H+	+
4	<i>Listeria</i>	<i>monocytogenes</i>	1973/2400	Puff pastry egg and ham (Quiche-lorraine)	36	+	H+	+
5	<i>Listeria</i>	<i>monocytogenes</i>	2407/3139	Tripes with tomatoes	31	+	H+	+
6	<i>Listeria</i>	<i>monocytogenes</i>	2760/3145	Raw bacon	44	+	H+	+
7	<i>Listeria</i>	<i>monocytogenes</i>	32.183	Croque-Monsieur	31	+	H+	+
8	<i>Listeria</i>	<i>monocytogenes</i>	38/181	Toulouse sausages	33	+	H+	+
9	<i>Listeria</i>	<i>monocytogenes</i>	5721/6179	Smoked bacon	49	+	H+	+
10	<i>Listeria</i>	<i>monocytogenes</i>	7111/7516	Pâté (Rillettes)	64	+	H+	+
11	<i>Listeria</i>	<i>monocytogenes</i>	850/109	Ready-To-Eat (RTE) food (deli salad with seafood)	34	+	H+	+
12	<i>Listeria</i>	<i>monocytogenes</i>	877/113	Environmental sample (pastry)	28	+	H+	+
13	<i>Listeria</i>	<i>monocytogenes</i>	913/1048	Black pudding	39	+	H+	+
14	<i>Listeria</i>	<i>monocytogenes</i>	A00C014	Sausage	37	+	H+	+
15	<i>Listeria</i>	<i>monocytogenes</i>	A00C022	Merguez	33	+	H+	+
16	<i>Listeria</i>	<i>monocytogenes</i>	A00C024	Sausage	21	+	H+	+
17	<i>Listeria</i>	<i>monocytogenes</i>	A00C036	Poultry (guinea)	41	+	H+	+
18	<i>Listeria</i>	<i>monocytogenes</i>	A00C039	Sausages	20	+	H+	+
19	<i>Listeria</i>	<i>monocytogenes</i>	A00C040	Cooked delicatessen (Museau)	36	+	H+	+
20	<i>Listeria</i>	<i>monocytogenes</i>	A00C041	Sausage	38	+	H+	+
21	<i>Listeria</i>	<i>monocytogenes</i>	A00C042	Raw sausage	32	+	H+	+

INCLUSIVITY								
Strain	Species	Reference	Origin	Inoculation level (cfu/225ml)	3M MDA 2 Demi Fraser broth 24h at 37°C			
					MDA2 - <i>Listeria monocytogenes</i>	Confirmation 100µl		
						ALOA	Palcam	
22	<i>Listeria</i>	<i>monocytogenes</i>	A00C043	Smoked Bacon	50	+	H+	+
23	<i>Listeria</i>	<i>monocytogenes</i>	A00C044	Poultry (duck)	34	+	H+	+
24	<i>Listeria</i>	<i>monocytogenes</i>	A00C052	RTE food (Osso bucco with turkey)	34	+	H+	+
25	<i>Listeria</i>	<i>monocytogenes</i>	A00C053	Gizzards	30	+	H+	+
26	<i>Listeria</i>	<i>monocytogenes</i>	A00C054	Beef heart	30	+	H+	+
27	<i>Listeria</i>	<i>monocytogenes</i>	A00C055	Raw sausages	43	+	H+	+
28	<i>Listeria</i>	<i>monocytogenes</i>	A00E008	Environmental sample	27	+	H+	+
29	<i>Listeria</i>	<i>monocytogenes</i>	A00E049	Environmental sample (smoked salmon)	41	+	H+	+
30	<i>Listeria</i>	<i>monocytogenes</i>	A00E082	Environmental sample (smoked salmon)	33	+	H+	+
31	<i>Listeria</i>	<i>monocytogenes</i>	A00L097	Milk	60	+	H+	+
32	<i>Listeria</i>	<i>monocytogenes</i>	A00M009	Smoked salmon	40	+	H+	+
33	<i>Listeria</i>	<i>monocytogenes</i>	A00M032	Smoked salmon	11	+	H+	+
34	<i>Listeria</i>	<i>monocytogenes</i>	A00M045	Smoked salmon	62	+	H+	+
35	<i>Listeria</i>	<i>monocytogenes</i>	A00M088	Smoked salmon	79	+	H+	+
36	<i>Listeria</i>	<i>monocytogenes</i>	Ad235	Poultry	45	+	H+	+
37	<i>Listeria</i>	<i>monocytogenes</i>	Ad253	Hard cheese	58	+	H+	+
38	<i>Listeria</i>	<i>monocytogenes</i>	Ad260	Semi hard cheese	27	+	H+	+
39	<i>Listeria</i>	<i>monocytogenes</i>	Ad265	Tongue	62	+	H+	+
40	<i>Listeria</i>	<i>monocytogenes</i>	Ad266	Poultry	26	+	H+	+
41	<i>Listeria</i>	<i>monocytogenes</i>	Ad267	Dry sausage	26	+	H+	+
42	<i>Listeria</i>	<i>monocytogenes</i>	Ad268	Cured ham	35	+	H+	+

INCLUSIVITY								
Strain	Species	Reference	Origin	Inoculation level (cfu/225ml)	3M MDA 2 Demi Fraser broth 24h at 37°C			
					MDA2 - <i>Listeria monocytogenes</i>	Confirmation 100µl		
						ALOA	Palcam	
43	<i>Listeria</i>	<i>monocytogenes</i>	Ad270	Fermented sausage	42	+	H+	+
44	<i>Listeria</i>	<i>monocytogenes</i>	Ad272	Fermented sausage	56	+	H+	+
45	<i>Listeria</i>	<i>monocytogenes</i>	Ad273	Cured delicatessen	27	+	H+	+
46	<i>Listeria</i>	<i>monocytogenes</i>	Ad274	Ready-to-eat food (Asiatic meal)	40	+	H+	+
47	<i>Listeria</i>	<i>monocytogenes</i>	Ad534	Fruits	54	+	H+	+
48	<i>Listeria</i>	<i>monocytogenes</i>	Ad544	Onion	43	+	H+	+
49	<i>Listeria</i>	<i>monocytogenes</i>	Ad546	Flour	83	+	H+	+
50	<i>Listeria</i>	<i>monocytogenes</i>	Ad623	Bread crumbs	50	+	H+	+

1 All the strains are wild strains isolated in Adria Developpement, Quimper, France.

2 Ad = Adria

3 A= Adria

4 C= Origin from Meat Meat products

5 M = Origin from Fish and Seafood

6 L = Origin from Milk and Dairy products

7 E = Origin from Production environment

8 H+ = Phosphy-Lipolysis halo

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**Table 3a. Exclusivity Study Results**

EXCLUSIVITY						
Strain	Species	Reference	Origin	Inoculation level (cfu/ml)	3M MDA2 – <i>Listeria monocytogenes</i>	
1	<i>Listeria</i>	<i>grayi</i>	Ad 1198	Smoked salmon	3.2 x 10 <sup>5</sup>	-

EXCLUSIVITY						
Strain	Species	Reference	Origin	Inoculation level (cfu/ml)	3M MDA2 – <i>Listeria monocytogenes</i>	
2	<i>Listeria</i>	<i>grayi</i>	Ad 1443	Pork meat sausages	4.9 x 10 <sup>5</sup>	-
3	<i>Listeria</i>	<i>innocua</i>	1	Smoked salmon	4.9 x 10 <sup>5</sup>	-
4	<i>Listeria</i>	<i>innocua</i>	Ad 658	Gorgonzola	5.9 x 10 <sup>5</sup>	-
5	<i>Listeria</i>	<i>ivanovii</i>	Ad 466	Raw veal meat	3.3 x 10 <sup>5</sup>	-
6	<i>Listeria</i>	<i>ivanovii</i>	Ad 662	Environment (dairy industry)	3.6 x 10 <sup>5</sup>	-
7	<i>Listeria</i>	<i>seeligeri</i>	Ad 649	Cheese	5.4 x 10 <sup>5</sup>	-
8	<i>Listeria</i>	<i>seeligeri</i>	BR1	Trout	5.1 x 10 <sup>5</sup>	-
9	<i>Listeria</i>	<i>welshimeri</i>	Ad1276	Environment (Slaughterhouse)	5.2 x 10 <sup>5</sup>	-
10	<i>Listeria</i>	<i>welshimeri</i>	Ad1175	Ready-to-eat-food	6.4 x 10 <sup>5</sup>	-
11	<i>Bacillus</i>	<i>cereus</i>	Ad 465	Salmon Terrine	7.2 x 10 <sup>4</sup>	-
12	<i>Bacillus</i>	<i>circulans</i>	Ad 760	Vegetables	2.0 x 10 <sup>4</sup>	-
13	<i>Bacillus</i>	<i>coagulans</i>	Ad 731	Dairy product	<2.0 x 10 <sup>3</sup> (opacity +)	-
14	<i>Bacillus</i>	<i>licheniformis</i>	Ad 978	Dairy product	1.6 x 10 <sup>4</sup>	-
15	<i>Bacillus</i>	<i>pumilus</i>	Ad 284	Ready-to-eat	1.7 x 10 <sup>5</sup>	-
16	<i>Brochotrix</i>	<i>campestris</i>	CIP 102920T	Environment	<2.0 x 10 <sup>3</sup> (opacity +)	-
17	<i>Carnobacterium</i>	<i>piscicola</i>	Ad 369	Raw milk	<2.0 x 10 <sup>3</sup> (opacity +)	-
18	<i>Enterococcus</i>	<i>durans</i>	Ad 149	Ham	<2.0 x 10 <sup>3</sup> (opacity +)	-
19	<i>Enterococcus</i>	<i>faecalis</i>	89L326	Soft cheese (Vacherin)	1.6 x 10 <sup>5</sup>	-
20	<i>Lactobacillus</i>	<i>brevis</i>	86L126	Ham	1.4 x 10 <sup>5</sup>	-
21	<i>Lactobacillus</i>	<i>curvatus</i>	Ad 380	Delicatessen	<2.0 x 10 <sup>3</sup> (opacity +)	-
22	<i>Lactobacillus</i>	<i>sakei</i>	Ad 473	Ham	<2.0 x 10 <sup>3</sup>	-

EXCLUSIVITY						
Strain	Species	Reference	Origin	Inoculation level (cfu/ml)	3M MDA2 – <i>Listeria monocytogenes</i>	
				(opacity +)		
23	<i>Leuconostoc</i>	<i>carosum</i>	Ad 411	Ham	<2.0 x 10 <sup>3</sup> (opacity +)	-
24	<i>Leuconostoc</i>	<i>citreum</i>	Ad 396	Ham	4.9 x 10 <sup>5</sup>	-
25	<i>Micrococcus</i>	<i>luteus</i>	Ad 432	Cocktail	<2.0 x 10 <sup>3</sup> (opacity +)	-
26	<i>Staphylococcus</i>	<i>aureus</i>	Ad 165	Smoked delicatessen	<2.0 x 10 <sup>3</sup> (opacity +)	-
27	<i>Staphylococcus</i>	<i>epidermidis</i>	Ad 931	Fruits	2.0 x 10 <sup>3</sup>	-
28	<i>Staphylococcus</i>	<i>haemolyticus</i>	Ad 989	Dairy product	2.8 x 10 <sup>4</sup>	-
29	<i>Streptococcus</i>	<i>bovis</i>	92L622	Cheese	2.0 x 10 <sup>3</sup>	-
30	<i>Streptococcus</i>	<i>salivarius</i>	Ad 441	Dairy product	<2.0 x 10 <sup>3</sup> (opacity +)	-

All the strains Cultures 1 through 30 are wild strains isolated in Adria Developpement, Quimper, France.

Ad = Adria

A= Adria

C= Origin from Meat Meat products

M = Origin from Fish and Seafood

L = Origin from Milk and Dairy products

E = Origin from Production environment

CIP = Collection of Pasteur Institute

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Table 3b. Exclusivity Study Results

EXCLUSIVITY						
Strain	Species	Reference	Origin	Level tested	3M MDA2 – <i>Listeria monocytogenes</i>	
31	<i>Listeria</i>	<i>aquatic</i>	ESL S10-1188	Running water, USA, Florida	1.37 x 10 <sup>7</sup>	±
32	<i>Listeria</i>	<i>booriae</i>	ESL A5-0281	Non-food-contact surface in a dairy processing plant, USA,	2.75 x 10 <sup>7</sup>	±

<u>EXCLUSIVITY</u>						
<u>Strain</u>	<u>Species</u>	<u>Reference</u>	<u>Origin</u>	<u>Level tested</u>	<u>3MMDA2 – <i>Listeria</i> <i>monocytogenes</i></u>	
			New York			
33	<i>Listeria</i>	<i>cornellensis</i>	ESL F6-969	Water, USA, Colorado	$1.80 \times 10^7$	=
34	<i>Listeria</i>	<i>fleischmannii</i> subsp. <i>coloradensis</i>	ESL S10-1203	grazing pasture – soil	$1.22 \times 10^8$	=
35	<i>Listeria</i>	<i>fleischmannii</i> subsp. <i>fleischmannii</i>	DSM 24998	hard cheese, Switzerland, Suisse Romande	$1.28 \times 10^8$	=
36	<i>Listeria</i>	<i>floridensis</i>	ESL S10-1187	running water, USA, Florida	$5.55 \times 10^7$	=
37	<i>Listeria</i>	<i>grandensis</i>	ESL F6-971	water, USA, Colorado	$3.05 \times 10^7$	=
38	<i>Listeria</i>	<i>ivanovii</i>	ATCC 19119	sheep, Bulgaria	$4.05 \times 10^7$	=
39	<i>Listeria</i>	<i>ivanovii</i> subsp. <i>londoniensis</i>	ATCC 49954	food, France	$7.40 \times 10^7$	=
40	<i>Listeria</i>	<i>newyorkensis</i>	ESL M6-0635	non-food-contact surface in a seafood processing plant, USA, New York	$7.00 \times 10^7$	=
41	<i>Listeria</i>	<i>riparia</i>	ESL S10-1204	running water, USA, Florida	$3.05 \times 10^7$	=
42	<i>Listeria</i>	<i>rocourtaie</i>	CIP 109804	pre-cut lettuce, Austria, Siezenheim	$7.30 \times 10^7$	=
43	<i>Listeria</i>	<i>weihenstephanensis</i>	LMG 26374	water plant <i>Lemma trisulca</i> from pond, Germany, Bavaria	$3.30 \times 10^5$	=

Cultures 31 through 43 were obtained from the laboratory of Dr. Martin Wiedmann at Cornell University, NY and from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany.

LMG = Laboratorium voor Microbiologie, Universiteit Gent (Laboratory of Microbiology, Department of Biochemistry and Microbiology, Faculty of Sciences of Ghent University, Belgium)

DSM = Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures)

ATCC = American Type Culture Collection

ESL = Food Safety Laboratory Cornell University, NY

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**Table 4a: Independent Study - Matrices and Inoculating Organisms**

Matrix	Inoculating Organism (Culture Condition)	Target Inoculum Level	# of Replicates	MDA2 Testing Time Points	Reference Method (Enrichment)
Beef Hot Dogs	<i>L. monocytogenes</i> ATCC <sup>1</sup> 7644 (heat-stressed)	0 CFU/Test Portion	5	24 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Deli Turkey	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19116 (heat-stressed)	0 CFU/Test Portion	5	24 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Queso Fresco	<i>L. monocytogenes</i> CWD <sup>2</sup> 1554 (heat-stressed)	0 CFU/Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Vanilla Ice Cream	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19114	0 CFU/Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
4% Milk Fat Cottage Cheese	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19112 & <i>L. welshimeri</i> ATCC <sup>1</sup> 35897	0 CFU/Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Whole Melon	<i>L. monocytogenes</i> FSL <sup>3</sup> J1-049	0 CFU/Test Portion	5	26 hours	FDA/BAM Chapter 10 (BLEB)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Raw Chicken Leg Pieces	Naturally Contaminated	Lot 1 & Lot 2	20 & 20	28 hours	USDA/FSIS MLG 8.09 (UVM)
Romaine Lettuce	<i>L. monocytogenes</i> ATCC <sup>1</sup> 49594	0 CFU/ Test Portion	5	24 hours	FDA/BAM Chapter 10 (BLEB)
		0.2-2 CFU/ Test Portion	20		
		2-5 CFU/ Test Portion	5		
3% Chocolate Whole Milk	<i>L. monocytogenes</i> ATCC <sup>1</sup> BAA-751	0 CFU/ Test Portion	5	24 hours	AOAC 993.12 (Selective Enrichment)
		0.2-2 CFU/ Test Portion	20		
		2-5 CFU/ Test Portion	5		
Sealed Concrete	<i>L. monocytogenes</i> ATCC <sup>1</sup> 19117	0 CFU/4" x 4" (100 cm <sup>2</sup> )	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/4" x 4" (100 cm <sup>2</sup> )	20		
		~100 CFU/4" x 4" (100 cm <sup>2</sup> )	5		
Plastic	<i>L. monocytogenes</i> ATCC <sup>1</sup> 51782 & <i>L. seeligeri</i> ATCC <sup>1</sup> 35967 & <i>10x Enterococcus faecalis</i> ATCC <sup>1</sup> 29212	0 CFU/1" x 1" (5 cm <sup>2</sup> )	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/1" x 1" (5 cm <sup>2</sup> )	20		
		~100 CFU/1" x 1" (5 cm <sup>2</sup> )	5		

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1 **Table 4a: Independent Study - Matrices and Inoculating Organisms (Continued)**

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Matrix	Inoculating Organism (Culture Condition)	Target Inoculum Level	# of Replicates	MDA2 Testing Time Points	Reference Method (Enrichment)
Stainless Steel	<i>L. monocytogenes</i> ATCC 19118 & <i>10x Enterococcus faecium</i> ATCC 19434	0 CFU/1" x 1" (5 cm2)	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/1" x 1" (5 cm2)	20		
		0 CFU/4" x 4" (100 cm2)	5		

3 <sup>1</sup> ATCC - American Type Culture Collection

4 <sup>2</sup> CWD - University of Vermont

5 <sup>3</sup> FSL - Cornell University

6 <sup>4</sup> Natural contamination was present during the background screen: 2 separate lots evaluated (20 replicates each)

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11 **Table 4b: Internal Study - Matrices and Inoculating Organisms**

Matrix	Inoculating Organism (Culture Condition)	Target Inoculum Level	# of Replicates	MDA2 Testing Time Points	Reference Method (Enrichment)
Raw chicken leg pieces	<i>L. monocytogenes</i> Ad <sup>1</sup> 668	0 CFU/Test Portion	5	28 hours	USDA/FSIS MLG 8.09 (UVM)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Bagged raw spinach	<i>L. monocytogenes</i> Ad 543	0 CFU/Test Portion	5	24 hours	ISO 11290-1/A1 (Half/Demi Fraser)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Cold smoked salmon	<i>L. monocytogenes</i> Ad 670	0 CFU/Test Portion	5	24 hours	FDA/BAM Chapter 10 (BLEB)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		
Cold smoked salmon	<i>L. monocytogenes</i> Ad 670	0 CFU/Test Portion	5	24 hours	ISO 11290-1/A1 (Half/Demi Fraser)
		0.2-2 CFU/Test Portion	20		
		2-5 CFU/Test Portion	5		

12 <sup>1</sup> Ad - Adria Developpement Culture Collection

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**Table 5a: Independent Study - Aerobic Plate Count and Background Results (Prior to Inoculation)**

<b>Matrix (Test Portion)</b>	<b>APC<sup>1</sup> (CFU/g)</b>	<b><i>Listeria</i> Pathogen Screen</b>
Beef Hot Dogs <sup>5</sup> (25 g)	6.0 x 10 <sup>3</sup>	0/5 Detected <sup>2</sup>
Deli Turkey (125 g)	1.5 x 10 <sup>3</sup>	0/5 Detected <sup>2</sup>
Raw Chicken Leg Pieces (25 g)	2.8 x 10 <sup>5</sup>	4/5 Detected <sup>2</sup>
Whole Melons	1.0 x 10 <sup>2</sup>	0/5 Detected <sup>3</sup>
Vanilla Ice Cream (25 g)	8.0 x 10 <sup>1</sup>	0/5 Detected <sup>4</sup>
Queso Fresco (25 g)	3.8 x 10 <sup>5</sup>	0/5 Detected <sup>4</sup>
4% Milk Fat Cottage Cheese (25 g)	1.7 x 10 <sup>4</sup>	0/5 Detected <sup>4</sup>
Beef Hot Dogs (25 g)	1.6 x 10 <sup>2</sup>	0/5 Detected <sup>2</sup>

<sup>1</sup> APC conducted in accordance with FDA/BAM Chapter 3

<sup>2</sup> *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines

<sup>3</sup> *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines

<sup>4</sup> *Listeria species* screen conducted following the AOAC 993.12 guidelines

<sup>5</sup> Matrix used to conduct Robustness and Lot-to-Lot testing

**Table 5b: Internal Study - Aerobic Plate Count and Background Results (Prior to Inoculation)**

Matrix (Test Portion)	APC <sup>1</sup> (CFU/g)	Background <i>Listeria</i>
Raw Chicken Leg Pieces (25 g)	5.4 x 10 <sup>5</sup>	None Detected
Bagged Raw Spinach (25 g)	8.0 x 10 <sup>6</sup>	None Detected
Cold Smoked Salmon (25 g)	4.0 x 10 <sup>2</sup>	None Detected

<sup>1</sup> APC conducted in accordance with FDA/BAM Chapter 3

<sup>2</sup> *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines

<sup>3</sup> *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines

<sup>4</sup> *Listeria species* screen conducted following the ISO 11290-1/1A guidelines

**Table 6: Inoculum Heat Stress Results**

Matrix	Test Portion Size	Inoculating Organism	Agar	CFU/ g	Percent Injury
Beef Hot Dogs <sup>1</sup>	25 g	<i>Listeria monocytogenes</i> ATCC 7644	TSA	1.8 x 10 <sup>8</sup>	58.3 %
			MOX	7.5 x 10 <sup>7</sup>	
Deli Turkey	125 g	<i>Listeria monocytogenes</i> ATCC 19116	TSA	2.6 x 10 <sup>9</sup>	68.5 %
			MOX	8.2 x 10 <sup>8</sup>	
Queso Fresco	25 g	<i>Listeria monocytogenes</i> ATCC CWD 1554	TSA	2.6 x 10 <sup>9</sup>	72.7%
			MOX	7.1 x 10 <sup>8</sup>	
Beef Hot Dogs <sup>2</sup>	25 g	<i>Listeria monocytogenes</i> ATCC 7644	TSA	2.6 x 10 <sup>9</sup>	73.8%
			MOX	6.8 x 10 <sup>8</sup>	

<sup>1</sup> - Testing for Robustness and Lot to Lot

<sup>2</sup> - Matrix Study

TSA: Trypticase soy agar

MOX: modified Oxford agar

**Table 7: Inoculum Summary Table for Whole Melons and Environmental Surfaces**

<b>Matrix</b>	<b>Whole Melons</b>	
<b>Inoculating Organism</b>	<i>Listeria monocytogenes</i> FSL J1-049	
<b>Low-Inoculum Level CFU<sup>a</sup>/Melon<sup>b</sup></b>	7	
<b>High-Inoculum Level CFU<sup>a</sup>/Test Melon<sup>b</sup></b>	55	
<b>Matrix</b>	<b>Stainless Steel</b>	
<b>Inoculating Organism</b>	<i>Listeria monocytogenes</i> ATCC 19118	<i>Enterococcus faecium</i> ATCC 19434
<b>Low-Inoculum Level CFU<sup>a</sup>/Test Area<sup>c</sup></b>	42	530
<b>High-Inoculum Level CFU<sup>a</sup>/Test Area<sup>c</sup></b>	400	4000
<b>Matrix</b>	<b>Sealed Concrete</b>	
<b>Inoculating Organism</b>	<i>Listeria monocytogenes</i> ATCC 19117	
<b>Low-Inoculum Level CFU<sup>a</sup>/Test Area<sup>c</sup></b>	35	
<b>High-Inoculum Level CFU<sup>a</sup>/Test Area<sup>c</sup></b>	360	
<b>Matrix</b>	<b>Plastic</b>	
<b>Inoculating Organism</b>	<i>Listeria monocytogenes</i> ATCC 51782	<i>Enterococcus faecalis</i> ATCC 29212
<b>Low-Inoculum Level CFU<sup>a</sup>/Test Area<sup>d</sup></b>	42	540
<b>High-Inoculum Level CFU<sup>a</sup>/Test Area<sup>d</sup></b>	420	3600

<sup>a</sup>CFU: aliquots of the inocula were plated in triplicate onto TSA and averaged

<sup>b</sup>Test Area: Whole Melon

<sup>c</sup>Test Area: 4" x 4" Surface Area

<sup>d</sup>Test Area: 1" x 1" Surface Area

**Table 8: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Candidate vs. Reference – POD Results (INTERNAL STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			USDA/FSIS MLG			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces (25 g)	<i>L. monocytogenes</i> Ad 668	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.91 (0.6, 1.39)	20	10	0.50	0.30, 0.70	12	0.60	0.39, 0.78	-0.10	-0.37, 0.19
			2.2	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			ISO 11290-1			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI							
Bagged Raw Spinach (25 g)	<i>L. monocytogenes</i> Ad 543	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.17 (0.74, 1.84)	20	9	0.45	0.26, 0.66	10	0.50	0.30, 0.70	-0.05	-0.34, 0.25
			2.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			FDA-BAM			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI							
Cold Smoked Salmon (25 g)	<i>L. monocytogenes</i> Ad 670	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.4 (0.18, 0.72)	20	10	0.50	0.30, 0.70	7	0.35	0.15, 0.60	0.15	-0.20, 0.47
			2.7	5	3	0.60	0.23, 0.88	5	1.00	0.57, 1.00	-0.40	-0.77, 0.12
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			ISO 11290-1			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI							
Cold Smoked Salmon (25 g)	<i>L. monocytogenes</i> Ad 670	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.4 (0.18, 0.72)	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.27, 0.36
			2.7	5	3	0.60	0.23, 0.88	4	0.80	0.38, 0.96	-0.20	-0.60, 0.31

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

**Table 9: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Deli Turkey (125 g)	<i>L. monocytogenes</i> ATCC 19116	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.85)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces (25 g): Lot 1	Naturally Contaminated	28 Hour	1.91 (1.23, 3.80)	20	17	0.85	0.64, 0.95	16	0.80	0.58, 0.92	0.05	-0.19, 0.29
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces (25 g): Lot 2	Naturally Contaminated	28 Hour	1.12 (0.68, 1.91)	20	10	0.50	0.30, 0.70	14	0.70	0.48, 0.85	-0.20	-0.45, 0.10
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Whole Melon	<i>L. monocytogenes</i> FSL J1-049	26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			7	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			55	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Vanilla Ice Cream (25 g)	<i>L. monocytogenes</i> ATCC 19114	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.59 (0.33, 0.97)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

**Table 10: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Romaine Lettuce (25 g)	<i>L. monocytogenes</i> ATCC 49594	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.40 (0.18, 0.70)	20	7	0.35	0.18, 0.57	6	0.30	0.15, 0.52	0.05	-0.23, 0.32
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Queso Fresco (25 g)	<i>L. monocytogenes</i> CWD 1554	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	11	0.55	0.34, 0.74	9	0.45	0.26, 0.66	0.10	-0.19, 0.37
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
4% Milk Fat Cottage Cheese (25 g)	<i>L. monocytogenes</i> ATCC 19112	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.55 (0.40, 0.92)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	-0.19, 0.37
			1.64 (0.84, 3.20)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
3% Chocolate Whole Milk (25 g)	<i>L. monocytogenes</i> ATCC BAA-751	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	9	0.45	0.26, 0.66	6	0.30	0.15, 0.52	0.15	-0.14, 0.41
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef Hot Dogs (25 g)	<i>L. monocytogenes</i> ATCC 7644	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	5	0.25	0.11, 0.47	6	0.30	0.15, 0.52	-0.05	-0.31, 0.22
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level



**Table 11: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Stainless Steel (225 mL)	<i>L. monocytogenes</i> ATCC 19118 & <i>Enterococcus faecium</i> ATCC 19434	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	8	0.40	0.22, 0.61	-0.10	-0.40, 0.13
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	8	0.40	0.22, 0.61	-0.10	-0.40, 0.13
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Sealed Concrete (100 mL)	<i>L. monocytogenes</i> ATCC 19117	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	3M MDA2			Reference			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Plastic (10 mL)	<i>L. monocytogenes</i> ATCC 51782 & <i>Enterococcus faecalis</i> ATCC 29212	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42& 540	20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42& 540	20	12	0.60	0.39, 0.78	10	0.50	0.30, 0.70	0.10	-0.19, 0.37
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>C</sub> = Difference between the confirmed candidate method result and reference method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

**Table 12: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INTERNAL STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>d</sub> <sup>CP</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces (25 g)	<i>L. monocytogenes</i> Ad 668	28 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.91 (0.6, 1.39)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
			2.2	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Bagged Raw Spinach (25 g)	<i>L. monocytogenes</i> Ad 543	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.17 (0.74, 1.84)	20	9	0.45	0.26, 0.66	10	0.50	0.30, 0.70	-0.05	-0.33, 0.24
			2.9	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cold Smoked Salmon (25 g)	<i>L. monocytogenes</i> Ad 670	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.4 (0.18, 0.72)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
			2.7	5	3	0.60	0.23, 0.88	3	0.60	0.23, 0.88	0.00	-0.46, 0.46

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

**Table 13: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Deli Turkey (125 g)	<i>L. monocytogenes</i> ATCC 19116	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.85)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces (25 g): Lot 1	Naturally Contaminated	28 Hours	1.91 (1.23, 3.80)	20	17	0.85	0.64, 0.95	17	0.85	0.64, 0.95	0.00	-0.23, 0.23
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Raw Chicken Leg Pieces (25 g): Lot 2	Naturally Contaminated	28 Hours	1.12 (0.68, 1.91)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Whole Melon	<i>L. monocytogenes</i> FSL J1-049	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			7	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
			55	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Vanilla Ice Cream (25 g)	<i>L. monocytogenes</i> ATCC 19114	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.59 (0.33, 0.97)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

**Table 14: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>C</sub> <sup>d</sup>	95% CI	X	POD <sub>R</sub> <sup>e</sup>	95% CI		
Romaine Lettuce (25 g)	<i>L. monocytogenes</i> ATCC 49594	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.40 (0.18, 0.70)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	MPN <sup>a</sup> / Test Portion	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>C</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Queso Fresco (25 g)	<i>L. monocytogenes</i> CWD 1554	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
4% Milk Fat Cottage Cheese (25 g)	<i>L. monocytogenes</i> ATCC 19112	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.55 (0.40, 0.92)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.28, 0.28
			1.64 (0.84, 3.20)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
3% Chocolate Whole Milk (25 g)	<i>L. monocytogenes</i> ATCC BAA-751	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Beef Hot Dogs (25 g)	<i>L. monocytogenes</i> ATCC 7644	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

**Table 15: 3M™ MDA 2 *Listeriamonocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)**

Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Stainless Steel (225 mL)	<i>L. monocytogenes</i> ATCC 19118 & <i>Enterococcus faecium</i> ATCC 19434	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 530	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0.00	-0.26, 0.26
			400 & 4000	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Sealed Concrete (100 mL)	<i>L. monocytogenes</i> ATCC 19117	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35	20	16	0.80	0.58, 0.92	15	0.75	0.53, 0.89	0.05	-0.21, 0.30
			360	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	Analysis Time Point	CFU <sup>a</sup> / Test Area	N <sup>b</sup>	Presumptive			Confirmed			dPOD <sub>CP</sub> <sup>f</sup>	95% CI <sup>g</sup>
					x <sup>c</sup>	POD <sub>CP</sub> <sup>d</sup>	95% CI	X	POD <sub>CC</sub> <sup>e</sup>	95% CI		
Plastic (10 mL)	<i>L. monocytogenes</i> ATCC 51782 & <i>Enterococcus faecalis</i> ATCC 29212	24 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 540	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
		26 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			42 & 540	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.28, 0.28
			590 & 3600	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

<sup>a</sup>CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

<sup>b</sup>N = Number of test portions

<sup>c</sup>x = Number of positive test portions

<sup>d</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials

<sup>e</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result POD values

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the diistically significant at the 5% level

**Table 16: Lot-to-Lot/Stability Study Results**

Expiration Date		Expiration Date		Expiration Date	
Lot #: 012715		Lot #: 081914		Lot #: 050114	
Sample #	Result	Sample #	Result	Sample #	Result
<i>Listeria monocytogenes</i> ATCC <sup>2</sup> 7644					
Low Level	6/10	Low Level	6/10	Low Level	6/10
Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5	Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5	Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5

<sup>1</sup>- All samples were analyzed from a common lysate

<sup>2</sup>ATCC- American Type Culture Collection

**Table 17: Robustness Results**

Robustness <sup>1</sup>			Robustness <sup>1</sup>		
13 Minute Lysis			13 Minute Lysis		
Sample #	18 µL <sup>2</sup> , 18 µL <sup>3</sup>	18 µL <sup>2</sup> , 22 µL <sup>3</sup>	Sample #	22 µL <sup>2</sup> , 18 µL <sup>3</sup>	22 µL <sup>2</sup> , 22 µL <sup>3</sup>
<i>Listeria monocytogenes</i> ATCC <sup>4</sup> 7644					
Low Level	6/10	6/10	Low Level	6/10	6/10
Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5	0/5	Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5	0/5
Robustness <sup>1</sup>			Robustness <sup>1</sup>		
17 Minute Lysis			17 Minute Lysis		
Sample #	18 µL <sup>2</sup> , 18 µL <sup>3</sup>	18 µL <sup>2</sup> , 22 µL <sup>3</sup>	Sample #	22 µL <sup>2</sup> , 18 µL <sup>3</sup>	22 µL <sup>2</sup> , 22 µL <sup>3</sup>
<i>Listeria monocytogenes</i> ATCC <sup>4</sup> 7644					
Low Level	6/10	6/10	Low Level	6/10	6/10
Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5	0/5	Uninoculated Target Organism ( <i>Enterococcus faecalis</i> ATCC29212)	0/5	0/5

<sup>1</sup>- All samples were analyzed from a common lysate

<sup>2</sup>-Volume of enrichment to lysis

<sup>3</sup>-Volume of lysis to assay

<sup>4</sup>ATCC- American Type Culture Collection