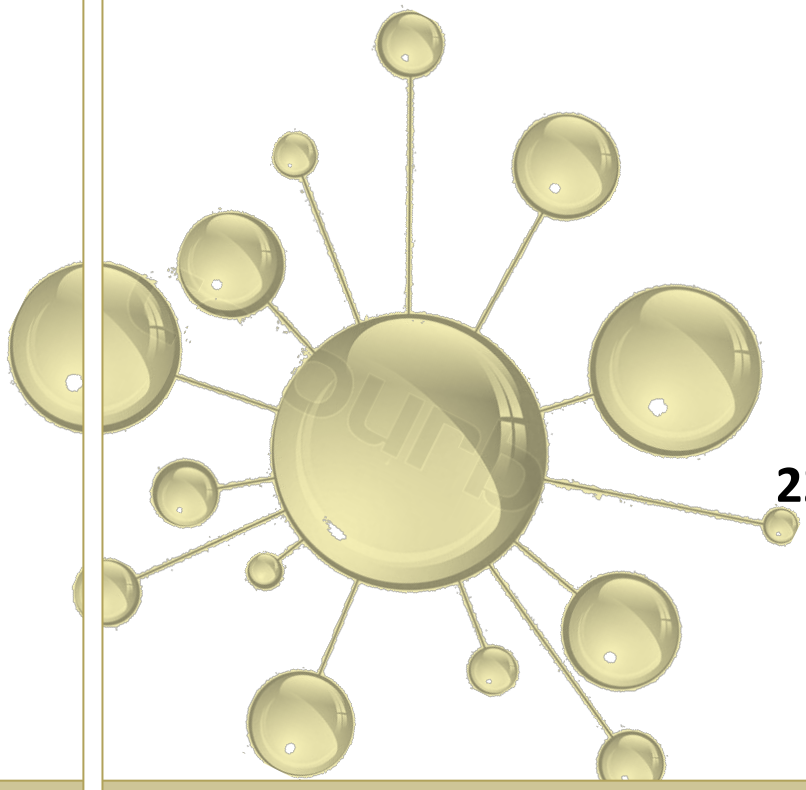
Official Methods of Analysis[SM] (OMA)

AOAC EXPERT REVIEW PANEL FOR MICROBIOLOGY FOR FOODS AND ENIVRONMENTAL 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

AOAC *OFFICIAL METHODS OF ANALYSIS*^{SM}

The *Official Methods of Analysis*^{SM} (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*^{SM} program by including an approach to First Action *Official Methods*^{SM} 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*^{SM} program by transitioning the conformity assessment component of the *Official Methods*^{SM} program into the AOAC Research Institute. The AOAC Research Institute now administers the AOAC *Official Methods*^{SM} 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*^{SM} program are now reviewed by Expert Review Panels for First Action AOAC *Official Methods of Analysis*^{SM} 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*^{SM} (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**

*Agenda is subject to change. V3

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

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.

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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

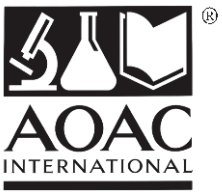
AOAC INTERNATIONAL
POLICY ON THE USE OF THE ASSOCIATION NAME, INITIALS, IDENTIFYING INSIGNIA, LETTERHEAD, AND BUSINESS CARDS

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.

* * * * * *

Adopted by the AOAC Board of Directors: September 24, 1989
Revised: June 13, 1991; February 26, 1992; March 21, 1995; October 1996

Official Methods of Analysis[SM] (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*[SM] (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.*

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 consideratio**n.**

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.

AOAC INTERNATIONAL
***Official Methods of Analysis*℠ Program**

Detection of *Listeria* Species in Selected Foodsand Environmental Surfaces by the 3M™ Molecular Detection Assay 2 - *Listeria*

and

Detection of *Listeria monocytogenes* in Selected Foodsand Environmental Surfaces by the 3M™ Molecular Detection Assay 2 - *Listeria monocytogenes*

Collaborative Study Protocol

OMA 2016-MONTH-XXX

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

Table of Contents

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.

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

Lisa Monteroso
Study Director
3M Food Safety Department
3M Center, Building 260-6B-01
St. Paul, MN 55144
Phone: 651-736-2913
Fax: 651-733-5819
Email: lmonteroso@mmm.com

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
ecrowley@qlaboratories.co

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[1]
- 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 2test 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)

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

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 4th 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 *L. monocytogenes* Levels	Test Portion Codes	Reference Method
Raw chicken breast *25g – 3M MDA2* *25g – USDA/FSIS MLG*	***L. monocytogenes*** **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		
Deli turkey *125g – 3M MDA2* *125g – USDA/FSIS MLG*	***L. monocytogenes*** **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:

$$(1-\frac{n_{select}}{n_{nonselect}})x100$$

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 Infectious 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 receive36 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 MDA2methods 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

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

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 with24-30 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)

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 20µL
4.5.2 *Multi-channel pipette*. – capable of 20µL
4.5.3 *Sterile pipette tips*. – capable of 20µL
4.5.4 *Stomacher®*. - Seward or equivalent
4.5.5 *Filter Stomacher® bags*. - Seward or equivalent
4.5.6 *Thermometer*. – calibrated range to include 100 ± 1°C range
4.5.7 *Incubators*. – capable of maintaining 37 ± 1°C
4.5.8 *Dry double block heater unit*. – capable of maintaining 100 ± 1°C; OR *a water bath* capable of maintaining 100 ± 1°C
4.5.9 *Freezer*. – capable of maintaining -10 to -20°C, for storing the 3M Molecular Detection Chill Block Tray
4.5.10 *Refrigerator*. – capable of maintaining 2-8°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 ± 30 seconds
4.6.1.1.2 Incubate 24-30 hr at 37 ± 1°C.

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 ± 0.2 min
4.6.1.2.2 Incubate 28-32 hr at 37 ± 1°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 (20-25 ºC) 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 µL enriched sample into a LS tube.
4.6.5 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.
4.6.6 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).
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 µL sample lysate from the upper portion of fluid in the LS tube into regent tube. Mix gently by pipetting up and down 5 times.
4.6.9 Transfer 20 µL of NC lysate into a reagent tube.
4.6.10 Transfer 20 µL 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.

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 with24-30 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.

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

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 ± 1°C range
5.5.7 *Incubators*. – capable of maintaining 37 ± 1°C
5.5.8 *Dry double block heater unit*. – capable of maintaining 100 ± 1°C; OR *a water bath* capable of maintaining 100 ± 1°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 **Deli 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 ± 0.2 min
5.6.1.1.2 Incubate 24-30 hr at 37 ± 1°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 ± 30 seconds
5.6.1.2.2 Incubate 28-32 hr at 37 ± 1°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 ºC) 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.

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 regent 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[2] 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 $dLPOD_C$ as the difference between the candidate and reference LPOD values, include CIs.

7.1.1.5.2 Estimate $dLPOD_{CP}$ 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_R, $LPOD_C$ and $dLPOD_C$ by level with 95% confidence intervals.

[2]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

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

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

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

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
ecrowley@qlaboratories.com

Important Information

1. Read the methods and Safety Instructions carefully. If you have any questions, contact one of the Study Directors. The 3M MDA2 -*Listeria* and 3M MDA2 -*Listeria monocytogenes* Assays are 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 Molecular Detection System and the 3M MDA2 -*Listeria* and 3M MDA2 -*Listeria monocytogenes;* retain the safety instructions for future reference. The 3M MDA2 -*Listeria* and 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 Molecular Detection System (instrument). Store the 3M MDA2 Assays 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 Assays 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 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.

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

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

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

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

Information about this Collaborative Study

Shipment Schedule

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

Test Portion Receipt

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	*Listeria*&*L. monocytogenes*
Raw chicken breast	USDA/FSIS MLG 8.09	UVM	Demi Fraser Broth with FAC	*Listeria*&*L. monocytogenes*

*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 ºC) 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.

1.6 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.

1.7 Place uncovered LS tubes in 3M MDS Heat block, heat 15±1 min at 100±1°C. LS Solution will change from pink (cool) to yellow (hot).

1.8 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.

1.9 Transfer 20 µL sample lysate from the upper portion of fluid in the LS tube into regent tube. Mix gently by pipetting up and down 5 times.

1.10 Transfer 20 µL of NC lysate into a reagent tube.

1.11 Transfer 20 µL of NC lysate into a Reagent Control (RC) tube.

1.12 Load capped tubes into speed loader tray and close lid.

1.13 Start assay.

1.14 **Validation Study Confirmation**

1.14.1 Confirm each test portion, regardless of presumptive result.

1.14.2 Transfer 0.1 ± 0.02 ml of the enriched sample to 10 ± 0.5 ml of Fraser Broth (FB). As per media preparation instructions, be sure that appropriate supplements have been added to the FB prior to inoculation. Incubate inoculated FB tubes at 35 ± 2°C for 26 ± 2 h.

1.14.3 Streak a MOX plate. Streak a loopful or a drop approximating 0.1 ml of the enriched sample over the surface of the plate. Incubate the MOX at 35 ± 2°C for 26 ±2 h.

1.14.4 Examine the MOX plates for colonies with morphology typical of *Listeria* spp. At 26 ± 2 h, suspect colonies are typically small (ca. 1 mm) and are surrounded by a zone of darkening due to esculin hydrolysis.

1.14.5 If suspect colonies are present on MOX, transfer suspect colonies to HL agar.

1.14.6 If no suspect colonies are evident, re-incubate the MOX plate for an additional 26 ± 2 hour.

1.14.7 After 26 ± 2 h of incubation, examine the FB for the potential presence of *Listeria spp.*, by visual examination of the broth for darkening due to esculin hydrolysis.

1.14.8 If any degree of FB darkening is evident, aseptically dispense a drop approximating 0.1 ± 0.02 ml of FB onto a MOX plate. Swab or streak 25-40% of the surface of the MOX plate with the FB inoculum. Use a loop to streak for isolation from the initial swab/streak quadrant onto the remainder of the plate. Incubate the MOX plate at 35 ± 2°C for 26 ± 2 h.

1.14.9 If no FB darkening is evident, re-incubate the FB at 35 ± 2°C until a total incubation time of 48 ± 2 h has been achieved.

1.14.10 Re-examine the FB for evidence of darkening after 48 ± 2 h of total incubation. If any degree of darkening is evident, swab, streak and incubate a MOX plate.

1.14.11 If no darkening of FB is evident and no suspect MOX and/or HL colonies have been demonstrated, the sample is considered negative for *Listeria* spp.

1.14.12 If suspect colonies are present on MOX from any source, streak for isolation on one or more HL agar plates. Incubate the streaked HL at 35 ± 2°C for 22 ± 4 h.

1.14.13 After incubation, examine the HL plate(s) against backlight for translucent colonies surrounded by a small zone of β-hemolysis.

1.14.14 If at least one suspect colony is clearly isolated, proceed to confirmatory testing. Hold all HL plates containing suspect colonies (room temperature or refrigeration) until confirmatory testing is complete.

1.14.15 If suspect colonies or β-hemolytic growth are present on HL but not clearly isolated, re-streak representative suspect colonies/growth onto one or more fresh HL plates and incubate at 35 ± 2°C for 22 ± 4 h.

1.14.16 Confirm ALL test portions according to the USDA FSIS MLG 8.09 biochemical/serological procedures. Biochemical tests will be performed using API or VITEK 2 GP. Please follow manufacturer's instructions when using any of these rapid methods.

USDA FSIS Reference Method

1.1 Add 125g test portion to 1125 mL UVM broth. Stomach (Seward 400 or equivalent) for 2± 0.2 minutes and incubate for 23-26h at 30±2°C.

1.2 Transfer 0.1 ± 0.02 ml of the enriched sample to 10 ± 0.5 ml of Fraser Broth (FB). As per media preparation instructions, be sure that appropriate supplements have been added to the FB prior to inoculation. Incubate inoculated FB tubes at 35 ± 2°C for 26 ± 2 h.

1.3 Streak a MOX plate. Streak a loopful or a drop approximating 0.1 ml of the enriched sample over the surface of the plate. Incubate the MOX at 35 ± 2°C for 26 ±2 h.

1.4 Examine the MOX plates for colonies with morphology typical of *Listeria* spp. At 26 ± 2 h, suspect colonies are typically small (ca. 1 mm) and are surrounded by a zone of darkening due to esculin hydrolysis.

1.5 If suspect colonies are present on MOX, transfer suspect colonies to HL agar.

1.6 If no suspect colonies are evident, re-incubate the MOX plate for an additional 26 ± 2 hour.

1.7 After 26 ± 2 h of incubation, examine the FB for the potential presence of *Listeria spp.*, by visual examination of the broth for darkening due to esculin hydrolysis.

1.8 If any degree of FB darkening is evident, aseptically dispense a drop approximating 0.1 ± 0.02 ml of FB onto a MOX plate. Swab or streak 25-40% of the surface of the MOX plate with the FB inoculum. Use a loop to streak for isolation from the initial swab/streak quadrant onto the remainder of the plate. Incubate the MOX plate at 35 ± 2°C for 26 ± 2 h.

1.9 If no FB darkening is evident, re-incubate the FB at 35 ± 2°C until a total incubation time of 48 ± 2 h has been achieved.

1.10 Re-examine the FB for evidence of darkening after 48 ± 2 h of total incubation. If any degree of darkening is evident, swab, streak and incubate a MOX plate.

1.11 If no darkening of FB is evident and no suspect MOX and/or HL colonies have been demonstrated, the sample is considered negative for *Listeria* spp.

1.12 If suspect colonies are present on MOX from any source, streak for isolation on one or more HL agar plates. Incubate the streaked HL at 35 ± 2°C for 22 ± 4 h.

1.13 After incubation, examine the HL plate(s) against backlight for translucent colonies surrounded by a small zone of β-hemolysis.

1.14 If at least one suspect colony is clearly isolated, proceed to confirmatory testing. Hold all HL plates containing suspect colonies (room temperature or refrigeration) until confirmatory testing is complete.

1.15 If suspect colonies or β-hemolytic growth are present on HL but not clearly isolated, re-streak representative suspect colonies/growth onto one or more fresh HL plates and incubate at 35 ± 2°C for 22 ± 4 h.

1.16 Confirm ALL test portions according to the USDA FSIS biochemical/serological procedures. Biochemical tests will be performed using API or VITEK 2 GP. Please follow manufacturer's instructions when using any of these rapid methods.

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

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

2.1 To each 25g test portion, add 475 mL Demi Fraser broth with ferric ammonium citrate (FAC). Homogenize each sample for two minutes ± 30 seconds and incubate 28-32 h at 37 ± 1°C.

2.2 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.

2.3 Equilibrate the LS tubes to room temperature (20-25 ºC) by setting LS tube overnight 16-18 hours. Or on the laboratory bench for at least 2 hours.

2.4 Invert the capped LS tubes to mix, up to 4 hours before use.

2.5 Transfer 20 µL enriched sample into a LS tube.

2.6 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.

2.7 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).

2.8 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.

2.9 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.

2.10 Transfer 20 µL of NC lysate into a reagent tube.

2.11 Transfer 20 µL of NC lysate into a Reagent Control (RC) tube.

2.12 Load capped tubes into speed loader tray and close lid.

2.13 Start assay.

2.14 **Validation Study Confirmation**

2.14.1 Confirm each test portion, regardless of presumptive result.

2.14.2 Transfer 0.1 ± 0.02 ml of the enriched sample to 10 ± 0.5 ml of Fraser Broth (FB). As per media preparation instructions, be sure that appropriate supplements have been added to the FB prior to inoculation. Incubate inoculated FB tubes at 35 ± 2°C for 26 ± 2 h.

2.14.3 Streak a MOX plate. Streak a loopful or a drop approximating 0.1 ml of the enriched sample over the surface of the plate. Incubate the MOX at 35 ± 2°C for 26 ±2 h.

2.14.4 Examine the MOX plates for colonies with morphology typical of *Listeria* spp. At 26 ± 2 h, suspect colonies are typically small (ca. 1 mm) and are surrounded by a zone of darkening due to esculin hydrolysis.

2.14.5 If suspect colonies are present on MOX, transfer suspect colonies to HL agar.

2.14.6 If no suspect colonies are evident, re-incubate the MOX plate for an additional 26 ± 2 hour.

2.14.7 After 26 ± 2 h of incubation, examine the FB for the potential presence of *Listeria spp.*, by visual examination of the broth for darkening due to esculin hydrolysis.

2.14.8 If any degree of FB darkening is evident, aseptically dispense a drop approximating 0.1 ± 0.02 ml of FB onto a MOX plate. Swab or streak 25-40% of the surface of the MOX plate with the FB inoculum. Use a loop to streak for isolation from the initial swab/streak quadrant onto the remainder of the plate. Incubate the MOX plate at 35 ± 2°C for 26 ± 2 h.

2.14.9 If no FB darkening is evident, re-incubate the FB at 35 ± 2°C until a total incubation time of 48 ± 2 h has been achieved.

2.14.10 Re-examine the FB for evidence of darkening after 48 ± 2 h of total incubation. If any degree of darkening is evident, swab, streak and incubate a MOX plate.

2.14.11 If no darkening of FB is evident and no suspect MOX and/or HL colonies have been demonstrated, the sample is considered negative for *Listeria* spp.

2.14.12 If suspect colonies are present on MOX from any source, streak for isolation on one or more HL agar plates. Incubate the streaked HL at 35 ± 2°C for 22 ± 4 h.

2.14.13 After incubation, examine the HL plate(s) against backlight for translucent colonies surrounded by a small zone of β-hemolysis.

2.14.14 If at least one suspect colony is clearly isolated, proceed to confirmatory testing. Hold all HL plates containing suspect colonies (room temperature or refrigeration) until confirmatory testing is complete.

2.14.15 If suspect colonies or β-hemolytic growth are present on HL but not clearly isolated, re-streak representative suspect colonies/growth onto one or more fresh HL plates and incubate at 35 ± 2°C for 22 ± 4 h.

2.14.16 Confirm ALL test portions according to the USDA FSIS MLG 8.09 biochemical/serological procedures. Biochemical tests will be performed using API or VITEK 2 GP. Please follow manufacturer's instructions when using any of these rapid methods.

USDA FSIS Reference Method

1.17 Add 25g test portion to 225 mL UVM broth. Stomach (Seward 400 or equivalent) for 2± 0.2 minutes and incubate for 20-26h at 30±2°C.

1.18 Transfer 0.1 ± 0.02 ml of the enriched sample to 10 ± 0.5 ml of Fraser Broth (FB). As per media preparation instructions, be sure that appropriate supplements have been added to the FB prior to inoculation. Incubate inoculated FB tubes at 35 ± 2°C for 26 ± 2 h.

1.19 Streak a MOX plate. Streak a loopful or a drop approximating 0.1 ml of the enriched sample over the surface of the plate. Incubate the MOX at 35 ± 2°C for 26 ±2 h.

1.20 Examine the MOX plates for colonies with morphology typical of *Listeria* spp. At 26 ± 2 h, suspect colonies are typically small (ca. 1 mm) and are surrounded by a zone of darkening due to esculin hydrolysis.

1.21 If suspect colonies are present on MOX, transfer suspect colonies to HL agar.

1.22 If no suspect colonies are evident, re-incubate the MOX plate for an additional 26 ± 2 hour.

1.23 After 26 ± 2 h of incubation, examine the FB for the potential presence of *Listeria spp.*, by visual examination of the broth for darkening due to esculin hydrolysis.

1.24 If any degree of FB darkening is evident, aseptically dispense a drop approximating 0.1 ± 0.02 ml of FB onto a MOX plate. Swab or streak 25-40% of the surface of the MOX plate with the FB inoculum. Use a loop to streak for isolation from the initial swab/streak quadrant onto the remainder of the plate. Incubate the MOX plate at 35 ± 2°C for 26 ± 2 h.

1.25 If no FB darkening is evident, re-incubate the FB at 35 ± 2°C until a total incubation time of 48 ± 2 h has been achieved.

1.26 Re-examine the FB for evidence of darkening after 48 ± 2 h of total incubation. If any degree of darkening is evident, swab, streak and incubate a MOX plate.

1.27 If no darkening of FB is evident and no suspect MOX and/or HL colonies have been demonstrated, the sample is considered negative for *Listeria* spp.

1.28 If suspect colonies are present on MOX from any source, streak for isolation on one or more HL agar plates. Incubate the streaked HL at 35 ± 2°C for 22 ± 4 h.

1.29 After incubation, examine the HL plate(s) against backlight for translucent colonies surrounded by a small zone of β-hemolysis.

1.30 If at least one suspect colony is clearly isolated, proceed to confirmatory testing. Hold all HL plates containing suspect colonies (room temperature or refrigeration) until confirmatory testing is complete.

1.31 If suspect colonies or β-hemolytic growth are present on HL but not clearly isolated, re-streak representative suspect colonies/growth onto one or more fresh HL plates and incubate at 35 ± 2°C for 22 ± 4 h.

1.32 Confirm ALL test portions according to the USDA FSIS biochemical/serological procedures. Biochemical tests will be performed using API or VITEK 2 GP. Please follow manufacturer's instructions when using any of these rapid methods.

Final Results

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

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

Appendix 8.2a – 125g Deli Turkey, 3M MDA2 Data Recording Form

Lab:
Analyst:

Analyst Start Date:
Analyst Completion Date:
Kit Lot Number:
APC Result:

Sample#	**MDA2 *Listeria* and MDA2 *Listeria monocytogenes***									
	MDA2 LM	MDA2 LS	MOX	FB	FB MOX	Catalase	Hemolysis	Gram rxn	VITEK/API	Final Results

Appendix 8.2b – Deli Turkey, USDA/FSIS MLG Data Recording Form

Lab:

Analyst:

Analyst Start Date:

Analyst Completion Date:

APC Result:

Sample#	MLG 8.09							
	MOX	FB	FB MOX	Catalase	Hemolysis	Gram rxn	VITEK/API	Final Results

Appendix 8.2c – Raw Chicken Breast, 3M MDA2 Data Recording Form

Lab:
Analyst:

Analyst Start Date:
Analyst Completion Date:
Kit Lot Number:
APC Result:

Sample#	MDA2 *Listeria* and MDA2 *Listeria monocytogenes*									
	MDA2 LM	MDA2 LS	MOX	FB	FB MOX	Catalase	Hemolysis	Gram rxn	VITEK/API	Final Results

Appendix 8.2d – Raw Chicken Breast, USDA/FSIS MLG Data Recording Form

Lab:

Analyst:

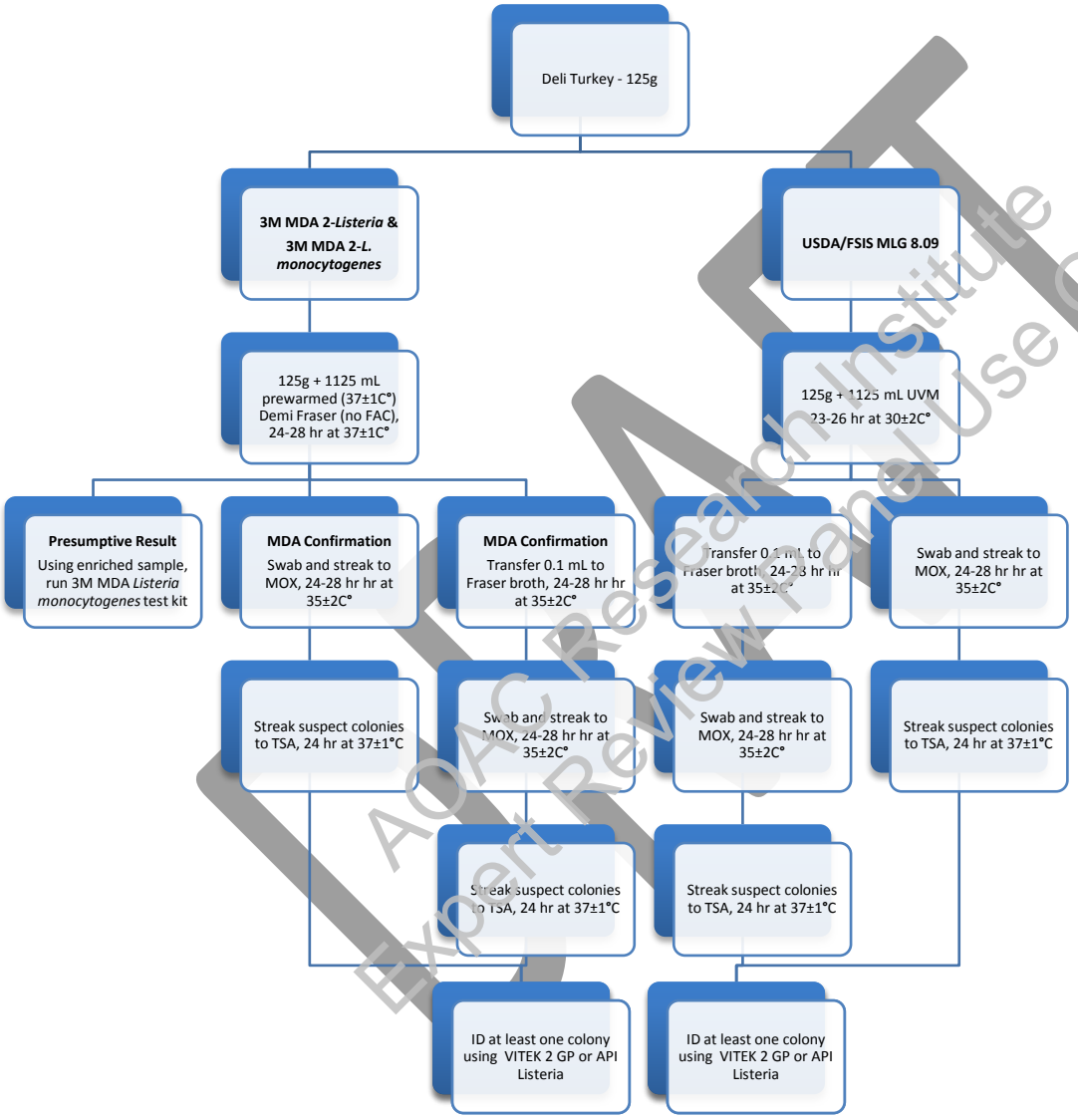
Analyst Start Date:

Analyst Completion Date:

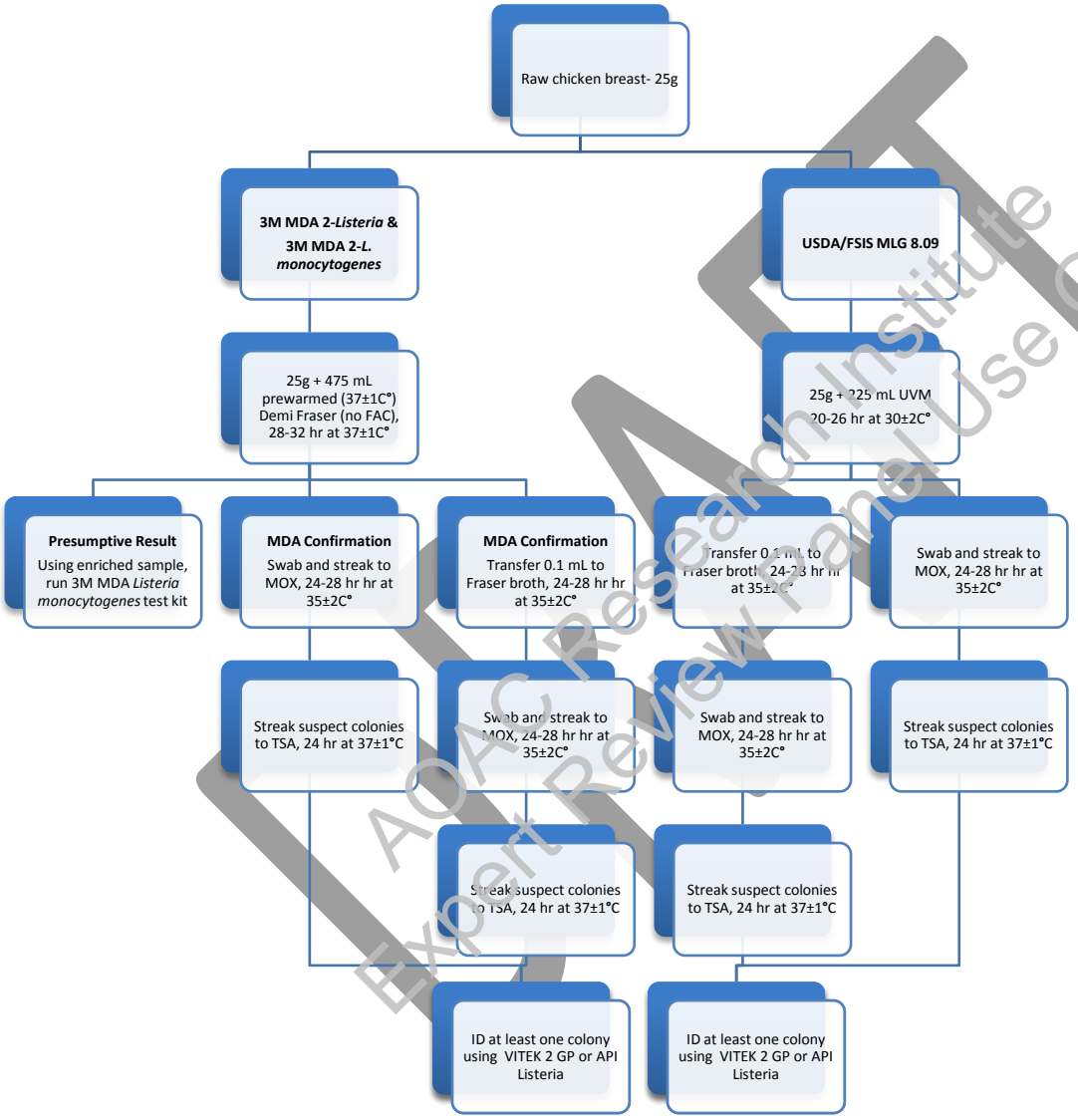
APC Result:

Sample#	MLG 8.09							
	MOX	FB	FB MOX	Catalase	Hemolysis	Gram rxn	VITEK/API	Final Results

Appendix 8.3a – Study Flow Diagram: Deli Turkey



Appendix 8.3b – Study Flow Diagram: Raw Chicken Breast



Appendix 8.4

Study Materials

Materials and reagents provided by the study sponsor

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

Enrichment

12. UVM
13. Demi Fraser broth with Ferric Ammonium Citrate (FAC)
14. Fraser broth with FAC

Selective agar

15. MOX

Confirmation

16. Blood agar (HL and SBA)
17. Catalase
18. Oxidase
19. Motility supplies
20. Tryptic Soy Agar with yeast extract
21. VITEK2 GP or API Listeria

Additional Supplies and Reagents required but not provided

1. Pipettes – capable of 20µL
2. Multi-channel (8-channel) pipette – capable of 20µL
3. Stomacher® – Seward or equivalent
4. Thermometer – calibrated range to include 100±1°C range
5. Dry double block heater unit – capable of maintaining 100 + 1°C; OR a water bath - capable of maintaining 100 + 1°C
6. Incubators – capable of maintaining 37 ± 1°C and 35 ± 1°C
7. Refrigerator – capable of maintaining 2-8°C, for storing the 3M Molecular Detection 2 Assay
8. Computer – compatible with the 3M Molecular Detection System (instrument)

Appendix 8.5

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

Collaborative Study
Collaborator Information Sheet

Please complete the information below and return to TBD.

Contact name:

Laboratory name:

Fax number:

Phone number:

Email address:

Mailing address:

Shipping address:

Appendix 8.6

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

Collaborative Study
Collaborator Comments Form

Collaborating Laboratory:

Date:

General Comments about Method:

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.

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
Listeria 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 immuno compromised, if exposed.

The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices, ISO 17025[4], or ISO 7218[5].

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

- 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.

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 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**, 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 ±0.2 minutes. Incubate at 37 ±1°C according to Table 2.
4. For raw dairy products, transfer 0.1 mL of the primary enrichment into 10 mL of Fraser Broth. Incubate at 37 ±1°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 Letheen 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^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 [(1)], USDA FSIS MLG [(2)] or ISO 18593 [(6)] 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: Enrichment protocols using Demi-Fraser Broth Enrichment

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature (°C)	Enrichment Time (hr)
Heat-processed, cooked, cured meats, poultry, seafood and fish Heat-processed / pasteurized dairy products Produce and vegetables Multi-component foods	25 g	225	37	24-30
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[a]
	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℠ # XXXXX

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 ± 1 °C according to AOAC Performance Tested SM Certificate #XXXXXX

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	
	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 ±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 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:

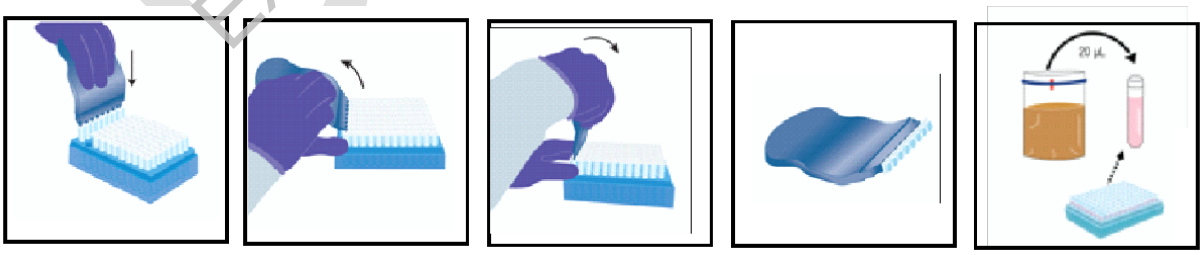
Transfer each enriched sample into an individual LS tube first. Transfer the NC **last**.

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 Table 2. 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.



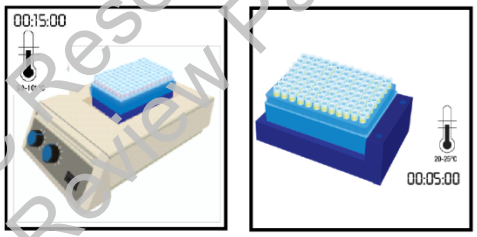
6. Repeat steps 4.1 to 4.6 as needed, for the number of samples to be tested.

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 ±1°C.
9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ±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.

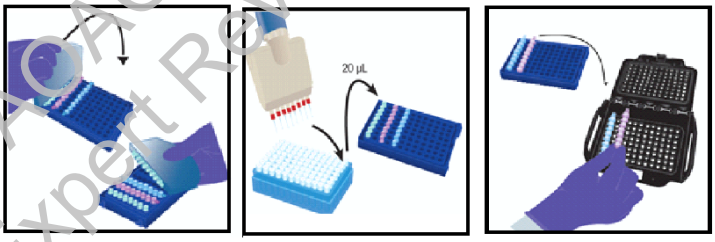


AMPLIFICATION

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 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.
   - 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 µL of NC lysate into a Reagent tube.
   - 5.6 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.
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 [1, 2, 3], 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 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. 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.

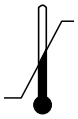
EXPLANATION OF PRODUCT LABEL SYMBOLS

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

Appendix 8.8

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 [1, 2, 3].

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. 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
Listeria monocytogenes 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 immuno compromised, if exposed.

The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices, ISO 17025[4], or ISO 7218[5].

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). 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

- 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.

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.

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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**, 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

5. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature.
6. 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.
7. Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ± 0.2 minutes. Incubate at 37 ±1°C according to Table 2.
8. For raw dairy products, transfer 0.1 mL of the primary enrichment into 10 mL of Fraser Broth. Incubate at 37 ±1°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 Letheen 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^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

[(1)], USDA FSIS MLG [(2)] or ISO 18593 [(6)] guidelines.

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

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 Heat-processed / pasteurized dairy products Produce and vegetables Multi-component foods	25 g	225	24-30
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

PREPARATION OF THE 3M™ MOLECULAR DETECTION SPEED LOADER TRAY

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

4. Launch the 3M™ Molecular Detection Software and log in.
5. Turn on the 3M Molecular Detection Instrument.
6. 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

5. 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.
6. Invert the capped tubes to mix. Proceed to next step within 4 hrs.
7. Remove the enrichment broth from the incubator.
8. One LS tube is required for each sample and the Negative Control (NC) (sterile enrichment medium) sample.

8.1 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.

8.2 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:

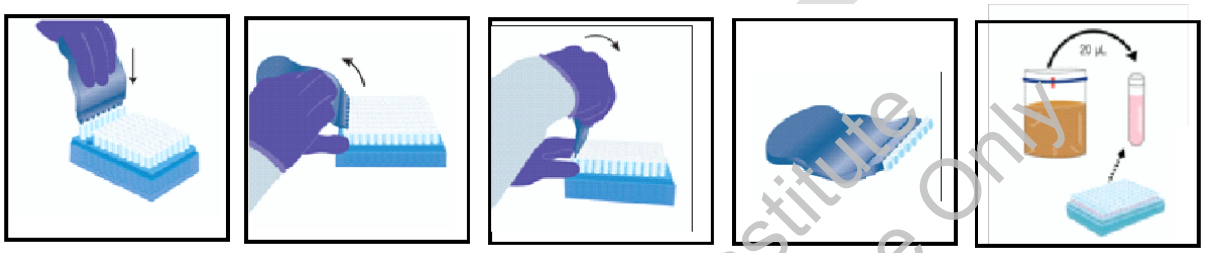
Transfer each enriched sample into an individual LS tube **first**. Transfer the NC **last**.

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 10uL.

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.

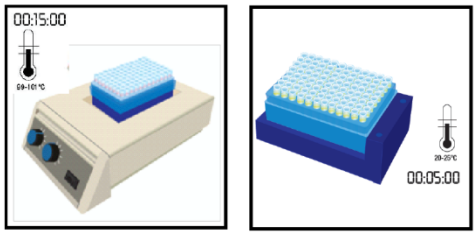
8. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100 ±1°C.

9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ± 2 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.



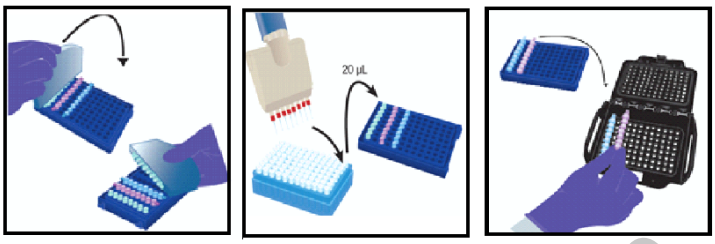
AMPLIFICATION

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.

16. 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.



17. Review and confirm the configured run in the 3M Molecular Detection Software.
18. Click the Start button in the software and select instrument for use. The selected instrument's lid automatically opens.
19. 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.
20. 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 [1, 2, 3], 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).

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 or contact your local 3M representative or distributor.

REFERENCES:

7. US Food and Drug Administration Bacteriological Analysis Manual. Chapter 10: Detection and Enumeration of *Listeria monocytogenes* in Foods. Section C-6. April 2011 Version.
8. 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.
9. 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.
10. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
11. ISO 7218. Microbiology of food and animal feeding stuffs – General rules for microbiological examination.
12. 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

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

9. Store at 4 to 8°C for up to 72 hours.
10. Prepare a stored sample for amplification by inverting 2-3 times to mix.
11. Decap the tubes.
12. Place the mixed lysate tubes on 3M Molecular Detection Heat Block Insert and heat at 100 ±1°C for 5 ±1 minutes.
13. 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.
14. Continue the protocol at the 'Amplification' section detailed above.

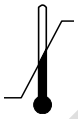
EXPLANATION OF PRODUCT LABEL SAMPLES


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.

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

Patrick Bird, Jonathan Flannery, Erin Crowley, James Agin, David Goins
Q Laboratories, Inc., 1400 Harrison Ave, Cincinnati, OH 45214

Lisa Monteroso
3M Food Safety Department, 3MCenter – Bldg. 260-6B-01, St. Paul, MN 55144

Collaborators: R. Brooks, J. Walia, F. Hernandez, D. Bosco, G. Trevino, A. Brandt, C. Lopez, E. Sjogren, M. Shekhawat, L. Ma, C. Timmons, C. Diaz Proano, A. Calle,Z. Metz, D. Baumler, R. Smith, D. Wood, E. Maranan, C. Chavarria,J. Miller, B. Schindler, C. Gies, E. Budge, Z. Geurin, A. Repeck, C. Zook, C. Barnes, B. Bastin, D. Isfort

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 from13laboratories located within the continental United Statesand 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 valuewith 95% confidence interval of 0.16, (0.04, 0.28) indicating astatisticallysignificant differencebetween the candidate and reference methods with a positive correlation in data indicating more recovery of the target analyte by the candidate method.

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]. Theorganism'sability to survive in extreme conditions make its presence in food a serious issue. In the past year, *Listeria*has been identified as the source ofseveral high profile outbreaks involving bagged leafy greens and ice cream [3]. The 3M™ Molecular Detection Assay(MDA) 2 - *Listeria*method,usinga combination of bioluminescence and isothermal amplification of nucleic

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

Prior to the collaborative study, the 3M MDA 2 - *Listeria* method was validated according to AOAC Guidelines[4] in a harmonized AOAC® Performance Tested Method℠ (PTM) study. The objective of the PTM study was to demonstrate that the 3M MDA 2-*Listeria* method could detect *Listeria*in a broad range of food matrices and environmental surfaces as claimed by the manufacturer. For the 3M MDA 2- *Listeria* PTM evaluation, 13 matrices were evaluated: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), and plastic (Enviroswab, 10 mL) environmental samples.

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

The purpose of this collaborative studywas to compare the reproducibility of the 3M MDA 2 - *Listeria*method 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*[5] for deli turkey (125 g) and raw chicken breast fillet.

Collaborative Study

Study Design

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

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

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

Preparation of Inocula and Test Portions

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

$$(1-\frac{n_{select}}{n_{nonselect}})x100$$

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

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

Test Portion Distribution

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

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 ±1°C. For the raw chicken breast fillettest 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 ±1°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, $POD_{CP,}$ the candidate confirmatory results (including false negative results), POD_{CC}, the difference in the candidate presumptive and confirmatory results, $dLPOD_{CP,}$ presumptive candidate results that confirmed positive (excluding false negative results), $POD_{C,}$ the reference method, POD_R, and the difference in the confirmed candidate and reference methods, $dLPOD_C$. A $dLPOD_C$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_r), the among laboratory repeatability standard deviation (s_L), the reproducibility standard deviation (s_R)and the P_T value were calculated. The s_r provides the variance of data within one laboratory, the s_L provides the difference in standard deviation between laboratories and the s_R provides the variance in data between different laboratories. The P_T value provides information on the homogeneity test of laboratory PODs [10].

AOAC Official Method 2016.xxx
*Listeria*speciesin SelectFoods and Environmental Surfaces
3M™ Molecular Detection Assay(MDA) 2- *Listeria* Method
First Action 2016

(Applicable to detection of *Listeria*speciesin 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 (3M™ Hydrated Sponge Stick with D/E, 225 mL & 100 mL), stainless steel (3M™ Hydrated Sponge Stick with D/E, 225 mL), and plastic (3M™ Enviroswab with Letheen, 10 mL) environmental samples.

*See Tables**2016.1A** and **2016.1B**for a summary of results of the inter-laboratory study.*
*See Tables **2016.2A** and **2016.2B** for detailed results of the inter-laboratory study*

A. Principle

The 3M™ Molecular Detection Assay (MDA) 2 - *Listeria* method is used with the 3M™ Molecular Detection System (MDS) for the rapid and specific detection of *Listeria* in enriched food and food process environmental samples. The 3M MDA2 -*Listeria*uses loop-mediated isothermal amplification of unique DNA target 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. Samples arepre-enriched in Demi Fraser broth with ferric ammonium citrate (FAC).

B. Apparatus and Reagents

Items (b)-(g) are available as the 3M™ Molecular Detection Assay (MDA) 2 - *Listeria* kit from 3M Food Safety (St. Paul, MN 55144-1000, USA).

- **(a)** *3M Molecular Detection System*(MDS100) – Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- **(b)** *3M Molecular Detection Assay 2 – Listeria reagent tubes*- 12 strips of 8 tubes. Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- **(c)** *Lysis Solution (LS) tubes* – 12 strips of 8 tubes.
- **(d)** *Extra caps* – 12 strips of 8 caps
- **(e)** *Reagent Control* – 8 reagent tubes
- **(f)** *Quick Start Guide*
- **(g)** *3M™ Molecular Detection Speed Loader Tray* - Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- **(h)** *3M™ Molecular Detection Chill Block Insert* - Available from 3M™ Food Safety (St. Paul, MN 55144-1000, USA).
- **(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 ± 1°C
(v) *Dry block heater unit*– capable of maintaining 100 ± 1°C
(w) *Incubators.* – Capable of maintaining 37 ± 1°C or 41.5 ± 1°C.
(x) *Freezer* – capable of maintaining -10 to -20°C, for storing the 3M Molecular Detection Chill Block Tray
(y) *Refrigerator* – capable of maintaining 2-8°C, for storing the 3M Molecular Detection Assay components
(z) *Computer* – compatible with the 3M™ Molecular Detection Instrument
(aa) *3M™ Enviroswab,* -- (hydrated with Letheen) Available from 3M Food Safety(Australia)
(bb) *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* 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 60 days. Do not use 3M Molecular Detection Assay 2 -*Listeria* past the expiration date.

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

Safety Precautions

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 the food and beverage industries. For example, 3M has not documented this product for testing drinking water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M MDA2 – *Listeria*has not been evaluated with all possible food products, food processes, testing protocols or with all possible strains of bacteria.

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

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.

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

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

Listeria monocytogenes is of particular concern for pregnant women, the aged and the infirmed. It is recommended that these concerned groups avoid handling this organism.After use, the enrichment medium and the 3M MDA 2 - *Listeria*tubes can potentially contain pathogenic materials. 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. 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.

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

D. Sample Enrichment

Foods

(a) Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature (20-25°C).

(b) 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.

(c) Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ±0.2 minutes. Incubate at 37 ±1°C according to Table A.

Environmental samples

(d) 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 Letheen 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. Another option is to transfer 10 µL of the NB enrichment in the LS tubes.

(e) The recommended size of the sampling area to verify the presence or absence of the pathogen on the surface is at least 100 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, USDA FSIS MLG or ISO 18593 [11] guidelines.

1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature (20-25°C).
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.

E. Preparation of the 3M™ Molecular Detection Speed Loader Tray

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

Specific Instructions for Validated Methods

AOAC® Performance Tested Method℠ #111501

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE
LICENSE NUMBER 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 2. 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 2).

Table 2: Enrichment protocols using Demi-Fraser Broth at 37 ± 1 °C according to AOAC Performance Tested ^{SM} 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

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 ±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

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 ±1°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.

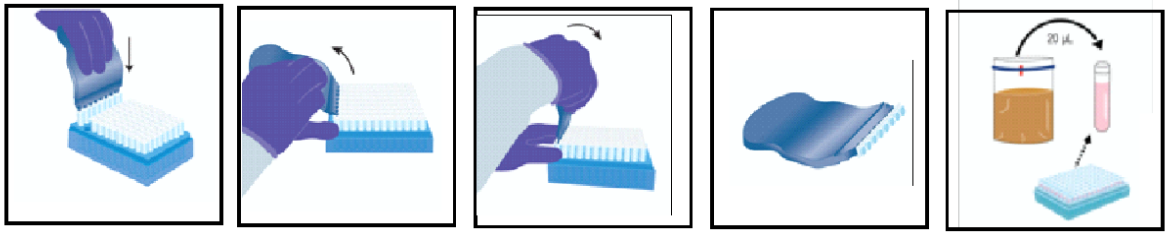
NOTE: The 3M Molecular Detection Instrument must reach and maintain temperature of 60°C before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 minutes and is indicated by an ORANGE light on the instrument's status bar. When the instrument 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**.

   - 4.4 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 µL 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.



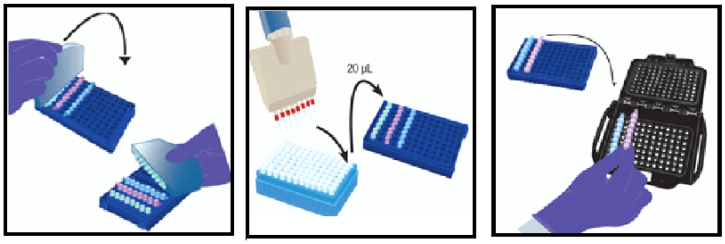
6. Repeat steps 4.1 to 4.6as needed, for the number of samples to be tested. When all samples have been transferred, then transfer **20 µL of NC into a LS tube**. Do not recap tubes.
7. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100 ±1°C. Place the rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ±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 (20-25°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 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.
   - 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 µL of NC lysate into a Reagent tube.
   - 4.7 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.
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* amplificationreagents 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.

Results of Collaborative Study

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

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

Results for the heat stress analysis of the inoculum for the deli turkey are presented in Table 2. Table 2016.1A and 2016.1B summarize the inter-laboratory results for all foods tested, including POD statistical analysis. As per criteria outlined in Appendix J of the AOAC Validation Guidelines, fractional positive results were obtained.Detailed results for each laboratory are presented in Tables 2016.2A and 2016.2B. For each matrix, the level of *Listeria* was determined by MPN on the day of initiation of analysis by the coordinating laboratory. MPN results are presented in Tables 2016.2A and 2016.2B.The individual laboratory and sample results are presented in Tables 1-2 of the Supplementary Materials. The APCresults for each collaborating are presented in Table3 of the Supplementary Materials

Deli Turkey (125 g Test Portions)

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

For the low inoculum level, 68 out of 132 test portions (POD_{CP} of 0.52) were reported as presumptive positive by the 3M MDA 2 – *Listeria*method with 66out of 132 test portions (POD_{CC} of 0.50) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria*method, 66out of 132 samples confirmed positive (POD_C of 0.50). For test portions evaluated by the USDA/FSIS MLG reference method, 60 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.04 with 95% confidence intervals of (-0.08, 0.17) was obtained between the candidate and reference method, indicating no statistical significant difference between the two methods. A $dLPOD_{CP}$ value of 0.02 with 95% confidence intervals of (-0.11, 0.14) was obtained between presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results.

For the high inoculum level, 132 out of 132 test portions (POD_{CP} of 1.00) were reported as presumptive positive by the 3M MDA 2 – *Listeria*method with 132out of 132 test portions (POD_{CC} of 1.00) confirming positive.For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria*method, 132out of 132 samples confirmed positive (POD_C of 1.00). For test portions evaluated by the USDA/FSISMLG reference method, 132 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between the candidate and reference method, indicating no statistical significant difference between the two methods. A $dLPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results.
For the un-inoculated controls, 0 out of 132 samples (POD_{CP} of 0.00) produced a presumptive positive result by the 3M MDA 2 - *Listeria*method with 0out of 132 test portions (POD_{CC} of 0.00) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria*method, 0out of 132 samples confirmed positive (POD_C of 0.00). For test portions evaluated by the USDA/FSIS MLG reference method, 0 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between the candidate and reference method, indicating no statistical significant difference between the two methods. A $dLPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.03, 0.00) was obtained between presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results.

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

Raw Chicken Breast Fillet (25 g Test Portions)

Raw chicken breast fillet test portions were inoculated at a low and high inoculum level and were analyzed for the detection of *Listeria* spp. Un-inoculated controls were included in each analysis. Laboratory 11 did not participate in the evaluation of this matrix. Laboratory 10 submitted data that indicated cross contamination of the inoculating organism in the un-inoculated control samples. Further analysis at the coordinating laboratory confirmed the cross contamination of the un-inoculated controls. Due to this issue, the data submitted from Laboratory 10 was not used in the statistical analysis. All other laboratories submitted data for both methods evaluated. The MPN levels obtained for this matrix, with 95% confidence intervals, were 0.66 CFU/test portion (0.51, 0.83) for the low level and 6.24 CFU/test portion (3.58, 10.88) for the high level.
For the low inoculum level, 88 out of 132 test portions (POD_{CP} of 0.67) were reported as presumptive positive by the 3M MDA 2 – *Listeria*method with 86out of 132 test portions (POD_{CC} of 0.65) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria*method, 85out of 132 test portions confirmed positive (POD_C of 0.64). For test portions evaluated by the USDA/FSIS MLGreference method, 64 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.16 with 95% confidence intervals of (0.04, 0.28) was obtained between the candidate and reference method, indicating a statistically

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

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

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

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

Discussion

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

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

Recommendations

It is recommended that the 3M Molecular Detection Assay 2 – *Listeria*method be adopted as Official First Action status for the detection of *Listeria* in selected foods: 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, stainless steel and plastic environmental samples.

Acknowledgements

We would like to extend a sincere thank you to the following collaborators for their dedicated participation in this study:

Robert Brooks- ATC Microbiology, LLC- North Little Rock, AR.
Jaspreet Walia & Francisco Hernandez – Certified Laboratories, Turlock, CA
David Bosco& Grizelda Trevino– Food Safety Net Services, Fresno, CA
Alex Brandt & Chris Lopez – Food safety Net Services, San Antonio, TX
Elizabeth Sjogren&Manish Shekhawat– Microbac Laboratories, Inc. Worcester, MA
Li Maria Ma, Chris Timmons, & Claudia Diaz Proano – Oklahoma St. University, Stillwater, OK
Alexandra Calle & David Campos – Texas Tech University, Lubbock, TX.
Zachary Metz&David Baumler, University of Minnesota, St. Paul, MN
Robbie Smith, Dianne Wood, Evelyn Maranan,&Carmen Chavarria Maple Leaf Foods– Guelph, ON, Canada
Ben Bastin– Microbiology R&D, Q Laboratories Inc., Cincinnati, OH
David Isfort– Microbiology-Food, Q Laboratories Inc., Cincinnati, OH
Jesse Miller, Bryan Schindler, Courtney Gies, Eric Budge, Zach Geurin & Alex Repeck - NSF International, Ann Arbor, MI
Cynthia Zook & Christina Barnes – 3M Food Safety, St. Paul, MN

We would like to extend a special thanks to the following team members at Q Laboratories, Inc. for their efforts during the collaborative study: M. Joseph Benzinger Jr., Allison Mastalerz, Megan Boyle,Alison DeShields, Jonathan Clifton, T. Shane Wilson, Meghan Daniel, Nicole Klass and Colleen Sweeney.

References

(1) United States Food and Drug Administration. (2008) *Guidance for Industry: Control of Listeria monocytogenes in Refrigerated or Frozen Ready-to-Eat Foods; Draft Guidance*http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/FoodProcessingHACCP/ucm073110.htm (Accessed March 2016)

(2) Jemmi, T., Stephan, R. (2006) Scientific and Technical Review of the Office International des Epizooties *Listeria monocytogenes*: *food-borne pathogen and hygiene indicator.*Vol. 25, No. 2, pp. 571-580

(3) Centers for Disease Control and Prevention. (2016) *Listeria Outbreaks*http://www.cdc.gov/listeria/outbreaks/(Accessed March 2016)

(4)*Official Methods of Analysis* of AOAC INTERNATIONAL (2012) 19th Ed., Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, AOAC INTERNATIONAL, Gaithersburg, MD, http://www.eoma.aoac.org/app_j.pdf(Accessed January 2016)

(5)United States Department of Agriculture Food Safety and Inspection Service. (2013) *Microbiological Laboratory Guidelines5.09*: *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and Environmental Samples*. (Accessed January 2016) http://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES

(6) *Official Methods of Analysis* of AOAC INTERNATIONAL (2015) *Enumeration of Aerobic Bacteria in Foods.*2015.13. (Accessed January2016)

(7) Least Cost Formulations, Ltd., MPN Calculator-Version 1.6, www.lcfltd.com/customer/LCFMPNCalculator.exe(Accessed March 2016)

(8) *Official Methods of Analysis* of AOAC INTERNATIONAL (2012) *Gram-Positive Bacteria Identification.* 2012.02. (Accessed January 2016)

(9) Wehling, P., LaBudde, R., Brunelle, S., Nelson, M. (2011) Journal of AOAC International*Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods.* Vol. 94, No. 1. pp. 335-347

(10) Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook (2013), http://lcfltd.com/aoac/aoac-binary-v2-3.xls (Accessed March, 2016)

(11) ISO 18593:2004 -*Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.*

Table 1: Participation of each Collaborating Laboratory[a]

Lab	Deli Turkey[a,b]	Raw Chicken Breast Fillet[a,b]
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

[a] Y= Collaborator analyzed the food type;[a]N= Collaborator did not analyze food type

Table 2: Heat-Stress Injury Results

Matrix	Test Organism[a]	CFU/MOX (Selective Agar)	CFU/TSA (Non-Selective Agar)	Degree Injury[b]
Deli Turkey	*Listeriamonocytogenes* ATCC 19115	9.1×10^8	2.5×10^9	60.8%

[a] ATCC- American Type Culture Collection
[b] Cultures were heat stressed for 10 minutes at 55°C in a recirculating water bath.

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

Method[a]	**3M™ MDA™2 - *Listeria***		
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[e]	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)[f]	0.00 (-0.03, 0.03)	0.04 (-0.08, 0.17)	0.00 (-0.03, 0.03)
dLPOD (Candidate Presumptive vs. Candidate Confirmed)[f]	0.00 (-0.03, 0.03)	0.02 (-0.11, 0.14)	0.00 (-0.03, 0.03)

[a] Results include 95% Confidence Intervals, [r] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation
[e] P Value = Homogeneity test of laboratory PODs; [f] 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* inRaw Chicken Breast Fillet (25g)

Method[a]	**3M™ MDA™2 -*Listeria***		
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[e]	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)[f]	0.01 (-0.02, 0.04)	0.16 (0.04, 0.28)	-0.01 (-0.04, 0.02)
dLPOD (Candidate Presumptive vs. Candidate Confirmed)[f]	0.01 (-0.03, 0.05)	0.02 (-0.10, 0.13)	-0.01 (-0.04, 0.02)

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

Table 2016.2A: Comparative Results for the Detection of *Listeria* in 125 g Deli TurkeyTest Portions by the 3M™ MDA 2 - *Listeria* 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[a]	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[a]	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		

[a]N/A – Laboratory did not participate or submit data for this matrix

[b] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation, [e] P_T Value = Homogeneity test of laboratory PODs

LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

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. 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[a]	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[a]	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		

[a] N/A – Laboratory did not participate or submit data for this matrix

[b] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation, [e] P_T Value = Homogeneity test of laboratory PODs

LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

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. 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[a]	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[a]	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		

[a] N/A – Laboratory did not participate or submit data for this matrix

[b] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation, [e] P_T Value = Homogeneity test of laboratory PODs
LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

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. 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[b]	12	0	0.00	12	2	0.17	12	0	0.00	12	0	0.00	0.00	-0.17
		11[a]	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		

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

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* 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[b]	12	6	0.50	12	8	0.66	12	6	0.50	12	5	0.42	0.08	-0.16
	MPN/ Test Portion	11[a]	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		

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

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* 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[b]	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
	MPN/ Test Portion	11[a]	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	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		

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

Supplementary Materials

Table 1. Individual Collaborator Results for Deli Turkey

	High Level Test Portions												Low Level Test Portions												Un-inoculated Test Portions											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
3M MDA 2 -*Listeria*																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-[b]	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
11	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-[b]	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
USDA/FSIS – MLG 8.09																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
9	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
11	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

[a] += *Listeria* species were detected in samples; -=*Listeria* species were not detected in sample; n/a- lab did not participate or submit data for the matrix;
[b] Sample was presumptive positive on 3M MDA 2 *Listeria* but confirmed negative indicating a false positive result;

Table 2. Individual Collaborator Results for Raw Chicken Breast Fillet

	High Level Test Portions												Low Level Test Portions												Un-inoculated Test Portions											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
3M MDA 2 - *Listeria*																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+[c]	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-[d]	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	-	-[d]	-[d]	+	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-[d]	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+[e]	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+[e]	+	-	-	-	-	-	-	-	-	-	-	-	-
10[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+[e]	+[e]	-	-	+	+[e]	-	-	-	-	-	-	-	-	-	-	+[e]
11	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
USDA/FSIS – MLG 8.09																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10[b]	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
11	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
12	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

[a] + = *Listeria* species were detected in samples; -=*Listeria* species were not detected in sample; n/a- lab did not participate or submit data for the matrix;
[b] Results were not used in statistical analysis due to laboratory error; [c] *Listeria innocua* recovered from natural contamination of the sample. [d]Sample was presumptive positive on 3M MDA 2 *Listeria* but confirmed negative indicating a false positive result; [e] Sample was presumptive negative on 3M MDA 2 *Listeria* but confirmed positive indicating a false negative result

Table 3: Results of Aerobic Plate Count for Collaborating Laboratories

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

[a] Samples analyzed by the 3M Petrifilm Rapid Aerobic Count Plate, AOAC OMA 2015.13

Figure 1A: POD Values of Candidate Method vs. Reference Method for125 gDeli Turkey

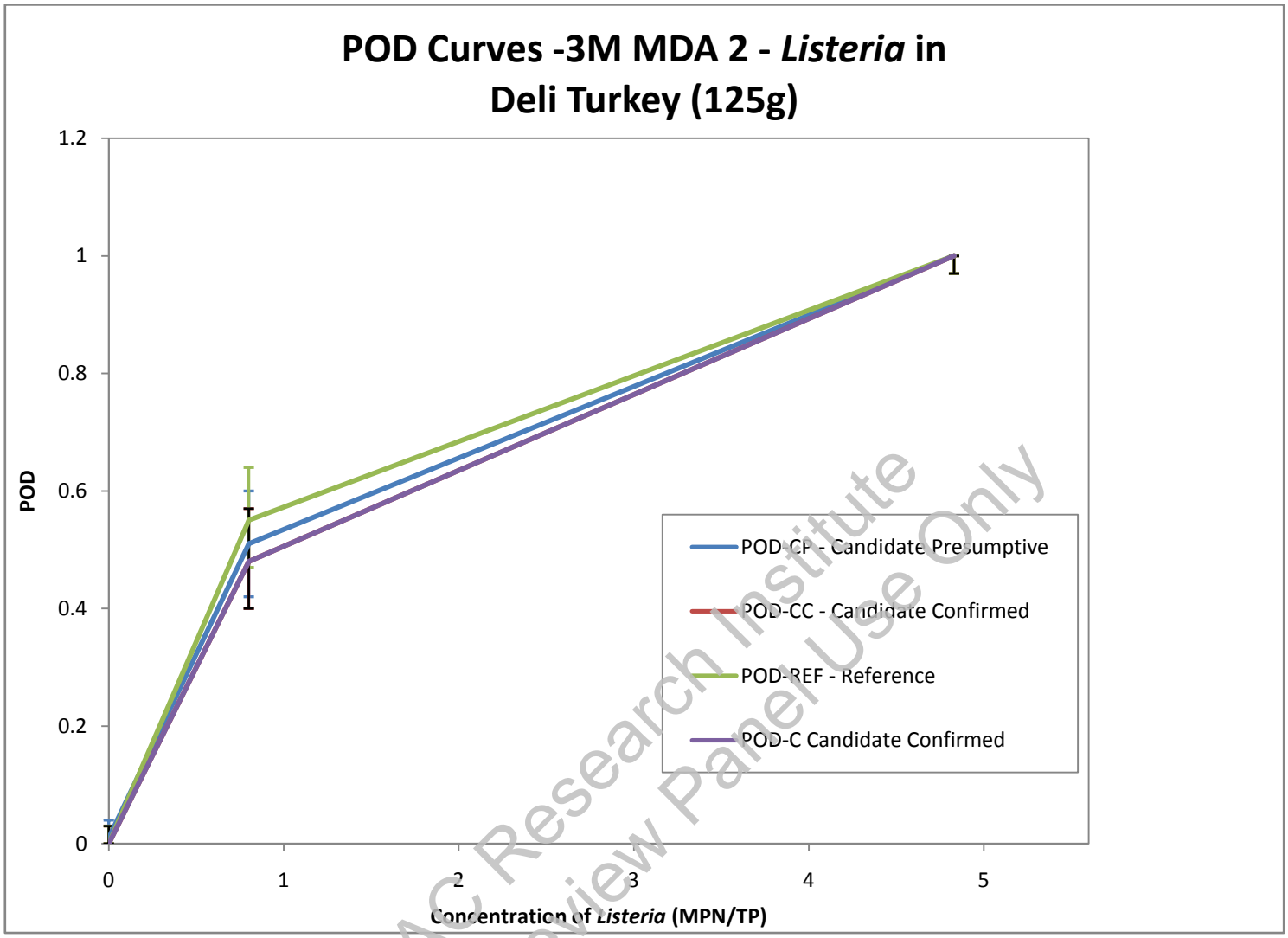


Figure 1B: dLPOD Values of Candidate Method vs. Reference Method for125 gDeli Turkey

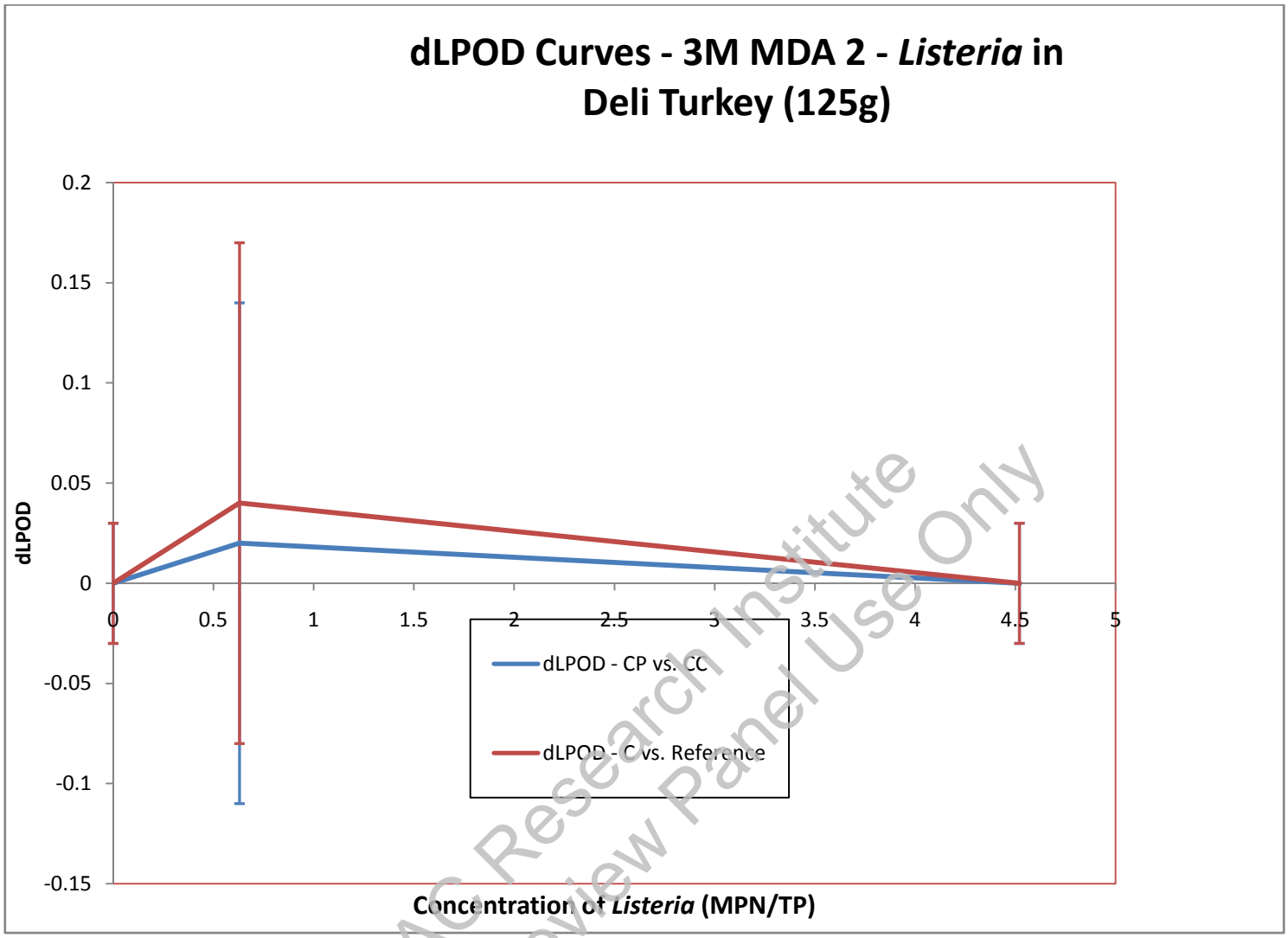


Figure 2A: POD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet

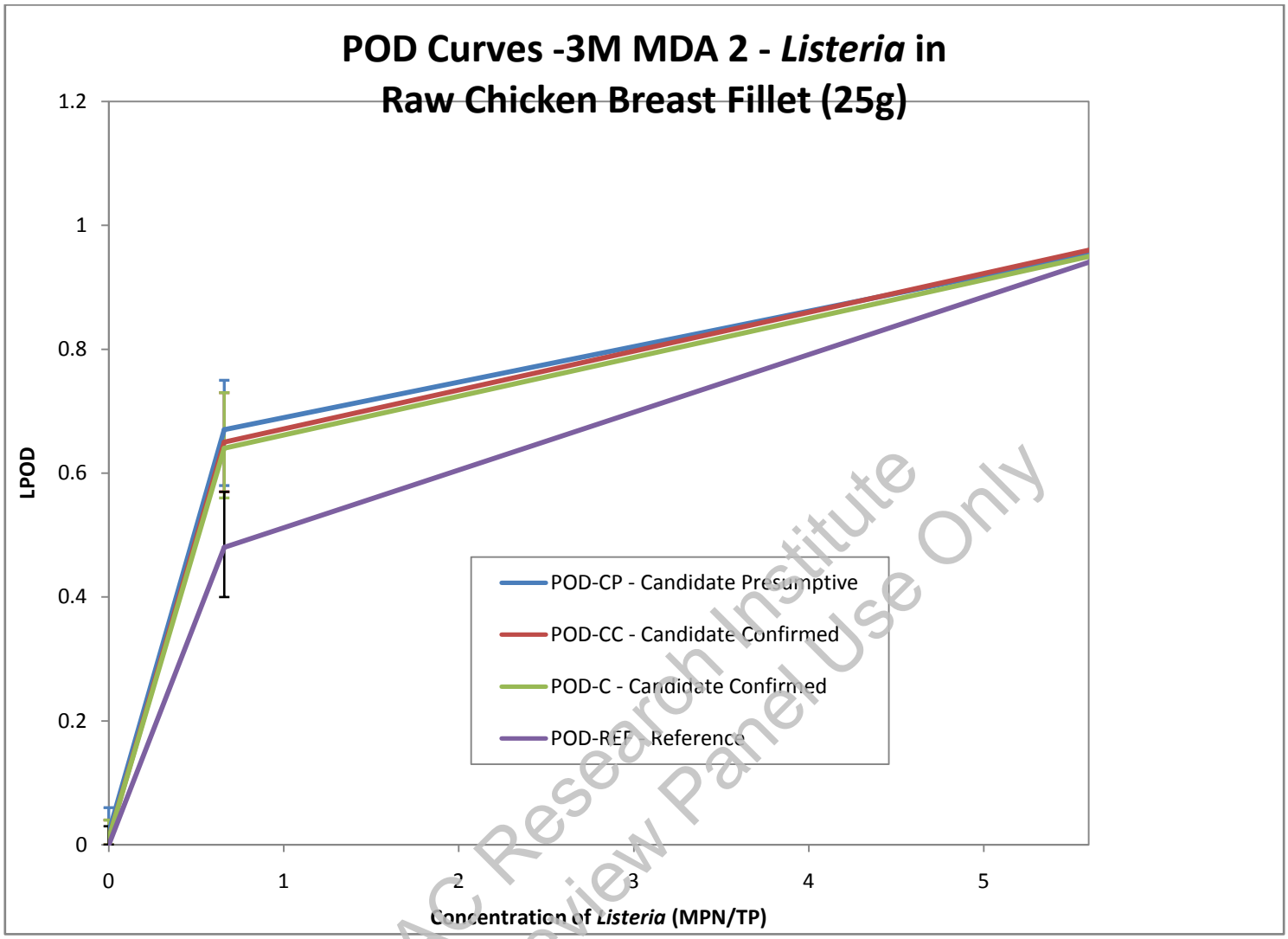
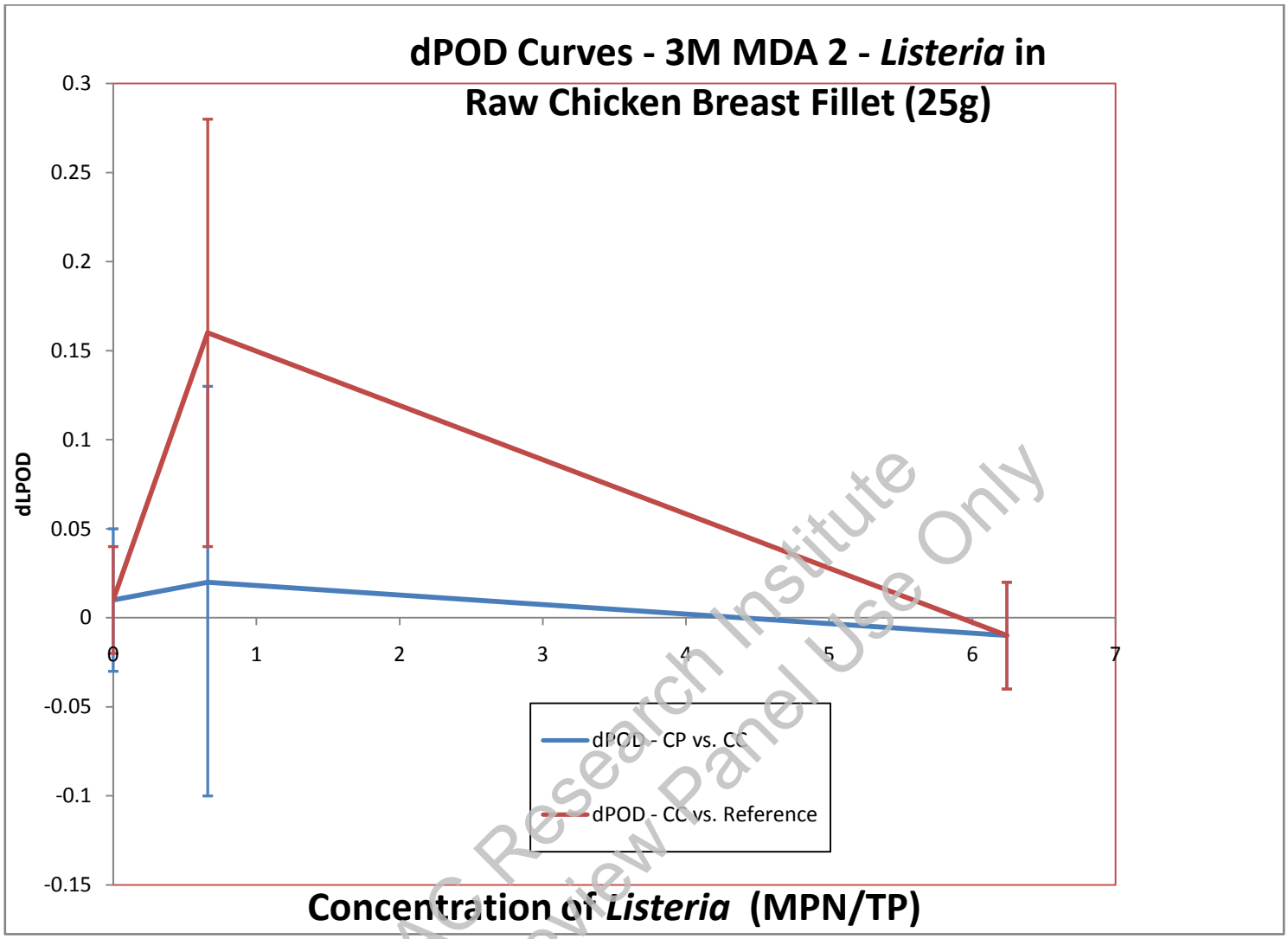


Figure 2B: dPOD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet



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* 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
Listeria 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[4], or ISO 7218[5].

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 thathave 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 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**, 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* tubescan 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 ±0.2 minutes. Incubate at 37 ±1°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 ±1°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 Letheen broth. It is recommended to sanitize the area after sampling.

WARNING: Should you select to use Neutralizing Buffer (NB) that contains aryl sulfonatecomplex 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 testingin 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^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 [(1)], USDA FSIS MLG [(2)] or ISO 18593 [(6)] 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 or3.

3. Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ±0.2 minutes. Incubate at 37 ±1°C for24-30 hours.

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 Heat-processed / pasteurized dairy products Produce and vegetables Multi-component foods	25 g	225	37	24-30
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[a]
	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℠#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℠ Certificate #111501 and AOAC Official Method℠ 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 DETECTIONCHILL 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 ±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 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:

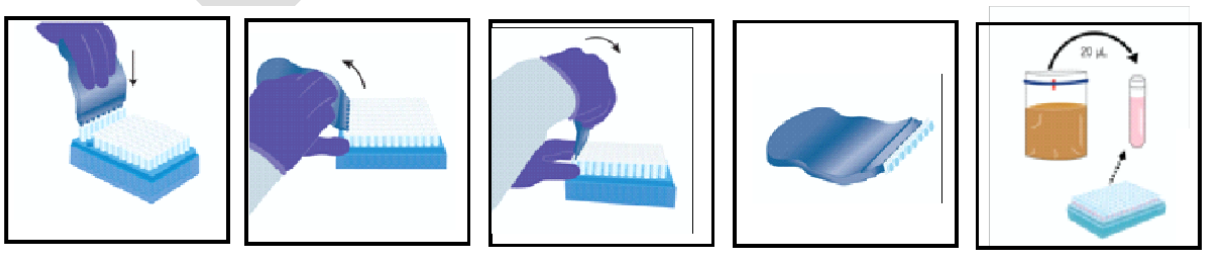
Transfer each enriched sample into an individual LS tube **first**. Transfer the NC **last**.

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 2or in Specific instructions for validated methods table 3.e.g. Raw dairy products use 10µL.

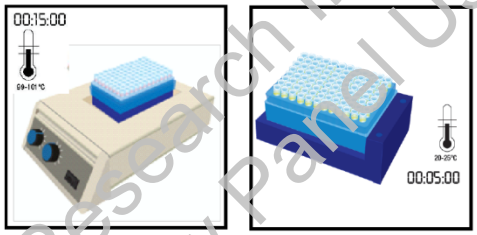
5. Repeat step 4.2 until each individual sample has been added to a corresponding LS tube in the strip.



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 ±1°C.
9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ±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 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.

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 µL of NC lysate into a Reagent tube.

5.6 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.

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 [1, 2, 3], 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* amplificationreagents 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 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

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

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

AOAC *Performance Tested Methods*℠ 111501

Abstract

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

Authors

Lisa Monteroso and DeAnn Benesh, 3M Food Safety Division
Ben Bastin, Jonathan Flannery, Erin Crowley, Patrick Bird, M. Joseph Benzinger, Jr., James Agin, David Goins, Q Laboratories, Inc.

Submitting Company

3M Company
Food Safety Department
3M Center, Bldg. 275-5W-05
St. Paul, MN 55144-1000

Independent Laboratory
Q Laboratories, Inc.
1400 Harrison Ave
Cincinnati, OH 45214

Reviewers
Yvonne Salfinger
Consultant
Denver, CO

Michael Brodsky
Brodsky Consultants
73 Donnamora Crescent
Thornhill, Ontario L3T 4K6
Canada

Reviewer
Tony Hitchins
FDA-Retired
Rockville, MD

Scope of Method

a) *Target organism.— Listeria* species (*L. monocytogenes, L. seeligeri, L. ivanovii, L. innocua, L. grayi, L. welshimeri, L. fleischmanii* subsp. *coloradensis, L. cornellensis, L. grandensis L. marthii*, and *L. riparia)*

b) *Matrixes*.— Raw chicken (leg pieces and fillets) (25 g), bagged raw spinach (25 g), cold smoked salmon (25 g), deli turkey (125 g), whole melon, vanilla ice cream (25 g), queso fresco (25 g), 4% milk fat cottage cheese (25 g), beef hot dogs (25 g), stainless steel (4” x 4”, sponge enriched in 225 mL), sealed concrete (4” x 4”, sponge enriched in 225 mL), plastic (1” x 1”, 3M™ Tecra™ Enviroswab [3M Australia Pty Ltd] enriched in 10 mL).

c) *Summary of Validated Performance Claims*.— Performance equivalent to that of the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM) Chapter 10 [1] for whole melon, cold smoked salmon, bagged raw spinach; the U.S. Department of Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (USDA-FSIS/MLG) 8.09 [2] for beef hot dogs, deli turkey, raw chicken , sealed concrete, plastic and stainless steel and AOAC 993.12 [3] for queso fresco, vanilla ice cream, 4% milk fat cottage cheese and for ISO 11290-1/A1 [4] for cold smoked salmon and bagged raw spinach.

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.

Principle of the Method

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 the laboratory's preferred method or as specified by local regulations.

General Information

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 that can 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.

Materials and Methods

Test Kit Information

a) *Kit Name.*— Molecular Detection Assay 2 – *Listeria*

b) Catalog Number.— MDA2LIS96

Test Kit Reagents - 3M MDA 2 - *Listeria* (96 tests)
a) *Lysis Solution (LS) tubes*. – 96 (12 strips of 8 tubes)
b) *Listeria Reagent tubes*. – 96 (12 strips of 8 tubes)
c) *Extra caps.* – 96 (12 strips of 8 caps)
d) *Reagent Control (RC).* – 16 (2 pouches of 8)
e) Quick Start Guide

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

Additional media
b) Oxford Agar (OX)
c)
d) Fraser Broth (FB)
e) Sheep Blood Agar (SBA)
f) PALCAM Agar
g) Ottaviani Agosti Agar (OAA)
h) Trypticase Soy Agar (TSA)
i) Trypticase Soy Agar with 0.6% Yeast Extract (TSA/YE)
j) De Man, Rogosa and Sharpe (MRS)
k) Modified Oxford Agar (MOX)
l) Brain Heart Infusion (BHI) broth
m) Horse Blood Overlay (HBO)
n) Buffered Listeria Enrichment Broth (BLEB)

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

h) *Dry double block heater unit.* – capable of maintaining 100 ± 1°C; or *a water bath* capable of maintaining 100 ± 1°C
i) *Refrigerator*. – capable of maintaining 2-8°C, for storing the 3M MDA2
j) *Computer*. – compatible with the 3M Molecular Detection System (instrument)

Safety Precautions

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.

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.
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.

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

Sample Preparation

3M recommends the use of Demi-Fraser Broth with FAC for the enrichment of food and environmental samples. Table 1 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

a) Allow the Demi-Fraser Broth enrichment medium (includes FAC) to equilibrate to ambient laboratory temperature.
b) Aseptically combine the enrichment medium and sample according to Table 1. For all meat and highly particulate samples, the use of filter bags is recommended.
c) Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ±0.2 minutes. Incubate at 37 ±1°C according to Table 1.

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 Letheen 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^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, 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 ~~is~~ transferred to separate lysis tubes using a new pipette tip after each sample transfer. Place uncovered samples on a dry double block heater r 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

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

Confirmation

Presumptive positive primary enrichment samples were confirmed by following the appropriate reference method confirmation, 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.

Validation Study

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

Internal Study

Inclusivity and Exclusivity

Methodology

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

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

Thirty frozen non-*Listeria* strain suspensions were thawed and sub-cultured in BHI broth overnight at 37°C ± 1°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 MDA 2 - *Listeria* method. See Table 3.

Results

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

Matrix Study

The method comparison study consisted of evaluating a total of 30 un-paired sample replicates for 10 matrixes, along with naturally contaminated raw chicken (leg pieces), tested at the independent laboratory, evaluating 20 sample replicates using two separate lots, see Table 4a. The raw chicken (leg pieces and fillets) analyzed at the internal lab was inoculated according to AOAC guidelines which are described below, see Table 4b. 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 the naturally contaminated raw chicken (leg pieces). The inoculum was prepared by transferring a single *Listeria* colony from Trypticase Soy Agar with 5% Sheep Blood (SBA) into BHI broth and incubating the culture at 35 ± 2°C for 24 ± 2 hours. Tables 4a and b presents the sample preparation guidelines for the matrix.

All matrixes 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 [10] 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 ± 1 minute at 50 ± 1°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 ± 1°C for 24 ± 2 hours and the colonies were counted. The degree of injury was estimated as:

$$(1-\frac{n_{select}}{n_{nonselect}})x100$$

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

For the inoculation of the whole melons, a single whole melon was placed into a large sterile bag and the blossom end of the melon was inoculated with 100 µL of the diluted *Listeria monocytogenes* culture. The liquid culture was then allowed to soak into the melon. The melon

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

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

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

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

The level of *Listeria monocytogenes* in the low level inoculum was determined by Most Probable Number (MPN) on the day of analysis by evaluating 5 x 50 g, 20 x 25 g (reference method test portions), and 5 x 10 g inoculated test samples. For the high inoculation level, the MPN was determined by examining 5 x 50 g, 5 x 25 g (reference method test portions) and 5 x 10 g . The level of *Listeria* in the low level inoculum for all 125 g test portions was determined by MPN by evaluating 5 x 250 g, 20 or 5 x 125 g (reference method test portions), and 5 x 50 g inoculated test samples. For the high inoculation level, the MPN was determined by examining 5 x 250 g, 5 x 125 g (reference method test portions), and 5 x 50 g inoculated test samples. Each test portion was enriched with the reference method enrichment broth at the reference method dilution scheme and analyzed by the reference method procedure. See Table A for details. The number of positives from the 3 test levels was used to calculate the MPN using the LCF MPN calculator (version 1.6) provided by AOAC RI. [11] (http://www.lcfltd.com/customer/LCFMPNCalculator.exe)

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

***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® 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 hours. After 24 hours of total incubation, the enriched samples were streaked to MOX agar plates and incubated at 35 ±1 °C for 24-48 hours. The enriched samples were re-incubated for an additional 24 hours at 30 ± 1 °C and then streaked to a second MOX agar plate which was incubated for 24-48 hours at 35 ± 1°C. MOX agar plates were examined for suspect colonies, and if present, at least 5 colonies were streaked to TSA containing 0.6% yeast extract (TSA/YE). The

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

AOAC 993.12 Listeria Reference Method

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

ISO 11290-1/A1 Reference Method

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

OAA agar plates, these suspect colonies were streaked to HBO and incubated at 37 ± 1°C for 18-24 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® GP Biochemical Identification following AOAC OMA 2013.02.

3M™ Molecular Detection Assay 2 - Listeria

All 25 g samples were analyzed by the 3M™ MDA 2 - *Listeria* were enriched with 225 mL of Demi-Fraser Broth with the addition of ferric ammonium citrate; all test portions were then homogenized by stomaching thoroughly for 2 ± 0.2 minutes and incubated at 37 ± 1 °C for 24-28 hours. For the 125 g deli turkey test portions, samples were enriched with 1125 mL of Demi-Fraser Broth with the addition of ferric ammonium citrate; test portions were then homogenized by stomaching thoroughly for 2 ± 0.2 minutes and incubated at 37 ± 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 with ferric ammonium citrate and incubated at 37 ± 1 °C for 26 hours. Stainless steel sponge samples were enriched with 225 mL of Demi-Fraser Broth with the addition of ferric ammonium citrate; samples were homogenized by hand thoroughly for 2 ± 0.2 minutes and incubated at 37 ±1 °C for 24 and26 hours. Sealed concrete environmental surface sponge samples were enriched with 100 mL of Demi-Fraser Broth with the addition of ferric ammonium citrate; samples were thoroughly homogenized by hand for 2 ± 0.2 minutes and incubated at 37°C for 24 and 26 hours. Plastic environmental surface swab samples were enriched with 10 mL of Demi-Fraser Broth with the addition of ferric ammonium citrate; samples were homogenized by vortexing for 2 ± 0.2 minutes and incubated at 37 ±1 °C for 24 and 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 in the 3M Molecular Detection Heat Block Insert and heated for15±1 minutes at 100 ± 1°C. Following the heat lysis, samples were transferred into the Chill Block Insert and placed on a 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. Also lysed was a sterile Demi Fraser Broth with FAC tube 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™ 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

procedure. There were 3 observed positives for the reference method. For the high inoculation level, there were 3 presumptive positives and 3 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 4 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 X.

Compared to ISO 11290-1/A1

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 6 presumptive positives and 6 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 9 observed positives for the reference method. For the high inoculation level, there were 3 presumptive positives and 3 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 4 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 X.

Cold smoked salmon

Compared to FDA-BAM

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 10 presumptive positives and 10 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 7 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 4 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 X.

Compared to ISO 11290-1/A1

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 10 presumptive positives and 10 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 8 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 4 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 X.

<u>*Independent Study using Demi Fraser with FAC brand Y.*</u>

Deli turkey (125 g)

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

procedure. There were 7 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.

Naturally Contaminated raw chicken leg pieces (25 g)

Lot 1

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

Lot 2

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

Whole melon

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 12 presumptive positives and 10 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure, resulting in 2 false positives. There were 8 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the FDA/BAM Chapter 10 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.

Vanilla ice cream (25 g)

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 7 presumptive positives and 7 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 7 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12 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.

Queso fresco (25 g)

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

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.

4% Milk fat cottage cheese (25 g)

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 12 presumptive positives and 12 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 9 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12 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.

Beef hot dog (25 g)

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 4 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure, resulting in 1 false negative. There were 6 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.

Stainless steel (sponge enriched in 225 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 5 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 8 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.

Sealed concrete (sponge enriched in 100 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.

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

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).

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 trouble shooting 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*

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."

Conclusion

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

References

1. Food and Drug Administration Bacteriological Analytical Manual Chapter 10: *Detection and Enumeration of Listeria monocytogenes in Foods*. February, 2013. (Accessed January 2015), http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm

2. United States Department of Agriculture Microbiological Laboratory Guidelines 8.09: *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and Environmental Samples*. May 1st, 2013. (Accessed January 2015)

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

3. Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., AOAC Official Method 993.12 *Listeria monocytogenes* in Milk and Dairy Products: Selective Enrichment and Isolation Method, First Action 1993, Final Action 1996

4. ISO 11290-1/A1 (2004) - Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: detection of *Listeria monocytogenes* in foods, http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=60313

5. U.S. Food and Drug Administration, and U.S. Food Safety and Inspection Service. 2003. Quantitative Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods. Available in Docket No. 1999N-1168, Vols. 23 through 28, and at Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods,

http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/FoodProcessingHACCP/ucm073110.htm

6. Doyle ME, Mazzotta AS, Wang T, Wiseman DW, and Scott VN. 2001. Heat resistance of *Listeria monocytogenes*. *Journal of Food Protection* 64 (3): 410-429.

7. ISO 18593. Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.

8. AOAC Research Institute *Performance Tested Methods*^{SM} *Program* validation outline protocol: *Comparative Evaluation of the 3M*^{TM} *Molecular Detection Assay Listeria monocytogenes 2 and of the 3M*^{TM} *Molecular Detection Assay Listeria* 2 (February 2015)

9. ISO 16140:2003. Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods.

10. U. S. Food and Drug Administration *Bacteriological Analytical Manual*, Chapter 3 *Aerobic Plate Count (*January 2001) (Accessed January 2015) http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.m

11. Least Cost Formulations, Ltd., MPN Calculator Version 1.6 www.lcfltd.com/customer/LCFMPNCalculator.exe (Accessed January 2015)

12. AOAC International, *Evaluation of the* VITEK 2 *Gram Positive (GP) Microbial Identification Test Card: Collaborative Study*, Journal of AOAC International, 95, 1425 (2012)

13. Wehling, P., LaBudde, R., Brunelle, S., Nelson, M. *Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods.* Journal of AOAC International, Vol. 94. No. 1, 2011

14. Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook Version 2.2 (2011) -http://lcfltd.com/aoac/aoac-binary-v2-2.xls(Accessed January 2015)

15. Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, AOAC INTERNATIONAL, Gaithersburg, MD, http://www.aoac.org/imis15_prod/AOAC_Docs/StandardsDevelopment/eoma_appendix_j.pdf (Accessed January 2015)

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

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		
							Confirmation 100µl	
						MDA 2 - *Listeria* spp	ALOA	PALCAM
1	*Listeria*	*monocytogenes*	1011/1410	Frozen broccoli	28	+	H+	+
2	*Listeria*	*monocytogenes*	153	Soft cheese (Munster)	39	+	H+	+
3	*Listeria*	*monocytogenes*	1973/2400	Egg and ham pastry (Quiche Lorraine)	38	+	H+	+
4	*Listeria*	*monocytogenes*	38/181	Toulouse sausages	33	+	H+	+
5	*Listeria*	*monocytogenes*	7111/7516	Pâté (Rillettes)	64	+	H+	+
6	*Listeria*	*monocytogenes*	913/1048	Black pudding	39	+	H+	+
7	*Listeria*	*monocytogenes*	A00C036	Poultry (guinea)	41	+	H+	+
8	*Listeria*	*monocytogenes*	A00C041	Sausage	38	+	H+	+
9	*Listeria*	*monocytogenes*	A00C044	Poultry (Duck)	34	+	H+	+
10	*Listeria*	*monocytogenes*	A00L097	Milk	60	+	H+	+
11	*Listeria*	*monocytogenes*	A00M009	Smoked salmon	40	+	H+	+
12	*Listeria*	*monocytogenes*	Ad 253	Semi-hard cheese	58	+	H+	+
13	*Listeria*	*monocytogenes*	Ad 266	Poultry	26	+	H+	+
14	*Listeria*	*monocytogenes*	Ad 270	Fermented sausage	42	+	H+	+
15	*Listeria*	*monocytogenes*	Ad 273	Cured delicatessen	27	+	H+	+
16	*Listeria*	*monocytogenes*	Ad 274	Ready-to-eat food (Asiatic meal)	40	+	H+	+
17	*Listeria*	*monocytogenes*	Ad 534	Fruits	54	+	H+	+
18	*Listeria*	*monocytogenes*	Ad 548	Environment (Seafood)	37	+	H+	+
19	*Listeria*	*monocytogenes*	Ad 623	Bread crumbs	50	+	H+	+
20	*Listeria*	*monocytogenes*	Ad 665	Raw milk	33	+	H+	+
21	*Listeria*	*grayi*	Ad 1198	Smoked salmon	158 (+25ml UHT milk)	+	H-	st
					1300	+	H-(2)	-
22					1300 (+25ml UHT milk)	+	H-	-
	Listeria	*grayi*	Ad 1443	Pork meat sausages	12(+25ml UHT milk)	+	H-	-
23	*Listeria*	*innocua*	1	Smoked salmon	40	+	H-	+
24	*Listeria*	*innocua*	Ad 658	Gorgonzola	28	+	H-	+
25	*Listeria*	*innocua*	Ad 65[illegible]	Brine	19	+	H-	+
26	*Listeria*	*innocua*	Ad 660	Bread crumbs	35	+	H-	+
27	*Listeria*	*innocua*	Ad 663	Environment (dairy industry)	25	+	H-	+
28	*Listeria*	*innocua*	Ad 671	Smocked bacon	10	+	H-	+
29	*Listeria*	*innocua*	Ad 661	Soft cheese (Pont L'Evêque)	15	+	H-	+
30	*Listeria*	*innocua*	Ad 659	Environment (dairy industry)	14	+	H-	+
31	*Listeria*	*ivanovii*	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		
							Confirmation 100µl	
						MDA 2 - *Listeria* spp	ALOA	PALCAM
32	*Listeria*	*ivanovii*	Ad 662	Environment (dairy industry)	20	+	H+	+
33	*Listeria*	*ivanovii*	BR11	Environment (fish)	29	+	H+	+
34	*Listeria*	*ivanovii*	Ad 1289	Raw milk cheese	30	+	H+	+
35	*Listeria*	*ivanovii*	Ad 1290	Milk powder	17	+	H+	+
36	*Listeria*	*ivanovii*	Ad 1291	Poultry	18	+	H+	+
37	*Listeria*	*ivanovii*	Ad 1288	Sheep milk	80	+	H+	+
38	*Listeria*	*ivanovii* subsp. *londoniensis*	CIP103466	Unknown	14	+	H-	+
39	*Listeria*	*seeligeri*	Ad 649	Cheese	29	+	H-	+
40	*Listeria*	*seeligeri*	Ad 651	Environment	36	+	H-	+
41	*Listeria*	*seeligeri*	Ad 652	Environment (dairy industry)	28	+	H-	+
42	*Listeria*	*seeligeri*	Ad 674	Soft cheese (Munster)	240	+	H-	H-
43	*Listeria*	*seeligeri*	BR1	Trout	28	+	H-	+
44	*Listeria*	*seeligeri*	BR18	Environment (fish)	36	+	H-	+
45	*Listeria*	*seeligeri*	CIP100100	Unknown	8	+	st	1col
46	*Listeria*	*welshimeri*	Ad1276	Environment (Slaughterhouse)	44	+	H-	+
47	*Listeria*	*welshimeri*	Ad1235	Beef meat	26	+	H-	+
48	*Listeria*	*welshimeri*	191424	Poultry	24	+	H-	+
49	*Listeria*	*welshimeri*	Ad 1175	Ready-to-eat-food	46	+	H-	+
50	*Listeria*	*welshimeri*	Ad 650	Poultry	33	+	H-	+

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.

Table 2b. Inclusivity Study Results

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

- 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 – *Listeria*
1	*Bacillus*	*cereus*	Ad 465	Salmon Terrine	$7{,}2\times10^{4}$	-
2	*Bacillus*	*circulans*	Ad 760	Vegetables	$1{,}0\times10^{4}$ (opacity +)	-
3	*Bacillus*	*coagulans*	Ad 731	Dairy product	$<2{,}0\times10^{3}$ (opacity +)	-
4	*Bacillus*	*licheniformis*	Ad 978	Dairy product	$<2{,}0\times10^{3}$ (opacity+)	-
5	*Bacillus*	*mycoïdes*	Ad 762	Milk	$5{,}6\times10^{4}$	-
6	*Bacillus*	*pseudomycoides*	Ad 765	Vegetables	$2{,}0\times10^{4}$	-
7	*Bacillus*	*pumilus*	Ad 284	Ready-to-eat	$1{,}7\times10^{5}$	-
8	*Bacillus*	*weihenstephanensis*	Ad 726	Egg product	$6{,}8\times10^{4}$	-
9	*Brochothrix*	*thermosphacta*	FN 15129	Trout	$8{,}5\times10^{5}$	-
10	*Brochrotrix*	*campestris*	CIP 102920T	Environment	$<2{,}0\times10^{3}$ (opacity +)	-
11	*Carnobacterium*	*divergens*	CIP 101029^{T}	Unknown	$>2{,}0\times10^{3}$ (opacity +)	-
12	*Carnobacterium*	*piscicola*	Ad 369	Raw milk	$<2{,}0\times10^{3}$ (opacity +)	-
13	*Enterococcus*	*durans*	Ad 149	Ham	$<2{,}0\times10^{3}$ (opacity +)	-
14	*Enterococcus*	*faecalis*	89L326	Soft cheese (Vacherin)	$1{,}6\times10^{5}$	-
15	*Lactobacillus*	*brevis*	86L126	Ham	$1{,}4\times10^{5}$	-
16	*Lactobacillus*	*curvatus*	Ad 380	Delicatessen	$1{,}7\times10^{5}$	-
17	*Lactobacillus*	*fermentum*	Ad 482	Tomatoes juice	$1{,}2\times10^{5}$	-
18	*Lactobacillus*	*sakei*	Ad 473	Ham	$4{,}9\times10^{5}$	-
19	*Lactococcus*	*lactis subsp cremoris*	Ad 137	Dairy product	$>2{,}0\times10^{3}$ (opacity +)	-

20	*Leuconostoc*	*carnosum*	Ad 411	Ham	$6,8x10^5$	-
21	*Leuconostoc*	*citreum*	Ad 396	Ham	$4,9x10^5$	-
22	*Micrococcus*	*luteus*	Ad 432	Cocktail	$<2,0x10^3$ (opacity+)	-
23	*Pediococcus*	*pentosaceus*	ATCC 33316	Unknown	$1,0x10^5$	-
24	*Propionibacterium*	*freundenreichii*	CNRZ 725	Dairy product	$<2,0x10^3$ (opacity +)	-
25	*Staphylococcus*	*aureus*	Ad 165	Smoked delicatessen	$1,3x10^5$	-
26	*Staphylococcus*	*aureus*	Ad 902	Nems	$8,2x10^4$	-
27	*Staphylococcus*	*epidermidis*	Ad 931	Fruits	$2,0x10^3$	-
28	*Staphylococcus*	*haemolyticus*	Ad 989	Dairy product	$2,8x10^4$	-
29	*Streptococcus*	*bovis*	92L622	Dairy product	$2,0x10^3$	-
30	*Streptococcus*	*salivarius*	Ad 441	Dairy product	$<2,0x10^3$ (opacity +)	-

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

CNRZ = National Centre for Zootechnical Research, Jouy-en-Josas, France
ATCC = American Type Culture Collection

1 **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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes* FSL[3] 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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes* CWD[2] 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	*L. monocytogenes* ATCC[1] 19112 & *L. welshimeri* ATCC[1] 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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes ATCC1 19118 & 10x Enterococcus faecium 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		
Sealed Concrete	*L. monocytogenes ATCC1 19117*	0 CFU/4" x 4" (100 cm2)	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/4" x 4" (100 cm2)	20		
		~100 CFU/4" x 4" (100 cm2)	5		
Plastic	*L. monocytogenes ATCC1 51782 & L. seeligeri ATCC1 35967 &*	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		
		~100 CFU/1" x 1" (5 cm2)	5		

	10x Enterococcus faecalis ATCC1 29212				

[1] ATCC - American Type Culture Collection
[2] CWD - University of Vermont
[3] FSL - Cornell University
[*] Natural contamination was present during the background screen: 2 separate lots evaluated (20 replicates each), *Listeria monocytogenes* and *Listeria innocua* were isolated.

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[2] leg pieces	*L. ivanovii* Ad[1] 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[3]	*L. ivanovii* 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[2] spinach	*Listeria seeligeri* 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[2] spinach	*Listeria seeligeri* 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[2] salmon	*Listeria innocua* 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[2] salmon	*Listeria innocua* 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		

[1] Ad – Adria Developement Culture Collection
[2] Tested using Demi Fraser brand (with FAC) X
[3] 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[1] (CFU/g)	Background *Listeria* (# positive/total tested)
Deli Turkey (125 g)	1.5×10^3	0/5[(2)]
Naturally Contaminated Raw Chicken Leg Pieces (25 g)	2.8×10^5	4/5[(2)]
Whole Melons	1.0×10^2	0/5[(3)]
Vanilla Ice Cream (25 g)	8.0×10^1	0/5[(4)]
Queso Fresco (25 g)	3.8×10^5	0/5[(4)]
4% Milk Fat Cottage Cheese (25 g)	1.7×10^4	0/5[(4)]
Beef Hot Dogs (25 g)	1.6×10^2	0/5[(2)]
Beef Hot Dogs[5] (25 g)	6.0×10^3	0/5[(2)]

[1] APC conducted in accordance with FDA/BAM Chapter 3
[2] *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines
[3] *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines
[4] *Listeria species* screen conducted following the AOAC 993.12 guidelines
[5] 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[1] (CFU/g)	Background *Listeria* (# positive/total tested)
Raw Chicken Leg Pieces (25 g)	4.0×10^5	0/5[(2)]
Raw chicken fillet (25 g)	1.4×10^5	0/5[(2)]
Bagged Raw Spinach (25 g)	9.3×10^6	0/5[(3,4)]
Cold Smoked Salmon (25 g)	2.0×10^2	0/5[(3,4)]

[1] APC conducted in accordance with FDA/BAM Chapter 3
[2] *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines
[3] *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines
[4] *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[1]	25 g	*Listeria monocytogenes* ATCC 7644	TSA	1.8×10^8	58.3 %
			MOX	7.5×10^7	
Deli Turkey	125 g	*Listeria monocytogenes* ATCC 19116	TSA	2.6×10^9	68.5 %
			MOX	8.2×10^8	
Queso Fresco	25 g	*Listeria monocytogenes* ATCC CWD 1554	TSA	2.6×10^9	72.7%
			MOX	7.1×10^8	
Beef Hot Dogs[2]	25 g	*Listeria monocytogenes* ATCC 7644	TSA	2.6×10^9	73.8%
			MOX	6.8×10^8	

[1]- Testing for Robustness and Lot to Lot
[2]- 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	*Listeria monocytogenes* FSL J1-049		
Low-Inoculum Level CFU[a]/Melon[b]	7		
High-Inoculum Level CFU[a]/Test Melon[b]	55		
Matrix	**Stainless Steel**		
Inoculating Organism	*Listeria monocytogenes* ATCC 19118	*Enterococcus faecium* ATCC 19434	
Low-Inoculum Level CFU[a]/Test Area[c]	42	530	
High-Inoculum Level CFU[a]/Test Area[c]	400	4000	
Matrix	**Sealed Concrete**		
Inoculating Organism	*Listeria monocytogenes* ATCC 19117		
Low-Inoculum Level CFU[a]/Test Area[c]	35		
High-Inoculum Level CFU[a]/Test Area[c]	360		
Matrix	**Plastic**		
Inoculating Organism	*Listeria monocytogenes* ATCC 51782	*Listeria seeligeri* ATCC 35967	*Enterococcus faecalis* ATCC 29212
Low-Inoculum Level CFU[a]/Test Area[d]	42	64	540
High-Inoculum Level CFU[a]/Test Area[d]	420	590	3600

[a] CFU: aliquots of the inocula were plated in triplicate onto TSA and averaged.
[b] Test Area: Whole Melon
[c] Test Area: 4" x 4" Surface Area
[d] 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[a]/ Test Portion	N[b]	3M MDA2			USDA/FSIS MLG			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Raw Chicken Leg Pieces[h] (25 g)	*L. ivanovii* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**3M MDA2**			**USDA/FSIS MLG**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Raw Chicken Fillet[i] (25 g)	*L. ivanovii* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**3M MDA2**			**FDA-BAM**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Bagged Raw Spinach[h] (25 g)	*L. seeligeri* 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**
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**3M MDA2**			**ISO 11290-1**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Bagged Raw Spinach[h] (25 g)	*L. seeligeri* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**3M MDA2**			**FDA-BAM**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Cold Smoked Salmon[h] (25 g)	*L. innocua* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**3M MDA2**			**ISO 11290-1**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Cold Smoked Salmon[h] (25 g)	*L. innocua* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_C = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD_R = Reference method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[h]Tested using Demi Fraser (with FAC) brand X
[i]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[a]/ Test Portion	N[b]	3M MDA2			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Deli Turkey (125 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Whole Melon	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Vanilla Ice Cream (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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[a]/ Test Portion	N[b]	3M MDA2			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Queso Fresco (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
4% Milk Fat Cottage Cheese (25 g)	*L. welshimeri* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Beef Hot Dogs (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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[a]/ Test Area	N[b]	3M MDA2			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Stainless Steel (225 mL)	*L. monocytogenes* ATCC 19118 & *Enterococcus faecium* 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[a]/ Test Area**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Sealed Concrete (100 mL)	*L. monocytogenes* 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[a]/ Test Area**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Plastic (10 mL)	*L. monocytogenes* ATCC 51782 & *Enterococcus faecalis* 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

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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[a]/ Test Portion	N[b]	Presumptive			Confirmed			$dPOD_{CP}$[f]	95% CI[g]
					x[c]	POD_{CP}[d]	95% CI	X	POD_{CC}[e]	95% CI		
Raw Chicken Leg Pieces[h] (25 g)	*L. ivanovii* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Raw Chicken Fillet[i] (25 g)	*L. ivanovii* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Bagged Raw Spinach[h] (25 g)	*L. seeligeri* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Cold Smoked Salmon[h] (25 g)	*L. innocua* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[h]Tested using Demi Fraser brand (with FAC) brand X.
[I]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[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Deli Turkey (125 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
Whole Melon	*L. monocytogenes* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
Vanilla Ice Cream (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCE MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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[a]/ Test Portion	N[b]	Presumptive			Confirmed			$dPOD_{CP}$[f]	95% CI[g]
					x[c]	POD_{CP}[d]	95% CI	X	POD_{CC}[e]	95% CI		
Queso Fresco (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
4% Milk Fat Cottage Cheese (25 g)	*L. welshimeri* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Beef Hot Dogs (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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) broth Y.

Table 15: 3M™ MDA 2 - *Listeria* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)

Matrix	Strain	Analysis Time Point	CFU[a]/ Test Area	N[b]	Presumptive			Confirmed			$dPOD_{CP}$[f]	95% CI[g]
					x[c]	POD_{CP}[d]	95% CI	X	POD_{CC}[e]	95% CI		
Stainless Steel (225 mL)	*L. monocytogenes* ATCC 19118 & *Enterococcus faecium* 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[a]/ Test Area**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Sealed Concrete (100 mL)	*L. monocytogenes* 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[a]/ Test Area**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Plastic (10 mL)	*L. monocytogenes* ATCC 51782 & *Enterococcus faecalis* 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

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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**
Listeria monocytogenes ATCC[2] 7644					
Low Level	6/10	Low Level	6/10	Low Level	6/10
Uninoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5	Uninoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5	Uninoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5

[1]All samples were analyzed from a common lysate
[2]ATCC- American Type Culture Collection

Table 17: Robustness Results

Robustness[1]			Robustness[1]		
13 Minute Lysis			13 Minute Lysis		
Sample #	**18 µL[2], 18 µL[3]**	**18 µL[2], 22 µL[3]**	**Sample #**	**22 µL[2], 18 µL[3]**	**22 µL[2], 22 µL[3]**
Listeria monocytogenes ATCC[4] 7644					
Low Level	6/10	6/10	Low Level	7/10	7/10
Uninoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5	0/5	Uninoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5	0/5
Robustness[1]			**Robustness[1]**		
17 Minute Lysis			**17 Minute Lysis**		
Sample #	**18 µL[2], 18 µL[3]**	**18 µL[2], 22 µL[3]**	**Sample #**	**22 µL[2], 18 µL[3]**	**22 µL[2], 22 µL[3]**
Listeria monocytogenes ATCC[4] 7644					
Low Level	6/10	6/10	Low Level	6/10	6/10
Uninoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5	0/5	Uninoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5	0/5

[1]All samples were analyzed from a common lysate
[2]Volume of enrichment to lysis
[3]Volume of lysis to assay
[4]ATCC- American Type Culture Collection

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

Patrick Bird, Jonathan Flannery, Erin Crowley, James Agin, David Goins
Q Laboratories, Inc., 1400 Harrison Ave, Cincinnati, OH 45214

Lisa Monteroso
3M Food Safety Department, 3MCenter – Bldg. 260-6B-01, St. Paul, MN 55144

Collaborators: R. Brooks, J. Walia, F. Hernandez, D. Bosco, G. Trevino, A. Brandt, C. Lopez, E. Sjogren, M. Shekhawat, L. Ma, C. Timmons, C. Diaz Proano,A. Calle,Z. Metz, D. Baumler, R. Smith, D. Wood, E. Maranan, C. Chavarria,J. Miller, B. Schindler, C. Gies, E. Budge, Z. Geurin, A. Repeck, C. Zook, C. Barnes, B. Bastin, D. Isfort

The 3M™ Molecular Detection Assay (MDA) 2 –*Listeria monocytogenes*usesloop-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 valuewith 95% confidence interval of 0.16, (0.04, 0.28) indicating astatisticallysignificant differencebetween the candidate and reference methods with a positive correlation in data indicating more recovery of the target analyte by the candidate method.

*Listeriamonocytogenes*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]. *Listeriamonocytogenes* 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

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

Prior to the collaborative study, the 3M MDA 2 - *Listeria monocytogenes* method was validated according to AOAC Guidelines[4] in a harmonized AOAC® Performance Tested Method℠ (PTM) study. The objective of the PTM study was to demonstrate that the 3M MDA 2-*Listeria monocytogenes* method could detect *Listeria monocytogenes*in a broad range of food matrices and environmental surfaces as claimed by the manufacturer. For the 3M MDA 2- *Listeria monocytogenes* PTM evaluation, 13 matrices were evaluated: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), and plastic (Enviroswab, 10 mL) environmental samples.

Additional PTM parameters (inclusivity, exclusivity, ruggedness, stability and lot-to-lot variability) tested in the PTM studies satisfied the performance requirements for PTM approval. The method was awarded PTM certification number 081501 on August 18th, 2015.

The purpose of this collaborative studywas to compare the reproducibility of the 3M MDA 2 - *Listeria monocytogenes*method 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*[5] for deli turkey (125 g) and raw chicken breast fillet.

Collaborative Study

Study Design

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

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

A detailed collaborative study packet outlining all necessary information related to the study including media preparation, test portion preparation and documentation of results was sent to each collaborating laboratory prior to the initiation of the study. A conference call was then

conducted to discuss the details of the collaborative study packet and answer any questions from the participating laboratories.

Preparation of Inocula and Test Portions

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

$$(1-\frac{n_{select}}{n_{nonselect}})x100$$

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

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

Test Portion Distribution

All samples were labeled with a randomized, blind-coded 3-digit number affixed to the sample container. Test portions were shipped on a Thursday via overnight delivery according to the Category B Dangerous Goods shipment regulations set forth by the International Air Transportations Association(IATA).The two matrices were shipped consecutively, with collaborating laboratories performing analysis on one matrix at a time. Upon receipt, samples were held by the collaborating laboratory at refrigeration temperature (2-8 °C) until the following Monday when analysis was initiatedafter a total equilibration time of 96 hours.All samples were packed with cold packs to target a temperature of < 7°C during shipment.
In addition to each of the test portions and a separate APCsample, collaborators received a test portion for each matrix labeled as 'temperature control'. Participants were instructed to obtain the temperature of this portion upon receipt of the package, document the results on the Sample

Receipt Confirmation form provided and fax or email it back to the study director.The shipment and hold timesof the inoculated test material had been verified as a quality control measure prior to study initiation.

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 monocytogenes* 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 monocytogenes* method, a 125 g portion was enriched with 975 mL of Demi-Fraser (DF) with ferric ammonium citrate (FAC) broth, homogenized for 2 minutes and incubated for 24-28 hours at 37 ±1°C. For the raw chicken breast fillettest portions analyzed by the 3M MDA 2 - *Listeria monocytogenes* method, a 25 g portion was enriched with 475 mL of DF, homogenized for 2 minutes and incubated for 28-32 hours at 37 ±1°C.

Following enrichment, samples were assayed by the 3M MDA 2 - *Listeria monocytogenes* 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 monocytogenes* 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 monocytogenes* 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 monocytogenes* 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, $POD_{CP,}$ the candidate confirmatory results (including false negative results), POD_{CC}, the difference in the candidate presumptive and confirmatory results, $dLPOD_{CP,}$ presumptive candidate results that confirmed positive (excluding false negative results), $POD_{C,}$ the reference method, POD_R, and the difference in the confirmed candidate and reference methods, $dLPOD_C$. A $dLPOD_C$confidence interval not containing the point zero would indicate a statistically significant difference between the 3M MDA 2 -*Listeria monocytogenes*and the reference methods at the 5 % probability level. In addition to POD, the repeatability standard deviation (s_r), the among laboratory repeatability standard deviation (s_L), the reproducibility standard deviation (s_R)and the P_T value were calculated. The s_r provides the variance of data within one laboratory, the s_L provides the difference in standard deviation between laboratories and the s_R provides the variance in data between different laboratories. The P_T value provides information on the homogeneity test of laboratory PODs [10].

AOAC Official Method 2016.xxx
Listeria monocytogenes in SelectFoods and Environmental Surfaces
3M™ Molecular Detection Assay(MDA) 2- *Listeria monocytogenes* Method
First Action 2016

(Applicable to detection of *Listeria monocytogenes* 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); raw chicken breast fillet (25g), concrete (3M™ Hydrated Sponge Stick with Dey-Engley [D/E] , 225 mL & 100 mL), stainless steel (3M Hydrated Sponge Stick with D/E, 225 mL), and plastic (3M™ Enviroswab with Letheen, 10 mL) environmental samples.

*See Tables **2016.1A** and **2016.1B** for a summary of results of the inter-laboratory study.*
*See Tables **2016.2A** and **2016.2B** for detailed results of the inter-laboratory study*

A. Principle

The 3M™ Molecular Detection Assay (MDA) 2 - *Listeria monocytogenes* method is used with the 3M™ Molecular Detection System (MDS) for the rapid and specific detection of *Listeria monocytogenes* in enriched food and food process environmental samples. The 3M MDA2 - *Listeria monocytogenes* uses loop-mediated isothermal amplification of unique DNA target 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. Samples are pre-enriched in Demi Fraser with ferric ammonium citrate broth.

B. Apparatus and Reagents

Items (b)-(g) are available as the 3M™ Molecular Detection Assay (MDA) 2 - *Listeria monocytogenes* kit from 3M Food Safety (St. Paul, MN 55144-1000, USA).

- **(a)** *3M Molecular Detection System* (MDS100) – Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- **(b)** *3M Molecular Detection Assay 2 – Listeria monocytogenes reagent tubes*- 12 strips of 8 tubes. Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- **(c)** *Lysis Solution (LS) tubes* – 12 strips of 8 tubes.
- **(d)** *Extra caps* – 12 strips of 8 caps
- **(e)** *Reagent Control* – 8 reagent tubes
- **(f)** *Quick Start Guide*
- **(g)** *3M™ Molecular Detection Speed Loader Tray* - Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- **(h)** *3M™ Molecular Detection Chill Block Insert* - Available from 3M™ Food Safety (St. Paul, MN 55144-1000, USA).
- **(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 ± 1°C
(v) *Dry block heater unit*– capable of maintaining 100 ± 1°C
(w) *Incubators.* – Capable of maintaining 37 ± 1°C or 41.5 ± 1°C.
(x) *Freezer* – capable of maintaining -10 to -20°C, for storing the 3M Molecular Detection Chill Block Tray
(y) *Refrigerator* – capable of maintaining 2-8°C, for storing the 3M Molecular Detection Assay components
(z) *Computer* – compatible with the 3M™ Molecular Detection Instrument
(aa) *3M™ Enviroswab,* -- (hydrated with Letheen) Available from 3M Food Safety(Australia)
(bb) *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°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 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 industries. For example, 3M has not documented this product for testing drinking water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M MDA2 – *Listeria monocytogenes*has not been evaluated with all possible food products, food processes, testing protocols or with all possible strains of bacteria.

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

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.

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

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

*Listeria monocytogenes*is of particular concern for pregnant women, the aged and the infirmed. It is recommended that these concerned groups avoid handling this organism.After use, the enrichment medium and the 3M MDA 2 - *Listeria monocytogenes*tubes can potentially contain pathogenic materials. 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. 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.

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

D. Sample Enrichment

Foods

(a) Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature (20-25^{o}C).

(b) 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.

(c) Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ±0.2 minutes. Incubate at 37 ±1°C according to Table A.

Environmental samples

(d) 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 Letheen 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. Another option is to transfer 10 µL of the NB enrichment in the LS tubes.

(e) The recommended size of the sampling area to verify the presence or absence of the pathogen on the surface is at least 100 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, USDA FSIS MLG or ISO 18593 [11] guidelines.

1. Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature (20-25°C).
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.

E. Preparation of the 3M™ Molecular Detection Speed Loader Tray

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

Specific Instructions for Validated Methods
AOAC® Performance Tested Method℠ # 081501

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 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 2. 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 2).

Table 2. Enrichment protocols using Demi-Fraser Broth at 37 ± 1 °C according to AOAC Performance Tested ^{SM} 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

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 ±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 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 ±1°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°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:

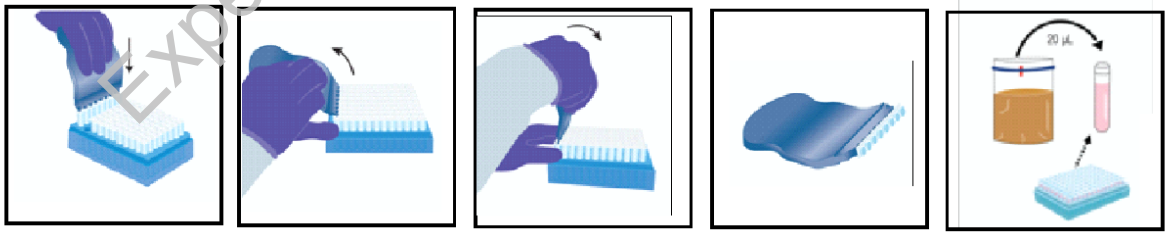
Transfer each enriched sample into individual LS tube **first**. Transfer the NC **last**.

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 **<u>20 µL of NC</u> 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

heat for 15 ±1 minutes. During heating, the LS solution will change from pink (cool) to yellow (hot).

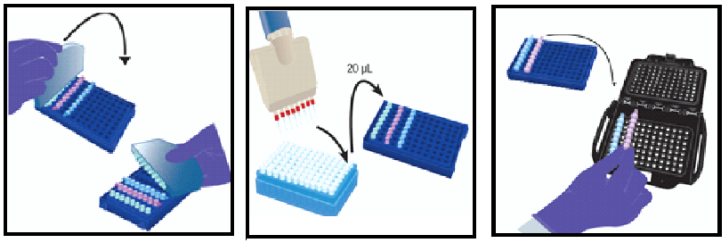
5. Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert at least 5 minutes and a maximum of 10 minutes. The 3M Molecular Chill Block Insert, used at ambient temperature (20-25°C) without the Molecular Detection Chill Block Tray, 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 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.
   - 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 µL of NC lysate into a Reagent tube.
   - 4.7 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.
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 monocytogenes*amplificationreagents 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.

Results of Collaborative Study

For this collaborative study, the 3M Molecular Detection Assay (MDA) 2 - *Listeria monocytogenes* method was compared to the USDA FSIS MLG 8.09 reference method for deli turkey and raw chicken breast fillet. A total of 13 laboratories throughout the United Statesand Canada participated in this study, with 11 laboratories submitting data for the deli turkey and 12 laboratories submitting data for the raw chicken breast fillet. See Table 1 for a summary of laboratory participation for each matrix. Each laboratory analyzed 36 test portions for each

method per matrix: 12 inoculated with a high level of *Listeria monocytogenes*, 12 inoculated with a low level of *Listeria monocytogenes*, and 12 un-inoculated controls.
A background screen of the matrix indicated an absence of indigenous *Listeria monocytogenes*in both matrices.Ten (10) replicate test portions (randomly sampled from 50% of the total packages used in the analysis) were screened for the presence of *Listeria monocytogenes*. All test portions produced negative results for the target analyte.
Results for the heat stress analysis of the inoculum for the deli turkey are presented in Table 2.
Table 2016.1A and 2016.1B summarize the inter-laboratory results for all foods tested, including POD statistical analysis. As per criteria outlined in Appendix J of the AOAC Validation Guidelines, fractional positive results were obtained.Detailed results for each laboratory are presented in Tables 2016.2A and 2016.2B. For each matrix, the level of *Listeria monocytogenes* was determined by MPN on the day of initiation of analysis by the coordinating laboratory. MPN results are presented in Tables 2016.2A and 2016.2B.The individual laboratory and sample results are presented in Tables 1-2 of the Supplementary Materials. The APCresults for each collaborating are presented in Table3 of the Supplementary Materials.

Deli Turkey (125 g Test Portions)

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

For the low inoculum level, 69 out of 132 test portions (POD_{CP} of 0.52) were reported as presumptive positive by the 3M MDA 2 – *Listeria monocytogenes*method with 66out of 132 test portions (POD_{CC} of 0.50) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria monocytogenes*method, 66out of 132 samples confirmed positive (POD_C of 0.50). For test portions evaluated by the USDA/FSIS MLG reference method, 60 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.04 with 95% confidence intervals of (-0.08, 0.17) was obtained between the candidate and reference method, indicating no statistically significant difference between the two methods. A $dLPOD_{CP}$ value of 0.02 with 95% confidence intervals of (-0.10, 0.15) was obtained between presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results.

For the high inoculum level, 132 out of 132 test portions (POD_{CP} of 1.00) were reported as presumptive positive by the 3M MDA 2 – *Listeria monocytogenes*method with 132out of 132 test portions (POD_{CC} of 1.00) confirming positive.For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria monocytogenes*method, 132out of 132 samples confirmed positive (POD_C of 1.00). For test portions evaluated by the USDA/FSISMLG reference method, 132 out of 132 test portions produced positive results. A $dLPOD_C$ value of

0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between the candidate and reference method, indicating no statistically significant difference between the two methods. A $dLPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results.

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

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

Raw Chicken Breast Fillet (25 g Test Portions)

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

For the low inoculum level, 86 out of 132 test portions (POD_{CP} of 0.65) were reported as presumptive positive by the 3M MDA 2 – *Listeria monocytogenes*method with 86out of 132 test portions (POD_{CC} of 0.65) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria monocytogenes*method, 85out of 132 test portions confirmed positive (POD_C of 0.64). For test portions evaluated by the USDA/FSIS MLGreference method, 64 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.16 with 95% confidence intervals of (0.04, 0.28) was obtained between the candidate and reference method, indicating a statistically significant difference between the two methods, with a positive correlation in data indicating more recovery of the target analyte by the candidate method. A $dLPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.12, 0.12) was obtained between

presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results.

For the high inoculum level, 129 out of 132 test portions (POD_{CP} of 0.98) were reported as presumptive positive by the 3M MDA 2 – *Listeria monocytogenes*method with 132out of 132 test portions (POD_{CC} of 1.00) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria monocytogenes*method, 129out of 132 samples confirmed positive (POD_C of 0.98). For test portions evaluated by the USDA/FSIS-MLG reference method, 132 out of 132 test portions produced positive results. A $dLPOD_C$ value of -0.02 with 95% confidence intervals of (-0.06, 0.01) was obtained between the candidate and reference method, indicating no statistically significant difference between the two methods. A $dLPOD_{CP}$ value of -0.02 with 95% confidence intervals of (-0.06, 0.01) was obtained between presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results.

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

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

Discussion

No negative feedback was provided by the collaborating laboratories in regard to the performance of the 3M MDA 2- *Listeria monocytogenes*method. For the raw chicken breast fillet, Laboratory 10 reported isolating *Listeria monocytogenes*from two un-inoculated control samples. The isolates were sent for further identification and it was determined that they were the same strain as the inoculating organism, indicating that cross contamination of the sample occurred. Due to the fact that cross contamination occurred, just cause for removal of the data was established and the data generated by Laboratory 10 was therefore not included in the statistical analysis.

Overall, the data generated during this evaluation demonstrates the reproducibility of this new method. For the deli turkey analysis, the POD statistical analysis indicated thatno statistically significant difference between the candidate method and the reference method or between the presumptive and confirmed results of the candidate method was obtained. For raw chicken

breastfillet, a statistically significant difference was observed between the reference and the alternative method. The dLPOD data indicated a positive correlation in data indicating more recovery of the target analyte by the candidate method. One possible contribution to the higher level of recovery observed with the 3M MDA 2 – *Listeria monocytogenes*method was the use of Demi-Fraser Broth for the candidate method. This enrichment media formulation is less selective than the modified University of Vermont Medium used in the USDA reference method and may have contributed to the higher level of recovery observed during the evaluation.
A second possible contribution to the higher level of recovery was the length of the primary enrichment. Test portions evaluated by the 3M MDA 2 – *Listeria monocytogenes*method were incubated for a minimum of 28 hours in the primary enrichment, while the USDA reference method had a maximum primary enrichment time of 26 hours. No statistically significant difference was observed between the candidate method presumptive and confirmed results for this matrix.

Recommendations

It is recommended that the 3M Molecular Detection Assay 2 – *Listeria monocytogenes*method be adopted as Official First Action status for the detection of *Listeria monocytogenes* in selected foods: 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), raw chicken breast fillet, concrete (sponge, 225 mL & 100 mL), stainless steel (sponge, 225 mL), and plastic (Enviroswab, 10 mL) environmental samples.

Acknowledgements

We would like to extend a sincere thank you to the following collaborators for their dedicated participation in this study:

Robert Brooks- ATC Microbiology, LLC- North Little Rock, AR.
Jaspreet Walia & Francisco Hernandez – Certified Laboratories, Turlock, CA
David Bosco& Grizelda Trevino – Food Safety Net Services, Fresno, CA
Alex Brandt & Chris Lopez – Food safety Net Services, San Antonio, TX
Elizabeth Sjogren&Manish Shekhawat– Microbac Laboratories, Inc. Worcester, MA
Li Maria Ma, Chris Timmons, & Claudia Diaz Proano – Oklahoma St. University, Stillwater, OK
Alexandra Calle & David Campos – Texas Tech University, Lubbock, TX.
Zachary Metz&David Baumler, University of Minnesota, St. Paul, MN
Robbie Smith, Dianne Wood, Evelyn Maranan,&Carmen Chavarria Maple Leaf Foods– Guelph, ON, Canada
Ben Bastin– Microbiology R&D, Q Laboratories Inc., Cincinnati, OH
David Isfort– Microbiology-Food, Q Laboratories Inc., Cincinnati, OH
Jesse Miller, Bryan Schindler, Courtney Gies, Eric Budge, Zach Geurin & Alex Repeck - NSF International, Ann Arbor, MI
Cynthia Zook & Christina Barnes – 3M Food Safety, St. Paul, MN

We would like to extend a special thanks to the following team members at Q Laboratories, Inc. for their efforts during the collaborative study: M. Joseph Benzinger Jr., Allison Mastalerz, Alison DeShields, Jonathan Clifton, T. Shane Wilson, Meghan Daniel, Nicole Klass and Colleen Sweeney.

References

(1) United States Food and Drug Administration. (2008) *Guidance for Industry: Control of Listeria monocytogenes in Refrigerated or Frozen Ready-to-Eat Foods; Draft Guidance*http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/FoodProcessingHACCP/ucm073110.htm (Accessed March 2016)

(2) European Food Safety Authority. (2015) *Listeria*http://www.efsa.europa.eu/en/topics/topic/listeria (Accessed March 2016)

(3) Centers for Disease Control and Prevention. (2016) *Listeria monocytogenes Outbreaks*http://www.cdc.gov/Listeria monocytogenes/outbreaks/(Accessed March 2016)

(4)*Official Methods of Analysis* of AOAC INTERNATIONAL. (2012) 19th Ed., Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, AOAC INTERNATIONAL, Gaithersburg, MD http://www.eoma.aoac.org/app_j.pdf(Accessed January 2016)

(5)United States Department of Agriculture Food Safety and Inspection Service. (2013) *Microbiological Laboratory Guidelines5.09*: *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and Environmental Samples*. (Accessed January 2016) http://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES

(6) *Official Methods of Analysis* of AOAC INTERNATIONAL (2015) *Enumeration of Aerobic Bacteria in Foods.*2015.13. (Accessed January2016)

(7) Least Cost Formulations, Ltd., MPN Calculator-Version 1.6, www.lcfltd.com/customer/LCFMPNCalculator.exe(Accessed March 2016)

(8) *Official Methods of Analysis* of AOAC INTERNATIONAL (2012) *Gram-Positive Bacteria Identification.* 2012.02. (Accessed January 2016)

(9) Wehling, P., LaBudde, R., Brunelle, S., Nelson, M. (2011) Journal of AOAC International*Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods.* Vol. 94, No. 1. pp. 335-347

(10) Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook (2013), http://lcfltd.com/aoac/aoac-binary-v2-3.xls (Accessed March, 2016)

(11) ISO 18593:2004 -*Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.*

Table 1: Participation of each Collaborating Laboratory

Lab	Deli Turkey[a,b]	Raw Chicken BreastFillet[a,b]
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

[a] Y= Collaborator analyzed the food type;[a]N= Collaborator did not analyze food type

Table 2: Heat-Stress Injury Results

Matrix	Test Organism[a]	CFU/MOX (Selective Agar)	CFU/TSA (Non-Selective Agar)	Degree Injury[b]
Deli Turkey	*Listeria monocytogenes* ATCC 19115	9.1×10^8	2.5×10^9	60.8%

[a] ATCC- American Type Culture Collection
[b] Cultures were heat stressed for 10 minutes at 55°C in a recirculating water bath.

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

Method[a]	**3M™ MDA™2 - *Listeria monocytogenes***		
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[e]	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)[f]	0.00 (-0.03, 0.03)	0.04 (-0.08, 0.17)	0.00 (-0.03, 0.03)
dLPOD (Candidate Presumptive vs. Candidate Confirmed)[f]	0.00 (-0.03, 0.03)	0.02 (-0.10, 0.15)	0.00 (-0.03, 0.03)

[a] Results include 95% Confidence Intervals, [r] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation
[e] P Value = Homogeneity test of laboratory PODs; [f] 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* inRaw Chicken Breast Fillet (25g)

Method[a]	**3M™ MDA™2 -*Listeria monocytogenes***		
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[e]	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)[f]	0.00 (-0.03, 0.03)	0.16 (0.04, 0.28)	-0.02 (-0.06, 0.01)
dLPOD (Candidate Presumptive vs. Candidate Confirmed)[f]	0.00 (-0.03, 0.03)	0.00 (-0.12, 0.12)	-0.02 (-0.06, 0.01)

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

Table 2016.2A: Comparative Results for the Detection of *Listeria monocytogenes* in 125 g Deli TurkeyTest Portions by the 3M™ MDA 2 - *Listeria 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[a]	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[a]	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		

[a]N/A – Laboratory did not participate or submit data for this matrix

[b] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation, [e] P_T Value = Homogeneity test of laboratory PODs

LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

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 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[a]	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[a]	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		

[a] N/A – Laboratory did not participate or submit data for this matrix

[b] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation, [e] P_T Value = Homogeneity test of laboratory PODs

LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

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 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[a]	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[a]	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		

[a] N/A – Laboratory did not participate or submit data for this matrix

[b] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation, [e] P_T Value = Homogeneity test of laboratory PODs
LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

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 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[b]	12	0	0.00	12	2	0.17	12	0	0.00	12	0	0.00	0.00	-0.17
		11[a]	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		

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

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 - *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[b]	12	6	0.50	12	8	0.66	12	6	0.50	12	5	0.42	0.08	-0.16
	MPN/ Test Portion	11[a]	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		

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

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 - *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[b]	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
	MPN/ Test Portion	11[a]	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		

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

Supplementary Materials

Table 1. Individual Collaborator Results for Deli Turkey

	High Level Test Portions												Low Level Test Portions												Un-inoculated Test Portions											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
3M MDA 2 -*Listeria monocytogenes*																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-[b]	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-[b]	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
11	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-[b]	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
USDA/FSIS – MLG 8.09																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
9	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
11	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

[a] += *Listeria monocytogenes*were detected in samples; -=*Listeria monocytogenes* were not detected in sample; n/a- lab did not participate or submit data for the matrix;
[b] Sample was presumptive positive on 3M MDA 2 *Listeria monocytogenes* but confirmed negative indicating a false positive result;

Table 2. Individual Collaborator Results for Raw Chicken Breast Fillet

	High Level Test Portions												Low Level Test Portions												Un-inoculated Test Portions											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
3M MDA 2 - *Listeria monocytogenes*																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6	+[d]	+[d]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-[c]	+	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+[d]	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+[d]	+	-	-	-	-	-	-	-	-	-	-	-	-
10[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+[d]	+[d]	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+[e]
11	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
USDA/FSIS – MLG 8.09																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10[b]	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
11	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
12	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

[a] + = *Listeria monocytogenes* were detected in samples; -=*Listeria monocytogenes* were not detected in sample; n/a- lab did not participate or submit data for the matrix;
[b] Results were not used in statistical analysis due to laboratory error; Sample was presumptive positive on 3M MDA 2 *Listeria monocytogenes* but confirmed negative indicating a false positive result;
[d] Sample was presumptive negative on 3M MDA 2 *Listeria monocytogenes* but confirmed positive indicating a false negative result

Table 3: Results of Aerobic Plate Count for Collaborating Laboratories

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

[a] Samples analyzed by the 3M Petrifilm Rapid Aerobic Count Plate, AOAC OMA 2015.13

Figure 1A: POD Values of Candidate Method vs. Reference Method for125 gDeli Turkey

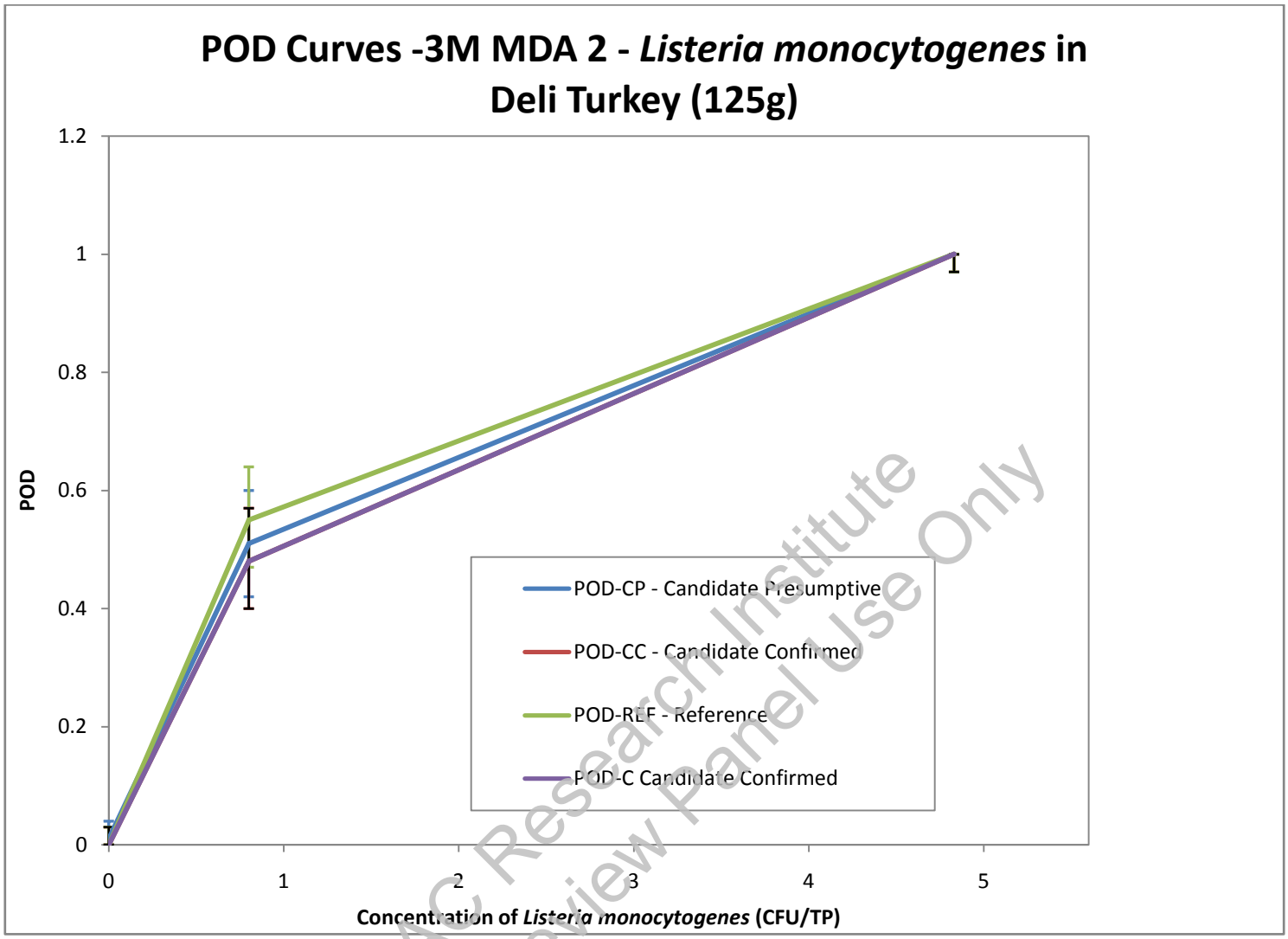


Figure 1B: dLPOD Values of Candidate Method vs. Reference Method for125 gDeli Turkey

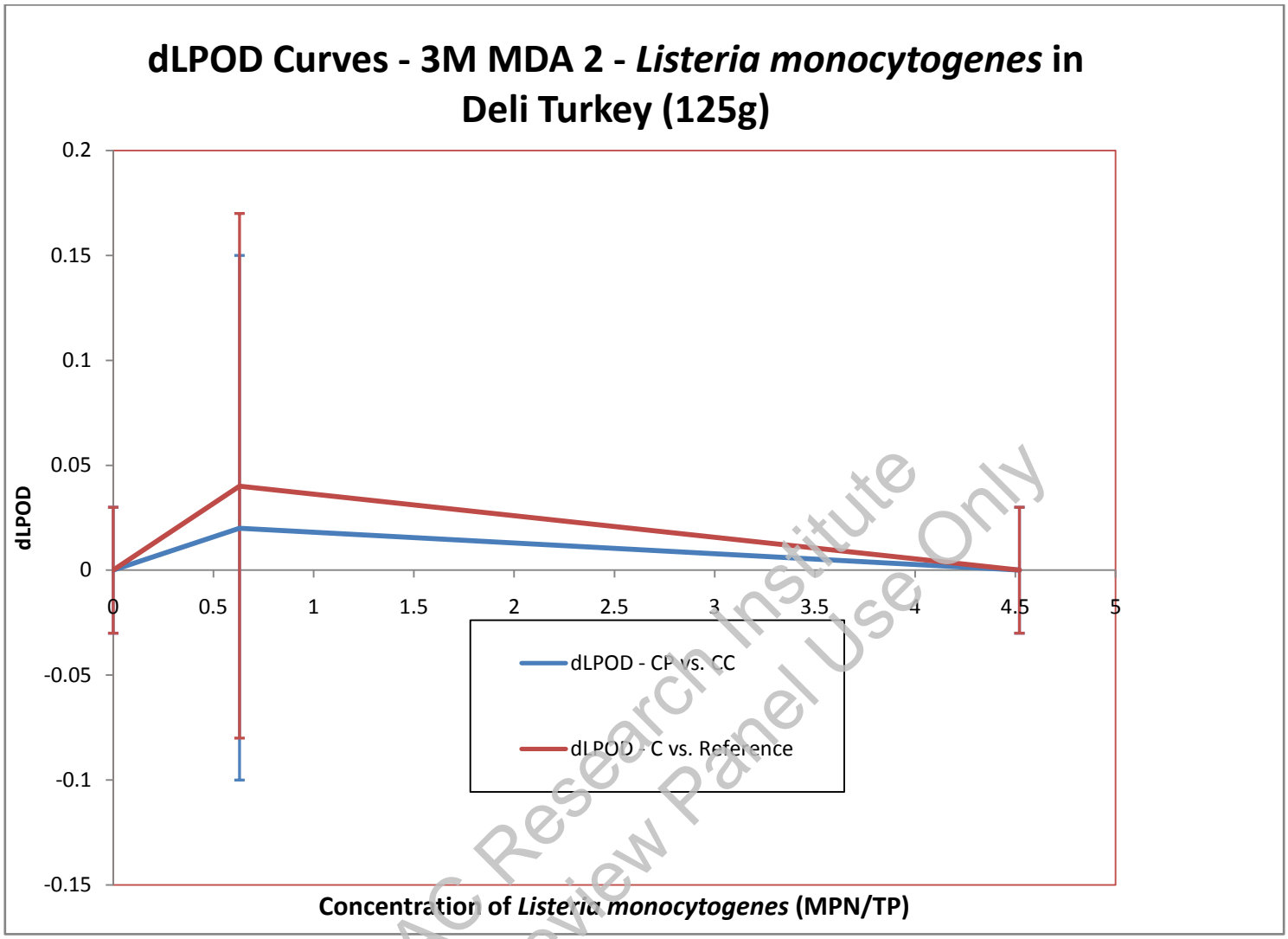


Figure 2A: POD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet

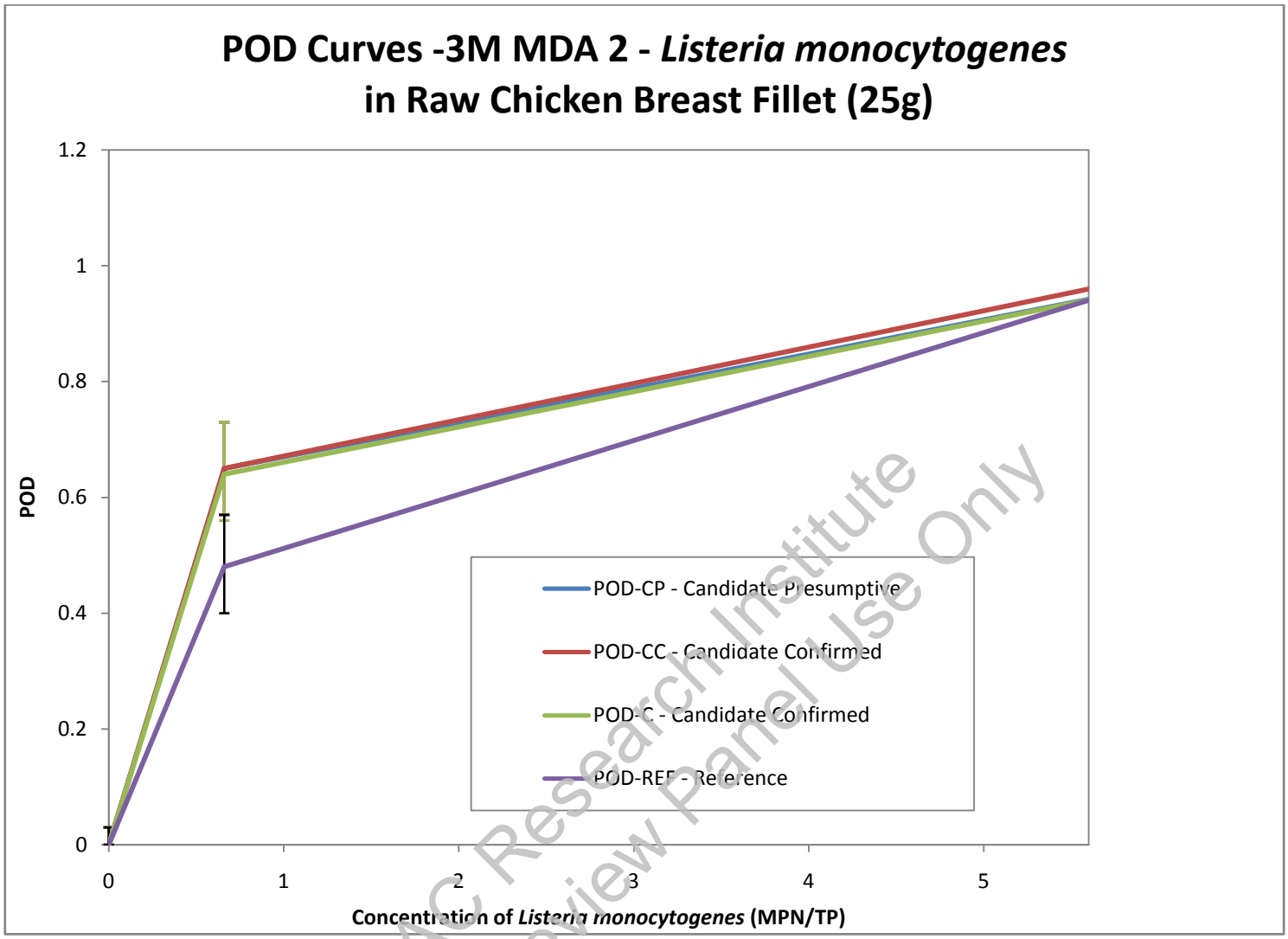
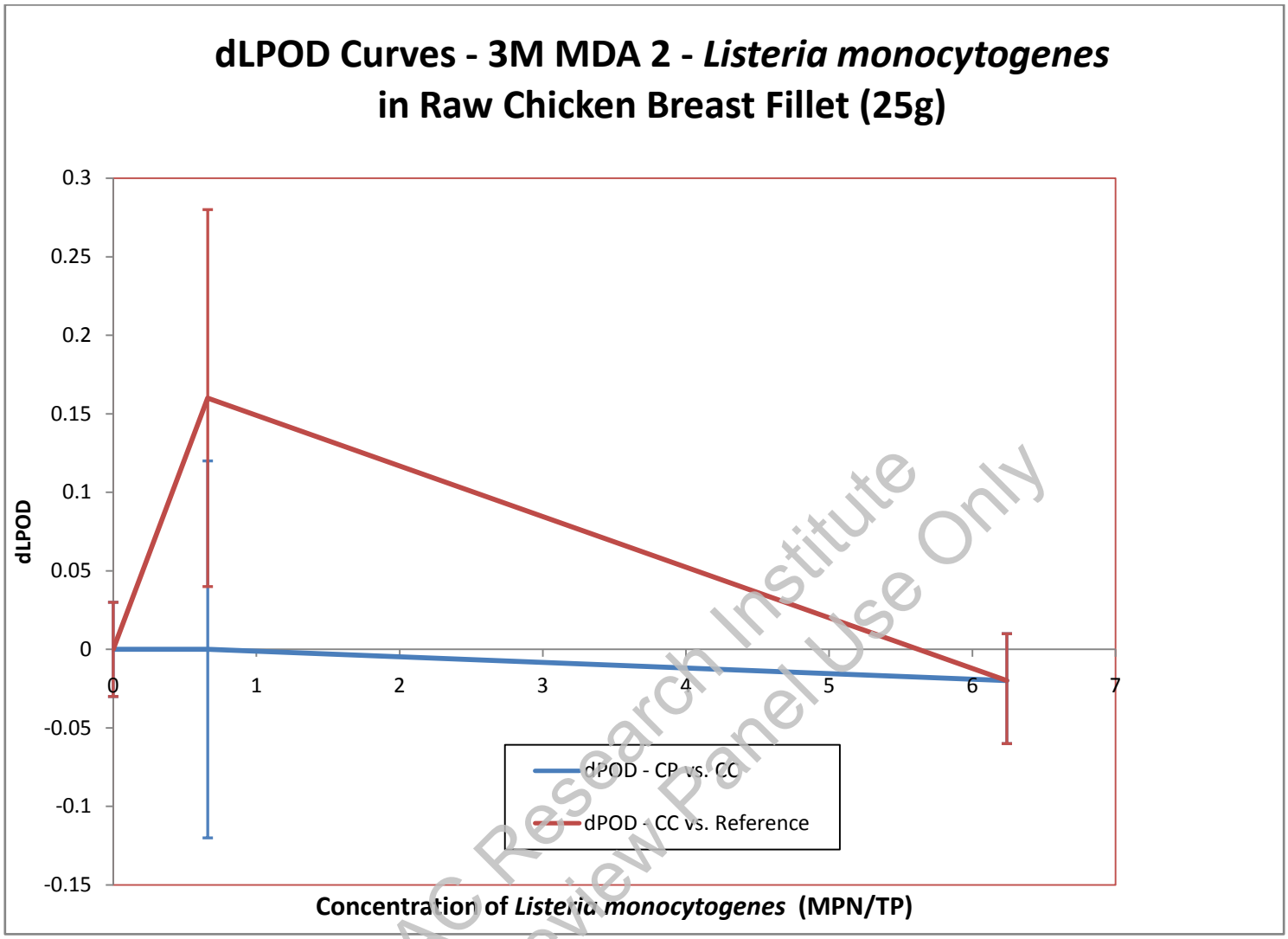


Figure 2B: dPOD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet



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 [1, 2, 3].

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
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 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
Listeria monocytogenes 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[4], or ISO 7218[5].

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 -*Listeriamonocytogenes* 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 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**, 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 ± 0.2 minutes. Incubate at 37 ±1°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 ±1°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 Letheen broth. It is recommended to sanitize the area after sampling.

WARNING: Should you select to use Neutralizing Buffer (NB) that contains aryl sulfonatecomplex 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 testingin 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^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 [(1)], USDA FSIS MLG [(2)] or ISO 18593 [(6)] 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 ± 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 andFraser Broth as needed

Sample Matrix	Sample Size	Enrichment Broth Volume (mL)	Enrichment Time (hr)
Heat-processed, cooked, cured meats, poultry, seafood and fish Heat-processed / pasteurized dairy products Produce and vegetables Multi-component foods	25 g	225	24-30
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 Fraser Broth	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℠# 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 ± 1 °C according to AOAC Performance Tested℠ Certificate #081501 and Official Method℠ 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 DETECTIONCHILL 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:

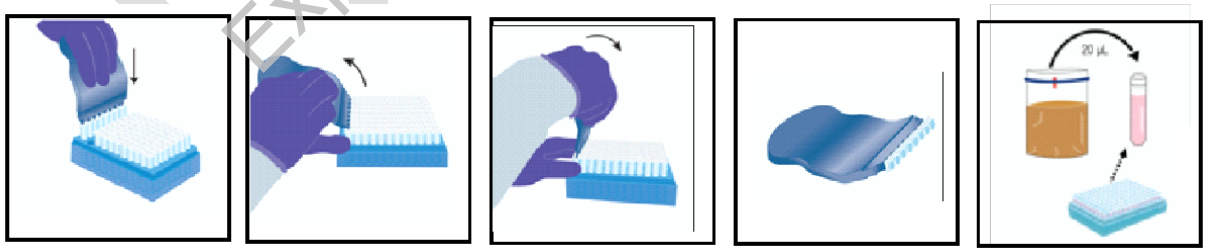
Transfer each enriched sample into an individual LS tube **first**. Transfer the NC **last**.

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 Table2or 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.



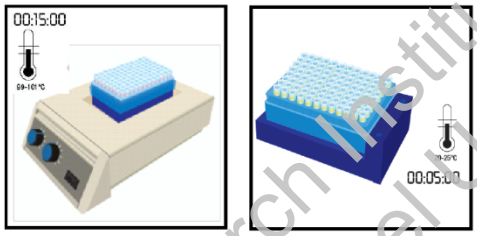
6. Repeat steps 4.1 to 4.6 as needed, for the number of samples to be tested.
7. When all samples have been transferred, transfer 20 µL of NC (sterile enrichment medium, e.g., Demi-Fraser Broth) into a LS tube. Do not use water as a NC.

8. Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100 ±1°C.
9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ±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 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.
   - 5.2 Repeat step 5.1 until individual Sample lysate has been added to a corresponding Reagent tube in the strip.

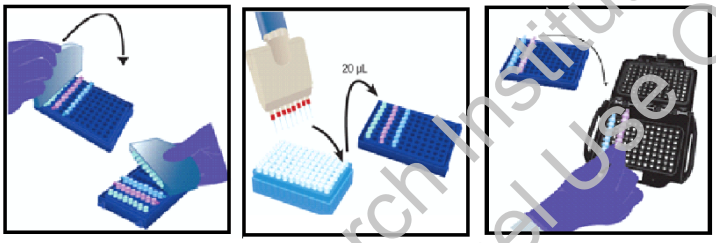
5.3 Cover the Reagent tubes with the provided extra caps and use the rounded side of the 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.

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 µL of NC lysate into a Reagent tube.

5.6 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.

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 [1, 2, 3], 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* amplificationreagents 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 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 8.09. 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

Submission Date	2016-05-04 12:36:15
First and Last Name	Lisa Monteroso
Organization	3M
E-mail	LPhillips@aoac.org
METHOD TITLE	OMAMAN-30 C/Method Safety Checklist for MDA 2 ListeriaM
Are any materials used or compounds formed that are explosive or flammable?	NO
Are there any side reactions that could occur that might produce flammable or explosive products or conditions?	NO
Are there any hazards created from electric or mechanical equipment?	NO
Are pressure differentials created that could result in an explosion or implosion?	NO
Would there be increased hazards if the reaction temperature were increased even modestly?	NO
Are special procedures required if a spill of the reaction mixture occurs?	NO
Is there a risk in producing a dangerous aerosol?	NO
Are special procedures required for the disposal of reagents or reaction products?	NO
Are there any potential hazards in handling or storage of reagents, test samples, or standards?	YES
Are there any other hazards that should be addressed regarding the method?	NO
Does your method use chlorinated solvents?	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

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

AOAC *Performance Tested Methods*℠ XXXXXX

Abstract

The 3M™ Molecular Detection Assay 2 (MDA2)–*Listeria monocytogenes* for the detection of *Listeria monocytogenes*was evaluated following the AOAC Research Institute *Performance Tested Methods*℠ program guidelines.The evaluation included inclusivity/exclusivity, lot-to-lot/stability, robustness and matrix studies. The 3M MDA2 –*Listeria monocytogenes*was compared to various reference methods for beef hot dogs, deli turkey, raw chicken leg pieces, sealed concrete, plastic, stainless steel, whole melons, bagged raw spinach, romaine lettuce, cold smoked salmon, queso fresco, 4% milk fat cottage cheese, 3% chocolate whole milk and vanilla ice cream in the matrix study.Twenty replicates of each food matrix were analyzed at a low inoculum level of 0.2-2 colony forming units (CFU)/test portion, five replicates were analyzed at a high level of 2-5 CFU/test portion, and five control replicates were analyzed at 0 CFU/test portion with the exception of raw chicken leg pieces. Naturally contaminated raw chicken leg pieces were evaluated using two separate lots consisting of twenty replicates for each lot. All environmental surfaces were analyzed using twenty replicates at a low inoculum level of ~50 CFU/standardtest area, five replicates were analyzed at a high level of ~100 CFU/standard test area, and five control replicates were analyzed at 0 CFU/standard test area. Based on the probability of detection statistical model, there were no statistically significant differences between the number of presumptive and confirmed positive samples or between the candidate and reference method.There were no unexpected results in the lot-to-lot/stability, robustness studies. In the inclusivity/exclusivity study, 50 target organisms and 30 non-target organisms were tested following the 3M MDA2 – *Listeria monocytogenes*method with all target organisms detected and none of the non-target organisms detected. The results of this evaluation demonstrate the specificity and sensitivity of the 3M MDA2 –*Listeria monocytogenes*and its ability to accurately detect *Listeria monocytogenes*in select food matrices and environmental surfaces, 24-30 hours post enrichment.

Authors

Lisa Monteroso and DeAnn Benesh, 3M Food Safety Division
Ben Bastin, Jonathan Flannery, Erin Crowley, Patrick Bird, M. Joseph Benzinger, Jr., James Agin, David Goins, Q Laboratories, Inc.

Submitting Company

3M Company
Food Safety Department
3M Center, Bldg. 275-5W-05
St. Paul, MN 55144-1000

Independent Laboratory

Q Laboratories, Inc.

1400 Harrison Ave
Cincinnati, OH 45214

Reviewers

Yvonne Salfinger
Consultant
Denver, CO

Michael Brodsky
Brodsky Consultants
73 Donnamora Crescent
Thornhill, Ontario L3T 4K6
Canada

Reviewer
Tony Hitchins
FDA-Retired
Rockville, MD

Scope of Method

a) *Target organism.— Listeria monocytogenes*

b) *Matrices.*— Beef hot dogs (25 g), deli turkey (125 g), queso fresco (25 g), vanilla ice cream (25 g), 4% milk fat cottage cheese (25 g), 3% chocolate whole milk (25 mL), whole melon, romaine lettuce (25 g), bagged raw spinach (25 g), cold smoked salmon (25 g), raw chicken leg pieces (25 g), sealed concrete (sponge enriched in 100 mL, 4” x 4”), plastic (Enviroswab enriched in 10 mL, 1” x 1”), stainless steel (sponge enriched in 225 mL, 4” x 4”).

c) *Summary of Validated Performance Claims.*— Performance equivalent to that of the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM) Chapter 10 [1] for whole melon, cold smoked salmon, romaine lettuce; the U.S. Department of Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (USDA-FSIS/MLG) 8.09 [2] for beef hot dogs, deli turkey, raw chicken leg pieces, sealed concrete, plastic and stainless steel and AOAC 993.12 [3] for queso fresco, vanilla ice cream, 4% milk fat cottage cheese, 3% chocolate whole milk and theISO 11290-1/A1 [4]for cold smoked salmon and bagged raw spinach.

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.

Principle of the Method

3M™ Molecular Detection Assay 2 –*Listeria monocytogenes*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 the laboratory'spreferred method or as specified by local regulations.

General Information

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 that can 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 3M MDA2–*Listeriamonocytogenes*was developed to provide rapid and specific detection of *Listeria monocytogenes*in enriched food samples and on food processing surfaces.

Materials and Methods

Test Kit Information

a) *Kit Name.*—Molecular Detection Assay 2 – *Listeriamonocytogenes*
b) Catalog Number.—MDA2LM96

Test Kit Reagents - 3M MDA2 – *Listeria monocytogenes*(96 tests)
a) *Lysis Solution (LS) tubes*. – 96 (12 strips of 8 tubes)
b) *Listeria monocytogenes Reagent tubes*. – 96 (12 strips of 8 tubes)
c) *Extra caps.* – 96 (12 strips of 8 caps)
d) *Reagent Control (RC).* – 16 (2 pouches of 8)
e) Quick Start Guide

Additional Supplies and Reagents

a) 3M Molecular Detection System (instrument), power supply, cord, USB cable and software
b) 3M Molecular Detection Speed Loader Tray
c) 3M Molecular Detection Chill Block Tray and Chill block insert
d) 3M Molecular Detection Heat Block Insert
e) 3M Molecular Detection Cap/Decap Tool [Reagent]
f) 3M Molecular Detection Cap/Decap Tool [Lysis]
g) Empty lysis tube rack
h) Empty reagent tube rack
i) Dey-Engley (D/E) Neutralizing Broth
j) Demi-Fraser broth (with additional of ferric ammonium citrate)

Additional media

a) Oxford Agar (OX)b) Sheep blood agar (SBA)
b) Fraser broth (FB)
c) Sheep blood agar (SBA)
d) PALCAM agar
e) Ottaviani Agosti Agar (OAA)
f) Trypticase soy agar (TSA)
g) Trypticase soy agar with 0.6% Yeast extract (TSA/YE)
h) De Man, Rogosa and Sharpe (MRS)
i) Modified Oxford agar (MOX)
j) Brain heart infusion (BHI) broth
k) Horse blood overlay (HBO)
l) Buffered Listeria enrichment broth (BLEB)

Apparatus

a) *Pipettes*. – capable of 20µL
b) *Multi-channel pipette*. – capable of 20µL
c) *Sterile pipette tips*. – capable of 20µL
d) *Stomacher®*. – Seward or equivalent
e) *Filter Stomacher® bags*. - Seward or equivalent
f) *Thermometer*. – calibrated range to include 100 ± 1°C range
g) *Incubators*. – capable of maintaining 37 ± 1°C
h) *Dry double block heater unit.* – capable of maintaining 100 ± 1°C; or*a water bath* capable of maintaining 100 ± 1°C
i) *Freezer*. – capable of maintaining -10 to -20°C, for storing the 3M Molecular Detection Chill Block Tray
j) *Refrigerator*. – capable of maintaining 2-8°C, for storing the 3M MDA2
k) *Computer*. – compatible with the 3M Molecular Detection System (instrument)

Safety Precautions

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 –*Listeriamonocytogenes*. Retain the safety instructions for future reference.

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

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

~~Enrichment~~Sample Preparation

3M recommends the use of Demi-Fraser Broth (with addition of ferric ammonium citrate) for the enrichment of food and environmental samples.Table 1 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

a) Allow the Demi-Fraser Broth enrichment medium to equilibrate to ambient laboratory temperature.
b) Aseptically combine the enrichment medium and sample according to Table 1. For all meat and highly particulate samples, the use of filter bags is recommended.
c) Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ±0.2 minutes. Incubate at 37 ±1°C according to Table 1.

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 Letheen broth. It is recommended to sanitize the area after sampling.

The recommended size of the sampling area for verifyingthe presence or absence of the pathogen on the surface is at least 100 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, USDA FSIS MLG or ISO 18593 (7) guidelines.

1. Allow the Demi-Fraser Broth enrichment medium 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 µLaliquot of each enriched sample are ~~is~~ transferred to separate lysis tubes using a new pipette tip aftereach sample transfer. Place uncovered samples on a dry bath incubator for 15 ±2minutes at 100 ± 1 °C. Following the heat lysis transfer samples to the 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 for the Negative Control (NC).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™MDA2 *Listeriamonocytogenes*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 –*Listeriamonocytogenes*amplificationreagents 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.

Confirmation

Presumptive positive samplesof primary enrichmentswere confirmed by following the appropriate reference method confirmation, 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.

Validation Study

Testing was conducted following the procedures outlined in the AOAC Research Institute *Performance Tested Methods℠Program* validation outline protocol: *Comparative Evaluation of the 3M™ Molecular Detection Assay 2-Listeria monocytogenes and of the 3M™ Molecular Detection Assay 2 Listeria* (February, 2015) [8]. The independent study involved a matrix study (9matrices), a lot-to-lot/stability evaluation, and a robustness evaluation. The internal study 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°C ± 1°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°C ± 1°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°C ± 1°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 ± 2°C for 24 ± 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 ± 1 minute at 50 ± 1°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 ± 1°C for 24 ± 2 hours and the colonies were counted. The degree of injury was estimated as:

$$(1-\frac{n_{select}}{n_{nonselect}})x100$$

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

For melons, a single whole melon was placed into a large sterile bag and the blossom end of the melon was inoculated with 100 µL of the diluted *Listeria monocytogenes*culture. The liquid culture was then allowed to soak into the melon. The melon was then inverted so that the inoculated end was on the bottom of the sterile bag. The bag was tied closed and held for 48-72 hours at 2-8 °C.

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

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

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

The level of *Listeria monocytogenes*in the low level inoculum and high level inoculum was determined by Most Probable Number (MPN) on the day of analysis by evaluating 5 x 50 g, 20 x 25 g (reference method test portions), and 5 x 10 g inoculated test samples for the low inoculation level and by examining 5 x 25 g, 5 x 5 g and 5 x 1 g for the high inoculation level. The level of *Listeriamonocytogenes*in the low level inoculum and high level inoculum for all 125 g test portions was determined by MPN by evaluating 5 x 250 g, 20 or 5 x 125 g (reference method test portions), and 5 x 50 g inoculated test samples. Each test portion was enriched with the reference method enrichment broth at the reference method dilution scheme and analyzed by the reference method procedure. The number of positives from the 3 test levels was used to calculate the MPN using the LCF MPN calculator (version 1.6) provided by AOAC RI. [10] (http://www.lcfltd.com/customer/LCFMPNCalculator.exe)

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® GP Biochemical Identification following AOAC OMA 2013.02. [11]

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 hours. After 24 hours of total incubation, the enriched samples were streaked to MOX agar plates and incubated at 35 ±1 °C for 24-48 hours. The enriched samples were re-incubated for an additional 24 hours at 30 ± 1 °C and then streaked to a second MOX agar plate which was incubated for 24-48 hours at 35 ± 1°C. MOX agar plates were examined for suspect colonies, and if present, at least 5 colonies were streaked to TSA containing 0.6% yeast extract (TSA/YE). The TSA/YE plates were incubated at 35 ± 1°C for 24-48 hours and then examined for purity. Pure colonies were tested for catalase reactivity and a Gram Stain was conducted. A pure *Listeria* colony was transferred to Trypticase Soy Broth containing 0.6% yeast extract (TSB/YE). The TSB/YE cultures were incubated at 25 ± 1 °C overnight, or until the broth was turbid, indicating sufficient growth. Catalase-positive organisms were stabbed into plates of 5% Sheep Blood Agar (SBA) and incubated at 35 ± 1°C for 24-48 hours. The TSB/YE tubes incubated at 25 ±1 °C were used to prepare a wet mount slide to determine motility pattern. After incubation, the SBA

plates were examined for hemolysis. Final confirmation was conducted using the VITEK® GP Biochemical Identification card following AOAC OMA 2013.02.

AOAC 993.12 Listeria Reference Method

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

ISO 11290-1/A1 Reference Method

For the ISO 11290-1/A1 reference method, 25 g test portions were enriched with 225 half Fraser broth. All test portions were mechanically stomached for two minutes. The test portions were incubated at 30 ± 1°C for 24 ± 3 hours. After incubation, 0.1 mL of the sample enrichment was transferred to 10 mL FB containing 0.1 mL of 5% ferric ammonium citrate and incubated at 37 ± 1°C for 48 ± 3 hours. After 48 ± 3 hours, a loopful of the sample secondary FB enrichment was streaked to PALCAM and OAA(Ottovani-Agosti Agar)and incubated at 37 ± 1°C for 24 ± 3 hours. A loopful of the sample primary enrichment was also streaked to PALCAM and OAAand incubated at 37 ± 1°C for 24 ± 3 hours. PALCAM and OAA agar plates were examined for the presence of suspect colonies. If no suspect colonies were present, the agar plate was re-incubated for an additional 18-24 hours at 37 ± 1°C. If no suspect colonies present the sample was determined to be negative for *Listeria*. If suspect colonies were present on the PALCAM or OAA agar plates, these suspect colonies were streaked to HBO and incubated at 37 ± 1°C for 18-24 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® GP Biochemical Identification following AOAC OMA 2013.02.

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 ± 0.2 minutes and incubated at 37 ±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 ± 0.2 minutes and incubated at 37 ±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 ±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 ± 0.2 minutes and incubated at 37 ±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 ± 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 ± 0.2 minutes and incubated at 37 ±1 °C for 24-26 hours.

Prior to analysis, lysis tubes were brought to room temperature (20-25 ^{o}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 ± 2minutes at 100 ± 1 ^{o}C. Following the heat lysis, samples were transferred to the sanitized laboratorybench 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

inoculum levels for each environmental surface are presented in Table 7. A summary of POD analyses [13] are presented in Tables 8-15. As per criteria outlined in Appendix J of the Official Methods of Analysis Manual [13], fractional positive results were obtained for all matrices.

Internal Study

Raw Chicken Leg Pieces (25 g) – 28 hour Primary Enrichment

For the low inoculation level of the MDA2 - *Listeria monocytogenes*assay, there were 10 presumptive positives and 10 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 12 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 positive following the reference method.Detailed results of the POD analyses are presented in Tables 8 and 12.

Bagged raw spinach

For the low inoculation level of the MDA2 - *Listeria monocytogenes*assay, there were 9 presumptive positives and 10 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure resulting in 1 false negative. There were 10 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 4 confirmed positives following the reference method.Detailed results of the POD analyses are presented in Tables 8 and 12.

Cold smoked salmon

Compared to FDA-BAM

For the low inoculation level of the MDA2 -*Listeria monocytogenes*assay, there were 10 presumptive positives and 10 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 7 observed positives for the reference method. For the high inoculation level, there were 3 presumptive positives and 3 confirmed positives following the FDA/BAM Chapter 10 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.

Compared to ISO 11290-1/A1

For the low inoculation level of the MDA2 - *Listeria monocytogenes*assay, there were 10 presumptive positives and 10 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 9 observed positives for the reference method. For the high inoculation level, there were 3 presumptive positives and 3 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 4 confirmed positives following the reference method.Detailed results of the POD analyses are presented in Tables 8 and 12.

Independent Study

Deli Turkey (125 g)

For the low inoculation level of the MDA2 - *Listeria monocytogenes*assay, there were 8 presumptive positives and 8 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 7 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.

Raw Chicken Leg Pieces (25 g)

Lot 1

For the lot 1 of the raw chicken leg pieces of the MDA2 - *Listeria monocytogenes*assay, there were 17 presumptive positives and 17 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 16 observed positives for the reference method. Detailed results of the POD analyses are presented in Tables 9 and 13.

Lot 2

For the lot 2 of the raw chicken leg pieces of the MDA2 -*Listeriamonocytogenes*assay, there were 10 presumptive positives and 10 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 14 observed positives for the reference method. Detailed results of the POD analyses are presented in Tables 9 and 13.

Whole Melon

For the low inoculation level of the MDA2 -*Listeria monocytogenes*assay, there were 10 presumptive positives and 10 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 8 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the FDA/BAM Chapter 10 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.

Vanilla Ice Cream (25 g)

For the low inoculation level of the MDA2 -*Listeria monocytogenes*assay, there were 7 presumptive positives and 7 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 7 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12 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.

Romaine lettuce (25 g)

For the low inoculation level of the MDA2 -*Listeria monocytogenes*assay, there were 7 presumptive positives and 7 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 6 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 5 confirmed positives following the reference method.Detailed results of the POD analyses are presented in Tables 10 and 14.

Queso Fresco (25 g)

For the low inoculation level of the MDA2 – *Listeriamonocytogenes*assay, there were 11 presumptive positives and 11 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 9 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives following the reference method.Detailed results of the POD analyses are presented in Tables 10 and 14.

4%Milk Fat Cottage Cheese (25 g)

For the low inoculation level of the MDA2 -*Listeria monocytogenes*assay, there were 10 presumptive positives and 10 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 8 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives following the reference method.Detailed results of the POD analyses are presented in Tables 10 and 14.

3% Chocolate Whole Milk (25 mL)

For the low inoculation level of the MDA2 -*Listeria monocytogenes*assay, there were 9 presumptive positives and 9 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 6 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives following the reference method.Detailed results of the POD analyses are presented in Tables 10 and 14.

Beef Hot Dog (25 g)

For the low inoculation level of the MDA2 - *Listeria monocytogenes*assay, there were 4 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure, resulting in 1 false negative. There were 6 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 10 and 14.

Stainless Steel (enriched in 225 mL)

Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level of the MDA2 - *Listeria monocytogenes*assay, there were 5 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 8 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 11 and 15.

Sealed Concrete (enriched in 100 mL)

24-hour primary enrichment - For the low inoculation level of the MDA2 – *Listeriamonocytogenes*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 11 and 15.

26-hour primary enrichment - For the low inoculation level of the MDA2 – *Listeriamonocytogenes*assay, there were 16 presumptive positives and 15 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure, resulting in 1 false positive. 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 11 and 15.

Plastic (enriched in 10 mL)

Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 12 presumptive positives and 12 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 10 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 11 and 15.

All five uninoculated control test portions were negative by both the MDA2 - *Listeria monocytogenes*assay and the corresponding reference method for each matrix above.

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

Methodology

Three lots of MDA2 –*Listeriamonocytogenes* test kits, 1 newly manufactured, 1 at the middle of its expiration, and 1 at or slightly beyond expiration was analyzed to determine the stability of the assay. For the MDA2 – *Listeriamonocytogenes* test kit, one *Listeria monocytogenes*ATCC 7644 isolate was analyzed at a fractional positive level (2-8 positives), and testing 10 replicates. One non-*Listeriamonocytogenes*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 MDA2 - *Listeria monocytogenes*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 Tables 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 17.For each parameter, one *Listeria monocytogenes*ATCC 7644isolate was analyzed at a fractional positive level (2-8 positives), testing 10 replicates. One non-*Listeriamonocytogenes*isolate, *Enterococcus faecalis*ATCC 29212, was analyzed at the growth level achieved in a non-selective broth, testing 5 replicates.

Results

For the robustness analysis of the MDA2 - *Listeria monocytogenes*assay, there were 6 presumptive positives out of 10 replicates for each variation evaluated. For the 5 control test portions, there were 0 presumptive positives out of 5 replicates. See Table 17 for robustness results.

Independent Laboratory Observations

The results of this study demonstrate the ability of the MDA2 *Listeria monocytogenes*to detect the presence of *Listeria monocytogenes*in various food matrices and select environmental

surfaces after 24-28 hours of a primary enrichment. The MDA2 *Listeria monocytogenes*offers the benefits of extremely high sensitivity and high specificity for the detection of *Listeria monocytogenes* while reducing the overall time to presumptive results. The MDA2 *Listeria monocytogenes* 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 foot print 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 trouble shooting more streamlined if any problems occur while testing is being conducted.

Discussion

There were no differences between the 24- and 26-hour enrichment time points for the environmental surfaces tested, therefore a 24-hour minimum enrichment will be recommended for these matrices.

Conclusion

The 3M MDA2 -*Listeria monocytogenes*AOAC PTM validation study included an inclusivity/exclusivity, lot-to-lot/stability, robustness and matrix studies. The 3M MDA2 – *Listeriamonocytogenes*was compared to the USDA/FSIS-MLG 8.09, the FDA-BAM Chapter 10, the AOAC 993.12 or the ISO 11290-1/A1 reference methods for the detection of *Listeriamonocytogenes*. There were nosignificant differences between the 3M MDA2 - *Listeriamonocytogenes*method and the corresponding reference method for any of the matrices evaluated. The 3M MDA2 -*Listeria monocytogenes*demonstrated reliability as a rapid and sensitive method for the detection of *Listeria monocytogenes*for the following matrices: beef hot dogs (25 g), deli turkey (125 g), queso fresco (25 g), vanilla ice cream (25 g), 4% milk fat cottage cheese (25 g), 3% chocolate whole milk (25 mL), whole melon, romaine lettuce (25 g), bagged raw spinach (25 g), cold smoked salmon (25 g), raw chicken leg pieces (25 g), sealed concrete (sponge enriched in 100mL), plastic (Enviroswabenriched in 10 mL), stainless steel (sponge enriched in 225 mL).

References

1. Food and Drug Administration Bacteriological Analytical Manual Chapter 10: *Detection and Enumeration of Listeria monocytogenes in Foods*. February, 2013. (Accessed January 2015)

2. United States Department of Agriculture Microbiological Laboratory Guidelines 8.09: *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and Environmental Samples*. May 1st, 2013. (Accessed January 2015)

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

3. AOAC Official Method 993.12 *Listeria monocytogenes*in Milk and Dairy Products: Selective Enrichment and Isolation Method, First Action 1993, Final Action 1996. (Accessed January 2015)

4. ISO 11290-1/A1 (2004) - Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes*- Part 1: detection of *Listeria monocytogenes*in foods

5. U.S. Food and Drug Administration, and U.S. Food Safety and Inspection Service. 2003. Quantitative Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods. Available in Docket No. 1999N-1168, Vols. 23 through 28, and at Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods

6. Doyle ME, Mazzotta AS, Wang T, Wiseman DW, and Scott VN. 2001. Heat resistance of *Listeria monocytogenes*. *Journal of Food Protection* 64 (3): 410-429.

7. ISO 18593. Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.

8. AOAC Research Institute *Performance Tested Methods*^{SM} *Program* validation outline protocol: *Comparative Evaluation of the 3M*™ *Molecular Detection Assay Listeria monocytogenes 2 and of the 3M*™ *Molecular Detection Assay Listeria* 2 (February 2015)

9. U. S. Food and Drug Administration *Bacteriological Analytical Manual*, Chapter 3 *Aerobic Plate Count (*January 2001) (Accessed January 2015) http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.m

10. Least Cost Formulations, Ltd., MPN Calculator Version 1.6 www.lcfltd.com/customer/LCFMPNCalculator.exe(Accessed January 2015)

11. AOAC International, *Evaluation of the* VITEK 2 *Gram Positive (GP) Microbial Identification Test Card: Collaborative Study*, Journal of AOAC International, 95, 1425 (2012)

12. Wehling, P., LaBudde, R., Brunelle, S., Nelson, M. *Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods.* Journal of AOAC International, Vol. 94, No. 1, 2011.

13. Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook Version 2.2 (2011) -http://lcfltd.com/aoac/aoac-binary-v2-2.xls(Accessed January 2015)

14. Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, AOAC INTERNATIONAL, Gaithersburg, MD,http://http://www.eoma.aoac.org/app_j.pdf (Accessed January 2015)

Table 1. Enrichment Protocols Using Demi-Fraser Broth Enrichmentat 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
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 **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 - *Listeria monocytogenes*	**Confirmation 100µl**	
							ALOA	**Palcam**
1	*Listeria*	*monocytogenes*	153	Soft cheese (Munster)	29	+	H+	+
2	*Listeria*	*monocytogenes*	1011/1410	Frozen broccoli	28	+	H+	+
3	*Listeria*	*monocytogenes*	1972/2399	Puff pastry with mushrooms	38	+	H+	+
4	*Listeria*	*monocytogenes*	1973/2400	Puff pastry egg and ham (Quiche-lorraine)	36	+	H+	+
5	*Listeria*	*monocytogenes*	2407/3139	Tripes with tomatoes	31	+	H+	+
6	*Listeria*	*monocytogenes*	2760/3145	Raw bacon	44	+	H+	+
7	*Listeria*	*monocytogenes*	32.183	Croque-Monsieur	31	+	H+	+
8	*Listeria*	*monocytogenes*	38/181	Toulouse sausages	33	+	H+	+
9	*Listeria*	*monocytogenes*	5721/6179	Smoked bacon	49	+	H+	+
10	*Listeria*	*monocytogenes*	7111/7516	Pâté (Rillettes)	64	+	H+	+
11	*Listeria*	*monocytogenes*	850/109	Ready-To-Eat (RTE) food (deli salad with seafood)	34	+	H+	+
12	*Listeria*	*monocytogenes*	877/113	Environmental sample (pastry)	28	+	H+	+
13	*Listeria*	*monocytogenes*	913/1048	Black pudding	39	+	H+	+
14	*Listeria*	*monocytogenes*	A00C014	Sausage	37	+	H+	+
15	*Listeria*	*monocytogenes*	A00C022	Merguez	33	+	H+	+
16	*Listeria*	*monocytogenes*	A00C024	Sausage	21	+	H+	+
17	*Listeria*	*monocytogenes*	A00C036	Poultry (guinea)	41	+	H+	+
18	*Listeria*	*monocytogenes*	A00C039	Sausages	20	+	H+	+
19	*Listeria*	*monocytogenes*	A00C040	Cooked delicatessen (Museau)	36	+	H+	+
20	*Listeria*	*monocytogenes*	A00C041	Sausage	38	+	H+	+
21	*Listeria*	*monocytogenes*	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 - *Listeria monocytogenes*	Confirmation 100µl	
							ALOA	Palcam
22	*Listeria*	*monocytogenes*	A00C043	Smoked Bacon	50	+	H+	+
23	*Listeria*	*monocytogenes*	A00C044	Poultry (duck)	34	+	H+	+
24	*Listeria*	*monocytogenes*	A00C052	RTE food (Ossobucco with turkey)	34	+	H+	+
25	*Listeria*	*monocytogenes*	A00C053	Gizzards	30	+	H+	+
26	*Listeria*	*monocytogenes*	A00C054	Beef heart	30	+	H+	+
27	*Listeria*	*monocytogenes*	A00C055	Raw sausages	43	+	H+	+
28	*Listeria*	*monocytogenes*	A00E008	Environmental sample	27	+	H+	+
29	*Listeria*	*monocytogenes*	A00E049	Environmental sample (smoked salmon)	41	+	H+	+
30	*Listeria*	*monocytogenes*	A00E082	Environmental sample (smoked salmon)	33	+	H+	+
31	*Listeria*	*monocytogenes*	A00L097	Milk	60	+	H+	+
32	*Listeria*	*monocytogenes*	A00M009	Smoked salmon	40	+	H+	+
33	*Listeria*	*monocytogenes*	A00M032	Smoked salmon	11	+	H+	+
34	*Listeria*	*monocytogenes*	A00M045	Smoked salmon	62	+	H+	+
35	*Listeria*	*monocytogenes*	A00M083	Smoked salmon	79	+	H+	+
36	*Listeria*	*monocytogenes*	Ad235	Poultry	45	+	H+	+
37	*Listeria*	*monocytogenes*	Ad253	Hard cheese	58	+	H+	+
38	*Listeria*	*monocytogenes*	Ad260	Semi hard cheese	27	+	H+	+
39	*Listeria*	*monocytogenes*	Ad265	Tongue	62	+	H+	+
40	*Listeria*	*monocytogenes*	Ad266	Poultry	26	+	H+	+
41	*Listeria*	*monocytogenes*	Ad267	Dry sausage	26	+	H+	+
42	*Listeria*	*monocytogenes*	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 - *Listeria monocytogenes*	Confirmation 100µl	
							ALOA	Palcam
43	*Listeria*	*monocytogenes*	Ad270	Fermented sausage	42	+	H+	+
44	*Listeria*	*monocytogenes*	Ad272	Fermented sausage	56	+	H+	+
45	*Listeria*	*monocytogenes*	Ad273	Cured delicatessen	27	+	H+	+
46	*Listeria*	*monocytogenes*	Ad274	Ready-to-eat food (Asiatic meal)	40	+	H+	+
47	*Listeria*	*monocytogenes*	Ad534	Fruits	54	+	H+	+
48	*Listeria*	*monocytogenes*	Ad544	Onion	43	+	H+	+
49	*Listeria*	*monocytogenes*	Ad546	Flour	83	+	H+	+
50	*Listeria*	*monocytogenes*	Ad623	Bread crumbs	50	+	H+	+

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
H+ = Phosphy-Lipolysis halo

Table 3. Exclusivity Study Results

EXCLUSIVITY						
Strain		Species	Reference	Origin	Inoculation level (cfu/ml)	3M MDA2 – *Listeria monocytogenes*
1	*Listeria*	*grayi*	Ad 1198	Smoked salmon	3.2×10^5	-

EXCLUSIVITY						
Strain		**Species**	**Reference**	**Origin**	**Inoculation level (cfu/ml)**	**3M MDA2 – *Listeria monocytogenes***
2	*Listeria*	*grayi*	Ad 1443	Pork meat sausages	4.9×10^5	-
3	*Listeria*	*innocua*	1	Smoked salmon	4.9×10^5	-
4	*Listeria*	*innocua*	Ad 658	Gorgonzola	5.9×10^5	-
5	*Listeria*	*ivanovii*	Ad 466	Raw veal meat	3.3×10^5	-
6	*Listeria*	*ivanovii*	Ad 662	Environment (dairy industry)	3.6×10^5	-
7	*Listeria*	*seeligeri*	Ad 649	Cheese	5.4×10^5	-
8	*Listeria*	*seeligeri*	BR1	Trout	5.1×10^5	-
9	*Listeria*	*welshimeri*	Ad1276	Environment (Slaughterhouse)	5.2×10^5	-
10	*Listeria*	*welshimeri*	Ad1175	Ready-to-eat-food	6.4×10^5	-
11	*Bacillus*	*cereus*	Ad 465	Salmon Terrine	7.2×10^4	-
12	*Bacillus*	*circulans*	Ad 760	Vegetables	2.0×10^4	-
13	*Bacillus*	*coagulans*	Ad 731	Dairy product	$<2.0 \times 10^3$ (opacity +)	-
14	*Bacillus*	*licheniformis*	Ad 978	Dairy product	1.6×10^4	-
15	*Bacillus*	*pumilus*	Ad 284	Ready-to-eat	1.7×10^5	-
16	*Brochrotrix*	*campestris*	CIP 102920T	Environment	$<2.0 \times 10^3$ (opacity +)	-
17	*Carnobacterium*	*piscicola*	Ad 369	Raw milk	$<2.0 \times 10^3$ (opacity +)	-
18	*Enterococcus*	*durans*	Ad 149	Ham	$<2.0 \times 10^3$ (opacity +)	-
19	*Enterococcus*	*faecalis*	89L326	Soft cheese (Vacherin)	1.6×10^5	-
20	*Lactobacillus*	*brevis*	86L126	Ham	1.4×10^5	-
21	*Lactobacillus*	*curvatus*	Ad 380	Delicatessen	$<2.0 \times 10^3$ (opacity +)	-
22	*Lactobacillus*	*sakei*	Ad 473	Ham	$<2.0 \times 10^3$(opacity	-

EXCLUSIVITY						
Strain		**Species**	**Reference**	**Origin**	**Inoculation level (cfu/ml)**	**3M MDA2 – *Listeria monocytogenes***
					+)	
23	*Leuconostoc*	*carnosum*	Ad 411	Ham	$<2.0 \times 10^3$ (opacity +)	-
24	*Leuconostoc*	*citreum*	Ad 396	Ham	4.9×10^5	-
25	*Micrococcus*	*luteus*	Ad 432	Cocktail	$<2.0 \times 10^3$ (opacity +)	-
26	*Staphylococcus*	*aureus*	Ad 165	Smoked delicatessen	$<2.0 \times 10^3$ (opacity +)	-
27	*Staphylococcus*	*epidermidis*	Ad 931	Fruits	2.0×10^3	-
28	*Staphylococcus*	*haemolyticus*	Ad 989	Dairy product	2.8×10^4	-
29	*Streptococcus*	*bovis*	92L622	Cheese	2.0×10^3	-
30	*Streptococcus*	*salivarius*	Ad 441	Dairy product	$<2.0 \times 10^3$ (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

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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes* CWD[2] 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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes* ATCC[1] 19112 & *L. welshimeri* ATCC[1] 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	*L. monocytogenes* FSL[3] 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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes ATCC1 19117*	0 CFU/4" x 4" (100 cm2)	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/4" x 4" (100 cm2)	20		
		~100 CFU/4" x 4" (100 cm2)	5		
Plastic	*L. monocytogenes ATCC1 51782& L. seeligeri ATCC1 35967& 10x Enterococcus faecalis ATCC1 29212*	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		
		~100 CFU/1" x 1" (5 cm2)	5		

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	*L. monocytogenes ATCC 19118 & 10x Enterococcus faecium 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		

[1] ATCC - American Type Culture Collection
[2] CWD - University of Vermont
[3] 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	*L. monocytogenes* Ad[1] 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	*L. monocytogenes* 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	*L. monocytogenes* 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	*L. monocytogenes* 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		

[1] Ad – Adria Developpement Culture Collection

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

Matrix (Test Portion)	APC[1] (CFU/g)	*Listeria* Pathogen Screen
Beef Hot Dogs[5] (25 g)	6.0×10^3	0/5 Detected[2]
Deli Turkey (125 g)	1.5×10^3	0/5 Detected[2]
Raw Chicken Leg Pieces (25 g)	2.8×10^5	4/5 Detected[2]
Whole Melons	1.0×10^2	0/5 Detected[3]
Vanilla Ice Cream (25 g)	8.0×10^1	0/5 Detected[4]
Queso Fresco (25 g)	3.8×10^5	0/5 Detected[4]
4% Milk Fat Cottage Cheese (25 g)	1.7×10^4	0/5 Detected[4]
Beef Hot Dogs (25 g)	1.6×10^2	0/5 Detected[2]

[1] APC conducted in accordance with FDA/BAM Chapter 3
[2] *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines
[3] *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines
[4] *Listeria species* screen conducted following the AOAC 993.12 guidelines
[5] 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[1] (CFU/g)	Background *Listeria*
Raw Chicken Leg Pieces (25 g)	5.4×10^5	None Detected
Bagged Raw Spinach (25 g)	8.0×10^6	None Detected
Cold Smoked Salmon (25 g)	4.0×10^2	None Detected

[1] APC conducted in accordance with FDA/BAM Chapter 3
[2] *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines
[3] *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines
[4] *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[1]	25 g	*Listeria monocytogenes* ATCC 7644	TSA	1.8×10^8	58.3 %
			MOX	7.5×10^7	
Deli Turkey	125 g	*Listeria monocytogenes* ATCC 19116	TSA	2.6×10^9	68.5 %
			MOX	8.2×10^8	
Queso Fresco	25 g	*Listeria monocytogenes* ATCC CWD 1554	TSA	2.6×10^9	72.7%
			MOX	7.1×10^8	
Beef Hot Dogs[2]	25 g	*Listeria monocytogenes* ATCC 7644	TSA	2.6×10^9	73.8%
			MOX	6.8×10^8	

[1]- Testing for Robustness and Lot to Lot
[2]- 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	*Listeria monocytogenes* FSL J1-049	
Low-Inoculum Level CFU[a]/Melon[b]	7	
High-Inoculum Level CFU[a]/Test Melon[b]	55	
Matrix	**Stainless Steel**	
Inoculating Organism	*Listeria monocytogenes* ATCC 19118	*Enterococcus faecium* ATCC 19434
Low-Inoculum Level CFU[a]/Test Area[c]	42	530
High-Inoculum Level CFU[a]/Test Area[c]	400	4000
Matrix	**Sealed Concrete**	
Inoculating Organism	*Listeria monocytogenes* ATCC 19117	
Low-Inoculum Level CFU[a]/Test Area[c]	35	
High-Inoculum Level CFU[a]/Test Area[c]	360	
Matrix	**Plastic**	
Inoculating Organism	*Listeria monocytogenes* ATCC 51782	*Enterococcus faecalis* ATCC 29212
Low-Inoculum Level CFU[a]/Test Area[d]	42	540
High-Inoculum Level CFU[a]/Test Area[d]	420	3600

[a] CFU: aliquots of the inocula were plated in triplicate onto TSA and averaged
[b] Test Area: Whole Melon
[c] Test Area: 4” x 4” Surface Area
[d] 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[a]/ Test Portion	N[b]	3M MDA2			USDA/FSIS MLG			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Raw Chicken Leg Pieces (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**ISO 11290-1**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Bagged Raw Spinach (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**FDA-BAM**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Cold Smoked Salmon (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**ISO 11290-1**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Cold Smoked Salmon (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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 *Listeria monocytogenes* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)

Matrix	Strain	Analysis Time Point	MPN[a]/ Test Portion	N[b]	3M MDA2			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Deli Turkey (125 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Whole Melon	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Vanilla Ice Cream (25 g)	*L. monocytogenes* 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, 0.92)	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

[a]MPN = Most Probable Number is calculated using the LCE MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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 *Listeria monocytogenes* Assay, Candidate vs. Reference – POD Results (INDEPENDENT STUDY)

Matrix	Strain	Analysis Time Point	MPN[a]/ Test Portion	N[b]	3M MDA2			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Romaine Lettuce (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Queso Fresco (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
4% Milk Fat Cottage Cheese (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
3% Chocolate Whole Milk (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Beef Hot Dogs (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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[a]/ Test Area	N[b]	3M MDA2			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Stainless Steel (225 mL)	*L. monocytogenes* ATCC 19118 & *Enterococcus faecium* 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[a]/ Test Area**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Sealed Concrete (100 mL)	*L. monocytogenes* 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[a]/ Test Area**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Plastic (10 mL)	*L. monocytogenes* ATCC 51782 & *Enterococcus faecalis* 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

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Raw Chicken Leg Pieces (25 g)	*L. monocytogenes* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
Bagged Raw Spinach (25 g)	*L. monocytogenes* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
Cold Smoked Salmon (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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 *Listeria monocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)

Matrix	Strain	Analysis Time Point	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Deli Turkey (125 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
Whole Melon	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
Vanilla Ice Cream (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCE MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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 *Listeria monocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)

Matrix	Strain	Analysis Time Point	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			$dPOD_C$[f]	95% CI[g]
					x[c]	POD_C[d]	95% CI	X	POD_R[e]	95% CI		
Romaine Lettuce (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}^{f}$**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Queso Fresco (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}^{f}$**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
4% Milk Fat Cottage Cheese (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}^{f}$**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
3% Chocolate Whole Milk (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_C$[f]**	**95% CI[g]**
					x[c]	**POD_C[d]**	**95% CI**	**X**	**POD_R[e]**	**95% CI**		
Beef Hot Dogs (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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 *Listeria monocytogenes* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)

Matrix	Strain	Analysis Time Point	CFU[a]/ Test Area	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Stainless Steel (225 mL)	*L. monocytogenes* ATCC 19118 & *Enterococcus faecium* 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[a]/ Test Area**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
Sealed Concrete (100 mL)	*L. monocytogenes* 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[a]/ Test Area**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
Plastic (10 mL)	*L. monocytogenes* ATCC 51782 & *Enterococcus faecalis* 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

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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
*Listeria monocytogenes*ATCC[2]7644					
Low Level	6/10	Low Level	6/10	Low Level	6/10
Uninoculated Target Organism (*Enterococcus faecalis*ATCC29212)	0/5	Uninoculated Target Organism (*Enterococcus faecalis*ATCC29212)	0/5	Uninoculated Target Organism (*Enterococcus faecalis*ATCC29212)	0/5

[1]- All samples were analyzed from a common lysate
[2]ATCC- American Type Culture Collection

Table 17: Robustness Results

Robustness[1]			Robustness[1]		
13 Minute Lysis			13 Minute Lysis		
Sample #	18 µL[2], 18 µL[3]	18 µL[2], 22 µL[3]	Sample #	22 µL[2], 18 µL[3]	22 µL[2], 22 µL[3]
*Listeria monocytogenes*ATCC 7644					
Low Level	6/10	6/10	Low Level	6/10	6/10
Uninoculated Target Organism (*Enterococcus faecalis*ATCC29212)	0/5	0/5	Uninoculated Target Organism (*Enterococcus faecalis*ATCC29212)	0/5	0/5
Robustness[1]			**Robustness[1]**		
17 Minute Lysis			**17 Minute Lysis**		
Sample #	**18 µL[2], 18 µL[3]**	**18 µL[2], 22 µL[3]**	**Sample #**	**22 µL[2], 18 µL[3]**	**22 µL[2], 22 µL[3]**
*Listeria monocytogenes*ATCC[4] 7644					
Low Level	6/10	6/10	Low Level	6/10	6/10
Uninoculated Target Organism (*Enterococcus faecalis*ATCC29212)	0/5	0/5	Uninoculated Target Organism (*Enterococcus faecalis*ATCC29212)	0/5	0/5

[1]- All samples were analyzed from a common lysate
[2]-Volume of enrichment to lysis
[3]-Volume of lysis to assay
[4]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 preesented?		
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 producct 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 variablity 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 Supplemantary Material and include it win 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 inujure 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 variablity 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.

	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.	Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.
	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.	Currently revising the PTMs to reflect the method of homognization. See above response.
	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.	Revised Table 2 to Table A. (five instances)
	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.	Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.
	ER4 Package Insert comments:	
	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.	Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.

	2. There should be an applicability statement, specific to AOAC, In the package insert.	Per AOAC website: http://www.aoac.org/imis15_prod/AOAC_Docs/SPDS/Formats_AOAC_collab.pdf 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 compenents 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	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 all the figures and tables pertinent?		
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
Could some be omitted and covered by a simple statement?		
ER1	NO	Thank You for your comments and review
ER2	NO	Thank You for your comments and review
ER3	NO	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	NO	Thank You for your comments and review

Are the references complete and correctly annotated?		
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
Does the method contain adequate safety precaution reference and/or statements?		
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
Recommendation: Do you recommend that the ERP adopt this method as an AOAC Official Method of Analysis (First Action status)?		
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, 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	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review

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

Patrick Bird, Jonathan Flannery, Erin Crowley, James Agin, David Goins
Q Laboratories, Inc., 1400 Harrison Ave, Cincinnati, OH 45214

Lisa Monteroso
3M Food Safety Department, 3MCenter – Bldg. 260-6B-01, St. Paul, MN 55144

Collaborators: R. Brooks, J. Walia, F. Hernandez, D. Bosco, G. Trevino, A. Brandt, C. Lopez, E. Sjogren, M. Shekhawat, L. Ma, C. Timmons, C. Diaz Proano, A. Calle,Z. Metz, D. Baumler, R. Smith, D. Wood, E. Maranan, C. Chavarria,J. Miller, B. Schindler, C. Gies, E. Budge, Z. Geurin, A. Repeck, C. Zook, C. Barnes, B. Bastin, D. Isfort

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 from13laboratories located within the continental United Statesand 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 the difference between methods was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the candidate and reference methods.~~For raw chicken breast fillet a dLPOD valuewith 95% confidence interval of 0.16, (0.04, 0.28) indicating astatisticallysignificant difference~~between the candidate and reference methods~~ an observed higher proportion of positive results by the candidate method than the reference method.~~with a positive correlation in data indicating more recovery of the target analyte by the candidate method.~~

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]. Theorganism'sability to survive in extreme conditions make its presence in food a serious issue. In the past year, *Listeria*has been identified as the source ofseveral high profile outbreaks

involving bagged leafy greens and ice cream [3].The 3M™ Molecular Detection Assay(MDA) 2 - *Listeria*method,usinga combination of bioluminescence and isothermal amplification of nucleic acid sequences,allows for the rapid and specific detection of *Listeria*species in a broad range of food types and environmental surfaces after 24 to ~~28~~32hoursof pre-enrichment. After enrichment, samples are evaluated using the 3M MDA 2 - *Listeria* on the 3M™ Molecular Detection System (MDS).Presumptive positive results are reported in real-time while negative results are displayed after completion of the assay in approximately 75 minutes.

Prior to the collaborative study, the 3M MDA 2 - *Listeria* method was validated according to AOAC Guidelines[4] in a harmonized AOAC® Performance Tested Method℠ (PTM) study. The objective of the PTM study was to demonstrate that the 3M MDA 2-*Listeria* method could detect *Listeria*in a broad range of food matrices and environmental surfaces as claimed by the manufacturer. For the 3M MDA 2- *Listeria* PTM evaluation, 13 matrices were evaluated: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), and plastic (Enviro~~s~~Swab, 10 mL) environmental samples.

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

The purpose of this collaborative studywas to compare the reproducibility of the 3M MDA 2 - *Listeria*method 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*[5] for deli turkey (125 g) and raw chicken breast fillet.

Collaborative Study

Study Design

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

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

A detailed collaborative study packet outlining all necessary information related to the study including media preparation, test portion preparation and documentation of results was sent to each collaborating laboratory prior to the initiation of the study. A conference call was then

conducted to discuss the details of the collaborative study packet and answer any questions from the participating laboratories.

Preparation of Inocula and Test Portions

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

$$(1-\frac{n_{select}}{n_{nonselect}})x100$$

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

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

Test Portion Distribution

All samples were labeled with a randomized, blind-coded 3-digit number affixed to the sample container. Test portions were shipped on a Thursday via overnight delivery according to the Category B Dangerous Goods shipment regulations set forth by the International Air Transportations Association(IATA).The two matrices were shipped consecutively, with collaborating laboratories performing analysis on one matrix at a time. Upon receipt, samples were held by the collaborating laboratory at refrigeration temperature (2-8 °C) until the following Monday when analysis was initiatedafter a total equilibration time of 96 hours.All samples were packed with cold packs to target a temperature of < 7°C during shipment.
In addition to each of the test portions and a separate APCsample, collaborators received a test portion for each matrix labeled as 'temperature control'.Participants were instructed to obtain the temperature of this portion upon receipt of the package, document the results on the Sample

Receipt Confirmation form provided and fax or email it back to the study director.The shipment and hold timesof the inoculated test material had been verified as a quality control measure prior to study initiation.

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 ±1°C. For the raw chicken breast fillettest 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 ±1°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]..POD statistical analysis was conducted using AOAC Binary Data Interlaboratory Study Workbook, Version 2.3 [10]. 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, $POD_{CP,}$ the candidate confirmatory results (excluding those with presumptive negative results~~including false negative results~~), POD_{CC}, the difference in the candidate presumptive and confirmatory results, $dLPOD_{CP,}$ presumptive candidate results that confirmed positive ((including those with presumptive negative results)~~excluding false negative results)~~, $POD_{C,}$ the reference method, POD_R, and the difference in the confirmed candidate and reference methods, $dLPOD_C$. A $dLPOD_C$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_r), the among laboratory repeatability standard deviation (s_L), the reproducibility standard deviation (s_R)and the P_T value were calculated. The s_r provides the ~~variance~~ variabilityof data within one laboratory, the s_L provides the difference in standard deviation between laboratories and the s_R provides the ~~variance~~ variabilityin data between different laboratories. The P_T value provides information on the homogeneity test of laboratory PODs~~[10]~~.

AOAC Official Method 2016.xxx
***Listeria*speciesin SelectFoods and Environmental Surfaces**
3M™ Molecular Detection Assay(MDA) 2- *Listeria* Method
First Action 2016

(Applicable to detection of *Listeria*speciesin 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 (3M™ Hydrated Sponge Stick with D/E, 225 mL & 100 mL), stainless steel (3M™ Hydrated Sponge Stick with D/E, 225 mL), and plastic (3M™ Enviro~~s~~Swab with Letheen, 10 mL) environmental samples.

*See Tables**2016.1A** and **2016.1B**for a summary of results of the inter-laboratory study.*
*See Tables **2016.2A** and **2016.2B** for detailed results of the inter-laboratory study*

A. Principle

The 3M™ Molecular Detection Assay (MDA) 2 - *Listeria* method is used with the 3M™ Molecular Detection System (MDS) for the rapid and specific detection of *Listeria* in enriched food and food process environmental samples. The 3M MDA2 -*Listeria*uses loop-mediated isothermal amplification of unique DNA target 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. Samples arepre-enriched in Demi Fraser broth with ferric ammonium citrate (FAC).

B. Apparatus and Reagents

Items (b)-(g) are available as the 3M™ Molecular Detection Assay (MDA) 2 - *Listeria* kit from 3M Food Safety (St. Paul, MN 55144-1000, USA).

- **(a)** *3M Molecular Detection System*(MDS100) – Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- **(b)** *3M Molecular Detection Assay 2 – Listeria reagent tubes*- 12 strips of 8 tubes. Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- **(c)** *Lysis Solution (LS) tubes* – 12 strips of 8 tubes.
- **(d)** *Extra caps* – 12 strips of 8 caps
- **(e)** *Reagent Control* – 8 reagent tubes
- **(f)** *Quick Start Guide*
- **(g)** *3M™ Molecular Detection Speed Loader Tray* - Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- **(h)** *3M™ Molecular Detection Chill Block Insert* - Available from 3M™ Food Safety (St. Paul, MN 55144-1000, USA).
- **(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 ± 1°C

(v) *Dry block heater unit*– capable of maintaining 100 ± 1°C

(w) *Incubators.* – Capable of maintaining 37 ± 1°C~~or 41.5 ± 1°C.~~

~~**(x)** *Freezer* – capable of maintaining -10 to -20°C, for storing the 3M Molecular Detection Chill Block Tray~~

~~(y)~~(x) *Refrigerator* – capable of maintaining 2-8°C, for storing the 3M Molecular Detection Assay components

~~(z)~~(y) *Computer* – compatible with the 3M™ Molecular Detection Instrument

~~(aa)~~(z) *3M™ Enviro~~s~~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* 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 60 days. Do not use 3M Molecular Detection Assay 2 -*Listeria* past the expiration date.

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

Safety Precautions

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 the food and beverage industries. For example, 3M has not documented this product for testing drinking water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M MDA2 – *Listeria*has not been

evaluated with all possible food products, food processes, testing protocols or with all possible strains of bacteria.

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

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.

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

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

Listeria monocytogenes is of particular concern for pregnant women, the aged and the infirmed. It is recommended that these concerned groups avoid handling this organism.After use, the enrichment medium and the 3M MDA 2 - *Listeria*tubes can potentially contain pathogenic materials.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. 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.

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

D. Sample Enrichment

Foods

(a) Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature (20-25^{o}C).

(b) Aseptically combine the enrichment medium and sample according to Table ~~2~~A. For all meat and highly particulate samples, the use of filter bags is recommended.

(c) Homogenize thoroughly by ~~blending,~~ stomaching, or hand mixing for 2 ±0.2 minutes. Incubate at 37 ±1°C according to Table A.

Environmental samples

(d) 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 Letheen 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. Another option is to transfer 10 µL of the NB enrichment in the LS tubes.

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(e) The recommended size of the sampling area to verify the presence or absence of the pathogen on the surface is at least 100 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, USDA FSIS MLG or ISO 18593 [11] guidelines.

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

E. Preparation of the 3M™ Molecular Detection Speed Loader Tray

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

Specific Instructions for Validated Methods

AOAC® Performance Tested Method℠#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 ~~2~~A. 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 ~~2~~A).

Table ~~2~~A: Enrichment protocols using Demi-Fraser Broth at 37 ± 1 °C according to AOAC Performance Tested ^{SM} 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[a]		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[b]	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

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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 ±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 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 ±1°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.

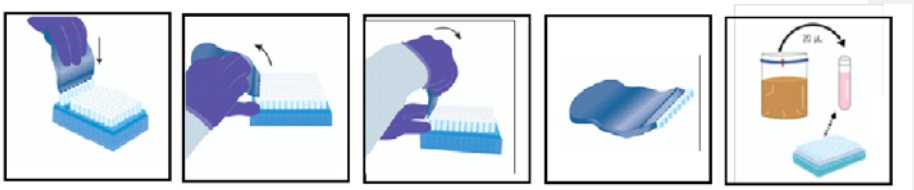
NOTE: The 3M Molecular Detection Instrument must reach and maintain temperature of 60°C before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 minutes and is indicated by an ORANGE light on the instrument's status bar. When the instrument 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±1°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 µL 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 4.6as 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.
7. 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 heat for 15 ±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 (20-25°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.
9. ~~6.~~ Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert.

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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 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.
   - 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 µL of NC lysate into a Reagent tube.

4.7 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.

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* amplificationreagents have a "background" relative light unit (RLU) reading.

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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.

Results of Collaborative Study

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

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

Results for the heat stress analysis of the inoculum for the deli turkey are presented in Table 2. The raw chicken breast fillet is not heat treated, therefore it was not necessary to injure the cells.Table 2016.1A and 2016.1B summarize the inter-laboratory results for all foods tested, including POD statistical analysis.As per criteria outlined in Appendix J of the AOAC Validation Guidelines, fractional positive results were obtained.Detailed results for each laboratory are presented in Tables 2016.2A and 2016.2B. For each matrix, the level of *Listeria* was determined by MPN on the day of initiation of analysis by the coordinating laboratory. MPN results are presented in Tables 2016.2A and 2016.2B.The individual laboratory and sample results are presented in Tables 1-2 of the Supplementary Materials. The APCresults for each collaborating are presented in Table5~~3~~ of the Supplementary Materials.

Deli Turkey (125 g Test Portions)

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

For the low inoculum level, 68 out of 132 test portions (POD_{CP} of 0.52) were reported as presumptive positive by the 3M MDA 2 – *Listeria*method with 66out of 132 test portions (POD_{CC} of 0.50) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria*method, 66out of 132 samples confirmed positive (POD_C of 0.50). For

test portions evaluated by the USDA/FSIS MLG reference method, 60 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.04 with 95% confidence intervals of (-0.08, 0.17) was obtained between the candidate and reference method, indicating the difference between methods was not statistically significant at the 0.05 probability level~~no statistical significant difference between the two methods.~~A $dLPOD_{CP}$ value of 0.02 with 95% confidence intervals of (-0.11, 0.14) was obtained between presumptive and confirmed results indicatingthe difference between presumptive and confirmed results was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the presumptive and confirmed results.~~

For the high inoculum level, 132 out of 132 test portions (POD_{CP} of 1.00) were reported as presumptive positive by the 3M MDA 2 – *Listeria*method with 132out of 132 test portions (POD_{CC} of 1.00) confirming positive.For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria*method, 132out of 132 samples confirmed positive (POD_C of 1.00). For test portions evaluated by the USDA/FSISMLG reference method, 132 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between the candidate and reference method, indicatingthe difference between methods was not statistically significant at the 0.05 probability level.~~no statistical significant difference between the two methods.~~A $dLPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between presumptive and confirmed results indicating the difference between presumptive and confirmed results was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the presumptive and confirmed results.~~

For the un-inoculated controls, 0 out of 132 samples (POD_{CP} of 0.00) produced a presumptive positive result by the 3M MDA 2 - *Listeria*method with 0out of 132 test portions (POD_{CC} of 0.00) confirming positive.For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria*method, 0out of 132 samples confirmed positive (POD_C of 0.00). For test portions evaluated by the USDA/FSIS MLG reference method, 0 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between the candidate and reference method, indicating the difference between methods was not statistically significant at the 0.05 probability level.~~no statistical significant difference between the two methods.~~ A $dLPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.03, 0.00) was obtained between presumptive and confirmed results indicatingthe difference between presumptive and confirmed results was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the presumptive and confirmed results.~~

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

Raw Chicken Breast Fillet (25 g Test Portions)

Raw chicken breast fillet test portions were inoculated at a low and high inoculum level and were analyzed for the detection of *Listeria* spp. Un-inoculated controls were included in each

analysis. Laboratory 11 did not participate in the evaluation of this matrix. Laboratory 10 submitted data that indicated cross contamination of the inoculating organism in the un-inoculated control samples. Further analysis at the coordinating laboratory confirmed the cross contamination of the un-inoculated controls. Due to this issue, the data submitted from Laboratory 10 was not used in the statistical analysis. All other laboratories submitted data for both methods evaluated.The MPN levels obtained for this matrix, with 95% confidence intervals, were 0.66 CFU/test portion (0.51, 0.83) for the low level and 6.24 CFU/test portion (3.58, 10.88) for the high level.

For the low inoculum level, 88 out of 132 test portions (POD_{CP} of 0.67) were reported as presumptive positive by the 3M MDA 2 – *Listeria*method with 86out of 132 test portions (POD_{CC} of 0.65) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria*method, 85out of 132 test portions confirmed positive (POD_C of 0.64). For test portions evaluated by the USDA/FSIS MLGreference method, 64 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.16 with 95% confidence intervals of (0.04, 0.28) was obtained between the candidate and reference method, indicating a statistically significant difference between the two methods, with anobserved higher proportion of positive results by the candidate method than the reference method.~~positive with a positive correlation in data indicating more recovery of the target analyte by the candidate method.~~A $dLPOD_{CP}$ value of 0.02 with 95% confidence intervals of (-0.10, 0.13) was obtained between presumptive and confirmed results indicating the difference between presumptive and confirmed results was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the presumptive and confirmed results.~~

For the high inoculum level, 131 out of 132 test portions (POD_{CP} of 0.99) were reported as presumptive positive by the 3M MDA 2 – *Listeria*method with 132out of 132 test portions (POD_{CC} of 1.00) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria*method, 131out of 132 samples confirmed positive (POD_C of 0.99). For test portions evaluated by the USDA/FSIS-MLG reference method, 132 out of 132 test portions produced positive results. A $dLPOD_C$ value of -0.01 with 95% confidence intervals of (-0.04, 0.02) was obtained between the candidate and reference method, indicatingthe difference between methods was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the two methods.~~ A $dLPOD_{CP}$ value of -0.01 with 95% confidence intervals of (-0.04, 0.02) was obtained between presumptive and confirmed results indicating the difference between presumptive and confirmed results was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the presumptive and confirmed results.~~

For the un-inoculated controls, 2 out of 132 samples (POD_{CP} of 0.02) produced a presumptive positive result by the 3M MDA 2 - *Listeria*method with 1 out of a 132 test portions (POD_{CC} of 0.01) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria*method, 1 out of 132 samples confirmed positive (POD_C of 0.01). For test portions evaluated by the USDA/FSIS-MLG reference method, 0 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.01 with 95% confidence intervals of (-0.02, 0.04) was obtained between the candidate and reference method, indicating the difference between methods was not statistically significant at the 0.05 probability level.~~no statistical~~

~~significant difference between the two methods.~~ A $dLPOD_{CP}$ value of 0.01 with 95% confidence intervals of (-0.03, 0.05) was obtained between presumptive and confirmed results indicating the difference between presumptive and confirmed results was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the presumptive and confirmed results.~~

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

Discussion

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

Overall, the data generated during this evaluation demonstrates the reproducibility of this new method. For the deli turkey analysis, the POD statistical analysis indicated the difference between the candidate method and reference method was not statistically significant at the 0.05 probability level. And that the difference between presumptive and confirmed candidate method was not statistically significant at the 0.05 probability level.~~thatno statistically significant difference between the candidate method and the reference method or between the presumptive and confirmed results of the candidate method was obtained.~~For raw chicken breast fillet, a statistically significant difference was observed between the reference and the alternative method. The dLPOD being significantly greater than zero showed an observed higher proportion of positive results by the candidate method than the reference method.~~The dLPOD data indicated a positive correlation in data indicating more recovery of the target analyte by the candidate method.~~ One possible contribution for the higher observed proportion positive results~~to the higher level of recovery~~ observed with the 3M MDA 2 – *Listeria* method was the use of Demi-Fraser Broth for the candidate method. This enrichment media formulation is less selective than the modified University of Vermont Medium used in the USDA reference method and may have contributed to the higher level of recovery observed during the evaluation. A second possible contribution ~~t~~for the higher observed proportion positive results~~o the higher level of recovery~~ was the length of the primary enrichment. Test portions evaluated by the 3M MDA 2 – *Listeria* method were incubated for a minimum of 28 hours in the primary enrichment, while the USDA reference method had a maximum primary enrichment time of 26 hours. No statistically

significant difference was observed between the candidate method presumptive and confirmed results for this matrix.

Recommendations

It is recommended that the 3M Molecular Detection Assay 2 – *Listeria*method be adopted as Official First Action status for the detection of *Listeria* in selected foods: 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, stainless steel and plastic environmental samples.

Acknowledgements

We would like to extend a sincere thank you to the following collaborators for their dedicated participation in this study:

Robert Brooks- ATC Microbiology, LLC- North Little Rock, AR.
Jaspreet Walia & Francisco Hernandez – Certified Laboratories, Turlock, CA
David Bosco& Grizelda Trevino– Food Safety Net Services, Fresno, CA
Alex Brandt & Chris Lopez – Food safety Net Services, San Antonio, TX
Elizabeth Sjogren&Manish Shekhawat– Microbac Laboratories, Inc. Worcester, MA
Li Maria Ma, Chris Timmons, & Claudia Diaz Proano – Oklahoma St. University, Stillwater, OK
Alexandra Calle & David Campos – Texas Tech University, Lubbock, TX.
Zachary Metz&David Baumler, University of Minnesota, St. Paul, MN
Robbie Smith, Dianne Wood, Evelyn Maranan,&Carmen Chavarria Maple Leaf Foods– Guelph, ON, Canada
Ben Bastin– Microbiology R&D, Q Laboratories Inc., Cincinnati, OH
David Isfort– Microbiology-Food, Q Laboratories Inc., Cincinnati, OH
Jesse Miller, Bryan Schindler, Courtney Gies, Eric Budge, Zach Geurin & Alex Repeck - NSF International, Ann Arbor, MI
Cynthia Zook & Christina Barnes – 3M Food Safety, St. Paul, MN

We would like to extend a special thanks to the following team members at Q Laboratories, Inc. for their efforts during the collaborative study: M. Joseph Benzinger Jr., Allison Mastalerz, Megan Boyle,Alison DeShields, Jonathan Clifton, T. Shane Wilson, Meghan Daniel, Nicole Klass and Colleen Sweeney.

References

(1) United States Food and Drug Administration. (2008) *Guidance for Industry: Control of Listeria monocytogenes in Refrigerated or Frozen Ready-to-Eat Foods; Draft Guidance*http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/FoodProcessingHACCP/ucm073110.htm (Accessed March 2016)

(2) Jemmi, T., Stephan, R. (2006) Scientific and Technical Review of the Office International des Epizooties *Listeria monocytogenes*: *food-borne pathogen and hygiene indicator*.Vol. 25, No. 2, pp. 571-580

(3) Centers for Disease Control and Prevention. (2016) *Listeria Outbreaks*http://www.cdc.gov/listeria/outbreaks/(Accessed March 2016)

(4)*Official Methods of Analysis* of AOAC INTERNATIONAL. (2012) 19th Ed., Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, AOAC INTERNATIONAL, Gaithersburg, MD, http://www.eoma.aoac.org/app_j.pdf(Accessed January 2016)

(5)United States Department of Agriculture Food Safety and Inspection Service. (2013) *Microbiological Laboratory Guidelines5.09*: *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and Environmental Samples*. (Accessed January 2016) http://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES

(6) *Official Methods of Analysis* of AOAC INTERNATIONAL (2015) *Enumeration of Aerobic Bacteria in Foods.*2015.13. (Accessed January2016)

(7) Least Cost Formulations, Ltd., MPN Calculator-Version 1.6, www.lcfltd.com/customer/LCFMPNCalculator.exe(Accessed March 2016)

(8) *Official Methods of Analysis* of AOAC INTERNATIONAL (2012) *Gram-Positive Bacteria Identification*. 2012.02. (Accessed January 2016)

(9) Wehling, P., LaBudde, R., Brunelle, S., Nelson, M. (2011) Journal of AOAC International*Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods.* Vol. 94, No. 1. pp. 335-347

(10) Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook (2013), http://www.aoac.org/iMIS15_Prod/AOAC/SD/SGR/AOAC_Member/SDCF/SDGRCF/SDSGM.aspx?hkey=086fdba4-32e2-43f0-8272-7d41a6603e87~~http://lcfltd.com/aoac/aoac-binary-v2-3.xls~~(Accessed March, 2016)

(11) ISO 18593:2004 -*Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.*

Table 1: Participation of each Collaborating Laboratory[a]

Lab	Deli Turkey[a,b]	Raw Chicken Breast Fillet[a,b]
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

[a] Y= Collaborator analyzed the food type;[a]N= Collaborator did not analyze food type

Table 2: Heat-Stress Injury Results

Matrix	Test Organism[a]	CFU/MOX (Selective Agar)	CFU/TSA (Non-Selective Agar)	Degree Injury[b]
Deli Turkey	*Listeriamonocytogenes* ATCC 19115	9.1×10^8	2.5×10^9	60.8%
Raw Chicken Breast Fillet	*Listeria monocytogenes* ATCC 7644	Not applicable	Not applicable	Raw chicken product is not heat treated so not necessary to injure the cells.

[a] ATCC- American Type Culture Collection

[b] 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* inDeli Turkey (125g)

Method[a]	**3M™ MDA™2 - *Listeria***		
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[e]	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)[f]	0.00 (-0.03, 0.03)	0.04 (-0.08, 0.17)	0.00 (-0.03, 0.03)

dLPOD (Candidate Presumptive vs. Candidate Confirmed)[f]	0.00 (-0.03, 0.03)	0.02 (-0.11, 0.14)	0.00 (-0.03, 0.03)

[a] Results include 95% Confidence Intervals, [r] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation
[e] P Value = Homogeneity test of laboratory PODs; [f] 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* inRaw Chicken Breast Fillet (25g)

Method[a]	**3M™ MDA™2 -*Listeria***		
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[e]	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)[f]	0.01	0.16	-0.01

	(-0.02, 0.04)	(0.04, 0.28)	(-0.04, 0.02)
dLPOD (Candidate Presumptive vs. Candidate Confirmed)[f]	0.01 (-0.03, 0.05)	0.02 (-0.10, 0.13)	-0.01 (-0.04, 0.02)

[a] Results include 95% Confidence Intervals, [r] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation

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

Table 2016.2A: Comparative Results for the Detection of *Listeria* in 125 g Deli TurkeyTest Portions by the 3M™ MDA 2 - *Listeria* 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[a]	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[a]	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		

[a]N/A – Laboratory did not participate or submit data for this matrix

[b]Repeatability Standard Deviation, [c]Among-Laboratory Standard Deviation, [d]Reproducibility Standard Deviation, [e] 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)

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. 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[a]	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[a]	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		

[a] N/A – Laboratory did not participate or submit data for this matrix

[b] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation, [e] 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)

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. 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[a]	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[a]	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		

[a] N/A – Laboratory did not participate or submit data for this matrix

[b] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation, [e] 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)

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. 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[b]	12	0	0.00	12	2	0.17	12	0	0.00	12	0	0.00	0.00	-0.17
		11[a]	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		

[a] N/A – Laboratory did not participate or submit data for this matrix; [b] Results were not used in statistical analysis due to deviation from testing protocol.
[c] Repeatability Standard Deviation, [d] Among-Laboratory Standard Deviation, [e] Reproducibility Standard Deviation, [f] 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)

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* 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[b]	12	6	0.50	12	8	0.66	12	6	0.50	12	5	0.42	0.08	-0.16
	MPN/ Test Portion	11[a]	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		

[a] N/A – Laboratory did not participate or submit data for this matrix; [b] Results were not used in statistical analysis due to deviation from testing protocol.
[c] Repeatability Standard Deviation, [d] Among-Laboratory Standard Deviation, [e] Reproducibility Standard Deviation, [f] 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)

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* 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[b]	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
	MPN/ Test Portion	11[a]	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	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		

[a]N/A – Laboratory did not participate or submit data for this matrix; [b] Results were not used in statistical analysis due to deviation from testing protocol.
[c]Repeatability Standard Deviation, [d]Among-Laboratory Standard Deviation, [e]Reproducibility Standard Deviation, [f] 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)

Table 3~~1~~. Individual Collaborator Results for Deli Turkey

	High Level Test Portions												Low Level Test Portions												Un-inoculated Test Portions											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
3M MDA 2 -*Listeria*																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-[b]	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
11	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-[b]	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
USDA/FSIS – MLG 8.09																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
9	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

[a] += *Listeria* species were detected in samples; -=*Listeria* species were not detected in sample; n/a- lab did not participate or submit data for the matrix;
[b] Sample was presumptive positive on 3M MDA 2 *Listeria* but confirmed negative indicating a false positive result;

Table ~~2~~4. Individual Collaborator Results for Raw Chicken Breast Fillet

	High Level Test Portions												Low Level Test Portions												Un-inoculated Test Portions											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
3M MDA 2 - *Listeria*																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+[c]	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-[d]	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	-	-[d]	-[d]	+	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-[d]	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+[e]	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+[e]	+	-	-	-	-	-	-	-	-	-	-	-	-
10[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+[e]	+[e]	-	-	+	+[e]	-	-	-	-	-	-	-	-	-	-	+[e]
11	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
USDA/FSIS – MLG 8.09																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10[b]	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
11	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
12	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

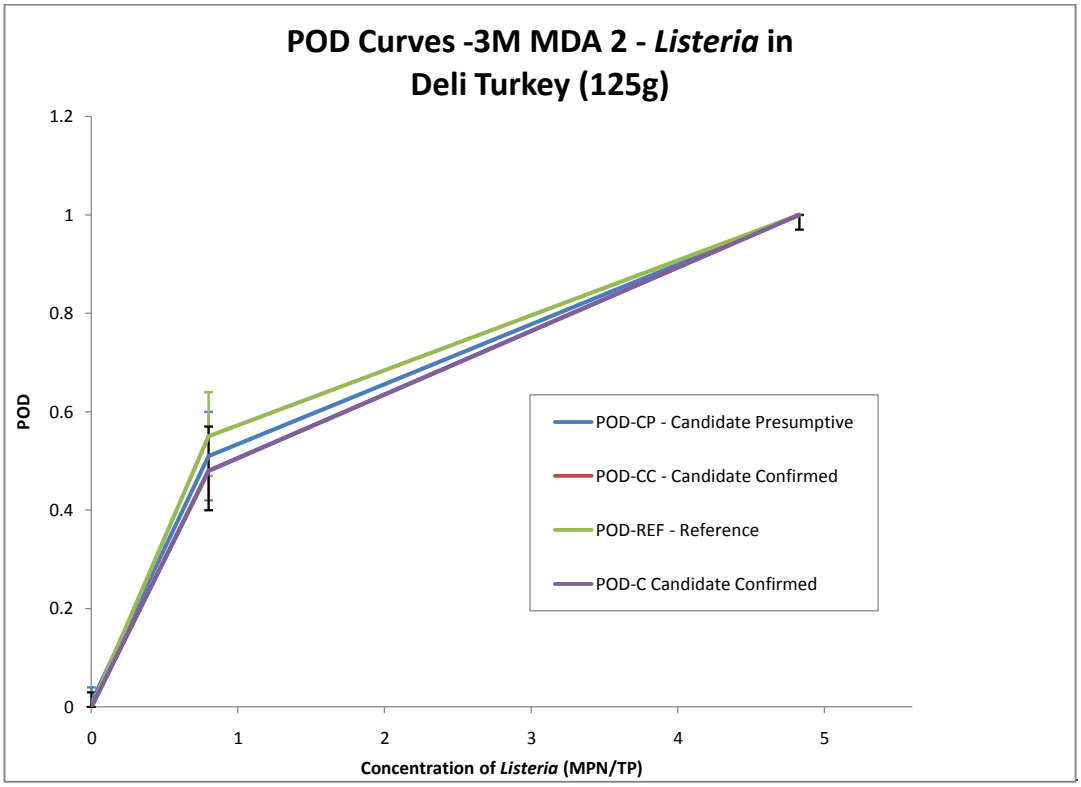
[a] + = *Listeria* species were detected in samples; -=*Listeria* species were not detected in sample; n/a- lab did not participate or submit data for the matrix;
[b] Results were not used in statistical analysis due to laboratory error; [c]*Listeria innocua* recovered from natural contamination of the sample. [d]Sample was presumptive positive on 3M MDA 2 *Listeria* but confirmed negative indicating a false positive result; [e] Sample was presumptive negative on 3M MDA 2 *Listeria* but confirmed positive indicating a false negative result

Table 53: Results of Aerobic Plate Count for Collaborating Laboratories

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

[a] Samples analyzed by the 3M Petrifilm Rapid Aerobic Count Plate, AOAC OMA 2015.13

Figure 1A: POD Values of Candidate Method vs. Reference Method for125 gDeli Turkey



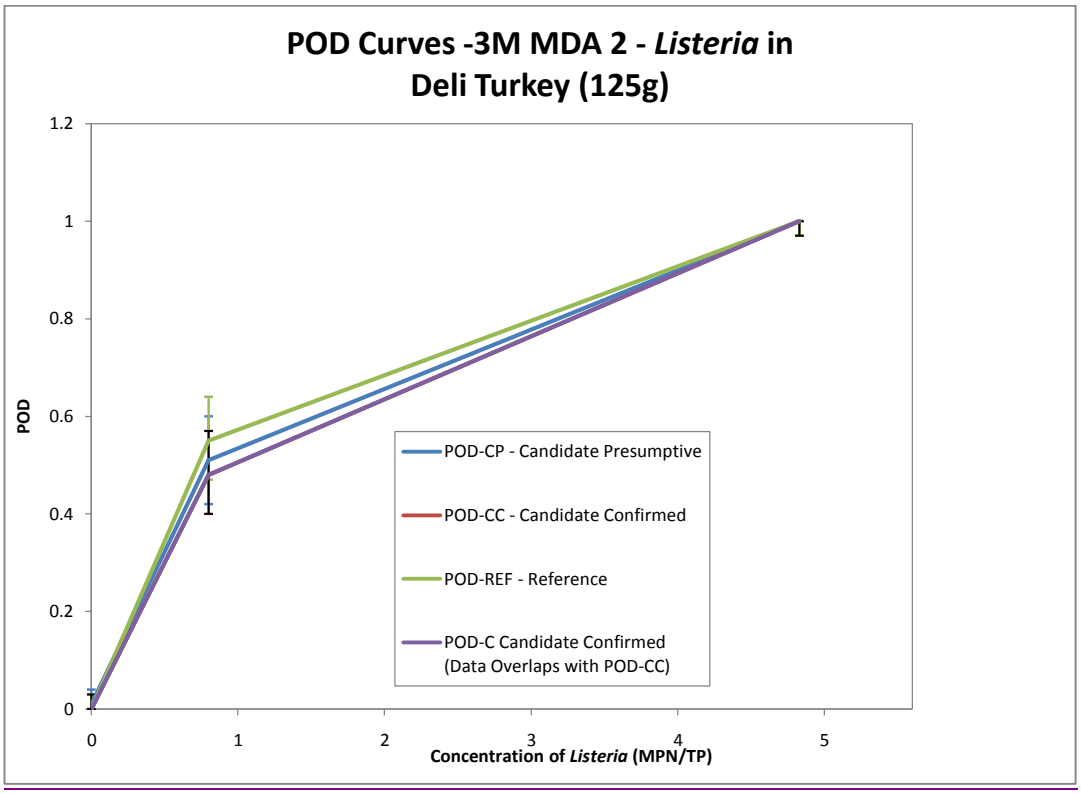


Figure 1B: dLPOD Values of Candidate Method vs. Reference Method for125 gDeli Turkey

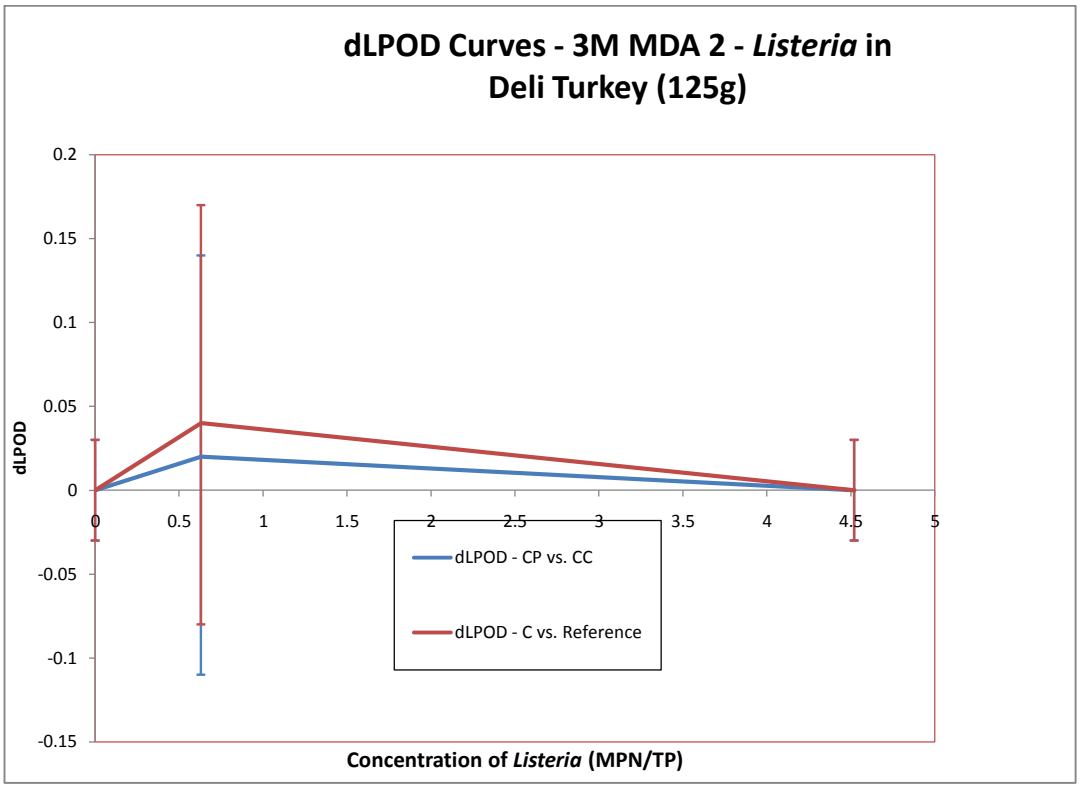
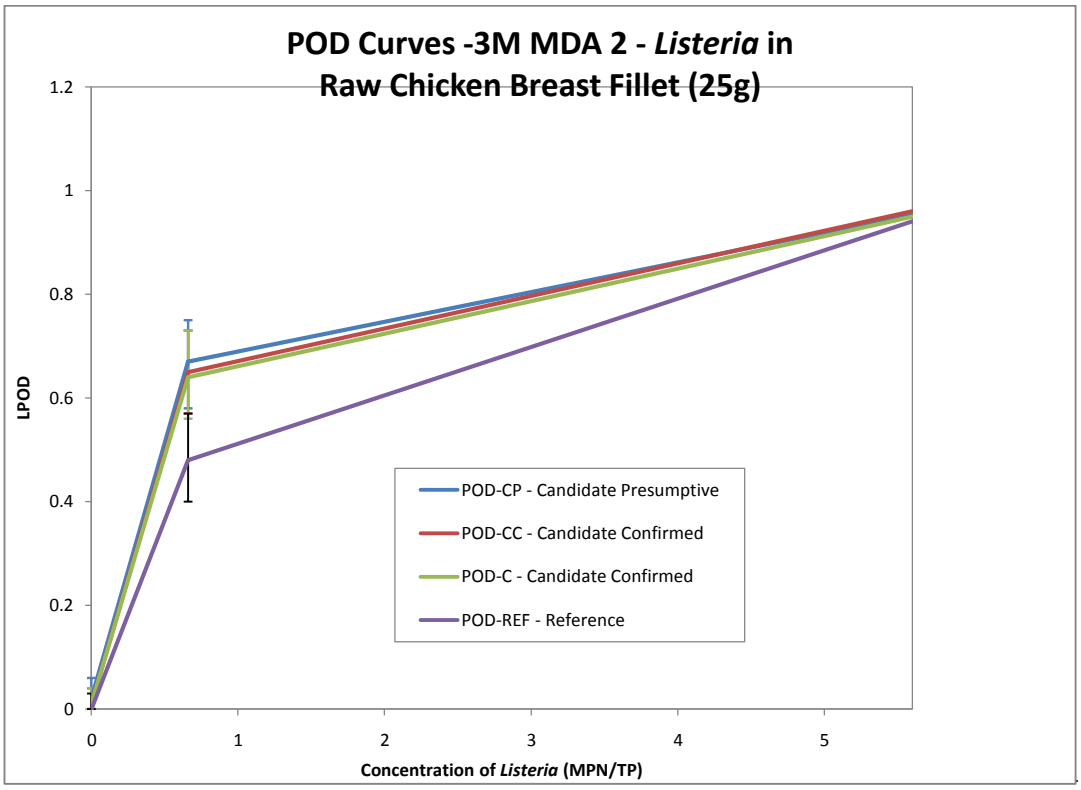


Figure 2A: POD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet


POD Curves -3M MDA 2 - *Listeria* in Raw Chicken Breast Fillet (25g)
LPOD
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
Concentration of *Listeria* (MPN/TP)
POD-CP - Candidate Presumptive
POD-CC - Candidate Confirmed
POD-C - Candidate Confirmed
POD-REF - Reference

1

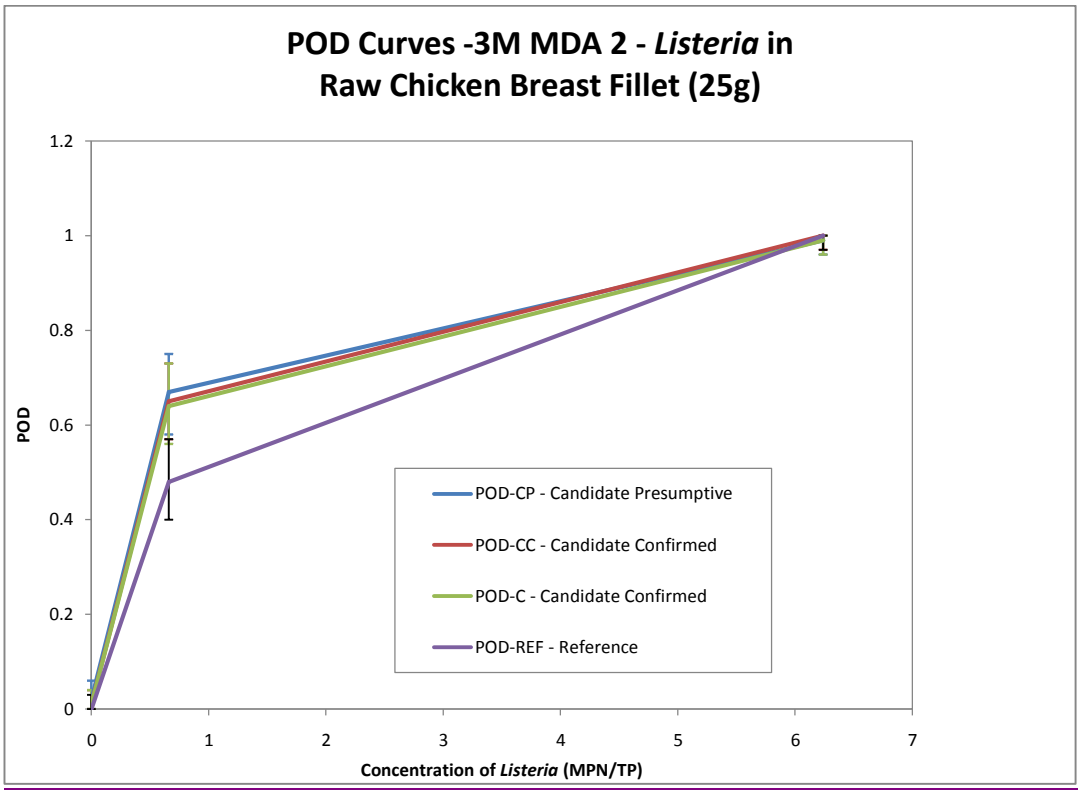
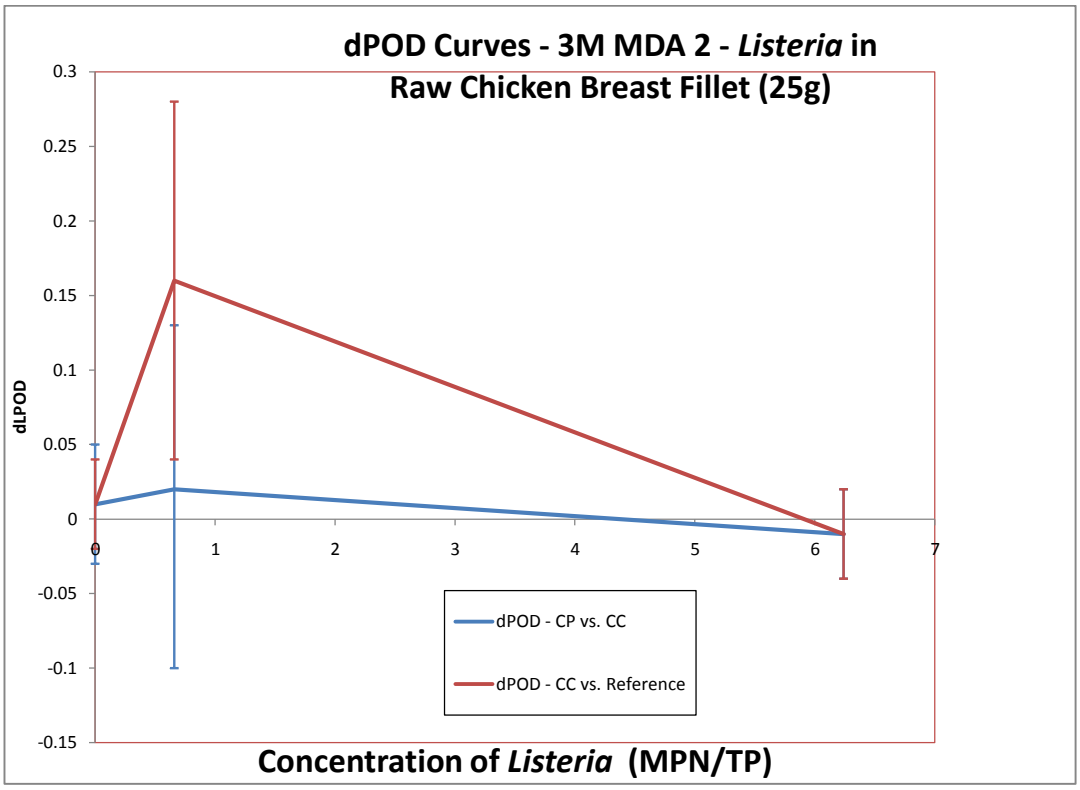


Figure 2B: dPOD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet


dPOD Curves - 3M MDA 2 - *Listeria* in Raw Chicken Breast Fillet (25g)
dLPOD
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
0
1
2
3
4
5
6
7
dPOD - CP vs. CC
dPOD - CC vs. Reference
Concentration of *Listeria* (MPN/TP)

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* with Demi-Fraser Brothand 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
Listeria 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[4], or ISO 7218[5]. The user shouldcomplete 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[6].

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 thathave 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 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**, 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* tubescan 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 ^{SM}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 ±0.2 minutes. Incubate at 37 ±1°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 ±1°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 Letheen broth. It is recommended to sanitize the area after sampling.

WARNING: Should you select to use Neutralizing Buffer (NB) that contains aryl sulfonatecomplex 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 testingin 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^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[1], USDA FSIS MLG[2] or ISO 18593[7] 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 or4.
3. Homogenize thoroughly by blending, stomaching, or hand mixing for 2 ±0.2 minutes. Incubate at 37 ±1°C for24-30 hours.

Table 2: Generalenrichment 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 Heat-processed / pasteurized dairy products Produce and vegetables Multi-component foods	25 g	225	37	24-30
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[a]
	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℠#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℠ 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[a]		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[b]	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[8] in comparison to ISO 11290-1[3]

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[3] and EN ISO 6887[9]

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 (±1°C)	Enrichment Time (hr)	Sample Analysis Volume[(a)]	Recommended Interruption Point
All food samples(excepted raw meats, raw seafood and raw dairy products)	25 g	225	37	24-30	20 µL	•DF broth up to 72 hr •lysate at -20°C •lysate at 4°C up to 72 hr
Environmental samples	25 g, 1 swab, or 1 wipe	225	37	24-30	20 µL	•DF broth up to 72 hr •lysate at -20°C
Specific Protocol 1	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature (±1°C)	Enrichment Time (hr)	Sample Analysis Volume (a) (µL)	Recommended Interruption Point
Raw meats and raw seafood	25 g	475	37	28-32	20 µL	•DF broth up to 72 hr •lysate at -20°C •lysate at 4°C up to 72 hr

Specific Protocol 2	Primary Enrichment (Demi-Fraser Broth)				Secondary Enrichment (Fraser Broth)				
	Sample Size	Enrichment Broth Volume (mL)	Enrichment Temperature (±1°C)	Enrichment Time (hr)	Sample Size	Enrichment Temperature (±1°C)	Enrichment Time (hr)	Sample Analysis Volume[(a)]	Recommended Interruption Point
Raw dairy products	25 g	225	37	20-24	Tranfer 0.1 mL into 10 mL Fraser Broth	37	20-24	10 µL	•DF broth upto 72 hr •lysate at -20°C

(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 DETECTIONCHILL 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 ±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 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:

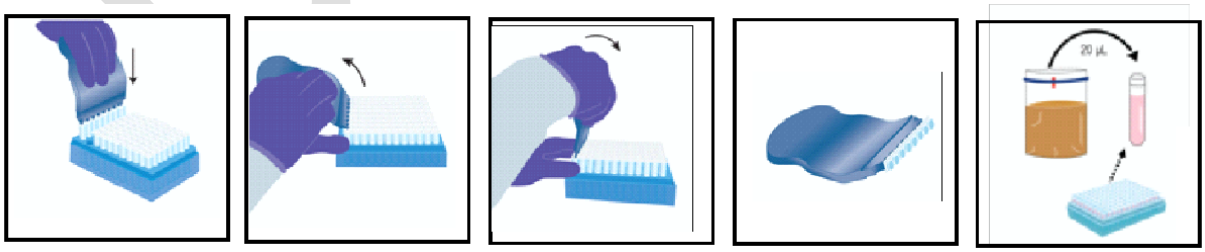
Transfer each enriched sample into an individual LS tube **first**. Transfer the NC **last**.

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).

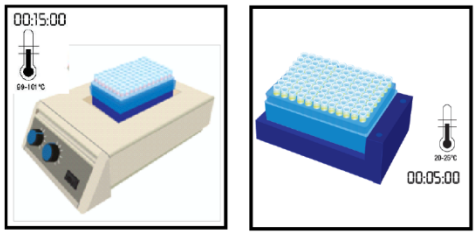
5. Repeat step 4.2 until each individual sample has been added to a corresponding LS tube in the strip.



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 ±1°C.
9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ±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 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.**

5.2 Repeat step 5.1 until individual Sample lysate has been added to a corresponding Reagent tube in the strip.

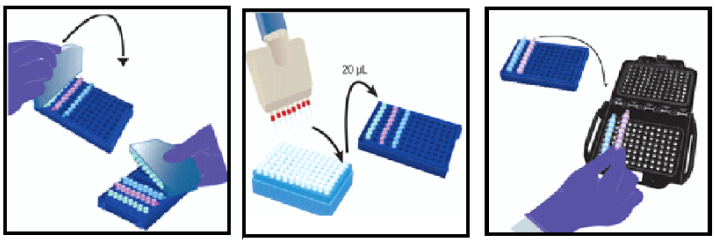
5.3 Cover the Reagent tubes with the provided extra caps and use the rounded side of the 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.

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 µL of NC lysate into a Reagent tube.

5.6 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.

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 [1, 2, 3], 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:

Option1: Using the ISO 11290-1[3] standard starting fromDemi-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[3].

Option 3: Using nucleic acid probes as described in EN ISO 7218[5] 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* amplificationreagents 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 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 8.08. 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

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

AOAC *Performance Tested Methods*℠ 111501

Abstract

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

Authors

Lisa Monteroso and DeAnn Benesh, 3M Food Safety Division
Ben Bastin, Jonathan Flannery, Erin Crowley, Patrick Bird, M. Joseph Benzinger, Jr., James Agin, David Goins, Q Laboratories, Inc.

Submitting Company

3M Company
Food Safety Department
3M Center, Bldg. 275-5W-05

Formatted: Font: Not Italic

St. Paul, MN 55144-1000

Independent Laboratory
Q Laboratories, Inc.
1400 Harrison Ave
Cincinnati, OH 45214

Reviewers
Yvonne Salfinger
Consultant
Denver, CO

Michael Brodsky
Brodsky Consultants
73 Donnamora Crescent
Thornhill, Ontario L3T 4K6
Canada

Reviewer
Tony Hitchins
FDA-Retired
Rockville, MD

Scope of Method

a) *Target organism.*— *Listeria* species (*L. monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. innocua*, *L. grayi*, *L. welshimeri*, *L. fleishmanii* subsp. coloradensis, *L. marthii*, *L. grandensis*, *L. cornellensis L. aquatic*, *L. newyorkensis*, *L. weihenstephanesis*, *L. baoriae*, *L. fleishmanii* subsp. fleishmanii, *L. ivanovii* subsp. londoniensis and *L. riparia*)~~(*L. monocytogenes, L. seeligeri, L. ivanovii, L. innocua, L. grayi, L. welshimeri, L. fleishmanii* subsp. *coloradensis, L. cornellensis, L. grandensis L. marthii*, and *L. riparia)*~~

b) *Matrixes.*— Raw chicken (leg pieces and fillets) (25 g), bagged raw spinach (25 g), cold smoked salmon (25 g), deli turkey (125 g), whole melon, vanilla ice cream (25 g), queso fresco (25 g), 4% milk fat cottage cheese (25 g), beef hot dogs (25 g), stainless steel (4” x 4”, sponge enriched in 225 mL), sealed concrete (4” x 4”, sponge enriched in 225 mL), plastic (1” x 1”, 3M™ ~~Tecra™~~ Enviro Ss~~wab~~ [3M Australia Pty Ltd] enriched in 10 mL).

c) *Summary of Validated Performance Claims.*— Performance equivalent to that of the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM) Chapter 10 [1] for whole melon, cold smoked salmon, bagged raw spinach; the U.S. Department of Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (USDA-FSIS/MLG) 8.09 [2] for beef hot dogs, deli turkey, raw chicken , sealed concrete, plastic and stainless steel and AOAC 993.12 [3] for queso fresco, vanilla ice cream, 4% milk fat cottage cheese and for ISO 11290-1/A1 [4] for cold smoked salmon and bagged raw spinach.

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.

Principle of the Method

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 the laboratory's preferred method or as specified by local regulations.

General Information

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 that can 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.

Materials and Methods

Test Kit Information

a) *Kit Name*.— Molecular Detection Assay 2 – *Listeria*
b) Catalog Number.— MDA2LIS96

Test Kit Reagents - 3M MDA 2 - *Listeria* (96 tests)
a) *Lysis Solution (LS) tubes.* – 96 (12 strips of 8 tubes)
b) *Listeria Reagent tubes*. – 96 (12 strips of 8 tubes)
c) *Extra caps.* – 96 (12 strips of 8 caps)
d) *Reagent Control (RC).* – 16 (2 pouches of 8)
e) Quick Start Guide

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

Additional media
b) Oxford Agar (OX)
c)
d) Fraser Broth (FB)
e) Sheep Blood Agar (SBA)
f) PALCAM Agar
g) Ottaviani Agosti Agar (OAA)
h) Trypticase Soy Agar (TSA)
i) Trypticase Soy Agar with 0.6% Yeast Extract (TSA/YE)
j) De Man, Rogosa and Sharpe (MRS)
k) Modified Oxford Agar (MOX)
l) Brain Heart Infusion (BHI) broth
m) Horse Blood Overlay (HBO)
n) Buffered Listeria Enrichment Broth (BLEB)

Apparatus
a) *Pipettes*. – capable of 20µL
b) *Multi-channel pipette*. – capable of 20µL
c) *Sterile pipette tips.* – capable of 20µL
d) *Stomacher*®. – Seward or equivalent

e) *Filter Stomacher® bags*. - Seward or equivalent
f) *Thermometer*. – calibrated range to include 100 ± 1°C range
g) *Incubators*. – capable of maintaining 37 ± 1°C
h) *Dry double block heater unit.* – capable of maintaining 100 ± 1°C; or *a water bath* capable of maintaining 100 ± 1°C
i) *Refrigerator*. – capable of maintaining 2-8°C, for storing the 3M MDA2
j) *Computer*. – compatible with the 3M Molecular Detection System (instrument)

Safety Precautions

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.

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.
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.

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

Sample Preparation
3M recommends the use of Demi-Fraser Broth with FAC for the enrichment of food and environmental samples. Table 1 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
a) Allow the Demi-Fraser Broth enrichment medium (includes FAC) to equilibrate to ambient laboratory temperature.
b) Aseptically combine the enrichment medium and sample according to Table 1. For all meat and highly particulate samples, the use of filter bags is recommended.

c) Homogenize thoroughly by ~~blending,~~ stomaching~~,~~ or hand mixing for 2 ±0.2 minutes. Incubate at 37 ±1°C according to Table 1.

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 Letheen 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^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, 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,~~vortexing or stomaching~~, or hand mixing~~ for 2 ±0.2 minutes. Incubate at 37 ±1°C for 24-30 hours.
4. Invert room temperature (20-25 ^oC) lysis tubes to mix. Proceed to next step within 4 hours. 20 µL aliquot of each enriched sample are ~~is~~ transferred to separate lysis tubes using a new pipette tip after each sample transfer. Place uncovered samples on a dry double block heater r for 15 ± 1 minutes at 100 ± 1 ^oC. 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.

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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.

Confirmation

Presumptive positive primary enrichment samples were confirmed by following the appropriate reference method confirmation, 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.

Validation Study

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

Internal Study

Inclusivity and Exclusivity

Methodology

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

~~Five~~ Thirteen additional *Listeria* strains were tested at 3M (St. Paul, MN). The ~~five~~ thirteen frozen *Listeria* strain suspensions were thawed, or lyophilized cultures were revived, and then streaked onto Sheep Blood Agar (SBA). The SBA plates were incubated at 37°C ± 1°C overnight. A single isolated colony from the SBA plate was sub-cultured in 10 mL of Demi Fraser Broth with FAC overnight at 37°C ± 1°C. The cultures were diluted using Demi Fraser broth with FAC, and the 3M MDA 2 – *Listeria* method was then performed. See Table 2b.

Thirty frozen non-*Listeria* strain suspensions were thawed and sub-cultured in BHI broth overnight at 37°C ± 1°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 MDA 2 - *Listeria* method. See Table 3.

Results

All ~~55~~ 63 *Listeria* strains were detected by the 3M MDA 2 - *Listeria* method. *L. grayi* recovery was enhanced when a food sample (Ultra-high-temperature [UHT] milk as described in ISO 16140 [9]) was added to the medium in the standard 1:10 dilution scheme. None of the 30 non-*Listeria* strains were detected. See Tables 2a, 2b and 3 for study details and results.

Matrix Study

The method comparison study consisted of evaluating a total of 30 un-paired sample replicates for 10 matrixes, along with naturally contaminated raw chicken (leg pieces), tested at the independent laboratory, evaluating 20 sample replicates using two separate lots, see Table 4a. The raw chicken (leg pieces and fillets) analyzed at the internal lab was inoculated according to AOAC guidelines which are described below, see Table 4b. 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 the naturally contaminated raw chicken (leg pieces). The inoculum was prepared by transferring a single *Listeria* colony from Trypticase Soy Agar with 5% Sheep Blood (SBA) into BHI broth and incubating the culture at 35 ± 2°C for 24 ± 2 hours. Tables 4a and b presents the sample preparation guidelines for the matrix.

All matrixes 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 [10] 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 ± 1 minute at 50 ± 1°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 ± 1°C for 24 ± 2 hours and the colonies were counted. The degree of injury was estimated as:

$$(1-\frac{n_{select}}{n_{nonselect}})x100$$

Where n_{select} = number of colonies on selective agar and $n_{nonselect}$ = number of colonies on non-selective agar. Using BHI broth as the diluent, the culture was diluted to a low level expected to yield fractional positive results (5-15 positive results) and a high level expected to yield all positive results. Following inoculation, a bulk lot of the matrix was homogenized by hand and

held for 48-72 hours at refrigerated temperature (2-8 °C) prior to analysis to allow time for the organism to equilibrate within the sample.

For the inoculation of the whole melons, a single whole melon was placed into a large sterile bag and the blossom end of the melon was inoculated with 100 µL of the diluted *Listeria monocytogenes* culture. The liquid culture was then allowed to soak into the melon. The melon was inverted so that the inoculated end was on the bottom of the sterile bag. The bag was tied closed and held for 48-72 hours at 2-8 °C.

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

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

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

The level of *Listeria monocytogenes* in the low level inoculum was determined by Most Probable Number (MPN) on the day of analysis by evaluating 5 x 50 g, 20 x 25 g (reference method test portions), and 5 x 10 g inoculated test samples. For the high inoculation level, the MPN was determined by examining 5 x 50 g, 5 x 25 g (reference method test portions) and 5 x 10 g . The level of *Listeria* in the low level inoculum for all 125 g test portions was determined by MPN by evaluating 5 x 250 g, 20 or 5 x 125 g (reference method test portions), and 5 x 50 g inoculated test samples. For the high inoculation level, the MPN was determined by examining 5 x 250 g, 5 x 125 g (reference method test portions), and 5 x 50 g inoculated test samples. Each test portion was enriched with the reference method enrichment broth at the reference method dilution scheme and analyzed by the reference method procedure. See Table A for details. The number of positives from the 3 test levels was used to calculate the MPN using the LCF MPN calculator (version 1.6) provided by AOAC RI. [11] (http://www.lcfltd.com/customer/LCFMPNCalculator.exe)

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

*****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® 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

hours. After 24 hours of total incubation, the enriched samples were streaked to MOX agar plates and incubated at 35 ±1 °C for 24-48 hours. The enriched samples were re-incubated for an additional 24 hours at 30 ± 1 °C and then streaked to a second MOX agar plate which was incubated for 24-48 hours at 35 ± 1°C. MOX agar plates were examined for suspect colonies, and if present, at least 5 colonies were streaked to TSA containing 0.6% yeast extract (TSA/YE). The TSA/YE plates were incubated at 35 ± 1°C for 24-48 hours and then examined for purity. Pure colonies were tested for catalase reactivity and a Gram Stain was conducted. A pure *Listeria* colony was transferred to Trypticase Soy Broth containing 0.6% yeast extract (TSB/YE). The TSB/YE cultures were incubated at 25 ± 1°C overnight, or until the broth was turbid, indicating sufficient growth. Catalase-positive organisms were stabbed into plates of 5% Sheep Blood Agar (SBA) and incubated at 35 ± 1°C for 24-48 hours. The TSB/YE tubes incubated at 25 ±1°C were used to prepare a wet mount slide to determine motility pattern. After incubation, the SBA plates were examined for hemolysis. Final confirmation was conducted using the VITEK® GP Biochemical Identification card following AOAC OMA 2013.02.

AOAC 993.12 Listeria Reference Method

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

ISO 11290-1/A1 Reference Method

For the ISO 11290-1/A1 reference method, 25 g test portions were enriched with 225 half Fraser broth. All test portions were mechanically stomached for two minutes. The test portions were incubated at 30 ± 1°C for 24 ± 3 hours. After incubation, 0.1 mL of the sample enrichment was transferred to 10 mL FB containing 0.1 mL of 5% ferric ammonium citrate and incubated at 37 ± 1°C for 48 ± 3 hours. After 48 ± 3 hours, a loopful of the sample secondary FB enrichment was streaked to PALCAM and Ottovani-Agosti Agar (OAA) and incubated at 37 ± 1°C for 24 ± 3

hours. A loopful of the sample primary enrichment was also streaked to PALCAM and OAA and incubated at 37 ± 1°C for 24 ± 3 hours. PALCAM and OAA agar plates were examined for the presence of suspect colonies. If no suspect colonies were present, the agar plate was re-incubated for an additional 18-24 hours at 37 ± 1°C. If no suspect colonies present the sample was determined to be negative for *Listeria.* If suspect colonies were present on the PALCAM or OAA agar plates, these suspect colonies were streaked to HBO and incubated at 37 ± 1°C for 18-24 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® GP Biochemical Identification following AOAC OMA 2013.02.

3M™ Molecular Detection Assay 2 - Listeria

All 25 g samples were analyzed by the 3M™ MDA 2 - *Listeria* were enriched with 225 mL of Demi-Fraser Broth with the addition of ferric ammonium citrate; all test portions were then homogenized by stomaching thoroughly for 2 ± 0.2 minutes and incubated at 37 ± 1 °C for 24-28 hours. For the 125 g deli turkey test portions, samples were enriched with 1125 mL of Demi-Fraser Broth with the addition of ferric ammonium citrate; test portions were then homogenized by stomaching thoroughly for 2 ± 0.2 minutes and incubated at 37 ± 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 with ferric ammonium citrate and incubated at 37 ± 1 °C for 26 hours. Stainless steel sponge samples were enriched with 225 mL of Demi-Fraser Broth with the addition of ferric ammonium citrate; samples were homogenized by hand thoroughly for 2 ± 0.2 minutes and incubated at 37 ±1 °C for 24 and26 hours. Sealed concrete environmental surface sponge samples were enriched with 100 mL of Demi-Fraser Broth with the addition of ferric ammonium citrate; samples were thoroughly homogenized by hand for 2 ± 0.2 minutes and incubated at 37°C for 24 and 26 hours. Plastic environmental surface swab samples were enriched with 10 mL of Demi-Fraser Broth with the addition of ferric ammonium citrate; samples were homogenized by vortexing for 2 ± 0.2 minutes and incubated at 37 ±1 °C for 24 and 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 in the 3M Molecular Detection Heat Block Insert and heated for15±1 minutes at 100 ± 1°C. Following the heat lysis, samples were transferred into the Chill Block Insert and placed on a 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. Also lysed was a sterile Demi Fraser Broth with FAC tube 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™ 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 procedure. There were 3 observed positives for the reference method. For the high inoculation level, there were 3 presumptive positives and 3 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 4 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 X.

Compared to ISO 11290-1/A1

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 6 presumptive positives and 6 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 9 observed positives for the reference method. For the high inoculation level, there were 3 presumptive positives and 3 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 4 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 X.

Cold smoked salmon

Compared to FDA-BAM

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 10 presumptive positives and 10 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 7 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 4 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 X.

Compared to ISO 11290-1/A1

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 10 presumptive positives and 10 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 8 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 4 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 X.

Independent Study using Demi Fraser with FAC brand Y.

Deli turkey (125 g)

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 8 presumptive positives and 8 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 7 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.

Naturally Contaminated raw chicken leg pieces (25 g)

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

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

Whole melon

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 12 presumptive positives and 10 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure, resulting in 2 false positives. There were 8 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the FDA/BAM Chapter 10 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.

Vanilla ice cream (25 g)

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 7 presumptive positives and 7 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 7 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12 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.

Queso fresco (25 g)

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

4% Milk fat cottage cheese (25 g)

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 12 presumptive positives and 12 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 9 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12 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.

Beef hot dog (25 g)

For the low inoculation level of the MDA 2 - *Listeria* assay, there were 4 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure, resulting in 1 false negative. There were 6 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.

Stainless steel (sponge enriched in 225 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 5 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 8 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.

Sealed concrete (sponge enriched in 100 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.

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 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

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).

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 trouble shooting 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 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."*

Conclusion

The 3M MDA 2 - *Listeria* AOAC PTM validation study included inclusivity/exclusivity, lot-to-lot/stability, robustness and matrix studies. The 3M MDA 2 - *Listeria* was compared to the USDA/FSIS-MLG 8.09, the FDA-BAM Chapter 10, the AOAC 993.12 or the ISO 11290-1/A1 reference methods for the detection of *Listeria*. There were no significant differences between the 3M MDA 2 - *Listeria* method and the corresponding reference method for any of the matrixes evaluated. The 3M MDA 2 - *Listeria* demonstrated reliability as a rapid and sensitive method for the detection of *Listeria* species for the following new matrixes: beef hot dogs (25 g), deli turkey (125 g), queso fresco (25 g), vanilla ice cream (25 g), 4% milk fat cottage cheese (25 g), whole melon, bagged raw spinach (25 g), cold smoked salmon (25 g), raw chicken (25 g), sealed concrete (sponge enriched in 225 mL), plastic (Enviro ~~s~~Swab enriched in 10 mL), stainless steel (sponge enriched in 225 mL).

References

1. Food and Drug Administration Bacteriological Analytical Manual Chapter 10: *Detection and Enumeration of Listeria monocytogenes in Foods.* February, 2013. (Accessed January 2015), http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm

2. United States Department of Agriculture Microbiological Laboratory Guidelines 8.09: *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and Environmental Samples.* May 1st, 2013. (Accessed January 2015) http://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES

3. Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., AOAC Official Method 993.12 *Listeria monocytogenes* in Milk and Dairy Products: Selective Enrichment and Isolation Method, First Action 1993, Final Action 1996

4. ISO 11290-1/A1 (2004) - Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: detection of *Listeria monocytogenes* in foods, http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=60313

5. U.S. Food and Drug Administration, and U.S. Food Safety and Inspection Service. 2003. Quantitative Assessment of the Relative Risk to Public Health from Foodborne *Listeria*

monocytogenes Among Selected Categories of Ready-to-Eat Foods. Available in Docket No. 1999N-1168, Vols. 23 through 28, and at Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods, http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/FoodProcessingHACCP/ucm073110.htm

6. Doyle ME, Mazzotta AS, Wang T, Wiseman DW, and Scott VN. 2001. Heat resistance of *Listeria monocytogenes*. *Journal of Food Protection* 64 (3): 410-429.
7. ISO 18593. Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.
8. AOAC Research Institute *Performance Tested Methods℠ Program* validation outline protocol: *Comparative Evaluation of the 3M™ Molecular Detection Assay Listeria monocytogenes 2 and of the 3M™ Molecular Detection Assay Listeria* 2 (February 2015)
9. ISO 16140:2003. Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods.
10. U. S. Food and Drug Administration *Bacteriological Analytical Manual*, Chapter 3 *Aerobic Plate Count (*January 2001) (Accessed January 2015) http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.m
11. Least Cost Formulations, Ltd., MPN Calculator Version 1.6 www.lcfltd.com/customer/LCFMPNCalculator.exe (Accessed January 2015)
12. AOAC International, *Evaluation of the* VITEK 2 *Gram Positive (GP) Microbial Identification Test Card: Collaborative Study*, Journal of AOAC International, 95, 1425 (2012)
13. Wehling, P., LaBudde, R., Brunelle, S., Nelson, M. *Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods.* Journal of AOAC International, Vol. 94, No. 1, 2011.
14. Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook Version 2.2 (2011) -http://lcfltd.com/aoac/aoac-binary-v2-2.xls(Accessed January 2015)
15. Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, AOAC INTERNATIONAL, Gaithersburg, MD, http://www.aoac.org/imis15_prod/AOAC_Docs/StandardsDevelopment/eoma_appendix_j.pdf (Accessed January 2015)

~~Table 1. Enrichment Protocols Using Demi-Fraser Broth Enrichment at 37 ± 1°C~~

Specific Instructions for Validated Methods

AOAC® Performance Tested Method℠ #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 1. Enrichment protocols using Demi-Fraser Broth at 37 ± 1 °C according to AOAC Performance Tested℠ 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[a]		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[b]	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

~~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~~

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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		
							Confirmation 100µl	
						MDA 2 - *Listeria* spp	ALOA	PALCAM
1	*Listeria*	*monocytogenes*	1011/1410	Frozen broccoli	28	+	H+	+
2	*Listeria*	*monocytogenes*	153	Soft cheese (Munster)	39	+	H+	+
3	*Listeria*	*monocytogenes*	1973/2400	Egg and ham pastry (Quiche Lorraine)	38	+	H+	+
4	*Listeria*	*monocytogenes*	38/181	Toulouse sausages	33	+	H+	+
5	*Listeria*	*monocytogenes*	7111/7516	Pâté (Rillettes)	64	+	H+	+
6	*Listeria*	*monocytogenes*	913/1048	Black pudding	39	+	H+	+
7	*Listeria*	*monocytogenes*	A00C036	Poultry (guinea)	41	+	H+	+
8	*Listeria*	*monocytogenes*	A00C041	Sausage	38	+	H+	+
9	*Listeria*	*monocytogenes*	A00C044	Poultry (Duck)	34	+	H+	+
10	*Listeria*	*monocytogenes*	A00L097	Milk	60	+	H+	+
11	*Listeria*	*monocytogenes*	A00M009	Smoked salmon	40	+	H+	+
12	*Listeria*	*monocytogenes*	Ad 253	Semi-hard cheese	58	+	H+	+
13	*Listeria*	*monocytogenes*	Ad 266	Poultry	26	+	H+	+
14	*Listeria*	*monocytogenes*	Ad 270	Fermented sausage	42	+	H+	+
15	*Listeria*	*monocytogenes*	Ad 273	Cured delicatessen	27	+	H+	+
16	*Listeria*	*monocytogenes*	Ad 274	Ready-to-eat food (Asiatic meal)	40	+	H+	+
17	*Listeria*	*monocytogenes*	Ad 534	Fruits	54	+	H+	+
18	*Listeria*	*monocytogenes*	Ad 548	Environment (Seafood)	37	+	H+	+
19	*Listeria*	*monocytogenes*	Ad 623	Bread crumbs	50	+	H+	+
20	*Listeria*	*monocytogenes*	Ad 665	Raw milk	33	+	H+	+
21	*Listeria*	*grayi*	Ad 1198	Smoked salmon	158 (+25ml UHT milk)	+	H-	st
					1300	+	H-(2)	-
22					1300 (+25ml UHT milk)	+	H-	-
	Listeria	*grayi*	Ad 1443	Pork meat sausages	12(+25ml UHT milk)	+	H-	-
23	*Listeria*	*innocua*	1	Smoked salmon	40	+	H-	+
24	*Listeria*	*innocua*	Ad 658	Gorgonzola	28	+	H-	+
25	*Listeria*	*innocua*	Ad 655	Brine	19	+	H-	+
26	*Listeria*	*innocua*	Ad 660	Bread crumbs	35	+	H-	+
27	*Listeria*	*innocua*	Ad 663	Environment (dairy industry)	25	+	H-	+
28	*Listeria*	*innocua*	Ad 671	Smocked bacon	10	+	H-	+
29	*Listeria*	*innocua*	Ad 661	Soft cheese (Pont L'Evêque)	15	+	H-	+
30	*Listeria*	*innocua*	Ad 659	Environment (dairy industry)	14	+	H-	+
31	*Listeria*	*ivanovii*	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		
							Confirmation 100µl	
						MDA 2 - *Listeria* spp	ALOA	PALCAM
32	*Listeria*	*ivanovii*	Ad 662	Environment (dairy industry)	20	+	H+	+
33	*Listeria*	*ivanovii*	BR11	Environment (fish)	29	+	H+	+
34	*Listeria*	*ivanovii*	Ad 1289	Raw milk cheese	30	+	H+	+
35	*Listeria*	*ivanovii*	Ad 1290	Milk powder	17	+	H+	+
36	*Listeria*	*ivanovii*	Ad 1291	Poultry	18	+	H+	+
37	*Listeria*	*ivanovii*	Ad 1288	Sheep milk	80	+	H+	+
38	*Listeria*	*ivanovii* subsp. *londoniensis*	CIP103466	Unknown	14	+	H-	+
39	*Listeria*	*seeligeri*	Ad 649	Cheese	29	+	H-	+
40	*Listeria*	*seeligeri*	Ad 651	Environment	36	+	H-	+
41	*Listeria*	*seeligeri*	Ad 652	Environment (dairy industry)	28	+	H-	+
42	*Listeria*	*seeligeri*	Ad 674	Soft cheese (Munster)	240	+	H-	H-
43	*Listeria*	*seeligeri*	BR1	Trout	28	+	H-	+
44	*Listeria*	*seeligeri*	BR18	Environment (fish)	36	+	H-	+
45	*Listeria*	*seeligeri*	CIP100100	Unknown	8	+	st	1col
46	*Listeria*	*welshimeri*	Ad1276	Environment (Slaughterhouse)	44	+	H-	+
47	*Listeria*	*welshimeri*	Ad1235	Beef meat	26	+	H-	+
48	*Listeria*	*welshimeri*	191424	Poultry	24	+	H-	+
49	*Listeria*	*welshimeri*	Ad 1175	Ready-to-eat-food	46	+	H-	+
50	*Listeria*	*welshimeri*	Ad 650	Poultry	33	+	H-	+

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.

Table 2b. Inclusivity Study Results

#	Listeria species	Reference	Origin	Level tested	MDA2LIS
51	*fleischmannii* subsp. *coloradensis*	FSL S10-1203	Running water, Grazing pasture Soil, USA, Florida	1.8e6 CFU/mL	+
52	*marthii*	FSL S4-120	Pristine environment, forest, NY	3.65e5 CFU/mL	+
53	*cornellensis*	FSL F6-969	Water, USA, Colorado	3.5e7 CFU/mL	+
54	*grandensis*	FSL F6-971	Water, USA, Colorado	1.95e7 CFU/mL	+
55	*riparia*	FSL S10-1204	Running water, FL	3.05e5 CFU/mL	+
56	*aquatica*	FSL S10-1188	Running water, USA, Florida	1.37e6 CFU/mL	+
57	*booriae*	FSL A5-0281	Non-food-contact surface in a dairy processing plant, USA, New York	2.75e5 CFU/mL	+
58	*fleishcmannii* subsp. fleishmannii	DSM 24998	Hard cheese, Switzerland, Suisse Romande	1.28e6 CFU/mL	+
59	*ivanovii*	ATCC 19119	Sheep, Bulgaria	4.50e5 CFU/mL	+
60	*ivanovii* subsp. Iondoniensis	ATCC 49954	Food, France	7.40e5 CFU/mL	+
61	*newyorkensis*	FSL M6-0635	Non-food-contact surface in a seafood processing plant, USA, New York	3.05e5 CFU/mL	+
62	*rocourtiae*	CIP 109804	Pre-cut lettuce, Austria, Siezenheim	7.30e5 CFU/mL	+
63	*weihenstephanesis*	LMG 26374	Water plant *Lemna trisulca* from pond, Germany, Bavaria	3.30e4 CFU/mL	+

- Table 2b are *Listeria* strains analyzed at 3M St. Paul, MN laboratory
- Cultures 51-5463 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* 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 – *Listeria*
1	*Bacillus*	*cereus*	Ad 465	Salmon Terrine	$7{,}2x10^4$	-
2	*Bacillus*	*circulans*	Ad 760	Vegetables	$1{,}0 \times 10^4$ (opacity +)	-
3	*Bacillus*	*coagulans*	Ad 731	Dairy product	$<2{,}0x10^3$ (opacity +)	-
4	*Bacillus*	*licheniformis*	Ad 978	Dairy product	$<2{,}0x10^3$ (opacity+)	-
5	*Bacillus*	*mycoïdes*	Ad 762	Milk	$5{,}6x10^4$	-
6	*Bacillus*	*pseudomycoides*	Ad 765	Vegetables	$2{,}0x10^4$	-
7	*Bacillus*	*pumilus*	Ad 284	Ready-to-eat	$1{,}7x10^5$	-
8	*Bacillus*	*weihenstephanensis*	Ad 726	Egg product	$6{,}8x10^4$	-
9	*Brochothrix*	*thermosphacta*	EN 15129	Trout	$8{,}5x10^5$	-
10	*Brochrotrix*	*campestris*	CIP 102920T	Environment	$<2{,}0x10^3$ (opacity +)	-
11	*Carnobacterium*	*divergens*	CIP 101029^T	Unknown	$>2{,}0x10^3$ (opacity +)	-
12	*Carnobacterium*	*piscicola*	Ad 369	Raw milk	$<2{,}0x10^3$ (opacity +)	-
13	*Enterococcus*	*durans*	Ad 149	Ham	$<2{,}0x10^3$ (opacity +)	-
14	*Enterococcus*	*faecalis*	89L326	Soft cheese (Vacherin)	$1{,}6x10^5$	-
15	*Lactobacillus*	*brevis*	86L126	Ham	$1{,}4x10^5$	-
16	*Lactobacillus*	*curvatus*	Ad 380	Delicatessen	$1{,}7x10^5$	-
17	*Lactobacillus*	*fermentum*	Ad 482	Tomatoes juice	$1{,}2x10^5$	-
18	*Lactobacillus*	*sakei*	Ad 473	Ham	$4{,}9x10^5$	-
19	*Lactococcus*	*lactis subsp cremoris*	Ad 137	Dairy product	$>2{,}0x10^3$ (opacity +)	-
20	*Leuconostoc*	*carnosum*	Ad 411	Ham	$6{,}8x10^5$	-
21	*Leuconostoc*	*citreum*	Ad 396	Ham	$4{,}9x10^5$	-
22	*Micrococcus*	*luteus*	Ad 432	Cocktail	$<2{,}0x10^3$ (opacity+)	-
23	*Pediococcus*	*pentosaceus*	ATCC 33316	Unknown	$1{,}0x10^5$	-
24	*Propionibacterium*	*freundenreichii*	CNRZ 725	Dairy product	$<2{,}0x10^3$ (opacity +)	-
25	*Staphylococcus*	*aureus*	Ad 165	Smoked delicatessen	$1{,}3x10^5$	-
26	*Staphylococcus*	*aureus*	Ad 902	Nems	$8{,}2x10^4$	-
27	*Staphylococcus*	*epidermidis*	Ad 931	Fruits	$2{,}0x10^3$	-
28	*Staphylococcus*	*haemolyticus*	Ad 989	Dairy product	$2{,}8x10^4$	-
29	*Streptococcus*	*bovis*	92L622	Dairy product	$2{,}0x10^3$	-
30	*Streptococcus*	*salivarius*	Ad 441	Dairy product	$<2{,}0x10^3$ (opacity +)	-

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

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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes* FSL[3] 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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes* CWD[2] 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	*L. monocytogenes* ATCC[1] 19112 & *L. welshimeri* ATCC[1] 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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes ATCC1 19118 & 10x Enterococcus faecium 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		
Sealed Concrete	*L. monocytogenes ATCC1 19117*	0 CFU/4" x 4" (100 cm2)	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/4" x 4" (100 cm2)	20		
		~100 CFU/4" x 4" (100 cm2)	5		
Plastic	*L. monocytogenes ATCC1 51782 & L. seeligeri ATCC1 35967 &*	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		
		~100 CFU/1" x 1" (5 cm2)	5		

	10x Enterococcus faecalis ATCC1 29212				

[1] ATCC - American Type Culture Collection
[2] CWD - University of Vermont
[3] FSL - Cornell University
[*] Natural contamination was present during the background screen: 2 separate lots evaluated (20 replicates each), *Listeria monocytogenes* and *Listeria innocua* were isolated.

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[2] leg pieces	*L. ivanovii* Ad[1] 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[3]	*L. ivanovii* 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[2] spinach	*Listeria seeligeri* 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[2] spinach	*Listeria seeligeri* 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[2] salmon	*Listeria innocua* 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[2] salmon	*Listeria innocua* 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		

[1] Ad – Adria Developpement Culture Collection
[2] Tested using Demi Fraser brand (with FAC) X
[3] 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[1] (CFU/g)	Background *Listeria* (# positive/total tested)
Deli Turkey (125 g)	1.5×10^3	0/5[(2)]
Naturally Contaminated Raw Chicken Leg Pieces (25 g)	2.8×10^5	4/5[(2)]
Whole Melons	1.0×10^2	0/5[(3)]
Vanilla Ice Cream (25 g)	8.0×10^1	0/5[(4)]
Queso Fresco (25 g)	3.8×10^5	0/5[(4)]
4% Milk Fat Cottage Cheese (25 g)	1.7×10^4	0/5[(4)]
Beef Hot Dogs (25 g)	1.6×10^2	0/5[(2)]
Beef Hot Dogs[5] (25 g)	6.0×10^3	0/5[(2)]

[1] APC conducted in accordance with FDA/BAM Chapter 3
[2] *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines
[3] *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines
[4] *Listeria species* screen conducted following the AOAC 993.12 guidelines
[5] 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[1] (CFU/g)	Background *Listeria* (# positive/total tested)
Raw Chicken Leg Pieces (25 g)	4.0×10^5	0/5[(2)]
Raw chicken fillet (25 g)	1.4×10^5	0/5[(2)]
Bagged Raw Spinach (25 g)	9.3×10^6	0/5[(3,4)]
Cold Smoked Salmon (25 g)	2.0×10^2	0/5[(3,4)]

[1] APC conducted in accordance with FDA/BAM Chapter 3
[2] *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines
[3] *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines
[4] *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[1]	25 g	*Listeria monocytogenes* ATCC 7644	TSA	1.8×10^8	58.3 %
			MOX	7.5×10^7	
Deli Turkey	125 g	*Listeria monocytogenes* ATCC 19116	TSA	2.6×10^9	68.5 %
			MOX	8.2×10^8	
Queso Fresco	25 g	*Listeria monocytogenes* ATCC CWD 1554	TSA	2.6×10^9	72.7%
			MOX	7.1×10^8	
Beef Hot Dogs[2]	25 g	*Listeria monocytogenes* ATCC 7644	TSA	2.6×10^9	73.8%
			MOX	6.8×10^8	

[1]- Testing for Robustness and Lot to Lot
[2]- 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	*Listeria monocytogenes* FSL J1-049		
Low-Inoculum Level CFU[a]/Melon[b]	7		
High-Inoculum Level CFU[a]/Test Melon[b]	55		
Matrix	**Stainless Steel**		
Inoculating Organism	*Listeria monocytogenes* ATCC 19118	*Enterococcus faecium* ATCC 19434	
Low-Inoculum Level CFU[a]/Test Area[c]	42	530	
High-Inoculum Level CFU[a]/Test Area[c]	400	4000	
Matrix	**Sealed Concrete**		
Inoculating Organism	*Listeria monocytogenes* ATCC 19117		
Low-Inoculum Level CFU[a]/Test Area[c]	35		
High-Inoculum Level CFU[a]/Test Area[c]	360		
Matrix	**Plastic**		
Inoculating Organism	*Listeria monocytogenes* ATCC 51782	*Listeria seeligeri* ATCC 35967	*Enterococcus faecalis* ATCC 29212
Low-Inoculum Level CFU[a]/Test Area[d]	42	64	540
High-Inoculum Level CFU[a]/Test Area[d]	420	590	3600

[a] CFU: aliquots of the inocula were plated in triplicate onto TSA and averaged
[b] Test Area: Whole Melon
[c] Test Area: 4" x 4" Surface Area
[d] 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[a]/ Test Portion	N[b]	3M MDA2			USDA/FSIS MLG			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Raw Chicken Leg Pieces[h] (25 g)	*L. ivanovii* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**3M MDA2**			**USDA/FSIS MLG**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Raw Chicken Fillet[i] (25 g)	*L. ivanovii* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**3M MDA2**			**FDA-BAM**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Bagged Raw Spinach[h] (25 g)	*L. seeligeri* 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**
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**3M MDA2**			**ISO 11290-1**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Bagged Raw Spinach[h] (25 g)	*L. seeligeri* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**3M MDA2**			**FDA-BAM**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Cold Smoked Salmon[h] (25 g)	*L. innocua* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**3M MDA2**			**ISO 11290-1**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Cold Smoked Salmon[h] (25 g)	*L. innocua* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]X = Number of positive test portions
[d]POD_C = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD_R = Reference method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[h]Tested using Demi Fraser (with FAC) brand X
[i]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[a]/ Test Portion	N[b]	3M MDA2			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Deli Turkey (125 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Whole Melon	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Vanilla Ice Cream (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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[a]/ Test Portion	N[b]	3M MDA2			Reference			$dPOD_C$[f]	95% CI[g]
					x[c]	POD_C[d]	95% CI	X	POD_R[e]	95% CI		
Queso Fresco (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**$dPOD_C$[f]**	**95% CI[g]**
					x[c]	**POD_C[d]**	**95% CI**	**X**	**POD_R[e]**	**95% CI**		
4% Milk Fat Cottage Cheese (25 g)	*L. welshimeri* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**$dPOD_C$[f]**	**95% CI[g]**
					x[c]	**POD_C[d]**	**95% CI**	**X**	**POD_R[e]**	**95% CI**		
Beef Hot Dogs (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_C = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD_R = Reference method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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[a]/ Test Area	N[b]	3M MDA2			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Stainless Steel (225 mL)	*L. monocytogenes* ATCC 19118 & *Enterococcus faecium* 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[a]/ Test Area**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Sealed Concrete (100 mL)	*L. monocytogenes* 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[a]/ Test Area**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Plastic (10 mL)	*L. monocytogenes* ATCC 51782 & *Enterococcus faecalis* 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

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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[a]/ Test Portion	N[b]	Presumptive			Confirmed			$dPOD_{CP}$[f]	95% CI[g]
					x[c]	POD_{CP}[d]	95% CI	X	POD_{CC}[e]	95% CI		
Raw Chicken Leg Pieces[h] (25 g)	*L. ivanovii* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Raw Chicken Fillet[i] (25 g)	*L. ivanovii* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Bagged Raw Spinach[h] (25 g)	*L. seeligeri* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Cold Smoked Salmon[h] (25 g)	*L. innocua* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[h]Tested using Demi Fraser brand (with FAC) brand X.
[i]Tested using Demi Fraser brand (with FAC) brand Z.

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Table 13: 3M™ MDA 2 - *Listeria* Assay, Presumptive vs. Confirmed – POD Results (INDEPENDENT STUDY)

Matrix	Strain	Analysis Time Point	MPN[a]/ Test Portion	N[b]	Presumptive			Confirmed			$dPOD_{CP}$[f]	95% CI[g]
					x[c]	POD_{CP}[d]	95% CI	X	POD_{CC}[e]	95% CI		
Deli Turkey (125 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Whole Melon	*L. monocytogenes* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Vanilla Ice Cream (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Queso Fresco (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
4% Milk Fat Cottage Cheese (25 g)	*L. welshimeri* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
Beef Hot Dogs (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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[a]/ Test Area	N[b]	Presumptive			Confirmed			$dPOD_{CP}$[f]	95% CI[g]
					x[c]	POD_{CP}[d]	95% CI	X	POD_{CC}[e]	95% CI		
Stainless Steel (225 mL)	*L. monocytogenes* ATCC 19118 & *Enterococcus faecium* 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[a]/ Test Area**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Sealed Concrete (100 mL)	*L. monocytogenes* 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[a]/ Test Area**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Plastic (10 mL)	*L. monocytogenes* ATCC 51782 & *Enterococcus faecalis* 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

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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**
Listeria monocytogenes ATCC[2] 7644					
Low Level	6/10	Low Level	6/10	Low Level	6/10
Un-inoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5	Un-inoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5	Un-inoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5

[1]All samples were analyzed from a common lysate
[2]ATCC- American Type Culture Collection

Table 17: Robustness Results

Robustness[1]			**Robustness[1]**		
13 Minute Lysis			**13 Minute Lysis**		
Sample #	**18 µL[2], 18 µL[3]**	**18 µL[2], 22 µL[3]**	**Sample #**	**22 µL[2], 18 µL[3]**	**22 µL[2], 22 µL[3]**
Listeria monocytogenes ATCC[4] 7644					
Low Level	6/10	6/10	Low Level	7/10	7/10
Un-inoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5	0/5	Un-inoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5	0/5
Robustness[1]			**Robustness[1]**		
17 Minute Lysis			**17 Minute Lysis**		
Sample #	**18 µL[2], 18 µL[3]**	**18 µL[2], 22 µL[3]**	**Sample #**	**22 µL[2], 18 µL[3]**	**22 µL[2], 22 µL[3]**
Listeria monocytogenes ATCC[4] 7644					
Low Level	6/10	6/10	Low Level	6/10	6/10
Un-inoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5	0/5	Un-inoculated Target Organism (*Enterococcus faecalis* ATCC 29212)	0/5	0/5

[1]All samples were analyzed from a common lysate
[2]Volume of enrichment to lysis
[3]Volume of lysis to assay
[4]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 preesented?		
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 producct 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 variablity 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 Supplemantary Material and include it win 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 inujure 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 variablity 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.

	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.	Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.
	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.	Currently revising the PTMs to reflect the method of homognization. See above response.
	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.	Revised Table 2 to Table A. (five instances)
	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.	Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.
	ER4 Package Insert comments:	
	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.	Added subscript/footnote explanations of which products were homogenized by stomaching, vortexing, and hand massaging.

	2. There should be an applicability statement, specific to AOAC, In the package insert.	Per AOAC website: http://www.aoac.org/imis15_prod/AOAC_Docs/SPDS/Formats_AOAC_collab.pdf 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 compenents 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	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 all the figures and tables pertinent?		
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
Could some be omitted and covered by a simple statement?		
ER1	NO	Thank You for your comments and review
ER2	NO	Thank You for your comments and review
ER3	NO	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	NO	Thank You for your comments and review

Are the references complete and correctly annotated?		
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
Does the method contain adequate safety precaution reference and/or statements?		
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
Recommendation: Do you recommend that the ERP adopt this method as an AOAC Official Method of Analysis (First Action status)?		
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, 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	Thank You for your comments and review
ER6	Yes	Thank You for your comments and review

Evaluation of the 3M™ Molecular Detection Assay(MDA) 2 -*Listeria monocytogenes*for the Detection of *Listeria monocytogenes*inSelectFoods and Environmental Surfaces: Collaborative Study

Patrick Bird, Jonathan Flannery, Erin Crowley, James Agin, David Goins
Q Laboratories, Inc., 1400 Harrison Ave, Cincinnati, OH 45214

Lisa Monteroso
3M Food Safety Department, 3MCenter – Bldg. 260-6B-01, St. Paul, MN 55144

Collaborators: R. Brooks, J. Walia, F. Hernandez, D. Bosco, G. Trevino, A. Brandt, C. Lopez, E. Sjogren, M. Shekhawat, L. Ma, C. Timmons, C. Diaz Proano,A. Calle,Z. Metz, D. Baumler, R. Smith, D. Wood, E. Maranan, C. Chavarria,J. Miller, B. Schindler, C. Gies, E. Budge, Z. Geurin, A. Repeck, C. Zook, C. Barnes, B. Bastin, D. Isfort

**The 3M™ Molecular Detection Assay (MDA) 2 –*Listeria monocytogenes*usesloop-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 the difference between methods was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the candidate and reference methods.~~ For raw chicken breast fillet a dLPOD valuewith 95% confidence interval of 0.16, (0.04, 0.28)indicating a statistically significant difference an observed higher proportion of positive results by the candidate method than the reference method.~~indicating astatisticallysignificant differencebetween the candidate and reference methods with a positive correlation in data indicating more recovery of the target analyte by the candidate method.~~

*Listeriamonocytogenes*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].

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Listeriamonocytogenes 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 Assay(MDA) 2 - *Listeria monocytogenes*method,usinga combination of bioluminescence and isothermal amplification of nucleic acid sequences,allows for the rapid and specific detection of *Listeria monocytogenes*in a broad range of food types and environmental surfaces after 24 to ~~28~~32hoursof pre-enrichment. After enrichment, samples are evaluated using the 3M MDA 2 - *Listeria monocytogenes* on the 3M™ Molecular Detection System (MDS).Presumptive positive results are reported in real-time while negative results are displayed after completion of the assay in approximately 75 minutes.

Prior to the collaborative study, the 3M MDA 2 - *Listeria monocytogenes* method was validated according to AOAC Guidelines[4] in a harmonized AOAC® Performance Tested Method℠ (PTM) study. The objective of the PTM study was to demonstrate that the 3M MDA 2-*Listeria monocytogenes* method could detect *Listeria monocytogenes*in a broad range of food matrices and environmental surfaces as claimed by the manufacturer. For the 3M MDA 2- *Listeria monocytogenes* PTM evaluation, 13 matrices were evaluated: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), and plastic (Enviro~~s~~Swab, 10 mL) environmental samples.

Additional PTM parameters (inclusivity, exclusivity, ruggedness, stability and lot-to-lot variability) tested in the PTM studies satisfied the performance requirements for PTM approval. The method was awarded PTM certification number 081501 on August 18th, 2015.

The purpose of this collaborative studywas to compare the reproducibility of the 3M MDA 2 - *Listeria monocytogenes*method 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*[5] for deli turkey (125 g) and raw chicken breast fillet.

Collaborative Study

Study Design

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

Twelve replicate samples from each of the three inoculation levels were analyzed by each method. Two sets of samples (72 total) were sent to each laboratory for analysis by 3M MDA 2 - *Listeria monocytogenes* and the USDA/FSIS MLG Chapter 8.09 reference method due to the different sample enrichment procedures for each method. Additionally, collaborators were sent a 60 g test portion and instructed to conduct atotal aerobic plate count (APC) using 3M™

Petrifilm™Rapid Aerobic Count Plate (AOAC Official Method 2015.13) [6] on the day samples were received for the purpose of determining the total aerobic ~~microbi~~microbe\-ppal load. A detailed collaborative study packet outlining all necessary information related to the study including media preparation, test portion preparation and documentation of results was sent to each collaborating laboratory prior to the initiation of the study. A conference call was then conducted to discuss the details of the collaborative study packet and answer any questions from the participating laboratories.

Preparation of Inocula and Test Portions

The *Listeria monocytogenes* cultures (ATCC 7644 and ATCC 19115) used in this evaluation were propagated onto Tryptic Soy Agar with 5% Sheep Blood (SBA) from a Q Laboratories frozen stock culture stored at -70°C. Each organism was incubated for 24 ± 2 hours at 35 ±1°C. Isolated colonies were picked to 10 mL of Brain Heart Infusion (BHI) broth and incubated for 18 ± 0.5 hours at 35 ±1°C. Raw chicken breast fillet was inoculated in bulk using the fresh, broth culture. Prior to inoculation of the deli turkey, the culture suspension was heat stressed at 55 ± 1°C in a water bath for 15 ±0.5minutes to obtain a percent injury of 50-80% (as determined by plating onto selective Modified Oxford agar (MOX) and non-selective tryptic soy agar with yeast (TSA/ye). The degree of injury was estimated as:

$$(1-\frac{n_{select}}{n_{nonselect}})x100$$

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

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

Test Portion Distribution

All samples were labeled with a randomized, blind-coded 3-digit number affixed to the sample container. Test portions were shipped on a Thursday via overnight delivery according to the Category B Dangerous Goods shipment regulations set forth by the International Air Transportations Association(IATA).The two matrices were shipped consecutively, with collaborating laboratories performing analysis on one matrix at a time. Upon receipt, samples were held by the collaborating laboratory at refrigeration temperature (2-8 °C) until the

following Monday when analysis was initiatedafter a total equilibration time of 96 hours.All samples were packed with cold packs to target a temperature of < 7°C during shipment. In addition to each of the test portions and a separate APCsample, collaborators received a test portion for each matrix labeled as 'temperature control'. Participants were instructed to obtain the temperature of this portion upon receipt of the package, document the results on the Sample Receipt Confirmation form provided and fax or email it back to the study director.The shipment and hold timesof the inoculated test material had been verified as a quality control measure prior to study initiation.

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 monocytogenes* 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 monocytogenes* method, a 125 g portion was enriched with 975 mL of Demi-Fraser (DF) with ferric ammonium citrate (FAC) broth, homogenized for 2 minutes and incubated for 24-~~28~~30 hours at 37 ±1°C. For the raw chicken breast fillettest portions analyzed by the 3M MDA 2 - *Listeria monocytogenes* method, a 25 g portion was enriched with 475 mL of DF, homogenized for 2 minutes and incubated for 28-32 hours at 37 ±1°C.

Following enrichment, samples were assayed by the 3M MDA 2 - *Listeria monocytogenes* 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 monocytogenes* 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 monocytogenes* 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 monocytogenes* 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].POD statistical analysis was conducted using AOAC Binary Data Interlaboratory Study Workbook, Version 2.3 [10]. 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, $POD_{CP,}$ the candidate confirmatory results (excluding those with presumptive negative results~~including false negative results~~), POD_{CC}, the difference in the candidate presumptive and confirmatory results, $dLPOD_{CP,}$ presumptive candidate results that confirmed positive ~~((~~including those with presumptive negative results)~~excluding false negative results)~~, $POD_{C,}$ the reference method, POD_R, and the difference in the confirmed candidate and reference methods, $dLPOD_C$. A $dLPOD_C$confidence interval not containing the point zero would indicate a statistically significant difference between the 3M MDA 2 -*Listeria monocytogenes*and the reference methods at the 5 % probability level. In addition to POD, the repeatability standard deviation (s_r), the among laboratory repeatability standard deviation (s_L), the reproducibility standard deviation (s_R)and the P_T value were calculated. The s_r provides the variability~~variance~~ of data within one laboratory, the s_L provides the difference in standard deviation between laboratories

and the s_R provides the variability~~variance~~ in data between different laboratories. The P_T value provides information on the homogeneity test of laboratory PODs.~~[10].~~

AOAC Official Method 2016.xxx
***Listeria monocytogenes*in SelectFoods and Environmental Surfaces**
3M™ Molecular Detection Assay(MDA) 2- *Listeria monocytogenes* Method
First Action 2016

(Applicable to detection of *Listeria monocytogenes*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); raw chicken breast fillet (25g), concrete (3M™ Hydrated Sponge Stick with Dey-Engley [D/E] , 225 mL & 100 mL), stainless steel (3M Hydrated Sponge Stick with D/E, 225 mL), and plastic (3M™ Enviro**sS**wab with Letheen, 10 mL) environmental samples.

*See Tables**2016.1A** and **2016.1B**for a summary of results of the inter-laboratory study.*
*See Tables **2016.2A** and **2016.2B** for detailed results of the inter-laboratory study*

A. Principle

The 3M™ Molecular Detection Assay (MDA) 2 - *Listeria monocytogenes* method is used with the 3M™ Molecular Detection System (MDS) for the rapid and specific detection of *Listeria monocytogenes* in enriched food and food process environmental samples. The 3M MDA2 - *Listeria monocytogenes*uses loop-mediated isothermal amplification of unique DNA target 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. Samples arepre-enriched in Demi Fraser with ferric ammonium citrate broth.

B. Apparatus and Reagents

Items (b)-(g) are available as the 3M™ Molecular Detection Assay (MDA) 2 - *Listeria monocytogenes* kit from 3M Food Safety (St. Paul, MN 55144-1000, USA).

- **(a)** *3M Molecular Detection System*(MDS100) – Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- **(b)** *3M Molecular Detection Assay 2 – Listeria monocytogenes reagent tubes*- 12 strips of 8 tubes. Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- **(c)** *Lysis Solution (LS) tubes* – 12 strips of 8 tubes.
- **(d)** *Extra caps* – 12 strips of 8 caps
- **(e)** *Reagent Control* – 8 reagent tubes
- **(f)** *Quick Start Guide*
- **(g)** *3M™ Molecular Detection Speed Loader Tray* - Available from 3M Food Safety (St. Paul, MN 55144-1000, USA).
- **(h)** *3M™ Molecular Detection Chill Block Insert* - Available from 3M™ Food Safety (St. Paul, MN 55144-1000, USA).
- **(i)** *3M™ Molecular Detection Heat Block Insert* - Available from 3M Food Safety

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(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 ± 1°C

(v) *Dry block heater unit*– capable of maintaining 100 ± 1°C

(w) *Incubators.* – Capable of maintaining 37 ± 1°C ~~or 41.5 ± 1°C.~~

~~**(x)** *Freezer* – capable of maintaining -10 to -20°C, for storing the 3M Molecular Detection Chill Block Tray~~

~~(y)~~**(x)** *Refrigerator* – capable of maintaining 2-8°C, for storing the 3M Molecular Detection Assay components

~~(z)~~**(y)** *Computer* – compatible with the 3M™ Molecular Detection Instrument

~~(aa)~~**(z)** *3M™ Enviro~~s~~ 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°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 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

industries. For example, 3M has not documented this product for testing drinking water, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M MDA2 – *Listeria monocytogenes*has not been evaluated with all possible food products, food processes, testing protocols or with all possible strains of bacteria.

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

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.

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

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

*Listeria monocytogenes*is of particular concern for pregnant women, the aged and the infirmed. It is recommended that these concerned groups avoid handling this organism.After use, the enrichment medium and the 3M MDA 2 - *Listeria monocytogenes*tubes can potentially contain pathogenic materials.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. 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.

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

D. Sample Enrichment

Foods

(a) Allow the Demi-Fraser Broth enrichment medium (includes ferric ammonium citrate) to equilibrate to ambient laboratory temperature (20-25^{o}C).

(b) Aseptically combine the enrichment medium and sample according to Table ~~2~~A. For

all meat and highly particulate samples, the use of filter bags is recommended.

(c) Homogenize thoroughly by ~~blending,~~ stomaching~~,~~ or hand mixing for 2 ±0.2 minutes. Incubate at 37 ±1°C according to Table A.

Environmental samples

(d) 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 Letheen 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. Another option is to transfer 10 µL of the NB enrichment in the LS tubes.

(e) The recommended size of the sampling area to verify the presence or absence of the pathogen on the surface is at least 100 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, USDA FSIS MLG or ISO 18593 [11] guidelines.

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

E. Preparation of the 3M™ Molecular Detection Speed Loader Tray

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

Specific Instructions for Validated Methods
AOAC® Performance Tested Method℠ # 081501

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 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 ~~2~~A. 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 ~~2~~A).

Table ~~2~~A. Enrichment protocols using Demi-Fraser Broth at 37 ± 1 °C according to AOAC Performance Tested [SM] 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[a]		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[b]	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

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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 ±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 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 ±1°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°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.

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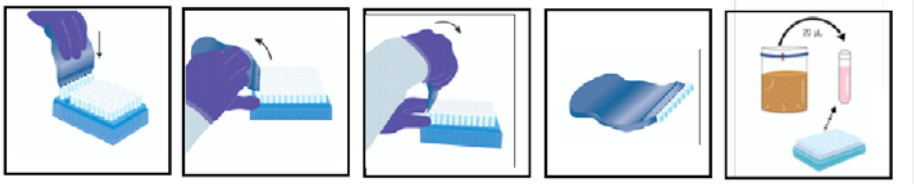

4.7 Repeat steps 4.1 to 4.4 as needed, for the number of samples to be tested. When all samples have been transferred, then transfer **20 µL of NC into a LS tube**. Do not recap tubes.

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 heat for 15 ±1 minutes. During heating, the LS solution will change from pink (cool) to yellow (hot).

5. 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 (20-25°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:

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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 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.

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 µL of NC lysate into a Reagent tube.

4.7 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.

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 monocytogenes*amplificationreagents 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.

Results of Collaborative Study

For this collaborative study, the 3M Molecular Detection Assay (MDA) 2 - *Listeria monocytogenes* method was compared to the USDA FSIS MLG 8.09 reference method for deli turkey and raw chicken breast fillet. A total of 13 laboratories throughout the United Statesand Canada participated in this study, with 11 laboratories submitting data for the deli turkey and 12 laboratories submitting data for the raw chicken breast fillet. See Table 1 for a summary of laboratory participation for each matrix. Each laboratory analyzed 36 test portions for each method per matrix: 12 inoculated with a high level of *Listeria monocytogenes*, 12 inoculated with a low level of *Listeria monocytogenes*, and 12 un-inoculated controls.
A background screen of the matrix indicated an absence of indigenous *Listeria monocytogenes*in both matrices.Ten (10) replicate test portions (randomly sampled from 50% of the total packages used in the analysis) were screened for the presence of *Listeria monocytogenes*. All test portions produced negative results for the target analyte.
Results for the heat stress analysis of the inoculum for the deli turkey are presented in Table 2. The raw chicken breast fillet is not heat treated, therefore it was not necessary to injure the cells.Table 2016.1A and 2016.1B summarize the inter-laboratory results for all foods tested, including POD statistical analysis. As per criteria outlined in Appendix J of the AOAC Validation Guidelines, fractional positive results were obtained.Detailed results for each laboratory are presented in Tables 2016.2A and 2016.2B. For each matrix, the level of *Listeria monocytogenes* was determined by MPN on the day of initiation of analysis by the coordinating laboratory. MPN results are presented in Tables 2016.2A and 2016.2B.The individual laboratory and sample results are presented in Tables 1-2 of the Supplementary Materials. The APCresults for each collaborating are presented in Table~~3~~5 of the Supplementary Materials.

Deli Turkey (125 g Test Portions)

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

For the low inoculum level, 69 out of 132 test portions (POD_{CP} of 0.52) were reported as presumptive positive by the 3M MDA 2 – *Listeria monocytogenes*method with 66out of 132 test portions (POD_{CC} of 0.50) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria monocytogenes*method, 66out of 132 samples confirmed

positive (POD_C of 0.50). For test portions evaluated by the USDA/FSIS MLG reference method, 60 out of 132 test portions produced positive results.A $dLPOD_C$ value of 0.04 with 95% confidence intervals of (-0.08, 0.17) was obtained between the candidate and reference method, indicating the difference between methods was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the two methods.~~A $dLPOD_{CP}$ value of 0.02 with 95% confidence intervals of (-0.10, 0.15) was obtained between presumptive and confirmed results indicating the difference between presumptive and confirmed methods was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the presumptive and confirmed results.~~

For the high inoculum level, 132 out of 132 test portions (POD_{CP} of 1.00) were reported as presumptive positive by the 3M MDA 2 – *Listeria monocytogenes*method with 132out of 132 test portions (POD_{CC} of 1.00) confirming positive.For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria monocytogenes*method, 132out of 132 samples confirmed positive (POD_C of 1.00).For test portions evaluated by the USDA/FSISMLG reference method, 132 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between the candidate and reference method, indicating the difference between methods was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the two methods~~.
A $dLPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between presumptive and confirmed results indicating the difference between presumptive and confirmed methods was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the presumptive and confirmed results.~~
For the un-inoculated controls, 0 out of 132 samples (POD_{CP} of 0.00) produced a presumptive positive result by the 3M MDA 2 - *Listeria monocytogenes*method with 0out of 132 test portions (POD_{CC} of 0.00) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria monocytogenes*method, 0out of 132 samples confirmed positive (POD_C of 0.00). For test portions evaluated by the USDA/FSIS MLG reference method, 0 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between the candidate and reference method, indicating the difference between methods was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the two methods.~~ A $dLPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.03, 0.00) was obtained between presumptive and confirmed results indicating the difference between presumptive and confirmed methods was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the presumptive and confirmed results.~~

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

Raw Chicken Breast Fillet (25 g Test Portions)

Raw chicken breast fillet test portions were inoculated at a low and high inoculum level and were analyzed for the detection of *Listeria monocytogenes*. Un-inoculated controls were included in

each analysis. Laboratory 11 did not participate in the evaluation of this matrix. Laboratory 10 submitted data that indicated cross contamination of the inoculating organism in the un-inoculated control samples. Further analysis at the coordinating laboratory confirmed the cross contamination of the un-inoculated controls. Due to this issue, the data submitted from Laboratory 10 was not used in the statistical analysis. All other laboratories submitted data for both methods evaluated. The MPN levels obtained for this matrix, with 95% confidence intervals, were 0.66 CFU/test portion (0.51, 0.83) for the low level and 6.24 CFU/test portion (3.58, 10.88) for the high level.

For the low inoculum level, 86 out of 132 test portions (POD_{CP} of 0~~.~~65) were reported as presumptive positive by the 3M MDA 2 – *Listeria monocytogenes*method with 86out of 132 test portions (POD_{CC} of 0.65) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria monocytogenes*method, 85out of 132 test portions confirmed positive (POD_C of 0.64). For test portions evaluated by the USDA/FSIS MLGreference method, 64 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.16 with 95% confidence intervals of (0.04, 0.28) was obtained between the candidate and reference method, indicating a statistically significant difference between the two methods, with a positive correlation in data indicating more recovery of the target analyte by the candidate method. A $dLPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.12, 0.12) was obtained between presumptive and confirmed results indicating the difference between presumptive and confirmed methods was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the presumptive and confirmed results.~~

For the high inoculum level, 129 out of 132 test portions (POD_{CP} of 0.98) were reported as presumptive positive by the 3M MDA 2 – *Listeria monocytogenes*method with 132out of 132 test portions (POD_{CC} of 1.00) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria monocytogenes*method, 129out of 132 samples confirmed positive (POD_C of 0.98). For test portions evaluated by the USDA/FSIS-MLG reference method, 132 out of 132 test portions produced positive results. A $dLPOD_C$ value of -0.02 with 95% confidence intervals of (-0.06, 0.01) was obtained between the candidate and reference method, indicating the difference between methods was not statistically significant at the 0.05 probability level.~~no statistically significant difference between the two methods.~~ A $dLPOD_{CP}$ value of -0.02 with 95% confidence intervals of (-0.06, 0.01) was obtained between presumptive and confirmed results indicating the difference between presumptive and confirmed methods was not statistically significant at the 0.05 probability level.~~indicating no statistically significant difference between the presumptive and confirmed results.~~

For the un-inoculated controls, 0 out of 132 samples (POD_{CP} of 0.00) produced a presumptive positive result by the 3M MDA 2 - *Listeria monocytogenes* method with 0 out of 132 test portions (POD_{CC} of 0.00) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *Listeria monocytogenes* method, 0 out of 132 samples confirmed positive (POD_C of 0.00). For test portions evaluated by the USDA/FSIS MLG reference method, 0 out of 132 test portions produced positive results. A $dLPOD_C$ value of 0.00 with 95% confidence intervals of (-0.03, 0.03) was obtained between the candidate and reference method, indicating the difference between methods was not statistically significant at the 0.05 probability level.~~no statistical significant difference between the two methods.~~ A $dLPOD_{CP}$ value of 0.00

with 95% confidence intervals of (-0.03, 0.00) was obtained between presumptive and confirmed results indicating the difference between presumptive and confirmed methods was not statistically significant at the 0.05 probability level.~~indicating no statistically significant difference between the presumptive and confirmed results.~~

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

Discussion

No negative feedback was provided by the collaborating laboratories in regard to the performance of the 3M MDA 2- *Listeria monocytogenes*method.For the raw chicken breast fillet, Laboratory 10 reported isolating *Listeria monocytogenes*from two un-inoculated control samples. The isolates were sent for further identification and it was determined that they were the same strain as the inoculating organism, indicating that cross contamination of the sample occurred. Due to the fact that cross contamination occurred, just cause for removal of the data was established and the data generated by Laboratory 10 was therefore not included in the statistical analysis.

Overall, the data generated during this evaluation demonstrates the reproducibility of this new method. For the deli turkey analysis, the POD statistical analysis indicated the difference between the candidate method and reference method was not statistically significant at the 0.05 probability level. And the~~thatno statistically significant difference between the candidate method and the reference methodor between the presumptive and confirmed results~~ difference between presumptive and confirmed candidate method was not statistically significant at the 0.05 probability level.~~of the candidate method was obtained.~~For raw chicken breast fillet, a statistically significant difference was observed between the reference and the alternative method. ~~The dLPOD data indicated~~ The dLPOD being significantly greater than zero showed an observed higher proportion of positive results by the candidate method than the reference method.~~a positive correlation in data indicating more recovery of the target analyte by the candidate method.~~One possible contributionfor the higher observed proportion positive results~~to the higher level of recovery~~ observed with the 3M MDA 2 – *Listeria monocytogenes*method was the use of Demi-Fraser Broth for the candidate method. This enrichment media formulation is less selective than the modified University of Vermont Medium used in the USDA reference method and may have contributed to the higher observed proportion positive results ~~level of recovery observed~~ during the evaluation.

A second possible contribution ~~to the higher~~for the higher observed proportion positive results~~level of recovery was~~ isthe length of the primary enrichment. Test portions evaluated by the 3M MDA 2 – *Listeria monocytogenes*method were incubated for a minimum of 28 hours in the primary enrichment, while the USDA reference method had a maximum primary enrichment time of 26 hours. No statistically significant difference was observed between the candidate method presumptive and confirmed results for this matrix.

Recommendations

It is recommended that the 3M Molecular Detection Assay 2 – *Listeria monocytogenes*method be adopted as Official First Action status for the detection of *Listeria monocytogenes* in selected foods: 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); raw chicken breast fillet, concrete (sponge, 225 mL & 100 mL), stainless steel (sponge, 225 mL), and plastic (Enviro-sSwab, 10 mL) environmental samples.

Acknowledgements

We would like to extend a sincere thank you to the following collaborators for their dedicated participation in this study:

Robert Brooks- ATC Microbiology, LLC- North Little Rock, AR.
Jaspreet Walia & Francisco Hernandez – Certified Laboratories, Turlock, CA
David Bosco& Grizelda Trevino– Food Safety Net Services, Fresno, CA
Alex Brandt & Chris Lopez – Food safety Net Services, San Antonio, TX
Elizabeth Sjogren&Manish Shekhawat– Microbac Laboratories, Inc. Worcester, MA
Li Maria Ma, Chris Timmons, & Claudia Diaz Proano – Oklahoma St. University, Stillwater, OK
Alexandra Calle & David Campos – Texas Tech University, Lubbock, TX.
Zachary Metz&David Baumler, University of Minnesota, St. Paul, MN
Robbie Smith, Dianne Wood, Evelyn Maranan,&Carmen Chavarria Maple Leaf Foods– Guelph, ON, Canada
Ben Bastin– Microbiology R&D, Q Laboratories Inc., Cincinnati, OH
David Isfort– Microbiology-Food, Q Laboratories Inc., Cincinnati, OH
Jesse Miller, Bryan Schindler, Courtney Gies, Eric Budge, Zach Geurin & Alex Repeck - NSF International, Ann Arbor, MI
Cynthia Zook & Christina Barnes – 3M Food Safety, St. Paul, MN

We would like to extend a special thanks to the following team members at Q Laboratories, Inc. for their efforts during the collaborative study: M. Joseph Benzinger Jr., Allison Mastalerz, Alison DeShields, Jonathan Clifton, T. Shane Wilson, Meghan Daniel, Nicole Klass and Colleen Sweeney.

References

(1) United States Food and Drug Administration. (2008) *Guidance for Industry: Control of Listeria monocytogenes in Refrigerated or Frozen Ready-to-Eat Foods; Draft Guidance*http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/FoodProcessingHACCP/ucm073110.htm (Accessed March 2016)

(2) European Food Safety Authority. (2015) *Listeria*http://www.efsa.europa.eu/en/topics/topic/listeria (Accessed March 2016)

(3) Centers for Disease Control and Prevention. (2016) *Listeria monocytogenes Outbreaks*http://www.cdc.gov/Listeria monocytogenes/outbreaks/(Accessed March 2016)

(4)*Official Methods of Analysis* of AOAC INTERNATIONAL. (2012) 19th Ed., Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, AOAC INTERNATIONAL, Gaithersburg, MD, http://www.eoma.aoac.org/app_j.pdf(Accessed January 2016)

(5)United States Department of Agriculture Food Safety and Inspection Service. (2013) *Microbiological Laboratory Guidelines5.09*: *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and Environmental Samples*. (Accessed January 2016) http://www.fsis.usda.gov/wps/wcm/connect/1710bee8-76b9-4e6c-92fc-fdc290dbfa92/MLG-8.pdf?MOD=AJPERES

(6) *Official Methods of Analysis* of AOAC INTERNATIONAL (2015) *Enumeration of Aerobic Bacteria in Foods.*2015.13. (Accessed January2016)

(7) Least Cost Formulations, Ltd., MPN Calculator-Version 1.6, www.lcfltd.com/customer/LCFMPNCalculator.exe(Accessed March 2016)

(8) *Official Methods of Analysis* of AOAC INTERNATIONAL (2012) *Gram-PositiveBacteria Identification.* 2012.02. (Accessed January 2016)

(9) Wehling, P., LaBudde, R., Brunelle, S., Nelson, M. (2011) Journal of AOAC International*Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods.* Vol. 94, No. 1. pp. 335-347

(10) Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook (2013), ~~http://lcfltd.com/aoac/aoac-binary-v2-3.xls~~http://www.aoac.org/iMIS15_Prod/AOAC/SD/SGR/AOAC_Member/SDCF/SDGRCF/SDSGM.aspx?hkey=086fdba4-32e2-43f0-8272-7d41a6603e87(Accessed March, 2016)

(11) ISO 18593:2004 -*Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.*

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Table 1: Participation of each Collaborating Laboratory

Lab	Deli Turkey[a,b]	Raw Chicken BreastFillet[a,b]
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

[a] Y= Collaborator analyzed the food type;[a]N= Collaborator did not analyze food type

Table 2: Heat-Stress Injury Results

Matrix	Test Organism[a]	CFU/MOX (Selective Agar)	CFU/TSA (Non-Selective Agar)	Degree Injury[b]
Deli Turkey	*Listeria monocytogenes* ATCC 19115	9.1×10^8	2.5×10^9	60.8%
Raw Chicken Breast Fillet	*Listeria monocytogenes* ATCC 7644	Not applicable	Not applicable	Raw chicken product is not heat treated so not necessary to injure the cells.

[a] ATCC- American Type Culture Collection

[b] 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* inDeli Turkey (125g)

Method[a]	**3M™ MDA™2 - *Listeria monocytogenes***		
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[e]	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)[f]	0.00 (-0.03, 0.03)	0.04 (-0.08, 0.17)	0.00 (-0.03, 0.03)

dLPOD (Candidate Presumptive vs. Candidate Confirmed)[f]	0.00 (-0.03, 0.03)	0.02 (-0.10, 0.15)	0.00 (-0.03, 0.03)

[a] Results include 95% Confidence Intervals, [r] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation
[e] P Value = Homogeneity test of laboratory PODs; [f] 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* inRaw Chicken Breast Fillet (25g)

Method[a]	**3M™ MDA™2 -*Listeria monocytogenes***		
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[e]	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)[f]	0.00	0.16	-0.02

	(-0.03, 0.03)	(0.04, 0.28)	(-0.06, 0.01)
dLPOD (Candidate Presumptive vs. Candidate Confirmed)[f]	0.00 (-0.03, 0.03)	0.00 (-0.12, 0.12)	-0.02 (-0.06, 0.01)

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

Table 2016.2A: Comparative Results for the Detection of *Listeria monocytogenes* in 125 g Deli TurkeyTest Portions by the 3M™ MDA 2 - *Listeria 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[a]	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[a]	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		

[a]N/A – Laboratory did not participate or submit data for this matrix

[b]Repeatability Standard Deviation, [c]Among-Laboratory Standard Deviation, [d]Reproducibility Standard Deviation, [e] 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)

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 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[a]	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[a]	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		

[a] N/A – Laboratory did not participate or submit data for this matrix

[b] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation, [e] 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)

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 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[a]	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[a]	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		

[a] N/A – Laboratory did not participate or submit data for this matrix

[b] Repeatability Standard Deviation, [c] Among-Laboratory Standard Deviation, [d] Reproducibility Standard Deviation, [e] 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)

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 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[b]	12	0	0.00	12	2	0.17	12	0	0.00	12	0	0.00	0.00	-0.17
		11[a]	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		

[a] N/A – Laboratory did not participate or submit data for this matrix; [b] Results were not used in statistical analysis due to deviation from testing protocol.
[c] Repeatability Standard Deviation, [d] Among-Laboratory Standard Deviation, [e] Reproducibility Standard Deviation, [f] 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)

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 - *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[b]	12	6	0.50	12	8	0.66	12	6	0.50	12	5	0.42	0.08	-0.16
	MPN/ Test Portion	11[a]	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		

[a] N/A – Laboratory did not participate or submit data for this matrix; [b] Results were not used in statistical analysis due to deviation from testing protocol.
[c] Repeatability Standard Deviation, [d] Among-Laboratory Standard Deviation, [e] Reproducibility Standard Deviation, [f] 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)

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 - *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[b]	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
	MPN/ Test Portion	11[a]	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		

[a]N/A – Laboratory did not participate or submit data for this matrix; [b]Results were not used in statistical analysis due to deviation from testing protocol.
[c]Repeatability Standard Deviation, [d]Among-Laboratory Standard Deviation, [e]Reproducibility Standard Deviation, [f] 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)

Supplementary Materials

Table 3~~1~~. Individual Collaborator Results for Deli Turkey

	High Level Test Portions												Low Level Test Portions												Un-inoculated Test Portions											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
3M MDA 2 -*Listeria monocytogenes*																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-[b]	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-[b]	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
11	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-[b]	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
USDA/FSIS – MLG 8.09																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
9	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
11	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

[a] += *Listeria monocytogenes*were detected in samples; -=*Listeria monocytogenes* were not detected in sample; n/a- lab did not participate or submit data for the matrix;
[b] Sample was presumptive positive on 3M MDA 2 -*Listeria monocytogenes* but confirmed negative indicating a false positive result;

Table 24. Individual Collaborator Results for Raw Chicken Breast Fillet

	High Level Test Portions												Low Level Test Portions												Un-inoculated Test Portions											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
3M MDA 2 - *Listeria monocytogenes*																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6	+[d]	+[d]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-[c]	+	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+[d]	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+[d]	+	-	-	-	-	-	-	-	-	-	-	-	-
10[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+[d]	+[d]	-	-	-	-	-	-	-	-	-	-	-	-	-	+[e]
11	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
USDA/FSIS – MLG 8.09																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10[b]	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
11	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
12	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

[a] + = *Listeria monocytogenes* were detected in samples; -= *Listeria monocytogenes* were not detected in sample; n/a- lab did not participate or submit data for the matrix;

[b] Results were not used in statistical analysis due to laboratory error, [c] Sample was presumptive positive on 3M MDA 2 - *Listeria monocytogenes* but confirmed negative indicating a false positive result;

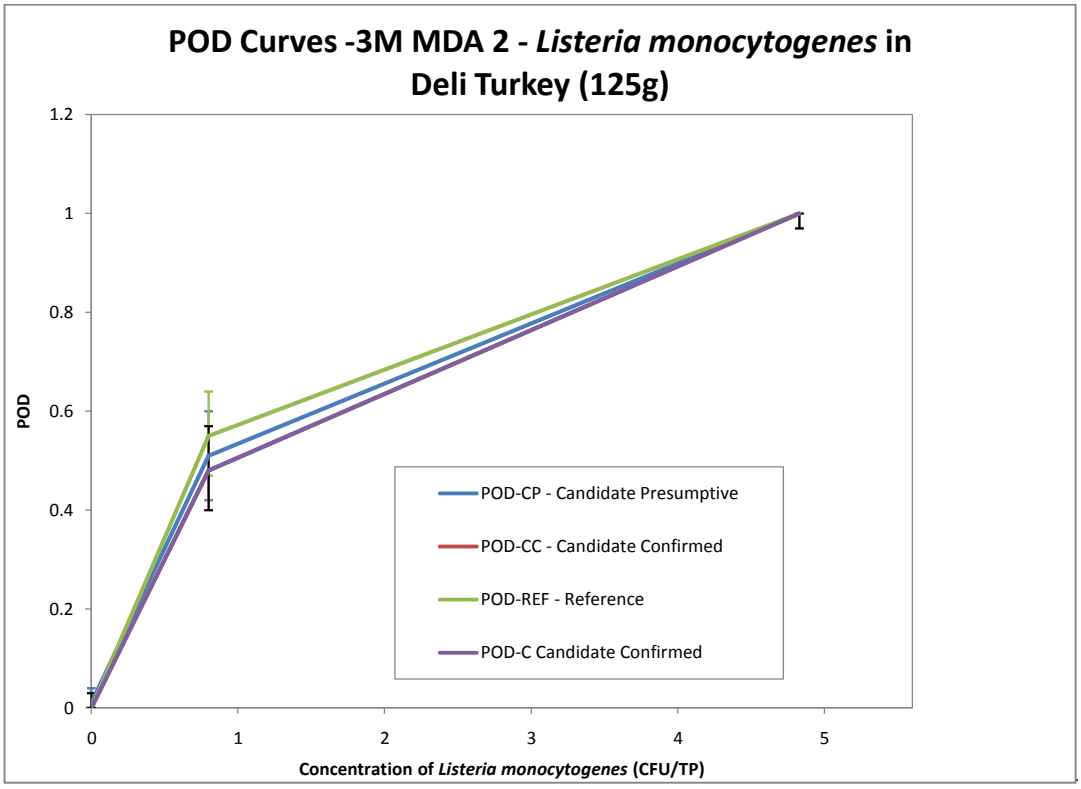
[d] Sample was presumptive negative on 3M MDA 2 - *Listeria monocytogenes* but confirmed positive indicating a false negative result

Table 35: Results of Aerobic Plate Count for Collaborating Laboratories

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

[a] Samples analyzed by the 3M Petrifilm Rapid Aerobic Count Plate, AOAC OMA 2015.13

Figure 1A: POD Values of Candidate Method vs. Reference Method for125 gDeli Turkey



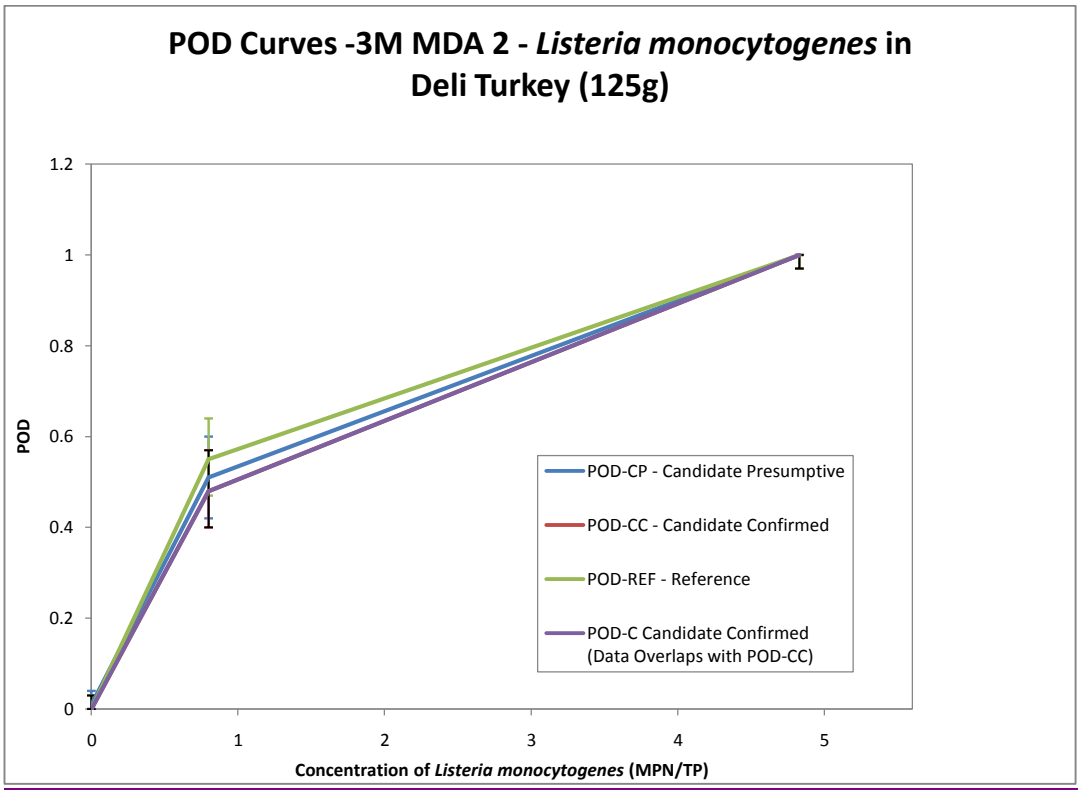


Figure 1B: dLPOD Values of Candidate Method vs. Reference Method for125 gDeli Turkey

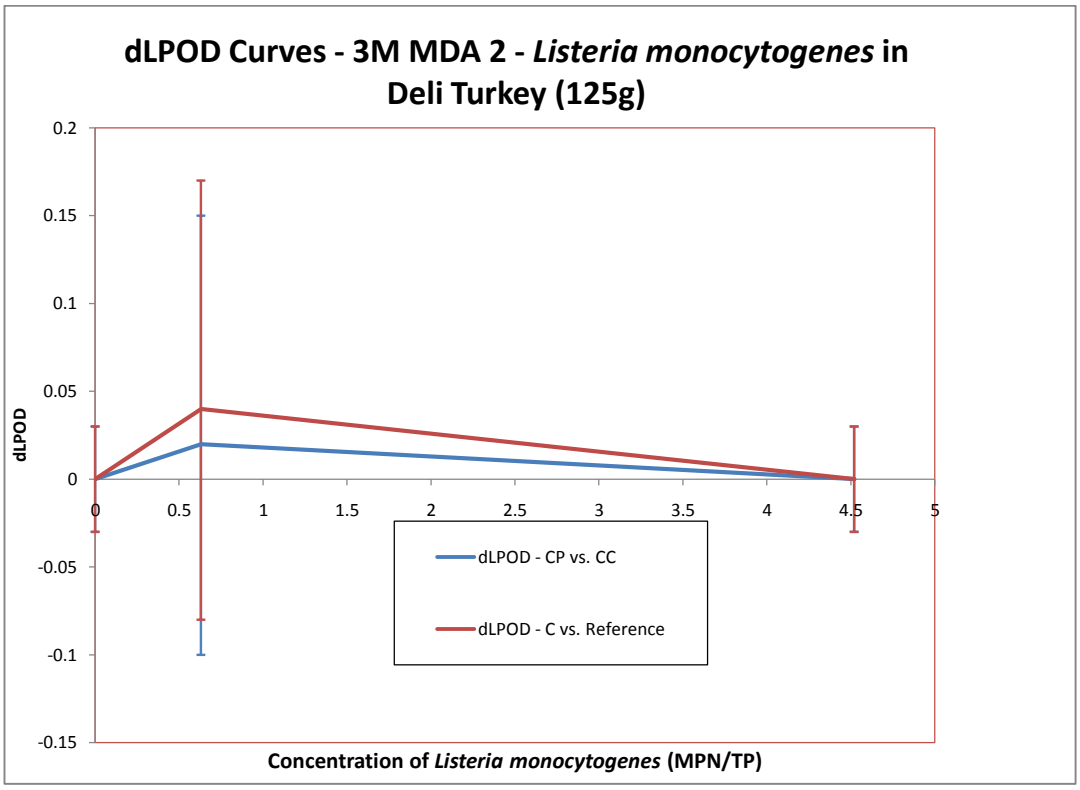
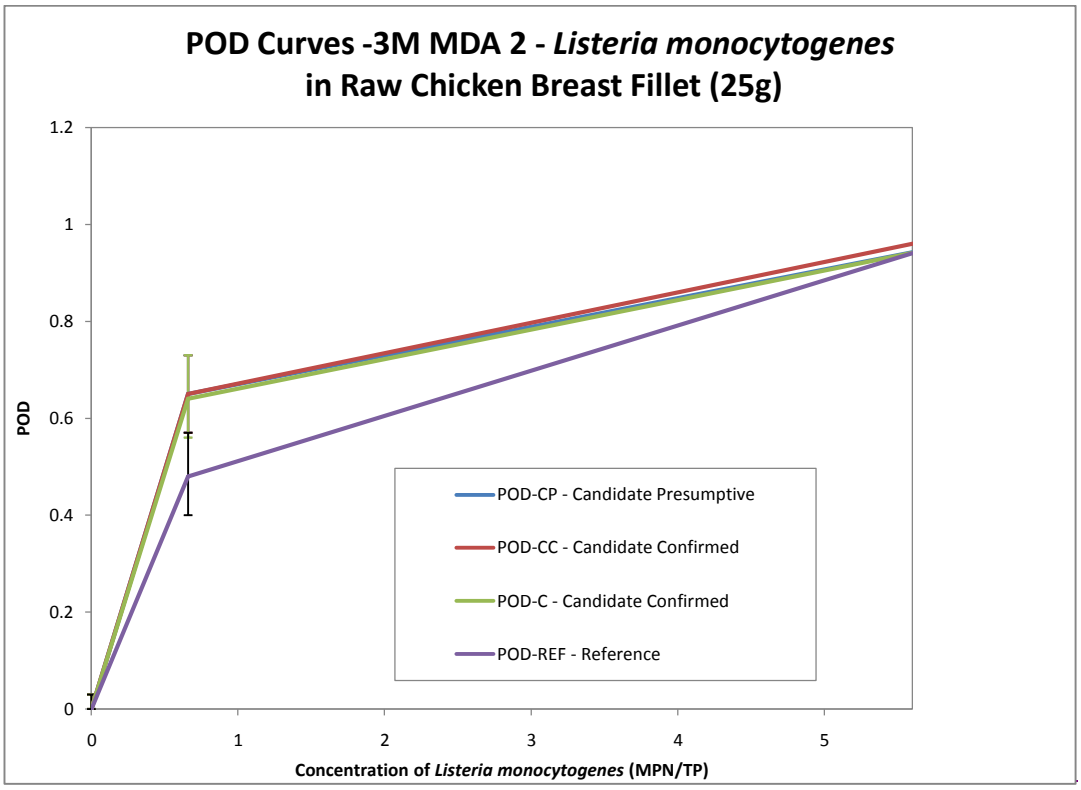


Figure 2A: POD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet


POD Curves -3M MDA 2 - Listeria monocytogenes
in Raw Chicken Breast Fillet (25g)
POD
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
Concentration of Listeria monocytogenes (MPN/TP)
POD-CP - Candidate Presumptive
POD-CC - Candidate Confirmed
POD-C - Candidate Confirmed
POD-REF - Reference

1

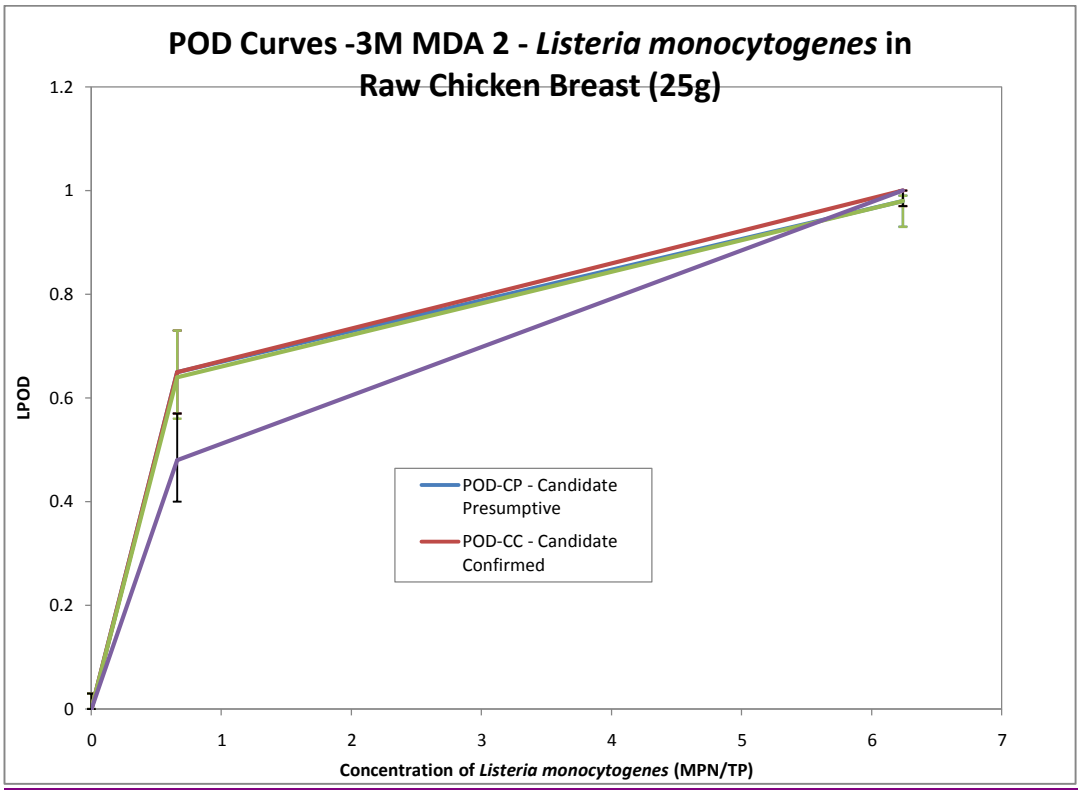
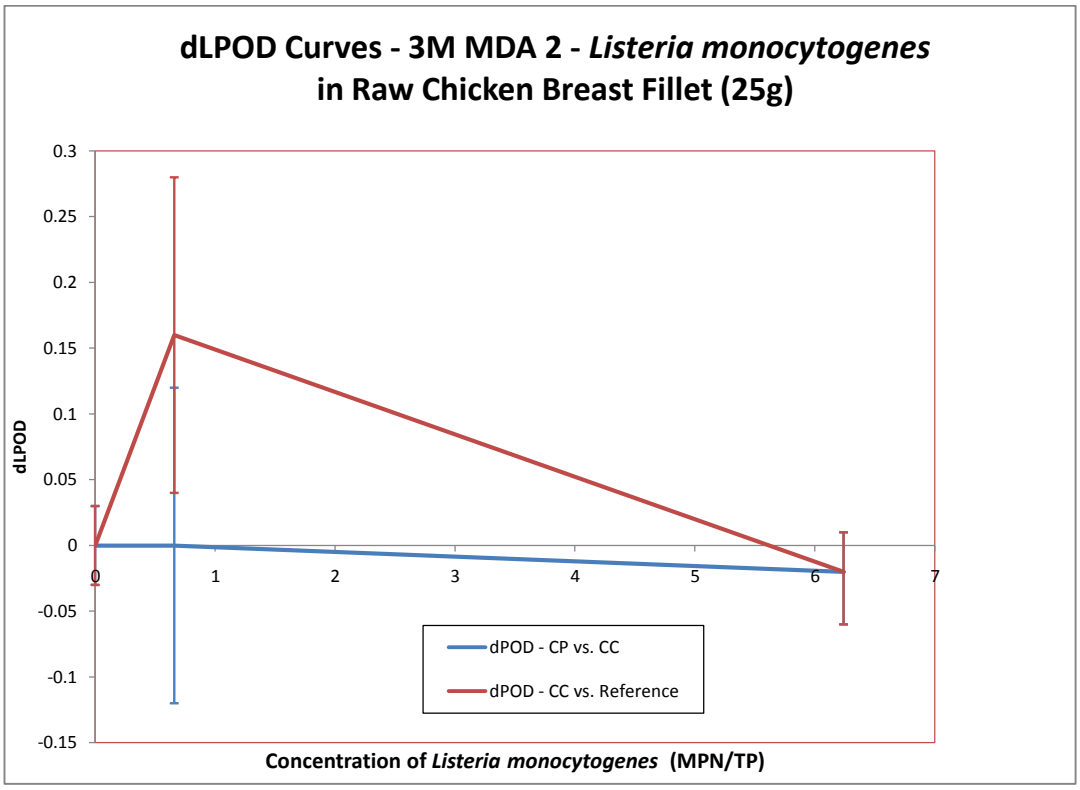


Figure 2B: dPOD Values of Candidate Method vs. Reference Method for Raw Chicken Breast Fillet


dLPOD Curves - 3M MDA 2 - Listeria monocytogenes in Raw Chicken Breast Fillet (25g)
dLPOD
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
0
1
2
3
4
5
6
7
dPOD - CP vs. CC
dPOD - CC vs. Reference
Concentration of Listeria monocytogenes (MPN/TP)

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 *Listeriamonocytogenes* 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 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
Listeria monocytogenes 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[4], or ISO 7218[5]. The user shouldcomplete 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[6].

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 *Listeriamonocytogenes* 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 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**, 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 ± 0.2 minutes. Incubate at 37 ±1°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 ±1°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 Letheen broth. It is recommended to sanitize the area after sampling.

WARNING: Should you select to use Neutralizing Buffer (NB) that contains aryl sulfonatecomplex 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 testingin 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^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 [1], USDA FSIS MLG [2] or ISO 18593 [7] 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 Heat-processed / pasteurized dairy products Produce and vegetables Multi-component foods	25 g	225	24-30
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

				Fraser Broth		

(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℠ # 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 ± 1 °C according to AOAC Performance Tested SM 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[(a)]		Whole melon	Enough volume to allow melon to float	26-30
e s ..	Stainless	1 sponge	225	24-30

	steel			
	Sealed concrete	1 sponge	100	24-30
	Plastic[(b)]	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 DETECTIONCHILL 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.

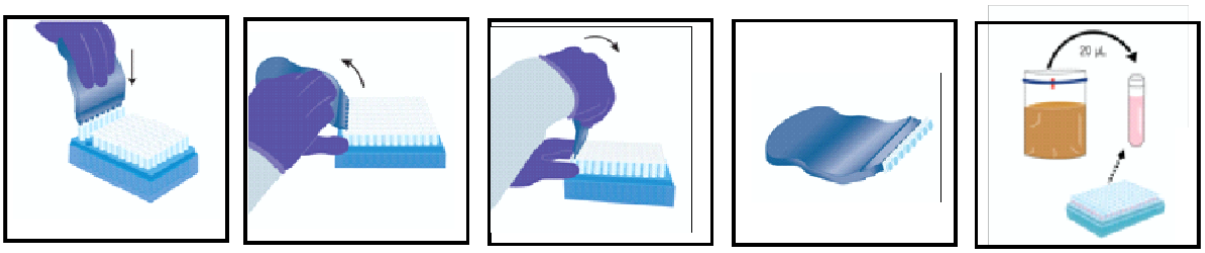
NOTE: The 3M Molecular Detection Instrument must reach and maintain temperature of 60°C before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 minutes and is indicated by an ORANGE light on the instrument's status bar. When the instrument 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:

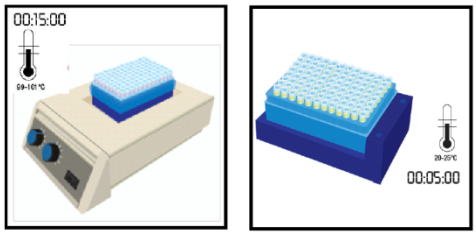
Transfer each enriched sample into an individual LS tube **first**. Transfer the NC **last**.

   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 ±1°C.
9. Place the uncovered rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ±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

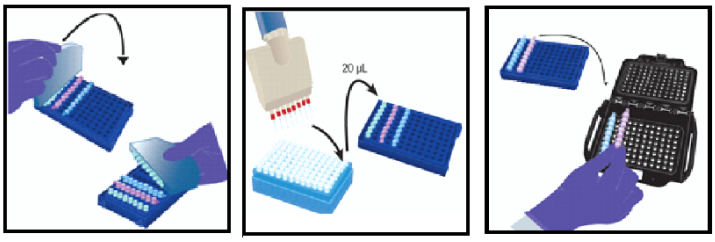
1. One Reagent tube is required for each sample and the NC.
   - 1.1 Reagent tube strips can be cut to desired tube number. Select the number of individual Reagent tubes or 8-tube strips needed.
   - 1.2 Place Reagent tubes in an empty rack.
   - 1.3 Avoid disturbing the reagent pellets from the bottom 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 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.
   - 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 µL of NC lysate into a Reagent tube.
   - 5.6 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.

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 [1, 2, 3], 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* amplificationreagents 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 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.

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

AOAC *Performance Tested Methods*℠ ~~XXXXXX~~081501

Abstract

The 3M™ Molecular Detection Assay 2 (MDA2)–*Listeria monocytogenes* for the detection of *Listeria monocytogenes*was evaluated following the AOAC Research Institute *Performance Tested Methods*℠program guidelines.The evaluation included inclusivity/exclusivity, lot-to-lot/stability, robustness and matrix studies. The 3M MDA2 –*Listeria monocytogenes*was compared to various reference methods for beef hot dogs, deli turkey, raw chicken leg pieces, sealed concrete, plastic, stainless steel, whole melons, bagged raw spinach, romaine lettuce, cold smoked salmon, queso fresco, 4% milk fat cottage cheese, 3% chocolate whole milk and vanilla ice cream in the matrix study.Twenty replicates of each food matrix were analyzed at a low inoculum level of 0.2-2 colony forming units (CFU)/test portion, five replicates were analyzed at a high level of 2-5 CFU/test portion, and five control replicates were analyzed at 0 CFU/test portion with the exception of raw chicken leg pieces. Naturally contaminated raw chicken leg pieces were evaluated using two separate lots consisting of twenty replicates for each lot. All environmental surfaces were analyzed using twenty replicates at a low inoculum level of ~50 CFU/standardtest area, five replicates were analyzed at a high level of ~100 CFU/standard test area,and five control replicates were analyzed at 0 CFU/standard test area. Based on the probability of detection statistical model, there were no statistically significant differences between the number of presumptive and confirmed positive samples or between the candidate and reference method.There were no unexpected results in the lot-to-lot/stability, robustness studies. In the inclusivity/exclusivity study, 50 target organisms and ~~30~~ 43non-target organisms were tested following the 3M MDA2 - *Listeriamonocytogenes*method with all target organisms detected and none of the non-target organisms detected. The results of this evaluation demonstrate the specificity and sensitivity of the 3M MDA2 -*Listeriamonocytogenes*and its ability to accurately detect *Listeria monocytogenes*in select food matrices and environmental surfaces, 24-30 hours post enrichment.

Authors

Lisa Monteroso and DeAnn Benesh, 3M Food Safety Division
Ben Bastin, Jonathan Flannery, Erin Crowley, Patrick Bird, M. Joseph Benzinger, Jr., James Agin, David Goins, Q Laboratories, Inc.

Submitting Company

3M Company
Food Safety Department
3M Center, Bldg. 275-5W-05
St. Paul, MN 55144-1000

Independent Laboratory

Q Laboratories, Inc.

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1400 Harrison Ave
Cincinnati, OH 45214

Reviewers
Yvonne Salfinger
Consultant
Denver, CO

Michael Brodsky
Brodsky Consultants
73 Donnamora Crescent
Thornhill, Ontario L3T 4K6
Canada

Reviewer
Tony Hitchins
FDA-Retired
Rockville, MD

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Scope of Method

a) *Target organism*.— *Listeriamonocytogenes*

b) *Matrices*.— Beef hot dogs (25 g), deli turkey (125 g), queso fresco (25 g), vanilla ice cream (25 g), 4% milk fat cottage cheese (25 g), 3% chocolate whole milk (25 mL), whole melon, romaine lettuce (25 g), bagged raw spinach (25 g), cold smoked salmon (25 g), raw chicken leg pieces (25 g), sealed concrete (sponge enriched in 100 mL, 4" x 4"), plastic (Enviro~~s~~Swab enriched in 10 mL, 1" x 1"), stainless steel (sponge enriched in 225 mL, 4" x 4").

~~c)~~ *Summary of Validated Performance Claims*.— Performance equivalent to that of the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM) Chapter 10 [1] for whole melon, cold smoked salmon, romaine lettuce; the U.S. Department of Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (USDA-FSIS/MLG) 8.09 [2] for beef hot dogs, deli turkey, raw chicken leg pieces, sealed concrete, plastic and stainless steeland AOAC 993.12 [3] for queso fresco, vanilla ice cream, 4% milk fat cottage cheese, 3% chocolate whole milk and theISO 11290-1/A1 [4]for cold smoked salmon and bagged raw 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.

Principle of the Method

3M™ Molecular Detection Assay 2 –*Listeriamonocytogenes*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 the laboratory'spreferred method or as specified by local regulations.

General Information

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 that can 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 3M MDA2–*Listeria monocytogenes*was developed to provide rapid and specific detection of *Listeriamonocytogenes*in enriched food samples and on food processing surfaces.

Materials and Methods

Test Kit Information

a) *Kit Name.*—Molecular Detection Assay 2 – *Listeriamonocytogenes*
b) Catalog Number.—MDA2LM96

Test Kit Reagents - 3M MDA2 - *Listeriamonocytogenes*(96 tests)
a) *Lysis Solution (LS) tubes*. – 96 (12 strips of 8 tubes)
b) *Listeriamonocytogenes Reagent tubes*. – 96 (12 strips of 8 tubes)
c) *Extra caps.* – 96 (12 strips of 8 caps)
d) *Reagent Control (RC).* – 16 (2 pouches of 8)
e) Quick Start Guide

Additional Supplies and Reagents

a) 3M Molecular Detection System (instrument), power supply, cord, USB cable and software
b) 3M Molecular Detection Speed Loader Tray
c) 3M Molecular Detection Chill Block Tray and Chill block insert
d) 3M Molecular Detection Heat Block Insert
e) 3M Molecular Detection Cap/Decap Tool [Reagent]
f) 3M Molecular Detection Cap/Decap Tool [Lysis]
g) Empty lysis tube rack
h) Empty reagent tube rack
i) Dey-Engley (D/E) Neutralizing Broth
j) Demi-Fraser broth (with additional of ferric ammonium citrate)

Additional media

a) Oxford Agar (OX)b) Sheep blood agar (SBA)
b) Fraser broth (FB)
c) Sheep blood agar (SBA)
d) PALCAM agar
e) Ottaviani Agosti Agar (OAA)
f) Trypticase soy agar (TSA)
g) Trypticase soy agar with 0.6% Yeast extract (TSA/YE)
h) De Man, Rogosa and Sharpe (MRS)
i) Modified Oxford agar (MOX)
j) Brain heart infusion (BHI) broth
k) Horse blood overlay (HBO)
l) Buffered Listeria enrichment broth (BLEB)

Apparatus

a) *Pipettes*. – capable of 20µL
b) *Multi-channel pipette*. – capable of 20µL
c) *Sterile pipette tips*. – capable of 20µL
d) *Stomacher*®. – Seward or equivalent
e) *Filter Stomacher® bags*. - Seward or equivalent
f) *Thermometer*. – calibrated range to include 100 ± 1°C range
g) *Incubators*. – capable of maintaining 37 ± 1°C
h) *Dry double block heater unit.* – capable of maintaining 100 ± 1°C; or*a water bath* capable of maintaining 100 ± 1°C
~~i) *Freezer*. – capable of maintaining -10 to -20°C, for storing the 3M Molecular Detection Chill Block Tray~~
~~j)~~i) *Refrigerator*. – capable of maintaining 2-8°C, for storing the 3M MDA2
~~k)~~j) *Computer*. – compatible with the 3M Molecular Detection System (instrument)

Safety Precautions

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 –*Listeriamonocytogenes*. Retain the safety instructions for future reference.

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

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

~~Enrichment~~ Sample Preparation

3M recommends the use of Demi-Fraser Broth (with addition of ferric ammonium citrate) for the enrichment of food and environmental samples.Table 1 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

a) Allow the Demi-Fraser Broth enrichment medium to equilibrate to ambient laboratory temperature.
b) Aseptically combine the enrichment medium and sample according to Table 1. For all meat and highly particulate samples, the use of filter bags is recommended.
c) Homogenize thoroughly by ~~blending,~~ stomaching~~,~~ or hand mixing for 2 ±0.2 minutes. Incubate at 37 ±1°C according to Table 1.

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 Letheen broth. It is recommended to sanitize the area after sampling.

The recommended size of the sampling area for verifyingthe presence or absence of the pathogen on the surface is at least 100 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, USDA FSIS MLG or ISO 18593 (7) guidelines.

1. Allow the Demi-Fraser Broth enrichment medium to equilibrate to ambient laboratory temperature.
2. Aseptically combine the enrichment medium and sample according to Table 1.
3. Homogenize thoroughly by ~~blending~~vortexing~~,~~ or stomaching~~, or hand mixing~~ for 2 ±0.2

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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 µLaliquot of each enriched sample are ~~is~~ transferred to separate lysis tubes using a new pipette tip aftereach sample transfer. Place uncovered samples on a dry bath incubator for 15 ±2minutes at 100 ± 1 °C. Following the heat lysis transfer samples to the 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 for the Negative Control (NC).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™MDA2 - *Listeriamonocytogenes*assay. Results are obtained within 75 minutes.

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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 –*Listeriamonocytogenes*amplificationreagents 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.

Confirmation

Presumptive positive samplesof primary enrichmentswere confirmed by following the appropriate reference method confirmation, 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.

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Validation Study

Testing was conducted following the procedures outlined in the AOAC Research Institute *Performance Tested Methods℠ Program* validation outline protocol: *Comparative Evaluation of the 3M™ Molecular Detection Assay 2-Listeria monocytogenes and of the 3M™ Molecular Detection Assay 2- Listeria* (February, 2015) [8]. The independent study involved a matrix study (9matrices), a lot-to-lot/stability evaluation, and a robustness evaluation. The internal study 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°C ± 1°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°C ± 1°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°C ± 1°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 (Table 3a).

An additional thirteen frozen non-*Listeria monocytogenes* strain suspensions were thawed, or lyophilized cultures were revived, and then streaked to sheep blood agar (SBA). Single isolated colonies were picked and sub-cultured in 10 mL Demi-Fraser broth with FAC for 24 hours at 37°C ± 1°C. The cultures were diluted with fresh Demi Fraser broth with FAC before testing using the 3M MDA2 - *Listeria monocytogenes* kit (Table 3b).

Results

All 50 *Listeria monocytogenes* strains were detected by the 3M MDA2 - *Listeria monocytogenes* method. None of the ~~30~~ 43 non- *Listeria monocytogenes* strains were detected. See Tables 2 and 3a, 3b 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 un-inoculated 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 ± 2°C for 24 ± 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 ± 1 minute at 50 ± 1°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)

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and Tryptic Soy agar (TSA). The agars were incubated at 35 ± 1°C for 24 ± 2 hours and the colonies were counted. The degree of injury was estimated as:

$$(1-\frac{n_{select}}{n_{nonselect}})x100$$

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

For melons, a single whole melon was placed into a large sterile bag and the blossom end of the melon was inoculated with 100 µL of the diluted *Listeria monocytogenes* culture. The liquid culture was then allowed to soak into the melon. The melon was then inverted so that the inoculated end was on the bottom of the sterile bag. The bag was tied closed and held for 48-72 hours at 2-8 °C.

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

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

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

The level of *Listeria monocytogenes* in the low level inoculum and high level inoculum was determined by Most Probable Number (MPN) on the day of analysis by evaluating 5 x 50 g, 20 x 25 g (reference method test portions), and 5 x 10 g inoculated test samples for the low inoculation level and by examining 5 x 25 g, 5 x 5 g and 5 x 1 g for the high inoculation level. The level of *Listeria monocytogenes*in the low level inoculum and high level inoculum for all 125 g test portions was determined by MPN by evaluating 5 x 250 g, 20 or 5 x 125 g (reference method test portions), and 5 x 50 g inoculated test samples. Each test portion was enriched with the reference method enrichment broth at the reference method dilution scheme and analyzed by the reference method procedure. The number of positives from the 3 test levels was used to calculate the MPN using the LCF MPN calculator (version 1.6) provided by AOAC RI. [10] (http://www.lcfltd.com/customer/LCFMPNCalculator.exe)

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® GP Biochemical Identification following AOAC OMA 2013.02. [11]

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 hours. After 24 hours of total incubation, the enriched samples were streaked to MOX agar plates and incubated at 35 ±1 °C for 24-48 hours. The enriched samples were re-incubated for an additional 24 hours at 30 ± 1 °C and then streaked to a second MOX agar plate which was incubated for 24-48 hours at 35 ± 1°C. MOX agar plates were examined for suspect colonies, and if present, at least 5 colonies were streaked to TSA containing 0.6% yeast extract (TSA/YE). The TSA/YE plates were incubated at 35 ± 1°C for 24-48 hours and then examined for purity. Pure

colonies were tested for catalase reactivity and a Gram Stain was conducted. A pure *Listeria* colony was transferred to Trypticase Soy Broth containing 0.6% yeast extract (TSB/YE). The TSB/YE cultures were incubated at 25 ± 1 °C overnight, or until the broth was turbid, indicating sufficient growth. Catalase-positive organisms were stabbed into plates of 5% Sheep Blood Agar (SBA) and incubated at 35 ± 1°C for 24-48 hours. The TSB/YE tubes incubated at 25 ±1 °C were used to prepare a wet mount slide to determine motility pattern. After incubation, the SBA plates were examined for hemolysis. Final confirmation was conducted using the VITEK® GP Biochemical Identification card following AOAC OMA 2013.02.

AOAC 993.12 Listeria Reference Method

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

ISO 11290-1/A1 Reference Method

For the ISO 11290-1/A1 reference method, 25 g test portions were enriched with 225 half Fraser broth. All test portions were mechanically stomached for two minutes. The test portions were incubated at 30 ± 1°C for 24 ± 3 hours. After incubation, 0.1 mL of the sample enrichment was transferred to 10 mLFB containing 0.1 mL of 5% ferric ammonium citrate and incubated at 37 ± 1°C for 48 ± 3 hours. After 48 ± 3 hours, a loopful of the sample secondary FB enrichment was streaked to PALCAM and OAA(Ottovani-Agosti Agar)and incubated at 37 ± 1°C for 24 ± 3 hours. A loopful of the sample primary enrichment was also streaked to PALCAM and OAAand incubated at 37 ± 1°C for 24 ± 3 hours. PALCAM and OAA agar plates were examined for the presence of suspect colonies. If no suspect colonies were present, the agar plate was re-incubated for an additional 18-24 hours at 37 ± 1°C. If no suspect colonies present the sample was determined to be negative for *Listeria.* If suspect colonies were present on the PALCAM or OAA agar plates, these suspect colonies were streaked to HBO and incubated at 37 ± 1°C for 18-

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24 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® GP Biochemical Identification following AOAC OMA 2013.02.

3M™ Molecular Detection Assay 2 –Listeria monocytogenes

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 ± 0.2 minutes and incubated at 37 ±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 ± 0.2 minutes and incubated at 37 ±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 ±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 ± 0.2 minutes and incubated at 37 ±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 ± 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 ± 0.2 minutes and incubated at 37 ±1 °C for 24-26 hours.

Prior to analysis, lysis tubes were brought to room temperature (20-25 ^{o}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 ± 2minutes at 100 ± 1 ^{o}C. Following the heat lysis, samples were transferred to the sanitized laboratorybench 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 inoculum levels for each environmental surface are presented in Table 7. A summary of POD analyses [13] are presented in Tables 8-15. As per criteria outlined in Appendix J of the Official Methods of Analysis Manual [13], fractional positive results were obtained for all matrices.

Internal Study

Raw Chicken Leg Pieces (25 g) – 28 hour Primary Enrichment

For the low inoculation level of the MDA2 - *Listeria monocytogenes*assay, there were 10 presumptive positives and 10 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 12 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 positive following the reference method.Detailed results of the POD analyses are presented in Tables 8 and 12.

Bagged raw spinach

For the low inoculation level of the MDA2 - *Listeria monocytogenes*assay, there were 9 presumptive positives and 10 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure resulting in 1 false negative. There were 10 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 4 confirmed positives following the reference method.Detailed results of the POD analyses are presented in Tables 8 and 12.

Cold smoked salmon

Compared to FDA-BAM

For the low inoculation level of the MDA2 -*Listeria monocytogenes*assay, there were 10 presumptive positives and 10 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 7 observed positives for the reference method. For the high inoculation level, there were 3 presumptive positives and 3 confirmed positives following the FDA/BAM Chapter 10 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.

Compared to ISO 11290-1/A1

For the low inoculation level of the MDA2 - *Listeria monocytogenes*assay, there were 10 presumptive positives and 10 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 9 observed positives for the reference method. For the high inoculation level, there were 3 presumptive positives and 3 confirmed positives following the ISO 11290-1/A1 reference method confirmation procedure. There were 4 confirmed positives following the reference method.Detailed results of the POD analyses are presented in Tables 8 and 12.

Independent Study

Deli Turkey (125 g)

For the low inoculation level of the MDA2 - *Listeria monocytogenes*assay, there were 8 presumptive positives and 8 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 7 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.

Raw Chicken Leg Pieces (25 g)

Lot 1

For the lot 1 of the raw chicken leg pieces of the MDA2 - *Listeria monocytogenes*assay, there were 17 presumptive positives and 17 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 16 observed positives for the reference method. Detailed results of the POD analyses are presented in Tables 9 and 13.

Lot 2

For the lot 2 of the raw chicken leg pieces of the MDA2 -*Listeriamonocytogenes*assay, there were 10 presumptive positives and 10 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 14 observed positives for the reference method. Detailed results of the POD analyses are presented in Tables 9 and 13.

Whole Melon

For the low inoculation level of the MDA2 -*Listeria monocytogenes*assay, there were 10 presumptive positives and 10 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 8 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the FDA/BAM Chapter 10 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.

Vanilla Ice Cream (25 g)

For the low inoculation level of the MDA2 -*Listeria monocytogenes*assay, there were 7 presumptive positives and 7 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 7 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12 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.

Romaine lettuce (25 g)

For the low inoculation level of the MDA2 -*Listeria monocytogenes*assay, there were 7 presumptive positives and 7 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 6 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the FDA/BAM Chapter 10 reference method confirmation procedure. There were 5 confirmed positives following the reference method.Detailed results of the POD analyses are presented in Tables 10 and 14.

Queso Fresco (25 g)

For the low inoculation level of the MDA2 – *Listeriamonocytogenes*assay, there were 11 presumptive positives and 11 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 9 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives following the reference method.Detailed results of the POD analyses are presented in Tables 10 and 14.

4%Milk Fat Cottage Cheese (25 g)

For the low inoculation level of the MDA2 - *Listeria monocytogenes*assay, there were 10 presumptive positives and 10 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 8 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives following the reference method.Detailed results of the POD analyses are presented in Tables 10 and 14.

3% Chocolate Whole Milk (25 mL)

For the low inoculation level of the MDA2 -*Listeria monocytogenes*assay, there were 9 presumptive positives and 9 confirmed positives following the AOAC 993.12 reference method confirmation procedure. There were 6 observed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the

AOAC 993.12 reference method confirmation procedure. There were 5 confirmed positives following the reference method.Detailed results of the POD analyses are presented in Tables 10 and 14.

Beef Hot Dog (25 g)

For the low inoculation level of the MDA2 - *Listeria monocytogenes*assay, there were 4 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure, resulting in 1 false negative. There were 6 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 10 and 14.

Stainless Steel (enriched in 225 mL)

Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level of the MDA2 - *Listeria monocytogenes*assay, there were 5 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 8 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 11 and 15.

Sealed Concrete (enriched in 100 mL)

24-hour primary enrichment - For the low inoculation level of the MDA2 – *Listeriamonocytogenes*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 11 and 15.

26-hour primary enrichment - For the low inoculation level of the MDA2 – *Listeriamonocytogenes*assay, there were 16 presumptive positives and 15 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure, resulting in 1 false positive. 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 11 and 15.

Plastic (enriched in 10 mL)

Results were identical after 24 and 26 hours of sample enrichment. For the low inoculation level of the MDA2 - *Listeria monocytogenes* assay, there were 12 presumptive positives and 12 confirmed positives following the USDA/FSIS MLG 8.09 reference method confirmation procedure. There were 10 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 11 and 15.

All five un-inoculated control test portions were negative by both the MDA2 - *Listeria monocytogenes*assay and the corresponding reference method for each matrix above.

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

Methodology
Three lots of MDA2 –*Listeria monocytogenes* test kits, 1 newly manufactured, 1 at the middle of its expiration, and 1 at or slightly beyond expiration was analyzed to determine the stability of the assay. For the MDA2 – *Listeria monocytogenes* 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 monocytogenes*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 MDA2 - *Listeria monocytogenes*assay, there were 6 presumptive positives out of 10 replicates for all three lots at the low inoculation level. For the 5 un-inoculated control test portions, there were 0 presumptive positives out of 5 replicates for all three lots.The five control test portions were negative. See Tables 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 17.For each parameter, one *Listeria monocytogenes* ATCC 7644isolate was analyzed at a fractional positive level (2-8 positives), testing 10 replicates. One non-*Listeriamonocytogenes* isolate, *Enterococcus faecalis* ATCC 29212, was analyzed at the growth level achieved in a non-selective broth, testing 5 replicates.

Results

For the robustness analysis of the MDA2 - *Listeria monocytogenes* assay, there were 6 presumptive positives out of 10 replicates for each variation evaluated. For the 5 control test

portions, there were 0 presumptive positives out of 5 replicates. See Table 17 for robustness results.

Independent Laboratory Observations

The results of this study demonstrate the ability of the MDA2 -*Listeria monocytogenes* to detect the presence of *Listeria monocytogenes* in various food matrices and select environmental surfaces after 24-28 hours of a primary enrichment. The MDA2 - *Listeria monocytogenes* offers the benefits of extremely high sensitivity and high specificity for the detection of *Listeria monocytogenes* while reducing the overall time to presumptive results. The MDA2 - *Listeria monocytogenes* 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 foot print 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 trouble shooting more streamlined if any problems occur while testing is being conducted.

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Discussion

There were no differences between the 24- and 26-hour enrichment time points for the environmental surfaces tested, therefore a 24-hour minimum enrichment will be recommended for these matrices.

Conclusion

The 3M MDA2 -*Listeria monocytogenes*AOAC PTM validation study included an inclusivity/exclusivity, lot-to-lot/stability, robustness and matrix studies. The 3M MDA2 – *Listeriamonocytogenes*was compared to the USDA/FSIS-MLG 8.09, the FDA-BAM Chapter 10, the AOAC 993.12 or the ISO 11290-1/A1 reference methods for the detection of *Listeriamonocytogenes*. There were nosignificant differences between the 3M MDA2 -*Listeriamonocytogenes*method and the corresponding reference method for any of the matrices evaluated. The 3M MDA2 -*Listeria monocytogenes*demonstrated reliability as a rapid and sensitive method for the detection of *Listeria monocytogenes*for the following matrices: beef hot dogs (25 g), deli turkey (125 g), queso fresco (25 g), vanilla ice cream (25 g), 4% milk fat cottage cheese (25 g), 3% chocolate whole milk (25 mL), whole melon, romaine lettuce (25 g), bagged raw spinach (25 g), cold smoked salmon (25 g), raw chicken leg pieces (25 g), sealed concrete (sponge enriched in 100mL), plastic (Enviro~~s~~Swab enriched in 10 mL), stainless steel (sponge enriched in 225 mL).

References

1. Food and Drug Administration Bacteriological Analytical Manual Chapter 10: *Detection and Enumeration of Listeria monocytogenes in Foods*. February, 2013. (Accessed January 2015)

2. United States Department of Agriculture Microbiological Laboratory Guidelines 8.09: *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and Environmental Samples*. May 1st, 2013. (Accessed January 2015)

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

3. AOAC Official Method 993.12 *Listeria monocytogenes* in Milk and Dairy Products: Selective Enrichment and Isolation Method, First Action 1993, Final Action 1996. (Accessed January 2015)

4. ISO 11290-1/A1 (2004) - Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: detection of *Listeria monocytogenes* in foods

5. U.S. Food and Drug Administration, and U.S. Food Safety and Inspection Service. 2003. Quantitative Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods. Available in Docket No. 1999N-1168, Vols. 23 through 28, and at Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods

6. Doyle ME, Mazzotta AS, Wang T, Wiseman DW, and Scott VN. 2001. Heat resistance of *Listeria monocytogenes*. *Journal of Food Protection* 64 (3): 410-429.

7. ISO 18593. Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.

8. AOAC Research Institute *Performance Tested Methods℠ Program* validation outline protocol: *Comparative Evaluation of the 3M™ Molecular Detection Assay Listeria monocytogenes 2 and of the 3M™ Molecular Detection Assay Listeria* 2 (February 2015)

9. U. S. Food and Drug Administration *Bacteriological Analytical Manual*, Chapter 3 *Aerobic Plate Count (*January 2001) (Accessed January 2015) http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.m

10. Least Cost Formulations, Ltd., MPN Calculator Version 1.6 www.lcfltd.com/customer/LCFMPNCalculator.exe(Accessed January 2015)

11. AOAC International, *Evaluation of the* VITEK 2 *Gram Positive (GP) Microbial Identification Test Card: Collaborative Study*, Journal of AOAC International, 95, 1425 (2012)

12. Wehling, P., LaBudde, R., Brunelle, S., Nelson, M. *Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods.* Journal of AOAC International, Vol. 94, No. 1, 2011.

13. Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook Version 2.2 (2011) -http://lcfltd.com/aoac/aoac-binary-v2-2.xls(Accessed January 2015)

14. Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, AOAC INTERNATIONAL, Gaithersburg, MD,http://http://www.eoma.aoac.org/app_j.pdf (Accessed January 2015)

~~**Table 1. Enrichment Protocols Using Demi-Fraser Broth Enrichmentat 37 ± 1 °C**~~

Specific Instructions for Validated Methods

AOAC® Performance Tested Method℠ # 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 SM 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[a]		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[b]	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

~~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~~

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 - *Listeria monocytogenes*	Confirmation 100µl	
							ALOA	Palcam
1	*Listeria*	*monocytogenes*	153	Soft cheese (Munster)	39	+	H+	+
2	*Listeria*	*monocytogenes*	1011/1410	Frozen broccoli	28	+	H+	+
3	*Listeria*	*monocytogenes*	1972/2399	Puff pastry with mushrooms	38	+	H+	+
4	*Listeria*	*monocytogenes*	1973/2400	Puff pastry egg and ham (Quiche-lorraine)	36	+	H+	+
5	*Listeria*	*monocytogenes*	2407/3139	Tripes with tomatoes	31	+	H+	+
6	*Listeria*	*monocytogenes*	2760/3145	Raw bacon	44	+	H+	+
7	*Listeria*	*monocytogenes*	32.183	Croque-Monsieur	31	+	H+	+
8	*Listeria*	*monocytogenes*	38/181	Toulouse sausages	33	+	H+	+
9	*Listeria*	*monocytogenes*	5721/6179	Smoked bacon	49	+	H+	+
10	*Listeria*	*monocytogenes*	7111/7516	Pâté (Rillettes)	64	+	H+	+
11	*Listeria*	*monocytogenes*	850/109	Ready-To-Eat (RTE) food (deli salad with seafood)	34	+	H+	+
12	*Listeria*	*monocytogenes*	877/113	Environmental sample (pastry)	28	+	H+	+
13	*Listeria*	*monocytogenes*	913/1048	Black pudding	39	+	H+	+
14	*Listeria*	*monocytogenes*	A00C014	Sausage	37	+	H+	+
15	*Listeria*	*monocytogenes*	A00C022	Merguez	33	+	H+	+
16	*Listeria*	*monocytogenes*	A00C024	Sausage	21	+	H+	+
17	*Listeria*	*monocytogenes*	A00C036	Poultry (guinea)	41	+	H+	+
18	*Listeria*	*monocytogenes*	A00C039	Sausages	20	+	H+	+
19	*Listeria*	*monocytogenes*	A00C040	Cooked delicatessen (Museau)	36	+	H+	+
20	*Listeria*	*monocytogenes*	A00C041	Sausage	38	+	H+	+
21	*Listeria*	*monocytogenes*	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 - *Listeria monocytogenes*	**Confirmation 100µl**	
							ALOA	**Palcam**
22	*Listeria*	*monocytogenes*	A00C043	Smoked Bacon	50	+	H+	+
23	*Listeria*	*monocytogenes*	A00C044	Poultry (duck)	34	+	H+	+
24	*Listeria*	*monocytogenes*	A00C052	RTE food (Osso bucco with turkey)	34	+	H+	+
25	*Listeria*	*monocytogenes*	A00C053	Gizzards	30	+	H+	+
26	*Listeria*	*monocytogenes*	A00C054	Beef heart	30	+	H+	+
27	*Listeria*	*monocytogenes*	A00C055	Raw sausages	43	+	H+	+
28	*Listeria*	*monocytogenes*	A00E008	Environmental sample	27	+	H+	+
29	*Listeria*	*monocytogenes*	A00E049	Environmental sample (smoked salmon)	41	+	H+	+
30	*Listeria*	*monocytogenes*	A00E082	Environmental sample (smoked salmon)	33	+	H+	+
31	*Listeria*	*monocytogenes*	A00L097	Milk	60	+	H+	+
32	*Listeria*	*monocytogenes*	A00M009	Smoked salmon	40	+	H+	+
33	*Listeria*	*monocytogenes*	A00M032	Smoked salmon	11	+	H+	+
34	*Listeria*	*monocytogenes*	A00M045	Smoked salmon	62	+	H+	+
35	*Listeria*	*monocytogenes*	A00M088	Smoked salmon	79	+	H+	+
36	*Listeria*	*monocytogenes*	Ad235	Poultry	45	+	H+	+
37	*Listeria*	*monocytogenes*	Ad253	Hard cheese	58	+	H+	+
38	*Listeria*	*monocytogenes*	Ad260	Semi hard cheese	27	+	H+	+
39	*Listeria*	*monocytogenes*	Ad265	Tongue	62	+	H+	+
40	*Listeria*	*monocytogenes*	Ad266	Poultry	26	+	H+	+
41	*Listeria*	*monocytogenes*	Ad267	Dry sausage	26	+	H+	+
42	*Listeria*	*monocytogenes*	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 - *Listeria monocytogenes*	Confirmation 100µl	
							ALOA	Palcam
43	*Listeria*	*monocytogenes*	Ad270	Fermented sausage	42	+	H+	+
44	*Listeria*	*monocytogenes*	Ad272	Fermented sausage	56	+	H+	+
45	*Listeria*	*monocytogenes*	Ad273	Cured delicatessen	27	+	H+	+
46	*Listeria*	*monocytogenes*	Ad274	Ready-to-eat food (Asiatic meal)	40	+	H+	+
47	*Listeria*	*monocytogenes*	Ad534	Fruits	54	+	H+	+
48	*Listeria*	*monocytogenes*	Ad544	Onion	43	+	H+	+
49	*Listeria*	*monocytogenes*	Ad546	Flour	83	+	H+	+
50	*Listeria*	*monocytogenes*	Ad623	Bread crumbs	50	+	H+	+

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 Production environment
H+ = Phosphy-Lipolysis halo

Table 3a. Exclusivity Study Results

EXCLUSIVITY						
Strain		Species	Reference	Origin	Inoculation level (cfu/ml)	3M MDA2 – *Listeria monocytogenes*
1	*Listeria*	*grayi*	Ad 1198	Smoked salmon	3.2×10^5	-

EXCLUSIVITY						
Strain		**Species**	**Reference**	**Origin**	**Inoculation level (cfu/ml)**	**3M MDA2 – *Listeria monocytogenes***
2	*Listeria*	*grayi*	Ad 1443	Pork meat sausages	4.9×10^5	-
3	*Listeria*	*innocua*	1	Smoked salmon	4.9×10^5	-
4	*Listeria*	*innocua*	Ad 658	Gorgonzola	5.9×10^5	-
5	*Listeria*	*ivanovii*	Ad 466	Raw veal meat	3.3×10^5	-
6	*Listeria*	*ivanovii*	Ad 662	Environment (dairy industry)	3.6×10^5	-
7	*Listeria*	*seeligeri*	Ad 649	Cheese	5.4×10^5	-
8	*Listeria*	*seeligeri*	BR1	Trout	5.1×10^5	-
9	*Listeria*	*welshimeri*	Ad1276	Environment (Slaughterhouse)	5.2×10^5	-
10	*Listeria*	*welshimeri*	Ad1175	Ready-to-eat-food	6.4×10^5	-
11	*Bacillus*	*cereus*	Ad 465	Salmon Terrine	7.2×10^4	-
12	*Bacillus*	*circulans*	Ad 760	Vegetables	2.0×10^4	-
13	*Bacillus*	*coagulans*	Ad 731	Dairy product	$<2.0 \times 10^3$ (opacity +)	-
14	*Bacillus*	*licheniformis*	Ad 978	Dairy product	1.6×10^4	-
15	*Bacillus*	*pumilus*	Ad 284	Ready-to-eat	1.7×10^5	-
16	*Brochrotrix*	*campestris*	CIP 102920T	Environment	$<2.0 \times 10^3$ (opacity +)	-
17	*Carnobacterium*	*piscicola*	Ad 369	Raw milk	$<2.0 \times 10^3$ (opacity +)	-
18	*Enterococcus*	*durans*	Ad 149	Ham	$<2.0 \times 10^3$ (opacity +)	-
19	*Enterococcus*	*faecalis*	89L326	Soft cheese (Vacherin)	1.6×10^5	-
20	*Lactobacillus*	*brevis*	86L126	Ham	1.4×10^5	-
21	*Lactobacillus*	*curvatus*	Ad 380	Delicatessen	$<2.0 \times 10^3$ (opacity +)	-
22	*Lactobacillus*	*sakei*	Ad 473	Ham	$<2.0 \times 10^3$	-

EXCLUSIVITY						
Strain		Species	Reference	Origin	Inoculation level (cfu/ml)	3M MDA2 – *Listeria monocytogenes*
					(opacity +)	
23	*Leuconostoc*	*carnosum*	Ad 411	Ham	$<2.0 \times 10^3$ (opacity +)	-
24	*Leuconostoc*	*citreum*	Ad 396	Ham	4.9×10^5	-
25	*Micrococcus*	*luteus*	Ad 432	Cocktail	$<2.0 \times 10^3$ (opacity +)	-
26	*Staphylococcus*	*aureus*	Ad 165	Smoked delicatessen	$<2.0 \times 10^3$ (opacity +)	-
27	*Staphylococcus*	*epidermidis*	Ad 931	Fruits	2.0×10^3	-
28	*Staphylococcus*	*haemolyticus*	Ad 989	Dairy product	2.8×10^4	-
29	*Streptococcus*	*bovis*	92L622	Cheese	2.0×10^3	-
30	*Streptococcus*	*salivarius*	Ad 441	Dairy product	$<2.0 \times 10^3$ (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 – *Listeria monocytogenes*
31	*Listeria*	*aquatic*	FSL S10-1188	Running water, USA, Florida	1.37×10^7	-
32	*Listeria*	*booriae*	FSL A5-0281	Non-food-contact surface in a dairy processing plant, USA,	2.75×10^7	-

EXCLUSIVITY						
Strain		Species	Reference	Origin	Level tested	3M MDA2 – *Listeria monocytogenes*
				New York		
33	*Listeria*	*cornellensis*	FSL F6-969	Water, USA, Colorado	1.80×10^7	-
34	*Listeria*	*fleischmannii* subsp. coloradensis	FSL S10-1203	grazing pasture – soil	1.22×10^8	-
35	*Listeria*	*fleischmannii* subsp. fleischmannii	DSM 24998	hard cheese, Switzerland, Suisse Romande	1.28×10^8	-
36	*Listeria*	*floridensis*	FSL S10-1187	running water, USA, Florida	5.55×10^7	-
37	*Listeria*	*grandensis*	FSL F6-971	water, USA, Colorado	3.05×10^7	-
38	*Listeria*	*ivanovii*	ATCC 19119	sheep, Bulgaria	4.05×10^7	-
39	*Listeria*	*ivanovii* subsp. Iondoniensis	ATCC 49954	food, France	7.40×10^7	-
40	*Listeria*	*newyorkensis*	FSL M6-0635	non-food-contact surface in a seafood processing plant, USA, New York	7.00×10^7	-
41	*Listeria*	*riparia*	FSL S10-1204	running water, USA, Florida	3.05×10^7	-
42	*Listeria*	*rocourtiae*	CIP 109804	pre-cut lettuce, Austria, Siezenheim	7.30×10^7	-
43	*Listeria*	*weihenstephanensis*	LMG 26374	water plant *Lemna trisulca* from pond, Germany, Bavaria	3.30×10^5	-

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
FSL = 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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes* CWD[2] 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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes* ATCC[1] 19112 & *L. welshimeri* ATCC[1] 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	*L. monocytogenes* FSL[3] 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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes* ATCC[1] 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	*L. monocytogenes ATCC1 19117*	0 CFU/4" x 4" (100 cm2)	5	24 and 26 hours	USDA/FSIS MLG 8.09 (UVM)
		~50 CFU/4" x 4" (100 cm2)	20		
		~100 CFU/4" x 4" (100 cm2)	5		
Plastic	*L. monocytogenes ATCC1 51782& L. seeligeri ATCC1 35967& 10x Enterococcus faecalis ATCC1 29212*	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		
		~100 CFU/1" x 1" (5 cm2)	5		

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	*L. monocytogenes ATCC 19118 & 10x Enterococcus faecium 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		

[1] ATCC - American Type Culture Collection
[2] CWD - University of Vermont
[3] 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	*L. monocytogenes* Ad[1] 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	*L. monocytogenes* 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	*L. monocytogenes* 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	*L. monocytogenes* 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		

[1] Ad – Adria Developpement Culture Collection

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

Matrix (Test Portion)	APC[1] (CFU/g)	*Listeria* Pathogen Screen
Beef Hot Dogs[5] (25 g)	6.0×10^3	0/5 Detected[2]
Deli Turkey (125 g)	1.5×10^3	0/5 Detected[2]
Raw Chicken Leg Pieces (25 g)	2.8×10^5	4/5 Detected[2]
Whole Melons	1.0×10^2	0/5 Detected[3]
Vanilla Ice Cream (25 g)	8.0×10^1	0/5 Detected[4]
Queso Fresco (25 g)	3.8×10^5	0/5 Detected[4]
4% Milk Fat Cottage Cheese (25 g)	1.7×10^4	0/5 Detected[4]
Beef Hot Dogs (25 g)	1.6×10^2	0/5 Detected[2]

[1] APC conducted in accordance with FDA/BAM Chapter 3
[2] *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines
[3] *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines
[4] *Listeria species* screen conducted following the AOAC 993.12 guidelines
[5] 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[1] (CFU/g)	Background *Listeria*
Raw Chicken Leg Pieces (25 g)	5.4×10^5	None Detected
Bagged Raw Spinach (25 g)	8.0×10^6	None Detected
Cold Smoked Salmon (25 g)	4.0×10^2	None Detected

[1] APC conducted in accordance with FDA/BAM Chapter 3
[2] *Listeria species* screen conducted following the USDA/FSIS MLG 8.09 guidelines
[3] *Listeria species* screen conducted following the FDA/BAM Chapter 10 guidelines
[4] *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[1]	25 g	*Listeria monocytogenes* ATCC 7644	TSA	1.8×10^8	58.3 %
			MOX	7.5×10^7	
Deli Turkey	125 g	*Listeria monocytogenes* ATCC 19116	TSA	2.6×10^9	68.5 %
			MOX	8.2×10^8	
Queso Fresco	25 g	*Listeria monocytogenes* ATCC CWD 1554	TSA	2.6×10^9	72.7%
			MOX	7.1×10^8	
Beef Hot Dogs[2]	25 g	*Listeria monocytogenes* ATCC 7644	TSA	2.6×10^9	73.8%
			MOX	6.8×10^8	

[1]- Testing for Robustness and Lot to Lot
[2]- 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	*Listeria monocytogenes* FSL J1-049	
Low-Inoculum Level CFU[a]/Melon[b]	7	
High-Inoculum Level CFU[a]/Test Melon[b]	55	
Matrix	**Stainless Steel**	
Inoculating Organism	*Listeria monocytogenes* ATCC 19118	*Enterococcus faecium* ATCC 19434
Low-Inoculum Level CFU[a]/Test Area[c]	42	530
High-Inoculum Level CFU[a]/Test Area[c]	400	4000
Matrix	**Sealed Concrete**	
Inoculating Organism	*Listeria monocytogenes* ATCC 19117	
Low-Inoculum Level CFU[a]/Test Area[c]	35	
High-Inoculum Level CFU[a]/Test Area[c]	360	
Matrix	**Plastic**	
Inoculating Organism	*Listeria monocytogenes* ATCC 51782	*Enterococcus faecalis* ATCC 29212
Low-Inoculum Level CFU[a]/Test Area[d]	42	540
High-Inoculum Level CFU[a]/Test Area[d]	420	3600

[a] CFU: aliquots of the inocula were plated in triplicate onto TSA and averaged
[b] Test Area: Whole Melon
[c] Test Area: 4" x 4" Surface Area
[d] 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[a]/ Test Portion	N[b]	3M MDA2			USDA/FSIS MLG			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Raw Chicken Leg Pieces (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**ISO 11290-1**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Bagged Raw Spinach (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**FDA-BAM**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Cold Smoked Salmon (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**ISO 11290-1**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Cold Smoked Salmon (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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[a]/ Test Portion	N[b]	3M MDA2			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Deli Turkey (125 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Whole Melon	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Vanilla Ice Cream (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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[a]/ Test Portion	N[b]	3M MDA2			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Romaine Lettuce (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Queso Fresco (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
4% Milk Fat Cottage Cheese (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
3% Chocolate Whole Milk (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Beef Hot Dogs (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_C = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD_R = Reference method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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[a]/ Test Area	N[b]	3M MDA2			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Stainless Steel (225 mL)	*L. monocytogenes* ATCC 19118 & *Enterococcus faecium* 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[a]/ Test Area**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Sealed Concrete (100 mL)	*L. monocytogenes* 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[a]/ Test Area**	**N[b]**	**3M MDA2**			**Reference**			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Plastic (10 mL)	*L. monocytogenes* ATCC 51782 & *Enterococcus faecalis* 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

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]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[a]/ Test Portion	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Raw Chicken Leg Pieces (25 g)	*L. monocytogenes* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
Bagged Raw Spinach (25 g)	*L. monocytogenes* 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
Matrix	**Strain**	**Analysis Time Point**	**MPN[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
Cold Smoked Salmon (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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[a]/ Test Portion	N[b]	Presumptive			Confirmed			$dPOD_{CP}$[f]	95% CI[g]
					x[c]	POD_{CP}[d]	95% CI	X	POD_{CC}[e]	95% CI		
Deli Turkey (125 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Whole Melon	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Vanilla Ice Cream (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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[a]/ Test Portion	N[b]	Presumptive			Confirmed			$dPOD_C$[f]	95% CI[g]
					x[c]	POD_C[d]	95% CI	X	POD_R[e]	95% CI		
Romaine Lettuce (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{C_P^f}$**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
Queso Fresco (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{C_P^f}$**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
4% Milk Fat Cottage Cheese (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_{C_P^f}$**	**95% CI[g]**
					x[c]	**POD_{CP}[d]**	**95% CI**	**X**	**POD_{CC}[e]**	**95% CI**		
3% Chocolate Whole Milk (25 g)	*L. monocytogenes* 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[a]/ Test Portion**	**N[b]**	**Presumptive**			**Confirmed**			**$dPOD_C$[f]**	**95% CI[g]**
					x[c]	**POD_C[d]**	**95% CI**	**X**	**POD_R[e]**	**95% CI**		
Beef Hot Dogs (25 g)	*L. monocytogenes* 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

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials
[f]$dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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[a]/ Test Area	N[b]	Presumptive			Confirmed			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	X	POD$_{CC}$[e]	95% CI		
Stainless Steel (225 mL)	*L. monocytogenes* ATCC 19118 & *Enterococcus faecium* 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[a]/ Test Area**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
Sealed Concrete (100 mL)	*L. monocytogenes* 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[a]/ Test Area**	**N[b]**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$[f]**	**95% CI[g]**
					x[c]	**POD$_{CP}$[d]**	**95% CI**	**X**	**POD$_{CC}$[e]**	**95% CI**		
Plastic (10 mL)	*L. monocytogenes* ATCC 51782 & *Enterococcus faecalis* 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

[a]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate
[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials
[e]POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values
[g]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**
Listeria monocytogenes ATCC[2]7644					
Low Level	6/10	Low Level	6/10	Low Level	6/10
Uninoculated Target Organism (*Enterococcus faecalis* ATCC29212)	0/5	Uninoculated Target Organism (*Enterococcus faecalis* ATCC29212)	0/5	Uninoculated Target Organism (*Enterococcus faecalis* ATCC29212)	0/5

[1]- All samples were analyzed from a common lysate
[2]ATCC- American Type Culture Collection

Table 17: Robustness Results

Robustness[1]			Robustness[1]		
13 Minute Lysis			13 Minute Lysis		
Sample #	**18 µL[2], 18 µL[3]**	**18 µL[2], 22 µL[3]**	**Sample #**	**22 µL[2], 18 µL[3]**	**22 µL[2], 22 µL[3]**
Listeria monocytogenes ATCC[4] 7644					
Low Level	6/10	6/10	Low Level	6/10	6/10
Uninoculated Target Organism (*Enterococcus faecalis* ATCC29212)	0/5	0/5	Uninoculated Target Organism (*Enterococcus faecalis* ATCC29212)	0/5	0/5

Robustness[1]			Robustness[1]		
17 Minute Lysis			17 Minute Lysis		
Sample #	**18 µL[2], 18 µL[3]**	**18 µL[2], 22 µL[3]**	**Sample #**	**22 µL[2], 18 µL[3]**	**22 µL[2], 22 µL[3]**
Listeria monocytogenes ATCC[4] 7644					
Low Level	6/10	6/10	Low Level	6/10	6/10
Uninoculated Target Organism (*Enterococcus faecalis* ATCC29212)	0/5	0/5	Uninoculated Target Organism (*Enterococcus faecalis* ATCC29212)	0/5	0/5

[1]- All samples were analyzed from a common lysate
[2]-Volume of enrichment to lysis
[3]-Volume of lysis to assay
[4]ATCC- American Type Culture Collection