AOAC Official Methods of Analysis℠ (OMA)

AOAC EXPERT REVIEW PANEL

FOR MICROBIOLOGY FOR FOODS AND ENVIRONMENTAL SURFACES

THURSDAY, JANUARY 19, 2017
8:30AM – 12:00PM
AOAC INTERNATIONAL 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*℠ (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

Thursday, January 19, 2017
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-35: Evaluation of the 3M™ Molecular Detection Assay(MDA) 2 -*E. coli* (including H7) for the Detection of *E. coli* O157:H7 Species in Selected Foods: Collaborative Study**
Co-Study Directors: Lisa Monteroso, 3M Food Safety, 3M Center, Building 260-06B-01, St. Paul, MN 55144 and Leslie Thompson, Vanguard Sciences, 224 N. Derby Lane, North Sioux City, SD 57049

2) **OMAMAN-36: Evaluation of the *mericon® E. coli* O157 Screen Plus and *mericon E. coli* STEC O-Type Pathogen Detection Assays in Select Foods: Collaborative Study**
Co-Study Directors: Marcia Armstrong, QIAGEN, 19300 Germantown Rd., Germantown, MD 20874 and Patrick Bird, 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. V1

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

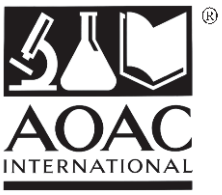
AOAC INTERNATIONAL
POLICY ON THE USE OF THE ASSOCIATION NAME, INITIALS, IDENTIFYING INSIGNIA, LETTERHEAD, AND BUSINESS CARDS

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)

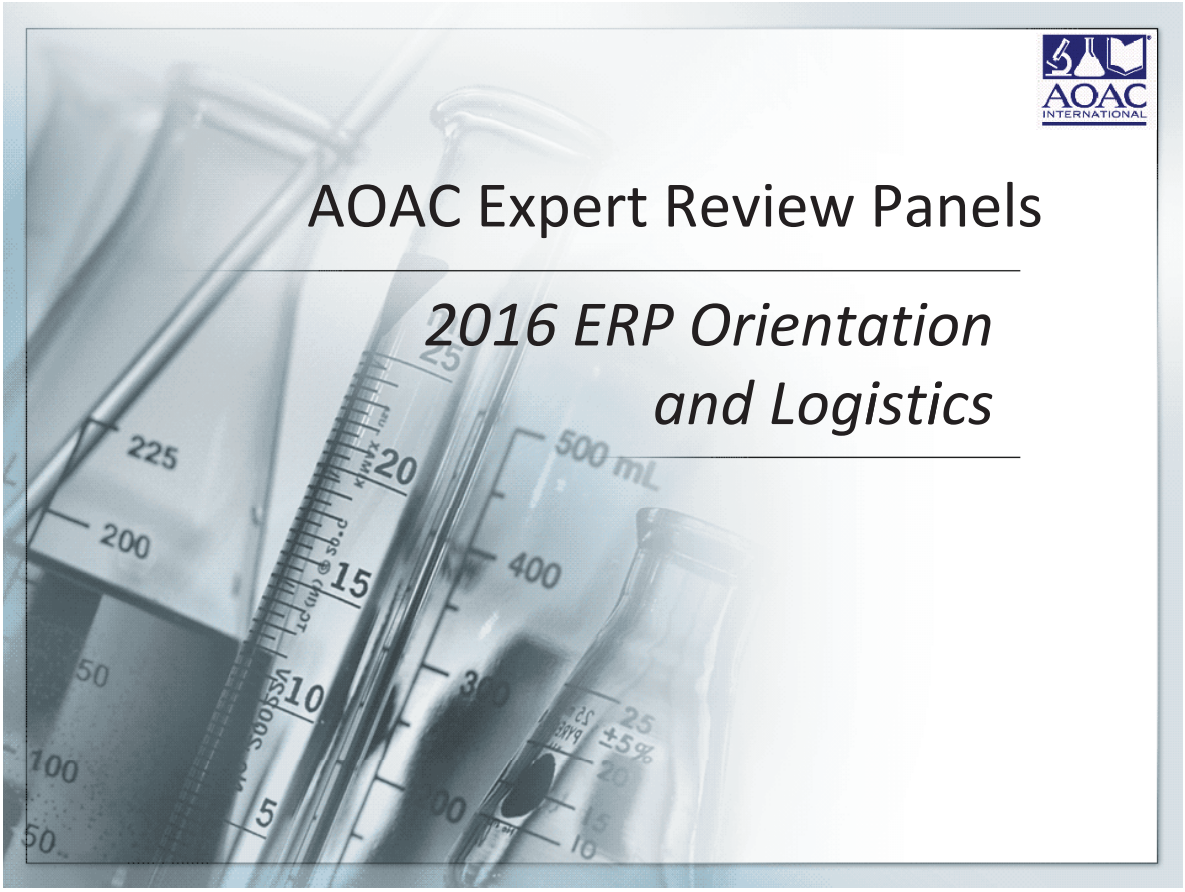
- Reviewers shall conduct in-depth review of method and any supporting information.
- In-depth reviews are completed electronically via the method review form. The method review form must be completed and submitted by the deadline date as provided.
- All reviews will be discussed during the Expert Review 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 Expert Review Panels

2016 ERP Orientation and Logistics

Session Syllabus

1. AOAC Method Submission
2. Recruitment of ERP Members
3. ERP Composition & Vetting Expertise
4. ERP Method Assignments
5. ERP Meeting
6. ERP Consensus
7. Post ERP Meeting
8. Publications
9. Method Modifications
10. First Action to Final Action
11. Documentation
12. Summary of Responsibilities

TRACT 1

METHOD SUBMISSION

Paths to AOAC *Official Methods*

- AOAC Official Methods through AOAC Standards Development
- AOAC Official Methods through AOAC Research Institute

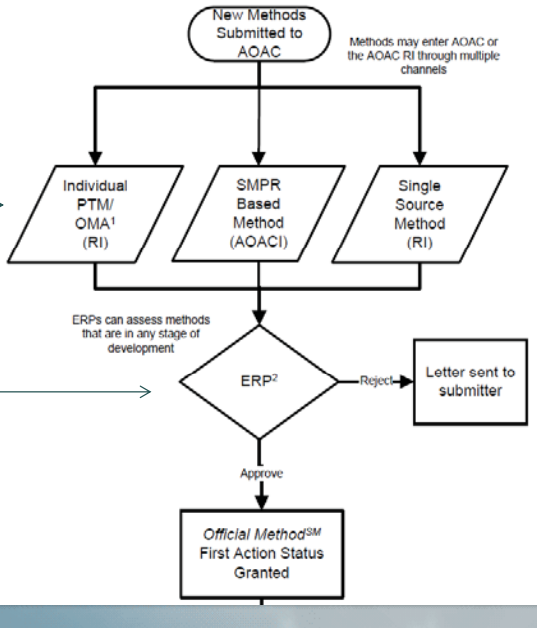
Road to First Action OMA Status

Terms:

- PTM – *Performance Tested Methods℠*
- RI – Research Institute
- ERP – Expert Review Panel
- OMB – Official Methods Board
- SP – Stakeholder Panel
- SMPR – Standard Method Performance Requirement

Three modes of entry and (program administration)

Expert Review Panels will review all methods for all three modes of entry.

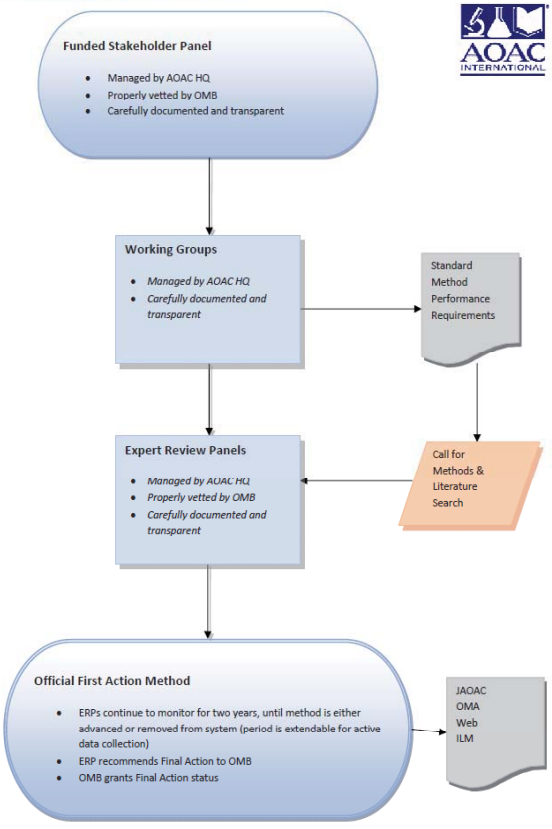


Note: Appeals process always available; see Alternative Pathway Guidelines for appeals process.

1 PTM certification previously issued, PTM reviewers will be ERP members
2 Unless otherwise provided for under a contractual agreement, AOAC will regularly convene ERPs twice a year: once during the Mid-Year Meeting and again during the Annual Meeting

Recap of the Overall Process for Methods Submitted in response to SMPRs or Call for Methods – aka "alternative pathway"

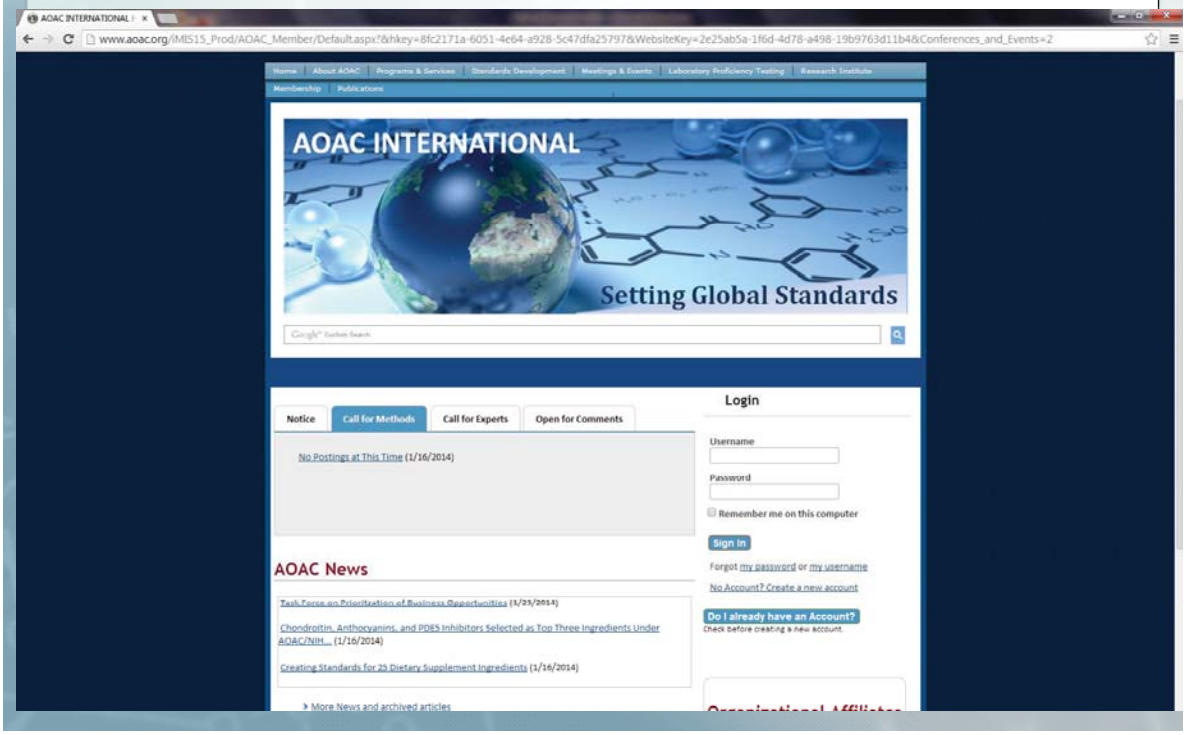
1. Allows AOAC to focus on projects addressing an urgent need of a critical mass of stakeholders.
2. Drives AOAC processes forward faster.
3. Assembles stakeholders (industry, government and academia) to neutral place to articulate and reach consensus on requirements and resolve conflicts.
4. Those requirements are codified and are published as "Standard Method Performance Requirements" (SMPRs).
5. Methods are solicited that purport to meet those requirements.
6. Expert review panels (ERPs) judge the methods against the SMPRs. Method(s) that best meet the SMPRs are adopted and designated "First Action" *Official Method of Analysis*.
7. Process for First Action status to Final Action status follows as the same process for all AOAC First Action *Official Methods*.



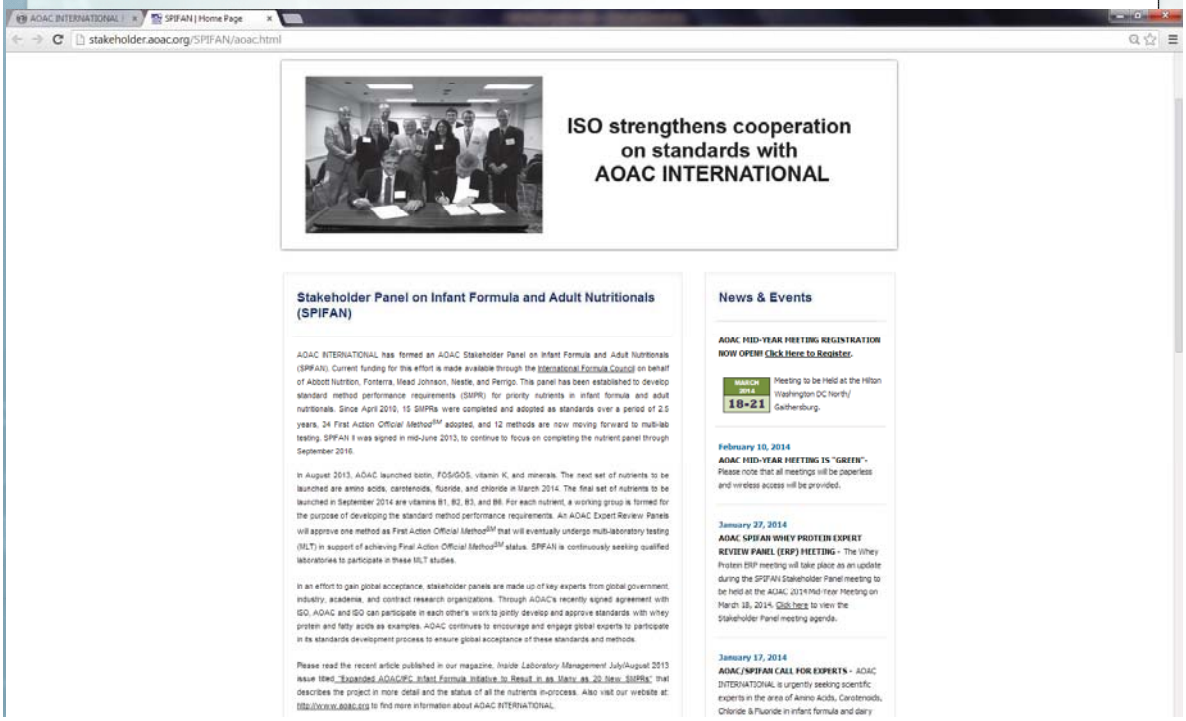
Method Submissions

- Method developers responding to an AOAC issued Call for Methods or to adopted standard method performance requirements (SMPRs) should submit their methods to AOAC INTERNATIONAL
- All other methods should be submitted to the AOAC Research Institute.
- Contact AOAC staff for details.

Calls for Methods



Call for Methods



STAKEHOLDER PANEL ON INFANT FORMULA & ADULT NUTRITIONALS (SPIFAN) NEWS

AOAC/SPIFAN CALL FOR CARNITINE METHODS EXTENDED

AOAC INTERNATIONAL invites method developers to submit Carnitine methods for consideration through the AOAC *Official Methods*℠ Program. Methods should meet or exceed the Standard Method Performance Requirement (SMPR). Click here to view Carnitine Call for Methods.

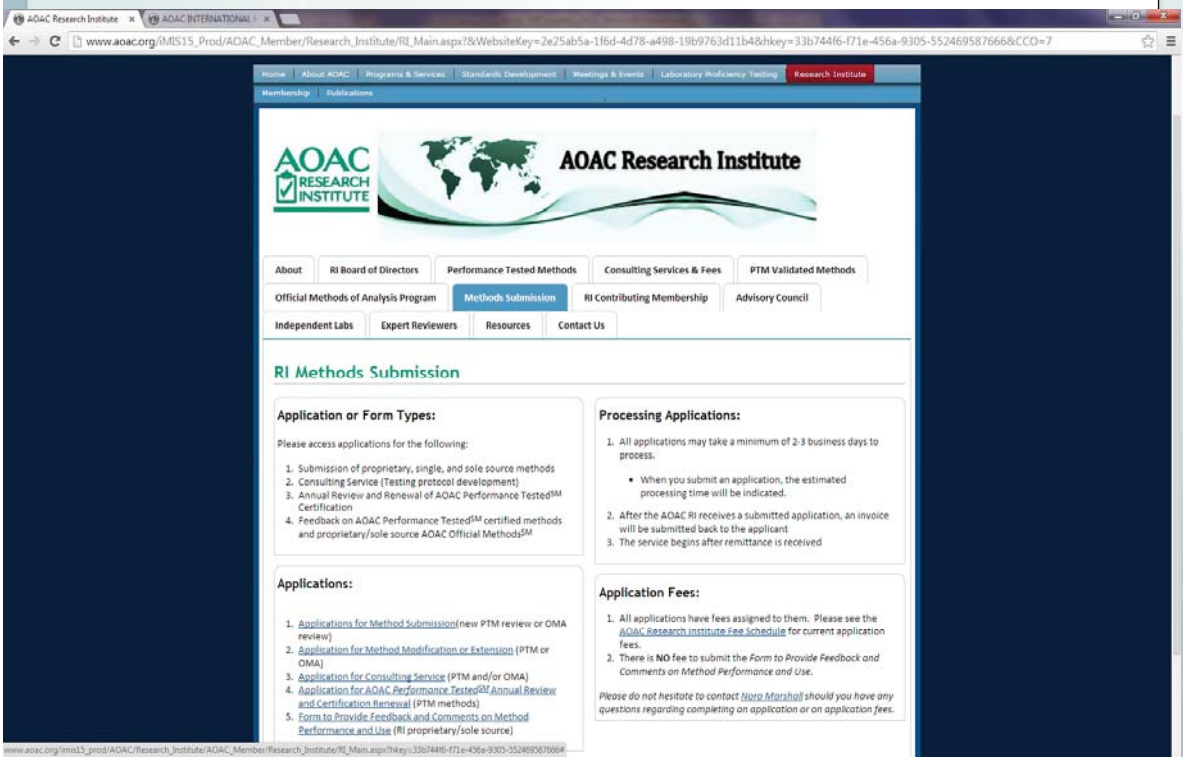
Interested method developers should provide a description and data demonstrating that the method will meet the SMPR. Click here to submit method(s). Deadline for submissions to be considered is **Friday, January 17, 2014**.

AOAC/SPIFAN CALL FOR EXPERTS

AOAC INTERNATIONAL is urgently seeking scientific experts in the area of Amino Acids, Carotenoids, Chloride & Fluoride in infant formula and dairy products to establish standard methods performance requirements (SMPRs). Click here to view Call for Experts.

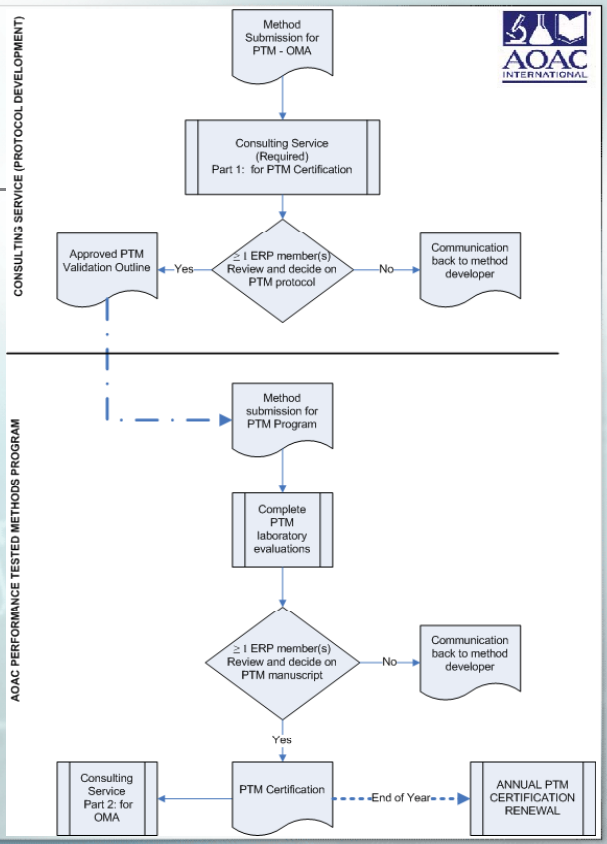
SPIFAN ACTIVITIES AT AOAC INTERNATIONAL MID-YEAR MEETING (March 18-19, 2014)

SOLE SOURCE OR PTM-OMA



PTM Overview for PTM-OMA Harmonized Process

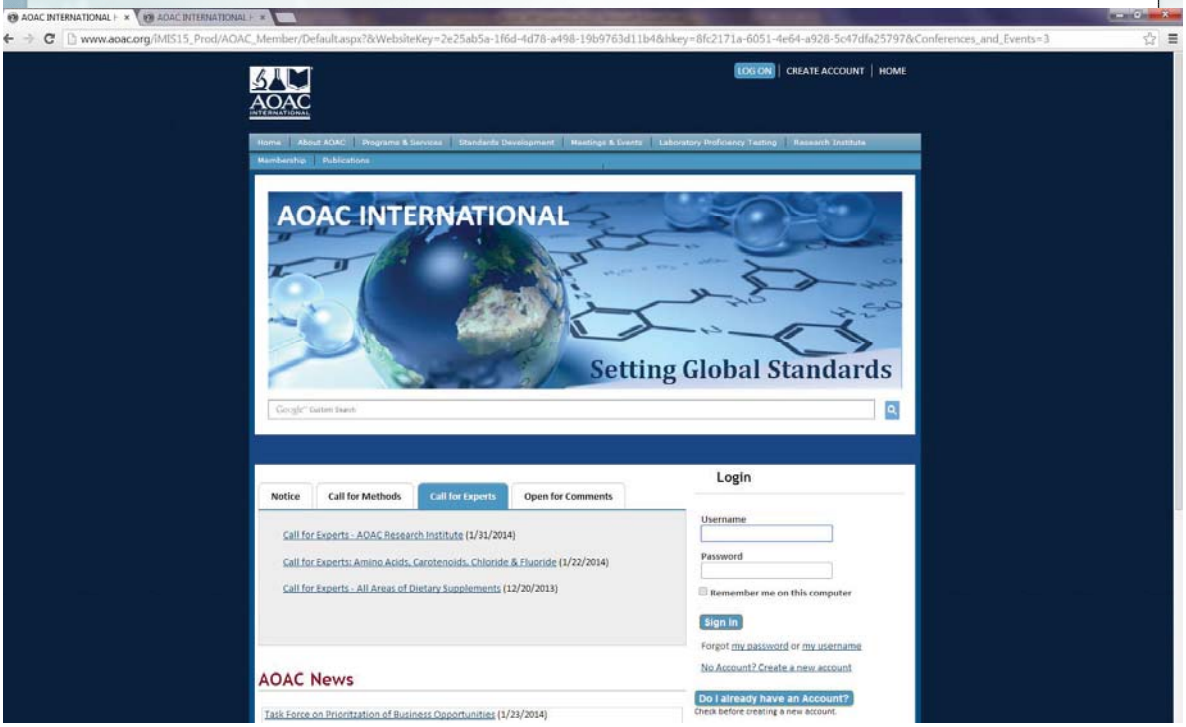
- Administered by the Research Institute in 2003.
- Well established and streamlined
- Original approved by consensus with the OAs, OMB, RI Board of Directors and AOAC INTERNATIONAL Board of Directors.
- ERP may be formed during Consulting Service.
- Criterion for OMA: manufacturer's method claims.



TRACT 2

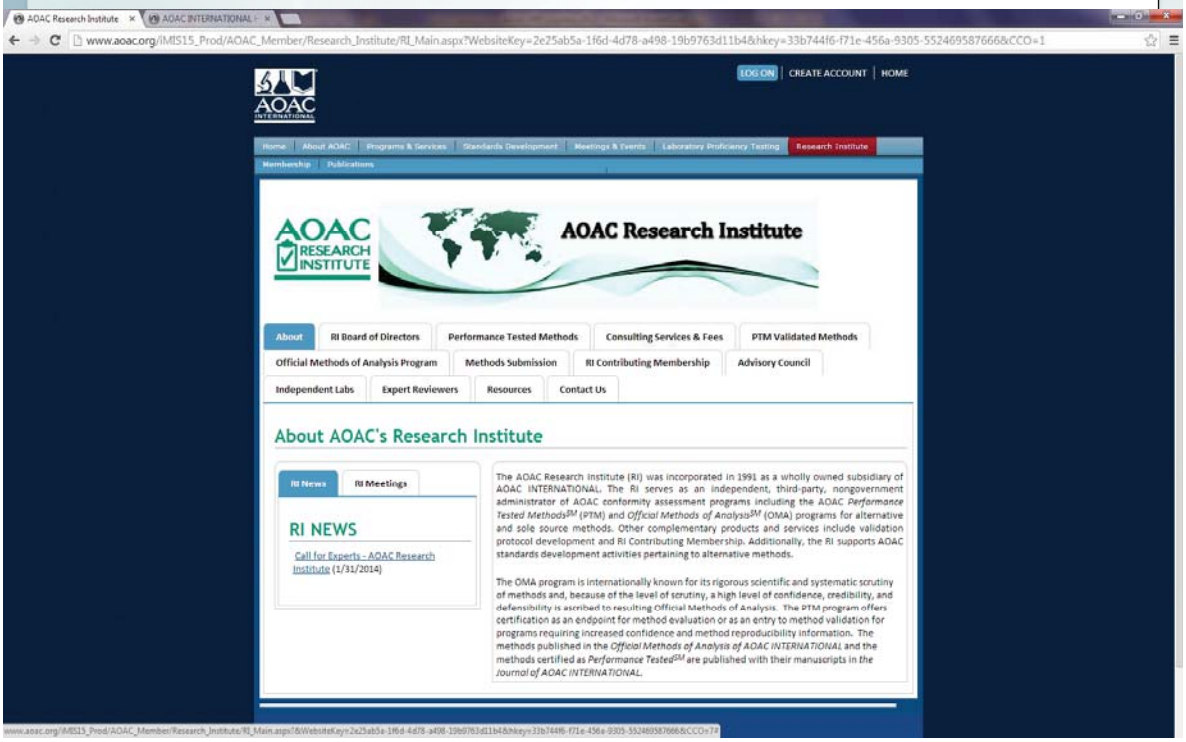
RECRUITMENT OF ERP MEMBERS

CALL FOR EXPERTS



CALL FOR EXPERTS

CALL FOR EXPERTS



Other Forms of Recruitment

- Official Methods Board
- Email Blasts to AOAC network
- Leveraging networks of Advisory Panel members, Working Group Members, AOAC Communities and Sections

REQUIREMENTS FOR ERP SERVICE

- Must have demonstrated expertise in the method, technology, analyte/matrix, etc… Be a subject matter expert.
- Must be able to attend ERP meetings
- Must be able to complete assigned reviews on time
- Must be prepared to speak on the method and share reviews during the meeting
- Must be proactive in tracking assigned First Action *Official Methods*
- Must be able to assist in peer reviewing paper for publication
- Must sign and submit AOAC Volunteer Acceptance Form

AOAC Policies & Procedures

- Policy on Antitrust
- Policy on Use of Association Name, Identifying Insignia, Letterhead, Business Cards
- Policy on Volunteer Conflict of Interest
- Expert Review Panel Policies and Procedures
- OMA Appendix G

AOAC Policies

- AOAC INTERNATIONAL Antitrust Policy
- AOAC INTERNATIONAL Policy On The Use Of The Association Name, Initials, Identifying Insignia, Letterhead, And Business Cards
- AOAC INTERNATIONAL Policy And Procedures On Volunteer Conflict Of Interest
- Volunteer Acceptance Form

Antitrust Responsibilities

- AOAC activities frequently involve cooperative undertakings and meetings where competitors may be present, it is important to emphasize the ongoing commitment of our members and the Association to full compliance with national and other antitrust laws
- Association's structure is fashioned and its programs are carried out in conformance with antitrust standards.
- An equal responsibility for antitrust compliance - which includes avoidance of even an appearance of improper activity - belongs to the individual.
  - The appearance of improper activity must be avoided because actual proof of misconduct is not required only whether misconduct can be inferred from the individual's activities.
- Compliance with AOAC policy and guidelines involves not only avoidance of antitrust violations, but avoidance of any behavior which might be perceived as such.

Antitrust Policy Document

- The document states antitrust laws in general terms, and 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 and activities.
- Signing the AOAC INTERNATIONAL Volunteer Acceptance Form means that the signer has read, understand and agrees to comply with the policy.

USE OF THE ASSOCIATION NAME, INITIALS, IDENTIFYING INSIGNIA, LETTERHEAD, AND BUSINESS CARDS

- to protect the reputation, image, legal integrity and property of the Association.
- "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.
- 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;
- Please review instructions on use and sanctions for violations.
- Signing the AOAC INTERNATIONAL Volunteer Acceptance Form means that the signer has read, understand and agrees to comply with the policy.

Volunteer Conflict Of Interest

- 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.

Volunteer Conflict Of Interest Policy Document

- Contains illustrations of apparent or direct conflicts of interest, but not all inclusive
- Contains guidance on Dos and Don'ts for volunteers
- Signing the AOAC INTERNATIONAL Volunteer Acceptance Form means that the signer has read, understand and agrees to comply with the policy.

TRACT 3

ERP COMPOSITION & VETTING EXPERTISE

ERP Composition

- Call for Experts or Volunteers is issued.
- Members must be vetted by AOAC Official Methods Board (OMB).
  - Demonstrated expertise
  - Diversity and balance of the overall expert review panel
- AOAC volunteer appointment
  - Serve at the pleasure of the President of AOAC INTERNATIONAL
- Additional members may be added.
- Can have non-voting members
- OMB assigns an OMB member to serve as a representative on each ERP

ERP SELECTION PROCESS

- AOAC paid consultants and AOAC staff should not act as Chairs of ERPs.
- Members of the BoD may act as voting members but it is recommended that they sit as non-voting members of the panel, unless the CSO can demonstrate that there are so few experts in the field available to the community that they are needed to move the project forward.
- Paid consultants of AOAC and AOAC staff may not serve as voting members on ERPs.
- If a single business location is represented by more than one person on an ERP, that location shall have only one vote.
- The Chair of the ERP must be a member of AOAC INTERNATIONAL.

Vetting Process

AOAC Chief Science Officer

- Reviews all candidates and supporting documentation for expertise
- Makes a recommendation for an ERP slate

Official Methods Board

- Reviews proposed recommended ERP slate
  - Expertise
  - Balance of panel
  - Conflicts of interest
- Renders decision on proposed ERP members and a Roster is formed.

TRACT 4

ERP METHOD ASSIGNMENTS

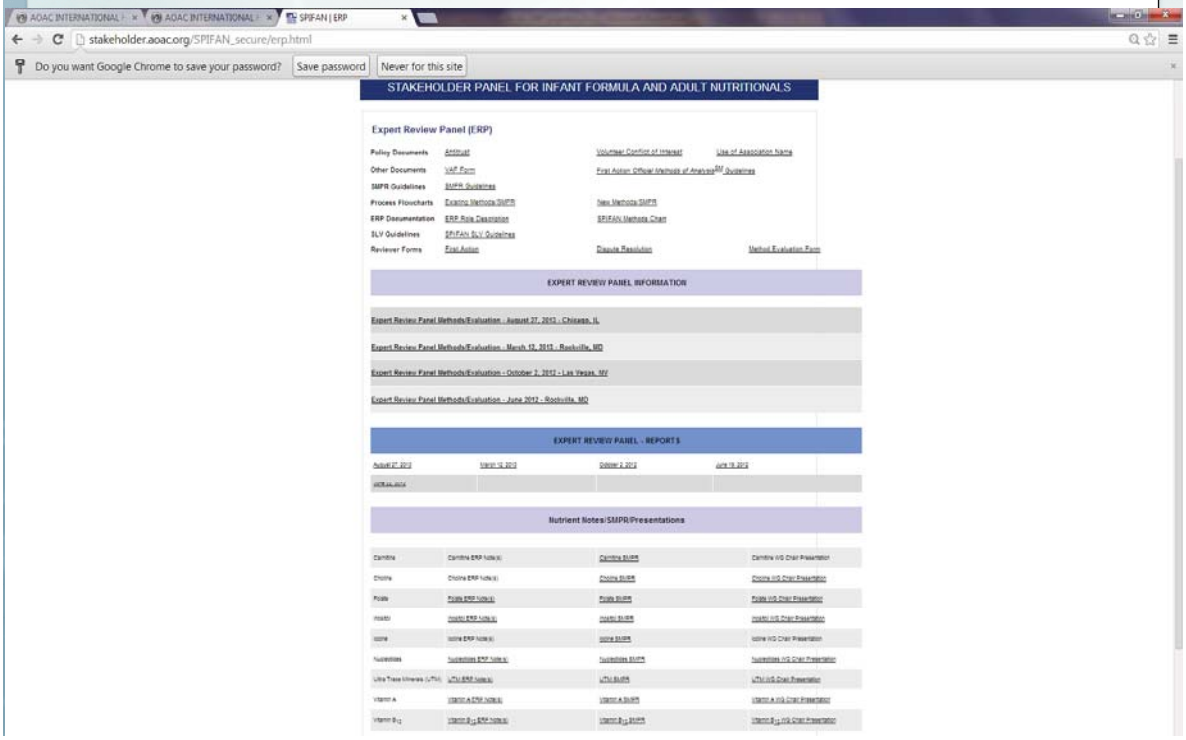
ERP Method Assignments

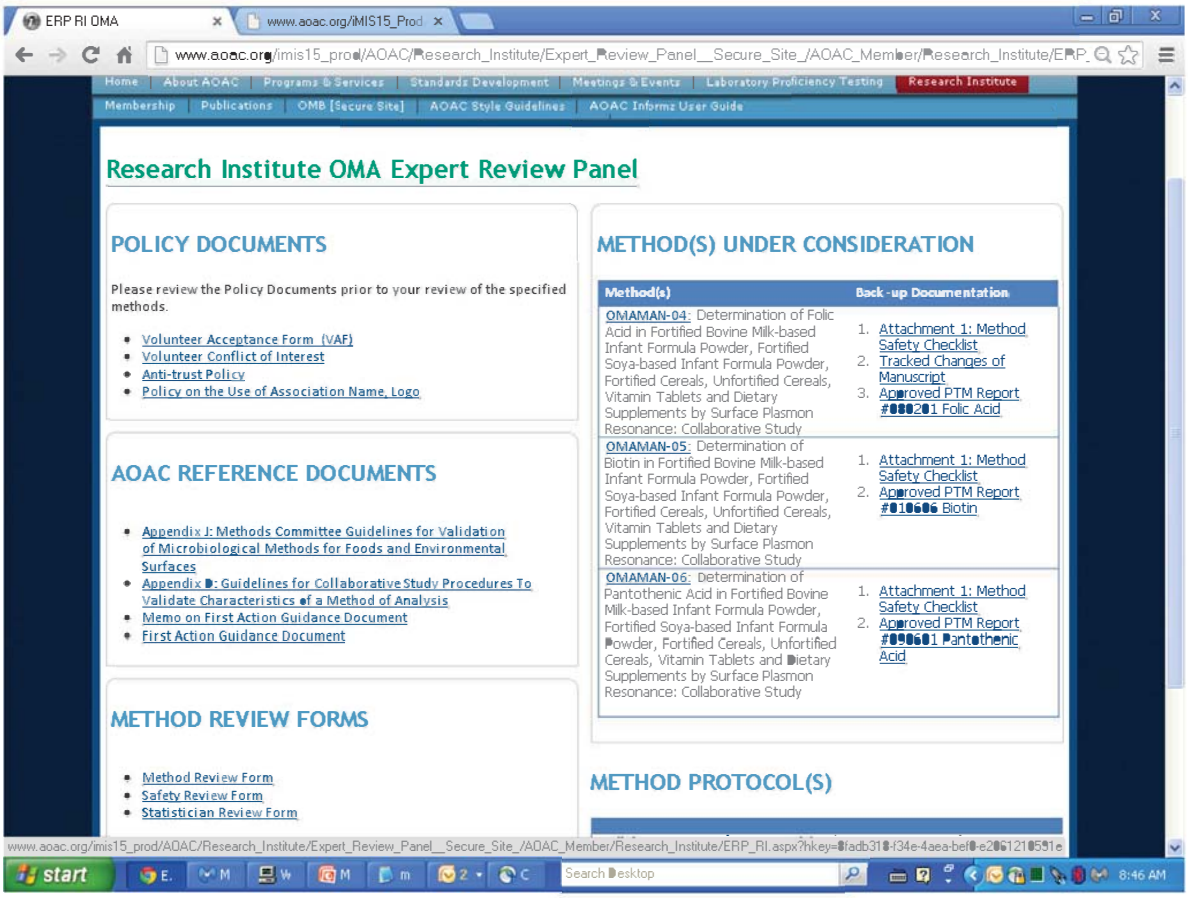
- A primary and secondary reviewer is assigned to every method.
  - In depth review via review form
  - Prepare to attend and speak on the method and make a recommendation for ERP discussion and consideration.
  - Review forms are completed and returned to AOAC staff in advance of the meeting.
- For Research Institute method submissions:
  - ERP members can participate in the Consulting Service conducting review of protocols – electronically.
- Members of both Committee on Safety and Committee on Statistics serve as advisory resources for all ERPs

ERP REVIEWS

- Primary and Secondary Reviewers or entire ERP (Research Institute ERPs) conduct in-depth review of method and any supporting information.
  - In-depth review is done electronically through password protected website access and is completed prior to the in-person meeting.
  - Deadlines for submission of reviews
  - Depending on the number of methods 15 to 30 days for review
  - Track and present feedback on assigned First Action *Official Methods*.
  - Present on the method during the meeting and can make the motion to adopt the method.
  - Can recommend additional feedback or information for Final Action consideration

ERP REVIEWS


stakeholder.aoac.org/SPIFAN_secure/erp.html
Do you want Google Chrome to save your password?
Save password
Never for this site
STAKEHOLDER PANEL FOR INFANT FORMULA AND ADULT NUTRITIONALS
Expert Review Panel (ERP)
EXPERT REVIEW PANEL INFORMATION
EXPERT REVIEW PANEL - REPORTS
Nutrient Notes/SMPR/Presentations


ERP RI OMA
www.aoac.org/imis15_prod/AOAC/Research_Institute/Expert_Review_Panel__Secure_Site_/AOAC_Member/Research_Institute/ERP
Home
About AOAC
Programs & Services
Standards Development
Meetings & Events
Laboratory Proficiency Testing
Research Institute
Membership
Publications
OMB [Secure Site]
AOAC Style Guidelines
AOAC Informz User Guide
Research Institute OMA Expert Review Panel
POLICY DOCUMENTS
Please review the Policy Documents prior to your review of the specified methods.
Volunteer Acceptance Form (VAF)
Volunteer Conflict of Interest
Anti-trust Policy
Policy on the Use of Association Name, Logo
AOAC REFERENCE DOCUMENTS
Appendix J: Methods Committee Guidelines for Validation of Microbiological Methods for Foods and Environmental Surfaces
Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis
Memo on First Action Guidance Document
First Action Guidance Document
METHOD REVIEW FORMS
Method Review Form
Safety Review Form
Statistician Review Form
METHOD(S) UNDER CONSIDERATION
Method(s)
Back-up Documentation
OMAMAN-04: Determination of Folic Acid in Fortified Bovine Milk-based Infant Formula Powder, Fortified Soya-based Infant Formula Powder, Fortified Cereals, Unfortified Cereals, Vitamin Tablets and Dietary Supplements by Surface Plasmon Resonance: Collaborative Study
1. Attachment 1: Method Safety Checklist
2. Tracked Changes of Manuscript
3. Approved PTM Report #080201 Folic Acid
OMAMAN-05: Determination of Biotin in Fortified Bovine Milk-based Infant Formula Powder, Fortified Soya-based Infant Formula Powder, Fortified Cereals, Unfortified Cereals, Vitamin Tablets and Dietary Supplements by Surface Plasmon Resonance: Collaborative Study
1. Attachment 1: Method Safety Checklist
2. Approved PTM Report #010606 Biotin
OMAMAN-06: Determination of Pantothenic Acid in Fortified Bovine Milk-based Infant Formula Powder, Fortified Soya-based Infant Formula Powder, Fortified Cereals, Unfortified Cereals, Vitamin Tablets and Dietary Supplements by Surface Plasmon Resonance: Collaborative Study
1. Attachment 1: Method Safety Checklist
2. Approved PTM Report #090601 Pantothenic Acid
METHOD PROTOCOL(S)
start
Search Desktop
8:46 AM

ERP REVIEWS

- **In your judgment, does the method sufficiently meet the Standard Method Performance Requirements (SMPR) or community-based guidance?**

- In your judgment, is the method scientifically sound and can be followed?
- In your judgment, what are the strengths and weaknesses of the method?
- In your judgment, how do the weaknesses weigh in your recommendation for the method?
- In your judgment, will the method serve well the stakeholder community that will use the method?
- In your judgment, what additional information may be needed to further support the method meeting the SMPR or community-based guidance?

TRACT 5

ERP MEETINGS

ERP Meetings

- ERPs will meet in person at a minimum of twice a year and up to four times per year:
  - AOAC Mid-Year meeting (DC metro area)
  - AOAC Annual Meeting.
  - 2 additional designated times for proprietary method Organziational Affiliates
- At the ERP meeting:
  - Primary and secondary reviewers **or entire ERP** will present their reviews and makes a motion/recommendation to the ERP whether or not to adopt the method as First Action OMA.
  - ERP discusses the method.
  - ERP renders a decision on First Action status.
  - ERP renders decisions on modifications to First Action methods only.
- If the method is adopted
  - ERP decides on what additional information is needed to recommend the method for Final Action status

ERP MEETINGS

- MEETINGS ARE HELD IN-PERSON, HOSTED BY AOAC
- A QUORUM IS THE PRESENCE OF SEVEN (7) MEMBERS OR 2/3 OF THE TOTAL VETTED ERP, WHICHEVER IS GREATER.

IF NO QUORUM, THEN NO MEETING!

ERP MEETINGS

- REVIEWERS PRESENT THEIR REVIEWS AND MAY INITIATE A MOTION TO ADOPT THE METHOD IF THEY CHOOSE
  - Chair recognizes the reviewers
  - Primary and secondary / ERP reviews are presented.
    - If in favor, they may make and second a motion to adopt or not adopt the method
    - Chair can then entertain discussion on the method
    - Chair can call for a vote once deliberation is complete

ERP MEETING - Discussions

- In your collective judgment, is the method scientifically sound and can be followed as written?
- **In your collective judgment, does the method sufficiently meet the Standard Method Performance Requirements (SMPR)?**
- In your collective judgment, what are the strengths and weaknesses of the method?
- In your collective judgment, do the weaknesses outweigh the strengths in your recommendation for the method?
- In your collective judgment, is the method safe and can it serve well the stakeholder community that will use the it?
- In your collective judgment, is additional information needed to before considering this method for First Action OMA status?

TRACT 6

ERP CONSENSUS

ERP CONSENSUS

- First Action Official Methods status is granted:
- Method 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.
- Method becomes First Action on the date when ERP decision is made.

ERP CONSENSUS

- The ERP may then reach consensus on any additional information that it needs to review to be able to make a recommendation for Final Action *Official Methods* status.
- This is a separate motion.

TRACT 7

POST ERP MEETING

Post ERP Meeting

- An ERP report with the decisions of the ERP will be drafted
  - Review and approval by ERP chair
  - Posted on website within 15 business days after the ERP meeting
- AOAC staff will send notification to method authors/submitters regarding outcomes on specific methods

TRACT 9

PUBLICATIONS

Publication of First Action Methods

- Any approved method(s) along with supporting manuscript(s) and documentation sent to AOAC Publications after the meeting.
  - AOAC Official Methods number assigned.
  - Method and method manuscript prepared for publication in the *Official Methods of Analysis of AOAC INTERNATIONAL* and in Journal of AOAC INTERNATIONAL
  - Updates on methods approved or status changes are published in the Inside Laboratory Management magazine and on the AOAC website

Format for AOAC *Official Methods of Analysis*

The language of the method should be concise and completely free from ambiguity. Conciseness is desirable, both to ensure clarity and to save space. Whenever there is a conflict between clarity and style, clarity is more important.

Present Tense and Imperative Mode

- Check sentences that do not begin with a verb and change them, if feasible, to the imperative mode (e.g. Pipet 10 mL..., Stir..., etc.). Exceptions are: use of adverb modifier ("Accurately weigh..."), prepositional clause ("For refined sugars, use..."), permissive statements ("Ferric hydroxide may be used..."), and statements in the "Principle" section.

Abbreviations

- Most abbreviations are the same as those used by Chemical Abstracts. Do not use abbreviations in titles and headings. See the *Definitions of Terms and Explanatory Notes*.

Repetition and Redundancy

- Eliminate repetition and redundancy as far as possible; use only for emphasis. Do not use "distilled" with water, "concentrated" with common acids, "95%" with alcohol, or "ACS" with reagents covered by ACS specifications. These are understood by definition.

Terminology, Formulae and Chemical Names

- For names of chemical compounds, use the spelling, hyphenation, and word division given in Chemical Abstracts. Use a national pharmacopeia for names for drugs. Use ISO nomenclature for pesticides and Codex nomenclature for names of food additives and color additives.

Consistency

- Watch for internal contradictions in the text: volumes that do not add up or that exceed the capacity of the container; too abrupt a transition from one operation to another (a line may be omitted); and impractical or impossible numbers (e.g., 100 g NaCl will not dissolve in 100 mL water).

Cross-references

- All new AOAC methods should be written as complete and self-contained as practical. Do not refer to other AOAC methods. If part of a procedure in an *Official Method*℠ is taken from material previously published elsewhere, incorporate those steps in the method rather than referring the analyst to another publication.

Definitions

- The section "Definition of Terms and Explanatory Notes," *Official Methods of Analysis of AOAC INTERNATIONAL*, is the basic guide to conventions and consistency.

Illustrations and Tables

- If symbols are used on the figure, include an explanation in the caption or text. Provide descriptive titles for tables. Explain any obscure headings in a footnote.

Bibliographic References

- Check all references for accuracy. Use standard Chemical Abstracts abbreviations for *Journal* titles. In general avoid references in method. Cite background references in the "Introduction" or "Discussion" section of the collaborative study manuscript -- not in the method. If part of a procedure in an *Official Method*℠ is taken from material previously published elsewhere, incorporate those steps in the method rather than referring the analyst to another publication.

Safety

- All methods must be reviewed for safety and potential hazards. Methods should automatically incorporate cross-references to the safety statement(s), or present questioned conditions to the attention of the Committee on Safety for resolution.
- Decisions regarding inclusion of safety statements should be practical, recognizing that overuse will be self-defeating.
- Methods that create toxic, obnoxious or environmentally hazardous fumes and wastes should contain practical directions for disposal.

Checking Edited Copy and Proofreading

- The author must review a copy of the original version and edited copy to ensure that there has been no change in meaning, to correct typographical errors, and to answer any questions posed by the editor. The author must review the typeset method for accuracy.

Revised October 2013

Online Technical Resources

Method Development, Optimization & Validation

- OMA - Appendix F - Guidelines for Standard Method Performance Requirements
- Homogeneity
- Guide for Writing Methods in AOAC Format
- Statistics Protocol Review Form
- OMA - Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis
- OMA - Appendix G: Procedures and Guidelines for the Use of AOAC Voluntary Consensus Standards to Evaluate Characteristics of a Method of Analysis
- OMA - Appendix I: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures
- OMA - Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces
- OMA - Appendix K: Guidelines for Dietary Supplements and Botanicals
- OMA - Appendix L: AOAC Recommended Guidelines for Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) Single-Laboratory Validation
- OMA - Appendix M - Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices
- Safety Checklist

Method Review

- Examples of Statistical Analysis
- Statistics Manuscript Review Form
- OMA - Appendix A: Standard Solutions and Reference Materials
- OMA - Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis
- OMA - Appendix H: Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods

Miscellaneous

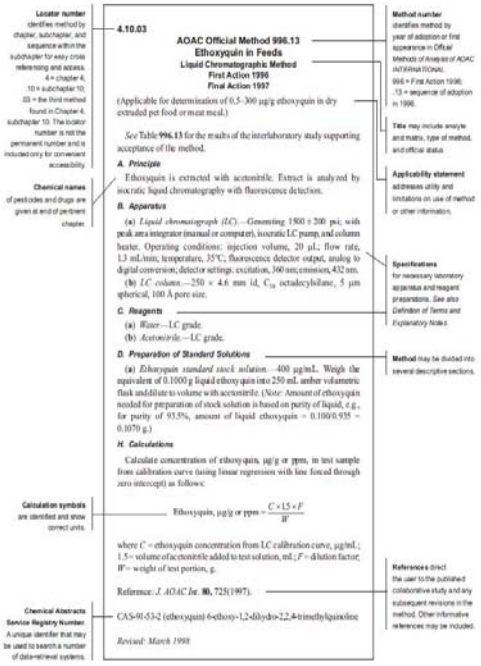
- Definition of Terms and Explanatory Notes
- OMA - Appendix B: Laboratory Safety
- OMA - Appendix E: Laboratory Quality Assurance
- OMA - Appendix C: Reference Tables

All resources are accessible at
http://www.aoac.org/vmethguidelines.htm

For questions, please contact:
P 301-924-7077 x157 E dmckenzie@aoac.org

Guide to Method Format

(Method shown is incomplete to allow space for description.)



4.10.03

AOAC Official Method 996.13
Ethoxyquin in Feeds
Liquid Chromatographic Method
First Action 1996
Final Action 1997

(Applicable for determination of 0.5–300 μg/g ethoxyquin in dry extruded pet food or meat meal.)

See Table **996.13** for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

Ethoxyquin is extracted with acetonitrile. Extract is analyzed by isocratic liquid chromatography with fluorescence detection.

B. Apparatus

(a) *Liquid chromatograph (LC).*—Generating 1500 ± 200 psi; with peak area integrator (manual or computer), isocratic LC pump, and column heater. Operating conditions: injection volume, 20 μL; flow rate, 1.3 mL/min; temperature, 35°C; fluorescence detector output, analog to digital conversion; detector settings: excitation, 360 nm; emission, 432 nm.

(b) *LC column.*—250 × 4.6 mm id, C_{18} octadecylsilane, 5 μm spherical, 100 Å pore size.

C. Reagents

(a) *Water.*—LC grade.

(b) *Acetonitrile.*—LC grade.

D. Preparation of Standard Solutions

(a) *Ethoxyquin standard stock solution.*—400 μg/mL. Weigh the equivalent of 0.1000 g liquid ethoxyquin into 250 mL amber volumetric flask and dilute to volume with acetonitrile. (*Note:* Amount of ethoxyquin needed for preparation of stock solution is based on purity of liquid, e.g., for purity of 93.5%, amount of liquid ethoxyquin = 0.100/0.935 = 0.1070 g.)

H. Calculations

Calculate concentration of ethoxyquin, μg/g or ppm, in test sample from calibration curve (using linear regression with line forced through zero intercept) as follows:

$$\text{Ethoxyquin, } \mu g/g \text{ or ppm} = \frac{C \times 1.5 \times F}{W}$$

where C = ethoxyquin concentration from LC calibration curve, μg/mL; 1.5 = volume of acetonitrile added to test solution, mL; F = dilution factor; W = weight of test portion, g.

Reference: *J. AOAC Int.* **80**, 725(1997).

CAS-91-53-2 (ethoxyquin) 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline

Revised: March 1998

Locator number identifies method by chapter, subchapter, and sequence within the subchapter for easy cross referencing and access. 4 = chapter 4, 10 = subchapter 10, 03 = the third method found in Chapter 4, subchapter 10. The locator number is not the permanent number and is included only for convenient accessibility.

Chemical names of pesticides and drugs are given at end of pertinent chapter.

Calculation symbols are identified and show correct units.

Chemical Abstracts Service Registry Number. A unique identifier that may be used to search a number of data-retrieval systems.

Method number identifies method by year of adoption or first appearance in Official Methods of Analysis of AOAC INTERNATIONAL. 996 = First Action 1996; .13 = sequence of adoption in 1996.

Title may include analyte and matrix, type of method, and official status.

Applicability statement addresses utility and limitations on use of method or other information.

Specifications for necessary laboratory apparatus and reagent preparations. *See also Definition of Terms and Explanatory Notes.*

Method may be divided into several descriptive sections.

References direct the user to the published collaborative study and any subsequent revisions in the method. Other informative references may be included.

The AOAC style used for preparing methods for publication in the *Official Methods of Analysis of AOAC INTERNATIONAL* includes the following essentials:

- ✓ Standardized format that follows the order of laboratory operations.
- ✓ Use of the imperative mode.
- ✓ Cross-references to identical reagents, apparatus, and operations.
- ✓ Use of standardized definitions, terminology, and style.
- ✓ Use of accepted abbreviations and simplifications.
- ✓ Use of SI units
- ✓ Methods should be written as complete and self-contained as practical.
- ✓ Normality should be referred in terms of Molarity.
- ✓ ppm should be changed to mg/kg or mg/L
- ✓ ppb should be changed to ng/g or ng/mL
- ✓ ppt should be changed to pg/g or pg/mL

FORMAT OF AOAC® *OFFICIAL METHODS of ANALYSIS OF AOAC INTERNATIONAL*

Title:
- ❖ Includes analyte being determined, type of matrix (matrices), and analytical technique used for analysis.

Applicability:
- ❖ Includes list of matrix(es) along with specific matrix types and range or limits of determination or detection.

Precautions:
- ❖ Makes an analyst aware of hazardous materials used in analysis.

Data Collection:
- ❖ Table(s) that presents performance parameters including matrices tested in a collaborative study, levels of analyte(s), % recovery, RSD_r, RSD_R, s_r, s_R, HORRAT, number of observations, etc

Principle:
- ❖ Explains scientific premise on which the method is operates specifically the mechanism of the analysis.

Apparatus:
- ❖ Lists the equipment that requires assembly or that has specifications critical to the method performance. Describe equipment in terms of performance characteristics.

Reagents:
- ❖ List the reagents with amounts and appropriate units needed to conduct the analysis and describe the reagents in terms of performance characteristics.

Sample and Test Portion Preparation:
- ❖ Describe the preparation of samples and the test portion.

Determination:
- ❖ Describes the actual analysis.

Calculations:
- ❖ Section that explains how to calculate final results; presented in a form of equation or description.

Other sections as needed

REFERENCING *AOAC® OFFICIAL METHODS*℠

When referencing *AOAC® Official Methods*℠, only the method number should be used as seen in the following example:

(1) *Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method **2008.01**

Revised October 2013

Publication of First Action Methods

NO OMA NUMBER ASSIGNED

UNTIL ALL DOCUMENTATION SUBMITTED

1. Method incorporating ERP revisions (preferably in AOAC Format)
2. Method Manuscript incorporating ERP revisions (in AOAC Format)
3. Signed AOAC Copyright Authorization form

Format for AOAC First Action *Official Methods* Manuscripts and Protocols

FORMAT FOR FIRST ACTION OMA MANUSCRIPTS

TITLE: Title of manuscript includes method title which includes the analyte(s), matrix(es), and analytical technique, if applicable. It may also include a *common* method name and ends with "Collaborative Study."

AUTHOR(S): Provides authors' full (e.g. no initials) names and contact information.

ABSTRACT:
- ✓ Specific information on the method and study.

INTRODUCTION:
- ✓ Information on why collaborative study was conducted, how many collaborators participated in the study, previous work done, and information on compound or process that was studied.

COLLABORATIVE STUDY:
- ✓ Information on matrices and number of test samples tested, test sample preparations, instructions for collaborators, etc.

METHOD:
- ✓ Written in AOAC style.

COLLABORATORS' COMMENTS:
- ✓ Any comments and suggestions received from collaborators and information on how they were addressed, e.g., incorporating instructions into the method, etc.

RESULTS AND DISCUSSION:
- ✓ Information on type of statistical analyses performed on raw data, reasons for rejecting some of the data, discussion of results with references to tables and figures, discussion of the method performance, etc.

RECOMMENDATION:
- ✓ Recommendation to adopt method First Action.

ACKNOWLEDGMENTS:
- ✓ Full names and addresses of all collaborators that participated in the study.

REFERENCES:
- ✓ Included all references cited in the text.

APPENDICES or **FIGURES AND TABLES:**
- ✓ Include any figures and tables that may make the manuscript and the performance of the method easier to understand and interpret.

Revised October 2013

Online Technical Resources

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- Methods and/or Procedures
- OMA - Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces
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- OMA - Appendix H: Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods

Miscellaneous
- Definition of Terms and Explanatory Notes
- OMA - Appendix B: Laboratory Safety
- OMA - Appendix E: Laboratory Quality Assurance
- OMA - Appendix C: Reference Tables

All resources are accessible at
http://www.aoac.org/vmethodguidelines.htm

For questions, please contact:
P 301-924-7077 x157 E dmckenzie@aoac.org

TRACT 8

FIRST ACTION TO FINAL ACTION STATUS

ERP Tracking

- Between First Action and Final Action:
  - The primary and secondary reviewers track the methods on behalf of the ERP over this time period.
    - Based on information from method authors, laboratories using the method, general community feedback, additional laboratory work
    - Are ERP recommendations being fulfilled?
    - Is the method meeting the standard criteria more closely?
    - How well is community guidance and OMB guidance being reflected?
  - Updates on the method are given by the primary and secondary reviewers during the ERP meetings.
  - At the end of two years, ERP makes a recommendation to OMB for Final Action status, repeal, or continuance.

Road to Final Action OMA Status

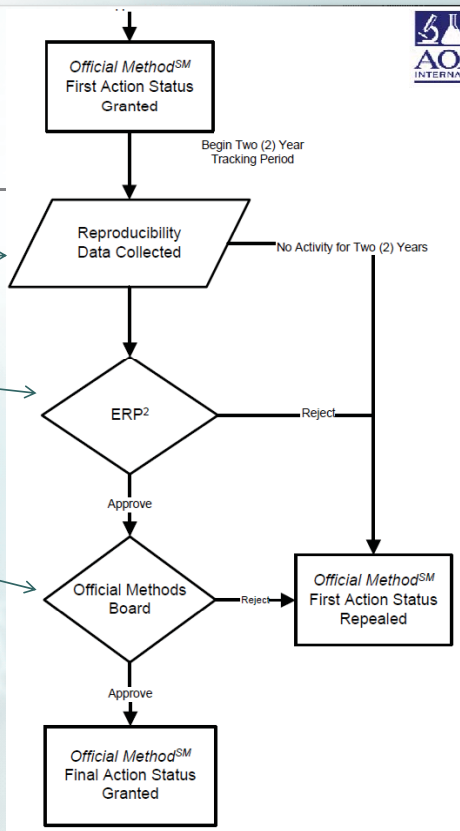
Method reproducibility must be demonstrated before Final Action consideration.

ERP determines if sufficient evidence merits a recommendation for Final Action status or repeal.

•Only the OMB promotes a method to "Final Action" status or repeal the method.

•Methods that did not meet the bar would be repealed.

•Same for all method submissions



Terms:

- PTM – *Performance Tested Methods℠*
- RI – Research Institute
- ERP – Expert Review Panel
- OMB – Official Methods Board
- SP – Stakeholder Panel
- SMPR – Standard Method Performance Requirement

Note: Appeals process always available; see Alternative Pathway Guidelines for appeals process.

1 PTM certification previously issued, PTM reviewers will be ERP members
2 Unless otherwise provided for under a contractual agreement, AOAC will regularly convene ERPs twice a year: once during the Mid-Year Meeting and again during the Annual Meeting

Path to Final Action

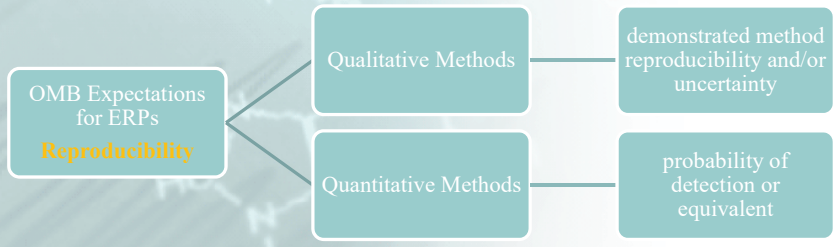
Review of ERP Method Recommendations

What to Expect from AOAC Official Method Board (OMB)

OMA, Appendix G

Further data indicative of adequate method reproducibility (between laboratory) performance to be collected. Data may be collected via a collaborative study or by proficiency or other testing data of similar magnitude.

- ERP is looking to verify if method reproducibility has been appropriately assessed and satisfactorily demonstrated



OMA, Appendix G

Two years maximum transition time (additional year(s) if ERP determines a relevant collaborative study or proficiency or other data collection is in progress).

2 yr tracking of method

- ERP verification of any changes to the method
- ERP recommendations implemented successfully
- ERP evaluation of any feedback on method and its performance

ERP Recommendations

- Move method to Final Action OMA status
- Repeal method from OMA
- Continuance of First Action OMA status

OMA, Appendix G

Method removed from Official First Action and OMA if no evidence of method use available at the end of the transition time.

First Action OMA Tracking

- Tracking period is ≤ 2 years and begins on the date of the ERP's decision to adopt a method for OMA First Action status.

No Use in 2 Years

- Repeal from OMA

OMA, Appendix G

Method removed from Official First Action and OMA if no data indicative of adequate method reproducibility is forthcoming as outlined above at the end of the transition time.

First Action OMA Tracking

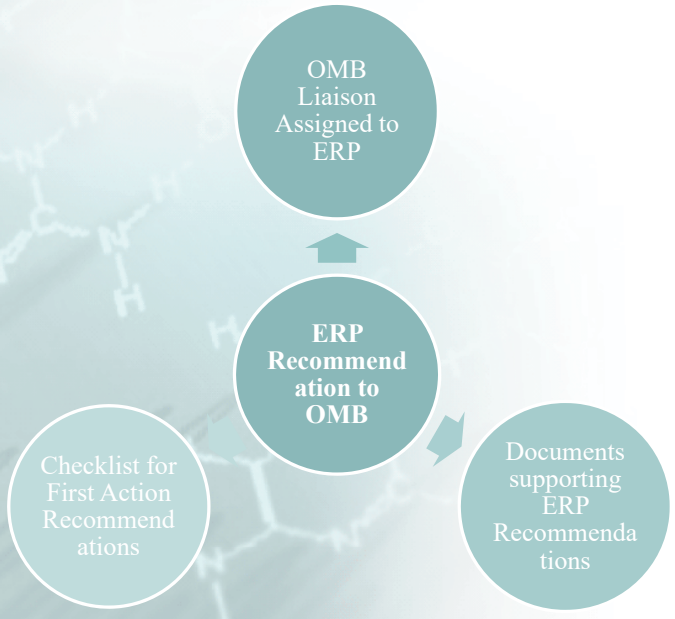
- Tracking period is ≤ 2 years and begins on the date of the ERP's decision to adopt a method for OMA First Action status.

No Demonstration of Method Reproducibility in ≤ 2 Years

- Repeal from OMA

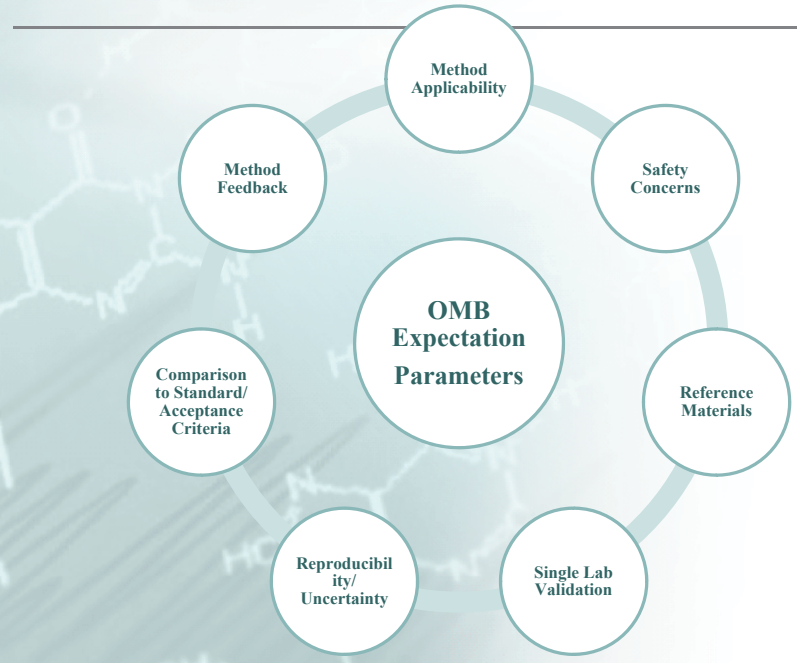
OMA, Appendix G

ERP to recommend Method to Official Final Action Status to the OMB.

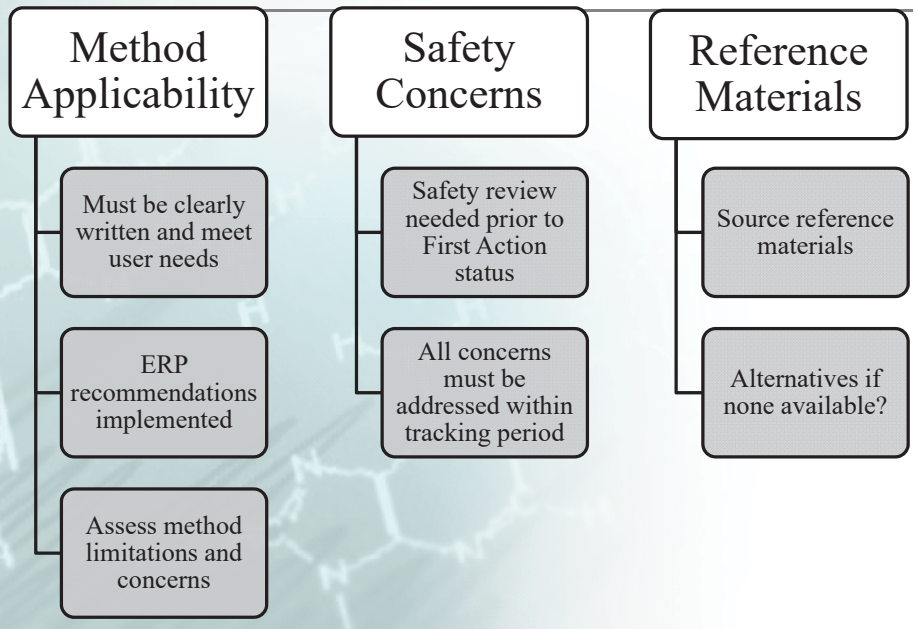


OMA, Appendix G

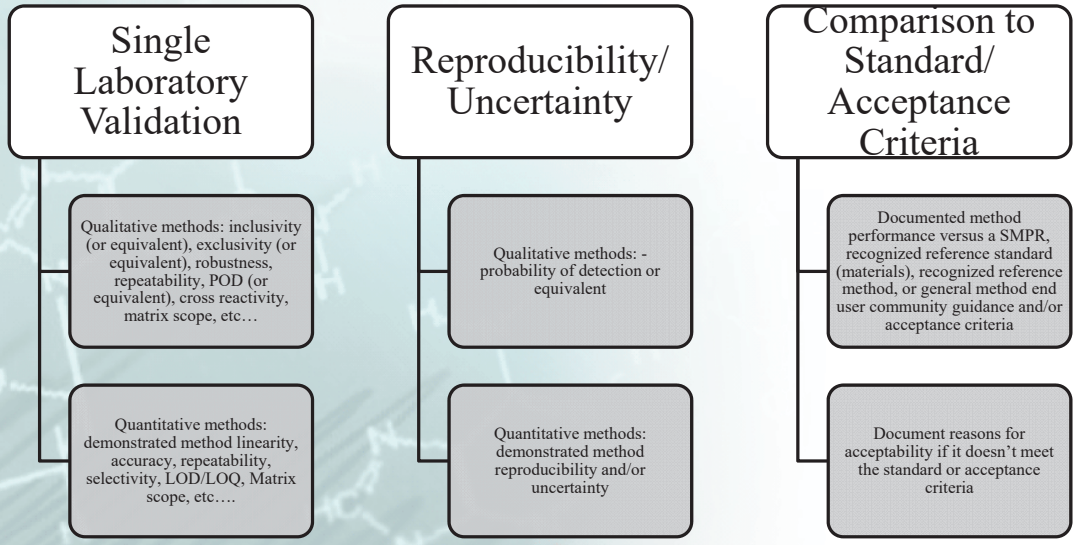
First Action to Final Action Methods: Guidance for AOAC Expert Review Panels



OMB Expectation Parameters



OMB Expectation Parameters



OMB Expectation Parameters

Method Feedback from End Users

Consider any positive or negative feedback on overall method performance, applicability, availability of reference materials, matrix scope, method component sourcing, robustness or ruggedness parameters.

Documentation Needed

- Method Safety Evaluation
- Reference Materials
- Evidence of Single Laboratory Validation or equivalent
- Evidence of Reproducibility Assessment
- Published First Action OMA
- Method Performance versus SMPR or acceptance criteria
- Final draft of First Action OMA to be considered for status update
- Rationale or Justification for Repeal or Continuance of First Action OMA

ERP Meetings

Quorum

Presence of 7 vetted ERP members

OR

Presence of 2/3 vetted ERP members

WHICHEVER IS GREATER

ERP Meetings

METHOD AUTHOR: present any method feedback obtained and any resulting changes to the method, any reproducibility information, any implemented ERP recommendations, final draft of method proposed for decision

ERP MEMBERS: present any method feedback obtained and discuss any resulting changes to the method, any reproducibility information, any implemented ERP recommendations, review and agree upon final draft of method proposed for decision, and make a recommendation to OMB.

CONSENSUS: 2/3 vote in favor of a motion. Abstentions do not count towards vote; in case of multiple abstentions. Staff will monitor and record consensus voting.

STAFF: Will organize and coordinate meeting, record ERP actions and decisions, draft ERP report and distribute after chair approval, work with chair and OMB liaison to complete checklist and assemble recommendation package for OMB.

TRACT 10

MODIFICATIONS

Modifications of Methods

- During First Action and Final Action, methods can be modified or extended to additional matrixes and/or analytes.

Submitting a Modification

Standards Development

- Contact staff and they will let you know the best way to submit the modification information and any additional requirements.
  - Staff will inform of the appropriate mechanism to submit a modification.
- Fully revised method manuscript and a revised version of the AOAC OMA method, both in OMA format, must be submitted.

Research Institute

- Submit request for modifying a method through the AOAC website.
  - AOAC > Research Institute > Method Submission
  - AOAC RI Application for Method Change or Modification
- Fully revised method manuscript and revised method, both in OMA format, must be submitted.

Processing Modifications

ERPs from Standard Development and Research Institute

- Review of the modification will undergo a preliminary review by at least the AOAC CSO.
  - Comments to be shared with method author.
- Original ERP reviewers will be assigned to review the method
- Method will be added to ERP agenda for their next meeting

Approval of Modifications

- If ERP approves a method modification including extensions, then the method begins a new two (2) year period.
- If the method modification is to correct an editorial error, then the method, then there is no change.

Method modifications require substantiation of the modification or extension with proof of method performance as deemed suitable by the EPR.

TRACT 11

DOCUMENTATION

Reports and Documentation

- AOAC staff or designee will capture the decisions and action items into an ERP report.
- The draft report will be sent back to the ERP Chair whose responsibility it is to sign off on the report once approved.
- The report is then distributed to the ERP.
- ERP is responsible for a drafting a written recommendation to the OMB for each method at a maximum of two years following adoption as First Action OMA
- Approved methods from the ERP meetings are published in the OMA and in the *Journal of AOAC INTERNATIONAL*.
- Meeting overviews are published in the AOAC *Inside Laboratory Management* magazine.

TRACT 12

SUMMARY OF RESPONSIBILITIES

Roles and Responsibilities

- Expert Review Panel:
  - Review methods and meet in person to discuss and render decisions on methods for First Action *Official Methods* status.
  - Track First Action *Official Methods*
  - Modify First Action methods if necessary
  - Make recommendations on First Action methods no more than 2 years after adoption to OMB.
- Official Methods Board:
  - Vet and approve ERP membership
  - Assign OMB liaison to be a resource to the ERP
  - Review ERP recommendations and render decisions (*Final Action, Repeal or remain First Action*) on First Action OMAs
- AOAC Staff
  - Coordinate the ERP and meetings, facilitate reviews, document ERP actions/decisions.
  - Issue necessary calls for experts and methods

Task Force on Communication/ ERP Best Practices

Recommendations for Staff

- Regularly debrief with ERP Chairs for input after meetings
- ERP background and training materials on website
- Offer orientation on a regular basis, to all ERP chairs and potential members, wider distribution of training materials
- Execute post training surveys
- Clearly outline expectations of reviewers prior to meeting: attendance is mandatory, cursory review of all methods to be discussed
- Encourage all method authors to attend ERP: helps process move smoothly and authors will only be privy to full discussion if they attend
- Establish a codification system in OMA for "dispute resolution methods" *
- Investigate ways to elevate the level of prestige for participation in an ERP.

** Project specific*

Task Force on Communication/ ERP Best Practices

Best Practices for ERP Chairs

1. Work closely with staff during the orientation period for ERP
2. Clearly understand consensus and quorum rules
3. Discourage abstentions unless a true conflict of interest is present; *use discretion as necessary when determining if a vote allows a method move forward.*
4. Encourage ERP reviewers to be fully prepared
5. Add brief orientation to ERP meeting agenda
6. Where in a stakeholder panel community requires only one method is desired, a 2 step process that considers multiple methods may be adopted as First Action and assessment of the best method is determined during follow up ERP meetings.
7. When considering methods for repeal, advise ERP members that repeal does not discredit method, it is simply a procedural determination that a method will not be moved forward.

Expert Review Panels

The ERPs review and approve appropriate methods (as submitted or modified) for adoption as First Action Official Methods or for further validation. ERPs also make recommendations regarding Final Action Official Methods status.

Expert Review Panels

- Must be supported by relevant stakeholders.
- Constituted for the review of methods, not for Standard Method Performance Requirements (SMPR) purposes or as an extension of a Working Group.
- Consist of a minimum of seven (7) members representing a balance of expert stakeholders. **Quorum is a minimum of 7 members present or 2/3 of the total vetted members, whichever is greater.**
- ERP constituency must be approved by the Official Methods Board (OMB).
- Holds transparent public meetings only.
- Remains in force as long as method in First Action Status.

First Action Official Method Status decision

- Must be made by an ERP constituted or reinstated post 2011-03-28 for First Action Official Method Approval (FAOMA).
- Must be made by an ERP vetted for FAOMA purposes by OMB post 2011-03-28.
- Method adopted by ERP must perform adequately against the SMPR set forth by the stakeholders. Or demonstrate performance or characteristics that meet the scope, applicability and/or claims of the method.
- Method 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 non-negative voting ERP members after due consideration
- Method becomes First Action Official Methods on date when ERP decision is made.
- Methods to be drafted into AOAC format by a knowledgeable AOAC staff member or designee in collaboration with the ERP and method author.
- Report of FAOMS decision complete with ERP report regarding decision including scientific background (references etc) to be published concurrently with method in traditional AOAC publication venues.

Method in First Action Status and Transitioning to Final Action Status

- Further data indicative of adequate method reproducibility (between laboratory) performance to be collected. Data may be collected via a collaborative study or by proficiency or other testing data of similar magnitude.
- Two years maximum transition time (additional year(s) if ERP determines a relevant collaborative study or proficiency or other data collection is in progress).
- Method removed from First Action Official Methods and OMA if no evidence of method use available at the end of the transition time.
- Method removed from First Action Official Methods and OMA if no data indicative of adequate method reproducibility is forthcoming as outlined above at the end of the transition time.
- ERP to recommend Method to Official Final Action Status to the OMB.
- OMB decision on First to Final Action Status

Revised October 2013

Online Technical Resources

Method Development, Optimization & Validation

- OMA - Appendix F - Guidelines for Standard Method Performance Requirements
- Homogeneity
- Guide for Writing Methods in AOAC Format
- Statistics Protocol Review Form
- OMA - Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis
- OMA - Appendix G: Procedures and Guidelines for the Use of AOAC Voluntary Consensus Standards to Evaluate Characteristics of a Method of Analysis
- OMA - Appendix I: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures
- OMA - Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces
- OMA - Appendix K: Guidelines for Dietary Supplements and Botanicals
- OMA - Appendix L: AOAC Recommended Guidelines for Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) Single-Laboratory Validation
- OMA - Appendix M - Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices
- Safety Checklist

Method Review

- Examples of Statistical Analysis
- Statistics Manuscript Review Form
- OMA - Appendix A: Standard Solutions and Reference Materials
- OMA - Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis
- OMA - Appendix H: Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods

Miscellaneous

- Definition of Terms and Explanatory Notes
- OMA - Appendix B: Laboratory Safety
- OMA - Appendix E: Laboratory Quality Assurance
- OMA - Appendix C: Reference Tables

All resources are accessible at
http://www.aoac.org/vmeth/guidelines.htm

For questions, please contact:
P 301-924-7077 x157 E dmckenzie@aoac.org

About Expert Review Panels (ERPs)

ERP OVERVIEW:

An Expert Review Panel (ERP) is assembled to review and adopt methods as Official First Action. ERPs will track Official Methods for two years or until such time as reproducibility has been demonstrated and cumulative feedback on method use and performance are obtained. ERPs will make a recommendation regarding Final Action method status for all OMAs to the Official Methods Board (OMB).

All ERP members are expected to serve with the highest integrity and without direct or indirect conflicts of interest. A method assignment can last two years. All members of the ERP are expected to actively participate in ERP meetings and to perform duties and reviews in timely fashion. All members should maintain strict adherence to review timelines and deadlines. AOAC staff documents ERP deliberations.

ESTABLISHING AN EXPERT REVIEW PANEL:

- AOAC staff issues a Call for Experts:
  - Based on voluntary consensus standards and methods submitted to AOAC INTERNATIONAL that may meet the standards.
  - Proprietary and sole source method developers submit individual methods to the AOAC Research Institute.
  - Candidates are asked to submit a CV or information that demonstrates expertise to AOAC staff if not already part of a recognized pool of experts.
- AOAC Chief Scientific Officer (CSO) reviews the documentation for the candidates and make recommends a slate for an expert review panel including the chair to the Official Methods Board.
- The candidate list and supporting documentation are forwarded to the Chair of the OMB who will assign the review to at least two OMB members.
- The OMB reviewers will review the candidates for expertise and perceived conflicts of interest and the OMB may then approve the members of the ERP. A Chair for the ERP is also approved.

EXPERT REVIEW PANEL (ERP):

- Review, discuss and demonstrate consensus on methods for Official First Action method status.
- Participate in the publications process of First Action methods.
- Track and discuss feedback all First Action methods for two years.
- Reach and demonstrate consensus on recommendations for Final Action method status.
- Actively participate in the broader stakeholder effort.

ERP CHAIR:

- Lead ERP discussions in the review and adoption of methods for First Action Official Methods.
- Participate in stakeholder panel activities.
- Review and approve ERP report.
- Work with AOAC staff, working groups and other stakeholder panels to ensure a thorough understanding of the standard method performance requirements and the methods to be assessed.
- Implement the OMB First Action to Final Action Guidelines with the ERP members.
- Advise and review First Action methods and post First Action publications.
- Represent the ERP in presenting the ERPs recommendation to the Official Methods Board regarding Final Action method status.

MECHANICS OF AN AOAC EXPERT REVIEW PANEL

- AOAC CSO assigns methods for review to the expert review panel members.
- For each method, 2 ERP members are assigned as primary and secondary reviewers and present at the ERP meeting.
- All members are expected to actively participate and review methods for First Action Official Method status - conducting thorough and prompt review of methods and being prepared to speak on assigned methods at ERP meetings
- The ERP chair and the 2 reviewers for each method are expected to participate in the publications peer review process for First Action methods.
- ERP reviewers track assigned methods that were adopted as First Action Official Methods and update ERP on method use during two year period between First Action and Final Action
  ERP members are expected to participant in the stakeholder panel activities and/or community at large .

- *ERPs can work with topic advisors (aka, subject matter experts)*
- *OMB can recognize a pool of experts from which ERP members can be selected*

Eligibility Criteria for Expert Reviewers

Be a key expert and/or thought leader of the method or priority under consideration.

- Demonstrated knowledge in the appropriate scientific disciplines.
- Demonstrated knowledge regarding data relevant to adequate method performance.
- Demonstrated knowledge of practical application of analytical methods to bona fide diagnostic requirements.

Be approved by the Official Methods Board

- Qualifications must be clearly described and submitted to AOAC headquarters.

Duties of Expert Reviewers

Members of the Pool of Experts will be called upon to serve on ERPs as needed and to review documents .These documents may include:

- Procedural documents on how methods will be selected and how single laboratory validation studies will be done;
- Methods submitted for consideration as First Action Official Methods;
- Methods submitted for selection for further validation studies;
- Protocols to be used for single laboratory validation studies;
- Selection of methods to be considered for full collaborative studies; and
- Validation study reports

Revised October 2013

Questions?

Thank you.

Evaluation of the 3M™ Molecular Detection Assay(MDA) 2 -*E. coli* (including H7) for the Detection of*E. coli* O157:H7speciesin SelectedFoods: Collaborative Study

Leslie K. Thompson, Cole Liska
Vanguard Sciences, 224 N. Derby Lane, North Sioux City, SD, 57049

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

Collaborators: C. Zuban, D. Metzger, H. Wright, E. Nix, M. Harrison, G. Hirsch, J. Schoeni, A. Winslow, C. Lopez, S. Johnson, D. Baumler, T. Dong, J. Miller, E. Budge, P. Bird, R. Fink, N.A. Jahan, M. Norbeck, S. Bom, M. Stein, M. Li, C. Diaz

The 3M™ Molecular Detection Assay (MDA) 2 –*E. coli* O157 (including H7)employs real-time isothermal amplification technology for the rapid and accurate detection of *E. coli* O157 (including H7) from enriched select food, feed and food process environmental samples.The 3M MDA 2 –*E. coli* O157 (including H7)was evaluated in a multi-laboratory collaborative study using an unpaired study design. The MDA 2–*E. coli* O157 (including H7)was compared to the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) Microbiology Laboratory Guidebook (MLG) Chapter 5.09*Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges*in raw ground beef (73% lean). Technicians from15laboratories located within the continental United Statesparticipated. 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 portionsproduced adLPODvalue with 95% confidence intervals of -0.11, (-0.22, 0.01) for raw ground beef indicating nostatisticallysignificant differencebetween the candidate and reference methods.

Escherichia coli O157:H7 is a gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms like cattle. Several foodborne outbreaks associated with beef as well as other food products such as produce have been attributed to *E. coli* O157:H7. Outbreaks are often associated with meat products. The organism has a minimum growth range between 6-7°C and therefore has potential for outgrowth in product when stored at the high end of refrigeration temperatures.The 3M™ Molecular Detection Assay(MDA) 2 –*E. coli* O157 (including H7)method uses a combination of bioluminescence and isothermal amplification of nucleic acid sequences to rapidly detect *E. coli* O157 (including H7)in select food matrices.The isothermal amplification is a molecular reaction conducted at a constant temperature, eliminating the need for temperature cycling and decreasing the time to results.
The 3M MDA 2- *E. coli* O157 (including H7) method allows for the rapid and specific detection of *E. coli* O157 (including H7)in select matrices after as little as 10 to 18 hoursof pre-enrichment using an ISO formulation of buffered peptone water (ISO BPW)[1].After enrichment, samples are evaluated using the 3M MDA 2 - *E. coli* O157 (including H7)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 60 minutes.
Prior to the collaborative study, the 3M MDA 2 –*E. coli* O157 (including H7) method was validated according to AOAC Guidelines[2] in pre-collaborative evaluation. The objective of the pre-collaborativeevaluationwas to demonstrate that the 3M MDA 2-*E. coli* O157 (including H7)method could detect *E. coli* O157 (including H7)in select food matrices as claimed by the manufacturer.For the 3M MDA 2 - *E. coli* O157 (including H7) pre-collaborative evaluation, four matrices were evaluated: raw ground beef (73% lean) (325g), frozen blueberries (25 g), fresh babyspinach (200g), and fresh sprouts (25 g). The frozen blueberries, fresh baby spinach and fresh sprouts were evaluated and compared to U.S. Food and Drug Administration Bacteriological Analytical Manual (FDA BAM) Chapter 4A: *Diarrheagenic Escherichia coli* [3]. All (100%) *E. coli* O157 strains were detected with the 3M MDA 2 – *E. coli* O157 (including H7). None of the (100%) non – *E. coli* O157 strains were detected by the 3M MDA 2 – *E. coli* O157 (including H7).

The purpose of this collaborative studywas to compare the reproducibility of the 3M MDA 2 -*E. coli* O157 (including H7)method to the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) -Microbiology Laboratory Guidebook (MLG) Chapter 5.09 Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges [4] for raw ground beef (73% lean).

Collaborative Study

Study Design

In this collaborative study, one matrix,325 g of raw ground beef(73% lean) wasevaluated through a qualitative, unpaired study design due to the different sample enrichments for the 3M MDA 2 – *E. coli* O157 (including H7) method and the USDA/FSIS MLG Chapter 5.09 reference method.The matrix was obtained from a local retailer and screened for the presenceof *E. coli* O157:H7by the appropriate reference method prior to analysis.The raw ground beef was artificially contaminated with non-heat stressed cells of *E. coli* O157:H7, American Type Culture Collection (ATCC) 43895®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 MDA2–*E. coli* O157 (including H7)or the USDA/FSIS MLG Chapter 5.09 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) [5]on the day samples testing was initiated 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. 3M Food Safety Technical Service provided on-site hands on training to 12 of the 15 participating laboratories prior to the start of the collaborative study.

Preparation of Inocula and Test Portions

The *E. coli* O157:H7cultureATCC43895 used in this evaluation were propagated onto Tryptic Soy Agar (TSA) from a Vanguard Sciences(collaborating laboratory) frozen stock culture stored at -70°C. The 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. The cultures were stored at 2-8°C for 24 h while the inoculum level was determined. Appropriate dilutions of each culture were prepared in Butterfield's Phosphate Diluent (BPD) 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. For the analysis of the raw ground beef, 25 g of inoculated test product was mixed with 300 g of un-inoculated test product to prepare 325 g test portions which were packaged in sterile Smasher®XLbags and shipped to collaborators.
A most probable number (MPN) was conducted by the coordinating laboratory on the day of the initiation of analysis using the USDA/FSIS-MLG 5.09 reference method for raw ground beef.The MPN was determined by analyzing 5 x 650 g test portions,thereference method test portions from the collaborating laboratories and 5 x 160 g test portions by the USDA/FSIS-MLG 5.09 reference method for the low level inoculated samples and the reference method test portions from the collaborating laboratories, 5 x 160 g, and 5 x 80 g test portions for the high samples. The MPN and 95% confidence intervals were calculated using the LCF MPN Calculator, Version 1.6, (www.lcftld.com/customer/LCFMPNCalculator.exe), provided by AOAC RI [6]. Confirmation of the samples was conducted according to the USDA/FSIS-MLG 5.09 reference method.

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). All raw ground beef samples were packed with cold packs to target a temperature of < 7°C during shipment.Upon receipt, raw ground beef 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.
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 measurethe temperature of this portion upon receipt of the package, record the results on the Sample Receipt Confirmation form provided and send the filled form back to the study directorby fax or email.

Test Portion Analysis

Collaborators were instructed to follow the appropriate preparation and analysis protocol for both the 3M MDA 2 – *E. coli* O157 (including H7) method and reference methods. 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 raw ground beef test portions by the 3M MDA 2 –*E. coli* O157 (including H7) method, a 325 g portion was enriched with 975 mL of pre-warmed (41.5 ±1°C) BPW ISO, manually homogenized by hand for 2 minutes and incubated for 10 hours at 41.5 ±1°C.

Following enrichment, samples were assayed by the 3M MDA 2 –*E. coli* O157 (including H7) method and, regardless of presumptive result, confirmed following the appropriate reference method. The matrix evaluated by

the 3M MDA 2 –*E. coli* O157 (including H7) method were compared to samples analyzed using the USDA/FSIS MLG reference method in an unpaired study design. All positive test portions were biochemically confirmed by the API 20E biochemical testor by the VITEK 2 GNbiochemical identification test, AOAC Official Method 2011.17 [7].

Statistical Analysis

Each collaborating laboratory recorded results for the reference method and the 3M MDA 2 –*E. coli* O157 (including H7) method on the data sheets provided. The data sheets were submitted to the study director at the end of testing for statistical analysis. Data was analyzed using the Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook (http://lcfltd.com/aoac/aoac-binary-v2-3.xls) provided by AOAC RI [8]. Data for each test portion size 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 –*E. coli* O157 (including H7)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 [9].

AOAC Official Method 2016.xx
***Escherichia coli* O157:H7 in Selected Foods**
3M™ Molecular Detection Assay(MDA) 2 –*E. coli* O157 (Including H7) Method
First Action 2016

(Applicable to detection of *Escherichia coli* O157 (including H7) in raw ground beef (73% lean), frozen blueberries, fresh sprouts, and fresh baby spinach

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

A. Principle

The 3M™ Molecular Detection Assay (MDA) 2 –*E. coli* O157 (including H7) method is used with the 3M™ Molecular Detection System (MDS) for the rapid and specific detection of *E. coli* O157 (including H7) in enriched foodsamples. The 3M MDA2 –*E. coli* O157 (including H7)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, 60 minutes. Samples are enriched in 3M™ Buffered Peptone Water (BPW) ISO formulation.

B. Apparatus and Reagents

Items (a)-(g) are available as the 3M™ Molecular Detection Assay (MDA) 2 – *E. coli* O157 (including H7) 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 – E. coli* O157 (including H7)*Catalog number:* MDA2ECO96,*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) *Buffered Peptone Water (ISO Formulation)* – Available from 3M Food Safety (St. Paul, MN 55144-1000, USA). (Formulation equivalent to ISO 6579:2002 Annex B or 3M equivalent)
(o) *Disposable pipette* – capable of 20 µL
(p) *Multi-channel (8-channel) pipette* - capable of 20 µL
(q) *Sterile filter tip pipette tips* - capable of 20 µL
(r) *Filter Stomacher® bags* – Seward or equivalent.
(s) *Stomacher®*– Seward or equivalent.
(t) *Partial immersion thermometer* – calibrated range to include 100 ± 1°C
(u) *Dry block heater unit*– capable of maintaining 100 ± 1°C
(v) *Incubators.* – Capable of maintaining 37 ± 1°C or 41.5 ± 1°C.
(w) *Refrigerator* – capable of maintaining 2-8°C, for storing the 3M Molecular Detection Assay components
(x) *Computer* – compatible with the 3M™ Molecular Detection Instrument
(y) *Modified TSB+Novobiocin* – Available from Mediabox™ Actero™ (Calgary, Alberta, Canada T2R 1J6)
(z) *OctoMACS™*
(aa) *Multistand to support OctoMACS*
(bb) *MACS® Large Cell Separation Columns*-Available from MiltenyBiotec (Gaithersburg, MD, 20878, USA)
(cc) *Dynabeads® anti-E. coli O157* (Dynal Inc., Lake Success, NY 11042, USA)
(dd) *Tryptic Soy Agar with 5% sheep blood (SBA)*-(TS-BD, Franklin Lakes, New Jersey 07417, USA; Sheep blood-Quad Five, Ryegate, Montana 59074, ,USA)
(ee) *Rainbow® Agar O157, Dehydrated* (Biolog Inc., Hayward California, 94545, USA)
(ff) *Novobiocin (5.0 mg/L)* (Alfa Aesar, Tewksbury, MA 01876, USA)
(gg) *Cefixime (0.05 mg/L)*(Sigma Aldrich, St. Louis, MO 63103, USA)
(hh) *Potassium Tellurite (*0.15 mg/L*)* (Oxoid, Hampshire RG24 8PW, UK)
(ii) *1N HCL* (Acros, New Jersey, USA)

(jj) *3M™ Petrifilm™ Rapid Aerobic Count* – Available from 3M Food Safety (St. Paul, MN, 55144-1000, USA)

(kk) *3M™ Rapid Plate Spreader* – Available from 3M Food Safety (St. Paul, MN, 55144-1000, USA)

C. General Instructions

(a) Store the 3M Molecular Detection Assay 2 –*E. coli* O157 (including H7) 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 - *E. coli* O157 (including H7) past the expiration date.

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

Safety Precautions

The 3M MDA 2 – *E. coli* O157 (including H7) 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 MDA 2 – *E. coli* O157 (including H7) 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 – *E. coli* O157 (including H7) 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 – *E. coli* O157 (including H7). Retain the safety instructions for future reference.

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 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.

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

D. Sample Enrichment

Foods

(a) Allow BPW ISO enrichment medium to pre-warm to 41.5±1°C. See Table A for matrix-specific enrichment protocols.

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

(c) Homogenize by stomacher, hand, or gentle agitation thoroughly for 2 ± 0.2 minutes. Incubate matrices according to the instructions provided in Table A.

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

Table A. 3M MDA 2 – *E. coli* O157 (including H7)enrichment protocolsusing pre-warmed BPW ISO at 41.5 ± 1°Caccording to AOAC *Official Method*℠ 2016.XX

Sample Matrix	Sample Size	Enrichment Broth Volume	Enrichment Time (hour)	Homogenized
Raw ground beef (73% lean)	325 g	975 mL	10-18	Manually by hand
Raw bagged spinach(a)	200 g	450 mL	18-24	Gently agitated by hand for 5 minutes
Fresh sprouts	25 g	225 mL	18-24	Stomach
Frozen blueberries(a)(b)	25 g	225 mL	18-24	Gently agitated by hand for 5 minutes

(a) Leafy produce and fruit samples should be gently agitated by hand for 5 minutes. Do not blend or stomach.
(b) Frozen samples should be equilibrated to 4-8°C before addition to enrichment broth.

F.Preparation of the 3M™ Molecular Detection Chill Block Insert

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

G. 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.

H. 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.

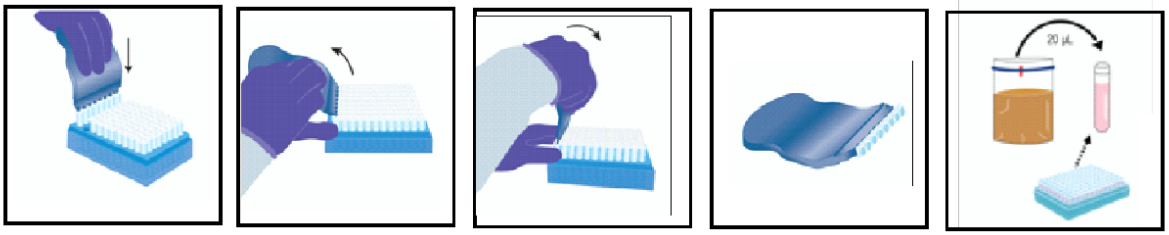
I. Lysis

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 hours.
3. Remove the enrichment broth from the incubator.
4. One LS tube is required for each sample and the Negative Control (NC) sample (sterile enrichment medium).
   - 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:

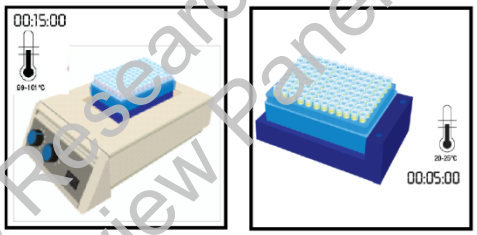
Transfer each enriched sample into individual LS tube **first**. Transfer the NC **last**.

   - 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 tubeunless otherwise indicated in the protocol table.

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. BPW) 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 3M 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.



J. 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.

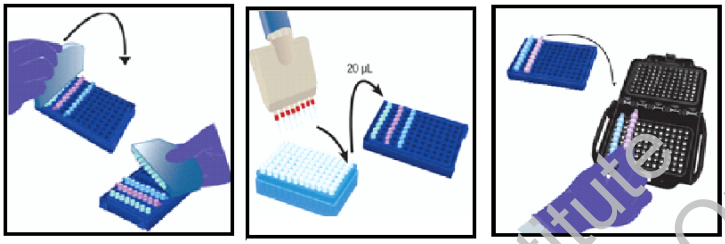
4.4 Cover the Reagent tubes with the provided extra cap and use the rounded side of the 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied.

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 <u>NC lysate</u> 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. 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 60 minutes, 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.

K. 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 confirmationbeginning with transfer from the primary BPW ISO enrichment to secondary enrichment broth(s), 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 - *E. coli* O157 (including H7) 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.

In the event of discordant results (presumptive positive with the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7), non-confirmed by one of the means described above, and in particular for the latex agglutination test), the laboratory must follow the necessary steps to ensure the validity of the results obtained.

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.

Results of Collaborative Study

For this qualitative, unpaired collaborative study, the 3M Molecular Detection Assay (MDA) 2 –*E. coli* O157 (including H7) method was compared to the USDA FSIS MLG 5.09 reference method for raw ground beef (325 g). A total of 15 laboratories throughout the United Statesparticipated in this study, with 10 laboratories submitting data for the raw ground beef. See Table 1 for a summary of laboratory participation. Each laboratory analyzed 36 test portions for each method: 12 inoculated with a high level of *E. coli* O157:H7, 12 inoculated with a low level of *E. coli* O157:H7, and 12 un-inoculated controls.
A background screen of the matrix indicated an absence of indigenous *E. coli* O157:H7 in the ground beef.Due to the amount of raw ground beef needed for the evaluation (~900lbs.), ten (10) replicate 325 g test portionswere screened for *E. coli* O157:H7. All test portions produced negative results for the target analyte.
The individual laboratory and sample results are presented in Table1 of the Supplementary Materials.
Table2016.1A summarizes the inter-laboratory results forthe ground beef, including POD statistical analysis [9].
As per criteria outlined in Appendix J of the AOAC Validation Guidelines, fractional positive results were obtained.For each matrix, the level of *E. coli* O157:H7was determined by MPN on the day of initiation of analysis by the coordinating laboratory. MPN results are presented in Tables 2016.2A.The APCresults for each collaborating laboratory are presented in Table 2of the Supplementary Materials.

Raw Ground Beef (73% Lean) (325 g Test Portions)

Raw ground beef test portions were inoculated at a low and high level and were analyzed (Table 1 of the Supplementary Materials) for the detection of *E. coli* O157:H7. Un-inoculated controls were included in each analysis. Laboratory 3 was unable to confirm their samples using the USDA method, Laboratory 7 reportedcontamination of the E buffer and was not able to return to the enrichments to re-confirm, Laboratory 10, 13, and 15 did not follow the alternative method such that results could not be used. All other laboratories submitted a full set of data for each method. The MPN levels obtained for this test portion, with 95% confidence

intervals, were 0.29 CFU/test portion(0.09,0.55) for the low inoculum level and 1.92 CFU/test portion (1.00, 3.42) for the high inoculum level.

For the low inoculum level, 32 out of 120 test portions (POD_{CP} of 0.27) were reported as presumptive positive by the 3M MDA 2 – *E. coli* O157 (including H7)method with 31 test portions (POD_{CC} of 0.26) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *E. coli* O157 (including H7) method, 29 samples confirmed positive (POD_C of 0.24). For test portions evaluated by the USDA/FSIS MLG reference method, 42 out of 120 test portions produced positive results (POD_R of 0.35). A $dLPOD_C$ value of -0.11 with 95% confidence intervals of (-0.22, 0.01) were 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 (-0.10, 0.12) were obtained between presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results.

For the high inoculum level, 97 out of 120 test portions (POD_{CP} of 0.81) were reported as presumptive positive by the 3M MDA 2 – *E. coli O157 (including H7)* method with 96test portions (POD_{CC} of 0.80) confirming positive.For samples that produced presumptive positive results on the 3M MDA 2 – *E. coli* O157 (including H7)method, 92 samples confirmed positive (POD_C of 0.77). For test portions evaluated by the USDA/FSIS MLG reference method, 95 out of 120 test portions produced positive results (POD_R of 0.79). A $dLPOD_C$ value of -0.025 with 95% confidence intervals of (-0.129, 0.080) wasobtained between the candidate and reference method, indicating no statistical significant difference between the two methods. A $dLPOD_{CP}$ value of 0.008 with 95% confidence intervals of (-0.092, 0.109) wasobtained between presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results.

For the un-inoculated controls, zero out of 120 samples (POD_{CP} of 0.00) produced a presumptive positive result by the 3M MDA 2 - *E. coli* O157 (including H7) method with 2test portions (POD_{CC} of 0.02) confirming positive. For samples that produced presumptive positive results on the 3M MDA 2 – *E. coli* O157 (including H7)method, zerosamples confirmed positive (POD_C of 0.00). For test portions evaluated by the USDA/FSIS MLG reference method, 3 out of 120 test portions produced positive results. Colonies isolated from the three test portions were further characterized utilizing partial sequencing of the 16S rRNA gene; isolates were not identified as *E. coli* O157:H7. A $dLPOD_C$ value of 0.017with 95% confidence intervals of (-0.059, 0.017) wasobtained between the confirmed 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 (-0.031, 0.031) wasobtained between presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results. POD statistical analysis for the un-inoculated test samples were conducted on the revised results (See Table 2016.2A) from laboratory 11 and 12 after 16S rRNA sequencing.

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

Discussion

While three laboratories experienced technical issues during the evaluation, no negative feedback was provided by the collaborating laboratories in regard to the performance of the 3M MDA 2- *E. coli* O157 (including H7) method.During the evaluation, three laboratories culturally confirmed*E. coli* O157:H7in their (blinded) un-inoculated control samples, see Table 2016.2A.The two test portions from Laboratory 5 were unavailable for further identification as the collaborative study plates had been disposed.However, laboratories 11 and 12 submitted their samples directly to a third party laboratory capable of performing 16S rRNA sequencing for additional identification.The inoculating strain *E. coli* O157:H7 ATCC 43895 was also analyzed as the control. Theprotocol and results for 16S rRNA sequencing can be found in Supplementary Material. Utilizing the Basic Local Alignment Search Tool (BLAST®) for nucleotides [10], the isolates were determined to 1) not be the inoculating strain *E. coli* O157:H7 ATCC 43895 and 2) not *E. coli*. Serological cross reactivity of other members of the microbial community for raw beef products has been documented due to the close relationship among species [11, 12]. This indicates that the 3M Molecular Detection Assay 2 – *E. coli* O157 (including H7) is more

specific and accurate for identifying*E. coli* O157:H7 in 325 g of raw ground beef than the USDA MLG cultural method.

Overall, the data generated during this evaluation demonstrates the reproducibility of the 3M Molecular Detection Assay 2 – *E. coli* O157 (including H7) method. For each matrix, the POD statistical analysis indicated that there is no statistically significant difference between the candidate method and the reference method or between the presumptive and confirmed results of the candidate method.

Recommendations

It is recommended that the 3M Molecular Detection Assay 2 – *E. coli* O157 (including H7)method be adopted as Official First Action status for the detection of *E. coli* O157:H7in selected foods: raw ground beef (73% lean), frozen blueberries, fresh sprouts, and fresh babyspinach.

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References

(1)ISO 6579. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. Annex B.

(2) *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 July 2016)

(3)U.S. Food and Drug Administration Bacteriological Analytical Manual, (2011)*Chapter 4A: Diarrheagenic*

Escherichia coli. (Accessed November 2015). http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070080.htm.

(4) United States Department of Agriculture Food Safety and Inspection Service. (2015) *Microbiological Laboratory Guidelines5.09*: *Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges* (Accessed July 2016)http://www.fsis.usda.gov/wps/wcm/connect/51507fdb-dded-47f7-862d-ad80c3ee1738/MLG-5.pdf?MOD=AJPERES

(5) *Official Methods of Analysis* of AOAC INTERNATIONAL (2015) *Enumeration of Aerobic Bacteria in Foods 3M™ Petrifilm™ Rapid Aerobic Count Plate.*2015.13. (Accessed July 2016)

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

(7) *Official Methods of Analysis* of AOAC INTERNATIONAL (2012) *AOAC Official Method 2011.17Salmonella, Escherichia coli,and Other Enterobacteriaceae VITEK® 2 Gram-Negative (GN)Biochemical Identification Method*(Accessed June 2016).

(8) Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook (2011), http://lcfltd.com/aoac/aoac-binary-v2-3.xls (Accessed July 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) Basic Local Alignment Search Tool (BLAST) nucleotide. https://blast.ncbi.nlm.nih.gov/Blast.cgi (Accessed September 2016)

(11) Uhitil,S., Jaksic, S., Petrak, T., Botka-Petrak, K. (2001) Journal of Food Protection *Presence of Escherichia coli O157:H7 in Ground Beef and Ground Baby Beef Meat.* Vol. 64, No. 6. Pp. 862-864

(12) Ercolini, D., Russo, F., Nasi, A. et al. (2009) Applied and Environmental Microbiology *Mesophilic and Psychrotropic Bacteria from Meat and Their Spoilage Potential in Vitro and in Beef.* Vol. 75, No. 7. Pp. 1990-2001.

Table 1: Participation of each Collaborating Laboratory[a]

Lab	Raw Ground Beef[a]
1	Y
2	Y
3	Y[b]
4	Y
5	Y
6	Y
7	Y[b]
8	Y
9	Y
10	Y[b]
11	Y
12	Y

13	Y[b]
14	Y
15	Y[b]

[a] Y= Collaborator analyzed the food type;[a]N= Collaborator did not analyze food type
[b] Results were not used in statistical analysis due to laboratory error

Table 2016.1A: Summary of Results for the Detection of *E. coli* O157:H7 in Raw Ground Beef (325g)

Method[a]	**3M™ MDA™ 2 *E. coli* O157 (including H7)**		
Inoculation Level	**Un-inoculated**	**Low**	**High**
Candidate Presumptive Positive/ Total # of Samples Analyzed	0/120	32/120	97/120
Candidate Presumptive POD (CP)	0.00 (0.00, 0.03)	0.27 (0.19, 0.35)	0.81 (0.67, 0.93)
s_r[b]	0.00 (0.00, 0.00)	0.45 (0.40, 0.52)	0.37 (0.33, 0.42)
s_L[c]	0.00 (0.00, 0.17)	0.00 (0.00, 0.16)	0.15 (0.07, 0.32)
s_R[d]	0.00 (0.00, 0.24)	0.45 (0.40, 0.52)	0.40 (0.35, 0.50)
P Value[e]	1.00	0.760	0.005
Candidate Confirmed Positive/ Total # of Samples Analyzed	2/120	31/120	96/120
Candidate Confirmed POD (CC)	0.02 (0.00, 0.06)	0.26 (0.18, 0.34)	0.80 (0.69, 0.91)
s_r	0.12 (0.11, 0.16)	0.45 (0.40, 0.52)	0.38 (0.34, 0.44)
s_L	0.04 (0.00, 0.09)	0.00 (0.00, 0.13)	0.13 (0.04, 0.29)
s_R	0.13 (0.12, 0.16)	0.45 (0.40, 0.51)	0.40 (0.36, 0.49)
P Value	0.0.031	0.920	0.018
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/120	29/120	92/120
Candidate Presumptive Positive that Confirmed POD (C)	0.00 (0.00, 0.03)	0.24 (0.16, 0.32)	0.77 (0.63, 0.91)
s_r	0.00 (0.00, 0.17)	0.44 (0.39, 0.51)	0.39 (0.34, 0.45)
s_L	0.00 (0.00, 0.17)	0.00 (0.00, 0.13)	0.18 (0.09, 0.37)
s_R	0.00 (0.00, 0.24)	0.44 (0.39, 0.50)	0.43 (0.38, 0.52)
P Value	1.00	0.908	0.002
Positive Reference Samples/ Total # of Samples Analyzed	0/120	42/120	95/120
Reference POD	0.00 (0.00, 0.03)	0.35 (0.26, 0.44)	0.79 (0.67, 0.92)
s_r	0.00 (0.00, 0.17)	0.48 (0.42, 0.52)	0.38 (0.34, 0.44)
s_L	0.00 (0.00, 0.17)	0.05 (0.00, 0.23)	0.15 (0.07, 0.33)
s_R	0.00 (0.14, 0.20)	0.48 (0.43, 0.52)	0.41 (0.37, 0.51)
P Value	1.00	0.342	0.01
dLPOD (Candidate vs. Reference)[f]	-0.017 (-0.059, 0.017)	-0.11 (-0.22, 0.01)	-0.025 (-0.129, 0.080)
dLPOD (Candidate Presumptive vs. Candidate Confirmed)[f]	-0.000 (-0.031, 0.031)	0.01 (-0.10, 0.12)	0.008 (-0.092, 0.109)

[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 *E. coli* O157:H7in Raw Ground Beef (73% Lean) by the 3M™ MDA 2 –*E. coli* O157 (including H7) Method vs. USDA/FSIS MLG Chapter 5.09 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 Ground Beef (73% Lean)/ Un-inoculated	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	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		4	12	0	0.00	NA	0	0.00	NA	0	0.00	12	0	0.00	0.00	0.00
		5	12	0	0.00	12	2	0.167	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[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		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	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		11	12	0	0.00	12	0[f]	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[f]	0.00	-0.00	0.00
		13[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		14	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Estimate		All	120	0	0.00	120	2	0.02	120	0	0.00	120	0	0.00	-0.000	0.017
LCL					0.00			0.00			0.00			0.00	-0.031	-0.059
UCL					0.03			0.06			0.03			0.03	0.031	0.017
s_r[b]					0.00			0.12			0.00			0.00		
LCL					0.00			0.11			0.00			0.00		
UCL					0.17			0.17			0.17			0.17		
s_L[c]					0.00			0.04			0.00			0.00		
LCL					0.00			0.00			0.00			0.00		
UCL					0.17			0.09			0.17			0.17		
s_R[d]					0.00			0.13			0.00			0.00		
UCL					0.00			0.12			0.00			0.00		
LCL					0.24			0.16			0.24			0.24		
P_T[e]					1.00			0.031			1.00			1.00		

[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, [f] Original results identified this test sample(s) as *E. coli* O157:H7; reversed due to 16S rRNA results, LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

Table 2016.2A (cont'd.): Comparative Results for the Detection of *E. coli* O157:H7in Raw Ground Beef (73% Lean) by the 3M™ MDA 2 - *E. coli* O157 (including H7) Method vs. USDA/FSIS MLG Chapter. 5.09in 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 Ground Beef (73% Lean)/ Low	1	12	2	0.167	12	2	0.167	12	2	0.167	12	2	0.167	0.00	0.00
		2	12	5	0.417	12	3	0.250	12	3	0.250	12	5	0.417	-0.167	0.167
		3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		4	12	2	0.167	NA	2	0.167	NA	2	0.167	12	4	0.333	-0.167	0.00
		5	12	2	0.167	NA	2	0.167	NA	2	0.167	NA	4	0.333	-0.167	0.00
		6	12	5	0.417	12	5	0.417	12	5	0.417	12	7	0.583	-0.167	0.00
		7[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		8	12	3	0.250	12	3	0.250	12	3	0.250	12	1	0.083	0.167	0.00
		9	12	3	0.250	12	3	0.250	12	3	0.250	12	4	0.333	-0.083	0.00
		10[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		11	12	4	0.333	12	3	0.250	12	3	0.250	12	4	0.333	-0.083	0.083
		12	12	4	0.333	12	4	0.333	12	4	0.333	12	5	0.417	-0.083	0.00
		13[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MPN/ Test Portion	14	12	2	0.167	12	4	0.333	12	2	0.167	12	6	0.500	-0.333	-0.167
		15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Estimate		All	120	32	0.27	120	31	0.26	120	29	0.24	120	42	0.35	-0.11	0.01
LCL	0.29				0.19			0.18			0.16			0.26	-0.22	-0.10
UCL	0.09-				0.35			0.34			0.32			0.44	0.01	0.12
s_r[b]	0.55				0.45			0.45			0.44			0.48		
LCL					0.40			0.40			0.39			0.42		
UCL					0.52			0.52			0.51			0.52		
s_L[c]					0.00			0.00			0.00			0.05		
LCL					0.00			0.00			0.00			0.00		
UCL					0.16			0.13			0.13			0.23		
s_R[d]					0.45			0.45			0.44			0.48		
UCL					0.40			0.40			0.39			0.43		
LCL					0.52			0.51			0.50			0.52		
P_T[e]					0.760			0.920			0.908			0.342		

[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 *Salmonella* in Raw Ground Beef (73% Lean) by the 3M™ MDA 2 - *E. coli* O157 (including H7) Method vs. USDA/FSIS MLG Chapter. 5.09 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 Ground Beef (73% Lean)/ High	1	12	6	0.500	12	6	0.500	12	6	0.500	12	8	0.667	-0.167	0.00
		2	12	12	0.917	12	11	0.917	11	11	0.917	12	11	0.917	0.00	0.083
		3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		4	12	12	1.000	12	12	1.000	12	12	1.000	12	9	0.750	0.250	0.00
		5	12	8	0.667	12	8	0.667	12	6	0.500	12	10	0.833	-0.333	0.00
		6	12	12	1.000	12	12	1.000	12	12	1.000	12	11	0.917	0.083	0.00
		7[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		8	12	11	0.917	12	11	0.917	12	11	0.917	12	12	1.000	-0.083	0.00
		9	12	11	0.917	12	11	0.917	12	11	0.917	12	11	0.917	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	7	0.583	12	8	0.667	12	6	0.500	12	10	0.833	-0.333	-0.083
		12	12	9	0.750	12	9	0.750	12	9	0.750	12	4	0.333	0.417	0.00
		13[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		14	12	9	0.750	12	8	0.667	12	8	0.667	12	9	0.750	-0.083	0.083
		15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Estimate		All	120	97	0.81	120	96	0.80	120	92	0.77	120	95	0.79	-0.025	0.008
LCL	1.92				0.67			0.69			0.63			0.67	-0.129	-0.092
UCL	1.00-3.42				0.93			0.91			0.91			0.92	0.080	0.109
s_r[b]					0.37			0.38			0.39			0.38		
LCL					0.33			0.34			0.34			0.34		
UCL					0.42			0.44			0.45			0.44		
s_L[c]					0.15			0.13			0.18			0.15		
LCL					0.07			0.04			0.09			0.07		
UCL					0.32			0.29			0.37			0.33		
s_R[d]					0.40			0.40			0.43			0.41		
UCL					0.35			0.36			0.38			0.37		
LCL					0.50			0.49			0.52			0.51		
P_T[e]					0.005			0.018			0.002			0.01		

[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

Supplementary Materials

Table 1. Individual Collaborator Results for Raw Ground Beef (73% Lean)

	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 -*E. coli O157 (including H7)*																																				
Lab[a]																																				
1	+	-	-	+	-	+	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	+	+	+	-[b]	+	+	+	+	+	+	+	+	-	-[b]	-	+	-	+	-	-	+	-	-	-[b]	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+[c]	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-
4	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
5	+[c]	+	+	+	-[b]	-	+	+[c]	+	-	+	-[b]	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+[c]	+[c]	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
8	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	+	-	-	-	+[c]	+[c]	+[c]	-[b]	-	-	+	-	-	+	-	-	+[c]	-	-	-	-	-	-[b]	-	-	-	-	-	-	-	-	-	-	-	-	-
11	+	+	+[c]	-	-	-	+	+	-[b]	+	+[c]	+	+	+	-	-	-	-	-	-	+	-[b]	-	-[d]	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	-	+	+	+	-	+	-	+	+	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
14	+	-	-	-[b]	+	+	+	+	+	+	+	-	+	-	+	-	-	+[c]	-	-	-	-	+[c]	-	-	-	-	-	-	-	-	-	-	-	-	-
15	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
USDA/FSIS - MLG 5.09																																				
Lab[a]																																				
1	+	+	+	-	+	+	+	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	-	+	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-
4	+	+	+	+	+	-	-	+	-	+	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10	-	+	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-	-
11	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	-	-	+	-	-	+	+	-	-	-	-	-	-	-	+	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-[d]	-	-	-[d]	-
13	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
14	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

[a] += *E. coli* O157 were detected in samples; -= *E. coli* O157 (including H7) 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 – *E. coli* O157 (including H7) but confirmed negative indicating a false positive result;
[c] Sample was presumptive negative on 3M MDA 2 - *E. coli* O157 (including H7) but confirmed positive indicating a false negative result; [d] Original results were positive, revised after 16S rRNA

Table 2: Results of Aerobic Plate Count for Collaborating Laboratories

Lab	Raw Ground Beef (CFU/g)
1	4.2×10^6
2	7.8×10^5
3	4.2×10^6
4	1.5×10^6
5	8.8×10^5
6	2.2×10^6
7	5.8×10^2
8	4.2×10^5
9	2.2×10^6
10	2.1×10^5
11	2.5×10^6
12	4.2×10^5
13	2.3×10^5
14	4.8×10^6
15	40

Figure 1A: POD Values of Candidate Method vs. Reference Method for 325 g Raw Ground Beef (73% lean)

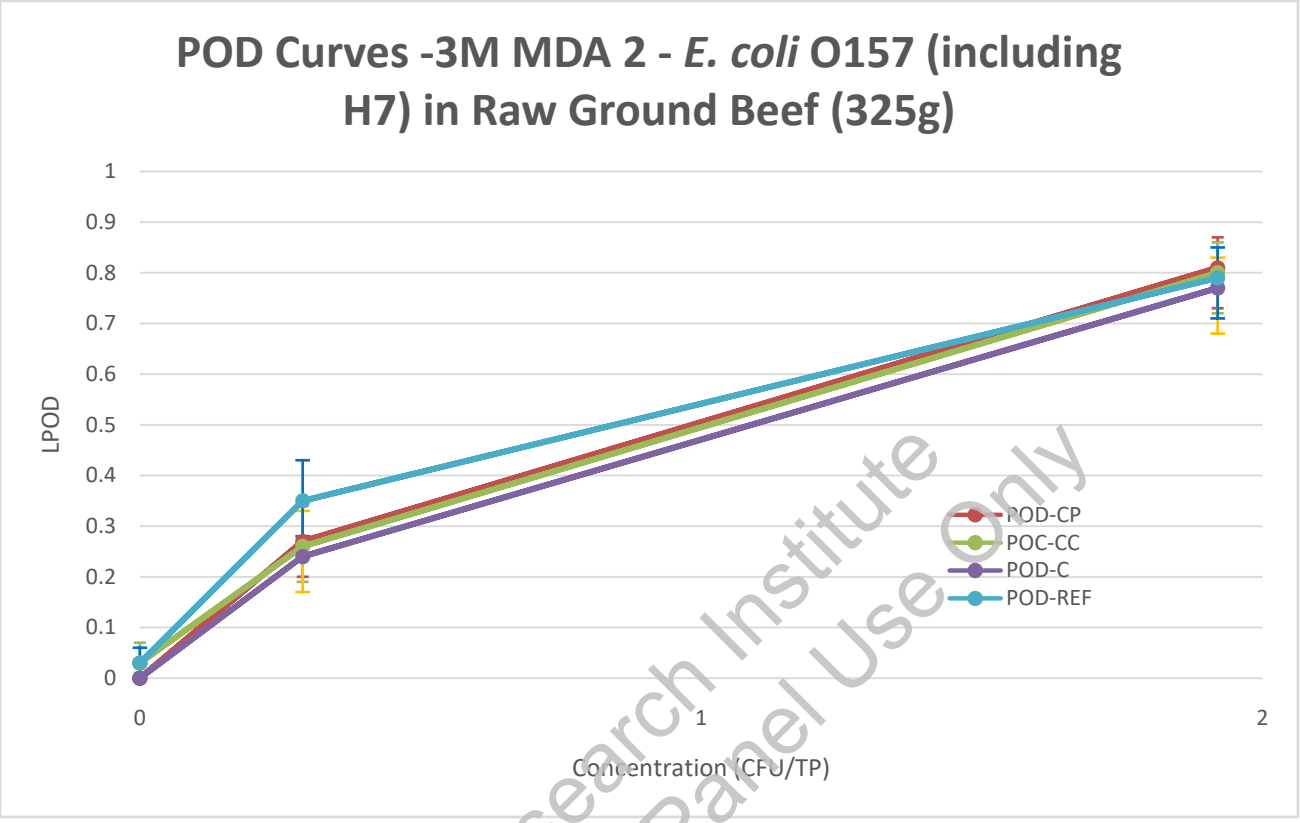
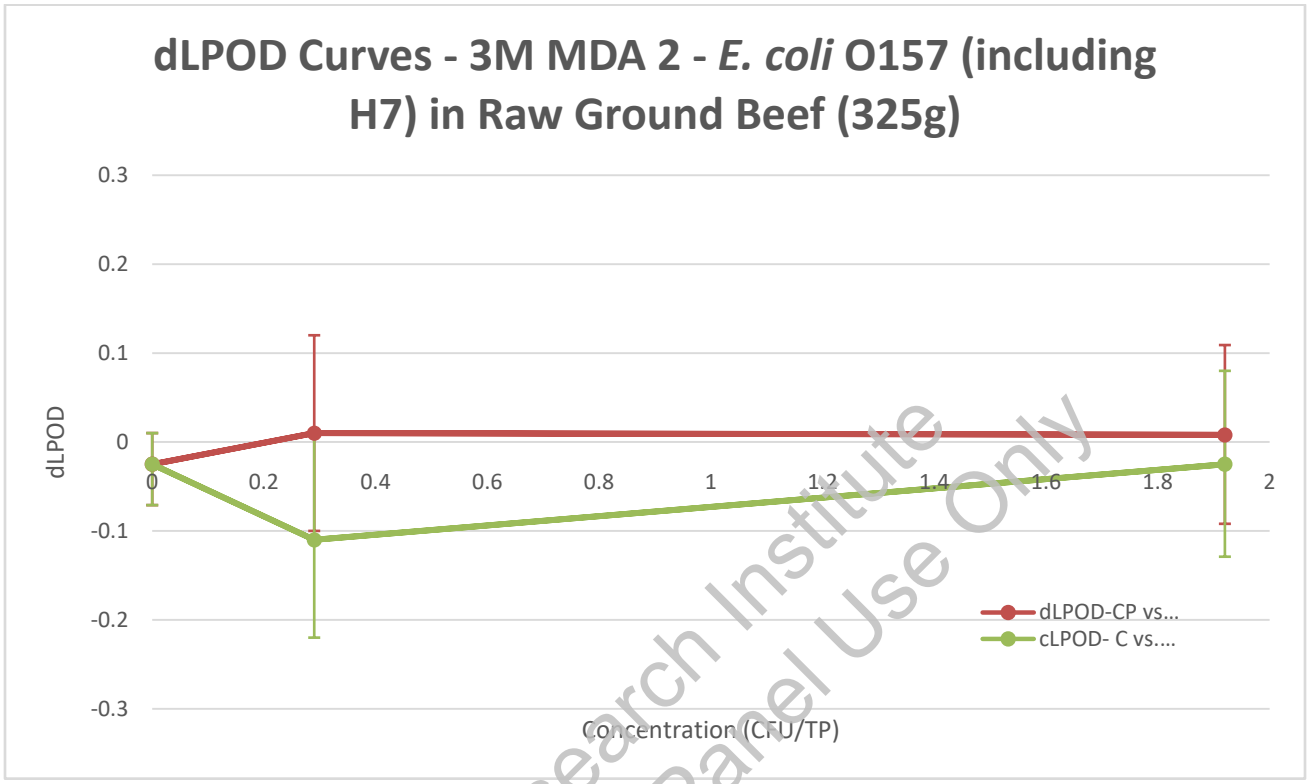


Figure 1B: dPOD Values of Candidate Method vs. Reference Method for 325 g Raw Ground Beef (73%)



Inclusivity/Exclusivity (Internal Studies)

Table 3: Source of Test Organisms Used for Testing of Inclusivity and Exclusivity

Strains	#s	Testing Lab
Inclusives	1 through 15	Q Laboratories Inc., (Cincinnati Ohio)
	16 through 50	3M (St. Paul, Minnesota)
Exclusives	ALL (1 through 50)	3M (St. Paul, Minnesota)

Inclusivity Methodology

Fifty frozen *E. coli* O157strain suspensions were thawed and sub-cultured on blood agar plates and incubated at 41.5 °C ± 1°C for 18-24 hours. Single isolated colonies were sub-cultured from agar intoBPW ISO broth and incubated for eight (8) hours at 41.5 °C ± 1°C. The cultures were diluted in fresh BPW ISO broth in order to obtain concentrations of approximately E+05 to E+06 CFU/mL and were then tested immediately using the 3M MDA2ECO96 kit.

ExclusivityMethodology

Fifty frozen non- *E. coli* O157strain suspensions were thawed and sub-cultured on blood agar plates and incubated at 37 ^{0}C for approximately 24 hours. Single isolated colonies were sub-cultured from agar into Tryptic® soy broth (TSB) broth and incubated at 35-37 ^{0}C for 18-24 hours.The cultures were diluted in fresh BPW ISO in order to obtain concentrations of approximately E+07 to E+08 CFU/mL and were then tested immediately using the 3M MDA2ECO96 kit.

Results

1. All 50 *E. coli* O157strains were correctly detected as positives by the 3MMDA2ECO96 kit/method.
2. None of the 50 non-*E. coli* O157strains were detected by the 3MMDA2ECO96 kit/method.
3. See Tables 4 and 5 for key to culture collection ID #s.
4. See Tables 5 and 6 for culture details and results.

Table 4. Culture Collection Key

Acronym	Collection / obtained from
ATCC	American Type Culture Collection
CDC	US Centers for Disease Control and Prevention
FDA	US Food and Drug Administration
FSD	3M Food Safety Culture Collection
MSU	Michigan State University STEC Center
NCTC	National Collection of Type Cultures
PSU	Pennsylvania State University *E. coli* Reference Center
QL	Q Laboratories, Inc., Cincinnati, Ohio 45214
TICC	3M Tecra™ Culture Collection

Table 5. *Escherichia coli* O157 – Inclusive Cultures

#	Serotype	ID #	Strain Information	MDA2ECO96 Result after 8 hours at 41.5 °C	CFU/mL tested
1	O157:H7	MSU# TW02302	Hamburger isolate	Pos	8.0E+05
2	O157:H7	MSU# DEC4A	Cow isolate	Pos	1.2E+05
3	O157:H7	MSU# DEC4C	Buffalo isolate	Pos	1.4E+05
4	O157:H7	QL# 14077.6	Ground beef isolate	Pos	3.0E+05
5	O157:H7	QL# 164673	Ground beef isolate	Pos	1.6E+05
6	O157:H7	QL# 2-202	Ground beef isolate	Pos	1.9 E+05
7	O157:H7	QL# 2-214	Beef trim isolate	Pos	7.0 E+05
8	O157:H7	QL# 2-701	Beef trim isolate	Pos	1.6 E+05
9	O157:H7	QL# 2-710	Beef trim isolate	Pos	2.8 E+05
10	O157:H7	QL# 14077.1	Meat isolate	Pos	2.5 E+05
11	O157:H7	QL# 14077.2	Meat isolate	Pos	4.4 E+05
12	O157:H7	QL# 14077.3	Salami isolate	Pos	7.8 E+05
13	O157:H7	QL# 14077.4	Apple cider isolate	Pos	1.2 E+05
14	O157:H7	QL# 14077.5	Outbreak isolate	Pos	5.8 E+05
15	O157:H7	QL# 14077.7	Ground beef isolate	Pos	3.6 E+05
16	O157:H7	ATCC 700728	Not available	Pos	1.01E+06
17	O157:H7	FSD 733	3M	Pos	6.10E+05
18	O157:H7	FSD 734	3M	Pos	6.80E+05
19	O157:H7	FSD 736	Not available	Pos	5.80E+05
20	O157:H7	ATCC 43894	Human feces, outbreak of hemorrhagic colitis, Michigan	Pos	6.50E+05
21	O157:H7	ATCC 35150	Feces, human	Pos	1.03E+06
22	O157:H7	3M RC43R	3M	Pos	4.90E+05
23	O157:H7	3M K20	3M	Pos	5.40E+05
24	O157:H7	ATCC BAA-1882	Bovine feces	Pos	4.10E+05
25	O157:H7	ATCC BAA-460	Human feces, 1996, Japan	Pos	5.40E+05
26	O157:H7	PSU 8.0416	Ground beef isolate	Pos	9.70E+05

27	O157:H7	PSU 8.0566	Ground beef isolate	Pos	1.01E+06
28	O157:H7	ATCC 700531	Not available	Pos	8.70E+05
29	O157:H7	ATCC 700599	Not available	Pos	9.20E+05
30	O157:H-	FSD 808	3M	Pos	8.40E+05
31	O157:H-	FSD 724	3M	Pos	6.30E+05
32	O157:H-	FSD 725	3M	Pos	9.15E+05
33	O157:H-	FSD 726	3M	Pos	9.80E+05
34	O157:H-	FSD 728	3M	Pos	8.40E+05
35	O157:H-	FSD 730	3M	Pos	8.00E+05
36	O157:H-	FSD 731	3M	Pos	7.90E+05
37	O157:N	PSU 93.0134	Ground beef isolate	Pos	9.50E+05
38	O157:N	PSU 93.0135	Ground beef isolate	Pos	1.01E+06
39	O157:N	PSU 93.0136	Ground beef isolate	Pos	7.00E+05
40	O157:N	PSU 93.0137	Ground beef isolate	Pos	9.40E+05
41	O157:H5	PSU 3.1177	Ground beef isolate	Pos	1.15E+06
42	O157:H5	PSU 3.1180	Ground beef isolate	Pos	6.70E+05
43	O157:H5	PSU 3.1178	Ground beef isolate	Pos	9.40E+05
44	O157:H12	PSU 7.1171	Ground beef isolate	Pos	1.09E+06
45	O157:H12	PSU 3.0370	Ground beef isolate	Pos	9.70E+05
46	O157:H29	PSU 3.1162	Ground beef isolate	Pos	7.50E+05
47	O157:H29	PSU 3.1163	Ground beef isolate	Pos	8.30E+05
48	O157:H42	PSU 6.1659	Ground beef isolate	Pos	1.01E+06
49	O157:NM	ATCC 700377	Not available	Pos	1.08E+06
50	O157	PSU 7.1274	Ground beef isolate	Pos	7.10E+05

Table 6. Non-*E. coli*O157 – Exclusive Cultures

#	Genus	Species	Serotype	ID #	MDA2ECO96 Results	CFU/ml tested
1	*Citrobacter*	*brakii*	n/a	ATCC 29063	Neg	9.00E+07
2	*Citrobacter*	*freundii*	n/a	ATCC 8090	Neg	7.30E+07
3	*Citrobacter*	*koseri*	n/a	ATCC 27156	Neg	1.09E+08
4	*Cronobacter*	*sakazakii*	n/a	ATCC 29544	Neg	1.35E+07
5	*Cronobacter*	*maloniticus*	n/a	(FDA) E615	Neg	8.35E+07
6	*Cronobacter*	*dublinensis*	n/a	CDC 0743-75	Neg	5.35E+07
7	*Cronobacter*	*condimenti*	n/a	(FDA) LMG 26250	Neg	9.85E+07
8	*Edwardsiella*	*tarda*	n/a	NCTC 10396	Neg	7.95E+07
9	*Enterobacter*	*amnigenus*	n/a	ATCC 51816	Neg	6.65E+07
10	*Enterobacter*	*cloacae*	n/a	ATCC 23355	Neg	5.15E+07
11	*Enterobacter*	*hormechei*	n/a	ATCC 700323	Neg	9.55E+07
12	*Escherichia*	*coli*	O103:H2	TICC 2431	Neg	5.30E+07
13	*Escherichia*	*coli*	O111:NM	TICC 2432	Neg	1.75E+07
14	*Escherichia*	*coli*	O91:H-	TICC 2480	Neg	2.85E+07
15	*Escherichia*	*coli*	O26:H11	TICC 2481	Neg	4.70E+07
16	*Escherichia*	*coli*	O91:H21	TICC 2483	Neg	6.30E+07
17	*Escherichia*	*coli*	O111:H2	TICC 2484	Neg	6.10E+05
18	*Escherichia*	*coli*	O118:H-	TICC 2485	Neg	4.95E+07
19	*Escherichia*	*coli*	O46:H16	TICC 2486	Neg	5.50E+07
20	*Escherichia*	*coli*	O88:H-	TICC 2487	Neg	7.20E+07
21	*Escherichia*	*coli*	O111:H-	TICC 2488	Neg	7.55E+07
22	*Escherichia*	*coli*	O:108	TICC 2929	Neg	4.95E+07
23	*Escherichia*	*coli*	O:121	TICC 2930	Neg	6.90E+07
24	*Escherichia*	*coli*	O:114	TICC 3128	Neg	9.60E+07
25	*Escherichia*	*coli*	O:55	TICC 3129	Neg	4.13E+08
26	*Escherichia*	*coli*	O:124	TICC 3130	Neg	7.40E+07

27	*Escherichia*	*coli*	n/a	ATCC 25922	Neg	5.90E+07
28	*Escherichia*	*coli*	n/a	ATCC 35421	Neg	4.05E+07
29	*Escherichia*	*coli*	n/a	ATCC 51813	Neg	5.75E+07
30	*Escherichia*	*coli*	n/a	ATCC 35401	Neg	4.65E+07
31	*Escherichia*	*coli*	n/a	ATCC 700609	Neg	4.25E+07
32	*Escherichia*	*hermanii*	n/a	TICC 3084	Neg	8.60E+07
33	*Escherichia*	*vulneris*	n/a	TICC 4479	Neg	3.85E+07
34	*Hafnia*	*alvei*	n/a	ATCC 51815	Neg	8.55E+07
35	*Klebsiella*	*oxytoca*	n/a	ATCC 51817	Neg	8.85E+07
36	*Klebsiella*	*oxytoca*	n/a	ATCC 700324	Neg	9.55E+07
37	*Klebsiella*	*pneumoniae*	n/a	ATCC 13882	Neg	1.22E+08
38	*Klebsiella*	*oxytoca*	n/a	ATCC 51817	Neg	8.85E+07
39	*Pantoea*	*agglomerans*	n/a	ATCC 53769	Neg	8.20E+06
40	*Proteus*	*mirabilis*	n/a	ATCC 25933	Neg	1.56E+08
41	*Proteus*	*mirabilis*	n/a	ATCC 29245	Neg	1.20E+08
42	*Proteus*	*vulgaris*	n/a	ATCC 13315	Neg	9.75E+07
43	*Salmonella*	*enterica*	Typhimurium	ATCC 51812	Neg	1.82E+08
44	*Salmonella*	*enterica*	Enteritidis	ATCC 13076	Neg	8.15E+07
45	*Serratia*	*ficaria*	n/a	TICC 4475	Neg	9.50E+07
46	*Serratia*	*liquefaciens*	n/a	ATCC 51814	Neg	5.25E+07
47	*Serratia*	*marcesens*	n/a	ATCC 14041	Neg	4.70E+07
48	*Shigella*	*sonnei*	n/a	ATCC 25931	Neg	7.80E+06
49	*Yersinia*	*enterocolitica*	n/a	ATCC 23715	Neg	9.00E+07
50	*Yersinia*	*kristensii*	n/a	ATCC 33639	Neg	5.95E+07

3M Molecular Detection Assay 2 – *E. coli* O157 (including H7) collaborative isolates needing additional identification by 16S rRNA sequencing.

Data generated by: NSF International,789 North Dixboro, Ann Arbor, MI 48105

Methodology

1. DNA Extraction for Sequencing

Sterile loops were used to collect sufficient mass of isolated colonies. Colonies were suspended in solution of 180 µL of Qiagen ATL buffer and 20 µL proteinase K. Samples were incubated overnight at 56 ± 1°C to complete lysis. The sample lysate was then purified via Qiagen silica-gel spin-columns and eluted with 10 mMTris HCL. The gDNA products were quantified via dsDNA Qubit fluorometry and purity was assessed using a NanoDrop N-1000 spectrophotometer; see Table 7 for quantification and quality control results.

2. Library Preparation for Sequencing

The sequencing libraries were prepared according to the QiaseqFX DNA Library kit protocol. Tris HCL buffer was used as the diluent for the input gDNA. A manual hand-pump centrifuge (RPI HandyFuge) was used for all 96-well plate centrifuge steps. A Bio-Analyzer was not available for the optional QC product cleanup.

Sample libraries were quantified via fluorometry, normalized, and pooled at 10pM according to the MiSeq V2 Reagent kit protocol. Illumina PhiX V3 Control DNA was diluted to 12.5 pM and spiked at 5% (vol/vol) of the library pool, as recommended for low-diversity libraries. The pooled libraries were loaded and sequenced using a MiSeq V2 500-cycle reagent kit with 250bp paired-end reads.

3. Data Handling and Analysis

Raw data sequences, in the form of paired FASTQ files, were first analyzed using the FastQC software (V0.11.5) to determine quality. Sequence reads were then submitted to a scripted pipeline, A5-Miseq (v20160825), which automates the data cleaning, alignment, and assembly. Finally RNAmmer (v1.2, server) was used to extract the 16S rRNA sequence from the draft assemblies. The isolated 16S sequence was provide in the form of FASTA files.

References

A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Coil D, Jospin G, Darling AE. Bioinformatics. 2015 15;31(4):587-9. Doi: 10.1093

Results

1. 16S rRNA SSU sequences. Provided in Appendix A. In addition to sequences provided in Appendix A, FASTA files are also being provided along with this report. However, due to the insecure nature of these FASTA files, they are being provided only as a courtesy.

Table 7. Isolate gDNA Quality Check Data

Isolate ID	ng/µL (Qubit)	A260/A280 (nanodrop)	A260/230 (Nanodrop)

Ec 43895	59.2	2.06	2.17
NSF153	30.3	2.04	2.01
Q111	22.7	2.18	2.19
Q134	27.7	2.01	2.09
Q144	26.0	2.07	2.14

Appendix A: 16s rRNA SSU sequences

Ec43895 /molecule=16s_rRNA

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

Detection of *E. coli* O157:H7 in Selected Foods by the 3M™ Molecular Detection Assay 2 (MDA2) – *E. coli* O157 (including H7): Collaborative Study Protocol

OMA 2016-May-XXXX

DRAFT DOCUMENT

Table of Contents

INTRODUCTION

The goal of this collaborative study is to estimate the following performance parameters of the 3M™ Molecular Detection Assay 2 (MDA2) - *E. coli* O157 (including H7): probability of detection (POD) and the difference in the POD at 3 contamination levels for both the candidate and reference methods. Test portions from 3 different contamination levels, including negative controls, will be sent to collaborators where they will perform the 3M MDA2 - *E. coli* O157:H7 method along with the corresponding reference method. Raw fresh ground beef will be tested with 325 g test portion sizes.

1.1 Description of the 3M™ MDA2 - *E. coli* O157 (including H7)

The 3M™ Molecular Detection Assay 2 - *E. coli* O157 (including H7) is used with the 3M™ Molecular Detection System for the rapid and specific detection of *E. coli* O157 (including H7) in enriched food, feed and food process 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.

The 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) 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 pharmaceutical, cosmetics, clinical or veterinary samples. The 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) has not been evaluated with all possible food products, food processes, testing protocols or with all possible strains of bacteria.

1.2 Summary of Pre-collaborative Study

The 3M MDA2 - *E. coli* O157 method was validated according to AOAC Guidelines (2012) in a pre-collaborative study. The aim of this study was to demonstrate that the 3M MDA2 - *E. coli* O157 method could detect *E. coli O157* in select foods as claimed by the manufacturer.

The 3M MDA2 - *E. coli* O157 was evaluated following the AOAC Official Method requirements: inclusivity/exclusivity and method comparison. The 3M MDA2 - *E. coli* O157 method was compared to the USDA/FSIS-MLG 5.09 *Detection, Isolation and Identification of Escherichia coli* O157:H7 *from Meat Products and Carcass and Environmental Sponges* for raw ground beef (325 g) and to FDA/BAM Chapter 4A *Diarrheagenic Escherichia coli* for frozen blueberries (25 g), sprouts (25 g) and fresh baby spinach (200 g). Overall the assay was equivalent to the reference methods based on the Probability of Detection (POD) analysis.

1.3 Study Director for Collaborative Study

The co-Study Directors for this collaborative study:

Lisa Monteroso
3M Food Safety Department
3M Center, Bldg. 275-5W-05
St. Paul, MN 55144-1000
Email: lmonteroso@mmm.com
Phone: 651-736-2913

Co-SD: TBD

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. Qualified laboratories, familiar with the Molecular Detection System, will act as collaborators. All collaborators have been, or will be, thoroughly trained by a 3M representative on the use of the Molecular Detection System.

A sample letter to collaborators can be found in Appendix 8.1. This letter will be sent to each collaborator prior to the start of the study.

3.0 Collaborative Study Design

a) The laboratory conducting the study will prepare the test portions. In this study, raw fresh ground beef (325 g) will be analyzed for *E. coli* O157:H7 following the 3M MDA2 - *E. coli* O157 protocol and appropriate reference method. The USDA-FSIS MLG Ch. 5.09 method will be used as the reference method.

b) Collaborators will receive 72 samples of a test matrix for analysis: 12 un-inoculated controls, 12 low level (target 0.2 – 2.0 cfu/test portion), and 12 high level (target 2 – 5 cfu/test portion) for each method – MDA2 and reference method. See text below and flow diagram 8.5 for the study details. Always reference the 3M MDA2 instructions for use and the MLG reference method for all details. Each laboratory will test all test portions. Each test matrix will be inoculated with an *E. coli* O157, randomized and blind-coded for analysis.

Table 1. Overview of Study Design

MATRIX	Inoculating organism	Target *E. coli* O157 Levels	# Test Portions (per method)	Test Portion Codes	Reference Method
Raw ground beef *325g*	***TBD***	0 cfu/test portion	12	Random number between 100-199	USDA/FSIS MLG Ch. 5.09
		0.2-2 cfu/test portion	12		
		2-5 cfu/test portion	12		

c) Study Schedule

a) All data should be sent to the SD by the 2nd week of study.

b) 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.

2) **Test Portion Preparation**

a) Fresh raw ground beef will be obtained from local retail outlets and screened for the presence of *E. coli* O157. 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.

b) **Preparation of test portions**. A liquid inoculum will be added drop-wise to a bulk quantity of ground beef for each inoculation level - 0.2-2 cfu/25g and 2-5 cfu/25g. The bulk quantity will then be mixed to achieve equal distribution of analyte throughout. Test portions (25g) will be pulled from this bulk lot and randomly assigned to the reference method or the 3M MDA2 - *E. coli* O157 (including H7) method. An additional 300g uncontaminated raw ground beef will be added to the 25g test portion resulting in a 325g test portion. The test portions will be stored at 2-8°C for 72-96 h prior to testing.

c) **Shipment of test portions.** Test portions will be packaged in leak-proof, insulated containers and shipped (according to the Dangerous Goods Regulations IATA for Infections Substances) by overnight carrier to arrive the day before initiation of analysis. 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 ground beef test portions will be stored at 2-8°C until they are analyzed.

d) **Microbiological analysis.** The test portion preparation is different for the 3M MDA2 - *E. coli* O157 and reference methods. Therefore, each collaborator will receive a separate test portion for each method. They will be instructed to test 325 g ground beef using the 3M MDA2 - *E. coli* O157 method and another 325 g ground beef using the MLG reference method. The Reference Method Confirmation procedures will be performed starting with the secondary enrichment steps. The recovered isolates will be identified by the API20E (Official Method 978.24) or the VITEK2 GN (Official Method 2011.17). The somatic (O) and flagellar (H) tests will also be performed.

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.

e) **Most Probable Number (MPN) Analysis.** The level of *E. coli* O157 will be determined by MPN on the day of analysis by evaluating 5 x 650 g, 20 x 325 g (using the reference method test portions) or 5 x 325 g and 5 x 160 g inoculated test samples. Each test portion will be 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 available at the AOAC RI website.

3) **3M™ Molecular Detection Assay 2 - *E. coli* O157 Method**

a) *Applicability*

For the detection of *E. coli* O157 from: raw ground beef (325 g), frozen blueberries (25 g), sprouts (25 g), fresh baby spinach (200 g).

b) *Test Kit Information*
   a) *Kit Name.*— 3M™ Molecular Detection Assay 2 – *E. coli* O157 (including H7)
   b) Catalog Number.— MDA2ECO96

c) *Test Kit Reagents* - 3M MDA2 - *E. coli O157* (including H7) (96 tests)
   a) *Lysis Solution (LS) tubes*. – 96 (12 strips of 8 tubes)
   b) *E. coli O157 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*

d) *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) 3M™ Enrichment Pouch with 225mL of Buffered Peptone Water (ISO formulation) and filter, available from 3M #QEBPWI225F
   j) Matrix Control, available from 3M #MDMC96NA

e) *Additional Media for Confirmation*
   a) Modified Rainbow agar (mRBA)
   b) Tryptic soy agar with 5% sheep blood (SBA)
   c) Dynal antibody coated paramagnetic beads
   d) Biochemical confirmation supplies
   e) Serological confirmation supplies

f) *Apparatus*
   a) *Pipettes*. – Capable of 20µL
   b) *Multi-channel pipette*. – Capable of 20µL
   c) *Sterile pipette tips.* – Capable of 20µL

d) *Serological Pipette Bulbs (Automatic Pipette)* – For sampling and delivering of 1 mL - 10 mL
e) *Serological pipettes*. – Aerosol resistant
f) *Stomacher®*. – Seward or equivalent
g) *Filter Stomacher® bags*. – Seward or equivalent
h) *Sterile collection sponge and swab*. – Environmental surface sampling
i) *Thermometer*. – Calibrated range to include 100 ± 1°C range
j) *Incubators*. – Capable of maintaining 35±2°C, 42±1°C and 41.5±1°C
k) *Dry Bath Incubator*. – Capable of maintaining a temperature of 100 ± 1 ºC
l) *Dry double block heater unit.* – Capable of maintaining 100 ± 1°C; or *a water bath* capable of maintaining 100 ± 1°C
m) *Refrigerator*. – capable of maintaining 2-8°C, for storing the 3M MDA2
n) *Computer*. – compatible with the 3M Molecular Detection System (instrument)

g) *Safety Precautions*

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.

The user should read, understand and follow all safety information in the instructions for the 3M Molecular Detection System and the 3M MDA2 – *E. coli* O157 (including H7). Retain the safety instructions for future reference.

h) *Sample Preparation*
a) *Foods* - 3M recommends the use of 3M BPW ISO for the enrichment of food 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.
(1) Pre-warm BPW ISO enrichment medium to 41.5 ± 1°C.
(2) Aseptically combine the enrichment medium and sample according to information below. For all meat and highly particulate samples, the use of filter bags is recommended.
(a) Ground beef – 325g + 975 mL BPW ISO (pre-warmed to 41.5 ± 1°C). Homogenize the sample for 2 ± 0.2 min. Incubate at 41.5 ±1°C for 10-18 hr.
(b) Sprouts – 25g + 225 mL 3M BPW ISO (pre-warmed to 41.5 ± 1°C). Homogenize the sample for 2 ± 0.2 min. Incubate at 41.5 ±1°C for 18-24 hr.
(c) Frozen whole blueberries – 25g + 225 mL 3M BPW ISO (pre-warmed to 41.5 ± 1°C). Gently agitate the sample for 2 ± 0.2 minutes (DO NOT STOMACH). Incubate at 41.5 ±1°C for 18-24 hr.
(d) Spinach – Add 200g + 450 mL BPW ISO (pre-warmed to 41.5 ± 1°C) in a sterile re-sealable plastic bag and gently agitate by hand. Incubate at 41.5 ±1°C for 18-24 hr.
(e) Run the 3M Molecular Detection Instrument following the 3M Molecular Detection System User Manual – Reserving enriched test portion for confirmation.

i) *Post-enrichment*

a) Invert room temperature (20-25 °C) lysis tubes to mix. Proceed to next step within 4 hours. A 20 µL aliquot of each enriched sample is transferred to separate lysis tubes using a new pipette tip after each sample transfer. Place uncovered samples in the 3M Molecular Detection Heat Block for 15 ± 2 minutes at 100 ± 1°C. During heating, the lysis samples will change from pink (cool) to yellow (hot). Following the heat lysis transfer samples place samples in the 3M Molecular Detection Chill Block Insert for 10 ± 1 minutes. The 3M Molecular Chill Block Insert, used at ambient temperature without the 3M Molecular Detection Chill Block Tray, should sit directly on the laboratory bench. When cool, the lysis solution will revert to a pink color.

b) 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 3M BPW ISO for the Negative Control (NC). Transfer a 20 µL aliquot to the NC and the Reagent Control (RC) tubes.

c) 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 - *E. coli* O157 (including H7) assay. Results are obtained within 60 minutes.

j) 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 MDA2 – *E. coli* O157 (including H7) 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.

k) Confirmation

All samples of primary enrichments 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.

4) **Reporting Raw Data**

a) Report data using the data report form in Appendix 8.2.

b) Upon completion the laboratory will fax or email the completed data form to the Study Director.

c) Copies of all related test results (data sheets and confirmation results) should be retained by the collaborating labs for a minimum of one year.

5) **Analyzing Raw Data**

a) The resulting data will be analyzed by two different calculations: probability of detection (POD).

b) **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[1] for complete analysis information on POD calculations.

a) Calculate:
(1) Estimate of repeatability - standard deviation (s_r)
(2) Determine POD values for each matrix and concentration - the number of positive outcomes divided by the total number of trials.
(3) Estimate of reproducibility – standard deviation of the laboratory POD values (s_{POD}) and confidence intervals.
(4) Cross laboratory probability of detection (LPOD) – LPODs by matrix and concentration with 95% confidence intervals for candidate method (presumptive and confirmed) results.
(5) Difference of cross-laboratory probability of detection (dLPOD) – difference probability of detection is the difference between any two LPOD values.
(a) Estimate $dLPOD_C$ as the difference between the candidate and reference LPOD values, include CIs.
(b) Estimate $dLPOD_{CP}$ as the difference between the presumptive and confirmed LPOD values, include CIs.
(c) **If the CI of a dLPOD does not contain zero then the difference is statistically significant**
(6) For each matrix, graph POD_R, $LPOD_C$ and $dLPOD_C$ by level with 95% confidence intervals.

6) **Appendices**

a) Instructions to Collaborators
b) Test Portion Data Report Forms
c) Study Materials
d) Flow Diagram – raw ground beef (325 g)

Appendix 8.1

Instructions to Collaborators

General Instructions about Collaborative Studies

[1] 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

Introduction

The purpose of this document is to provide detailed instructions for performing the collaborative study for the 3M™ Molecular Detection Assay 2 - *E. coli* O157 (including H7).

The trial will be conducted by co-Study Directors, Lisa Monteroso and TBD. 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 co-Study Directors:

Lisa Monteroso
3M Food Safety Department
3M Center, Bldg. 275-5W-05
St. Paul, MN 55144-1000
Email: lmonteroso@mmm.com
Phone: 651-736-2913

Co-SD: Leslie Thompson
Vanguard Scienes
Sioux Falls, SD

Important Information

1. Read the methods carefully. If you have any questions, contact one of the co-Study Directors.

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 - *E. coli* O157 (including H7) 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 products 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 express courier to arrive the day before initiation of analysis. If the test portions do not arrive by the normal delivery time one day prior to analysis, please contact a co-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 will be sent directly from 3M.**

Proposed analysis schedule

Food	Date Shipped	Date Tested
Raw ground beef *325 g*	Thursday	Monday

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 products should arrive cold to the touch and should be stored at 4°C (refrigerated) until the day of analysis.

Test Portion Analysis

Collaborators will be provided with enough test portion to test using the 3M MDA2 - *E. coli* O157 (including H7) method and the reference method. For raw ground beef testing, collaborators will receive: 36 x 325g (3M) and 36 x 325g (MLG). See Appendix 8.5 for detailed instructions on analysis. 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. 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 sheet provided.

1. **3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) Method**
   1.1. Pre-warm 3M buffered peptone water BPW ISO enrichment medium to the incubation temperature: 41.5 ±1°C. For meat and highly particulate samples, filter bags are recommended.
   1.2. Aseptically add 325 g test portion to 975 mL BPW ISO. Homogenize the sample for 2 ± 0.2 min. Incubate at 41.5 ±1°C. **After 10 hr**, pull a sample from ground beef enrichment to run the 3M Molecular Detection Instrument following the 3M Molecular Detection System User Manual – Reserving enriched test portion for confirmation.
   1.3. **Confirmation of alternative method -** All samples, regardless of presumptive result, must be confirmed starting with step 2.3 below.
2. **USDA/FSIS Reference Method, MLG online Chapter 5.09 – *E. coli* O157:H7**
   2.1. Add 325g into 975 mL mTSB. Stomach each test portion for approximately two minutes. Incubate at 42±1°C for 15-24 h.
   2.2. Perform the screening test for *E. coli* O157:H7/non-motile following the manufacturer instructions for the screening method listed in USDA FSIS MLG 5A.

2.3. For all test portions, follow the isolation procedure using Dynal #710.04 *E.coli* O157:H7 immunomagnetic beads as described in USDA/FSIS.
2.4. Prepare the beads as described by USDA/FSIS, spread plate dilutions of the bead suspension (with and without acid treatment) onto modfied Rainbow® Agar. Incubate for 20-24 h at 35±2°C.
2.5. Confirm a minimum of 1 typical colony from all potential positive test portions with typical colonies following the identification and confirmation procedures as per USDA FSIS.

Final Results

All results are to be recorded on the data sheets provided. For each test portion, record the results reported by the 3M method along with the confirmation results for both the 3M MDA2 - *E. coli* O157 (including H7) and reference methods. Immediately upon completion of each food type, the data sheet should be faxed or emailed to TBD, co-Study Director.

1 **3M MDA2 - *E. coli O157* (including H7) Collaborative Study Data Report Form, Raw Ground Beef 325g (**3M MDA2**)**

Lab: Analyst start date:
Analyst: Analyst completion date:
Biochemical ID method: 3M MDA2 lot number:

3M MDA2 *E. coli* O157 (including H7) Method							
Sample #	**3M MDA2 presumptive**	**Isolation**		**ID**			
		mRBA	SBA	biochemical ID	Somatic O	Flagellar H	Final result

+ if typical colonies/reaction present, - if no typical colonies/reaction present

2

1 **3M MDA2 - *E. coli O157* (including H7) Collaborative Study Data Report Form, Raw Ground Beef 325g** (USDA/MLG)

Lab: Analyst start date:
Analyst: Analyst completion date:
Biochemical ID method: APC:

USDA/FSIS MLG							
Sample #	**Rapid Screen**	**Isolation**		**ID**			
		mRBA	SBA	biochemical ID	Somatic O	Flagellar H	Final result

+ if typical colonies/reaction present, - if no typical colonies/reaction present

2

Appendix 8.3

Study Materials

Materials provided by collaborator

a) *Pipettes*. – Capable of 20µL
b) *Multi-channel pipette*. – Capable of 20µL
c) *Sterile pipette tips.* – Capable of 20µL
d) *Serological Pipette Bulbs (Automatic Pippette)* – For sampling and delivering of 1 mL - 10 mL
e) *Serological pipettes*. – Aerosol resistant
f) *Stomacher®*. – Seward or equivalent
g) *Filter Stomacher® bags*. – Seward or equivalent
h) *Thermometer*. – Calibrated range to include 100 ± 1°C range
i) *Incubators*. – Capable of maintaining 42 ± 1°C, 35 ± 2°C, and 41.5 ± 1°C
j) *Dry Bath Incubator*. – Capable of maintaining a temperature of 100 ± 1 ºC
k) *Dry double block heater unit.* – Capable of maintaining 100 ± 1°C; or *a water bath* capable of maintaining 100 ± 1°C
l) *Refrigerator*. – capable of maintaining 2-8°C, for storing the 3M MDA2
m) *Computer*. – compatible with the 3M Molecular Detection System (instrument)

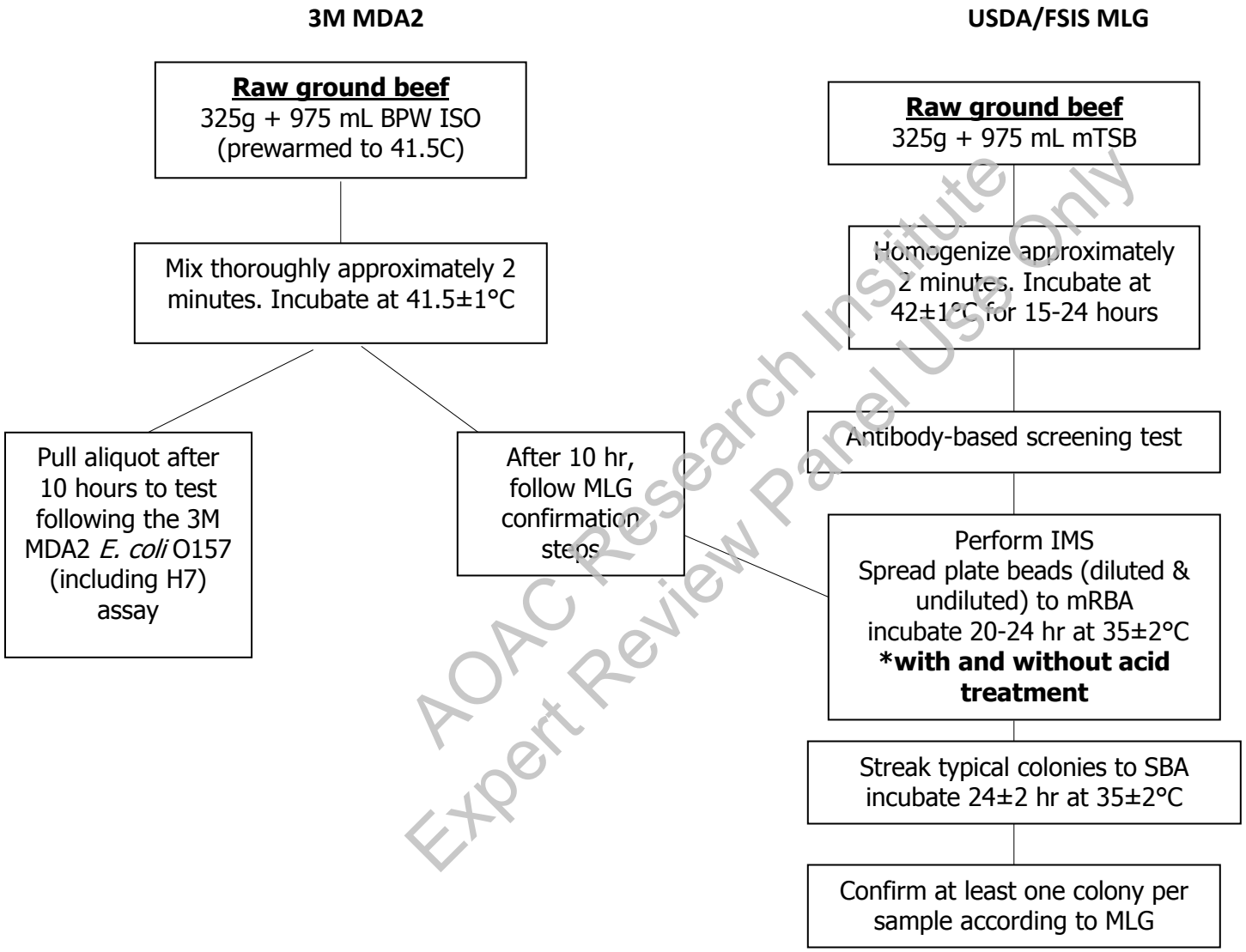
Materials and reagents provided by the study sponsor

a) 3M MDA2 – *E. coli O157* (including H7) Test Kits
b) 3M Molecular Detection System (instrument), power supply, cord, USB cable and software
c) 3M Molecular Detection Speed Loader Tray
d) 3M Molecular Detection Chill Block Tray and Chill block insert
e) 3M Molecular Detection Heat Block Insert
f) 3M Molecular Detection Cap/Decap Tool [Reagent]
g) 3M Molecular Detection Cap/Decap Tool [Lysis]
h) Empty lysis tube rack
i) Empty reagent tube rack
j) 3M™ Enrichment Pouch with 225 mL Buffered Peptone Water (ISO formulation) and filter, available from 3M #QEBPWI225
k) Matrix Control, available from 3M #MDMC96NA

Media and Reagents

a) Modified tryptone soya broth (mTSB)
b) Modified Rainbow agar (mRBA)
c) Tryptic soy agar with 5% sheep blood (SBA)
d) Antibody screening test
e) Dynal antibody coated paramagnetic beads
f) 1N hydrochloric acid (HCl)
g) Biochemical confirmation supplies
h) Serological confirmation supplies

Appendix 8.4 – Flow Diagram for Raw Ground Beef (325g)



3M™ Molecular Detection Assay 2 - *E. coli* O157 (including H7)

MDA2ECO96

PRODUCT DESCRIPTION AND INTENDED USE

The 3M™ Molecular Detection Assay 2 - *E. coli* O157 (including H7)is used with the 3M™ Molecular Detection System for the rapid and specific detection of *E. coli* O157 (including H7) in enriched foodand feed 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 - *E. coli*O157 (including H7) 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 environmental, pharmaceutical, cosmetics, clinical or veterinary samples. The 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7)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, 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 - *E. coli* O157 (including H7)with Buffered Peptone Water ISO.

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 - *E. coli* O157 (including H7) 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
E. coli O157 (including H7) Reagent tubes	Pinktubes	96 (12 strips of 8 tubes)	Lyophilized specific amplification and detection mix	Ready to use
Extra caps	Pink 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 a sterile enrichment medium, e.g., BPW ISO. 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 - *E. coli* O157 (including H7).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 - *E. coli* O157 (including H7) in the diagnosis of conditions in humans or animals.

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[7].

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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.
- Use medium pre-warmed to 41.5 ±1°C. Do not allow the medium to drop below the incubation temperature range during sample preparation.
- Store the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) as indicated on the package and in the product instructions.
- Always use the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) by the expiration date.
- Use the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7) with food, feed and food process environmental samples that have been validated internally or by a third party.
- Use the3M Molecular Detection Assay 2 - *E. coli* O157 (including H7)only with surfaces, sanitizers, protocols and bacterial strains that have been validated internally or by a third party.
- For an environmental sample containing Neutralizing Buffer (NB) 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 includeNeutralizing Buffer with aryl sulfonate complex: BPPFV10NB, RS96010NB, RS9604NB, SSL10NB, XSLSSL10NB, HS10NB and HS1195 10NB.

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

- 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.

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 - *E. coli* O157 (including H7)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 - *E. coli* O157 (including H7)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 - *E. coli* O157 (including H7) 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 - *E. coli* O157 (including H7) 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 general enrichment protocols for food.

It is the user's responsibility to validate alternate sampling protocols ordilution ratios to ensure this test method meets the user's criteria.

Foods

1. Pre-warm BPW ISO enrichment medium to 41.5 ±1°C. (See Tables 2 & 3)
2. Aseptically combine the enrichment medium and sample. For all meat and highly particulate samples, the use of filter bags is recommended.
3. Homogenize all matrices except leafy produceand fruit, thoroughly by blending, stomaching, or hand mixing for 2 ±0.2 minutes. Incubate as outlined in the appropriate protocol table. (See Tables 2& 3)

Table 2. General enrichment protocols

Sample Matrix[a]	Sample Size	Enrichment Broth Volume	Enrichment Temperature (±1°C)	Enrichment Time (hour)
Raw beef including ground/mince and trim	325 g	975 mL BPW ISO (pre-warmed)	41.5	10-18
Raw meats including raw beef, pork, poultry, lamb, bison	25 g	225 mL BPW ISO (pre-warmed)	41.5	8-18
Leafy produce[b]	200 g	450 mL BPW ISO (pre-warmed)	41.5	18-24
Other foods including fruit[b], vegetables, fruit/vegetable juices, fresh herbs, raw seafood, raw eggs, raw milk, cookie dough, processed meats	25 g	225 mL BPW ISO (pre-warmed)	41.5	18-24
Walnuts or nut mixes containing walnuts (this protocol is appropriate for other nuts including pecans, almonds, pistachios, cashews, chestnuts,	25 g	225 mL reconstituted non-fat dry milk	41.5	18-24

(a) Frozen samples should be equilibrated to 4-8°C before addition to enrichment broth.
(b) Leafy produce and fruit samples should be gently agitated by hand for 5 minutes. Do not blend or stomach.

Table 3. Enrichment protocols using pre-warmed BPW ISO at 41.5 ± 1°C according to AOAC® *Official Methods*℠ 2016.xxx

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Sample Matrix	Sample Size	Enrichment Broth Volume	Enrichment Time (hour)	Homogenized
Raw ground beef (73% lean)	325 g	975 mL	10-18	Manually by hand
Raw bagged spinach[a]	200 g	450 mL	18-24	Gently agitated by hand for 5 minutes
Fresh sprouts	25 g	225 mL	18-24	Stomach
Frozen blueberries[a][b]	25 g	225 mL	18-24	Gently agitated by hand for 5 minutes

(a) Leafy produce and fruit samples should be gently agitated by hand for 5 minutes. Do not blend or stomach.
(b) Frozen samples should be equilibrated to 4-8°C before addition to enrichment broth.

NOTES:

The recommended protocol interruption points are after enrichment or after sample lysis. Enrichment broth or sample lysate can be stored at 2-8°C for up to 72 hours. After removing the enrichment broth

from storage, resume testing from Step 1 in the **LYSIS** section. After removing the sample lysate from storage, resume testing from Step 7 in the **LYSIS** section.

PREPARATION OF THE 3M™ MOLECULAR DETECTION SPEED LOADER TRAY

1. Wet a cloth or disposable towel 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 Insert directly on the laboratory bench: The 3M Molecular Detection Chill Block Tray is not used. Use the 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, 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 3MMOLECULAR 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

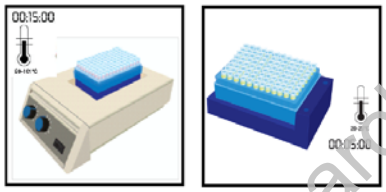
1. Allow the lysis solution (LS) tubes to warm up by setting the rack at room temperature (20-25°C) overnight (16-18 hours). Alternatives to equilibrate the LS tubes to room temperature are to set the LS tubes on the laboratory bench for at least 2 hours, incubate the LS tubes in a 37 ±1°C incubator 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 hours.
3. Remove the enrichment broth from the incubator.
4. One LS tube is required for each sample and the Negative Control (NC) sample (sterile enrichment medium).
   - 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.
     - 4.5.1 For processing of retained lysate, see Appendix A.
   - 4.6 Transfer 20 µL of sample into a LS tube unless otherwise indicated in the protocol table (example protocol 5).
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.BPW) 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 3M 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 4.2 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 steps 4.1 to 4.4 as needed, for the number of samples to be tested.

5.5 When all sample lysates have been transferred, repeat 4.1 to 4.4 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 60 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 analysed 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 BPW ISO enrichment to secondary enrichment broth(s), 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 - *E. coli* O157 (including H7)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.

In the event of discordant results (presumptive positive with the 3M Molecular Detection Assay 2 - *E. coli* O157 (including H7), non-confirmed by one of the means described above, and in particular for the latex agglutination test), the laboratory must follow the necessary steps to ensure the validity of the results obtained.

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 samples

1. To store a heat-treated lysate, re-cap the lysis tube with a clean cap (see "LYSIS", 4.5)
2. To store an enriched sample, incubate for a minimum of 18 hours prior to storage.
3. Store at 4 to 8°C for up to 72 hours
4. Prepare a stored sample for amplification by inverting 2-3 times to mix.
5. Decap the tubes.
6. Place the mixed lysate tubes on 3M Molecular Detection Heat Block Insert and heat at 100 ±1°C for 5 ±1 minutes.
7. 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.
8. Continue the protocol at the 'Amplification' section detailed above.

REFERENCES:

1. US Food and Drug Administration Bacteriological Analytical Manual. Chapter 4A: Diarrheagenic *Escherichia coli*. November 2015.
2. US Department of Agriculture (USDA) FSIS Microbiology Laboratory Guidebook 5.09. Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges. Effective Date: 15 January 2015.
3. ISO 16654:2001 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157.
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 6887. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.
7. Installation Qualification (IQ)/Operational Qualification (OQ) 3M™ Molecular Detection System. 3M Food Safety.

EXPLANATION OF PRODUCT5 LABEL SYMBOLS

www.3M.com/foodsafety/symbols

AOAC OMA Method Safety Checklist

Submission Date	2016-12-06 12:18:28
First and Last Name	Lisa Monteroso
Organization	3M Food Safety
E-mail	LPhillips@aoac.org
METHOD TITLE	Evaluation of the 3M™ Molecular Detection Assay (MDA) 2 -E. coli 2 (including H7) for the Detection of E. coli O157:H7 species in Selected Foods
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?	YES
Is there a risk in producing a dangerous aerosol?	NO
Are special procedures required for the disposal of reagents or reaction products?	YES
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

LABORATORY SERVICES • AUDITING • CONSULTING • EDUCATION • RESEARCH

FINAL REPORT

May 10, 2016

EVALUATION STUDY

Validation of 3M™ Molecular Detection Assay for *Escherichia coli* O157 in Frozen Blueberries

PREPARED BY

Aaron Pleitner

Research Scientist

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STUDY SPONSOR

The 3M™ Company

1.0 OBJECTIVE

The objective of this study was to validate the 3M™ Molecular Detection Assay (MDA) for detection of *Escherichia coli* O157 in a frozen blueberry food matrix. The Food Safety Net Services (FSNS) San Antonio Laboratory worked to validate the MDA system, considered here as the 'candidate method', using a 25 g sample size for frozen blueberries. The study followed the format for the validation of a candidate method using the experimental setup and Probability of Detection (POD) statistical analysis that are provided in the 2012 version of the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces document (1). For the purposes of this study, the candidate method results were compared with the analysis of a 25 g sample size for frozen blueberries, performed by reference method outlined via the 2014 version of the U.S. Food and Drug Administration Bacteriological Analytical Manual (FDA BAM) (2).

2.0 METHODS AND MATERIALS

2.1 Acquisition and Initial Analysis of Food Matrices

As mentioned in Section 1.0, the food matrix of frozen blueberries were tested in the validation of the MDA. The FSNS San Antonio Laboratory acquired blueberries from various sources, to be mixed prior to use. The frozen blueberries were thawed at refrigeration temperatures prior to inoculation. Prior to inoculation, a 25 g sample was removed and analyzed for *E. coli* O157 according to the FDA BAM for *E. coli* O157 (2, 3), as outlined in Section 2.4.

2.2 Preparation of E. coli *O157:H7 Inoculum*

As stated, the objective of this study was to determine the ability of the MDA to detect the presence of *E. coli* O157:H7 in frozen blueberries. The inoculum used for this validation study consisted of the following strain of *E. coli* O157:H7:

- *Escherichia coli* serotype O157:H7 ATCC 700599

Prior to each day of the experiment, an individual culture was prepared by streaking loopfuls of culture from -80˚C freezer stocks onto Tryptic Soy Agar (TSA; Becton, Dickinson and Company, Franklin Lakes, NJ) and incubating aerobically for 24 h at 35 ± 2°C. One isolated colony from the TSA plate was then transferred into Tryptic Soy Broth (TSB; Becton, Dickinson and Company) and incubated aerobically at 35 ± 2°C for 18-24 h to achieve stationary phase growth. A 1:9 dilution of the TSB culture was made in Butterfield's Phosphate Buffer (BPB; Made In-House) and the transmittance at 560 nm was measured using a Spectronic™ 200 Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA). This suspension served as the inoculum for *E. coli* O157:H7 and the concentrations of

cells were verified on each day of the experiment by serially diluting in BPB and plating onto Sorbitol MacConkey Agar (SMAC).

2.3 Inoculation of Food Samples

After the *E. coli* O157:H7 culture was prepared, individual portions of 25 g for frozen blueberry matrices were inoculated. As outlined in Section 2.1, *E. coli* O157:H7 ATCC 700599 was used for the frozen blueberry matrix. Three portion types were prepared for each testing method. Each individual portion was placed into a sterile Whirl-Pak bag. This included a high inoculum level (Portion 1), a low inoculum level (Portion 2) and un-inoculated samples (Portion 3). A 5 and 0.5 CFU/sample was targeted for the high and low inoculum levels, respectively. As stated in Section 2.2, the inoculum concentration was enumerated at the time of inoculation. Samples consisting of 425 g (blueberries) were removed from each inoculated portion (i.e. Portion 1 and Portion 2) in order to perform a five tube-three level Most Probable Number (MPN) analysis on each portion/matrix as described in Section 2.6. After inoculation, the food samples were then stored at -20°C for 14 d to stabilize. Once this stabilization period is complete, testing will commence. A total of 20 samples were prepared for Portion 2, 5 samples were prepared for Portion 1 and 5 samples were prepared for Portion 3.

2.4 Analysis of Reference Method Sub-Portions

After the 14 d stabilization period post-inoculation, the samples were subjected to the corresponding reference method according to the FDA BAM testing procedure (2). The 25 g sample were combined with 225 mL of mBPWp and placed at 37°C ± 1°C for 5 h to incubate. No stomaching or blending was performed. The mixing of the enrichment broth and blueberries was performed gently with efforts to limit any bursting of blueberries. Following incubation, 1 mL of ACV supplement was added and incubated at 42°C ± 1°C for 18-24 h. Following overnight enrichments, the sample was serially diluted in BPB. Appropriate dilutions were plated, in duplicate, onto Tellurite Cefixime-Sorbitol MacConkey Agar (TC-SMAC, Becton, Dickinson and Company, Franklin Lakes, NJ) along with one Chromogenic Selective Agar (ChromAgar, R&F Laboratories, Downers Grove, IL). Plates were incubated at 37°C ± 1°C for 18-24 h. Typical colony morphology was used to identify probable positive for O157. Typical colonies were picked and tested for latex agglutination (Remel kit) and streaked onto TSA + Yeast Extract (TSAYE) with a ColiComplete disc placed in the heaviest streak area. Plates were incubated at 37°C ± 1°C for 18-24 h. Isolates were confirmed with commercial antisera (RIM® *E. coli* O157:H7 Latex Test or equivalent test) followed by VITEK for *E. coli* identification.

2.5 Analysis of Candidate Method Sub-Portions

After the 14 d stabilization period post-inoculation, the samples were subjected to the following enrichment procedures and testing on the MDA. 3M™ provided all 3M™ consumable supplies involved with the MDA test. In brief, 3M™ Buffered Peptone Water (BPW) was pre-heated to 41.5°C ± 1°C. The 25 g sample was added to 225 mL 3M™ BPW, gently agitated and incubated at 41.5°C ± 1°C for 18 h. The mixing of the enrichment broth and blueberries was performed gently with efforts to limit any bursting of blueberries. The enriched samples were then subject to analysis as outlined by the instructions for use for the 3M™ Molecular Detection Assay 2 – *E. coli* O157 test kit. The sample results obtained after completing the MDA were considered the 'screening stage' result for the candidate method. Concurrent with the transfer and testing on the MDA, aliquots of the enriched samples were transferred and subjected to the testing outlined in Section 2.4 for cultural confirmation. The results obtained after completing this methodology were considered the 'confirmation stage' result for the candidate method.

2.6 Most Probable Number Analysis

As mentioned above in Section 2.3, samples of each portion were inoculated with two different levels of *E. coli* O157 (i.e. Portion 1, and Portion 2) and used for performing a five tube-three level MPN analysis to determine the concentration of *E. coli* O157 inoculated into the portion. This analysis was setup after the 14 d stabilization period in order to mimic all handling and treatment of matrices. From each 300 g sample of each portion, five 50 g samples and five 10 g samples were weighed into sterile Whirl-Pak bags and hydrated with 425 mL and 90 mL of mBPWp, respectively. Each sample was then processed according to the reference method procedures described above in Section 2.4. Note: for the MPN test samples, the set of five samples (high inoculum) and twenty samples (low inoculum) for the reference testing were also used (25 g, respectively). Samples that confirm positive for this procedure were noted and the LCF MPN Calculator Version 1.6 (Least Cost Formulations, Ltd., Virginia Beach, VA) was used to calculate the MPN/g and 95% confidence intervals for each portion using the mass of each sample and the number of reference method confirmed positives at each level of the MPN setup.

2.7 Probability of Detection Statistical Analysis

After results from the "screening" and "confirmation" stages were obtained for the candidate method and "confirmed" results were obtained for the reference method, the data was statistically analyzed according to the POD methods of the 2012 version of the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces document (1). The POD for the candidate method's "screening stage" (presumptive) results (POD_{CP}) were calculated for each level of inoculation, and were compared with the POD for

the candidate method's "confirmation stage" (confirmed) results (POD_{CC}) at each level of inoculation in order to attain a difference in POD for the two stages ($dPOD_{CP}$) at each level of inoculation. A 95% confidence interval was also calculated for each $dPOD_{CP}$ value that was calculated, and was used to determine whether the "screening stage" results is significantly different from the "confirmation stage" results for each level of inoculation. According to the methodology, if the 95% confidence interval of a $dPOD_{CP}$ value does not contain zero, then the difference between the POD_{CP} and the POD_{CC} of the candidate method at that level of inoculation is statistically significant at the 5% level. In addition to these analyses, the rate of true positives (samples with a "screening stage" positive result and "confirmation stage" positive result), the rate of true negatives (samples with a "screening stage" negative result and "confirmation stage" negative result), the rate of false positives (samples with a "screening stage" positive result and "confirmation stage" negative result), and the rate of false negatives (samples with a "screening stage" negative result and "confirmation stage" positive result) were calculated. Additionally, PODs were calculated for the reference method's "confirmed" results (POD_R) at each level of inoculation and were compared to the PODs that were calculated for each level of inoculation for the candidate method where the "screening stage" and "confirmation stage" results were both positive for a particular sample (POD_C). The difference in POD for the two values at each level of inoculation were calculated ($dPOD_C$) along with its corresponding 95% confidence interval. As noted above, if the 95% confidence interval of a $dPOD_C$ value does not contain zero, then the difference between the POD_C of the candidate method and the POD_R of the reference method at that level of inoculation is statistically significant at the 5% level.

3.0 RESULTS

The objective of this study was to validate the 3M™ MDA for the detection of *E. coli* O157:H7 in frozen blueberries. The inoculum concentration of *E. coli* O157:H7 was determined from Most Probable Number analysis, along with the 95% confidence intervals, as shown in Table 1. These inoculation levels targeted were chosen in order to meet the targeted POD values for the different inoculation levels. The concentrations achieved through inoculation were found to be 1.700×10^{-2} MPN/g for the high inoculation level and 1.305×10^{-2} MPN/g for the low inoculation level, according to the MPN analysis (Table 1).

All data obtained for the candidate method testing of frozen blueberries, 'screening stage' and 'confirmation stage' (25 g frozen blueberry analyzed by 3M™ MDA) is provided in Table 2. All data obtained for the reference method testing of frozen blueberries (25 g frozen blueberry analyzed by the FDA BAM Ch. 4A) is provided in Table 3. The number of true positives (samples with a

positive ‘screening stage’ and a positive ‘confirmation stage’), true negatives (samples with a negative ‘screening stage’ and a negative ‘confirmation stage’), false positive (samples with a positive ‘screening stage’ but a negative ‘confirmation stage’) and false negatives (samples with a negative ‘screening stage’ but a positive ‘confirmation stage’) for the candidate method testing of frozen blueberries is provided in Table 4. The AOAC fractional positive criterion (POD between 0.25 and 0.75) was met, providing evidence that the inoculation procedure was successful for the frozen blueberry matrix. Additionally, no false positives or false negatives were identified when comparing the ‘screening stage’ and ‘confirmation stage’ of the candidate method.

As described in Section 2.7, the results presented here were statistically analyzed according to the POD methods of the 2012 version of the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces document (1). The POD_{CP}, POD_{CC}, $dPOD_{CP}$, POD_{C}, POD_{R}, and $dPOD_{C}$ values calculated at each level of inoculation for the candidate method and the reference method testing of spinach, and their corresponding 95% confidence intervals, are provided below in Table 5. Review of the POD calculation outcomes for the frozen blueberry candidate method (25 g Frozen Blueberries Analyzed via the 3M™ MDA) and reference method (25 g Frozen Blueberries Analyzed via the FDA BAM Ch. 4A), shows that the 95% confidence intervals contained 0.000 at all inoculation levels for the $dPOD_{CP}$, comparing the ‘screening stage’ and ‘confirmation stage’ of the candidate method, and the $dPOD_{C}$, comparing the candidate method and reference method. Because of this, for the candidate method, the POD_{CP} values were not significantly different from their corresponding POD_{CC} values. Based on this outcome, it can be stated that the probability of detecting a positive result in the “screening stage” of the candidate method was not significantly different from the probability of detecting a positive result in its “confirmation stage” at any of the inoculation levels tested. Additionally, for the candidate method versus the reference method, the POD_{C} values were not significantly different from their corresponding POD_{R} values. Based on these outcomes, by fulfilling the criteria to contain 0.000 within the 95% confidence intervals, it can be stated that the probability of detecting a positive result in the candidate method was not significantly different from the probability of detecting a positive results in the reference method.

Table 1. Concentration of* E. coli *O157:H7 Inoculum Levels per Gram of Blueberries, Determined through Most Probable Number Analysis

Food Matrix/Inoculation Level	Sample Size	Sample Number	Number of Positives	MPN/g[1]	95% CI[1]
Blueberries/High	50	5	0	1.700×10^{-2}	7.598×10^{-3}, 3.804×10^{-2}
	25	5	5		
	10	5	1		
Blueberries/Low	50	5	0	1.305×10^{-2}	6.655×10^{-3}, 2.237×10^{-2}
	25	20	8		
	10	5	1		

1. MPN/g and 95% Confidence Interval Values were generated from the LCF MPN Calculator Version 1.6

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Table 2. Candidate Method Testing Results for Frozen Blueberries Enrichment 'Screening Stage' and 'Confirmation Stage' (25 g Frozen Blueberry Analyzed via the 3M™ MDA)

Sample Number	Inoculum Level	3M MDS 18 h	CT-SMAC #1	CT-SMAC #2	Rainbow	O Serology	TSAYE	H Serology	VITEK	Confirmation
1	**LOW**	=	=	=	=	=	=	=		**=**
2	**LOW**	=	=	=	=	=	=	=		**=**
3	**LOW**	+	+	+	+	+	+	+	*E. coli*	**+**
4	**LOW**	+	+	+	+	+	+	+	*E. coli*	**+**
5	**LOW**	+	+	+	+	+	+	+	*E. coli*	**+**
6	**LOW**	=	=	=	=	=	=	=		**=**
7	**LOW**	=	=	=	=	=	=	=		**=**
8	**LOW**	=	=	=	=	=	=	=		**=**
9	**LOW**	=	=	=	=	=	=	=		**=**
10	**LOW**	+	+	+	+	+	+	+	*E. coli*	**+**
11	**LOW**	=	=	=	=	=	=	=		**=**
12	**LOW**	=	=	=	=	=	=	=		**=**
13	**LOW**	+	+	+	+	+	+	+	*E. coli*	**+**
14	**LOW**	=	=	=	=	=	=	=		**=**
15	**LOW**	=	=	=	=	=	=	=		**=**
16	**LOW**	=	=	=	=	=	=	=		**=**
17	**LOW**	+	+	+	+	+	+	+	*E. coli*	**+**
18	**LOW**	+	+	+	+	+	+	+	*E. coli*	**+**
19	**LOW**	=	=	=	=	=	=	=		**=**
20	**LOW**	+	+	+	+	+	+	+	*E. coli*	**+**
1	**HIGH**	+	+	+	+	+	+	+	*E. coli*	**+**
2	**HIGH**	+	+	+	+	+	+	+	*E. coli*	**+**
3	**HIGH**	+	+	+	+	+	+	+	*E. coli*	**+**
4	**HIGH**	+	+	+	+	+	+	+	*E. coli*	**+**
5	**HIGH**	+	+	+	+	+	+	+	*E. coli*	**+**
1	**UNINOC**	=	=	=	=	=	=	=		**=**
2	**UNINOC**	=	=	=	=	=	=	=		**=**
3	**UNINOC**	=	=	=	=	=	=	=		**=**
4	**UNINOC**	=	=	=	=	=	=	=		**=**
5	**UNINOC**	=	=	=	=	=	=	=		**=**

Table 3. Reference Method Testing Results for Frozen Blueberries (25 g Frozen Blueberry Analyzed via the FDA BAM Ch. 4A)

Sample Number	Inoculum Level	CT-SMAC #1	CT-SMAC #2	Rainbow	O Serology	TSAYE	H Serology	VITEK	Confirmation
1	**LOW**	+	+	+	+	+	+	*E. coli*	**+**
2	**LOW**	=	=	=	=	=	=		**=**
3	**LOW**	=	=	=	=	=	=		**=**
4	**LOW**	=	=	=	=	=	=		**=**
5	**LOW**	=	=	=	=	=	=		**=**
6	**LOW**	+	+	+	+	+	+	*E. coli*	**+**
7	**LOW**	+	+	+	+	+	+	*E. coli*	**+**
8	**LOW**	=	=	=	=	=	=		**=**
9	**LOW**	=	=	=	=	=	=		**=**
10	**LOW**	=	=	=	=	=	=		**=**
11	**LOW**	+	+	+	+	+	+	*E. coli*	**+**
12	**LOW**	=	=	=	=	=	=		**=**
13	**LOW**	+	+	+	+	+	+	*E. coli*	**+**
14	**LOW**	=	=	=	=	=	=		**=**
15	**LOW**	+	+	+	+	+	+	*E. coli*	**+**
16	**LOW**	+	+	+	+	+	+	*E. coli*	**+**
17	**LOW**	=	=	=	=	=	=		**=**
18	**LOW**	=	=	=	=	=	=		**=**
19	**LOW**	+	+	+	+	+	+	*E. coli*	**+**
20	**LOW**	=	=	=	=	=	=		**=**
1	**HIGH**	+	+	+	+	+	+	*E. coli*	**+**
2	**HIGH**	+	+	+	+	+	+	*E. coli*	**+**
3	**HIGH**	+	+	+	+	+	+	*E. coli*	**+**
4	**HIGH**	+	+	+	+	+	+	*E. coli*	**+**
5	**HIGH**	+	+	+	+	+	+	*E. coli*	**+**
1	**UNINOC**	=	=	=	=	=	=		**=**
2	**UNINOC**	=	=	=	=	=	=		**=**
3	**UNINOC**	=	=	=	=	=	=		**=**
4	**UNINOC**	=	=	=	=	=	=		**=**
5	**UNINOC**	=	=	=	=	=	=		**=**

Table 4. Quantities and Percentages of True Positive, True Negatives, False Positives, and False Negatives for the Candidate Method Testing of Frozen Blueberries

Inoculation Level	Final Result[1]	Total	Percentage (%)
High	True Positive	5	100
	True Negative	0	0
	False Negative	0	0
	False Positive	0	0
Low	True Positive	8	40
	True Negative	12	60
	False Negative	0	0
	False Positive	0	0
Un-inoculated	True Positive	0	0
	True Negative	5	100
	False Negative	0	0
	False Positive	0	0

1. TRUE POSITIVE, ""Screening Stage"" Positive Result-"Confirmation Stage" Positive Result; TRUE NEGATIVE, "Screening Stage" Negative Result-"Confirmation Stage" Negative Result; FALSE POSITIVE, "Screening Stage" Positive Result-"Confirmation Stage" Negative Result; FALSE NEGATIVE, "Screening Stage" Negative Result-"Confirmation Stage" Positive Result

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Table 5. POD_{CP}, POD_{CC}, and $dPOD_{CP}$ Values for the Candidate Method (25 g Frozen Blueberries Analyzed via the 3M™ MDA) and POD_R, POD_C, and $dPOD_C$ Values for Candidate Method vs. the Reference Method (25 g Frozen Blueberries Analyzed via the FDA BAM Ch. 4A)[1]

Method / Inoculation Level	Measure	Value	95% CI
Candidate Method Screening vs. Confirmation High Level Inoculation	POD_{CP}	1.000	(0.5655, 1.000)
	POD_{CC}	1.000	(0.5655, 1.000)
	$dPOD_{CP}$	0.000	(-0.434, 0.434)
Candidate Method Screening vs. Confirmation Low Level Inoculation	POD_{CP}	0.400	(0.2188, 0.6134)
	POD_{CC}	0.400	(0.2188, 0.6134)
	$dPOD_{CP}$	0.000	(-0.280, 0.280)
Candidate Method Screening vs. Confirmation Un-inoculated	POD_{CP}	0.000	(0.000, 0.4345)
	POD_{CC}	0.000	(0.000, 0.4345)
	$dPOD_{CP}$	0.000	(-0.434, 0.434)
Reference Method vs. Candidate Method High Level Inoculation	POD_R	1.000	(0.5655, 1.000)
	POD_C	1.000	(0.5655, 1.000)
	$dPOD_C$	0.000	(-0.434, 0.434)
Reference Method vs. Candidate Method Low Level Inoculation	POD_R	0.400	(0.2188, 0.6134)
	POD_C	0.400	(0.2188, 0.6134)
	$dPOD_C$	0.000	(-0.280, 0.280)
Reference Method vs. Candidate Method Un-inoculated	POD_R	0.000	(0.000, 0.4345)
	POD_C	0.000	(0.000, 0.4345)
	$dPOD_C$	0.000	(-0.434, 0.434)

1. POD_{CP}, Probability of Detection for Candidate Method "Screening Stage" Positive Results; POD_{CC}, Probability of Detection for Candidate Method "Confirmation Stage" Results; $dPOD_{CP}$, POD_{CP} Minus POD_{CC}; POD_R, Probability of Detection for Reference Method Confirmed Results; POD_C, Probability of Detection for Candidate Method Confirmed Results (i.e. Combined "Screening Stage" and "Confirmation Stage" Results); $dPOD_C$, POD_R Minus POD_C. 95% Confidence Interval Values are Generated from the AOAC International POD Calculator.

4.0 DISCUSSION

The purpose of this study was to validate the 3M™ MDA for detection of *E. coli* O157:H7 in frozen blueberries. This study followed the format for the validation of a candidate method using the experimental setup and Probability of Detection (POD) statistical analysis provided in the February 24, 2012 version of the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces document (1).

Overall, based on the evidence presented here, the 3M™ MDA is a valid method for the detection of *E. coli* O157:H7 in frozen blueberries. The POD statistical analysis of the results for the candidate method and the reference method identified that these methods are not significantly different from each other. Additionally, no false positives or false negatives were identified by comparing the 'screening stage' and the 'confirmation stage' of the candidate method. This validation is accurate for the target sample size (25 g) tested.

5.0 REFERENCES

1. **Brunelle S, LaBudde R, Nelson M, Wehling P**. 2012. AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environ-mental Surfaces. Available at: http://www.aoac.org/imis15_prod/AOAC_Docs/StandardsDevelopment/AOAC_Validation_Guidelines_for_Food_Microbiology-Prepub_version.pdf. Accessed: 11/30/15.
2. **Feng P, Weagant SD, Jinneman, K.** 2011. U.S. Food and Drug Administration Bacteriological Analytical Manual, Chapter 4A: Diarrheagenic *Escherichia coli*. Available at: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070080.htm. Accessed: 11/30/15.

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6.0 PROPOSAL APPROVAL

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05/10/16

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LABORATORY SERVICES • AUDITING • CONSULTING • EDUCATION • RESEARCH

FINAL REPORT

February 22, 2016

EVALUATION STUDY

Validation of 3M™ Molecular Detection Assay for *Escherichia coli* O157 in Various Food Matrices

PREPARED BY

Aaron Pleitner

Research Scientist

Food Safety Net Services, Ltd.

STUDY SPONSOR

The 3M Company

1.0 OBJECTIVE

The objective of this study was to validate the 3M™ Molecular Detection Assay (MDA) for detection of *Escherichia coli* O157 in two different food matrices (sprouts and spinach). The Food Safety Net Services (FSNS) San Antonio Laboratory worked to validate the MDA system, considered here as the 'candidate method', a 25 g sample size for sprouts and a 200 g sample size for the spinach matrices. The study followed the format for the validation of a candidate method using the experimental setup and Probability of Detection (POD) statistical analysis that are provided in the 2012 version of the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces document (1). For the purposes of this study, the candidate method results were compared with the analysis of 200 g sample sizes for spinach and 25 g sample sizes for sprouts, performed by reference methods outlined via the 2014 version of the U.S. Food and Drug Administration Bacteriological Analytical Manual (FDA BAM) (2).

2.0 METHODS AND MATERIALS

2.1 Acquisition and Initial Analysis of Food Matrices

As mentioned in Section 1.0, three different food matrices were tested in the validation of the MDA. The FSNS San Antonio Laboratory acquired sprouts and spinach from either commercial grocers or producers. Multiple lots and producers were sourced for each matrix, whereupon the products were mixed in order to make an independent lot. All samples were stored at refrigeration temperatures until the inoculation and testing commenced. Prior to inoculation, one 25 g sample of each matrix was removed and analyzed for *E. coli* O157 according to the FDA BAM as outlined in Section 2.4.

2.2 Preparation of E. coli *O157:H7 Inoculum*

As stated, the objective of this study was to determine the ability of the MDA to detect the presence of *E. coli* O157:H7 in various food matrices. The inoculum used for this validation study consisted of the following strain of *E. coli* O157:H7:

- *Escherichia coli* serotype O157:H7 ATCC 35150

Prior to each day of the experiment, an individual culture was prepared by streaking loopfuls of culture from -80˚C freezer stocks onto Tryptic Soy Agar (TSA; Becton, Dickinson and Company, Franklin Lakes, NJ) and incubating aerobically for 24 h at 35 ± 2°C. One isolated colony from each TSA plate was then transferred into Tryptic Soy Broth (TSB; Becton, Dickinson and Company) and incubated aerobically at 35 ± 2°C for 24 h to achieve stationary phase growth. Cells were harvested by centrifuging aliquots of the stationary phase TSB culture at maximum

speed for 10 min in a Model 16K Microcentrifuge (Bio-Rad Laboratories, Inc., Hercules, CA). The supernatant of each centrifuged aliquot was removed and the pelleted cells were re-suspended in Butterfield's Phosphate Buffer (BPB; Made In-House From Various Ingredients). The cells re-suspended in BPB were centrifuged a second time, and the supernatant was removed once again. The pelleted cells were re-suspended once more in BPB. These final solutions of cells re-suspended in BPB were adjusted to a concentration of ~8.00 $\log_{10}$ CFU/ml based on the transmittance of the suspension as determined using a Spectronic™ 200 Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA). This suspension served as inoculum for *E. coli* O157:H7 and the concentrations of cells in each were verified on each day of the experiment by serially diluting in BPB and plating onto Sorbitol MacConkey Agar (SMAC).

2.3 Inoculation of Food Samples

After the *E. coli* O157:H7 culture was prepared, the two different food matrices were prepared in individual portions of 25 g and 200 g for sprouts and spinach matrices, respectively. Three portion types were prepared for each testing method, for each matrix. Each individual portion was placed into a sterile Whirl-Pak bag. This included a high inoculum level (Portion 1), a low inoculum level (Portion 2) and un-inoculated samples (Portion 3). After inoculation, all samples for each inoculum level were removed from each Whirl-Pak bag, combined and homogenized in one large batch unit and re-aliquoted into the appropriate portion. In sprouts and spinach, a 7.5 and 0.75 CFU/sample was targeted for the high and low inoculum levels, respectively. As stated in Section 2.2, the inoculum concentration was enumerated at the time of inoculation. Minimum volumes consisting of 300 g (sprouts) and 555 g (spinach) were also removed from each food matrix and each inoculated portion (i.e. Portion 1 and Portion 2) in order to perform a five tube-three level Most Probable Number (MPN) analysis on each portion/matrix as described in Section 2.6. After inoculation, the food samples were stored at 4°C for 48 h to stabilize. Once this stabilization period was complete, testing commenced. For each food matrix, a total of 20 samples were prepared for Portion 2, 5 samples were prepared for Portion 1 and 5 samples were prepared for Portion 3.

2.4 Analysis of Reference Method Sub-Portions

After the 48 h stabilization period post-inoculation, the samples were subjected to the corresponding reference method according to the FDA BAM testing procedures (2).

For the raw spinach matrix, categorized as 'Leafy Produce', the 200 g sample was combined with 450 mL of pre-heated (37°C) mBPWp and placed at 37°C ± 1°C for 5 h to incubate. No stomaching or blending was performed. Following incubation, 2 mL of ACV supplement was added (1 mL per 225 mL mBPWp) and incubated at 42°C ± 1°C for 18-24 h. For the raw sprout matrix, categorized as 'Cilantro or Parsley', the 25 g sample was combined with 225 mL of mBPWp and placed at 37°C ± 1°C for 5 h to incubate. No stomaching or blending was performed. Following incubation, 1 mL of ACV supplement was added and incubated at 42°C ± 1°C for 18-24 h. Following overnight enrichments, the sample was serially diluted in BPB. Appropriate dilutions were plated, in duplicate, onto Tellurite Cefixime-Sorbitol MacConkey Agar (TC-SMAC, Becton, Dickinson and Company, Franklin Lakes, NJ) along with one mRBA plate. Plates were incubated at 37°C ± 1°C for 18-24 h. Typical colony morphology was used to identify probable positive for O157. Typical colonies were picked and tested for latex agglutination (Remel kit) and streaked onto TSA + Yeast Extract (TSAYE) with a ColiComplete disc placed in the heaviest streak area. Plates were incubated at 37°C ± 1°C for 18-24 h. Isolates were confirmed with commercial antisera (RIM® *E. coli* O157:H7 Latex Test or equivalent test) followed by VITEK for *E. coli* identification.

2.5 Analysis of Candidate Method Sub-Portions

After the 48 h stabilization period post-inoculation, the samples were subjected to the following enrichment procedures and testing on the MDA. 3M provided all 3M consumable supplies involved with the MDA test. 3M Buffered Peptone Water (BPW) was pre-heated to 41.5°C ± 1°C prior to enriching all matrices. For the raw sprouts, the 25 g sample was added to 225 mL 3M BPW and immediately incubated at 41.5°C ± 1°C for 18 h. For the raw spinach, to each 200 g sample, 450 mL of pre-warmed BPW was added and immediately incubated at 41.5°C ± 1°C for 18 h. The sprout and spinach samples were not homogenized or stomached during preparation. The enriched samples were then subject to analysis as outlined by the instructions for use for the 3M™ Molecular Detection Assay 2 – *E. coli* O157 test kit. The sample results obtained after completing the MDA were considered the 'screening stage' result for the candidate method. Concurrent with the transfer and testing on the MDA, aliquots of the enriched samples were transferred and subjected to the testing outlined in Section 2.4 for cultural confirmation. The results obtained after completing this methodology were considered the 'confirmation stage' result for the candidate method.

2.6 Most Probable Number Analysis

As mentioned above in Section 2.3, samples for each portion, for each food matrix were inoculated with *E. coli* O157:H7 (i.e. Portion 1, and Portion 2; sprouts, spinach) and used for performing a five tube-three level MPN analysis to determine the concentration of *E. coli* O157 inoculated into the portion and matrix. This

analysis was setup after the 48 hr stabilization period in order to mimic all handling and treatment of matrices.

For spinach, five 100 g samples, five 10 g samples and five 1 g samples were weighed into sterile Whirl-Pak bags and hydrated with 900 mL, 90 mL and 9 mL of mBPWp, respectively. For sprouts, five 50 g samples and five 10 g samples were weighed into sterile Whirl-Pak bags and hydrated with 450 mL and 90 mL of mBPWp, respectively. The samples were then processed according to the reference method procedures described above in Section 2.4. Note: for sprouts MPN test samples, the second set of five samples for the reference testing will be used (25 g, respectively). Samples that confirmed positive for this procedure were noted and the LCF MPN Calculator Version 1.6 (Least Cost Formulations, Ltd., Virginia Beach, VA) was used to calculate the MPN/g and 95% confidence intervals for each portion using the mass of each sample and the number of reference method confirmed positives at each level of the MPN setup.

2.7 Probability of Detection Statistical Analysis

After results from the "screening" and "confirmation" stages were obtained for the candidate method and "confirmed" results were obtained for the reference method, the data was analyzed according to the POD methods of the 2012 version of the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces document (1). The POD for the candidate method's "screening stage" (presumptive) results (POD_{CP}) was calculated for each level of inoculation, and compared with the POD for the candidate method's "confirmation stage" (confirmed) results (POD_{CC}) at each level of inoculation in order to attain a difference in POD for the two stages ($dPOD_{CP}$) at each level of inoculation. A 95% confidence interval was also calculated for each $dPOD_{CP}$ value calculated, and was used to determine whether the "screening stage" results was significantly different from the "confirmation stage" results for each level of inoculation. According to the methodology, if the 95% confidence interval of a $dPOD_{CP}$ value does not contain zero, then the difference between the POD_{CP} and the POD_{CC} of the candidate method at that level of inoculation is statistically significant at the 5% level. In addition to these analyses, the rate of true positives (samples with a "screening stage" positive result and "confirmation stage" positive result), the rate of true negatives (samples with a "screening stage" negative result and "confirmation stage" negative result), the rate of false positives (samples with a "screening stage" positive result and "confirmation stage" negative result), and the rate of false negatives (samples with a "screening stage" negative result and "confirmation stage" positive result) were calculated. Additionally, PODs were calculated for the reference method's "confirmed" results (POD_R) at each level of inoculation and were compared to the PODs that were calculated for each level of inoculation for the candidate method where the "screening stage" and

"confirmation stage" results were both positive for a particular sample (POD_C). The difference in POD for the two values at each level of inoculation were calculated ($dPOD_C$) along with its corresponding 95% confidence interval. As noted above, if the 95% confidence interval of a $dPOD_C$ value does not contain zero, then the difference between the POD_C of the candidate method and the POD_R of the reference method at that level of inoculation is statistically significant at the 5% level.

3.0 RESULTS

The objective of this study was to validate the 3M™ Molecular Detection Assay (MDA) for the detection of *E. coli* O157:H7 in three different food matrices. The inoculum concentration of *E. coli* O157:H7 in each food matrix was determined from Most Probable Number analysis, along with the 95% confidence intervals, as shown in Table 1.

All data obtained for the candidate method testing of spinach, 'screening stage' and 'confirmation stage' (200 g spinach analyzed by the 3M™ MDA) is provided in Table 2. All data obtained for the reference method testing of spinach (200 g spinach analyzed by the FDA Bam Ch. 4A) is provided in Table 3. The number of true positives (samples with a positive 'screening stage' and a positive 'confirmation stage'), true negatives (samples with a negative 'screening stage' and a negative 'confirmation stage'), false positive (samples with a positive 'screening stage' but a negative 'confirmation stage') and false negatives (samples with a negative 'screening stage' but a positive 'confirmation stage') for the candidate method testing of spinach is provided in Table 4. As described in Section 2.7, the results presented here were statistically analyzed according to the POD methods of the 2012 version of the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces document (1). The POD_{CP}, POD_{CC}, $dPOD_{CP}$, POD_C, POD_R, and $dPOD_C$ values calculated at each level of inoculation for the candidate method and the reference method testing of spinach, and their corresponding 95% confidence intervals, are provided below in Table 5.

All data obtained for the candidate method testing of sprouts, 'screening stage' and 'confirmation stage' (25 g sprouts analyzed by the 3M™ MDA) is provided in Table 6. All data obtained for the reference method testing of sprouts (25 g sprouts analyzed by the FDA Bam Ch. 4A) is provided in Table 7. The number of true positives (samples with a positive 'screening stage' and a positive 'confirmation stage'), true negatives (samples with a negative 'screening stage' and a negative 'confirmation stage'), false positive (samples with a positive 'screening stage' but a negative 'confirmation stage') and false negatives (samples with a negative 'screening stage' but a positive 'confirmation stage') for the candidate method testing of sprouts is provided in Table 8. As described in Section 2.7, the results

presented here were statistically analyzed according to the POD methods of the 2012 version of the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces document (1). The POD_{CP}, POD_{CC}, $dPOD_{CP}$, POD_C, POD_R, and $dPOD_C$ values calculated at each level of inoculation for the candidate method and the reference method testing of sprouts, and their corresponding 95% confidence intervals, are provided below in Table 9.

Table 1. Concentration of* E. coli *O157:H7 Inoculum Level per Gram of Matrix, Determined through Most Probable Number Analysis

Food Matrix/Inoculation Level	Sample Size	Sample Number	Number of Positives	MPN/g[1]	95% CI[1]
Spinach/High	100	5	4	2.584×10^{-2}	1.107×10^{-2}, 6.028×10^{-2}
	10	5	1		
	1	5	2		
Sprouts/High	50	5	3	4.740×10^{-2}	2.494×10^{-2}, 9.008×10^{-2}
	25	5	5		
	10	5	3		

1. MPN/g and 95% Confidence Interval Values were generated from the LCF MPN Calculator Version 1.6

Table 2. Candidate Method Testing Results for Spinach Matrix 'Screening Stage' and 'Confirmation Stage' (200 g Spinach Analyzed via the 3M™ MDA)

Sample Number	Inoculum Level	3M MDS 18 h	CT-SMAC #1	CT-SMAC #2	Rainbow	O Serology	H Serology	VITEK	Confirmation
1	**LOW**	+	+	+	+	+	+	E. coli O157	+
2	**LOW**	=	=	=	=	=			=
3	**LOW**	=	=	=	=	=			=
4	**LOW**	=	=	=	=	=			=
5	**LOW**	=	=	=	=	=			=
6	**LOW**	=	=	=	=	=			=
7	**LOW**	=	=	=	=	=			=
8	**LOW**	+	+	+	+	+	+	E. coli O157	+
9	**LOW**	=	=	=	=	=			=
10	**LOW**	=	=	=	=	=			=
11	**LOW**	=	=	=	=	=			=
12	**LOW**	=	=	=	=	=			=
13	**LOW**	+	+	+	+	+	+	E. coli O157	+
14	**LOW**	+	+	+	+	+	+	E. coli O157	+
15	**LOW**	=	=	=	=	=			=
16	**LOW**	=	=	=	=	=			=
17	**LOW**	=	=	=	=	=			=
18	**LOW**	+	+	+	+	+	+	E. coli O157	+
19	**LOW**	+	+	+	+	+	+	E. coli O157	+
20	**LOW**	+	+	+	+	+	+	E. coli O157	+
1	**HIGH**	+	+	+	+	+	+	E. coli O157	+
2	**HIGH**	+	+	+	+	+	+	E. coli O157	+
3	**HIGH**	+	+	+	+	+	+	E. coli O157	+
4	**HIGH**	+	+	+	+	+	+	E. coli O157	+
5	**HIGH**	+	+	+	+	+	+	E. coli O157	+
1	**UNINOC**	=	=	=	=	=			=
2	**UNINOC**	=	=	=	=	=			=
3	**UNINOC**	=	=	=	=	=			=
4	**UNINOC**	=	=	=	=	=			=
5	**UNINOC**	=	=	=	=	=			=

Table 3. Reference Method Testing Results for Spinach Matrix (200 g Spinach Analyzed via the FDA BAM Ch. 4A)

Sample Number	Inoculum Level	CT-SMAC #1	CT-SMAC #2	Rainbow	O Serology	H Serology	VITEK	Confirmation
1	**LOW**	+	+	+	+	+	E. coli O157	+
2	**LOW**	=	=	=	=			=
3	**LOW**	+	+	+	+	+	E. coli O157	+
4	**LOW**	+	+	+	+	+	E. coli O157	+
5	**LOW**	+	+	+	+	+	E. coli O157	+
6	**LOW**	=	=	=	=			=
7	**LOW**	+	+	+	+	+	E. coli O157	+
8	**LOW**	=	=	=	=			=
9	**LOW**	+	+	+	+	+	E. coli O157	+
10	**LOW**	+	+	+	+	+	E. coli O157	+
11	**LOW**	=	=	=	=			=
12	**LOW**	+	+	+	+	+	E. coli O157	+
13	**LOW**	=	=	=	=			=
14	**LOW**	+	+	+	+	+	E. coli O157	+
15	**LOW**	=	=	=	=			=
16	**LOW**	=	=	=	=			=
17	**LOW**	=	=	=	=			=
18	**LOW**	=	=	=	=			=
19	**LOW**	=	=	=	=			=
20	**LOW**	=	=	=	=			=
1	**HIGH**	+	+	+	+	+	E. coli O157	+
2	**HIGH**	+	+	+	+	+	E. coli O157	+
3	**HIGH**	+	+	+	+	+	E. coli O157	+
4	**HIGH**	+	+	+	+	+	E. coli O157	+
5	**HIGH**	+	+	+	+	+	E. coli O157	+
1	**UNINOC**	=	=	=	=			=
2	**UNINOC**	=	=	=	=			=
3	**UNINOC**	=	=	=	=			=
4	**UNINOC**	=	=	=	=			=
5	**UNINOC**	=	=	=	=			=

Table 4. Quantities and Percentages of True Positive, True Negatives, False Positives, and False Negatives for the Candidate Method Testing of Spinach

Inoculation Level	Final Result[1]	Total	Percentage (%)
High	True Positive	5	100
	True Negative	0	0
	False Negative	0	0
	False Positive	0	0
Low	True Positive	7	35
	True Negative	13	65
	False Negative	0	0
	False Positive	0	0
Un-inoculated	True Positive	0	0
	True Negative	5	100
	False Negative	0	0
	False Positive	0	0

1. TRUE POSITIVE, ""Screening Stage"" Positive Result-"Confirmation Stage" Positive Result; TRUE NEGATIVE, "Screening Stage" Negative Result-"Confirmation Stage" Negative Result; FALSE POSITIVE, "Screening Stage" Positive Result-"Confirmation Stage" Negative Result; FALSE NEGATIVE, "Screening Stage" Negative Result-"Confirmation Stage" Positive Result

Table 5. POD_{CP}, POD_{CC}, and $dPOD_{CP}$ Values for the Candidate Method (200 g Spinach Analyzed via the 3M™ MDA) and POD_R, POD_C, and $dPOD_C$ Values for Candidate Method vs. the Reference Method (200 g Spinach Analyzed via the FDA BAM Ch. 4A)

Method / Inoculation Level	Measure	Value	95% CI
Candidate Method Screening vs. Confirmation High Level Inoculation	POD_{CP}	1.000	(0.5655, 1.000)
	POD_{CC}	1.000	(0.5655, 1.000)
	$dPOD_{CP}$	0.000	(-0.434, 0.434)
Candidate Method Screening vs. Confirmation Low Level Inoculation	POD_{CP}	0.350	(0.1812, 0.5672)
	POD_{CC}	0.350	(0.1812, 0.5672)
	$dPOD_{CP}$	0.000	(-0.275, 0.275)
Candidate Method Screening vs. Confirmation Un-inoculated	POD_{CP}	0.000	(0.000, 0.4345)
	POD_{CC}	0.000	(0.000, 0.4345)
	$dPOD_{CP}$	0.000	(-0.434, 0.434)
Reference Method vs. Candidate Method High Level Inoculation	POD_R	1.000	(0.5655, 1.000)
	POD_C	1.000	(0.5655, 1.000)
	$dPOD_C$	0.000	(-0.434, 0.434)
Reference Method vs. Candidate Method Low Level Inoculation	POD_R	0.450	(0.2582, 0.6579)
	POD_C	0.350	(0.1812, 0.5672)
	$dPOD_C$	-0.100	(-0.368, 0.190)
Reference Method vs. Candidate Method Un-inoculated	POD_R	0.000	(0.000, 0.4345)
	POD_C	0.000	(0.000, 0.4345)
	$dPOD_C$	0.000	(-0.434, 0.434)

POD_{CP}, Probability of Detection for Candidate Method "Screening Stage" Positive Results; POD_{CC}, Probability of Detection for Candidate Method "Confirmation Stage" Results; $dPOD_{CP}$, POD_{CP} Minus POD_{CC}; POD_R, Probability of Detection for Reference Method Confirmed Results; POD_C, Probability of Detection for Candidate Method Confirmed Results (i.e. Combined "Screening Stage" and "Confirmation Stage" Results); $dPOD_C$, POD_R Minus POD_C. 95% Confidence Interval Values are Generated from the AOAC International POD Calculator.

Table 6. Candidate Method Testing Results for Sprouts Matrix 'Screening Stage' and 'Confirmation Stage' (25 g Sprouts Analyzed via the 3M™ MDA)

Sample Number	Inoculum Level	3M MDS 18 h	CT-SMAC #1	CT-SMAC #2	Rainbow	O Serology	H Serology	VITEK	Confirmation
1	LOW	=	=	=	=	=			=
2	LOW	+	+	+	+	+	+	E. coli O157	+
3	LOW	=	=	=	=	=			=
4	LOW	=	=	=	=	=			=
5	LOW	=	=	=	=	=			=
6	LOW	+	+	+	+	+	+	E. coli O157	+
7	LOW	+	+	+	+	+	+	E. coli O157	+
8	LOW	+	+	+	+	+	+	E. coli O157	+
9	LOW	=	=	=	=	=			=
10	LOW	=	=	=	=	=			=
11	LOW	=	=	=	=	=			=
12	LOW	+	+	+	+	+	+	E. coli O157	+
13	LOW	+	+	+	+	+	+	E. coli O157	+
14	LOW	=	=	=	=	=			=
15	LOW	+	+	+	+	+	+	E. coli O157	+
16	LOW	+	+	+	+	+	+	E. coli O157	+
17	LOW	=	=	=	=	=			=
18	LOW	=	=	=	=	=			=
19	LOW	+	+	+	+	+	+	E. coli O157	+
20	LOW	+	+	+	+	+	+	E. coli O157	+
1	HIGH	+	+	+	+	+	+	E. coli O157	+
2	HIGH	=	=	=	=	=			=
3	HIGH	+	+	+	+	+	+	E. coli O157	+
4	HIGH	+	+	+	+	+	+	E. coli O157	+
5	HIGH	+	+	+	+	+	+	E. coli O157	+
1	UNINOC	=	=	=	=	=			=
2	UNINOC	=	=	=	=	=			=
3	UNINOC	=	=	=	=	=			=
4	UNINOC	=	=	=	=	=			=
5	UNINOC	=	=	=	=	=			=

Table 7. Reference Method Testing Results for Sprouts Matrix (25 g Sprouts Analyzed via the FDA BAM Ch. 4A)

Sample Number	Inoculum Level	CT-SMAC #1	CT-SMAC #2	Rainbow	O Serology	H Serology	VITEK	Confirmation
1	**LOW**	=	=	=	=			=
2	**LOW**	=	=	=	=			=
3	**LOW**	+	+	+	+	+	E. coli O157	+
4	**LOW**	+	+	+	+	+	E. coli O157	+
5	**LOW**	=	=	=	=			=
6	**LOW**	+	+	+	+	+	E. coli O157	+
7	**LOW**	=	=	=	=			=
8	**LOW**	=	=	=	=			=
9	**LOW**	=	=	=	=			=
10	**LOW**	=	=	=	=			=
11	**LOW**	=	=	=	=			=
12	**LOW**	=	=	=	=			=
13	**LOW**	+	+	+	+	+	E. coli O157	+
14	**LOW**	=	=	=	=			=
15	**LOW**	=	=	=	=			=
16	**LOW**	+	+	+	+	+	E. coli O157	+
17	**LOW**	=	=	=	=			=
18	**LOW**	=	=	=	=			=
19	**LOW**	+	+	+	+	+	E. coli O157	+
20	**LOW**	+	+	+	+	+	E. coli O157	+
1	**HIGH**	+	+	+	+	+	E. coli O157	+
2	**HIGH**	+	+	+	+	+	E. coli O157	+
3	**HIGH**	+	+	+	+	+	E. coli O157	+
4	**HIGH**	+	+	+	+	+	E. coli O157	+
5	**HIGH**	+	+	+	+	+	E. coli O157	+
1	**UNINOC**	=	=	=	=			=
2	**UNINOC**	=	=	=	=			=
3	**UNINOC**	=	=	=	=			=
4	**UNINOC**	=	=	=	=			=
5	**UNINOC**	=	=	=	=			=

Table 8. Quantities and Percentages of True Positive, True Negatives, False Positives, and False Negatives for the Candidate Method Testing of Sprouts

Inoculation Level	Final Result[1]	Total	Percentage (%)
High	True Positive	4	80
	True Negative	1	20
	False Negative	0	0
	False Positive	0	0
Low	True Positive	10	50
	True Negative	10	50
	False Negative	0	0
	False Positive	0	0
Un-inoculated	True Positive	0	0
	True Negative	5	100
	False Negative	0	0
	False Positive	0	0

1. TRUE POSITIVE, ""Screening Stage"" Positive Result-"Confirmation Stage" Positive Result; TRUE NEGATIVE, "Screening Stage" Negative Result-"Confirmation Stage" Negative Result; FALSE POSITIVE, "Screening Stage" Positive Result-"Confirmation Stage" Negative Result; FALSE NEGATIVE, "Screening Stage" Negative Result-"Confirmation Stage" Positive Result

Table 9. POD_{CP}, POD_{CC}, and $dPOD_{CP}$ Values for the Candidate Method (25 g Sprouts Analyzed via the 3M™ MDA) and POD_R, POD_C, and $dPOD_C$ Values for Candidate Method vs. the Reference Method (25 g Sprouts Analyzed via the FDA BAM Ch. 4A)

Method / Inoculation Level	Measure	Value	95% CI
Candidate Method Screening vs. Confirmation High Level Inoculation	POD_{CP}	0.800	(0.3755, 1.000)
	POD_{CC}	0.800	(0.3755, 1.000)
	$dPOD_{CP}$	0.000	(-0.469, 0.469)
Candidate Method Screening vs. Confirmation Low Level Inoculation	POD_{CP}	0.500	(0.2993, 0.7007)
	POD_{CC}	0.500	(0.2993, 0.7007)
	$dPOD_{CP}$	0.000	(-0.284, 0.284)
Candidate Method Screening vs. Confirmation Un-inoculated	POD_{CP}	0.000	(0.000, 0.4345)
	POD_{CC}	0.000	(0.000, 0.4345)
	$dPOD_{CP}$	0.000	(-0.434, 0.434)
Reference Method vs. Candidate Method High Level Inoculation	POD_R	1.000	(0.5655, 1.000)
	POD_C	0.800	(0.3755, 1.000)
	$dPOD_C$	-0.200	(-0.624, 0.278)
Reference Method vs. Candidate Method Low Level Inoculation	POD_R	0.350	(0.1812, 0.5672)
	POD_C	0.500	(0.2993, 0.7007)
	$dPOD_C$	0.150	(-0.146, 0.412)
Reference Method vs. Candidate Method Un-inoculated	POD_R	0.000	(0.000, 0.4345)
	POD_C	0.000	(0.000, 0.4345)
	$dPOD_C$	0.000	(-0.434, 0.434)

POD_{CP}, Probability of Detection for Candidate Method "Screening Stage" Positive Results; POD_{CC}, Probability of Detection for Candidate Method "Confirmation Stage" Results; $dPOD_{CP}$, POD_{CP} Minus POD_{CC}; POD_R, Probability of Detection for Reference Method Confirmed Results; POD_C, Probability of Detection for Candidate Method Confirmed Results (i.e. Combined "Screening Stage" and "Confirmation Stage" Results); $dPOD_C$, POD_R Minus POD_C. 95% Confidence Interval Values are Generated from the AOAC International POD Calculator.

4.0 DISCUSSION

The purpose of this study was to validate the 3M™ Molecular Detection Assay (MDA) for detection of *Escherichia coli* O157:H7 in sprouts and spinach. This study followed the format for the validation of a candidate method using the experimental setup and Probability of Detection (POD) statistical analysis provided in the February 24, 2012 version of the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces document (1). Portions of spinach (200 g sample size) and sprouts (25

g sample size) were inoculated with *E. coli* O157:H7 at a concentration aimed to achieve a targeted level of contamination. The inoculation levels targeted were chosen in order to meet the targeted POD values for the different inoculation levels. The concentrations achieved through inoculation were found to be 2.584 x 10^{-2} MPN/g for the high inoculation level of spinach and 4.740 x 10^{-2} MPN/g for the high inoculation level of sprouts, according to the MPN analysis (Table 2). In addition to this, the AOAC fractional positive criterion (POD between 0.25 and 0.75) was met, providing evidence that the inoculation procedure was successful for spinach and sprouts (Table 5, Table 9).

Review of the POD calculation outcomes for the spinach candidate method (200 g Spinach Analyzed via the 3M™ MDA) and reference method (200 g Spinach Analyzed via the FDA BAM Ch. 4A) shows that the 95% confidence intervals contained 0.000 at all inoculation levels for the $dPOD_{CP}$, comparing the 'screening stage' and 'confirmation stage' of the candidate method, and the $dPOD_{C}$, comparing the candidate method and reference method (Table 5). Because of this, for the candidate method, the POD_{CP} values were not significantly different from their corresponding POD_{CC} values. Based on this outcome, it can be stated that the probability of detecting a positive result in the "screening stage" of the candidate method was not significantly different from the probability of detecting a positive result in its "confirmation stage" at any of the inoculation levels tested. Additionally, for the candidate method versus the reference method, the POD_{C} values were not significantly different from their corresponding POD_{R} values. Based on these outcomes, by fulfilling the criteria to contain 0.000 within the 95% confidence intervals, it can be stated that the probability of detecting a positive result in the candidate method was not significantly different from the probability of detecting a positive results in the reference method. Thus, the candidate method and the reference method can be considered equivalent, based on the AOAC International guidelines. Also, the lack of false positive or false negatives (Table 4) when comparing the 'screening stage' and the 'confirmation stage' of the candidate method further supports the adequacy of the method.

Review of the POD calculation outcomes for the sprout candidate method (25 g Spinach Analyzed via the 3M™ MDA) and reference method (25 g Spinach Analyzed via the FDA BAM Ch. 4A), shows that the 95% confidence intervals contained 0.000 at all inoculation levels for the $dPOD_{CP}$, comparing the 'screening stage' and 'confirmation stage' of the candidate method, and the $dPOD_{C}$, comparing the candidate method and reference method (Table 9). Because of this, for the candidate method, the POD_{CP} values were not significantly different from their corresponding POD_{CC} values. Based on this outcome, it can be stated that the probability of detecting a positive result in the "screening stage" of the candidate method was not significantly different from the probability of detecting a

positive result in its “confirmation stage” at any of the inoculation levels tested. Additionally, for the candidate method versus the reference method, the POD_C values were not significantly different from their corresponding POD_R values. Based on these outcomes, by fulfilling the criteria to contain 0.000 within the 95% confidence intervals, it can be stated that the probability of detecting a positive result in the candidate method was not significantly different from the probability of detecting a positive results in the reference method. Thus, the candidate method and the reference method can be considered equivalent, based on the AOAC International guidelines. Also, the lack of false positive or false negatives (Table 8) when comparing the ‘screening stage’ and the ‘confirmation stage’ of the candidate method further supports the adequacy of the method.

Overall, based on the results of this study, the 3M™ Molecular Detection Assay (MDA) is a valid method for the detection of *Escherichia coli* O157:H7 in 25 g sprouts and 200 g spinach. The candidate methods explored here with the targeted sample sizes produce equivalent results in both the ‘screening stage’ and the ‘confirmation stage’ and produce equivalent results when compared with the FDA BAM reference methods, typically employed for these matrices.

5.0 REFERENCES

1. **Brunelle S, LaBudde R, Nelson M, Wehling P**. 2012. AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environ-mental Surfaces. Available at: http://www.aoac.org/imis15_prod/AOAC_Docs/StandardsDevelopment/AOAC_Validation_Guidelines_for_Food_Microbiology-Prepub_version.pdf. Accessed: 11/30/15.
2. **Feng P, Weagant SD, Jinneman, K.** 2011. U.S. Food and Drug Administration Bacteriological Analytical Manual, Chapter 4A: Diarrheagenic *Escherichia coli*. Available at: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070080.htm. Accessed: 11/30/15.
3. **United States Department of Agriculture, Food Safety Inspection Service, Office of Public Health Science**. 2014. Microbiology Laboratory Guidebook Method 5.09: Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Product and Carcass and Environmental Sponges. Available at: http://www.fsis.usda.gov/wps/wcm/connect/51507fdb-dded-47f7-862d-ad80c3ee1738/MLG-5pdf?MOD=AJPERES. Accessed: 11/30/2015.

6.0 DATA DISPOSITION

Copies of raw data and final reports from this study will be archived at Food Safety Net Services, Ltd., 199 W. Rhapsody, San Antonio, TX 78216, and shall be kept confidential as per the Agreement between FSNS and 3M™.

7.0 PROPOSAL APPROVAL

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2/22/16

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Independent Laboratory Evaluation of the
3M™ Molecular Detection Assay 2 - *E. coli* O157:H7
for the Detection of *E. coli* O157:H7 in Raw Ground Beef

The 3M™ Molecular Detection Assay 2 (MDA 2) – *E. coli* O157 (including H7) employs real-time isothermal technology for the rapid and accurate detection of *E. coli* O157 (including H7) from select food matrices. This study included a method comparison evaluation of the 3M MDA 2 – *E. coli* O157:H7 to the USDA/FSIS-MLG 5.09 *Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges* for the detection of *E. coli* O157:H7 in raw ground beef (73% lean). The 3M MDA 2 – *E. coli* O157:H7 was evaluated for its ability to accurately detect *E. coli* O157:H7 from raw ground beef after 10 hours of incubation.

In the method comparison study, the 3M™ MDA 2 - *E. coli* O157:H7 demonstrated no statistically significant differences between presumptive and confirmed results ($dPOD_{CP}$) or between candidate and reference method results ($dPOD_C$) for the evaluation of the raw ground beef matrix.

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Scope of Methods

(a) Target organism – E. coli O157

(b) Matrix

1) Raw Ground Beef (73% lean) (325 gram test portion)

(c) Summary of Validated Performance Claims

The 3M MDA 2 - *E. coli* O157 is considered equivalent to the USDA/FSIS-MLG 5.09 reference method [1] for the detection of *E. coli* O157 in raw ground beef (73% lean).

Definitions

(a) Probability of Detection

Probability of Detection (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. There are several POD measures that can be calculated, e.g., POD_R (reference method POD), POD_C (confirmed candidate method POD), POD_{CP} (candidate method presumptive result POD), POD_{CC} (candidate method confirmation result POD) and dPOD, the difference between any two POD values.

General Information

Most *E. coli* bacteria are harmless; however, some produce a toxin (Shiga toxin) that can cause serious illness, including bloody diarrhea, hemolytic anemia, renal failure, and sometimes death. For example, a recent outbreak occurred on February 25, 2016, by a company called Jack & the Green Sprouts, Inc. recalled all alfalfa and alfalfa onion sprout products due to nine people infected with the outbreak strain of Shiga toxin-producing *Escherichia coli* O157 [2]. Like generic *E. coli*, toxin-producing Shiga-toxigenic *Escherichia coli* (STEC) are Gram-negative, rod-shaped bacteria, but are characterized by the production of Shiga toxins (*stx 1, stx 2,* and *eae*). Depending on the reference cited, there are approximately 200 to 400 STEC serotypes, many of which have not been implicated in human illness; however, a subset of STEC called Enterohemorrhagic *Escherichia coli* (EHEC) includes only those that cause serious illness. Serotype O157:H7 is the prototypic EHEC strain [3].

Principle of the Method

3M™ Molecular Detection Assay 2 - *E. coli* O157 is used with the 3M™ Molecular Detection System for the rapid and specific detection of *E. coli* O157 in enriched food samples. The 3M Molecular Detection Assay uses a 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 appropriate reference method.

Materials and Methods

Test Kit Information

(a) MDA 2 – *E. coli* O157 Test Kits

(b) MDA 2 Software

(c) 3M BPW ISO Formulation

(d) Matrix Control Kits

Additional Supplies and Reagents

(a) 3M Molecular Detection System (instrument)

(b) 3M Molecular Detection Speed Loader Tray

(c) 3M Molecular Detection Heat Block Insert

(d) 3M Molecular Detection Cap/Decap Tool [Reagent]

(e) 3M Molecular Detection Cap/Decap Tool [Lysis]

(f) Buffered Peptone Water, ISO formulation (ISO BPW)

Apparatus

(a) Top Loading Balance – capable of weighing and measuring 25 – 2000 grams.

(b) Filter laboratory blender bags.

(c) Laboratory paddle blender - Seward 3500 or equivalent: for sample homogenization.

(d) Incubators – capable of maintaining 41.5 ± 1 °C

(e) Dry bath incubators – capable of maintaining 100 ± 1 °C.

(f) Calibrated Thermometers – capable of measuring, 41.5 ± 1 °C, and 100 ± 1 °C.

(g) Serological Pipette Bulbs (Automatic Pipette) – For sampling and delivering of 1 mL - 10 mL.

(h) Serological pipettes – Aerosol resistant.

(i) Microcentrifuge – For 1.5 mL microcentrifuge tubes.

(j) Adjustable, variable volume single pipette capable of sampling and delivering 10-1000 µL.

(k) Adjustable, variable volume multichannel pipette capable of sampling and delivering 30 µL.

(l) Micropipette tips – Aerosol resistant.

(m) Sterile Gloves

(n) Felt-tip marker.

(o) Refrigerator – Capable of maintaining 2-8 °C.

(p) Freezer – Capable of maintaining -20 °C.

(q) Calibrated Timer

Reference Materials

Organisms used in the method comparison study were obtained from the following sources:

(a) American Type Culture Collection (ATCC) — Manassas, VA. *www.atcc.org.*

Safety Precautions

Enrichment

Shiga-toxin producing *Escherichia coli* is a Biosafety Level 2 organism. Biological samples such as enrichments have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes. Wear appropriate protective equipment which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities utilizing the appropriate safety equipment (for example, physical containment devices). Individuals should be trained in accordance with applicable regulatory and company/institution requirements

before working with potentially infectious materials. All enrichment broths should be sterilized following any culture based confirmatory steps.

General Preparation

(a) 975 ± 19.5 mL of pre-warmed (41.5 ± 1° C) 3M ISO BPW enrichment media

(b) Use aseptic techniques

(c) Use filter laboratory bags during enrichment to minimize particulates

(d) Separate work areas for the following: media preparation, sample preparation, and pathogen detection

(e) Clean the work stations with a disinfectant of choice before and after use. (Sodium hypochlorite solution, phenol solution, Quaternary ammonium solution, etc.)

(f) Do not reuse kit disposables

(g) Change pipette tips in between samples

(h) Wear personal protective equipment (PPE)

DNA Lysis

(a) Allow the lysis solution tubes to warm to room temperature (20-25° C)

(b) Pre-warm dry bath incubator to 100 ± 1°C.

(c) Transfer enriched sample into an individual lysis tube.

DNA Amplification

(a) Use of aseptic technique

(b) Change pipette tips between samples

(c) Use gloves and protective laboratory wear

(d) Do not touch any molecular equipment and supplies without wearing gloves

(e) Ensure that the reagent tubes are completely sealed with caps

Sample Preparation

(a) *Performing Pre-Enrichment:* 3M MDA 2 - *E. coli* O157

1) *Raw Ground Beef*
A 325 g test portion is aseptically combined with 975 ± 19.5 mL (1 L) of pre-warmed (41.5 ± 1°C) ISO-BPW in a filtered enrichment bags.

2) Homogenize for 2 ± 0.2 minutes.

3) Incubate at 41.5 ± 1°C for 10 hours.

4) Following incubation, a sample aliquot will be removed to be prepared by 3M MDA 2 - *E. coli* O157 method.

Lysis Workflow Procedures

(a) *Lysis 3M MDA 2 - E. coli O157 process*

1) Allow the lysis solution (LS) tubes to warm to room temperature (20-25° C) over night (16 – 18 hours). Alternatives to equilibrate the LS tubes at 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 the next step within 4 hours.

3) Remove the enrichment broth from the incubator.

4) Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip- one strip at a time.

5) Discard the LS cap.

6) Transfer 20 µL of sample into a LS tube.

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

8) When all samples have been transferred, transfer 20 µL of Negative Control (sterile enrichment medium) into a LS tube.

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

10) 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).

11) Remove the uncovered rack of LS tubes from the heating block and allow to cool, when cool the lysis solution will revert to a pink color.

(b) *Amplification: 3M MDA 2 - E. coli O157 process*

1) One Reagent tube is required for each sample and the negative control (NC).

2) Select one 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 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 to decap the Reagent tubes, one Reagent tube strip at a time. Discard cap.

6) Transfer 20 µL of Sample Lysate from the upper ½ 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.

7) Repeat step 6 until individual Sample lysate has been added to a corresponding Reagent tube in the strip.

8) 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.

9) Repeat steps 6 – 8 as needed, for the number of samples to be tested.

10) When all sample lysates have been transferred, repeat steps 6 – 8 to transfer 20 µL of NC lysate into a Reagent tube.

11) 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.

12) Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader Tray. Close and latch the 3M Molecular Detection Speed Loader Tray and lid.

13) Review and confirm the configured run in the 3M Molecular Detection Software.

14) Select the Start button in the software and select the instrument for use. The selected instrument's lid automatically opens.

15) 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 60 minutes, although positives may be detected sooner.

16) All samples must be confirmed from the enrichment broth for the presence of *E. coli* O157 using appropriate approved reference methods.

Method Comparison Study

This study included a method comparison evaluation of the 3M MDA 2 – *E. coli* O157:H7 compared to the USDA/FSIS-MLG 5.09 *Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges* for the detection of *E. coli* O157:H7 in raw ground beef (73% lean). The food matrix, raw ground beef (72% lean 27% fat), was purchased from a local distributor, prescreened for natural contamination of the target analyte and analyzed for total aerobic count by the FDA/BAM Chapter 3 method [4]. Following the screening, raw ground beef was inoculated with *E. coli* O157:H7 as indicated in Table A. The method comparison study consisted of evaluating a total of 30 un-paired sample replicates for the matrix. 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). All samples analyzed by the 3M MDA 2 - *E. coli* O157, regardless of presumptive results, were culturally confirmed by the reference method after10 hours of incubation.

Table A: Matrix and Inoculating Organism

Matrix/Test Portion Size	Inoculating Organism	Target Inoculum Level	# of Replicates	Testing Time Point/Incubation Temperature	Reference Method (Enrichment)
Raw Ground Beef 73% lean (325 gram)	*E. coli* O157:H7 ATCC[1] 43895	0 CFU/ Test Portion	5	10 hours at 41.5°C	USDA/FSIS MLG 5.09 (mTSB + CAA)
		0.2-2 CFU/ Test Portion	20		
		2-5 CFU/ Test Portion	5		

[1] ATCC - American Type Culture Collection

The inoculating organism was prepared by transferring a single colony from trypticase soy agar with 5% sheep blood (SBA) into brain heart infusion (BHI) broth and incubated at 35 ± 2°C for 24 ± 2 hours. Following incubation, the culture was diluted to a target level using BHI broth as the diluent. For the inoculated food matrix, bulk portions were spiked and homogenized in large sterile stainless steel containers. Sterile spatulas were used to mix the bulk portions to ensure the inoculum was evenly distributed. Prior to analysis, the organism was allowed to equilibrate in the matrix for 48 – 72 hours at 4°C. To prepare the test portions, 25 g of inoculated matrix was combined with 300 g of uninoculated matrix.

The level of *E. coli* in the low level inoculum for the 325 g test portions were determined by Most Probable Number (MPN) on the day of analysis by evaluating 5 x 650 g, 20 x 325 g (reference method test portions), and 5 x 130 g inoculated test samples. The level of *E. coli* in the high level inoculum for the 325 g test portions were determined by MPN on the day of analysis by evaluating 5 x 325 g (reference method test portions), 5 x 130 g, and 5 x 65 g inoculated test samples. The test portion size is presented below in Table B. Each test portion was enriched with the reference method enrichment medium and analyzed by the reference method procedure. The number of positives from the 3 test levels were used to calculate the MPN using the LCF MPN calculator (version 1.6) provided by AOAC RI. [5] (http://www.lcfltd.com/customer/LCFMPNCalculator.exe)

Table B: MPN Test Portion Sizes

Reference Method Test Portion	Inoculation Level	MPN Test Portions		
325 g	Low	5 x 650 g	20 x 325 g*	5 x 130 g
	High	5 x 325 g*	5 x 130 g	5 x 65 g

*****Test portions from reference method

USDA/FSIS-MLG 5.09: Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges

The raw ground beef test portions were prepared as outlined in the study protocol. Following equilibration of the microorganism in the matrix, test portions consisting of 325 ± 32.5 g were enriched with 975 ± 19.5 mL of pre-warmed modified tryptic soy broth with the addition of casamino acids (mTSB + CAA), homogenized for 2 minutes and incubated 15-24 hours at 42 ± 1°C. After incubation, the primary enrichment from each sample was screened using a USDA/FSIS-MLG 5.09 validated lateral flow device test system. Regardless of the screening result, all samples were subjected to isolation by immunomagnetic separation (IMS) by transferring 1.0 mL aliquots of the primary enrichment to a microcentrifuge tube containing a 50 µL suspension of *E. coli* O157 immunomagnetic (paramagnetic) beads. The solution was placed onto a Labquake™ agitator and rotated for 10 to 15 minutes at 18-30°C. After rotation the bead and sample solution was transferred to a MACS® large cell separation (ferromagnetic) column and

was washed four times with E buffer (pre-warmed to 18°C) before the final elute was collected with 1 mL of E buffer into a sterile tube. Following the IMS procedure, a 1:10 dilution and a 1:100 dilution of each IMS suspension in E Buffer, were spread plated onto modified Rainbow® agar (mRBA).

A 450 µL aliquot of each remaining IMS suspension was transferred into a microcentrifuge tube and mixed with 25 µL of a 1 N HCl solution. The microcentrifuge tubes were vortexed and placed onto a Labquake agitator and rotated for 1 hour at 18 - 30°C. After rotating, 475 µL of E buffer was added to each sample tube. The acid treated IMS suspension and a 1:10 dilution of this suspension in E Buffer were plated onto mRBA. All mRBA plates were incubated for 20 - 24 hours at 35 ± 2°C. After incubation, plates were observed for typical colonies. The mRBA plates containing typical colonies were tested for O157 latex agglutination and up to 5 isolated colonies were streaked to SBA and incubated for 16 - 24 hours at 35 ± 2°C. After incubation, SBA plates were observed for purity. Isolated colonies from SBA were confirmed positive by conducting a H7 latex agglutination test and for the presence of Shiga-toxins using the USDA approved Real-Time PCR assay. Final biochemical confirmations were obtained by VITEK 2 GN Biochemical Identification following AOAC OMA 2011.17. [6]

Confirmation

All samples analyzed by the 3M™ MDA 2 - *E. coli* O157, regardless of presumptive result, were confirmed by procedures outlined in the reference method for 10 hour sample sets. Final confirmation was achieved by VITEK® 2 GN Biochemical Identification, AOAC OMA 2011.17.

Results

Method Comparison

As per criteria outlined in Appendix J of the Official Methods of Analysis Manual, fractional positive results were obtained [7] for the raw ground beef matrix. A summary of the method comparison results are presented in Table C. The pre-evaluation pathogen screen results and aerobic plate count (APC) results are presented in Table 1 of the Appendix. A summary of the MPN results are presented in Table 2 of the Appendix. A detailed summary of results is presented in Table 3 of the Appendix.

The probability of detection (POD) was calculated as the number of positive outcomes divided by the total number of trials [8]. The POD was calculated for the candidate presumptive results, $POD_{CP,}$ the candidate confirmatory results, POD_{CC}, the difference in the candidate presumptive and confirmatory results, $dPOD_{CP,}$ presumptive candidate results that confirmed positive, $POD_{C,}$ the reference method, POD_R, and the difference in the confirmed candidate and reference methods, $dPOD_C$. The POD analysis between the 3M™ MDA 2 - *E. coli*

O157 and the reference method indicated that there was no significant difference at the 5% level between the number of positive results by the two methods. The POD analysis between the 3M™ MDA 2 - *E. coli* O157 presumptive and confirmed results indicated that there was no significant difference at the 5% level. A summary of POD analyses [9] are presented in Tables 4-7 of the Appendix.

Table C: Summary of Results

Matrix	Raw Ground Beef (73% lean 27% fat)		
Method	3M™ MDA 2 - *E. coli* O157		USDA/FSIS MLG 5.09
Result	Presumptive	Confirmed	
Time Point	10 Hours	10 Hours	
Uninoculated	0/5	0/5	0/5
Low	11/20	11/20	7/20
High	5/5	5/5	5/5

Raw Ground Beef- 10 Hour Enrichment

For the low inoculation level of the 3M™ MDA 2 - *E. coli* O157, there were 11 presumptive positives and 11 confirmed positives following the USDA/FSIS MLG 5B.05 reference method. There were 7 confirmed positives for the reference method. For the high inoculation level, there were 5 presumptive positives and 5 confirmed positives following the USDA/FSIS MLG 5.09 reference method. There were 5 confirmed positives following the reference method.

For the low inoculation level, a $dPOD_C$ value of 0.20 was obtained with a 95% confidence interval of (-0.10, 0.46), indicating no statistically significant difference between the candidate and reference method. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no statistically significant difference between the candidate presumptive and the USDA/FSIS MLG 5.09 reference method confirmed results.

For the high inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate and reference method. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no statistically significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 5 and 7 of the Appendix.

Discussion

The 3M™ MDA 2 - *E. coli* O157 accurately detected *E. coli* O157:H7 from raw ground beef at 10 hour time point. The MDA 2 - *E. coli* offers the benefits of extremely high sensitivity and high specificity for the detection of *E. coli* O157 while reducing the overall time to presumptive results. The MDA 2 - *E. coli* O157 also reduces the total analyst time required to obtain final results by implementing a single heat lysis step and cool step, followed by a single transfer to molecular reaction tubes. The small foot print of the Molecular Detection System requires minimal space, making it easy to move the instrument or pair additional MDS units to perform multiple MDA runs concurrently. The updated software is user friendly with easy to interpret results. Also, the ability to examine Relative Light Unit (RLU) curves makes troubleshooting more streamline if problems occur during testing. There was no significant, statistical difference observed at the 10 hour time point for the evaluation of raw ground beef.

Conclusion

The data from the study, within the statistical uncertainty, support the product claims of the 3M™ MDA 2 - *E. coli* O157 in the selected food matrix.

The results obtained by the POD analysis of the method comparison study demonstrated that there were no statistically significant differences between the number of positive samples detected by the candidate and the reference method for the raw ground beef food matrix for 10 hour time point.

References

[1] U.S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook, Revision 5.09 *Isolation and Identification of Escherichia*

coli O157:H7 from Meat Products and Carcass and Environmental Sponges (2015) (Accessed January 2016) http://www.fsis.usda.gov/wps/wcm/connect/51507fdb-dded-47f7-862d-ad80c3ee1738/MLG-5.pdf?MOD=AJPERES

[2] Centers for Disease and Control Prevention www.cdc.gov (accessed March 2016)

[3] Feng, Peter. Enterohemorrhagic Escherichia coli (EHEC). Bad Bug Book – Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins, 2nd Ed. 2011

[4] U. S. Food and Drug Administration *Bacteriological Analytical Manual*, Chapter 3 *Aerobic Plate Count (*January 2001) (Accessed January 2016) http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.htm

[5] Least Cost Formulations, Ltd., MPN Calculator Version 1.6 (Accessed January 2016) www.lcfltd.com/customer/LCFMPNCalculator.exe

[6] AOAC International, *Evaluation of the VITEK 2 Gram-Negative (GN) Microbial Identification Test Card: Collaborative Study,* Journal of AOAC International, 95, 3, (2012).

[7] 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)

[8] 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. pp. 335-347

[9] Least Cost Formulations, Ltd., AOAC Binary Data Interlaboratory Study Workbook Version 2.3 (2014) -http://lcfltd.com/aoac/aoac-binary-v2-2.xls (Accessed January 2016)

APPENDIX

Table 1: Aerobic Plate Count and Background Results (Prior to Inoculation)

Matrix	APC[1] (CFU/g)	*E. coli* O157:H7 Pathogen Screen[2]

Raw Ground Beef	3.2 x 10^3	0/5

[1] APC conducted in accordance with FDA/BAM Chapter 3
[2] *E. coli* O157:H7 screen conducted using USDA/FSIS MLG 5.09 lateral flow device.

Table 2: MPN Summary Table for Raw Ground Beef

Raw Ground Beef (73% lean, 27% fat) *E. coli* O157:H7 ATCC 43895					
Low Level Inoculum (0.2-2 MPN/Test Portion)					
	A	**B**	**C**	**D**	**E**
5 x 650 g	-	-	+	+	+
5 x 130 g	-	+	-	-	+
20 x 325 g (Reference Samples)	**7/20**				
MPN/Test portion	**0.49**				
Low Conf. Limit MPN/Test Portion	**0.25**				
High Conf. Limit MPN/Test Portion	**0.85**				
High Level Inoculum (2-5 MPN/Test Portion)					
	A	**B**	**C**	**D**	**E**
5 x 130 g	+	+	+	+	+
5 x 65 g	+	-	+	+	-
5 x 325 g (Reference Samples)	**5/5**				
MPN/Test portion	**2.97**				
Low Conf. Limit MPN/Test Portion	**1.30**				
High Conf. Limit MPN/Test Portion	**6.81**				

[*] MPN was calculated for the low level inoculation for raw ground beef using five 650 g, five 130 g and the 20 low level reference method samples (325 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI http://www.lcfltd.com/customer/LCFMPNCalculator
[*] MPN was calculated for the high level inoculation for raw ground beef using five 130 g, five 65 g and the 5 high level reference method samples (325 g) using the LCF MPN Calculator version 1.6 provided by AOAC-RI http://www.lcfltd.com/customer/LCFMPNCalculator

Table 3: Detailed Results for the 3M™ MDA 2 - *E. coli* O157 in Raw Ground Beef (73% Lean)

Raw Ground Beef *E. coli* O157:H7 ATCC 43895

Low Level 0.49 (0.25, 0.85) MPN/Test Portion			
Sample #	**3M MDA 2 - *E. coli* O157**		**USDA/FSIS MLG 5.09**
	presumptive	**confirmed**	
1	+	+	+
2	-	-	-
3	+	+	+
4	+	+	-
5	+	+	-
6	-	-	-
7	-	-	+
8	-	-	-
9	+	+	-
10	+	+	+
11	-	-	-
12	+	+	-
13	-	-	-
14	+	+	-
15	-	-	+
16	+	+	+
17	-	-	-
18	-	-	-
19	+	+	+
20	+	+	-
Total	**11/20**	**11/20**	**7/20**
High Level 2.97 (1.30, 6.81) MPN/Test Portion			
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+
Total	**5/5**	**5/5**	**5/5**
Uninoculated			
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
Total	**0/5**	**0/5**	**0/5**

Table 4: 3M MDA 2 - *E. coli* O157, Presumptive vs. Confirmed – POD Results

Matrix	Strain	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 Ground Beef	*E. coli* O157:H7 ATCC 43895	10 Hour	-	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	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
			2.97 (1.30, 6.81)	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 5: 3M MDA 2 - *E. coli* O157, Candidate vs. Reference – POD Results

Matrix	Strain	Time Point	MPN[a]/ Test Portion	N[b]	Candidate			Reference			dPOD$_{C}$[f]	95% CI[g]
					x[c]	POD$_{C}$[d]	95% CI	X	POD$_{R}$[e]	95% CI		
Raw Ground Beef	*E. coli* O157:H7 ATCC 43895	10 Hour	-	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	11	0.55	0.34, 0.74	7	0.35	0.18, 0.57	0.20	-0.10, 0.46
			2.97 (1.30, 6.81)	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

LABORATORY SERVICES • AUDITING • CONSULTING • EDUCATION • RESEARCH

FINAL REPORT

May 10, 2016

EVALUATION STUDY

Validation of 3M™ Molecular Detection Assay for *Escherichia coli* O157:H7 in Ground Beef

PREPARED BY

Aaron Pleitner

Research Scientist

Food Safety Net Services, Ltd.

STUDY SPONSOR

The 3M Company

1.0 OBJECTIVE

The objective of this study was to validate the 3M™ Molecular Detection Assay (MDA) for detection of *Escherichia coli* O157 in ground beef with an 18 hr enrichment time. The Food Safety Net Services (FSNS) San Antonio Laboratory worked to validate the MDA system, considered here as the 'candidate method', using a 325 g sample size for ground beef with an 18 hr enrichment. The study followed the format for the validation of a candidate method using the experimental setup and Probability of Detection (POD) statistical analysis that are provided in the 2012 version of the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces document (1). For the purposes of this study, the candidate method results were compared with the analysis of 325 g sample sizes for ground beef performed by reference methods outlined via the 2015 version of the United States Department of Agriculture Food Safety and Inspection Service Microbiological Laboratory Guidebook (USDA FSIS MLG) (2).

2.0 METHODS AND MATERIALS

2.1 Acquisition and Initial Analysis of Ground Beef

As mentioned in Section 1.0, ground beef was tested in the validation of the MDA. The FSNS San Antonio Laboratory acquired raw intact whole muscle beef from either commercial grocers or producers. Multiple lots and producers were sourced, whereupon the products were mixed in order to make an independent lot. The raw intact whole muscle beef was ground in-house at the FSNS San Antonio Laboratory. All samples were stored at refrigeration temperatures until the inoculation and testing commenced. Prior to inoculation, one 25 g sample was removed and analyzed for *E. coli* O157 according to the USDA FSIS MLG methods for *E. coli* O157 (2), as outlined in Section 2.4.

2.2 Preparation of E. coli *O157:H7 Inoculum*

As stated, the objective of this study was to determine the ability of the MDA to detect the presence of *E. coli* O157:H7 in ground beef. The inoculum used for this validation study consisted of the following strain of *E. coli* O157:H7:

- *Escherichia coli* serotype O157:H7 ATCC 35150

Prior to each day of the experiment, an individual culture was prepared by streaking loopfuls of culture from -80˚C freezer stocks onto Tryptic Soy Agar (TSA; Becton, Dickinson and Company, Franklin Lakes, NJ) and incubating aerobically for 24 h at 35 ± 2°C. One isolated colony from each TSA plate was then transferred into Tryptic Soy Broth (TSB; Becton, Dickinson and Company) and incubated aerobically at 35 ± 2°C for 24 h to achieve stationary phase growth. Cells were

harvested by centrifuging aliquots of the stationary phase TSB culture at maximum speed for 10 min in a Model 16K Microcentrifuge (Bio-Rad Laboratories, Inc., Hercules, CA). The supernatant of each centrifuged aliquot was removed and the pelleted cells were re-suspended in Butterfield's Phosphate Buffer (BPB; Made In-House From Various Ingredients). The cells re-suspended in BPB were centrifuged a second time, and the supernatant was removed once again. The pelleted cells were re-suspended once more in BPB. These final solutions of cells re-suspended in BPB were adjusted to a concentration of ~8.00 $\log_{10}$ CFU/ml based on the transmittance of the suspension as determined using a Spectronic™ 200 Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA). This suspension served as inoculum for *E. coli* O157:H7 and the concentrations of cells in each were verified on each day of the experiment by serially diluting in BPB and plating onto Sorbitol MacConkey Agar (SMAC).

2.3 Inoculation of Food Samples

After the *E. coli* O157:H7 culture was prepared, the ground beef was prepared in individual portions of 325 g. Three portion types were prepared for each testing method. Each individual portion was placed into a sterile Whirl-Pak bag. This included a high inoculum level (Portion 1), a low inoculum level (Portion 2) and un-inoculated samples (Portion 3). After inoculation, all samples for each inoculum level were removed from each Whirl-Pak bag, combined and homogenized in one large batch unit and re-aliquoted into the appropriate portion. In ground beef, a 5 and 0.5 CFU/sample was targeted for the high and low inoculum levels, respectively. As stated in Section 2.2, the inoculum concentration was enumerated at the time of inoculation. A minimum volume consisting of 4,060 g of ground beef was also removed from each inoculated portion level (i.e. Portion 1 and Portion 2) in order to perform a five tube-three level Most Probable Number (MPN) analysis on each portion as described in Section 2.6. After inoculation, the food samples were stored at 4°C for 48 h to stabilize. Once this stabilization period was complete, testing commenced. A total of 20 samples were prepared for Portion 2, 5 samples were prepared for Portion 1 and 5 samples were prepared for Portion 3.

2.4 Analysis of Reference Method Sub-Portions

After the 48 h stabilization period post-inoculation, the samples were subjected to the corresponding reference method according to USDA FSIS MLG testing procedures (2). The 325 g ground beef sample was combined with 975 mL of pre-heated (42°C) mTSB broth and hand massaged in order to homogenize. The samples were then incubated for 15-24 h at 42 $\pm$ 1°C. In brief, the following procedure followed the USDA MLG method (2). Following the enrichment, 5 $\pm$ 1 mL of each enriched culture was passed through a 40 µm cell strainer. At least 1 mL of this filtrate was added to a prepared immunomagnetic bead suspension

(Dynal® anti-*E. coli* O157 antibody-coated paramagnetic beads; Dynal Inc., Lake Success, NY). The sample tubes were then agitated for 10-15 min at 18-30°C. The sample-bead suspensions were filtered, processed and washed as described in the USDA MLG method (2). A 1:10 and 1:100 dilution of each treated bead suspension was prepared with E-Buffer. Once the dilutions were prepared, 0.1 mL of each were spread onto modified Rainbow® Agar (mRBA, Biolog Inc., Hayward, CA) plates and incubated at 35°C ± 2°C for 20-24 h. Each sample was also subjected to acid treatment as outlined and plated onto mRBA as well. Colonies exhibiting typical colony morphologies were then subject to latex agglutination assays (RIM® *E. coli* O157:H7 Latex Test (Remel, Lenexa, KS). Latex positive colonies were streaked onto TSA with 5% Sheep Blood (SBA) plates and incubated at 35°C ± 2°C for 16-24 h. Colonies with typical morphologies were then subject to biochemical testing using VITEK® 2 GN cards (bioMerieux Vitek, Inc., Hazelwood, MO) along with O157 and H7 antigen confirmations by latex agglutination testing by RIM® *E. coli* O157:H7 Latex Test. Positive samples were designated as such by the serological positive for 'O157' and biochemical identifications.

2.5 Analysis of Candidate Method Sub-Portions

After the 48 h stabilization period post-inoculation, the samples were subjected to the following enrichment procedures and testing on the MDA. 3M provided all 3M consumable supplies involved with the MDA test. 3M Buffered Peptone Water (BPW) was pre-heated to 41.5°C ± 1°C prior to enriching all samples. For raw ground beef, the 325 g sample was added to 975 mL 3M BPW. The sample was homogenized for 2 min and incubated at 41.5°C ± 1°C for 18 h. Aliquots from both time points were subject to confirmation procedures outlined in Section 2.4. The enriched samples were then subject to analysis as outlined by the instructions for use for the 3M™ Molecular Detection Assay 2 – *E. coli* O157 test kit. The sample results obtained after completing the MDA were considered the 'screening stage' result for the candidate method. Concurrent with the transfer and testing on the MDA, aliquots of the enriched samples were transferred and subjected to the testing outlined in Section 2.4 for cultural confirmation. The results obtained after completing this methodology were considered the 'confirmation stage' result for the candidate method.

2.6 Most Probable Number Analysis

As mentioned above in Section 2.3, samples for each portion were inoculated with *E. coli* O157:H7 (i.e. Portion 1, and Portion 2) and used for performing a five tube-three level MPN analysis to determine the concentration of *E. coli* O157 inoculated into the portion. This analysis was setup after the 48 hr stabilization period in order to mimic all handling and treatment of samples.

For ground beef, five 650 g samples and five 162 g samples were weighed into sterile Whirl-Pak bags and hydrated with 1,955 mL and 486 mL, respectively, of mTSB. The samples were then processed according to the reference method procedures described above in Section 2.4. Note: for the MPN test samples, the second set of five samples for the reference testing were used. Samples that confirmed positive for this procedure were noted and the LCF MPN Calculator Version 1.6 (Least Cost Formulations, Ltd., Virginia Beach, VA) was used to calculate the MPN/g and 95% confidence intervals for each portion using the mass of each sample and the number of reference method confirmed positives at each level of the MPN setup.

2.7 Probability of Detection Statistical Analysis

After results from the "screening" and "confirmation" stages were obtained for the candidate method and "confirmed" results were obtained for the reference method, the data was analyzed according to the POD methods of the 2012 version of the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces document (1). The POD for the candidate method's "screening stage" (presumptive) results (POD_{CP}) was calculated for each level of inoculation, and compared with the POD for the candidate method's "confirmation stage" (confirmed) results (POD_{CC}) at each level of inoculation in order to attain a difference in POD for the two stages ($dPOD_{CP}$) at each level of inoculation. A 95% confidence interval was also calculated for each $dPOD_{CP}$ value calculated, and was used to determine whether the "screening stage" results was significantly different from the "confirmation stage" results for each level of inoculation. According to the methodology, if the 95% confidence interval of a $dPOD_{CP}$ value does not contain zero, then the difference between the POD_{CP} and the POD_{CC} of the candidate method at that level of inoculation is statistically significant at the 5% level. In addition to these analyses, the rate of true positives (samples with a "screening stage" positive result and "confirmation stage" positive result), the rate of true negatives (samples with a "screening stage" negative result and "confirmation stage" negative result), the rate of false positives (samples with a "screening stage" positive result and "confirmation stage" negative result), and the rate of false negatives (samples with a "screening stage" negative result and "confirmation stage" positive result) were calculated. Additionally, PODs were calculated for the reference method's "confirmed" results (POD_R) at each level of inoculation and were compared to the PODs that were calculated for each level of inoculation for the candidate method where the "screening stage" and "confirmation stage" results were both positive for a particular sample (POD_C). The difference in POD for the two values at each level of inoculation were calculated ($dPOD_C$) along with its corresponding 95% confidence interval. As noted above, if the 95% confidence interval of a $dPOD_C$ value does not contain zero, then the difference between the POD_C of the candidate method and the POD_R of the

reference method at that level of inoculation is statistically significant at the 5% level.

3.0 RESULTS

The objective of this study was to validate the 3M™ Molecular Detection Assay (MDA) for the detection of *E. coli* O157:H7 in ground beef. The inoculum concentration of *E. coli* O157:H7 was determined from Most Probable Number analysis, along with the 95% confidence intervals, as shown in Table 1.

All data obtained for the candidate method testing of ground beef enriched for 18 hr, 'screening stage' and 'confirmation stage' (325 g ground beef analyzed by the 3M™ MDA) is provided in Table 2. All data obtained for the reference method testing of ground beef (325 g ground beef analyzed by the USDA FSIS MLG Method 5.09) is provided in Table 3. The number of true positives (samples with a positive 'screening stage' and a positive 'confirmation stage'), true negatives (samples with a negative 'screening stage' and a negative 'confirmation stage'), false positive (samples with a positive 'screening stage' but a negative 'confirmation stage') and false negatives (samples with a negative 'screening stage' but a positive 'confirmation stage') for the candidate method testing of ground beef enriched for 18 hr is provided in Table 4. As described in Section 2.7, the results presented here were statistically analyzed according to the POD methods of the 2012 version of the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces document (1). The POD_{CP}, POD_{CC}, $dPOD_{CP}$, POD_{C}, POD_{R}, and $dPOD_{C}$ values calculated at each level of inoculation for the candidate method and the reference method testing of ground beef, and their corresponding 95% confidence intervals, are provided below in Table 5 for the ground beef samples enriched for 18 hr.

Table 1. Concentration of* E. coli *O157:H7 Inoculum Level per Gram of Matrix, Determined through Most Probable Number Analysis

Food Matrix/Inoculation Level	Sample Size	Sample Number	Number of Positive	MPN/g[1]	95% CI[1]
Ground Beef/Low	650	5	1	1.739×10^{-3}	9.882×10^{-4}, 2.808×10^{-3}
	325	20	11		
	162	5	2		
Ground Beef/High	650	5	5	1.538×10^{9}	6.318×10^{-3}, 1.5380^{9}
	325	5	5		
	162	5	5		

1. MPN/g and 95% Confidence Interval Values were generated from the LCF MPN Calculator Version 1.6

Table 2. Candidate Method Testing Results for Ground Beef Matrix 18hr Enrichment 'Screening Stage' and 'Confirmation Stage' (325 g Ground Beef Analyzed via the 3M™ MDA)

Sample Number	Inoculum Level	3M MDS 18 h	Rainbow	Rainbow Acid Treated	O Serology	SBA	H Serology	VITEK	Confirmation
1	**LOW**	=	=	=	=				=
2	**LOW**	+	+	+	+	+	+	E.coli O157	+
3	**LOW**	+	+	+	+	+	+	E.coli O157	+
4	**LOW**	+	+	+	+	+	+	E.coli O157	+
5	**LOW**	=	=	=	=				=
6	**LOW**	+	+	+	+	+	+	E.coli O157	+
7	**LOW**	+	+	+	+	+	+	E.coli O157	+
8	**LOW**	=	=	=	=				=
9	**LOW**	=	=	=	=				=
10	**LOW**	+	+	+	+	+	+	E.coli O157	+
11	**LOW**	=	=	=	=				=
12	**LOW**	=	=	=	=				=
13	**LOW**	+	+	+	+	+	+	E.coli O157	+
14	**LOW**	=	=	=	=				=
15	**LOW**	+	+	+	+	+	+	E.coli O157	+
16	**LOW**	+	+	+	+	+	+	E.coli O157	+
17	**LOW**	=	=	=	=				=
18	**LOW**	=	=	=	=				=
19	**LOW**	=	=	=	=				=
20	**LOW**	=	=	=	=				=
1	**UNINOC**	=	=	=	=				=
2	**UNINOC**	=	=	=	=				=
3	**UNINOC**	=	=	=	=				=
4	**UNINOC**	=	=	=	=				=
5	**UNINOC**	=	=	=	=				=
1	**HIGH**	+	+	+	+	+	+	E.coli O157	+
2	**HIGH**	+	+	+	+	+	+	E.coli O157	+
3	**HIGH**	+	+	+	+	+	+	E.coli O157	+
4	**HIGH**	+	+	+	+	+	+	E.coli O157	+
5	**HIGH**	+	+	+	+	+	+	E. coli O157	+

Table 3. Reference Method Testing Results for Ground Beef Matrix (325 g Ground Beef Analyzed via the USDA FSIS MLG Method 5.09)

Sample Number	Inoculum Level	Rainbow	Rainbow Acid Treated	O Serology	SBA	H Serology	VITEK	Confirmation
1	**LOW**	=	=	=				=
2	**LOW**	=	=	=				=
3	**LOW**	+	+	+	+	+	E. coli O157	+
4	**LOW**	+	+	+	+	+	E. coli O157	+
5	**LOW**	=	=	=				=
6	**LOW**	+	+	+	+	+	E. coli O157	+
7	**LOW**	+	+	+	+	+	E. coli O157	+
8	**LOW**	=	=	=				=
9	**LOW**	=	=	=				=
10	**LOW**	+	+	+	+	+	E. coli O157	+
11	**LOW**	+	+	+	+	+	E. coli O157	+
12	**LOW**	=	=	=				=
13	**LOW**	=	=	=				=
14	**LOW**	+	+	+	+	+	E. coli O157	+
15	**LOW**	+	+	+	+	+	E. coli O157	+
16	**LOW**	=	=	=				=
17	**LOW**	+	+	+	+	+	E. coli O157	+
18	**LOW**	+	+	+	+	+	E. coli O157	+
19	**LOW**	=	=	=				=
20	**LOW**	+	+	+	+	+	E. coli O157	+
1	**UNINOC**	=	=	=				=
2	**UNINOC**	=	=	=				=
3	**UNINOC**	=	=	=				=
4	**UNINOC**	=	=	=				=
5	**UNINOC**	=	=	=				=
1	**HIGH**	+	+	+	+	+	E. coli O157	+
2	**HIGH**	+	+	+	+	+	E. coli O157	+
3	**HIGH**	+	+	+	+	+	E. coli O157	+
4	**HIGH**	+	+	+	+	+	E. coli O157	+
5	**HIGH**	+	+	+	+	+	E. coli O157	+

Table 4. Quantities and Percentages of True Positive, True Negatives, False Positives, and False Negatives for the Candidate Method Testing of Ground Beef, 18hr Enrichment

Inoculation Level	Final Result[1]	Total	Percentage (%)
High	True Positive	5	100
	True Negative	0	0
	False Negative	0	0
	False Positive	0	0
Low	True Positive	9	45
	True Negative	11	55
	False Negative	0	0
	False Positive	0	0
Un-inoculated	True Positive	0	0
	True Negative	5	100
	False Negative	0	0
	False Positive	0	0

1. TRUE POSITIVE, ""Screening Stage"" Positive Result-"Confirmation Stage" Positive Result; TRUE NEGATIVE, "Screening Stage" Negative Result-"Confirmation Stage" Negative Result; FALSE POSITIVE, "Screening Stage" Positive Result-"Confirmation Stage" Negative Result; FALSE NEGATIVE, "Screening Stage" Negative Result-"Confirmation Stage" Positive Result

Table 5. POD_{CP}, POD_{CC}, and $dPOD_{CP}$ Values for the Candidate Method (325 g Ground Beef 18 hr Enrichment Analyzed via the 3M™ MDA) and POD_R, POD_C, and $dPOD_C$ Values for Candidate Method vs. the Reference Method (325 g Ground Beef 18 hr Enrichment Analyzed via the USDA FSIS MLG Method 5.09)

Method / Inoculation Level	Measure	Value	95% CI
Candidate Method Screening vs. Confirmation High Level Inoculation	POD_{CP}	1.000	(0.5655, 1.000)
	POD_{CC}	1.000	(0.5655, 1.000)
	$dPOD_{CP}$	0.000	(-0.434, 0.434)
Candidate Method Screening vs. Confirmation Low Level Inoculation	POD_{CP}	0.450	(0.2582, 0.6579)
	POD_{CC}	0.450	(0.2582, 0.6579)
	$dPOD_{CP}$	0.000	(-0.283, 0.283)
Candidate Method Screening vs. Confirmation Un-inoculated	POD_{CP}	0.000	(0.000, 0.4345)
	POD_{CC}	0.000	(0.000, 0.4345)
	$dPOD_{CP}$	0.000	(-0.434, 0.434)
Reference Method vs. Candidate Method High Level Inoculation	POD_R	1.000	(0.5655, 1.000)
	POD_C	1.000	(0.5655, 1.000)
	$dPOD_C$	0.000	(-0.434, 0.434)
Reference Method vs. Candidate Method Low Level Inoculation	POD_R	0.550	(0.3421, 0.7418)
	POD_C	0.450	(0.2582, 0.6579)
	$dPOD_C$	-0.100	(-0.371, 0.194)
Reference Method vs. Candidate Method Un-inoculated	POD_R	0.000	(0.000, 0.4345)
	POD_C	0.000	(0.000, 0.4345)
	$dPOD_C$	0.000	(-0.434, 0.434)

POD_{CP}, Probability of Detection for Candidate Method "Screening Stage" Positive Results; POD_{CC}, Probability of Detection for Candidate Method "Confirmation Stage" Results; $dPOD_{CP}$, POD_{CP} Minus POD_{CC}; POD_R, Probability of Detection for Reference Method Confirmed Results; POD_C, Probability of Detection for Candidate Method Confirmed Results (i.e. Combined "Screening Stage" and "Confirmation Stage" Results); $dPOD_C$, POD_R Minus POD_C. 95% Confidence Interval Values are Generated from the AOAC International POD Calculator.

4.0 DISCUSSION

The purpose of this study was to validate the 3M™ Molecular Detection Assay (MDA) for detection of *Escherichia coli* O157:H7 in ground beef with an 18 hr enrichment time. This study followed the format for the validation of a candidate method using the experimental setup and Probability of Detection (POD) statistical analysis provided in the February 24, 2012 version of the AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces document (1). Portions of ground beef (325 g sample size) were inoculated with *E. coli* O157:H7 at a concentration aimed to achieve a targeted level of contamination. The inoculation levels targeted were chosen in order to meet the targeted POD values for the different inoculation levels. The concentrations achieved through inoculation were found to be 1.739×10^{-3} MPN/g for the low inoculation level of ground beef and 1.538×10^{9} MPN/g for the high inoculation level (Table 1). In addition to this, the AOAC fractional positive criterion (POD between 0.25 and 0.75) was met, providing evidence that the inoculation procedure was successful for ground beef with an 18 hr enrichment time (Table 4).

Review of the POD calculation outcomes for the 18 hr ground beef enrichment candidate method (325 g Ground Beef Analyzed via the 3M™ MDA) and reference method (325 g Ground Beef Analyzed via the USDA FSIS MLG Method 5.09) shows that the 95% confidence intervals contained 0.000 at all inoculation levels for the $dPOD_{CP}$, comparing the 'screening stage' and 'confirmation stage' of the candidate method, and the $dPOD_{C}$, comparing the candidate method and reference method (Table 5). Because of this, for the candidate method, the POD_{CP} values were not significantly different from their corresponding POD_{CC} values. Based on this outcome, it can be stated that the probability of detecting a positive result in the "screening stage" of the candidate method was not significantly different from the probability of detecting a positive result in its "confirmation stage" at any of the inoculation levels tested. Additionally, for the candidate method versus the reference method, the POD_{C} values were not significantly different from their corresponding POD_{R} values. Based on these outcomes, by fulfilling the criteria to contain 0.000 within the 95% confidence intervals, it can be stated that the probability of detecting a positive result in the candidate method was not significantly different from the probability of detecting a positive results in the reference method. Thus, the candidate method and the reference method can be considered equivalent, based on the AOAC International guidelines. Also, the lack of false positive or false negatives (Table 4) when comparing the 'screening stage' and the 'confirmation stage' of the candidate method further supports the adequacy of the method.

Overall, based on the results of this study, the 3M™ Molecular Detection Assay (MDA) is a valid method for the detection of *Escherichia coli* O157:H7 in 325 g ground beef (18 hr enrichment). The candidate methods explored here with the targeted sample sizes produce equivalent results in both the 'screening stage' and the 'confirmation stage' and produce equivalent results when compared with the USDA MLG reference method, typically employed for ground beef.

5.0 REFERENCES

1. **Brunelle S, LaBudde R, Nelson M, Wehling P**. 2012. AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environ-mental Surfaces. Available at: http://www.aoac.org/imis15_prod/AOAC_Docs/StandardsDevelopment/AOAC_Validation_Guidelines_for_Food_Microbiology-Prepub_version.pdf. Accessed: 11/30/15.
2. **United States Department of Agriculture, Food Safety Inspection Service, Office of Public Health Science**. 2014. Microbiology Laboratory Guidebook Method 5.09: Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Product and Carcass and Environmental Sponges. Available at: http://www.fsis.usda.gov/wps/wcm/connect/51507fdb-dded-47f7-862d-ad80c3ee1738/MLG-5pdf?MOD=AJPERES. Accessed: 11/30/2015.

6.0 DATA DISPOSITION

Copies of raw data and final reports from this study will be archived at Food Safety Net Services, Ltd., 199 W. Rhapsody, San Antonio, TX 78216, and shall be kept confidential as per the Agreement between FSNS and 3M™.

7.0 PROPOSAL APPROVAL

Prepared By:

5/10/16

Aaron Pleitner, Ph.D.
Research Scientist
Food Safety Net Services, Ltd.

Date

Approved By:

Study Sponsors – The 3M Company

Lisa Monteroso
Regulatory Affairs Specialist
3M Health Care

Date

Evaluation of the *mericon*® E. coli O157 Screen Plus and *mericon* E. coli STEC O-Type Pathogen Detection AssaysinSelectFoods: Collaborative Study

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The QIAGEN *mericon*®E. coliO157 Screen Plus and *mericon*E. coliSTEC O-Type Pathogen Detection Assays employ Real-Time (RT) PCR technology incorporating a HotStart Taq DNA Polymerase for the rapid, accurate detection of *Escherichia coli* O157 and the Big 6 (non-O157 Shiga toxin-producing*E. coli* (STEC)) from select food types. Using a paired study design, the *mericon* E. coliO157 Screen Plus Pathogen Detection Assay and the*mericon* E. coliSTEC O-Type Pathogen Detection Assay were compared to the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) Microbiology Laboratory Guidebook (MLG) Chapter 5.09*Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges*for the detection of *E. coli* O157:H7 in raw ground beef (73% lean). Both *mericon* assays were evaluated using themanual and automated DNA extraction method.Thirteen (13) technicians from5laboratories located within the continental United Statesparticipated in the collaborative study. Three levels of contamination were evaluated: 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 intervalof 0.00, (-0.12, 0.12) for the *mericon* E. coliO157 Screen Plus with manual and automated extraction and *mericon* E. coliSTEC O-Type with manual extraction and -0.01, (-0.13, 0.10) for the *mericon* E. coliSTEC O-Type with automated extraction. The dLPOD resultsindicate no statistically significant difference between the candidate methods and reference method.

Most *E. coli* bacteria are harmless; however, some produce a toxin (Shiga toxin) that can cause serious illness, including hemolytic anemia or renal failure, and can sometimes lead to death. Like generic *E. coli*, Shiga-toxigenic *Escherichia coli* (STEC) are Gram-negative, rod-shaped bacteria that are characterized by the production of Shiga toxins (*stx1, stx 2*) and the virulence

factor intimin*eae* [1]. Depending on the reference cited, there are approximately 200 to 400 STEC serotypes, many of which have not been implicated in human illness; however, a subset of STEC called enterohemorrhagic *Escherichia coli* (EHEC) includes only those that cause serious illness [2]. EHEC outbreaks have been traced to many different types of foods such as the following: raw ground meats, unpasteurized ("raw") milk, unpasteurized fruit juice, lettuce, spinach, sprouts, and more recently, commercially manufactured frozen cookie dough. Infections caused by EHEC can progress into life-threatening problems with children and immuno-compromised people being at especially high risk[1].

QIAGEN's *mericon*E. coli O157 Screen Plus and *mericon*E. coliSTEC O-TypePathogen Detection Assaysaremultiplex PCR assays designed to provide both a screening and confirmation assay. The *mericon*E. coliO157 Screen Plus is designed to detect the presence of *E. coli* O157 and pertinent virulence genes *stx*1/2 and *eae.* The *mericon*E. coliSTEC O-Typeis designed to confirm the presence of *E. coli* O157:H7 and the big six non-O157 STEC strains (*E. coli* O26, O45, O103, O111, O121 and O145). The assays can be utilized with DNA extracted following a manual or automated procedure on the QIAsymphony QS/AS instrument. Final prepared PCR samples are analyzed using the Rotor-Gene Q cycler and its software.
Prior to the collaborative study, both the *mericon*E. coliO157 Screen Plus and *mericon*E. coliSTEC O-Type Pathogen Detection Assays with manual and automated DNA extractionwere validated according to current AOAC Guidelines[3] in a harmonized AOAC® Performance Tested Method℠ (PTM) study. The objective of thestudy was to demonstrate that the *mericon* methods could detect and confirm the presence of both *E. coli* O157 and non-O157 STECin select food matrices as claimed by the manufacturer. The assays, using both the manual and automated DNA extraction procedures, were evaluated for 3 matrices:fresh spinach (25g), raw ground beef (73% lean) (325g)and raw beef trim (325g).

Additional PTM parameters (inclusivity, exclusivity, ruggedness, stability and lot-to-lot variability) were tested and satisfied the performance requirements for AOAC PTM approval. The *mericon*E. coli O157Screen Plus Pathogen Detection Assay was awarded PTM certification number 101503 and the *mericon*E. coli STEC O-Type Pathogen Detection Assay was awarded PTM certification number 101504 on October23rd, 2015.

The purpose of this collaborative studywas to compare the reproducibility of both the*mericon*E. coliO157 Screen Plus Pathogen Detection Assay and *mericon*E. coliSTEC O-Type Pathogen Detection Assay with manual and automated DNA extraction method to the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) -Microbiology Laboratory Guidebook (MLG)Chapter 5.09 *Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges*[4] for 73% lean raw ground beef (325 g).

Collaborative Study

Study Design

In this collaborative study, one matrix, raw ground beef (73% lean), wasevaluated. The matrix was obtained from a local retailer and screened for the presenceof *Escherichia coli* O157by the USDA/FSIS MLG 5.09 reference method. The raw ground beef was artificially contaminated with fresh unstressed cells of *Escherichia coli* O157:H7, American Type Culture Collection (ATCC) 43895,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. Due to a common primary enrichment, 1 set of 325 gram samples (36 total) wassent to each participating technician for analysis by both candidate methods (*mericon*E. coliO157 Screen Plus Pathogen Detection Assay and *mericon*E. coliSTEC O-Type Pathogen Detection Assay) and reference method. The candidate methods were evaluated using both the manual and automated DNA extraction 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) [5] 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.

Preparation of Inoculum and Test Portions

The *Escherichia coli* O157:H7ATCC 43895 culture used in this evaluation was propagated onto Tryptic Soy Agar with 5% Sheep Blood (SBA) from a Q Laboratories frozen stock culture stored at -70°C. The 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.Appropriate 1:10 dilutions were prepared in Butterfield's Phosphate Diluent (BPD) based on previously established growth curves for both low and high inoculation levels. Bulk portions of the matrix were inoculated with the diluted liquid inoculum and mixed thoroughly to ensure an even distribution of microorganisms. For the preparation of test portions, 25 g of inoculated test product was mixed with 300 g of un-inoculated test product to prepare 325 g test portions which were packaged in sterile Whirl-Pak® bags and shipped to collaborators.
To determine the level of *Escherichia coli* O157:H7 in the matrix, 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 5.09 reference method. The MPN was determined by analyzing 5 x 650 g test portions,thereference method test portions from the collaborating laboratories and 5 x 160 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)[6].

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).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. A single set of test portions (36) was enriched following a paired study design with the candidate method utilizing two sample preparation techniques, manual and automated DNA extraction methods. Each collaborator received 36 test portions (12 high, 12 low and 12 un-inoculated controls). Thetest portions (325 g) were enriched with 975 mL of modified tryptic soy broth(includes casamino acids), homogenized for 2 minutes and incubated for 10 hours at 42 ±1°C. After 10 hours, a subsample of each enrichment was removed for analysis by the *mericon* methods and the remaining sample was incubated for an additional 5-14 hours (a total incubation time of 15-24 hours) at 42 ± 1°C.

Following the 10 hour enrichment, samples were assayed by the *mericon*E. coli O157Screen Plus Pathogen Detection Assay(using both the manual and automated DNA extraction procedure methods) and then by the*mericon*E. coliSTEC O-Type Pathogen Detection Assay (using both manual and automated DNA extraction method procedures). Regardless of presumptive results (from both the screening assay and the O-Type assay) obtained from both assays and extraction procedures,all test portions were confirmed following the USDA/FSIS MLG 5.09 reference method after a total incubation time of 15-24 hours, beginning with a screen using an USDA/FSIS approved lateral flow device. All positive test portions were evaluated for the presence of O157 and H7 antigens by latex agglutination, for the presence of *stx* 1/2 and *eae* genes by a USDA/FSIS approved PCR test and biochemically confirmed by the API 20 E or VITEK 2 GNbiochemical identification test, AOAC Official Method 2011.17[7].

Statistical Analysis

Each collaborating laboratory recorded results for both *mericon*E. coli O157Screen Plus Pathogen Detection Assay and *mericon*E. coliSTEC O-Type Pathogen Detection Assay, using both the manual and automated DNA extraction procedures on the data sheets provided. The data sheets were submitted to the study director at the end of testing for statistical analysis. Data for each contamination level was analyzed using the probability of detection (POD)statistical model [8]. 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 *mericon*E. coliO157 Screen Plus Pathogen Detection Assay or*mericon*E. coliSTEC O-Type Pathogen Detection Assay, and the reference methodat 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 [9].

AOAC Official Method 2016.xxx
Escherichia coli O157:H7 and *Escherichia coli* non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC)in SelectFoodsQIAGEN *mericon*®E. coliO157 Screen Plus and *mericon*®E. coliSTEC O-Type Pathogen DetectionAssays
First Action 2016

Applicable to detection of *Escherichia coli* O157:H7 and *Escherichia coli* non-O157 STECin fresh spinach (25g), raw ground beef (70% lean) (325g), and raw beef trim (325g).

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

A. Principle

QIAGEN's *mericon*E. coliO157 Screen Plus and *mericon*E. coliSTEC O-Type *Pathogen Detection Assays* aremultiplex PCR assays that screen and confirm the presence of *E. coli* O157 and non-O157 STEC in food matrices. Each *mericon*PCR Assay includes a PCR primer set for a pathogen-specific target sequence, probes labeled with four distinct fluorescent dyes (*mericon* E. coliO157 Screen Plus) or three distinct fluorescent dyes (*mericon* E. coliSTEC O-Type), positive control DNA, and all of the reagents necessary to perform the analysis. The Multiplex PCR Master Mix included in each kit contains QIAGEN proprietary technology, including HotStart Taq *plus* DNA Polymerase, patented multiplex PCR technology such as Factor MP, and fast cycling technology including Q-bond. Extracted DNA is analyzed using the Rotor-Gene Q® cycler which uses proprietary software to detect the presence of target DNA.
QIAGEN, 19300 Germantown Rd., Germantown, MD 20874

B. Apparatus and Reagents

Items are available from QIAGEN (Germantown, MD, 20874, USA).

- **(a)** Rotor-Gene Q5 Plex system (cat. 9001570)- For automated detection of pathogens with a notebook computer containing Rotor-Gene Q Software version 2.3.1.49.
- **(b)** QIAsymphony SP/AS instrument(cat. 9001297 and cat. 9001301)– For automated sample preparation, DNA extraction, and PCR preparation.
- **(c)** *mericon*E. coli O157 Screen Plus Pathogen Detection Assay (24) (cat. 290403)
- **(d)** *mericon*E. coli STEC O-Type Pathogen Detection Assay (24) (cat. 290233)
- **(e)** *mericon*DNA Bacteria Kit (100) (cat. 69525)
- **(f)** QIAsymphony®*mericon*DNA Bacteria Kit (360) (cat. 931156)
- **(g)** 72-Well Rotor (cat. 9018903) - For holding 0.1 mL Strip Tubes and Caps.
- **(h)** Locking Ring 72-Well Rotor (cat. 9018904) - For locking 0.1 mL Strip Tubes and Caps.
- **(i)** Rotor- Disc 72 Rotor (cat. 9018899) - For holding Rotor- Disc 72 (cat. 981303 or 981301).
- **(j)** Rotor-Disc 72 Locking Ring (cat. 9018900) - For locking Rotor-Disc
- **(k)** Insert, 2.0 mL v2, samplecarrier. (24), Qsym (cat. 9242083)
- **(l)** Cooling Adapter, EMT, v2, Qsym (cat. 920730)
- **(m)**Sample Prep Cartridges, 8-well (cat. 997002)
- **(n)** 8-Rod Covers (cat. 997004)
- **(o)** Filter-Tips, 1500 µL (cat. 997024)

(p) Elution Microtubes CL with cap strips (cat. 19588)
(q) Cooling Adapter, Reagent Holder 1, (cat. 9018090)
(r) Adapter 2 x Rotor-Disc, Qsym (cat. 9242204)
(s) Rotor-Disc 72 Loading Block (cat. 9018910)
(t) Rotor-Disc Heat Sealing Film (cat. 981604 (600) / 981601 (60))
(u) Rotor-Disc Heat Sealer(cat. 9018898 (110V); *QIAGEN* cat. 9019725 (230V))
(v) Filter-Tips, 200 µL (cat. 990332)
(w) Filter-Tips, 50 µL (cat. 997120)
(x) Disposable pipette – capable of 10-1000 µL
(y) Sterile filter tip pipette tips- capable of 10-1000 µL
(z) Top loading balance – Capable of weighing and measuring 25-2000 grams
(aa) Filter Stomacher® bags – Seward or equivalent.
(bb) Stomacher®3500 – Seward or equivalent.
(cc) Thermometer – calibrated range to include 100 ± 1°C
(dd) Dry block heater unit– capable of maintaining 100 ± 1°C
(ee) Microcentrifuge – Capable of holding 1.5-2 mL microcentrifuge tubes
(ff) Vortex – For sample mixing
(gg) Incubators. – Capable of maintaining 42 ± 1°C.
(hh) Freezer –capable of maintaining -10 to -20°C, for storing the purified DNA for long term storage and purified genomic DNA
(ii) Refrigerator – capable of maintaining 2-8° C, for storing the purified viral DNA for up to 24 hours prior to analysis

C. General Instructions

(a) Store the *mericon*E. coliO157 Screen Plus and *mericon*E. coliSTEC O-TypePathogen Detection Assaysat 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 the *mericon* E. coliPCR kit at -30 °C to -15 °C. Do not use either of the *mericon* kits past the expiration date.

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

Safety Precautions

*mericon*E.coli O157 Screen Plusand *mericon* E.coli STEC O-Type Pathogen Detection Assays and *mericon* DNA Bacteria Kit

All chemicals should be considered potentially hazardous. When working with chemicals, always wear a suitable lab coat and disposable gloves. Product usage should follow good laboratory practices.

QIAsymphony*mericon* Bacteria Kit

The buffers in the reagent cartridge contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is

spilled, clean with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite solution. Reagents in this kit are highly flammable and are harmful by inhalation, skin contact and swallowing.

Real-Time PCR System

Improper use of the Rotor-Gene Q may cause personal injuries or damage to the instrument. The Rotor-Gene Q must only be operated by qualified personnel who have been appropriately trained. Servicing of the Rotor-Gene Q must only be performed by QIAGEN Field Service Specialists.

Enrichment

Shiga-toxin producing *Escherichia coli* is a Biosafety Level 2 organism. Biological samples such as enrichments have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes. Wear appropriate protective equipment which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities utilizing the appropriate safety equipment (for example, physical containment devices). Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials. All enrichment broths should be sterilized following any culture based confirmatory steps.

The user should read, understand and follow all safety information in the instructions for the *mericon*E. coliO157 Screen Plus and *mericon*E. coliSTEC O-TypePathogen Detection Assays. 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.

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 modified tryptic soy broth enrichment medium (includes casamino acids) to equilibrate to 42 ± 1 °C.

(b) Aseptically combine the enrichment medium and sample according to Table 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 42 ± 1 °C according to Table A.

Table A: Sample Enrichment and Incubation

Matrix	Sample Size	Enrichment Media and Volume	Incubation Conditions
Fresh Spinach	25 g	mTSB/225 mL	42 ± 1°C for 10 hours
Raw Ground Beef (73% Lean)	325 g	mTSB/975 mL	42 ± 1°C for 10 hours
Raw Beef Trim	325 g	mTSB/975 mL	42 ± 1°C for 10 hours

E. Preparing Samples

(a) *Manual mericonDNA Extraction Method*

1) Gently mix all enriched samples to disperse *E. coli* organisms throughout the sample. Transfer 1 mL of enriched sample to a sterile 2 mL screw cap tube and centrifuge for 5 minutes at 13,000 x *g*.

2) Discard the supernatant with a pipettor, taking care not to disrupt the pellet.

3) Add 200 µL Fast Lysis Buffer to the bacterial pellet and vortex the tube to resuspend the pellet.

4) Place the microcentrifuge tube into a dry bath incubator (100 ± 1°C) for 10 minutes.

5) Remove the sample and allow it to cool to room temperature (15-24 °C) for 2 minutes.

6) Centrifuge the tube at 13,000 x *g* for 5 minutes.

7) For raw ground beef (73% lean), dilute the extracted DNA 1:10 using sterile nuclease-free water.

8) Prepare the reconstituted assay by adding 1040 µL (96 sample kit) or 130 µl (24 sample kit) of the Multiplex PCR master mix to a vial of *mericon*® Assay and mix by pipetting up and down 2-3 times.

9) Prepare a positive control by re-suspending the lyophilized Positive Control DNA in 200 µL of QuantiTect® Nucleic Acid Dilution Buffer and mix.

10) Prepare the appropriate number of strip tubes by transferring 10 µL of reconstituted assay to reaction tubes. Pipette 10 µL of extracted sample DNA into each sample strip tube and mix by pipetting up and down 2-3 times.

11) Prepare the positive control tube by pipetting 10 µL of the Positive Control DNA into a strip tube containing 10 µL of reconstituted assay. Prepare the negative control by pipetting 10 µL of RNase-free water into a strip tube with 10 µL of reconstituted assay.

12) Seal the strip tubes with the strip tube caps.

13) Load sample tubes into the 72-well rotor and load into the Rotor-Gene-Q and initiate analysis immediately.

(b) *Automated QIAsymphony® DNA Extraction Method*

1) Ensure the "Waste" drawer is prepared properly, and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag, if necessary.

2) Load an elution microtube rack (EMTR) into the "Eluate" drawer and perform an inventory scan of the "Eluate" drawer.

3) Load the QIAsymphony*mericon* Bacteria Kit and consumables into the "Reagents and Consumables" drawer. Select the "R+C" button in the touchscreen to open the screen that shows the consumables status ("Consumables/8-RodCovers/Tubes/ Filter-Tips/Reagent Cartridges"). Select the "Scan Bottle" button to scan the bar code of the TopElute bottle with the handheld bar code scanner. Select the "OK" button.

4) Perform an inventory scan of the "Reagents and Consumables" drawer.

5) Transfer 500 µL of the enriched sample into a 2 mL screw cap tube. Place the samples into the appropriate tube carrier and load them into the "Sample" drawer.

6) Using the touch screen, enter the required information for each batch of samples to be processed.

7) Choose elution volumes according to Table B.

Table B: Matrix Manual Dilution and Automated Elution Volumes

Matrix	Manual *mericon*® Method DNA Dilution	Automated *QIAsymphony*® Method DNA Elution Volume
Fresh Spinach	1:10	400 µL
Raw Ground Beef (73% Lean)	1:10	400 µL
Raw Beef Trim	1:10	400 µL

8) Select the "Run" button to start the purification procedure.

9) When sample processing is complete, perform a direct transfer of the elution rack to the QIAsymphony AS via the transfer module (integrated operation). Select "Transfer" to transfer the elution rack from slot 1 of the QIAsymphony SP to slot 2 of the QIAsymphony AS.

10) For independent transfer, open the eluate drawer, select the "complete" button on the main QIAsymphony SP page to remove the eluate tray.

11) If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips immediately after the end of the last protocol run to avoid evaporation.

12) Discard used sample tubes, plates, and waste according to your local safety regulations and replace the tip disposal bag.

13) Close the instrument drawers, and proceed with assay setup on the QIAsymphony AS.

Automated QIAsymphony Assay Setup

1) Insert the tip chute into its position on the right hand side in the front part of the QIAsymphony AS module.

2) Install an empty tip disposal bag in the bag holder under the "Assays" drawer.

3) Switch user interface from sample preparation to assay setup by selecting the assay setuptab.

4) Start the assay definition process.

5) For integrated operation (elution rack is automatically transferred from the QIAsymphony SP into the AS module) the “Sample Rack(s)” screen will appear directly.

6) All stored sample information (sample status, sample ID, sample volume, and rack ID) is transferred to the QIAsymphony AS module together with the elution rack and will automatically complete the required information in the “Sample Rack(s)” screen of the assay setup user interface.

7) If the assay setup is independent from a former QIAsymphony SP run, select the rack file of the corresponding QIAsymphony SP run or select the rack type of your elution rack for the highlighted “Sample” position (slot 2) and then either manually type in the “Rack ID” of the elution rack or choose “Automatic ID” for a new ID.

8) In the “Assay Selection” screen, select the Assay Parameter Set(s) to use in the run.

9) In the “Assay Assignment” screen, assign the Assay Parameter Sets to samples.

10) In the “Assay Rack(s)” screen, define the assay rack ID. Either type in the assay rack ID manually or choose “Automatic ID” for a new ID.

11) The cooling of samples and reagents will start automatically. Check the temperature of the cooling positions.

12) The “Loading Information” screen displays the working table of the QIAsymphony AS module with all previously defined sample and reagent rack types in the designated positions. The required position of the PCR reaction adapter is displayed as well as information on the required filter-tip types and number.

13) Place the reconstituted *mericon*E. coli O157Screen Plus or the *mericon*E.coliSTEC O-Type Pathogen Detection Assays, the reconstituted Positive Control(s) and the Negative Control(s), without lids, into the appropriate positions of the precooled reagent adapters.

14) Open the “Eluate and Reagents” and “Assays” drawers.

15) Load the prepared reagent adapter into slot 3 of the “Eluate and Reagents” drawer according to the illustration in the “Loading Information” screen. Place the Rotor-Disc in the appropriate adapter and load the adapter into the designated slot of the “Assays” drawer.

16) Load disposable filter-tips into the “Eluate and Reagents” and “Assays” drawers, according to the required number of each tip type.

17) Close the “Eluate and Reagents” and “Assays” drawers.

18) Upon closing each drawer, select “Yes” to start the inventory scan for each drawer.

19) Select “Queue”. Monitoring of the cooling starts.

20) Select “Run” to start the run.

21) After the run is finished, select “Remove” in the assay setup “Overview” screen. Open the “Assays” drawer and unload the Rotor-Disc 72.

22) Place the Rotor-Disc sealing film over the Rotor-Disc 72; place it into the Rotor-Disc heat sealer. Sealing is complete in about 2 minutes. Remove the excess film from the Rotor-Disc 72 and transfer to the Rotor-Disc 72 Rotor. In the “Sample Rack Layout” screen of the assay setup user interface, the elution rack in slot 2 is pictured.

F. Analysis

(a) *Prepare Equipment*

1) Turn on the Rotor-Gene Q instrument and attached computer. Load the Rotor-Gene Q Series Software. Start an empty run to warm up the Rotor-Gene Q.

   i. Run a Disc
   ii. Load Samples

2) Manual *mericon*DNA Extraction Method

   i. Load the QIAGEN strip tubes into the 72-Well Rotor strip tube ring. Snap the locking ring in place over the strip tubes, and place into the Rotor-Gene Q. When running strip tubes, balance the ring using the empty strip tubes.

3) Automated QIAsymphonyDNA Extraction Method

   i. Load the QIAGEN 72 Rotor-Disk into the 72-Rotor Disk ring. Snap the locking ring in place over the Rotor Disk, and place into the Rotor-Gene Q.

4) Start Instrument Run

   i. Software Version 2.3.1 (Build 49)

   Follow the Rotor-Gene software instructions. Verify that the correct setting is selected for each preparation method: the Rotor-Disc 72 rotor is selected for the QIAsymphony (automated preparation method), and the

72 count strip tubes for the manual preparation method. Ensure that the locking ring is in place and select "Next". Verify that the reaction volume is 20 µL and select "Next". Select "Next" after verifying the temperature profile. Select "Start Run" to start the Rotor-Gene Q instrument.

5) When the run is complete, click "Save" and remove the samples from the instrument.

G. Interpretation and Test Result Report

(a) Viewing results

1) Software Version 2.3.1 (Build 49)

i. When the run is complete, select "Analysis" located on the top of the main page of the software. In the "Analysis" box, import the analysis settings to each channel by activating the "Quantitation Analysis" window for the channel and select the respective file from your directory with the "Import" function. Select "Ignore First" and ignore the first 10 cycles for all four channels. For all channels (Green, Crimson, Yellow, and Orange) select "Take Off Point Adjustment". Adjust the settings so that if the take off point was calculated before cycle 15, then cycle 20 is used as the take of point, then select "OK". Set the threshold for channels Green, Crimson, and Yellow to 0.035 and set the threshold for channel Orange to 0.08.

(b) Printing and Exporting Results

To export the results to Excel, go to the "File" menu, followed by "Save As" and then "Excel Analysis Sheet". The results will be saved in a .csv format. The results may be saved to the notebook computer or an external flash drive. The results may be printed by opening the "Report Browser" window, choosing a report, and select "Show." Choose "Print" on the report window.

Results of Collaborative Study

This collaborative study involved a method comparison evaluation of the *mericon*E. coliO157 Screen Plus and *mericon*E. coliSTEC O-Type Pathogen Detection Assays to the USDA FSIS MLG 5.09 reference method for raw ground beef. A total of five laboratories throughout the continental United States participated in this study. Four of the laboratories had 3 separate analysts participate and the fifth laboratory had one participant. Twelve of the 13 total participants submitted data. One participant, identified as Laboratory #2, did not incubate their test portions for the correct duration. When the error was determined, no further sample analysis was conducted and no data was submitted. Each participant analyzed 36 paired test portions for each assay: 12 inoculated with a high level of *E. coli* O157:H7, 12 inoculated with a low level of *E. coli* O157:H7, and 12 un-inoculated controls.

A background screen of the matrix indicated an absence of indigenous *E. coli* O157:H7. Ten (10) replicate test portions (randomly sampled from 50% of the total packages used in the analysis) were screened for the presence of *E. coli* O157:H7. All test portions produced negative results for the target analyte.

Table 2016.1A and 2016.1D summarize the inter-laboratory results for each assay (screening and confirmatory) for both manual and automated preparation steps. 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 2016.2D. The level of *E. coli* O157:H7 was determined by MPN on the day of initiation of analysis by the coordinating laboratory. The MPN levels obtained, with a 95% confidence interval, were 0.62 CFU/test portion (0.48, 0.77) for the low inoculum level and 5.13 CFU/test portion (3.45, 7.62) for the high inoculum level.MPN results are presented in the second column of Tables 2016.2A and 2016.2D. The individual laboratory and sample results are presented in Tables 1-2 of the Supplementary Materials. The APC results for each collaborating are presented in Table 3 of the Supplementary Materials.

Raw Ground Beef – *mericon* E. coli O157Screen Plus with Manual Preparation

For the low inoculum level, 69 out of 144 test portions (POD_{CP} of 0.48) were reported as presumptive positive by the *mericon*E. coliO157Screen Plus with 65 out of 144 test portions (POD_{CC}& POD_R of 0.45 due to paired sample enrichment) confirming positive. For samples that produced presumptive positive results on the *mericon*E. coliO157Screen Plus, 65 out of 144 samples confirmed positive (POD_C of 0.45, value include only presumptive positive results that confirmed positive). A $dLPOD_C$ value of 0.00 with 95% confidence interval of (-0.12, 0.12) was obtained between the candidate and reference method, indicating no statistically significant difference between the two methods. A $dLPOD_{CP}$ value of 0.03 with 95% confidence intervals of (-0.09, 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, 144 out of 144 test portions (POD_{CP} of 1.00) were reported as presumptive positive by the *mericon*E. coliO157Screen Plus with 144 out of 144 test portions (POD_{CC}& POD_R of 1.00) confirming positive. For samples that produced presumptive positive results on the *mericon* E. coliO157Screen Plus, 144 out of 144 samples confirmed positive (POD_C of 1.00). A $dLPOD_C$ value of 0.00 with 95% confidence interval 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, 3 out of 144 samples (POD_{CP} of 0.02) produced a presumptive positive result by the *mericon* E. coli O157Screen Plus with 0 out of 144 test portions (POD_{CC}& POD_R of 0.00) confirming positive. For samples that produced presumptive positive results on the *mericon* E. coliO157Screen Plus, 0 out of 144 samples confirmed positive (POD_C of 0.00).

A $dLPOD_C$ value of 0.00 with 95% confidence interval 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 0.02 with 95% confidence intervals of (-0.01, 0.06) 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 Ground Beef – *mericon* E. coliSTEC O-Type with Manual Preparation

For the low inoculum level, 66 out of 144 test portions (POD_{CP} of 0.46) were reported as presumptive positive by the *mericon* E. coliSTEC O-Type with 65 out of 144 test portions (POD_{CC}& POD_R of 0.45) confirming positive. For samples that produced presumptive positive results on the *mericon* E. coliSTEC O-Type, 65 out of 144 samples confirmed positive (POD_C of 0.45). A $dLPOD_C$ value of 0.00 with 95% confidence interval of (-0.12, 0.12) 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 (-0.11, 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, 144 out of 144 test portions (POD_{CP} of 1.00) were reported as presumptive positive by the *mericon* E. coliSTEC O-Type with 144 out of 144 test portions (POD_{CC}& POD_R of 1.00) confirming positive. For samples that produced presumptive positive results on the *mericon* E. coliSTEC O-Type, 144 out of 144 samples confirmed positive (POD_C of 1.00). A $dLPOD_C$ value of 0.00 with 95% confidence interval 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 144 samples (POD_{CP} of 0.00) produced a presumptive positive result by the *mericon* E. coliSTEC O-Type with 0 out of 144 test portions (POD_{CC}& POD_R of 0.00) confirming positive. For samples that produced presumptive positive results on the *mericon* E. coliSTEC O-Type, 0 out of 144 samples confirmed positive (POD_C of 0.00). A $dLPOD_C$ value of 0.00 with 95% confidence interval 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.

Detailed results of the POD statistical analysis are presented in Table 2016.2Band Figures 2A-2B.

Raw Ground Beef – *mericon* E. coliO157**Screen Plus with Automated Preparation**

For the low inoculum level, 69 out of 144 test portions (POD_{CP} of 0.48) were reported as presumptive positive by the *mericon* E. coliO157Screen Plus with 65 out of 144 test portions (POD_{CC}& POD_R of 0.45) confirming positive. For samples that produced presumptive positive results on the *mericon* E. coli O157Screen Plus, 65 out of 144 samples confirmed positive (POD_C of 0.45). A $dLPOD_C$ value of 0.00 with 95% confidence interval of (-0.12, 0.12) was obtained between the candidate and reference method, indicating no statistically significant difference between the two methods. A $dLPOD_{CP}$ value of 0.03 with 95% confidence intervals of (-0.09, 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, 144 out of 144 test portions (POD_{CP} of 1.00) were reported as presumptive positive by the *mericon* E. coliO157Screen Plus with 144 out of 144 test portions (POD_{CC}& POD_R of 1.00) confirming positive. For samples that produced presumptive positive results on the *mericon* E. coli O157Screen Plus, 144 out of 144 samples confirmed positive (POD_C of 1.00). A $dLPOD_C$ value of 0.00 with 95% confidence interval 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, 2 out of 144 samples (POD_{CP} of 0.01) produced a presumptive positive result by the *mericon* E. coliO157Screen Plus with 0 out of 144 test portions (POD_{CC}& POD_R of 0.00) confirming positive. For samples that produced presumptive positive results on the *mericon* E. coliO157Screen Plus, 0 out of 144 samples confirmed positive (POD_C of 0.00). A $dLPOD_C$ value of 0.00 with 95% confidence interval 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 0.01 with 95% confidence intervals of (-0.01, 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 2016.2Cand Figures 3A-3B.

Raw Ground Beef – *mericon* E. coliSTEC O-Type with Automated Preparation

For the low inoculum level, 63 out of 144 test portions (POD_{CP} of 0.44) were reported as presumptive positive by the *mericon* E. coliSTEC O-Type with 65 out of 144 test portions (POD_{CC}& POD_R of 0.45) confirming positive. For samples that produced presumptive positive results on the *mericon* E. coliSTEC O-Type, 63 out of 144 samples confirmed positive (POD_C of 0.44). A $dLPOD_C$ value of -0.01 with 95% confidence interval of (-0.13, 0.10) 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 (-0.13,

0.10) was obtained between presumptive and confirmed results indicating no statistically significant difference between the presumptive and confirmed results.

For the high inoculum level, 141 out of 144 test portions (POD_{CP} of 0.98) were reported as presumptive positive by the *mericon* E. coliSTEC O-Type with 144 out of 144 test portions (POD_{CC}& POD_R of 1.00) confirming positive. For samples that produced presumptive positive results on the *mericon* E. coliSTEC O-Type, 141 out of 144 samples confirmed positive (POD_C of 0.98). A $dLPOD_C$ value of -0.02 with 95% confidence interval 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, 1 out of 144 samples (POD_{CP} of 0.01) produced a presumptive positive result by the *mericon* E. coliSTEC O-Type with 0 out of 144 test portions (POD_{CC}& POD_R of 0.00) confirming positive. For samples that produced presumptive positive results on the *mericon* E. coliSTEC O-Type, 0 out of 144 samples confirmed positive (POD_C of 0.00; value). A $dLPOD_C$ value of 0.00 with 95% confidence interval 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 0.01 with 95% confidence intervals of (-0.02, 0.04) 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 2016.2Dand Figures 4A-4B.

Discussion

No negative feedback was provided with regard to the manual preparation of the assay or the performance of *mericon* assays on the Rotor Gene Q thermocycler. Two technicians (Laboratory 6 & 11) experienced issues with the automated extraction procedure. Further investigation into the issues indicated the problems were a result with unfamiliarity of the instrument which led to the software, and or mechanical, errors. Based on the feedback from the collaborators, it is recommended that analysts using the automated extraction platform be well versed in PCR analysis and receive a thorough training prior to use of the automated platform.

Overall, the data generated during this evaluation demonstrates the reproducibility of this new method. No statistically significant differences were observed between the presumptive methods (*mericon* E. coliO157Screen Plus and *mericon* E. coliSTEC O-Type) and the confirmed results. A few false positive results were observed for both the manual and automated procedures. Some of the false positive results observed for the *mericon* E. coliO157Screen Plus assay were due to the detection of the virulence genes for STEC and not for the detection of E. coliO157.

Subsequently, these samples were screened as negative on the *mericon* E. coliSTEC O-Type assay. These results indicate that these two assays when run in combination, can detect the potential presence of pathogenic genes, but at the same time screen the sample negative for *E. coli* O157 and the Top Six STEC. Additionally, a few of the false positive results obtained had high CT values (>36 CT). These false positive results could be attributed to cross reactivity with background flora that is sometimes observed with matrices with high background counts, or could be the resultof cross contamination from the samples that were inoculated at a high level that were processed prior right before or after the sample. No false negative results were observed for samples analyzed by either assay with manual extraction or with the screening assay and automated extraction. Minimal false negative results (5 false negative results out of 432 total samples) were observed for the STEC O-Type assay with automated extraction. Three of the false negative results occurred on a reanalysis of the test sample after the initial sample produced an invalid result (no internal CT value was observed). This may indicate that an error in the sample processing occurred due to the sample containing high levels of particulate. Due to availability of reagents, the reanalysis also occurred several days later, and an increase in background flora may have resulted in the non-detection of the target analyte.

Recommendations

It is recommended that the *mericon* E. coliO157Screen Plus and *mericon* E. coliSTEC O-Type method be adopted as Official First Action status for the detection of *E. coli* O157 and the Top Six STEC (*E. coli* O26, O45, O103, O111, O121 and O145 in selected foods: raw ground beef (73%) (325 g), raw beef trim (375 g) and spinach (25 g).

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References

(1) Feng, Peter. (2011) The United States Food and Drug Administration Bad Bug Book – Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins *Enterohemorrhagic Escherichia coli (EHEC).* 2nd Ed. Pp. 69-81

(2) Centers for Disease Control and Prevention. (2015) *E. coli (Escherichia coli)*http://www.cdc.gov/ecoli/general/index.html#what-are-shiga-toxin(Accessed August 2016)

(3) *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 August 2016)

(4)United States Food and Drug Administration, Food Safety Inspection Service, Office of Public Health Science (2015) Microbiology Laboratory Guidebook 5.09: *Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges.* Pp. 1-14 http://www.fsis.usda.gov/wps/wcm/connect/51507fdb-dded-47f7-862d-ad80c3ee1738/MLG-5.pdf?MOD=AJPERES(Accessed June 2016)

(5) Journal of AOAC INTERNATIONAL (2016) *Evaluation of the 3M Petrifilm Rapid Aerobic Count Plate for the Enumeration of Aerobic Bacteria: Collaborative Study, First Action 2015.13.* Vol. 99, No. 3, pp. 664-675(12)

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

(7) AOAC International, *Evaluation of the VITEK 2 Gram-Negative (GN) Microbial Identification Test Card: Collaborative Study,* Journal of AOAC International, 95, 3, (2012).

(8) 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

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

Table 1: Participation of each Collaborating Laboratory[a]

Participant	Raw Ground Beef[b]
1	Y
2	N
3	Y
4	Y
5	Y
6	Y
7	Y
8	Y
9	Y
10	Y
11	Y
12	Y
13	Y

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

Table 2016.1A:Summary of Results for the Detection of *E. coli* O157:H7in 73% Lean Raw Ground Beef (325g)

Method[a]	***mericon*E. coli O157Screen Plus**		
Extraction Method	**Manual**		
Inoculation Level	**Un-inoculated**	**Low**	**High**
Candidate Presumptive Positive/ Total # of Samples Analyzed	3/144	69/144	144/144
Candidate Presumptive POD (CP)	0.02 (0.01, 0.06)	0.48 (0.40, 0.56)	1.00 (0.97, 1.00)
s_r[b]	0.14 (0.13, 0.16)	0.50 (0.45, 0.52)	0.00 (0.00, 0.16)
s_L[c]	0.00 (0.00, 0.15)	0.00 (0.00, 0.18)	0.00 (0.00, 0.16)
s_R[d]	0.14 (0.13, 0.16)	0.50 (0.45, 0.52)	0.00 (0.00, 0.22)
P Value[e]	0.6042	0.5820	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/144	65/144	144/144
Candidate Confirmed POD (CC)	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 (0.45, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.22)	0.51 (0.46, 0.52)	0.00 (0.00, 0.22)
P Value	1.0000	0.8479	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/144	65/144	144/144
Candidate Presumptive Positive that Confirmed POD (C)	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 (0.45, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.22)	0.51 (0.46, 0.52)	0.00 (0.00, 0.22)
P Value	1.0000	0.8479	1.0000
Positive Reference Samples/ Total # of Samples Analyzed	0/144	65/144	144/144
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 (0.45, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.22)	0.51 (0.46, 0.52)	0.00 (0.00, 0.22)
P Value	1.0000	0.8479	1.0000
dLPOD (Candidate vs. Reference)[f]	0.00 (-0.03, 0.03)	0.00(-0.12, 0.12)	0.00 (-0.03, 0.03)
dLPOD (Candidate Presumptive vs. Candidate Confirmed)[f]	0.02(-0.01, 0.06)	0.03(-0.09, 0.15)	0.00 (-0.03, 0.03)

[a] Results include 95% Confidence Intervals, [b] 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 *E. coli* O157:H7in 73% Lean Raw Ground Beef (325g)

Method[a]	***mericon*®E. coli STEC O-Type**		
Extraction Method	**Manual**		
Inoculation Level	**Un-inoculated**	**Low**	**High**
Candidate Presumptive Positive/ Total # of Samples Analyzed	0/144	66/144	144/144
Candidate Presumptive POD (CP)	0.00(0.00, 0.03)	0.46(0.37, 0.54)	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, 0.22)	0.51 (0.46, 0.52)	0.00 (0.00, 0.22)
P Value[e]	1.0000	0.7951	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/144	65/144	144/144
Candidate Confirmed POD (CC)	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 (0.45, 0.52)	0.00(0.00, 0.16)
s_L	0.00(0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
s_R	0.00(0.00, 0.22)	0.51 (0.46, 0.52)	0.00 (0.00, 0.22)
P Value	1.0000	0.8479	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/144	65/144	144/144
Candidate Presumptive Positive that Confirmed POD (C)	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 (0.45, 0.52)	0.00(0.00, 0.16)
s_L	0.00(0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
s_R	0.00(0.00, 0.22)	0.51 (0.46, 0.52)	0.00 (0.00, 0.22)
P Value	1.0000	0.8479	1.0000
Positive Reference Samples/ Total # of Samples Analyzed	0/144	65/144	144/144
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 (0.45, 0.52)	0.00(0.00, 0.16)
s_L	0.00(0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
s_R	0.00(0.00, 0.22)	0.51 (0.46, 0.52)	0.00 (0.00, 0.22)
P Value	1.0000	0.8479	1.0000
dLPOD (Candidate vs. Reference)[f]	0.00 (-0.03, 0.03)	0.00(-0.12, 0.12)	0.00 (-0.03, 0.03)
dLPOD (Candidate Presumptive vs. Candidate Confirmed)[f]	0.00 (-0.03, 0.03)	0.01(-0.11, 0.13)	0.00 (-0.03, 0.03)

[a] Results include 95% Confidence Intervals, [b] 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 2016.1C:Summary of Results for the Detection of *E. coli* O157:H7in 73% Lean Raw Ground Beef (325g)

Method[a]	***mericon* E. coli O157 Screen Plus**		
Extraction Method	**Automated QIAsymphony**		
Inoculation Level	**Un-inoculated**	**Low**	**High**
Candidate Presumptive Positive/ Total # of Samples Analyzed	2/144	69/144	144/144
Candidate Presumptive POD (CP)	0.01(0.00, 0.05)	0.48(0.40, 0.56)	1.00(0.97, 1.00)
s_r[b]	0.12(0.11, 0.16)	0.51(0.45, 0.52)	0.00(0.00, 0.16)
s_L[c]	0.00(0.00, 0.04)	0.00(0.00, 0.18)	0.00 (0.00, 0.16)
s_R[d]	0.12(0.11, 0.13)	0.51 (0.46, 0.52)	0.00 (0.00, 0.22)
P Value[e]	0.5178	0.6436	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/144	65/144	144/144
Candidate Confirmed POD (CC)	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 (0.45, 0.52)	0.00(0.00, 0.16)
s_L	0.00(0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.22)	0.51 (0.46, 0.52)	0.00 (0.00, 0.22)
P Value	1.0000	0.8479	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/144	65/144	144/144
Candidate Presumptive Positive that Confirmed POD (C)	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 (0.45, 0.52)	0.00(0.00, 0.16)
s_L	0.00(0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.22)	0.51 (0.46, 0.52)	0.00 (0.00, 0.22)
P Value	1.0000	0.8479	1.0000
Positive Reference Samples/ Total # of Samples Analyzed	0/144	65/144	144/144
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 (0.45, 0.52)	0.00(0.00, 0.16)
s_L	0.00(0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.22)	0.51 (0.46, 0.52)	0.00 (0.00, 0.22)
P Value	1.0000	0.8479	1.0000
dLPOD (Candidate vs. Reference)[f]	0.00 (-0.03, 0.03)	0.00(-0.12, 0.12)	0.00 (-0.03, 0.03)
dLPOD (Candidate Presumptive vs. Candidate Confirmed)[f]	0.01(-0.01, 0.05)	0.03(-0.09, 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 2016.1D:Summary of Results for the Detection of *E. coli* O157:H7in 73% Lean Raw Ground Beef (325g)

Method[a]	***mericon* E. coli STEC O-Type**		
Extraction Method	**Automated QIAsymphony**		
Inoculation Level	**Un-inoculated**	**Low**	**High**
Candidate Presumptive Positive/ Total # of Samples Analyzed	1/144	63/144	141/144
Candidate Presumptive POD (CP)	0.01(0.00, 0.04)	0.44(0.35, 0.52)	0.98(0.94, 0.99)
s_r[b]	0.08(0.07, 0.16)	0.51(0.45, 0.52)	0.14(0.13, 0.16)
s_L[c]	0.00(0.00, 0.03)	0.00(0.00, 0.16)	0.00 (0.00, 0.15)
s_R[d]	0.08(0.08, 0.10)	0.51 (0.46, 0.52)	0.14(0.13, 0.16)
P Value[e]	0.4368	0.8105	0.6042
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/144	65/144	144/144
Candidate Confirmed POD (CC)	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 (0.45, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.22)	0.51 (0.46, 0.52)	0.00 (0.00, 0.22)
P Value	1.0000	0.8479	1.0000
Candidate Confirmed Positive/ Total # of Samples Analyzed	0/144	63/144	141/144
Candidate Presumptive Positive that Confirmed POD (C)	0.00 (0.00, 0.03)	0.44 (0.35, 0.52)	0.98 (0.94, 0.99)
s_r	0.00 (0.00, 0.16)	0.51 (0.45, 0.52)	0.14 (0.13, 0.16)
s_L	0.00 (0.00, 0.16)	0.00 (0.00, 0.16)	0.00 (0.00, 0.15)
s_R	0.00 (0.00, 0.22)	0.51 (0.46, 0.52)	0.14 (0.13, 0.16)
P Value	1.0000	0.8105	0.6042
Positive Reference Samples/ Total # of Samples Analyzed	0/144	65/144	144/144
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 (0.45, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.22)	0.51 (0.46, 0.52)	0.00 (0.00, 0.22)
P Value	1.0000	0.8479	1.0000
dLPOD (Candidate vs. Reference)[f]	0.00 (-0.03, 0.03)	-0.01(-0.13, 0.10)	-0.02(-0.06, 0.01)
dLPOD (Candidate Presumptive vs. Candidate Confirmed)[f]	0.01(-0.02, 0.04)	-0.01(-0.13, 0.10)	-0.02(-0.06, 0.01)

[a] Results include 95% Confidence Intervals, [b] 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 *E. coli* O157 in 325 g Raw Ground Beef(73% Lean) Test Portions by the *mericon* E. coli O157Screen Plususing manual extraction vs. USDA/FSIS MLG 5.09 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 Ground Beef	1	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		2[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		3	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		4	12	1	0.08	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.08
		5	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		6	12	1	0.08	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.08
		7	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		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	12	1	0.08	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.08
		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	144	3	0.02	144	0	0.00	144	0	0.00	144	0	0.00	0.00	0.21
LCL					0.01			0.00			0.00			0.00	-0.03	-0.01
UCL					0.06			0.03			0.03			0.03	0.03	0.06
s_r[b]					0.14			0.00			0.00			0.00		
LCL					0.13			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					0.05			0.16			0.16			0.16		
s_R[d]					0.14			0.00			0.00			0.00		
UCL					0.13			0.00			0.00			0.00		
LCL					0.16			0.22			0.22			0.22		
P_T[e]					0.6042			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 *E. coli* O157 in 325 g Raw Ground Beef (73% Lean) Test Portions by the *mericon* E. coliO157Screen Plususing manual extraction vs. USDA/FSIS MLG 5.09 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 Ground Beef	1	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		2[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		3	12	7	0.58	12	7	0.58	12	7	0.58	12	7	0.58	0.00	0.00
		4	12	6	0.50	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.00
		5	12	6	0.50	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.08
		6	12	4	0.33	12	4	0.33	12	4	0.33	12	4	0.33	0.00	0.00
		7	12	7	0.58	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.08
		8	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		9	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		10	12	9	0.75	12	8	0.67	12	8	0.67	12	8	0.67	0.00	0.08
	MPN/ Test Portion	11	12	7	0.58	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.08
		12	12	3	0.25	12	3	0.25	12	3	0.25	12	3	0.25	0.00	0.00
		13	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
Estimate	0.62	All	144	69	0.48	144	65	0.43	144	65	0.45	144	65	0.45	0.00	0.03
LCL	0.48				0.40			0.37			0.37			0.37	-0.12	-0.09
UCL	0.77				0.56			0.54			0.54			0.54	0.12	0.15
s_r[b]					0.50			0.51			0.51			0.51		
LCL					0.45			0.45			0.45			0.45		
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					0.18			0.15			0.15			0.15		
s_R[d]					0.50			0.51			0.51			0.51		
UCL					0.45			0.46			0.46			0.46		
LCL					0.52			0.52			0.52			0.52		
P_T[e]					0.5820			0.8479			0.8479			0.8479		

[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 *E. coli* O157 in 325 g Raw Ground Beef (73% Lean) Test Portions by the *merican* E. coliO157Screen Plus using manual extraction vs. USDA/FSIS MLG 5.09 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 Ground Beef / MPN/ Test Portion	1	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		2[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		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	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		10	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		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	5.13	All	144	144	1.00	144	144	1.00	144	144	1.00	144	144	1.00	0.00	0.00
LCL	3.45				0.97			0.97			0.97			0.97	-0.03	-0.03
UCL	7.62				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					0.22			0.22			0.22			0.22		
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 *E. coli* O157 in 325 g Raw Ground Beef (73% Lean) Test Portions by the *mericon* E. coliSTEC O-Typeusing manual extraction vs. USDA/FSIS MLG 5.09 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 Ground Beef	1	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		2[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		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	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	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		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	144	0	0.00	144	0	0.00	144	0	0.00	144	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					0.22			0.22			0.22			0.22		
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 *E. coli* O157 in 325 g Raw Ground Beef (73% Lean) Test Portions by the *merlicon* E. coliSTEC O-Typeusing manual extraction vs. USDA/FSIS MLG 5.09 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 Ground Beef	1	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		2[a]	12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		3	12	7	0.58	12	7	0.58	12	7	0.58	12	7	0.58	0.00	0.00
		4	12	6	0.50	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.00
		5	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		6	12	4	0.33	12	4	0.33	12	4	0.33	12	4	0.33	0.00	0.00
		7	12	6	0.50	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.00
		8	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		9	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		10	12	8	0.66	12	8	0.66	12	8	0.66	12	8	0.66	0.00	0.00
	MPN/ Test Portion	11	12	7	0.58	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.08
		12	12	3	0.25	12	3	0.25	12	3	0.25	12	3	0.25	0.00	0.00
		13	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
Estimate	0.62	All	144	66	0.46	132	65	0.45	132	65	0.45	132	65	0.45	0.00	0.01
LCL	0.48				0.37			0.37			0.37			0.37	-0.12	-0.11
UCL	0.77				0.54			0.54			0.54			0.54	0.12	0.13
s_r[c]					0.51			0.51			0.51			0.51		
LCL					0.45			0.45			0.45			0.45		
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					0.16			0.16			0.16			0.16		
s_R[e]					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[f]					0.7951			0.8479			0.8479			0.8479		

[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 *E. coli* O157 in 325 g Raw Ground Beef (73% Lean) Test Portions by the *mericon* E. coliSTEC O-Typeusing manual extraction vs. USDA/FSIS MLG 5.09 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 Ground Beef	1	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		2[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		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	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		10	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
	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	5.13	All	144	144	1.00	144	144	1.00	144	144	1.00	144	144	1.00	0.00	0.00
LCL	3.45				0.97			0.97			0.97			0.97	-0.03	-0.03
UCL	7.62				1.00			1.00			1.00			1.00	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					0.22			0.22			0.22			0.22		
P_T[f]					1.000			1.000			1.000			1.000		

[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 2016.2C: Comparative Results for the Detection of *E. coli* O157 in 325 g Raw Ground Beef (73% Lean) Test Portions by the *mericon* E. coliO157Screen Plususing automated extraction vs. USDA/FSIS MLG 5.09 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 Ground Beef	1	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		2[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		3	12	1	0.08	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.08
		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	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	12	1	0.08	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.08
		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	144	2	0.01	144	0	0.00	144	0	0.00	144	0	0.00	0.00	0.01
LCL					0.00			0.00			0.00			0.00	-0.03	-0.01
UCL					0.05			0.03			0.03			0.03	0.03	0.05
s_r[b]					0.12			0.00			0.00			0.00		
LCL					0.11			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					0.04			0.16			0.16			0.16		
s_R[d]					0.12			0.00			0.00			0.00		
UCL					0.11			0.00			0.00			0.00		
LCL					0.13			0.22			0.22			0.22		
P_T[e]					0.5178			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 2016.2C (cont'd.): Comparative Results for the Detection of *E. coli* O157 in 325 g Raw Ground Beef (73% Lean) Test Portions by the *merican* E. coliO157Screen Plususing automated extraction vs. USDA/FSIS MLG 5.09 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 Ground Beef	1	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		2[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		3	12	8	0.66	12	7	0.58	12	7	0.58	12	7	0.58	0.00	0.08
		4	12	6	0.50	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.00
		5	12	6	0.50	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.08
		6	12	4	0.33	12	4	0.33	12	4	0.33	12	4	0.33	0.00	0.00
		7	12	7	0.58	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.08
		8	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		9	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		10	12	8	0.66	12	8	0.66	12	8	0.66	12	8	0.66	0.00	0.00
	MPN/ Test Portion	11	12	7	0.58	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.08
		12	12	3	0.25	12	3	0.25	12	3	0.25	12	3	0.25	0.00	0.00
		13	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
Estimate	0.62	All	144	69	0.48	144	65	0.43	144	65	0.45	144	65	0.45	0.00	0.03
LCL	0.48				0.40			0.37			0.37			0.37	-0.12	-0.09
UCL	0.77				0.56			0.54			0.54			0.54	0.12	0.15
s_r[b]					0.51			0.51			0.51			0.51		
LCL					0.45			0.45			0.45			0.45		
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					0.18			0.15			0.15			0.15		
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]					0.6436			0.8479			0.8479			0.8479		

[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 2016.2C (cont'd.): Comparative Results for the Detection of *E. coli* O157 in 325 g Raw Ground Beef (73% Lean) Test Portions by the *merican* E. coliO157Screen Plus using automated extraction vs. USDA/FSIS MLG 5.09 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 Ground Beef / MPN/ Test Portion	1	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		2[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		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	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		10	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		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	5.13	All	144	144	1.00	144	144	1.00	144	144	1.00	144	144	1.00	0.00	0.00
LCL	3.45				0.97			0.97			0.97			0.97	-0.03	-0.03
UCL	7.62				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					0.22			0.22			0.22			0.22		
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 2016.2D: Comparative Results for the Detection of *E. coli* O157 in 325 g Raw Ground Beef (73% Lean) Test Portions by the *mericon* E. coliSTEC O-Typeusing automated extraction vs. USDA/FSIS MLG 5.09 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 Ground Beef	1	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		2[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		3	12	1	0.08	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.08
		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	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	12	0	0.00	12	0	0.00	12	0	0.00	12	0	0.00	0.00	0.00
		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	144	1	0.01	144	0	0.00	144	0	0.00	144	0	0.00	0.00	0.01
LCL					0.00			0.00			0.00			0.00	-0.03	-0.02
UCL					0.04			0.03			0.03			0.03	0.03	0.04
s_r[c]					0.08			0.00			0.00			0.00		
LCL					0.07			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					0.03			0.16			0.16			0.16		
s_R[e]					0.08			0.00			0.00			0.00		
UCL					0.08			0.00			0.00			0.00		
LCL					0.10			0.22			0.22			0.22		
P_T[f]					0.4368			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 2016.2D (cont'd.): Comparative Results for the Detection of *E. coli* O157 in 325 g Raw Ground Beef (73% Lean) Test Portions by the *mericon* E. coliSTEC O-Typeusing automated extraction vs. USDA/FSIS MLG 5.09 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 Ground Beef	1	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		2[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		3	12	7	0.58	12	7	0.58	12	7	0.58	12	7	0.58	0.00	0.00
		4	12	6	0.50	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.00
		5	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		6	12	4	0.33	12	4	0.33	12	4	0.33	12	4	0.33	0.00	0.00
		7	12	6	0.50	12	6	0.50	12	6	0.50	12	6	0.50	0.00	0.00
		8	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		9	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
		10	12	8	0.66	12	8	0.66	12	8	0.66	12	8	0.66	0.00	0.00
	MPN/ Test Portion	11	12	4	0.33	12	6	0.50	12	4	0.33	12	6	0.50	0.00	-0.17
		12	12	3	0.25	12	3	0.25	12	3	0.25	12	3	0.25	0.00	0.00
		13	12	5	0.42	12	5	0.42	12	5	0.42	12	5	0.42	0.00	0.00
Estimate	0.62	All	144	63	0.44	144	65	0.45	144	63	0.44	144	65	0.45	-0.01	-0.01
LCL	0.48				0.35			0.37			0.35			0.37	-0.13	-0.13
UCL	0.77				0.52			0.54			0.52			0.54	0.10	0.10
s_r[c]					0.51			0.51			0.51			0.51		
LCL					0.45			0.45			0.45			0.45		
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					0.16			0.15			0.16			0.15		
s_R[e]					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[f]					0.8105			0.8479			0.8105			0.8479		

[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 2016.2D (cont'd.): Comparative Results for the Detection of *E. coli* O157 in 325 g Raw Ground Beef (73% Lean) Test Portions by the *merlcon* E. coliSTEC O-Typeusing automated extraction vs. USDA/FSIS MLG 5.09 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 Ground Beef / MPN/ Test Portion	1	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		2[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		3	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		4	12	11	0.92	12	12	1.00	12	11	0.92	12	12	1.00	-0.08	-0.08
		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	11	0.92	12	12	1.00	12	11	0.92	12	12	1.00	-0.08	-0.08
		9	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		10	12	12	1.00	12	12	1.00	12	12	1.00	12	12	1.00	0.00	0.00
		11	12	11	0.92	12	12	1.00	12	11	0.92	12	12	1.00	0.00	-0.08
		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	5.13	All	144	141	0.98	144	144	1.00	144	141	0.98	144	144	1.00	-0.02	-0.02
LCL	3.45				0.94			0.97			0.94			0.97	-0.06	-0.06
UCL	7.62				0.99			1.00			0.99			1.00	0.01	0.01
s_r[c]					0.14			0.00			0.14			0.00		
LCL					0.13			0.00			0.13			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			0.16			0.05			0.16		
s_R[e]					0.14			0.00			0.14			0.00		
UCL					0.13			0.00			0.13			0.00		
LCL					0.16			0.22			0.16			0.22		
P_T[f]					0.6042			1.000			0.6042			1.000		

[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

1

Table 1. Individual Collaborator Results for Raw Ground Beef (73% Lean) – Manual Extraction

	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
***mericon* E. coliO157 Screen Plus**																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-[b]	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	+	-	+	+	-[b]	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	-	-	-	+	-	-[b]	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-[b]	-	+	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
10	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	-[b]	-	+	+	-	-	-	-[b]	-	-	-	-	-	-	-	-	-
11	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	-	+	+	-[b]	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***mericon* E. coliSTEC O-Type**																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
11	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	-	+	+	-[b]	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] += *E. coli* O157 was detected in samples; -=*E. coli* O157 was not detected in sample; n/a- lab did not participate or submit data for the matrix;
[b] Sample was presumptive positive but confirmed negative indicating a false positive result;

Table 2. Individual Collaborator Results for Raw Ground Beef (73% Lean) – Automated Extraction

	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
***mericon* E. coli O157Screen Plus**																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-[b]	+	+	-	-	-	+	-	-	-	-	-	-	-	-[b]	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	+	-	+	+	-[b]	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	-[b]	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
10	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-[b]	-	-	-	-
11	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	-	+	+	-[b]	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***mericon* E. coliSTEC O-Type**																																				
Lab[a]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-[b]	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	+[c]	+	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
6	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
8	+	+	+	+	+	+	+	+[c]	+	+	+	+	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
11	+	+	+	+	+	+	+	+	+[c]	+	+	+	-	-	+	-	+	+	-	-	-	+[c]	+[c]	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

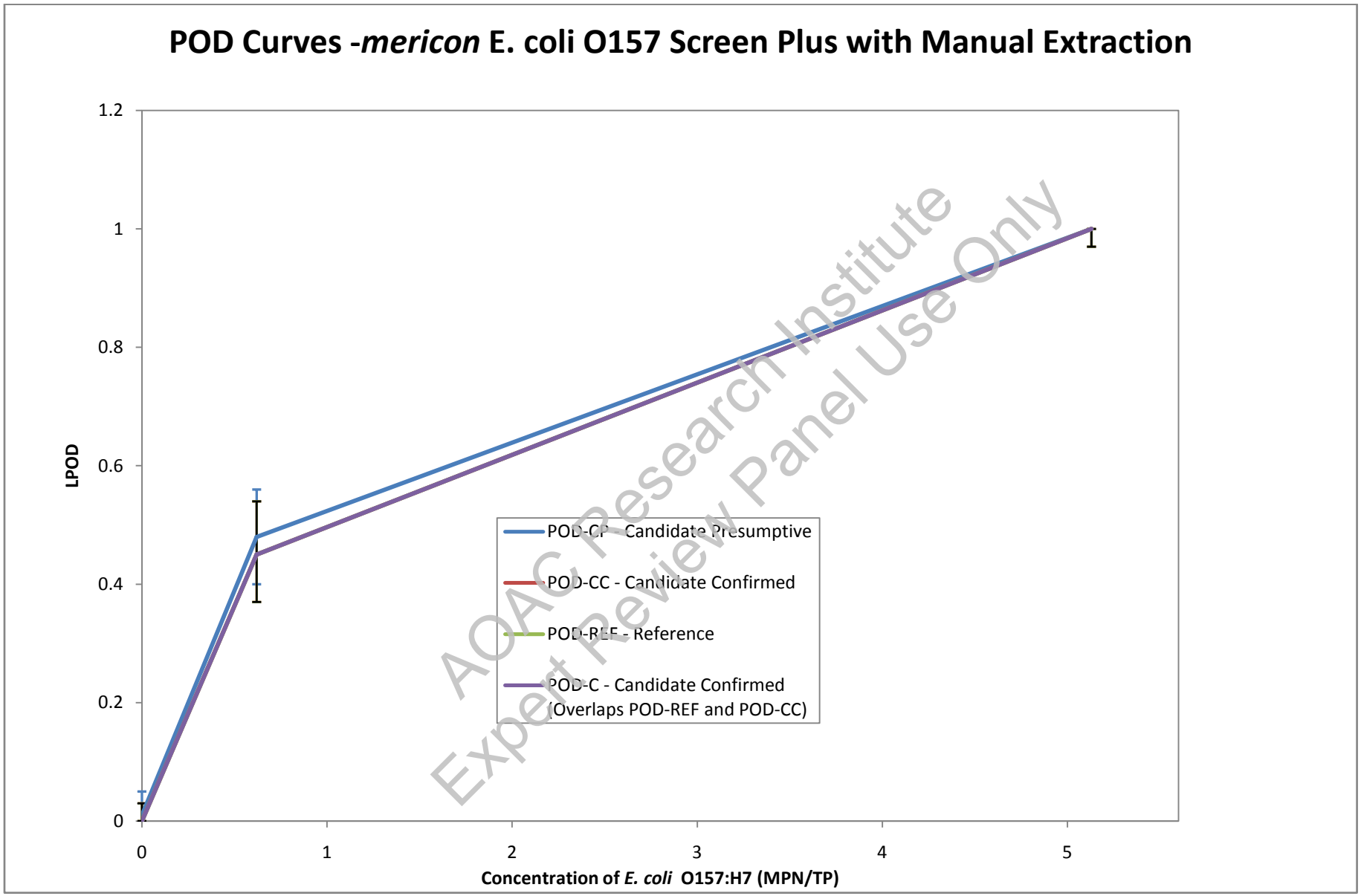
[a] += *E. coli* O157 was detected in sample; -=*E. coli* O157 was not detected in sample; n/a- lab did not participate or submit data for the matrix;
[b] Sample was presumptive positive but confirmed negative indicating a false positive result;
[c] Sample was presumptive negative but confirmed positive indicating a false negative result

Table 3: Results of Aerobic Plate Count for Collaborating Laboratories

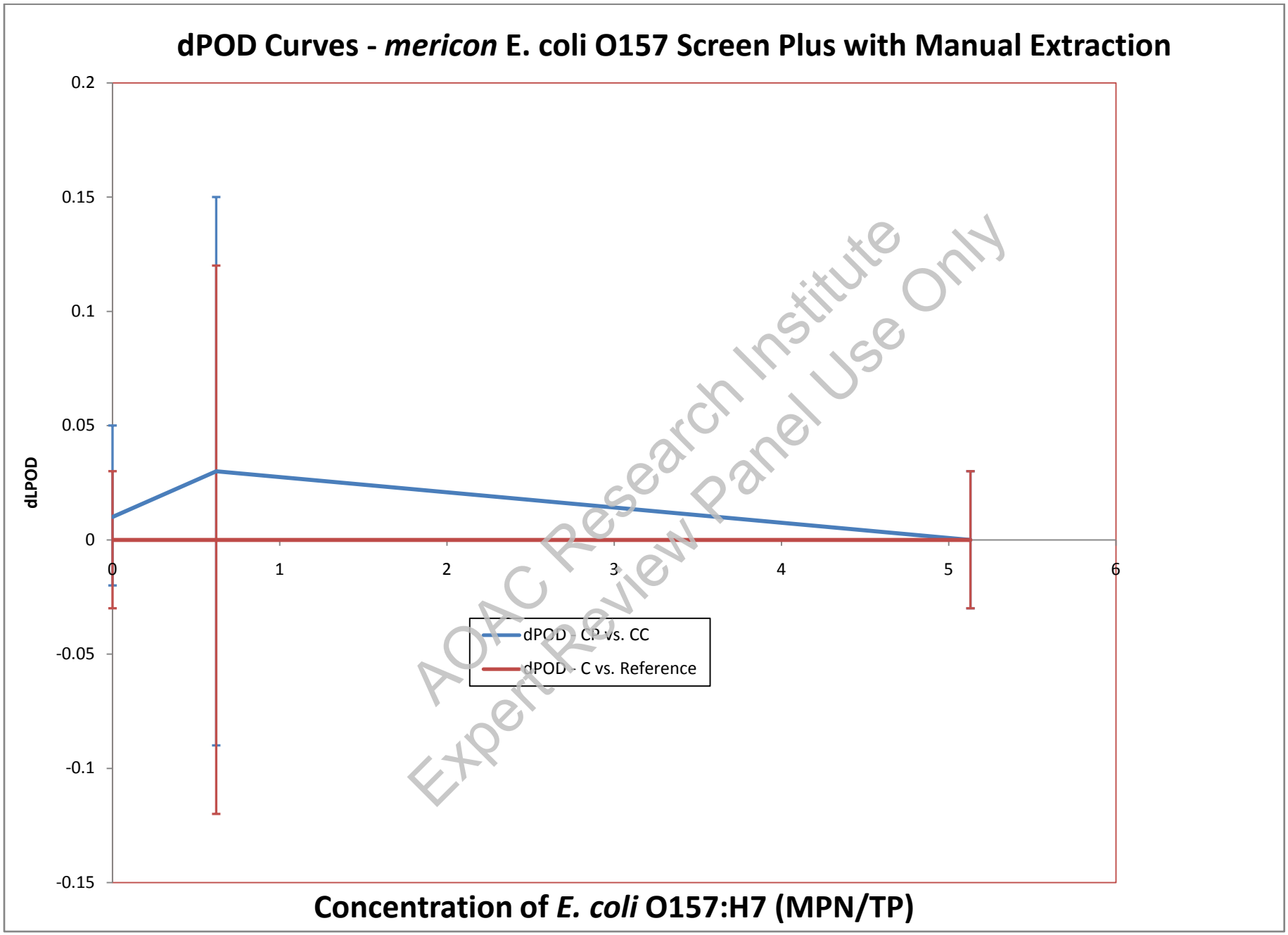
Lab	Raw Ground Beef (CFU/g)[a]
1	6.0×10^3
2	NA
3	6.7×10^6
4	1.9×10^2
5	5.7×10^5
6	1.2×10^5
7	9.6×10^6
8	2.4×10^3
9	2.3×10^6
10	1.2×10^7
11	1.3×10^2
12	1.1×10^5
13	6.6×10^5

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

Figure 1A: POD Values of *mericon* E. coli O157Screen Pluswith Manual Extraction and USDA/FSIS-MLG 5.09 Reference Method



1 **Figure 1B: dLPOD Values of *mericon* E. coli O157Screen Pluswith Manual Extraction and USDA/FSIS-MLG 5.09 Reference Method**



2

Figure 2A: POD Values of *mericon* E. coliSTEC O-Typewith Manual Extraction and USDA/FSIS-MLG 5.09 Reference Method

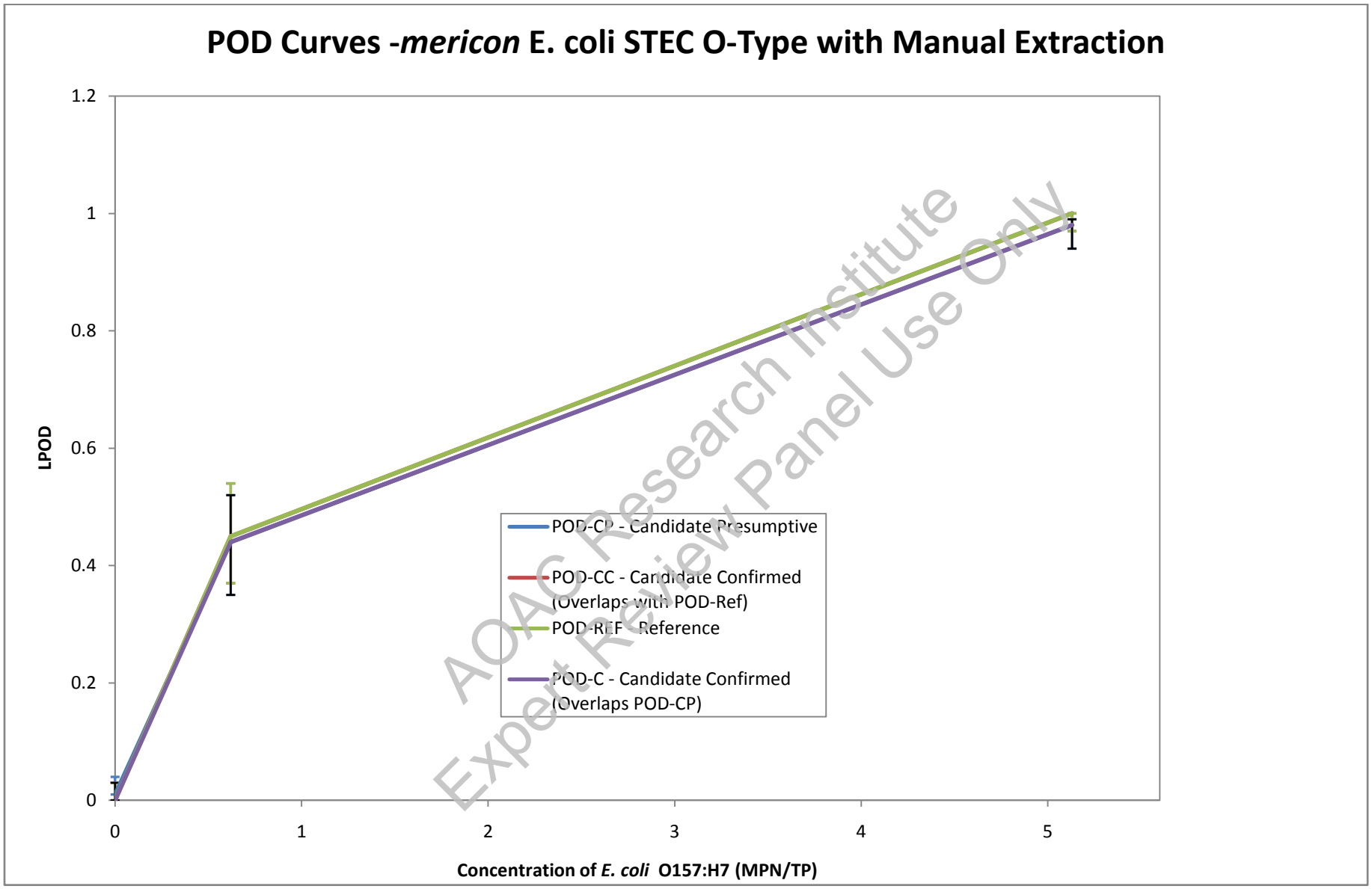


Figure 2B: dLPOD Values of *mericon* E. coliO157 Screen Pluswith Manual Extraction and USDA/FSIS-MLG 5.09 Reference Method

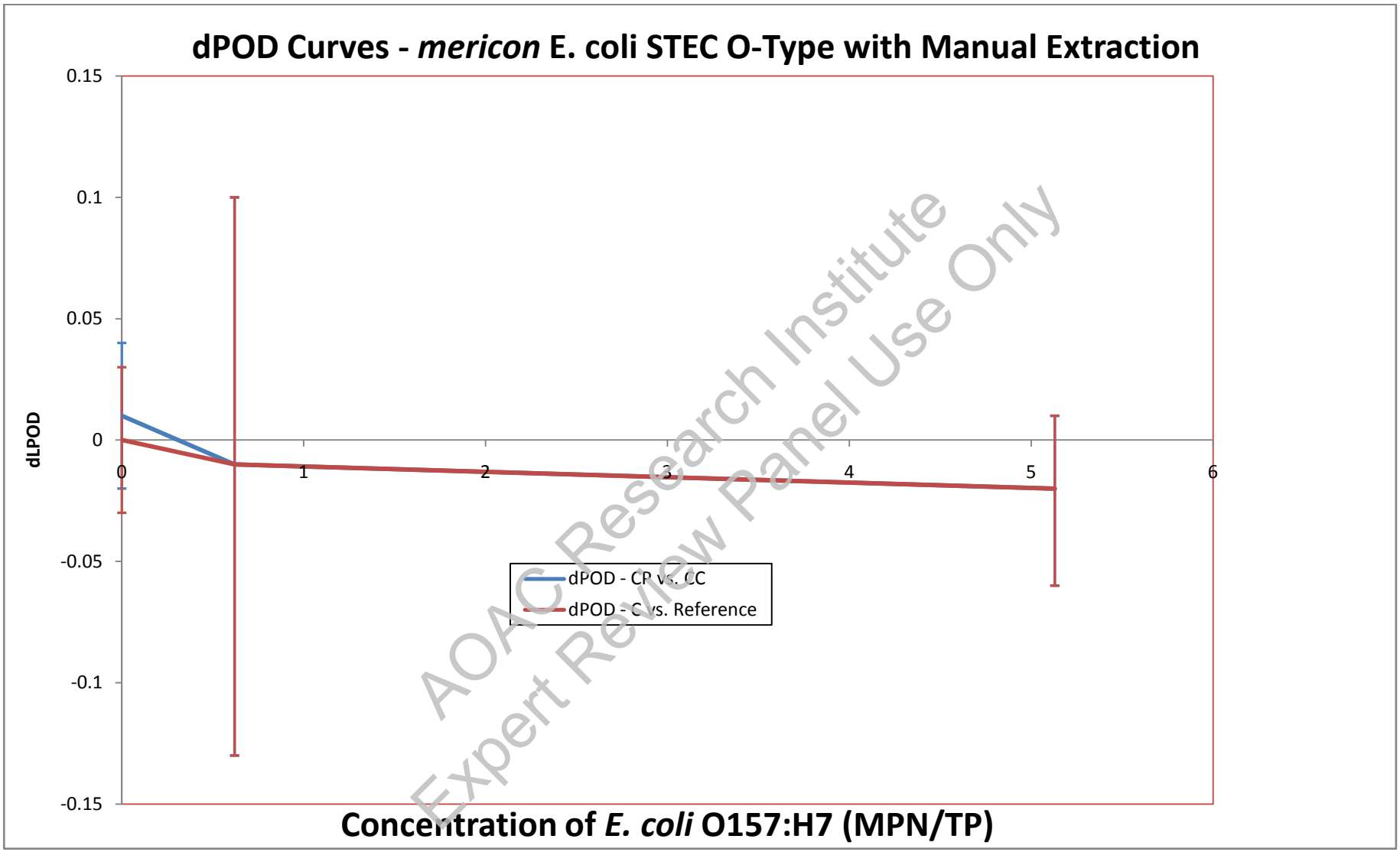


Figure 3A: POD Values of *mericon* E. coli O157Screen Pluswith Automated Extraction and USDA/FSIS-MLG 5.09 Reference Method

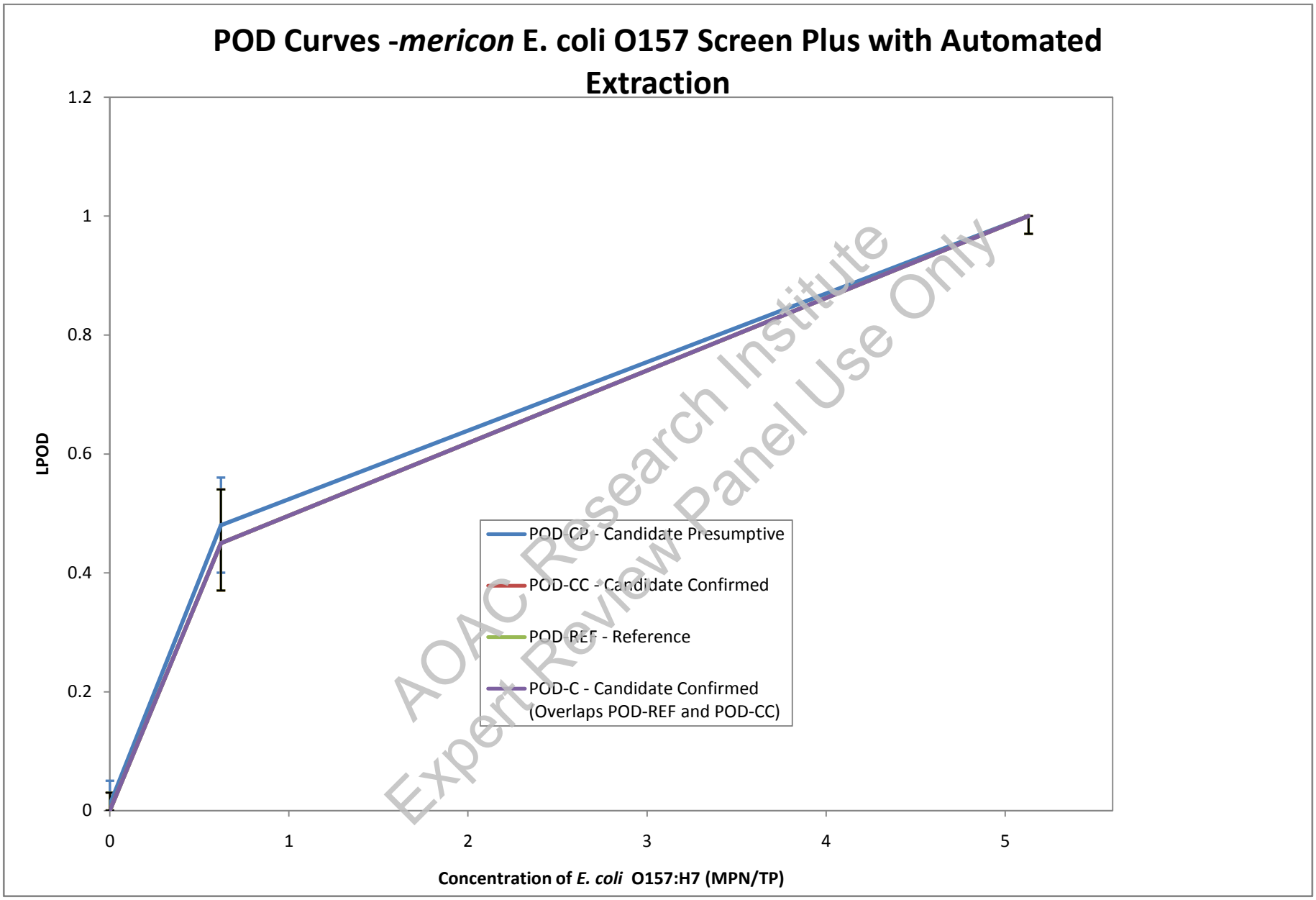


Figure 3B: dLPOD Values of *mericon* E. coli O157Screen Pluswith Automated Extraction and USDA/FSIS-MLG 5.09 Reference Method

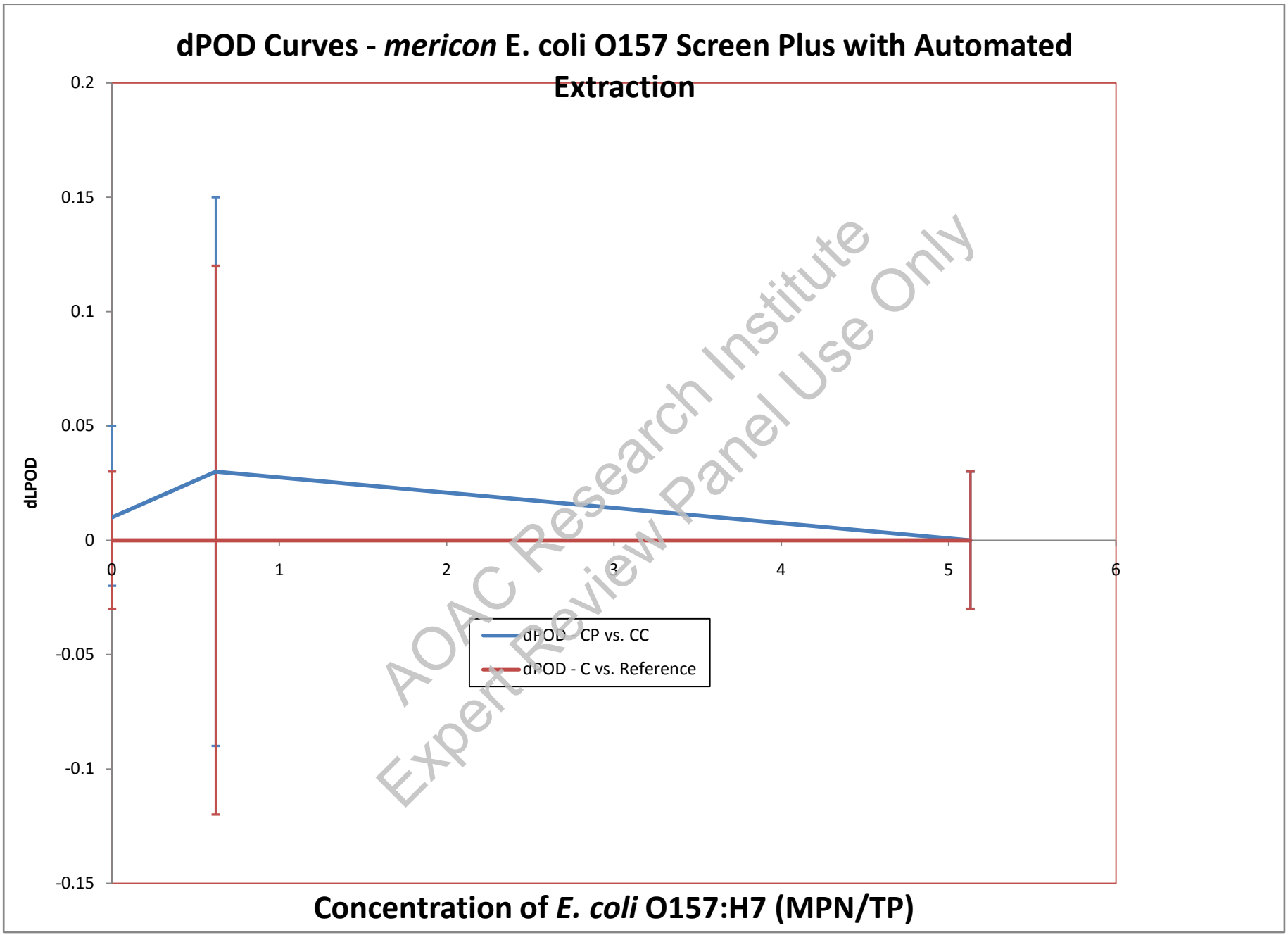


Figure 4A: POD Values of *mericon* E. coliSTEC O-Typewith Automated Extraction and USDA/FSIS-MLG 5.09 Reference Method

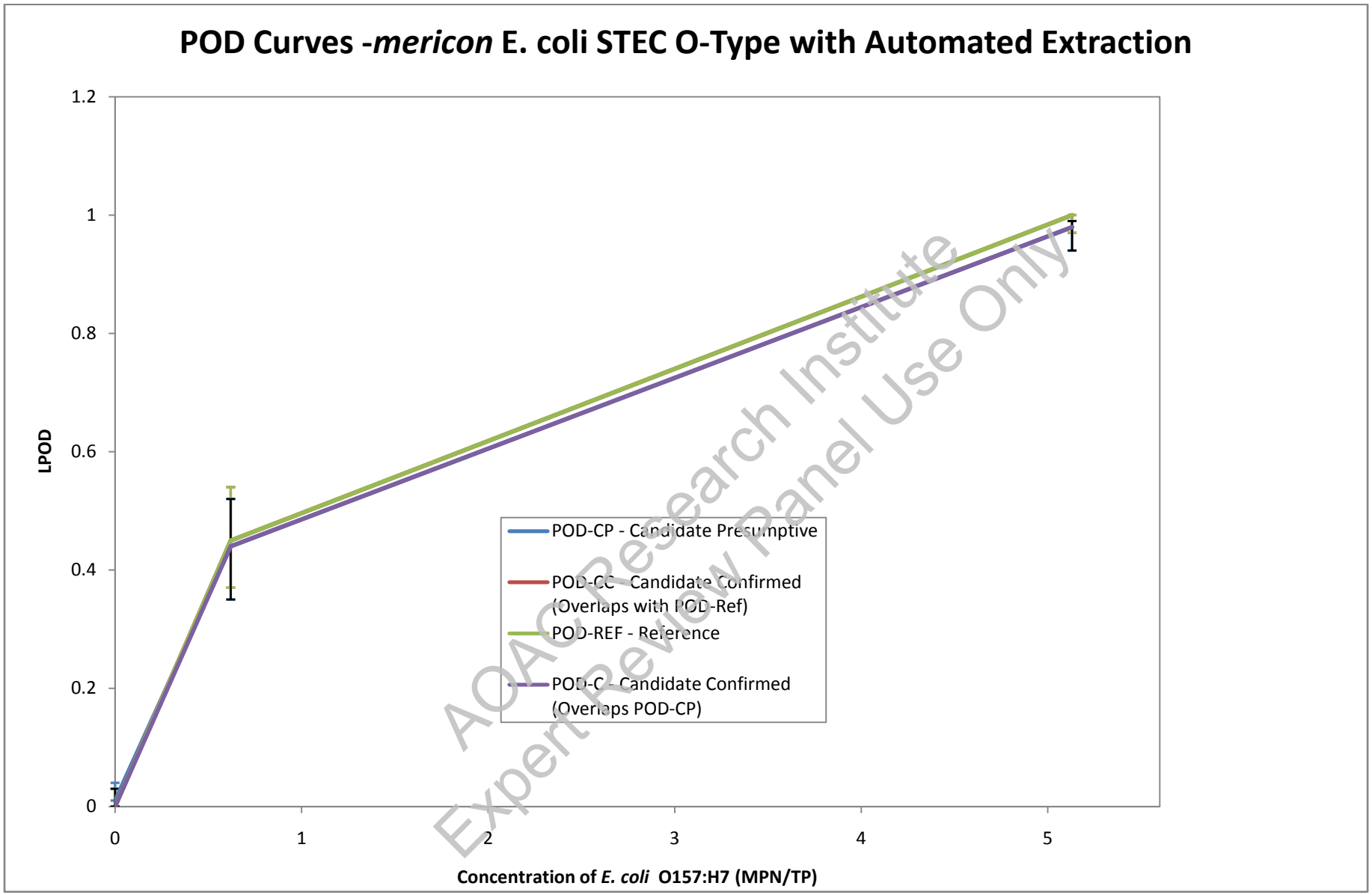
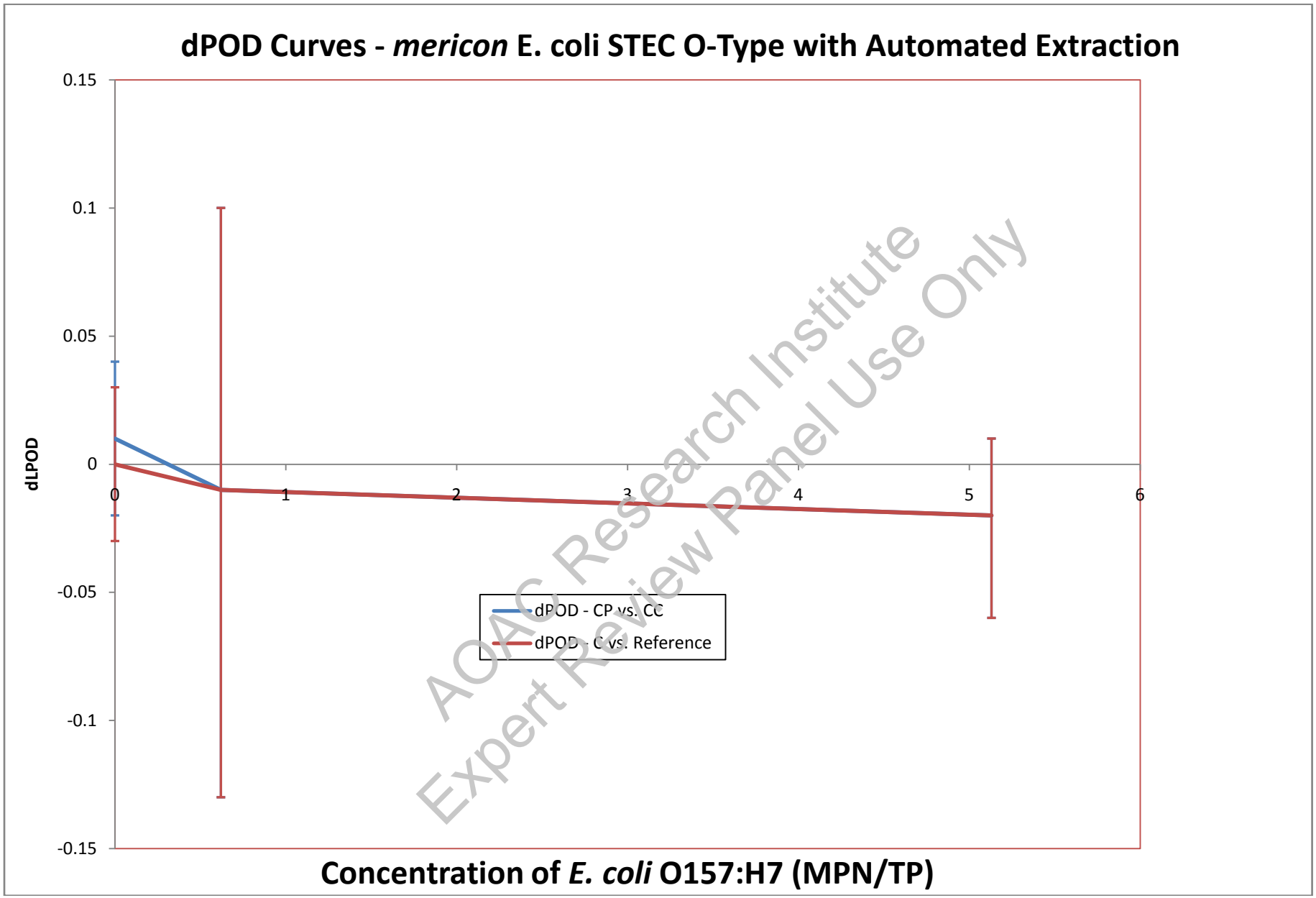


Figure 4B: dLPOD Values of *mericon* E. coliSTEC O-Typewith Automated Extraction and USDA/FSIS-MLG 5.09 Reference Method



AOAC Research Institute
*Official Methods of Analysis*SM Program

Detection of STECs in Selected Foods by the*mericon*®E. coli O157 Screen Plusand*mericon*® E. coli STEC O-Type Pathogen Detection Workflow: Collaborative Study Protocol

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Version 7

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1.0 Introduction

The goal of this collaborative study is to estimate the reproducibility of the QIAGEN *mericon*® E. coli O157 Screen Plus and *mericon*® E. coli STEC O-type Pathogen Detection workflow and to compare the performance of the workflow to the US FSIS MLG 5.09 reference method for detection of *E. coli* O157:H7 in ground beef. Performance will be measured by POD statistical analyses, repeatability, and reproducibility as described in the current AOAC microbiology guidelines. The OMA claim will be limited to ground beef and beef trim.Raw ground beef (325 g test portions) will be inoculated with *E. coli*O157:H7and compared to FSIS MLG 5.09 in a paired study. Randomized blind coded test portions of the high, low and uninoculated batches will be sent to collaborators who will perform the*mericon*workflowand the referencemethod. Collaborators will have the option to send samples to QLabs for cultural confirmation. Collaborators will report raw data and QLabs will perform the statistical analyses.

1.1 Description of the *mericon*STEC workflow

The *mericon*E. coli O157 Screen Plus and E. coli STEC O-Type methodsaremultiplex PCR assaysthat amplify specific DNA fragments from pathogenic *E. coli* in food and include an internal control. The internal control provides data regarding the presence of inhibitors in the tested samples and the overall quality of the PCR run.

These assays were developed for use on the Rotor-Gene Q. The *mericon* E. coli O157 Screen Plus kit and the *mericon* E. coli STEC O-type kit are not compatible with other real time cyclers. The PCR Master Mix utilizes HotStarTaq*Plus* DNA Polymerase, patented multiplex PCR technology such as Factor MP, and fast cycling technology including Q-Bond®. The assays include an internal control and are used for detecting *E. coli* O157 and STECs, respectively, in raw ground beef, rawbeef trim and spinach after 10 ± 1hours enrichment.

The methods usemodified Tryptone Soy Brothplus casamino acids (mTSB + CAA) for enrichment of test portions. A 325-g food sample (ground beef or beef trim) is added to 975 mL of pre-warmed mTSB + CAA. All samples are homogenized by stomaching at 230 rpm (2 min ± 10 s for ground beef and beef trim) and incubated at 42 ± 1°C for 10 ± 1 h. There are two DNA preparation procedures that can be utilized – the manual *mericon* DNA Bacteria Kit and the automated QIAsymphony*mericon* BacteriaKit. For the manual *mericon* method, a 1-mL aliquot of enrichment is centrifuged and the pellet is resuspended in Lysis Buffer and heat-treated. After a second centrifugation, a 10-µL aliquot of the supernatant is used in the PCR reaction. For the QIAsymphony method, a 0.5-mL aliquot of enrichment is loaded onto the instrument. The instrument processes 0.4 mL by heat lysis and bead-based DNA extraction and purification. A 10-µL aliquot of the resultant purified DNA is automatically loaded into a PCR reaction on a Rotor-Disc-72.

PCR cycling parameters are consistent across all *mericon* pathogen detection assays. The internal control must be uninhibited for the run to be valid. If the run is valid, the test sample results are interpreted as positive or negative depending on whether the fluorescence exceeds a

preset threshold. Inhibited samples are retested at a 1/10 dilution. The *mericon* E. coli Screen Plus targets the *stx1*/*stx2*, *O157*, and *eae* genes. The *mericon*E. coli STEC O-Type targets the big six O-groups (O26, O45, O103, O111, O121, and O145) and O157:H7. It does not distinguish between the big six O-groups.

Confirmation of presumptive results is carried out according to MLG 5.09 (for *E. coli* O157:H7 in ground beef or beef trim) or MLG 5B.05 (for non-O157 STEC in ground beef or beef trim).

1.2 Summary of PTM Study

The *mericon* methods were validated according to the current AOAC Guidelines (2012) and granted PTM certification for detection of *E. coli* O157 and non-O157 STECs in ground beef, beef trim, and spinach. The *mericon*®E. coliO157 Screen PlusPathogen Detection Assay was assigned PTM #101503 and the *mericon*®E. coliSTEC O-typePathogen Detection Assay was assigned PTM #101504. The two methods were co-validated and compared to the FSIS MLG 5.09: *Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges* for raw ground beef (70% lean), or the FSIS MLG 5B.05: *Detection and Isolation of non-O157 Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products and Carcass and Environmental Sponges* for raw beef trim, and FDA BAM Chapter 4A: *Diarrheagenic Escherichia coli* for fresh spinach. Raw ground beef was inoculated with *E. coli* O157:H7 and raw beef trim with *E. coli* O26 and the studies were carried out with paired 325 g test portions enriched in modified Tryptic Soy Broth with casamino acids (mTSB + CAA) at 42 ± 1°C. The *mericon* methods were performed at 10 ± 1 h incubation time, while the MLG methods were performed at 15-24 h incubation time. Fresh spinach was inoculated with *E. coli* O111 and the study design was unpaired with the *mericon* methods using 25 g test portions enriched in mTSB + CAA at 42 ± 1°C for 10 ± 1 h and the BAM reference method using 200 g test portions (prepared from 25 g of the inoculated spinach mixed with 175 g of uninoculated spinach) in modified Buffered Peptone Water with pyruvate (mBPWp) without homogenization at 37 ± 1°C for 5 h, followed by addition of selective agents, and incubation at 42 ± 1°C for 18-24 h.

Table 1 shows a summary of the PTM study and proposed collaborative study. *Note that spinach is not claimed in the OMA method*. The ground beef and beef trim use the same enrichment conditions and test portion size, so only ground beef will be tested in the collaborative study.

Table 1. Comparison of PTM study and proposed Collaborative study

Matrix	*mericon* Test Portion Size	PTM Study		Collaborative Study - Proposed	
		Organism	Reference Method	Organism	Reference Method
Spinach	25 g	*E. coli* O111	BAM Chapter 4A	NC	NC
Ground beef	325 g	*E. coli* O157:H7	MLG 5.09	*E. coli* O157:H7	MLG 5.09
Beef trim	325 g	*E. coli* O26	MLG 5B.05	NA	NA

NC = Not Claimed for OMA; NA = Not applicable (not included in the collaborative study)

1.3 Co-Study Directors for the Collaborative Study

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2.0 Collaborators

A minimum of 10 valid data sets are needed for the successful completion of this study, so 12-15 collaborators will be solicited to participate. Qualified analysts, familiar with the *mericon*® Pathogen Detection System, will act as collaborators. All collaborators have been, or will be, thoroughly trained by a QIAGEN representative on the use of the *mericon*® System (QIAsymphony SP/AS and Rotor-Gene Q).

QLabs will provide 3 collaborators. Additional collaborator sites could include Vanguard, FSNS, Covance, Aemtek, Microbac, Silliker, Tyson or Marshfield. Each site must have a large Stomacher or similar homogenization equipment and preferably will be able to confirm all test portion sets at that site. In the event a site cannot accommodate the confirmation of all test portion sets, they must be able to ship infectious samples to QLabs for confirmation. QLabs can accommodate the confirmation of up to 3 test portion sets in addition to the 3 test portions sets from their own laboratory. A sample letter to collaborators can be found in Appendix 8.1. This letter will be sent to each collaborator prior to the start of the study. In addition, a conference call will be held with collaborators to review the study protocol in detail and answer any questions before the start of the study.

3.0 Collaborative Study Design

3.1 The study will include 4-5 test sites, with no more than 3 collaborators at each site. The collaborators at each site will share the use of a *mericon*® system (QIAsymphony SP/AS and Rotor-Gene Q). The three collaborators may be three analysts employed at that site, but working independently.

3.2 The *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-Type methods will be validated together as one workflow and will comprise one Official Method. The method will include both manual and automated sample prep and assay setup options and will be applicable to ground beef and beef trim. Spinach will not be included in the OMA claim.

3.3 One matrixis proposed. Fresh raw ground beef (325 g test portion size) artificially inoculated with *E. coli*O157:H7 will be analyzed in a paired study design by the *mericon* workflow and the FSIS MLG 5.09 reference method.

3.4 Collaborators will each receive1test portion set as described in Table 2. All test portions within a set will be randomized and blind coded so that the collaborators do not know which test portions are from which contamination level. The randomization will be different for each collaborator. In addition, collaborators will receive 1 portion of ground beef for aerobic plate count.

Table 2. Test portion set for each collaborator

Matrix	Test Portion Size	Number of Test Portions	Number per Level	Method(s)
Ground beef	325	36	12 uninoc. 12 low 12 high	*mericon* and MLG 5.09 (Paired)

3.5 Study schedule

3.5.1 All media and reagents as outlined in Appendix 8.2 will be prepared by QLabs and shipped to collaborator sites to arrive no later than the Wednesday before shipment of test portions.

3.5.2 Test kits and Study Materials as outlined in Appendix 8.2 will be shipped to collaborators by QIAGEN to arrive no later than the Wednesday before shipment of test portions.

3.5.3 Test portions will be shipped refrigerated by overnight courier to each test site to arrive on a Friday. The specific date will be determined once collaborators and sites are confirmed.

3.5.4 All collaborators will begin analyses on the following Monday. Only the coordinating laboratory will conduct MPN analyses.

3.5.5 Data forms will be completed and emailed to the co-Study Director within 2 weeks of initiation of analyses.

Table 3. Study Schedule (Specific Dates TBD)

Collaborator Activity	Receipt of Media and Reagents	Receipt of Test Kits and Materials	Receipt of Test Portions Set	Initiation of Analyses	Submission of Data Report Form
Date	Week 1, Wednesday	Week 1, Wednesday	Week 1, Friday	Week 2, Monday	Week 4, Monday

4.0 Test Portion Preparation and Analysis

4.1 Test Portion Preparation

4.1.1 The Coordinating Laboratory, Q Laboratories, will screen fresh raw ground beef for natural STEC contamination using the *mericon* E. coli O157 Screen Plus and

FSIS MLG 5.09 methods. If natural contamination is found and it is high enough to yield fractional positives, it can be used for the collaborative study. If the level is too low, the lot can be temperature abused or alternative lots can be screened. If no natural contamination is found, the laboratory will inoculate one bulk amount of ground beef at a low level to achieve fractional positives (25-75% positives) and one bulk amount at a high level designed to produce all positive results(e.g., 2-3 fold higher inoculum than the low level). The ground beef will be inoculated with a fresh culture of a type strain or well-characterized strain of pathogenic *E. coli* O157. Each inoculated bulk will be kneaded to achieve a distribution of pathogen as close to homogeneous as possible. Twenty five-g aliquots of inoculated matrixwill be placed into individual bags containing 300 g uninoculated ground beef to create the 325 g test portions. Each bag will be kneaded to mix, though homogeneity is not required. Once bagged and labeled (see 4.1.2), all test portions will be chilled to 2-8°C to stabilize the inoculum. Test portions will be shipped at 2-8°C on the day of inoculation (on a Thursday to arrive Friday) and stored at 2-8°C at the collaborator site until Monday. By the time analyses begin on Monday, the stabilization time will be ~96 h.

4.1.2 Test portions designated for each particular collaborator will be uniquely randomized and blind-coded for that collaborator. For example, Collaborator 1 will receive 36 randomized test portions of ground beef comprised of 12 uninoculated test portions, 12 low level test portions, and 12 high level test portions labeled 101-136. Likewise, Collaborator 2 will have a unique randomization and test portions will be labeled 201-236. Thus, the collaborators at each site can easily distinguish their test portions from the other collaborators' test portions at that site.

4.1.3 In addition, each collaborator will receive one uninoculated test portion of each matrix for determination of APC on the day of initiation of analyses.

4.2 Shipment of Test Portions

4.2.1 Test portions will be packaged in leak-proof insulated containers and shipped (according to the Dangerous Goods Regulations IATA for Infections Substances) by overnight carrier to arrive the Friday before initiation of analysis. All 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 the temperature of samples upon receipt. Upon arrival, the temperature of the temperature control sample will be recorded and the remaining test portions will be stored at 2-8°C until they are analyzed.

4.3 Analysis of Test Portions – See Appendix 8.3

4.3.1 The candidate and reference methods use the same enrichment procedure for ground beef, differing only in the time of enrichment. Therefore, each collaborator will receive only one set of ground beef test portions and one portion for aerobic plate count. Each collaborator will analyze test portions as follows.

4.3.2 Addeach 325-g test portion to 975 mL pre-warmed (42°C) modified Tryptone Soy Broth with casaminoacids (mTSB+CAA) in a filtered stomacher bag, homogenizewith paddle blender for 2.0 ± 10 sec., and enrich at 42±1°C (Appendix 8.3).

4.3.2.1 At sites with multiple collaborators, the enrichment setup should be staggered by at least two hours between collaborators in order to stagger usage of the shared RotorGene Q.

4.3.3 Prepare sample and serial decimal dilutions for aerobic plate count (APC) according to BAM Chapter 3. Plate the appropriate dilutions in duplicate on plate count agar using the pour plate technique. Incubate plates inverted for 48 ± 2 h at 35±1°C. Count plates and report APCs as described.

4.3.4 At 10±1 h, mix the enrichment bags by hand and remove two aliquots as follows. The same analyst shall perform the manual and automated procedures. After removing aliquots, return the enrichment bags to the incubator. Refer to the OMA draft method for method details.

4.3.4.1 Pipette 1.0 mL of enrichment into a 2 mL microtube for processing by the manual *mericon* DNA Bacteria Kit and manual assay setup.

4.3.4.2 Pipette 500 µL of enrichment into a 2 mL microtube for processing with the automated QIAsymphony*mericon* Bacteria Kit and automated QIAsymphony assay setup.

4.3.4.3 Test the resulting DNA preps by the *mericon* E. coli O157 Screen Plus assay following the method workflow.Record the C_T values from each channel on the Data Report Form.

4.3.4.4 Determine and record the “Next Action” for each enrichment. Refer to the draft OMA *mericon* method for details. Three “Next Action” results are possible.

4.3.4.4.1 For those DNA preps yielding “Additional testing required”, proceed to the *mericon* E. coli STEC O-type assay.

4.3.4.4.2 Those DNA preps yielding “No further action” in the screening assay will be considered negative and no further testing using the *mericon*kit will be performed.

4.3.4.4.3 Those DNA preps yielding “Dilute sample and repeat test” are inhibited and should be diluted 1/10 with RNase-free water and retested in the *mericon* E. coli O157 Screen Plus assay.

4.3.4.5 For those DNA preps from 4.3.4.4.1 (Additional testing required), perform the manual and/or automated assay setup as appropriate and analyze in the *mericon* E. coli O157 Screen Plus Assay following the method workflow. Record the C_T values from each channel on the Data Report Form.

4.3.4.6 Determine and record the "Next Action" for each enrichment. Refer to the draft OMA *mericon* method for details. There are again three results possible.

4.3.4.6.1 Those DNA preps yielding "Additional testing required" are positive for STEC, either *E. coli* O157:H7 (orange channel positive) or *E. coli*serogroups O26, O45, O103, O111, O121, or O145 (Green channel positive).

4.3.4.6.2 Those DNA preps yielding "No further action" are negative for STEC, including *E. coli* O157:H7, O26, O45, O103, O111, O121, and O145.

4.3.4.6.3 Those DNA preps yielding "Dilute sample and repeat test" are inhibited and should be diluted 1/10 with RNase-free water and retested in the *mericon* E. coli STEC O-Type assay.

4.3.4.7 Regardless of *mericon* results, proceed to step 4.3.5 for confirmation of all enrichments using the MLG 5.09 reference method for *E. coli* O157:H7.

4.3.5 At 15-24 h, mix the enrichment bags by hand and remove an aliquot for processing according to MLG 5.09 as summarized below.Refer to MLG 5.09 for details. [Alternatively, if pre-arranged, remove a 20-mL aliquot of each enrichment and place into appropriate screw-cap tubes for shipment to QLabs for confirmation.Follow instructions provided by QLabs for shipment. Make a note on the Data Report Form of the date when enrichment aliquots were shipped.]

4.3.5.1 Performa screening assay specified in MLG 5A.04. Record which screening assay was used and the results.

4.3.5.2 Regardless of the screening assay results, perform anti-O157 IMS on an aliquot from each enrichment bag.

4.3.5.3 After performing IMS, plate acid-treated and untreated IMS beads onmRBA. Test resultant typical colonies by latex agglutinationfor O157 and transfer up to 5 positive colonies to SBA.

4.3.5.4 Test isolated colonies on SBA by latex agglutination for O157 and H7, confirmfor toxin production byBAX STEC Screen Assay or Premier EHEC EIA, and determine biochemical ID by VITEK 2 GN or API20E.

4.3.5.5 Record all results on the Data Report Form.

4.4 Most Probable Number (MPN) Analysis

4.4.1 The low and high contamination levels will be estimated by MPN analysis on the day of initiation of the study using the MLG 5.09reference method. MPN determination will be performed in the Coordinating Laboratory only. The following analyses will be performed:

Table 4. MPN Test Portions for Low and High Inoculation Levels

Matrix	Inoculation Level	Large Test Portions	Medium Test Portions	Small Test Portions	Reference Method
Ground beef	Low	5 x 650 g	180 x 325 g*	5 x 130 g	MLG 5.09
	High	180 x 325 g*	5 x 130 g	5 x 65 g	

*Test portions from matrix study, 12 replicates per collaborator

4.4.2 The number of positives from the large portions, medium portions and small portions are used to calculate the MPN/test portion for each inoculated level of each matrix. The MPN calculator (http://www.lcfltd.com/customer/LCFMPNCalculator.exe) is used to determine the MPN values and 95% confidence intervals. *Note 1: Data must be entered in test portion size order from large to small. Note 2: The MPN calculator is a Microsoft Windows application and is not supported by the Mac Operating System.*

4.5 Analysis of QC Test Portion Sets

4.5.1 The Coordinating Laboratory will retain two Quality Control Test Portion Sets, QC Set 1 and QC Set 2. These sets are identical to the collaborator test portion sets and will be randomized and blind coded with test portions labeled QC 101-136 and QC 201-236, respectively.

4.5.2 QC Set 1 will be analyzed on the day of shipment to check the fractional positive level. Testing will include manual sample prep, manual assay setup and the *mericon* E. coli Screen Plus Assay. No *mericon* STEC O-Type Assay or confirmations will be performed unless unexpected results occur (e.g., a positive in the uninoculated test portions). These data are used only to gauge whether the fractional positive criteria will be met in the study and to ensure no cross contamination of the uninoculated test portions has occurred.

4.5.3 QC Set 2 will be the "official" test portion set analyzed by the Coordinating Laboratory on the day the collaborators initiate analyses and will follow section 4.3 above.The Coordinating Laboratory will analyze 2 additional collaborator test portion sets, for a total of 3 test portion sets at that site.

5.0 Candidate Method

AOAC Official Method XXXX.XX
STEC in Selected Foods
PCR with Manual and Automated Sample Preparation
First Action XXXX

(Applicable for detection of Shiga toxin-producing *E. coli* (STEC), including serogroups O26, O45, O103, O111, O121, O145 and O157:H7, in fresh raw ground beef and fresh raw beef trim.)

See Table XXXX.XXA for the results of the interlaboratorystudy supporting acceptance of the method.

A. Principle

QIAGEN's*mericon*E.coliO157 Screen Plus and *mericon*E.coliSTEC O-TypePathogen Detection Assays are multiplex PCR assays that amplify both a specific DNA target and an internal control with high specificity. The internal control provides data regarding the presence of inhibitors in the tested samples and the overall quality of the PCR run. Each*mericon*PCR Assay includes a PCR primer set for a pathogen-specific target sequence, probes labeled with four distinct fluorescent dyes (*mericon*E. coliO157 Screen Plus) or three distinct fluorescent dyes (*mericon* E. coliSTEC O-Type), positive control DNA, and all of the reagents necessary to perform the analysis. The Multiplex PCR Master Mix included in each kit contains QIAGEN proprietary technology, including HotStarTaq*Plus* DNA polymerase, patented multiplex PCR technology such as Factor MP, and fast cycling technology including Q-bond.

The Rotor-Gene Q cycler produces raw data files that are further interpreted by the software using mathematical algorithms to characterize samples. The software guides the user through each step and provides simplicity for beginners as well as an experimental platform for advanced users.

B. Test Kits and Components

(**a**)*mericon® E. coli O157 Screen Plus Kit.*-QIAGEN #290403, 24 tests, includes assay components, RNase-free water, and multiplex PCR master mix.

(**b**) *mericon®E. coli STEC O-Type Kit.*-QIAGEN #290233, 24 tests, includes assay components, RNase-free water, and multiplex PCR master mix.

(**c**) *mericon® DNA Bacteria Kit.*-QIAGEN #69525, includes Fast Lysis buffer, product insert, and quick start protocol.

(**d**) *QIAsymphonymericon® Bacteria Kit.*-QIAGEN #931156, includes reagent cartridge, piercing lid, top elute fluid, reuse seal set, product insert, and quick start protocol.

C. Apparatus

(**a**) *Incubator*.-Capable of maintaining 42 ± 1°C.

(**b**) *Top-loading balance*.-Capable of weighing 1-2000 g.

(**c**) *Laboratory paddle blender*.-Seward 3500 or equivalent.

(**d**) *Vortex mixer*.-Multiple laboratory suppliers (MLS).

(**e**) *Microcentrifuge*.-For 1.5-2.0 mL microcentrifuge tubes.

(**f**) *Pipettors*.-Adjustable, capable of delivering 10-100 µL and 100-1000 µL.

(**g**) *Dry bath incubator*.-Capable of maintaining 100 ± 1°C.

(**h**) *Calibrated thermometer*.-Capable of measuring 100 ± 1°C.

(**i**) *Thermocycler*.-RotorGene Q®, QIAGEN #9001640, with notebook computer containing Rotor-Gene Q® software version 2.3.1.49 or later.

(**j**) *QIAsymphony® SP*.-QIAGEN #9001297, for automated DNA extraction.

(**k**) *QIAsymphony® AS*.-QIAGEN #9001301, for automated PCR assay setup.

(**l**) *Rotor*.-QIAGEN #9018903, 72 wells, for holding 0.1 mL strip tubes and caps.

(**m**) *Locking ring for 72 well rotor*.-QIAGEN #9018904, for locking strip tubes and caps in 72-well rotor.

(**n**) *Rotor-Disc 72 Rotor*.-QIAGEN #9018899.

(**o**) *Locking ring for Rotor-Disc 72*.-QIAGEN #9018900.

(**p**) *Reagent cartridge holder (2)*.-QIAGEN #997008.

(**q**) *Sample carrier insert, 2 mL v2*.-QIAGEN #9242083, secondary tube adapter for 2 mL screw-cap tubes.

(**r**) *Cooling adapter*.-QIAsymphony EMT v2, QIAGEN #920730.

(**s**) *Sample preparation cartridges*.-8-well, QIAGEN #997002.

(**t**) *8-Rod covers*.-QIAGEN #997004.

(**u**) *Elution microtubes*.-CL with cap strips, QIAGEN #19588.

(**v**) *Reagent holder cooling adapter*.-QIAGEN #9018090.

(**w**) *Adapter*.-2 x Rotor-Disc, for use with QIAsymphony, QIAGEN #9242204.

(**x**) *Rotor-Disc 72 loading block*.-QIAGEN #9018910.Store refrigerated to ensure that PCR setup is performed under stable thermal conditions.

(**y**) *Rotor-Disc 72*QIAGEN #981301 (24) or # 981303 (240)

(**z**) *Rotor-Disc heat sealing film*.-QIAGEN #981601 (60) or #981604 (600).

(**aa**) *Rotor-Disc heat sealer*.-QIAGEN #9018898 (110 V) or #9019725 (230 V).

(**bb**) *Filter tips*.-1500 µL, QIAGEN #997024.

(**cc**) *Filter tips*.-200 µL, QIAGEN #990332.

(**dd**) *Filter tips*.-50 µL, QIAGEN #997120.

D. Additional Supplies, Reagents and Media

(**a**) *Filter laboratory blender bags*.-MLS.

(**b**) *Serological pipettes* – Aerosol resistant

(**c**) *Micropipette tips,* aerosol resistant.-MLS.

(**d**) *Microtubes, 2.0 mL, with caps.*-MLS.

(**e**) *Modified tryptone soy broth with casamino acids (mTSB + CAA)*.-prepared according to FSIS MLG 1.08, or equivalent commercial formula.

(**f**) *Water.*-Nuclease-free PCR grade, or equivalent, MLS.

E. Safety Precautions

(**a**) *mericon E. coli O157 Screen Plus Kit, mericon E. coli STEC O-Type Kit, and mericon DNA Bacteria Kit.*-All chemicals should be considered potentially hazardous. When working with chemicals, always wear a suitable lab coat and disposable gloves. Product usage should follow good laboratory practices.

(**b**) *QIAsymphonymericonBacteria Kit.*-The buffers in the reagent cartridge contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite solution. Reagents in this kit are highly flammable and are harmful by inhalation, skin contact and swallowing.

(**c**) *Real-Time PCR System*.-Improper use of the Rotor-Gene Q may cause personal injuries or damage to the instrument. The Rotor-Gene Q must only be operated by qualified personnel who have been appropriately trained. Servicing of the Rotor-Gene Q must only be performed by QIAGEN Field Service Specialists.

(**d**) *Enrichment.*-Shiga-toxin producing *E. coli* is a Biosafety Level 2 organism. Biological samples such as enrichments have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes. Wear appropriate protective equipment, which includes but is not limited to protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities utilizing the appropriate safety equipment (for example, physical containment devices). Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials. All enrichment broths should be sterilized following any culture-based confirmatory steps.

F. General Preparation

(**a**) *General.*-(1) Use aseptic techniques. (2) Use Filter bags during enrichment to minimize particulates. (3) Clean the workstations with a disinfectant of choice (e.g., sodium hypochlorite solution, phenol solution, Quaternary ammonium solution, etc.) before and after use. *Note: Do not use sodium hypochlorite for cleaning the QIAsymphony.* (4) Separate work areas for the following: media preparation, sample preparation, DNA lysis, and DNA amplification and detection. (5) Do not reuse kit disposables. (6) Change pipette tips between samples. (7) Wear personal protective equipment.

(**b**) *DNA Lysis.*-(1) Pre-warm heat block to 100 ± 1°C. (2) Change pipette tips between sample transfers. (3) Do not disrupt the pellet when discarding the supernatant. (4) Be sure to allow the samples to cool to room temperature (24 ± 2°C) after the heat lysis procedure.

(**c**) *DNA Amplification*.-(1) Store PCR loading block at 2-5 °C. (2) Use aseptic techniques. (3) Change pipette tips between samples. (4) Change pipette tips for the PCR master mix and the DNA samples. (5) Use gloves and protective laboratory wear. (6) Do not touch any PCR equipment or supplies without wearing gloves. (7) In case of contamination, laboratory benches and pipettes can be decontaminated with a 1/10 dilution of commercial bleach solution.

(**d**) *Enrichment medium.*-Pre-warm mTSB + CAA to 42 ± 1°C.

G. Test Portion Enrichment

(**a**) *Raw ground beef and raw beef trim.*-Add a 325 g test portion to 975 mL mTSB + CAApre-warmed to 42 ± 1°C. Homogenize in a paddle blender for 2.0 min ± 10 sec and incubate at 42 ± 1°C for 10 ± 1 h.

H. DNA Extraction and Assay Setup

(**a**) *Manual mericonDNA Bacteria Kit method*.-(1) Gently mix enriched test portions to disperse organisms throughout the enrichment broth. (2) Transfer 1.0 mL of enrichment culture to a sterile 2 mL screw cap tube and centrifuge for 5 minutes at 13,000 x*g*. (3) Discard the supernatant with a pipettor, taking care not to disrupt the pellet. (4) Add 200 µL Fast Lysis Buffer to the bacterial pellet, tightly cap the tube, and resuspend the pellet by brief, vigorous vortex mixing. (5) Place the microcentrifuge tube into a heat block at 100 ± 1°C for 10 min ± 10 sec. (6) Remove the tube and allow it to cool to 15-24°C for 2 min ± 10 sec. (7) Centrifuge the tube at 13,000 x *g* for 5 min. (8) Transfer 100 µL of the supernatant to a fresh 1.5 mL microcentrifuge tube. This is the extracted DNA. (9) Dilute an aliquot of the extracted DNA 1/10 with RNase-free water.

(**b**) *Manual Assay Setup.*-(1) Place the desired number of PCR 72-well strip tubes into the adapters of the cooling block for the Rotor-Gene Q. (2) Reconstitute *mericon* Assay by adding 130 µL Multiplex PCR Master Mix (tube(s) with blue lid) to each vial of *mericon* Assay (yellow lid). (3) Add 10 µL reconstituted*mericon* Assay to each strip tube. (4) Setup sample reactions by adding 10 µL sample DNA to each appropriate strip tube. (5) Setup control reaction by adding 10 µLRNase-free water to the negative PCR control strip tube. (6) Proceed to Analysis.

(**c**) *Automated QIAsymphony Bacteria Kit method.*-(1) Gently mix enriched test portions to disperse organisms throughout the enrichment broth. (2) Transfer 500 µL of each enrichment culture into sterile 2 mL microtubes. (3) Close all the drawers and hoods of the QIAsymphony SP/AS instrument. (4) Switch on the instrument and wait until the “Sample Preparation” screen appears and the initialization procedure as finished. (5) Log in to the instrument. (6) Ensure the “Waste” drawer is prepared properly and perform an inventory scan of the “Waste” drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary. (7) Load the required elution rack into the “Eluate” drawer and perform an inventory scan of the “Eluate” drawer. (8) Load the required reagent cartridge(s) and consumables into the “Reagents and Consumables” drawer. (9) Press the “R+C” button in the touchscreen to open the screen that shows the consumables status (“Consumables/8-RodCovers/Tubes/Filter-tips/Reagent Cartridges”). Press the “Scan Bottle” button to scan the bar code of the TopElute bottle with the handheld bar code scanner. Press the “OK” button. (10) Perform an inventory scan of the “Reagents and Consumables” drawer. (11) Place the samples into the appropriate tube carrier and load them into the

"Sample" drawer without tube lids. (12) Using the touchscreen, enter the required information for each batch of samples to be processed. (13) Choose elution volume of 400 µL. (14) Press the "Run" button to start the purification procedure. (15) When sample processing is compete, perform a direct transfer of the elution rack to the QIAsymphony AS via the transfer module (integrated operation). Press "Transfer" to transfer the elution rack from slot 1 of the QIAsymphony SP to slot 2 of the QIAsymphony AS. (16) If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips immediately after the end of the last protocol run to avoid evaporation. (17) Discard used sample tubes, plates, and waste according to local safety regulations and replace the tip disposal bag. (18) Close the instrument drawers and proceed with assay setup on the QIAsymphony AS.

(**d**) *QIAsymphony AS Assay Setup*.-(1) Reconstitute *mericon* Assay by adding 130 µL Multiplex PCR Master Mix (tube(s) with blue lid) to each vial of *mericon* Assay (yellow lid). Mix by pipetting up and down 5 times or by quick vortex mixing and centrifuge briefly. Transfer the reconstituted *mericon* Assay to a labeled, fresh 2 mL microtube. (2) Insert the tip chute into its position on the right hand side in the front part of the QIAsymphony AS module. (3) Install an empty tip disposal bag in the bag holder under the "assays" drawer. (4) Switch user interface from sample preparation to assay setup. (5) Start the assay definition process. (6) For integrated operation (elution rack is automatically transferred from the QIAsymphony SP into the AS module) the "Sample Rack(s)" screen will appear directly. (i) All stored information(sample status, sample ID, sample volume, and rack ID) is transferred to the QIAsymphony AS module together with the elution rack and will automatically complete the required information in the "Sample Rack(s)" screen of the assay setup user interface. (7) For assay setup independent from a former QIAsymphony SP run, select the rack file of the corresponding QIAsymphony SP run or select the rack type of the elution rack for the highlighted "Sample" position (slot 2) and then either manually type in the "Rack ID" of the elution rack or choose "Automatic ID" for a new ID. (8) In the "Sample Rack Layout" screen of the assay setup user interface, the elution tack in slot 2 is pictured. (i) For integrated operation, or for independent operation incombination with a loaded rack file, sample IDs and sample volumes are automatically assigned to the corresponding positions. (ii) For independent operation without a rack file, select the positions to be processed from the elution rack. Define the highlighted positions as "Sample" then reselect the defined samples and assign sample volumes. (9) In the "Assay Selection" screen, select the Assay Parameter Set(s) to use in the run. (10) In the "Assay Assignment" screen, assign the Assay Parameter Sets to samples. (11) In the "Assay Rack(s)" screen, define the assay rack ID. Either type in the assay rack ID manually or choose "Automatic ID" for a new ID. (12) Cooling of samples and reagents will start automatically. Check the temperature of the cooling positions. (13) The "Loading Information" screen displays the working table of the QIAsymphony AS module with all previously defined sample and reagent rack types in the designated positions. The required position of the PCR reaction adapter is displayed as well as information on the required filter-tip types and number. (14) Place the reconstituted *mericon* Assay(s), the reconstituted Positive Control(s), and the reconstituted Negative Control(s), without lids, into the appropriate positions of the precooled reagent adapters. (15) Open the "Eluate and Reagents" and "Assays" drawers. Load the prepared reagent adapter into slot 3 of the "Eluate and Reagents" drawer according to the illustration on the "Loading Information" screen. Place the Rotor-Disc in the appropriate adapter and load the adapter into the designated slot of the "Assays" drawer. (16) Load disposable filter-tips into the "Eluate and Reagents"

and "Assays" drawers, according to the required number of each tip type. (17) Close the "Eluate and Reagents" and "Assays" drawers. Upon closing each drawer, press "Yes" to start the inventory scan. (18) Press "Queue". Monitoring of the cooling starts. (19) Press "Run" to start the run. (20) After the run is finished, press "Remove" in the assay setup "Overview" screen. Open the "Assays drawer and unload the PCR adapter. (21) Download the result and cycler files via the QIAsymphony Management Console (QMC). (22) Proceed to Analysis.

I. Analysis

(**a**) Seal the Rotor-Disc after automated assay setup or close the strip tubes after manual assay setup. Place Rotor-Disc or strip tubes in the respective rotor and make sure to apply the locking ring. If using tubes, fill the empty positions in the rotor with empty strip tubes. Place the rotor in the reaction chamber of the Rotor-Gene Q. (**b**) Open the Rotor-Gene Software. It is recommended to use the template file provided. In the Advanced Wizard, select "Open a Template in Another Folder…" and load the files "*mericon* E. coli O157 Screen Plus" and "*mericon* E. coli STEC O-Type". *Note: If you copy the template files "mericon E. coli O157 Screen Plus" and "mericon E. coli STEC O-Type" in the Rotor-GeneQ Templates and in the Quick Start Templates folders, the template will appear directly in the Quick Start and Advanced Wizard windows*. (**c**) For manual cycling setup, select "Empty Run" and click "New". *Note: It is recommended to use the template files provided to facilitate the reaction setup. When using template files, the settings may already be those described in the next step. In this case, click to the next screen*. (**d**) Select the correct rotor and confirm the locking ring is attached by checking the check box. Click "Next" to continue. (**e**) Ensure that the reaction volume is set to 20 µL. Click "Next" to continue. (**f**) Click "Edit Profile" and program the Rotor-Gene Q according to Table XXXX.XXB. Click "OK" to close the window and return to the Wizard. (**g**) To set the gain optimization settings for Green, Crimson, Yellow, and Orange channels, click "Gain Optimization". Select the 4 channels in the drop-down menu and click "Add". (**h**) In the dialog box that opens, confirm the standard settings. Click "Perform Optimization Before 1st Acquisition". Then close the window. *Note: Make sure that the tube at position 1 is not empty, since the gain optimization will be performed on this tube*. (**i**) Start the PCR run.

Table XXXX.XXB. Cycling Protocol for Rotor-Gene Q

Step	Time	Temp.	Comment
Initial PCR activation	5 min	95°C	Activation of HotStarTaq*Plus* DNA Polymerase
3-Step cycling			
Denaturation	15 sec	95°C	
Annealing[a]	15 sec	60°C	Data collection at 60°C for green, crimson, orange, and yellow channels
Extension	10 sec	72°C	
Number of cycles = 40			

[a]Gain optimization before first acquisition at 60°C for green, crimson, orange, and yellow channels.

J. Interpretation and Test Result Report

(**a**) Open the run file using the Rotor-Gene Q Software. Go to "File", followed by "Open", and then "Browse" to locate the saved file.

(**b**) *mericon E. coli O157 Screen Plus.*-(1) Click "Analysis" to edit the analysis parameters. Open the Quantitation Analysis windows under "Analysis". (2) To import analysis settings to each channel, activate the "Quantitation Analysis" window for the channel and select the respective template file fro the directory with the "Import" function. (3) To setup the analysis parameters manually, set the following parameters. (i) Click "Ignore First" and ignore the first 10 cycles for all 4 channels. (ii) For all channels, click "Take Off Point Adjustment" and adjust the settings so that if the take off point was calculated before cycle 15, then cycle 20 is used as the take off point. Click "OK". (iii) Set the threshold for channels Green, Crimson, and Yellow to 0.035. (iv) Set the threshold for channel Orange to 0.08. (4) To export the results to Excel®, go to the "File" menu, followed by "Save As", and then "Excel Analysis Sheet". The results will be saved as comma separated values (*.csv) format. (5) To create a printable report, go to "Reports" and create a "Quantitation (Full Report)" for each channel.

(**c**) *mericon E. coli STEC O-Type.*-(1) Edit analysis parameters by clicking "Analysis". (2) Click "Ignore First" to ignore the first 10 cycles when calculating the threshold for all 3 channels. (3) Adjust the take off point by clicking "Take Off Point Adjustment" and "OK". (4) Do not activate "Slope Correct". (5) Set the threshold for the Green and Yellow channels to 0.035. (6) Set the threshold for the Orange channel to 0.08. (6) To export the results to Excel®, go to the "File" menu, followed by "Save As", and "Excel Analysis Sheet". (7) To create a printable report, go to "Reports" and create a "Quantitation (Full Report)" for each channel.

(**d**) *Analyzing the results.*-(1) Presence or absence of pathogen DNA is determined based on amplification of the target sequence. A positive result is visible as a final point on the fluorescence curve that lies clearly above the threshold. (2) Possible outcomes are summarized in Table XXXX.XXC. (3) In the event of a PCR inhibited internal Control (C_T≥30.01 or No C_T) and a positive sample result, repeating the test is not necessary. (4) In the event of a PCR inhibited Internal Control (C_T≥30.01 or No C_T) and a negative or indeterminate sample result, dilute the extracted sample 1/10 with RNase-free water and repeat the test.

Table XXXX.XXC. Summary of Possible Outcomes

Sample	Internal Control (IC)	Result
C_T<38	C_T 24-30	Sample is positive (+)
C_T <38	C_T≥30.01 Or No C_T	Sample is positive (+)
C_T 38.01-40	C_T 24-30	Sample is indeterminate; repeat test
No C_T	C_T 24-30	Sample is negative (-)
No C_T	C_T≥30.01 Or No C_T	IC invalid, PCR inhibited; dilute sample and repeat test

(**e**) *Interpretation of mericon E. coli O157 Screen Plus Results.*-(1) Results are interpreted according to Table XXXX.XXD. The presence of DNA sequences due to the O157 serotype and/or the virulence factors (*stx1/stx2* and *eae*) indicate the presumptive presence of pathogenic *E. coli* (STEC). (2) No further action means the sample was negative for pathogenic *E. coli* and no additional testing is needed. (3) Additional testing required means the sample was presumptive positive for pathogenic *E. coli* and should be tested by the *mericon* E. coli STEC O-Type assay for O157 and non-O157 STEC serotypes. (4) An invalid IC with any target reactions negative indicates inhibition of the PCR reaction and the sample should be diluted 1/10 with RNase-free water and retested.

Table XXXX.XXD. *mericon* E. coli O157 Screen Plus Results and Next Actions

Channel Outcome				Next Action
Orange O157	**Green *stx1/stx2***	**Crimson *eae***	**Yellow IC**	
-	-	-	Valid	No further action
-	+	-	Valid	No further action
-	-	+	Valid	No further action
+	-	-	Valid	Additional testing required
-	+	+	Valid	Additional testing required
+	+	+	Valid	Additional testing required
-	-	-	Invalid	Dilute sample and repeat test

(**f**) *Interpretation of mericon E. coli STEC O-Type Results.*-(1) Results are interpreted according to Table XXXX.XXE. The presence of DNA sequences due to the O157 serotype and/or one of the big six non-O157 STEC serotypes indicates the presence of pathogenic *E. coli* (STEC). (2) No further action means the sample was negative for pathogenic *E. coli* and no additional testing is needed. (3) Additional testing required means the sample was positive for pathogenic *E. coli* and should be confirmed according to the cultural confirmation procedures in K. (3) An invalid IC with either target reaction negative indicates inhibition of the PCR reaction and the sample should be diluted 1/10 with RNase-free water and retested.

Table XXXX.XXE. *mericon* E. coli STEC O-Type Results and Next Actions

Channel Outcome			Next action
Orange O157:H7	**Green O26, O45, O103, O111, O121, O145**	**Yellow IC**	
-	-	Valid	No further action
-	+	Valid	Additional testing required
+	-	Valid	Additional testing required
+	+	Valid	Additional testing required
-	-	Invalid	Dilute sample and repeat test

K. Confirmation

(**a**) *Cultural confirmation of fresh raw ground beef or fresh raw beef trim*.-Follow cultural isolation and confirmation procedures described in the US Food Safety Inspection Service Microbiology Laboratory Guidebook (FSIS MLG) 5.09: *Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges* for *E. coli* O157:H7 or FSIS MLG 5B.05: *Detection and Isolation of non-O157 Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products and Carcass and Environmental Sponges* for *E. coli* O26, O45, O103, O111, O121 or O145.

6.0 Reporting Raw Data

6.1 Data will be reported using the data reporting form in Appendix 8.2.

6.2 Each collaborator will complete the data reporting form and email it to the co-Study Director as indicated on the form. Collaborators will either complete the confirmations or ship samples to QLabs for confirmation. If the latter, the date of shipment shall be indicated on the data report form.

6.3 Each collaborator or collaboration site must retain all related test results (data sheets and confirmation results) for a minimum of one year.

6.4 Collaborators may also submit a comment form (Appendix 8.4).

6.5 A table reporting all raw data from all collaborators must be included in the collaborative study report. All submitted collaborator comments must be included in the collaborative study report.

7.0 Analyzing Raw Data

7.1 Each concentration level must be analyzed and reported separately. Data may be excluded due to an assignable cause if sufficient justification is provided.

7.2 Using the AOAC POD Calculator(http://lcfltd.com/AOAC/aoac-binary-v2-3.xls), determine to following parameters for each contamination level:

- 7.2.1 Repeatability Standard Deviation - s_r
- 7.2.2 Among Laboratories Standard Deviation - s_L
- 7.2.3 Homogeneity test of laboratory PODs – p-value
- 7.2.4 Reproducibility Standard Deviation - s_R
- 7.2.5 $LPOD_{CP}$ with 95% confidence interval
- 7.2.6 $LPOD_{CC}$ with 95% confidence interval
- 7.2.7 $dLPOD_{CP}$ ($LPOD_{CP} – LPOD_{CC}$) with 95% confidence interval
- 7.2.8 $LPOD_C$ with 95% confidence interval
- 7.2.9 $LPOD_R$ with 95% confidence interval
- 7.2.10 $dLPOD_C$($LPOD_C$ - $LPOD_R$) with 95% confidence interval

7.3 Compare the p-value from the homogeneity test to 0.1 to determine whether the variability between laboratories (s_L) is significant.

7.4 If the 95% confidence interval on $dLPOD_{CP}$ or $dLPOD_{C}$does not contain zero, then the difference is statistically significant.

7.5 Optionally, the data can be analyzed by RLOD statistics as prescribed by the revised ISO 16140-2. A spreadsheet calculator is available for download at http://standards.iso.org/iso/16140/.

8.0 Appendices

Appendix 8.1

Instructions to Collaborators
QIAGEN *mericon*® STEC Workflow Collaborative Study

General Instructions about Collaborative Studies

Introduction

The purpose of this document is to provide detailed instructions for performing the collaborative study for the *mericon*® E. coli O157 Screen Plus and *mericon*® E. coli STEC O-type Detection Workflow.

The study will be conducted by co-Study Directors Marcia Armstrong and Erin Crowley. The Study Directors are responsible for providing method-specific training, providing test portions, clarifying procedures, collating results, and submitting a report to AOAC INTERNATIONAL. Should any questions arise before or during the course of the study, please direct them immediately to one of the co-Study Directors:

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Important Information

1. Read the methods carefully. If you have any questions, contact one of the co-Study Directors.
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 *mericon* supplies are on hand.
3. Begin the analyses 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 the shipment, confirm the contents with the Study Director by faxing or emailing 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 analyses 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 instructions and indicate which results to report. A space is provided on the Data Report Form to indicate any deviations to the methods or the instructions to collaborators. Please include any criticisms, suggested improvements, or general comments about the products on the Collaborators' Comments Form provided. Any results that were derived from modified protocols should be included but must be clearly indicated. Results and comments should be returned to the Study Director immediately upon completion of the study.

Information about this Collaborative Study

Shipment Schedule and Receipt of Study Materials and Test Portions

All test kits, study materials, media and reagentsas outlined in Appendix 8.2 should be received by Wednesday of Week 1. If all collaborators are not on-site at the time of arrival, a designated site coordinator shall receive the materials. Collaborators or site coordinators shall inventory all test kits,study materials, media and reagents against the packing lists upon arrival to ensure all materials have arrived. Contact co-Study Director Marcia Armstrong if any test kits or study materials have not arrived by Wednesday.Contact co-Study Director Erin Crowley if enrichment media and IMS reagents have not arrived by Wednesday.

Test portions will be shipped by overnight courieron Thursday of Week 1 to arrive on Friday. If all collaborators are not on-site at the time of arrival, a designated site coordinator shall receive the packages. Each package shall be examined for potential damage. The collaborator or site coordinator must alert co-Study Director Erin Crowley if any packages appear compromised or if the contents are not cold to the touch. Each package will contain a clearly labeled temperature control sample. The collaborator or site coordinator should quickly take a temperature measurement of the core of this sample and record the temperature reading on the Data Report Form. If the temperature of this control sample is outside the range 2-8°C, co-Study Director Erin Crowley should be alerted. Each package of ground beef should contain 36 test portions labeled X01-X36, where X is a number between 1 and 16, unique for each collaborator so you can distinguish your test portions from the test portions for other collaborators at the same site. In addition, there should be one additional test portion in each package designated for aerobic plate count determination. Inventory the test portions to be sure all are present and alert co-Study Director Erin Crowley if any test portions are missing.The test portions should be stored at 2-8°C until initiation of the study on the following Monday.

Table 1. Study Schedule (Specific Dates TBD)

Collaborator Activity	Receipt of Media and Reagents	Receipt of Test Kits and Materials	Receipt of Test Portions Set	Initiation of Analyses	Submission of Data Report Form
Date	Week 1, Wednesday	Week 1, Wednesday	Week 1, Friday	Week 2, Monday	Week 4, Monday

Safety Precautions

1. *mericon E. coli O157 Screen Plus Kit, mericon E. coli STEC O-Type Kit, and mericon DNA Bacteria Kit.*-All chemicals should be considered potentially hazardous. When working with chemicals, always wear a suitable lab coat and disposable gloves. Product usage should follow good laboratory practices.
2. *QIAsymphonymericonBacteria Kit.*-The buffers in the reagent cartridge contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite solution. Reagents in this kit are highly flammable and are harmful by inhalation, skin contact and swallowing.
3. *Real-Time PCR System*.-Improper use of the Rotor-Gene Q may cause personal injuries or damage to the instrument. The Rotor-Gene Q must only be operated by qualified personnel who have been appropriately trained. Servicing of the Rotor-Gene Q must only be performed by QIAGEN Field Service Specialists.
4. *Enrichment.*-Shiga-toxin producing *E. coli* is a Biosafety Level 2 organism. Biological samples such as enrichments have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes. Wear appropriate protective equipment, which includes but is not limited to protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities utilizing the appropriate safety equipment (for example, physical containment devices). Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials. All enrichment broths should be sterilized following any culture-based confirmatory steps.

Test Portion Analysis

On Monday, all collaborators shall initiate the enrichment of test portions according to the *mericon* and reference methods. A copy of the detailed methods will be provided for you. Note that the ground beef is a paired study, so the same enrichments are used for the *mericon* and MLG methods. Refer to the Study Flowchart in Appendix 8.3 for an overview of the matrix study and methods. All test portions are pre-weighed. An additional uninoculatedtest portion is labeled for aerobic plate count (APC) determination. Initiate the APC determination using the FDA BAM method on the same day as the study test portions.

Ground beef analysis (see Flowchart in Appendix 8.3)

1. Prewarm modified Tryptic Soy Broth with casamino acids (mTSB+CAA) to 42°C.
2. Place each pre-weighed325 g test portion into a filtered stomacher bag.
3. Add 975 mL pre-warmed mTSB+CAA to each bag.
4. Homogenize with a paddle blender at 230 rpm for 2.0 min ± 10 sec.
5. Incubate all bags at 42 ± 1°C.
    5.1 At sites with multiple collaborators, the enrichment setup should be staggered by at least two hours between collaborators in order to stagger usage of the shared RotorGene Q.
6. Prepare sample and serial decimal dilutions for aerobic plate count (APC) according to BAM Chapter 3. Plate the appropriate dilutions in duplicate on plate count agar using the pour plate technique. Incubate plates inverted for 48 ± 2 h at 35± 1°C. Count plates and report APCs as described.
7. At 10 ± 1 h, mix the enrichment bags by hand and transfer and process two aliquots as follows for the *mericon* method. The same analyst shall perform the manual and automated procedures. After removing aliquots, return the enrichment bags to the incubator. Refer to the OMA draft method for method details.
    7.1 Pipette 1.0 mL of enrichment into a 2 mL microtube for processing by the manual *mericon* DNA Bacteria Kit and manual assay setup.
    7.2 Pipette 500 µL of enrichment into a 2 mL microtube for processing with the automated QIASymphony*mericon* Bacteria Kit and automated QIAsymphony assay setup.
    7.3 Test the resulting DNA samples by the *mericon* E. coli O157 Screen Plus assay following the method workflow.Record the C_T values from each channel on the Data Report Form.
    7.4 Determine and record the "Next Action" for each enrichment. Refer to the draft OMA *mericon* method for details. Three "Next Action" results are possible.
        7.4.1 Test those DNA preps yielding "Additional testing required" in the screening assay by the *mericon* E. coli STEC O-type assay as described in the method workflow.
        7.4.2 Those DNA preps yielding "No further action" in the screening assay will be considered negative by the *mericon* method and no further testing will be performed.
        7.4.3 Those DNA preps yielding "Dilute sample and repeat test" are inhibited and should be diluted 1/10 with RNase-free water and retested in the *mericon* E. coli O157 Screen Plus assay.
    7.5 For those DNA preps from 7.4.1 (Additional testing required), perform the manual and/or automated assay setup as appropriate and analyze in the *mericon* E. coli O157 Screen Plus Assay following the method workflow. Record the C_T values from each channel on the Data Report Form.
    7.6 Determine and record the "Next Action" for each enrichment. Refer to the draft OMA *mericon* method for details. There are again three results possible.

7.6.1 Those DNA preps yielding "Additional testing required" are positive for STEC, either *E. coli* O157:H7 (orange channel positive) or *E. coli*serogroups O26, O45, O103, O111, O121, or O145 (Green channel positive).

7.6.2 Those DNA preps yielding "No further action" are negative for STEC, including *E.coli* O157:H7, O26, O45, O103, O111, O121, and O145.

7.6.3 Those DNA preps yielding "Dilute sample and repeat test" are inhibited and should be diluted 1/10 with RNase-free water and retested in the *mericon* E. coli STEC O-Type assay.

7.7 Regardless of *mericon* results, proceed to step 8 for confirmation of all enrichments using the MLG 5.09 reference method for *E. coli* O157:H7.

8. At 15-24 h, mix the enrichment bags by hand and remove an aliquot for processing according to MLG 5.09. [Alternatively, if pre-arranged, transfer a 20-mL aliquotof each enrichmentinto appropriate screw-cap tubes for shipment to QLabs for confirmation. Follow instructions provided by QLabs for shipment. Make a note on the Data Report Form of the date when enrichment aliquots were shipped.]

8.1 Performa screening assay specified in MLG 5A.04. Record which screening assay was used and the results.

8.2 Regardless of the screening assay results, perform anti-O157 IMS on an aliquot from each enrichment bag.

8.3 After performing IMS, plate acid-treated and untreated IMS beads onmRBA. Test resultant typical colonies by latex agglutination for O157 and transfer up to 5 positive colonies to SBA.

8.4 Test isolated colonies on SBA by latex agglutination for O157 and H7, confirmfor toxin production by BAX STEC Screen Assay or Premier EHEC EIA, and determine biochemical ID by VITEK 2 GN or API20E.

8.5 Record all results on the Data Report Form.

Reporting Results

Complete the Data Report Form (included in your packet as Excel file Appendix 8.5) for each test portion, noting any deviations. Once analyses are complete, finalize the Data Report Form, electronically sign and date the form indicating your QC check of the data, and submit it by email to co-Study Director Erin Crowley.

Collaborator Comment Form

At the completion of the study, please fill out the Collaborator Comment Form (Appendix 8.4) to submit any comments you may have regarding the *mericon* method or the conduct of the study. Completed forms should be returned by email to co-Study Director Erin Crowley.

Appendix 8.2

Study Materials and Apparatus

QIAGEN *mericon*® STEC Workflow Collaborative Study

Materials provided by collaborator

Apparatus (standard lab apparatus):

(a) Incubator.-Capable of maintaining 42 ± 1°C.
(b) Top-loading balance.-Capable of weighing 1-2000 g.
(c) Laboratory paddle blender.-Seward 3500 or equivalent.
(d) Vortex mixer.- Multiple laboratory suppliers (MLS).
(e) Microcentrifuge.-For 1.5-2.0 mL microcentrifuge tubes.(minimum of 100 required for MLG confirmation)
(f) Pipettors.-Adjustable, capable of delivering 10-100 µL and 100-1000 µL.
(g) Dry bath incubator.-Capable of maintaining 100 ± 1°C.
(h) Calibrated thermometer.-Capable of measuring 100 ± 1°C.
(i) Miltenyi Biotech Octomacs magnets or equivalent (required for MLG confirmation)
(j) Labquake agitator or equivalent (required for MLG confirmation)

Consumables:

(a) Serological pipettes – Aerosol resistant
(b) Micropipette tips, aerosol resistant.-MLS.
(c) Microtubes, 2.0 mL, with caps.-MLS.
(d) Water.-Nuclease-free PCR grade, or equivalent, MLS.
(e) Sterile Petri Dishes.-100 x 15 mm, MLS.

Materials provided by QIAGEN

Kits supplied by QIAGEN:

(a) mericon® E. coliO157 Screen Plus Pathogen Detection Assay (24), CAT# 290403
(b) mericon® E. coliSTEC O-Type Pathogen Detection Assay (24), CAT# 290233
(c) mericon® DNA Bacteria Kit (100), CAT#69525
(d) QIAsymphony® *mericon®* DNA Bacteria Kit, CAT# 931156

Apparatus and accessories supplied by QIAGEN:

(a) Thermocycler.-RotorGene Q®, QIAGEN #9001640, with notebook computer containing Rotor-Gene Q® software version 2.3.1.49 or later.
(b) QIAsymphony® SP.-QIAGEN #9001297, for automated DNA extraction.
(c) QIAsymphony® AS.-QIAGEN #9001301, for automated PCR assay setup.
(d) Rotor.-QIAGEN #9018903, 72 wells, for holding 0.1 mL strip tubes and caps.
(e) Locking ring for 72 well rotor.-QIAGEN #9018904, for locking strip tubes and caps in 72-well rotor.

(f) Rotor-Disc 72 Rotor.-QIAGEN #9018899.
(g) Locking ring for Rotor-Disc 72.-QIAGEN #9018900.
(h) Reagent cartridge holder (2).-QIAGEN #997008.
(i) Sample carrier insert, 2 mL v2.-QIAGEN #9242083, secondary tube adapter for 2 mL screw-cap tubes.
(j) Cooling adapter.-QIAsymphony EMT v2, QIAGEN #920730.
(k) Reagent holder cooling adapter.-QIAGEN #9018090.
(l) Adapter.-2 x Rotor-Disc, for use with QIAsymphony, QIAGEN #9242204.
(m) Rotor-Disc 72 loading block.-QIAGEN #9018910. Store refrigerated to ensure that PCR setup is performed under stable thermal conditions.
(n) Rotor-Disc heat sealer.-QIAGEN #9018898 (110 V) or #9019725 (230 V).

Consumables supplied by QIAGEN:

(a) Rotor-Disc 72 QIAGEN #981301 (24) or # 981303 (240)
(b) Rotor-Disc heat sealing film.-QIAGEN #981601 (60) or #981604 (600).
(c) Sample preparation cartridges.-8-well, QIAGEN #997002.
(d) 8-Rod covers.-QIAGEN #997004.
(e) Elution microtubes.-CL with cap strips, QIAGEN #19588.
(f) Filter tips.-1500 µL, QIAGEN #997024.
(g) Filter tips.-200 µL, QIAGEN #990332.
(h) Filter tips.-50 µL, QIAGEN #997120.

Materials provided by QLabs:

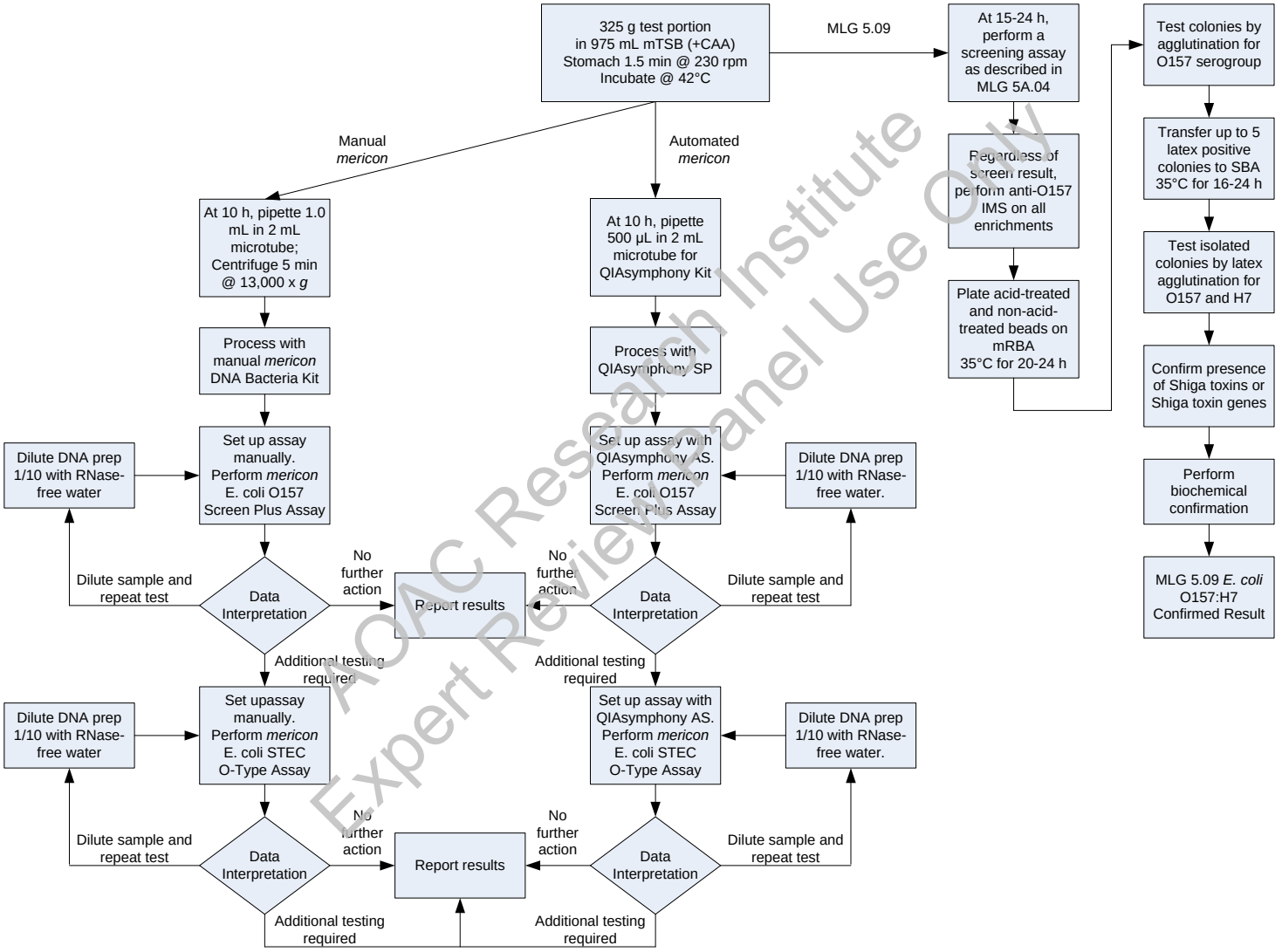
(a) Sample sets packaged in filter stomacher bags
(b) Dehydrated enrichment media (mTSB + CAA)
(c) Prepared IMS reagents (E-Buffer)
(d) Large Cell Separation Column
(e) 50 mL Conical Vials
(f) 0.45 µm Cell Strainers
(g) Rainbow Agar
(h) Cefiximine
(i) Potassium Tellurite Solution
(j) Novobiocin Solution
(k) DuPont Nutrition and Health BAX Systems STEC PCR Screening Assay
(l) Remel O157 Latex Agglutination Test Kit
(m) VITEK 2 GN or API 20 E Biochemical Identification Kits
(n) Prepared Sheep Blood Agar

Methods provided by QLabs

1. Draft OMA *mericon* workflow - *STEC in Selected Foods, PCR with Manual and Automated Sample Preparation*
2. FSIS MLG 5.09 –*Detection, Isolation, and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges (2015)*
3. FSIS MLG 5A.04–*FSIS Procedure for the Use of Escherichia coli O157:H7 Screening Tests for Meat Products and Carcass and Environmental Sponges (2014)*
4. BAM Chapter 3 – *Aerobic Plate Count (2001)*

Appendix 8.3

Collaborative Study Flowchart - QIAGEN *mericon*® STEC Workflow – Ground Beef



Appendix 8.4

Collaborator Comment Form

Collaborator:

Site:

Comments about the Method:

Comments about the Conduct of the Study:

August 2015

Product Insert for the AOAC-RI PTM-certified Automated and Manual mericon® E. coli O157 Screen Plus and mericon® E. coli STEC O-type Detection Workflow

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 101503

mericon® E. coli O157 Screen Plus

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 101504

mericon® E. coli STEC O-type

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents

DNA extraction — automated workflow

QIAsymphony® mericon Bacteria Kit	**(360)**
Catalog no.	**931156**
Number of reactions	**360**
Reagent Cartridge*	2
Piercing Lid	2
TopElute Fluid	60 ml
Reuse Seal Set†	2
Product Insert	1
Quick-Start Protocol	1

* Contains guanidine salts. Not compatible with disinfectants containing bleach. See page 8 for safety information.

† A Reuse Seal Set contains 8 Reuse Seal Strips.

DNA extraction — manual workflow

mericon® DNA Bacteria Kit	
Catalog no.	**69525**
Number of preps	**100**
Fast Lysis Buffer	2 x 25 ml
Product Insert	1
Quick-Start Protocol	1

Real-time PCR — automated and manual workflows

mericon® E. coli O157 Screen Plus Kit		**(24)**
Catalog no.		**290403**
Number of preps		**24**
Yellow	*mericon* Assay*	2 x 12 reactions
	RNase-free water	1.9 ml
Blue	Multiplex PCR Master Mix†	2 x 130 µl
Quick-Start Protocol		1

* Contains target-specific primers and probes, as well as the internal control (IC).

† Contains HotStarTaq® Plus DNA Polymerase, dedicated multiplex real-time PCR buffer, and dNTP mix (dATP, dCTP, dGTP, dTTP).

mericon® E. coli STEC O-Type Kit		**(24)**
Catalog no.		**290233**
Number of reactions		**24**
Yellow	*mericon* Assay*	2 x 12 reactions re
	RNase-free water	1.9 ml
Blue	Multiplex PCR Master Mix†	2 x 130 µl
Quick-Start Protocol	1	

* Contains target-specific primers and probes, as well as the internal control (IC).

† Contains HotStarTaq® *Plus* DNA Polymerase, dedicated multiplex real-time PCR buffer, and dNTP mix (dATP, dCTP, dGTP, dTTP).

Storage and Shelf Life

The QIAsymphony *mericon* Bacteria Kit should be stored at room temperature (15–25°C). Do not store the reagent cartridges at temperatures below 15°C. When stored properly, the kit is stable until the expiration date stated on the kit box. Partially used reagent cartridges can be stored for a maximum of 1 month. If a reagent cartridge is partially used, reseal all troughs with the provided Reuse Seal Strips. To avoid reagent evaporation, the reagent cartridge should be open for a maximum of 48 hours (including run times) at ambient temperature. Fast Lysis Buffer should be stored dry at room temperature (15–25°C). Under these conditions, the kit remains stable for 2 years.

The *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type assays are shipped on dry ice. The Multiplex PCR Master Mix should be stored immediately at –15°C to –30°C upon receipt. All remaining kit components not reconstituted should be stored at 2–8°C and protected from light. Stored under these conditions and handled correctly, assay performance remains unaffected until the date of expiration printed on the quality control label inside the kit box or envelope. Reconstituted reagents of *mericon* Pathogen Detection Assays should be dispensed into aliquots to avoid more than 5 freeze–thaw cycles, and stored at 2–8˚C for short-term storage (1 month) or at –20˚C for long-term storage.

Intended Use

Products for the automated and manual *mericon* Pathogen Detection workflows are intended for molecular biology applications in food, animal feed, water, and pharmaceutical product testing. These products are not intended for the diagnosis, prevention, or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN® kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to regional guidelines that have been developed for working with pathogens and recombinant DNA.

Intended User

The automated and manual *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type detection workflows are designed to be used by qualified users in microbiology laboratories for the determination of the presence or

absence of *E. coli* serotype O157 and the *E.* coli associated virulence genes *eae*, *stx1* and *stx2* *(mericon* E. coli O157 Screen Plus*)* and the detection of *E. coli serotype* O157:H7 and the *E.* coli non-O157:H7 serotypes O26, O45, O103, O111, O121 and O145 (*mericon* E. coli STEC O-type kit) in selected food products.

Applicability

The *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type detection workflows have been evaluated in an independent laboratory for use with the following food matrices: Ground beef, raw beef trim and spinach. The protocol includes preparation of an enrichment culture, followed by a manual or automated purification of *E. coli* DNA, and real-time PCR assay for presence or absence of pathogen using the *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type detection assays on the Rotor-Gene Q.

Environmental Factors

To allow for optimal real-time PCR detection quality using the Rotor-Gene Q, the instrument should be installed in a temperature-controlled, draft-free laboratory. Temperature should not be below 68°F (20°C) and should not fluctuate during the performance of the PCR assay. If the ambient temperature is below 68°F (20°C), it is recommended to preheat the Rotor-Gene Q at 95°C for 20 minutes before the run.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in a convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Work with samples potentially contaminated with *E. coli* should be performed in laboratories meeting Biosafety Level 2 (BSL2) regulations.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

The buffers in the reagent cartridge contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. Reagents in this kit are highly flammable and are harmful by inhalation, skin contact and swallowing.

For safety information regarding the instruments, see the relevant instrument user manual.

Discard sample and assay waste according to your local safety regulations.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Technical Assistance

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover) or on the web: http://b2b.qiagen.com/about-us/contact/global-contacts/subsidiaries/ or

http://b2b.qiagen.com/about-us/contact/global-contacts/distributors-and-importers/

Technical Service in North America can be reached at 1-800-362-7737 and at QIAGEN GmbH at +49-2103-29-12400.

Please also refer to the handbooks for the kits and user manuals for the instruments for comprehensive Troubleshooting Guides. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Principle of the assays

QIAGEN's *mericon. coli O157 Screen Plus* and *mericon* E. coli STEC O-type Pathogen Detection assays are multiplex PCR assays that amplify specific DNA fragments from pathogenic *E. coli* in food and an internal control. The internal control provides data regarding the presence of inhibitors in the tested samples and the overall quality of the PCR run.

Each *mericon* Assay is an optimized mixture of PCR primer sets for target sequences specific for pathogenic *E. coli* and an internal control (IC), plus probes labeled with four distinct fluorescent dyes (see Table 1 for the *mericon* E. coli O157 Screen Plus Assay) or three fluorescent dyes (see Table 2 for the *mericon* E. Coli STEC O-type Assay). In addition, each kit includes internal control DNA and all reagents necessary to perform the analysis.

These assays were developed for use on the Rotor-Gene Q. The *mericon* E. coli O157 Screen Plus Kit and *mericon* E. coli STEC O-type are not compatible with other Real Time cyclers. The Multiplex PCR Master Mix included in each kit contains QIAGEN proprietary technology, including HotStarTaq plus DNA Polymerase, patented multiplex PCR technology such as Factor MP, and fast cycling technology including Q-bond. [1]

Table1. Targets and channels of the mericon E. coli O157 Screen Plus Assay

	Green channel: FAM™ (495/520 nm)	**Orange channel: ROX™ NHS Ester (588/608 nm)**	**Crimson channel: Cy®5.5 (685/707 nm)**	**Yellow channel: MAX™ NHS Ester (524/557 nm)**
mericon E. coli O157 Screen Plus Assay	*stx1/stx2*	O157	*eae*	Internal control (IC)

Table 2. Targets and channels of the mericon E. coli STEC O-Type Assay

	Green Channel FAM™ (495/520 nm)	**Orange Channel ROX™ NHS Ester (588/608 nm)**	**Yellow Channel MAX™ NHS Ester (524/557 nm)**
mericon E. coli STEC O-Type Assay	O26, O45, O103, O111, O121, O145	O157:H7	Internal Control (IC)

Data Analysis

The Rotor-Gene Q cycler produces raw data files that are further interpreted by the software using mathematical algorithms to characterize samples. The software guides the user through each step and provides simplicity for beginners as well as an experimental platform for advanced users. [2]

General Precautions for Real-Time PCR Assays

The *mericon* E. coli O157 Screen Plus and *mericon E. coli* STEC O-type pathogen detection assays involve DNA detection by PCR. Care must be taken to avoid contamination of the PCR reactions.

It is extremely important to include at least one negative control that lacks the template nucleic acid in every PCR setup to detect possible contamination.

General physical and chemical precautions

- Store and extract positive materials (specimens, positive controls, and amplicons) separately from all other reagents, and add them to the reaction mix in a spatially separated facility.
- Use a separate set of pipets for the PCR Master Mix and the DNA samples. Use of pipet tips with hydrophobic filters is strongly recommended.
- Use gloves and protective laboratory wear. Do not touch any PCR equipment and supplies (e.g., rotors, loading blocks, tubes, pipets) without wearing gloves.
- In case of contamination, laboratory benches, apparatus, and pipets can be decontaminated by cleaning them with a 1/10 dilution of a commercial bleach solution. Afterwards, the benches and pipets should be rinsed with distilled water.
- All materials and media possibly potentially containing pathogens should be autoclaved for 20 min at 120°C prior to disposal.

Equipment and Reagents to Be Supplied by User

Automated Workflow

For the preparation of E. coli O157 Screen Plus and E. coli STEC O-type food enrichment cultures

- Modified Tryptic Soy Broth (mTSB) with casaminoacids (see MLG Appendix 1.08, p 21, revision 08).
- Lab paddle blender (e.g., Stomacher 3500,, Seward)*
- Filter homogenizer bags (e.g., VWR, cat. no. 129-9874)

- Balance*
- Incubators
- Vortex
- Microcentrifuge
- Precision Pipettors – For sampling and delivering of 10 – 1000 µL
- Dry bath incubator – Capable of maintaining 100 ± 1°C
- Calibrated Thermometer – Capable of measuring 100 ± 1°C

For sample preparation

- QIAsymphony SP instrument (cat. no. 9001297)*
- QIAsymphony *mericon* Bacteria Kit (cat. no. 931156)

* Ensure that all instruments have been checked and calibrated according to manufacturer's recommendations

Accessories and adapters for the QIAsymphony SP

- Reagent Cartridge Holder (2) (cat. no. 997008)
- Insert, 2.0 ml v2, sample carrier. (24), Qsym (cat. no. 9242083)
- Cooling Adapter, EMT, v2, Qsym (cat. no. 9020730)

Consumables for the QIAsymphony SP

- Sample Prep Cartridges, 8-well (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Microtubes 2 ml, PP, CB with screw-caps(cat no. 990382)
- Filter-Tips, 1500 µl (cat. no. 997024)
- Elution Microtubes CL with cap strips (cat. no. 19588)
- Tip disposal bags (cat. no. 9013395)

For assay set-up

- QIAsymphony AS instrument (cat. no. 9001301)*
- *mericon*E. coli O157 Screen Plus (290403) and *mericon* E. coli STEC O-type Kit (Cat no 290233 or 290235)

Accessories and adapters for the QIAsymphony AS

- Cooling Adapter, Reagent Holder 1, Qsym (cat. no. 9018090)

For use with the Rotor-Gene Q 72 Rotor-Disc® (cat. no. 9018899)

- Adapter 2 x Rotor-Disc, Qsym (cat. no. 9242204)
- Rotor-Disc 72 Loading Block (cat. no. 9018910)
- Rotor-Disc 72 (cat. no. 981303 [240]/981301 [24])
- Rotor-Disc Heat Sealing Film (cat. no. 981604 [600]/981601 [60])
- Rotor-Disc Heat Sealer (cat. no. 9018898 [110 V]; cat. no. 9019725 [230 V])
- Rotor-Disc 72 Locking Ring (cat. no. 9018900)

Consumables for the QIAsymphony AS

- Filter-Tips, 200 µl (cat. no. 990332)
- Filter-Tips, 50 µl (cat. no. 997120)
- Micro tubes 2 ml, PP, CB with screw-caps (cat.no. 990382)
- Tip disposal bags (cat. no. 9013395)

* Ensure that all instruments have been checked and calibrated according to the manufacturer's recommendations

Manual Workflow

For the preparation of E. coli O157 Screen Plus and E. coli STEC O-type food enrichment cultures (see Table 4 for validated matrix portions and culture volumes)

- Ground Beef, Raw Beef Trim, Spinach (modified Tryptic Soy Broth (mTSB) with Casaminoacids - see MLG Appendix 1.08, p 21, revision 08.
- Lab paddle blender (e.g., Stomacher 3500, Seward)*
- Filter homogenizer bags (e.g., VWR, cat. no. 129-9874)
- Balance*
- Incubators
- Vortex

* Ensure that all instruments have been checked and calibrated according to the manufacturer's recommendations.

- Microcentrifuge
- Precision Pipettors – For sampling and delivering of 10 – 1000 µL
- Dry bath incubator – Capable of maintaining 100 ± 1°C
- Calibrated Thermometer – Capable of measuring 100 ± 1°C

For sample preparation

- *mericon* DNA Bacteria Kit (cat. no. 69525)
- Vortexer
- SafeSeal Micro tubes 2 ml (Sarstedt, cat. no. 72.695) or microcentrifuge tubes with screw caps (2 ml)
- Microcentrifuge with rotor for 1.5 ml or 2 ml tubes
- Thermomixer* or heating block* suitable for 1.5 or 2 ml tubes and capable of attaining a temperature of 100°C. Alternatively, a water bath may be used.
- Pipets and pipet tips

For assay set-up

- Pipets and filter pipet tips

For use with the Rotor-Gene Q 72-Well Rotor (cat. no. 9018903)

- Loading Block, RG Strip Tubes 72, Qsym (cat. no. 9018092)
- Strip Tubes and Caps, 0.1 ml (cat. no. 981103)
- Locking Ring 72-Well Rotor (cat. no. 9018904)

Real-Time PCR

- Rotor-Gene Q 5-plex instrument (e.g., cat. no. 9001640)*
- Rotor-Gene Q software version 2.3 with assay package 3.0.4

Specifications of the AOAC-RI PTM-Certified mericon E. coli O157 Screen Plus and mericon E. coli STEC O-type Detection Workflows

The automated and manual *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type workflows are being submitted for AOAC-RI PTM Certification. The specifications for these workflows can be found in Tables 3, 4, and 5. For PCR assay setup, elution volumes of the automated workflow, and eluate dilutions of the manual workflow, are given in Table 6

Table 3. Overview of the specifications

AOAC-RI PTM-certified specification	Details
Target	*E. coli* strains containing virulence factors *stx1/stx2, eae, and/or O157* (*E. coli O157 Screen Plus* assay) *and E coli* with STEC O-type O157:H7 or the non-O157 STEC O-types O26, O45, O103, O111, O121, or O145 (*E. coli* STEC O-type assay).
DNA extraction Kit	**Automated workflow**: QIAsymphony *mericon* Bacteria Kit **Manual workflow**: *mericon* DNA Bacteria Kit
Real-time PCR assay	*mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type Kit
Enrichment broth	modified Tryptic Soy Broth (mTSB) with casaminoacids (prewarmed)
Enrichment temperature	42 ±1°C
Enrichment time	10 ± 1 hours
Homogenizer bag	Filter bag
Sample matrices	Ground Beef Raw Beef Trim Spinach

Table 4. Validated matrix portions and culture volumes

Matrix	Sample amount	Culture volume
Ground Beef	325 g	975 ml
Raw Beef Trim	325 g	975 ml
Spinach	25 g	225 ml

Table 5. Limit of detection

Workflow segment	Limit of detection*
Overall automated and manual workflow	10^3 cfu/ml
mericon E. coli O157 Screen Plus and *mericon* E. coli STEC O-type kit	10 copies/reaction

*Limit of Detection is derived from method development studies

Table 6. Sample volumes for mericon assay setup

Matrix	Manual workflow eluate dilution	Automated workflow elution volume
Ground Beef	1:10	400 µl
Raw Beef Trim	1:10	400 µl
Spinach	1:10	400 µl

Confirmation of Positive Results:

All positive *mericon* E. coli O157 Screen Plus and *mericon* E. coli STEC O-type results are presumptive positives and require confirmation by a reference method starting with the secondary selective enrichment. Appropriate standard methods for the determination of *E. coli O157 Screen Plus* and *mericon* E. coli STEC O-type include USDA/FSIS MLG 5.

Protocol: Preparation of mericon E. coli O157 Screen Plus and mericon E. coli STEC O-type Enrichment Cultures – Food Matrices

Procedure

1. Add 25 g (325 g) of the potentially contaminated food sample to a filter homogenizer bag and add 225 ml (975 ml) pre-warmed mTSB plus casaminoacids. (see Table 4).
2. Homogenize the food sample using a lab paddle blender at 230 rpm for 1.5 min ±10 sec (spinach) or 2.0 min ±10 sec for raw ground beef or raw beef trim. Then, seal the homogenizer bag and incubate the homogenate at 42 ± 1°C for 10 ± 1 hour.
3. Automated workflow: After incubation of the enrichment culture, dispense 500 µl aliquots into 2 ml microtubes and start the automated QIAsymphony DNA extraction protocol.

   Manual workflow: After incubation of the enrichment culture, dispense 1 ml aliquots into 2 ml SafeSeal or screw cap tubes and start the manual DNA extraction protocol.

Automated Workflow

Protocol: Automated isolation of bacterial DNA on the QIAsymphony SP

Procedure

1. Close all the drawers and hoods of the QIAsymphony SP/AS instrument.
2. Switch on the instrument and wait until the “Sample Preparation” screen appears and the initialization procedure has finished.
3. Log in to the instrument.

4. Ensure the "Waste" drawer is prepared properly, and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag, if necessary.
5. Load the required elution rack into the "Eluate" drawer and perform an inventory scan of the "Eluate" drawer.
6. Load the required reagent cartridge(s) and consumables into the "Reagents and Consumables" drawer.
7. Press the "R+C" button in the touchscreen to open the screen that shows the consumables status ("Consumables/8-RodCovers/Tubes/ Filter-Tips/Reagent Cartridges"). Press the "Scan Bottle" button to scan the bar code of the TopElute bottle with the handheld bar code scanner. Press the "OK" button.
8. Perform an inventory scan of the "Reagents and Consumables" drawer.
9. Place the samples into the appropriate tube carrier and load them into the "Sample" drawer.
10. Using the touchscreen, enter the required information for each batch of samples to be processed.
11. Choose elution volumes according to Table 6.

Table 6. Elution volumes for mericon assay setup

Matrix	Elution volume
Ground Beef	400 µl
Raw Beef Trim	400 µl
Spinach	400 µl

12. Press the "Run" button to start the purification procedure.
13. When sample processing is complete, perform a direct transfer of the elution rack to the QIAsymphony AS via the transfer module (integrated operation). Press "Transfer" to transfer the elution rack from slot 1 of the QIAsymphony SP to slot 2 of the QIAsymphony AS.
14. If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips immediately after the end of the last protocol run to avoid evaporation.
15. Discard used sample tubes, plates, and waste according to your local safety regulations and replace the tip disposal bag.

16. **Close the instrument drawers, and proceed with assay setup on the QIAsymphony AS (page 21).**
17. **Clean the QIAsymphony SP during the assay setup on the QIAsymphony AS, or later.**

Note: For daily maintenance, remove the waste bottle, tip park station, tip chute, tip guards, and magnetic-head guards and soak these in a glyoxal and quaternary ammonium salt-based disinfectant (e.g., gigasept® instru AF) for at least 15 minutes. Rinse with water and wipe dry with paper towels. Wipe the QIAsymphony SP worktable and touch screen with an ethanol-based disinfectant (e.g., mikrozid®) then wipe with a damp cloth and dry with a paper towel. For more information, please refer to the QIAsymphony Instrument User Manuals.

Protocol: Assay setup on the QIAsymphony AS

Things to do before starting

- **24 sample kit**: Add 130 µl Multiplex PCR Master Mix (tube[s] with blue lid) to each vial of *mericon* Assay (yellow lid). Transfer the reconstituted *mericon* Assay to a labeled, fresh 2 ml microtube. Mix by pipetting up and down 5 times or by quick vortexing, and centrifuge briefly

Procedure

1. **Insert the tip chute into its position on the right hand side in the front part of the QIAsymphony AS module.**
2. **Install an empty tip disposal bag in the bag holder under the "Assays" drawer.**
3. **Switch user interface from sample preparation to assay setup.**
4. **Start the assay definition process.**
5. **For integrated operation (elution rack is automatically transferred from the QIAsymphony SP into the AS module) the "Sample Rack(s)" screen will appear directly.**
6. **All stored sample information (sample status, sample ID, sample volume, and rack ID) is transferred to the QIAsymphony AS module together with the elution rack and will automatically complete the required information in the "Sample Rack(s)" screen of the assay setup user interface.**
7. **If the assay setup is independent from a former QIAsymphony SP run, select the rack file of the corresponding QIAsymphony SP run or select**

the rack type of your elution rack for the highlighted "Sample" position (slot 2) and then either manually type in the "Rack ID" of the elution rack or choose "Automatic ID" for a new ID.

8. **In the "Sample Rack Layout" screen of the assay setup user interface, the elution rack in slot 2 is pictured.**

   For integrated operation, or for independent operation in combination with a loaded rack file, sample IDs and sample volumes are automatically assigned to the corresponding positions.

   For independent operation without a rack file, select the positions to be processed from the elution rack. Define the highlighted positions as "Sample" then reselect the defined samples and assign sample volumes.

9. **In the "Assay Selection" screen, select the Assay Parameter Set(s) to use in the run.**
10. **In the "Assay Assignment" screen, assign the Assay Parameter Sets to samples.**
11. **In the "Assay Rack(s)" screen, define the assay rack ID. Either type in the assay rack ID manually or choose "Automatic ID" for a new ID.**
12. **The cooling of samples and reagents will start automatically. Check the temperature of the cooling positions.**
13. **The "Loading Information" screen displays the working table of the QIAsymphony AS module with all previously defined sample and reagent rack types in the designated positions. The required position of the PCR reaction adapter is displayed as well as information on the required filter-tip types and number.**
14. **Place the reconstituted mericon Assay(s), the reconstituted Positive Control(s) and the Negative Control(s), without lids, into the appropriate positions of the precooled reagent adapters.**
15. **Open the "Eluate and Reagents" and "Assays" drawers.**
16. **Load the prepared reagent adapter into slot 3 of the "Eluate and Reagents" drawer according to the illustration in the "Loading Information" screen. Place the Rotor-Disc in the appropriate adapter and load the adapter into the designated slot of the "Assays" drawer.**
17. **Load disposable filter-tips into the "Eluate and Reagents" and "Assays" drawers, according to the required number of each tip type.**
18. **Close the "Eluate and Reagents" and "Assays" drawers.**
19. **Upon closing each drawer, press "Yes" to start the inventory scan for each drawer.**

20. Press “Queue”. Monitoring of the cooling starts.
21. Press “Run” to start the run.
22. After the run is finished, press “Remove” in the assay setup “Overview” screen. Open the “Assays” drawer and unload the PCR assay adapter.
23. Download the result and cycler files via the QIAsymphony Management Console (QMC).
24. Proceed to “Protocol: PCR and data analysis on the Rotor-Gene Q”, page 25.
25. Perform the regular maintenance/cleaning of the QIAsymphony AS during the PCR run on the Rotor-Gene Q, or later.

    For more information about regular cleaning procedures, please refer to the QIAsymphony Instrument User Manuals.

Manual Workflow

Protocol: Manual isolation of DNA using the mericon DNA Bacteria Kit

Things to do before starting

- Prewarm a Thermomixer or heating block to 100°C for use in step 4.

Procedure

1. Pipet 1 ml enrichment culture into a 2 ml microcentrifuge SafeSeal or screw-cap tube (not supplied) and centrifuge at 13,000 x g for 5 minutes.
2. Discard the supernatant using a pipet, taking care to not disrupt the pellet.
3. Add 200 µl Fast Lysis Buffer to the bacterial pellet, tightly cap the tube, and resuspend the pellet by brief, vigorous vortexing.
4. Place the microcentrifuge tube into a heating block or thermal shaker (800 rpm) set to 100 ± 1°C. Heat the sample for 10 min ± 10 sec.
5. Remove the sample and allow it to cool to room temperature (15–24°C) for 2 min ± 10 sec..
6. Centrifuge the tube at 13,000 x g for 5 minutes.
7. Transfer 100 µl of the supernatant to a fresh 1.5 ml microcentrifuge tube. For the PCR reaction, use an aliquot of the collected supernatant diluted according to Table 5.

Table 7. Sample volumes for mericon manual assay setup

Matrix	DNA dilution
Ground Beef	1:10
Raw Beef Trim	1:10
Spinach	1:10

Protocol: Manual assay setup

Things to do before starting

- Please refer to "General Precautions for Real-Time PCR Assays", page 12.
- PCR loading block should be stored refrigerated to ensure that PCR setup is performed under stable thermal conditions.

  24 sample kit: Add 130 µl Multiplex PCR Master Mix (tube[s] with blue lid) to each vial of *mericon* Assay (yellow lid). Mix by pipetting up and down 5 times or by quick vortexing, and centrifuge briefly

Procedure

1. **Place the desired number of PCR 72-well strip tubes into the adapters of the cooling block for the Rotor-Gene Q.**
2. **Set up the sample and control reactions according to Table 8.**
3. **Add reconstituted assay to the tubes first, then add the Sample DNA or controls.**

Table 8. Setup of sample and control reactions

Component	Sample	Negative PCR control
Reconstituted *mericon* Assay	10 µl	10 µl
Sample DNA	10 µl	–
RNase-free water	–	10 µl
Total volume	20 µl	20 µl

Real-Time PCR

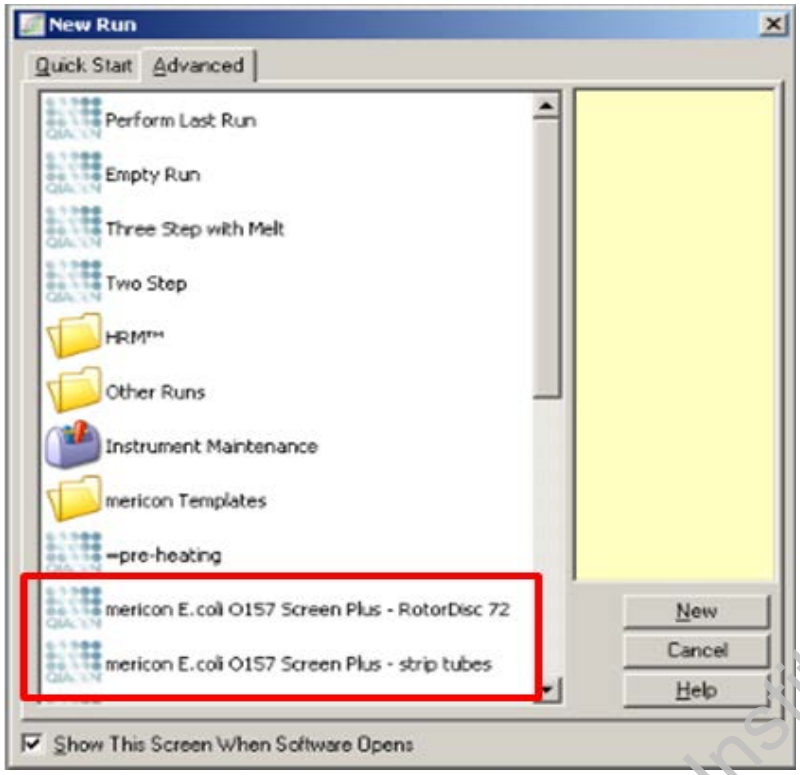
Protocol: PCR and data analysis on the Rotor-Gene Q using the open mode software

Procedure

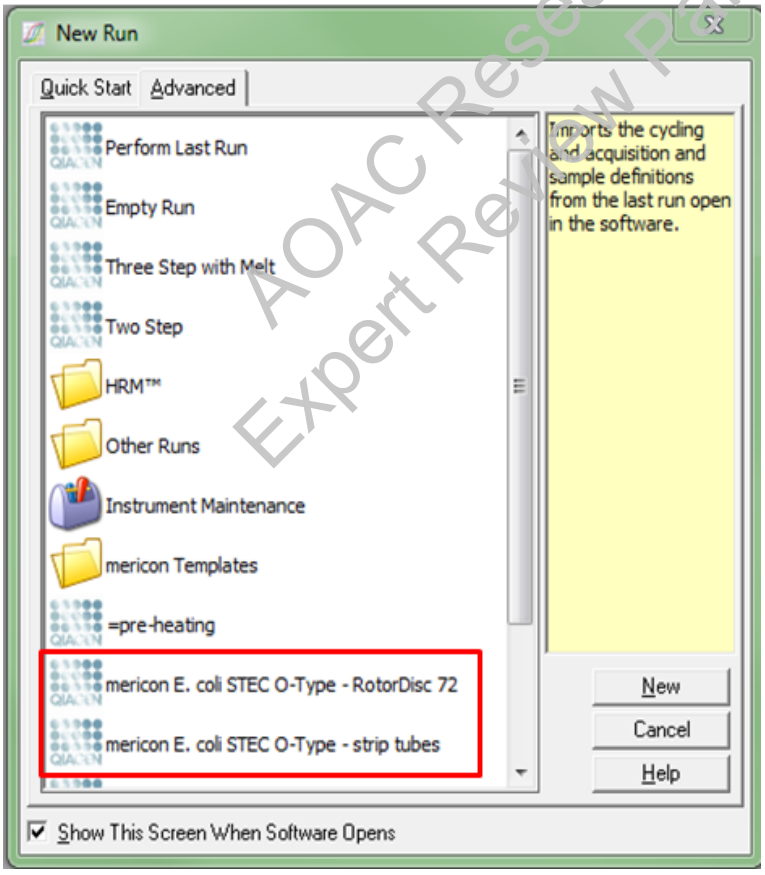
1. **Seal the Rotor-Disc after automated PCR setup, or close the strip tubes after manual PCR setup. Place Rotor-Disc or strip tubes in the respective rotor and make sure to apply the locking ring. If you are using tubes, fill the empty positions in the rotor with empty Strip Tubes. Place the rotor in the reaction chamber of the Rotor-Gene Q.**
2. **Open the Rotor-Gene Software. We recommend using the template file provided. In the Advanced Wizard, select "Open A Template In Another Folder…" and load the files "mericon E. coli O157 Screen Plus" and "mericon E. coli STEC O-Type".**

   If you copy the template files "*mericon* E. coli O157 Screen Plus" and "*mericon* E. coli STEC O-Type" in the Rotor-Gene Q Templates and in the Quick Start Templates folders, the template will appear directly in the Quick Start and Advanced Wizard windows.
3. **To setup cycling manually, select "Empty Run" and click "New".**

   We recommend using the provided template files to facilitate the reaction setup. When using template files, the settings may already be those described in the next step. In this case, click to the next screen

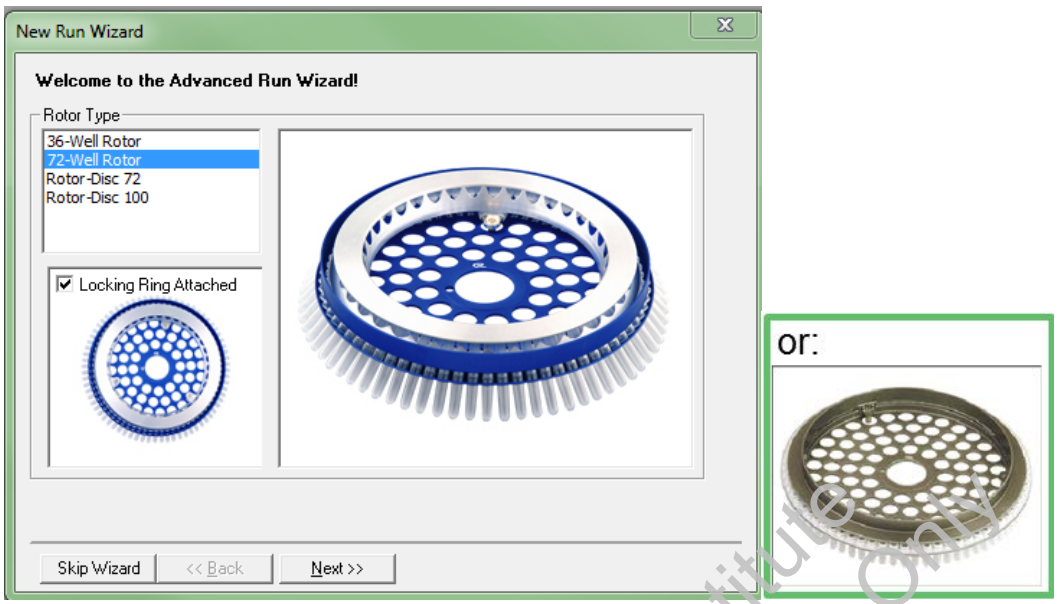


Select the *mericon* E. coli O157 Screen Plus assay.

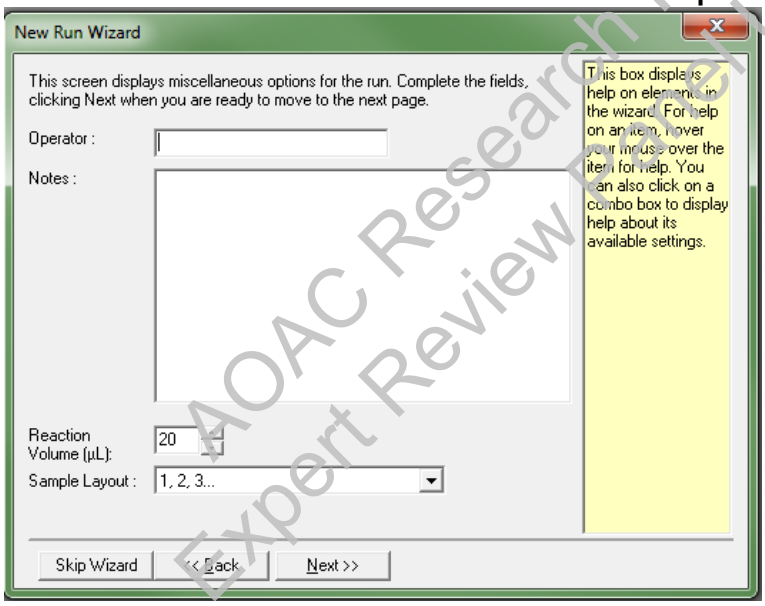


Select the *mericon* E. coli STEC O-type assay.

4. **Select the correct rotor and confirm the locking ring is attached by checking the check box. Click "Next" to continue.**



5. **Ensure that the reaction volume is set to 20 µl.**



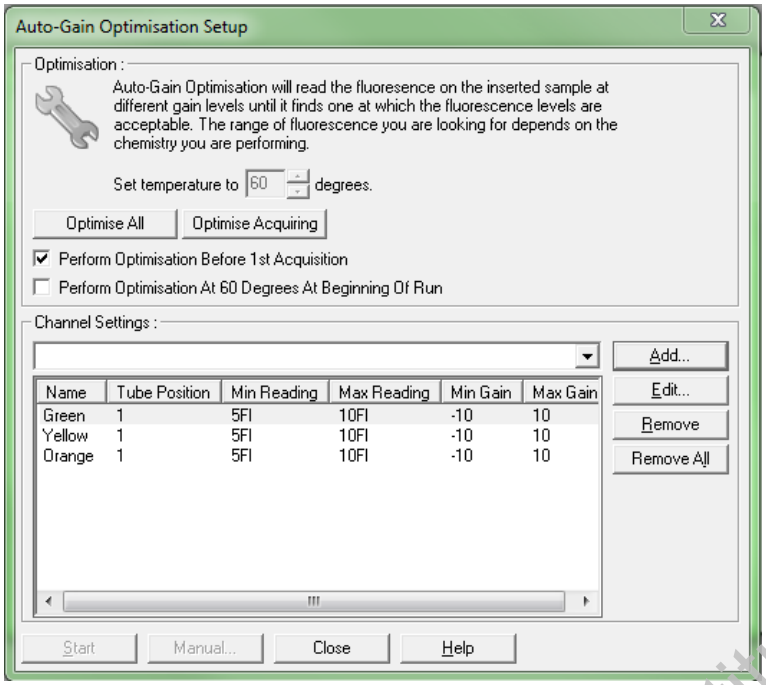
6. **Click "Next" to continue.**
7. **Click "Edit Profile" and program the Rotor-Gene Q according to Table 9**
   Data acquisition should be performed during the annealing step at 60°C.

Table 9. Cycling protocol for Rotor-Gene Q

Step	Time	Temperature	Comment
Initial PCR activation step	5 min	95°C	Activation of HotStarTaq *Plus* DNA Polymerase
3-step cycling			
Denaturation	15 s	95°C	Data collection at 60°C for green, crimson, orange and yellow channels
Annealing*	15 s	60°C*	
Extension	10 s	72°C	
Number of cycles	40		
*Gain optimization before first acquisition at 60°C for green, crimson, orange and yellow channels			

8. **Click "OK" to close the window and return to the Wizard.**
9. **To set the gain optimization settings for the Green, Yellow, and Orange Channels click "Gain Optimisation".**
10. **Select the 3 channels in the drop-down menu and click "Add".**
11. **In the dialog box that opens, confirm the standard settings. Click "Perform Optimisation Before 1st Acquisition". Then close the window.**

    Make sure that the tube at position 1 is not empty, since the gain optimization will be performed on this tube.



12. **Start the PCR run.**

Data Analysis on the Rotor-Gene Q

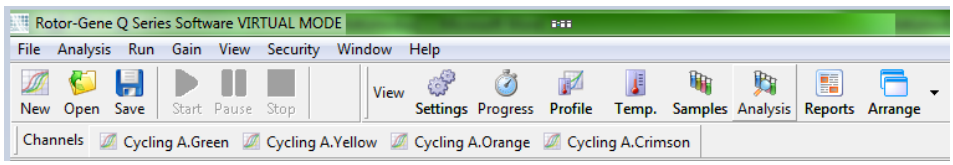
Note: Optimal analysis settings are a prerequisite for accurate real-time PCR data. Always use the following analysis settings.

Procedure

Open the run file using the Rotor-Gene Q Software. Go to "File", followed by "Open", and then "Browse" to locate the saved file.

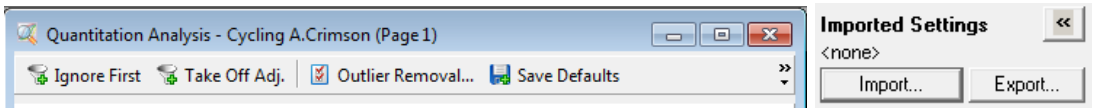
mericon E. Coli O157 Screen Plus:

1. **Click "Analysis" to edit analysis parameters.**

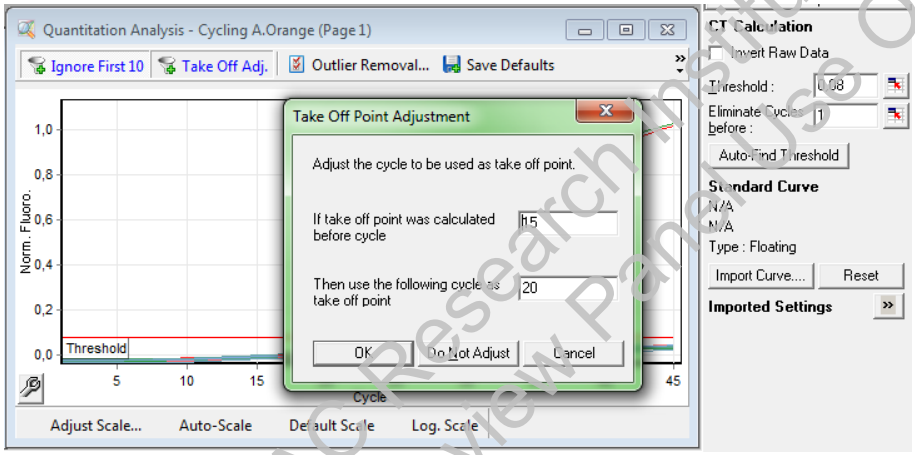


Open the Quantitation Analysis windows under "Analysis".

2. To import the analysis settings to each channel, activate the "Quantitation Analysis" window for the channel and select the respective template file from your directory with the "Import" function.



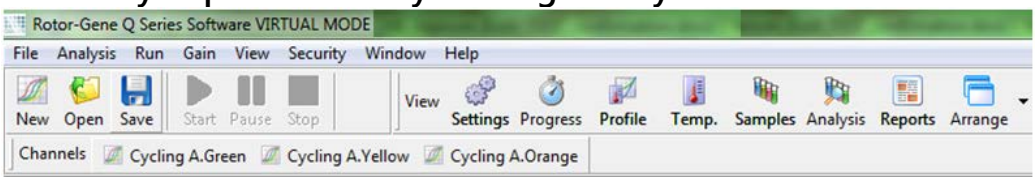
3. To set up the analysis parameters manually, continue with step 4.
4. Click "Ignore First" and ignore the first 10 cycles for all four channels.
5. For all channels (Green, Crimson, Yellow, and Orange), click "Take Off Point Adjustment".
6. Adjust the settings so that if the take off point was calculated before cycle 15, then cycle 20 is used as the take off point. Click "OK".



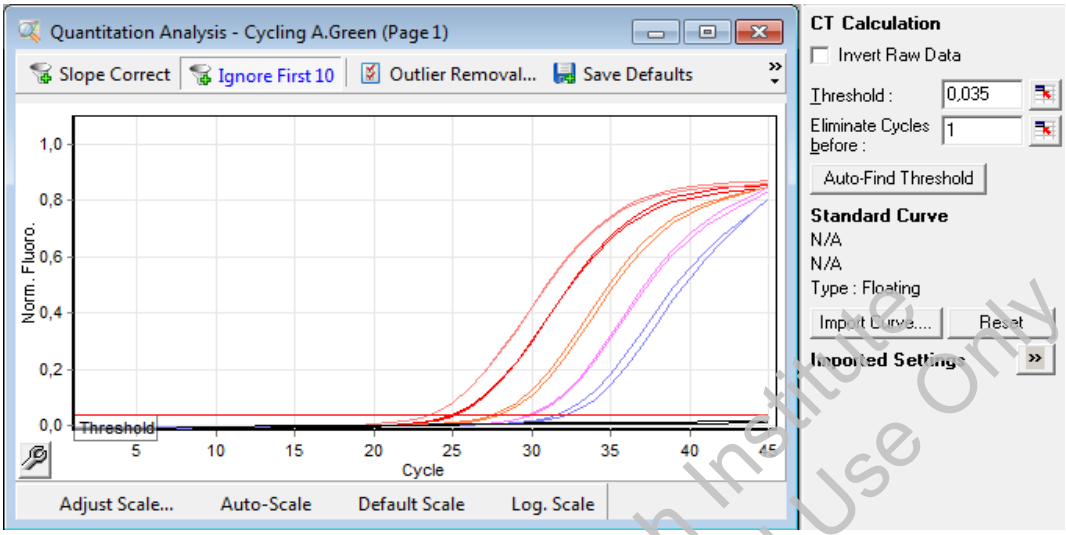
7. Set the threshold for channels Green, Crimson, and Yellow to 0.035.
8. Set the threshold for channel Orange to 0.08.
9. To export the results to Excel®, go to the "File" menu, followed by "Save As" and then "Excel Analysis Sheet". The results will be saved in *.csv format.

For mericon E. coli STEC O-type:

1. Edit analysis parameters by clicking "Analysis".



2. Click “Ignore First” to ignore the first 10 cycles when calculating the threshold for all 3 channels. Adjust the take-off point by clicking “Take Off Point Adjustment” and “OK”. Do not activate “Slope Correct”. Set the threshold for the Green and Yellow Channels to 0.035, and for the Orange Channel to 0.08



3. To export the results to Excel, go to the “File” menu, followed by “Save As” and “Excel Analysis Sheet”. To create a printable report go to “Reports” and create a “Quantitation (Full Report)” for each channel.

Analyzing the Results

Determining the presence or absence of pathogen DNA is carried out based on the amplification of the target sequence and is visualized in real time on the amplification plot generated by the application software of the real-time PCR instrument used. A positive result is visible as a final point on the fluorescence curve that lies clearly above the threshold. Figures 1 – 3 are examples of possible outcomes, which are summarized in Table 10, (page 32).

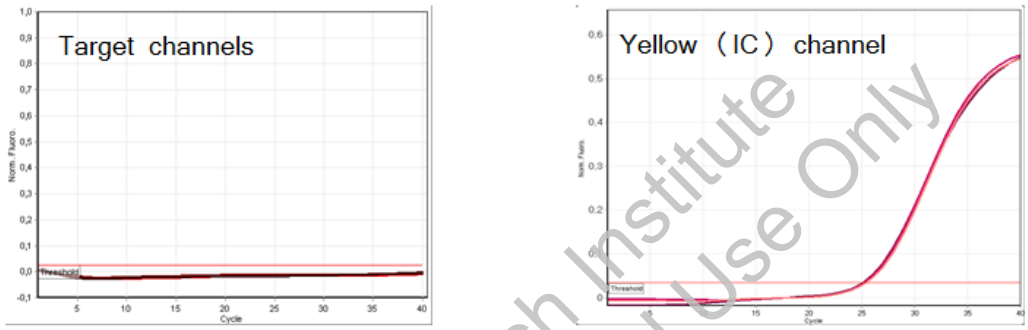


Figure 1. The sample is negative for tested pathogen. The 3 sample curves in the target channels (left) are at the baseline and below a preset threshold. The corresponding curves of the internal control in the yellow channel (right) are above the threshold and have a CT value of 24–30, indicating that the PCR was successful and not inhibited.

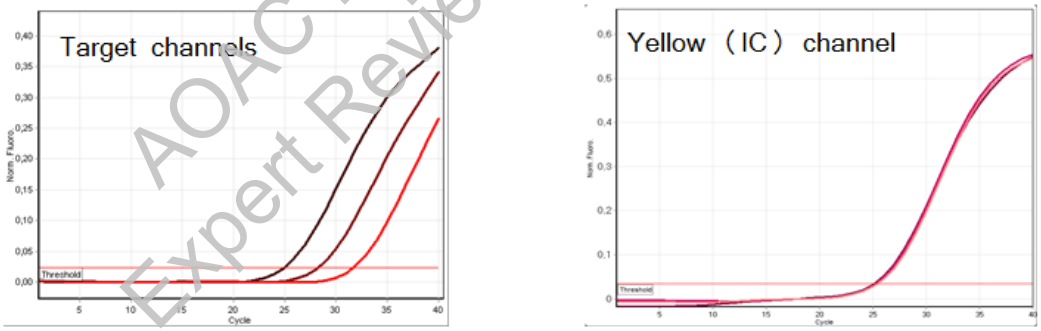


Figure 2. The sample is positive for tested pathogen. The 3 sample curves in the target channel (left) are above a preset threshold indicating the presence of pathogen DNA. The corresponding curves of the internal control in the yellow channel (right) are above the threshold and have a CT value of 24–30, indicating that the PCR was successful and not inhibited.

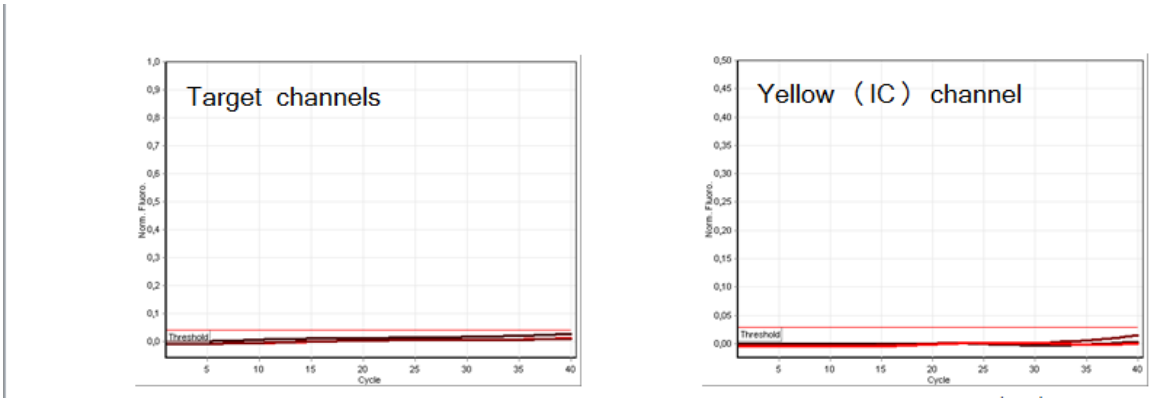


Figure 3. The PCR is inhibited. No amplification of the three samples in the green channel (left) or the internal control in the yellow channel (right). All curves lie along the baseline and do not exceed a preset threshold.

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Table 10. Summary of possible outcomes

Amplification of sample	Amplification of internal control	Result
C_T <38	C_T 24–30	Sample is positive
C_T 38.01–40	C_T 24–30	Sample is indeterminate; repeat test
No C_T	C_T 24–30	Sample is negative
No C_T	C_T ≥30.01 or No C_T	IC invalid, PCR inhibited; dilute sample and repeat test

Partial inhibition of the PCR due to the presence of detectable but tolerable concentrations of inhibitors in the samples is typically indicated by a shift of the internal control to higher C_T values. As a guideline, the uninhibited internal control should give a C_T value ranging between 24 and 30. A cycle threshold above 30 indicates inhibition.

In the event of a PCR inhibited internal control channel and a positive target result, repeating the test is not necessary.

In the event of inhibition PCR inhibited internal control channel and a negative target result, dilute the extracted samples 1:0 with RNase-free water and repeat the test.

If DNA template concentration is very high, a shift of the Internal Control to lower cycle values might occur, which does not influence its sensitivity toward PCR inhibitors or amplification of the target DNA.

Test scheme for presence of E. coli O157 and virulence factors eae and/or stx1/stx2 DNA

The *mericon* E. coli O157 Screen Plus assay screens for the *E. coli* O157, *eae*, and *stx1/stx2* genes. This serves to identify situations in which virulence factors are present and in which further studies to confirm the presence of the *E. coli* O157:H7 serotype, and the non-O157 serotypes O26, O45, O103, O111, O121, and O145 are required. Table11 indicates the further actions required with each possible combination of test results.

Table 11. Test results and next actions

No.	Orange O157	Green *stx1/stx2*	Crimson *eae*	Yellow IC	Next action
1	–	–	–	Valid	No further action
2	–	+	–	Valid	No further action
3	–	–	+	Valid	No further action
4	+	–	–	Valid	Additional testing required
5	–	+	+	Valid	Additional testing required
6	+	+	+	Valid	Additional testing required
7	–	–	–	Invalid	Dilute sample and repeat test

Several different test results are possible for the screening assay:

1. If the sample curves in the target channels (green, orange, and crimson) are at the baseline and below a preset threshold, the samples are negative for *E. coli* O157, *eae*, and *stx1*/*stx2*. No further action is required.
2. If the sample curve for the *stx1*/*stx2* targets is above a preset threshold and the sample curves for *eae* and O157 are at the baseline, the test result is negative for *E. coli* virulence factor DNA.
3. If the sample curve for the *eae* target is above a preset threshold and the sample curves for *stx1*/*stx2* and O157 are at the baseline, the test result is negative for *E. coli* virulence factor DNA.
4. If the sample curve in the orange channel (O157) is above a preset threshold, this indicates the presence of *E. coli* O157 DNA. As a consequence, additional testing is required.
5. If the sample curves in the green and crimson channels (*stx1*/*stx2* and *eae*) are above a preset threshold, this indicates the presence of the *E. coli* virulence factor DNA and the likelihood that the samples will be positive for the non-O157 serotypes, O26, O45, O103, O111, O121, and O145. As a consequence, additional testing is required.
6. If the sample curves in the green, orange, and crimson channels (*stx1*/*stx2* and *eae*) are above a preset threshold, this indicates the presence of the *E. coli* virulence factor DNA and the likelihood that the samples will be positive for O157:H7 or the non-O157 serotypes, O26, O45, O103, O111, O121, and O145. As a consequence, additional testing is required.
7. If IC is invalid, the sample might contain PCR inhibitors. In this case the test needs to be repeated with diluted sample.

Test Scheme for Presence or Absence of Suspected E. coli DNA

The *mericon* E. coli STEC O-type assay detects the presence of *E. coli* O157:H7 and non-O157:H7 serotype (O26, O45, O103, O111, O121, and O145) DNA. This serves to confirm a positive result from the *mericon* E. coli O157 Screen Plus Assay. A confirmed positive food sample cannot be cleared. It might be harmful for the customer and additional testing by authorized laboratories is required. Table 12 indicates the further actions required with each possible combination of test results.

Table 12. Test results and next actions

No.	O157:H7 Orange Channel	non-O157:H7 serotypes Green Channel	IC Yellow Channel	Next action
1	–	–	Valid	No further action
2	–	+	Valid	Additional testing required
3	+	–	Valid	Additional testing required
4	+	+	Valid	Additional testing required
5	–	–	Invalid	Dilute sample and repeat test

Several different test results are possible for the *mericon* E. coli STEC O-Type assay:

1. If the sample curves in the target channels (Green and Orange) are at the baseline and below a preset threshold, the samples are negative for *E. coli* O157:H7 and the non-O157:H7 serotypes (O26, O45, O103, O111, O121, and O145). No further action is required.
2. If the sample curve for the *E. coli* non-O157:H7 serotypes (O26, O45, O103, O111, O121, and O145) is above a preset threshold and the sample curve for O157:H7 is at the baseline, the test result is positive and additional testing is required.
3. If the sample curve for *E. coli* O157:H7 is above a preset threshold and the sample curve for *E. coli* non-O157:H7 serotypes (O26, O45, O103, O111, O121, and O145) is at the baseline, the test result is positive and additional testing is required.
4. If the sample curves for *E. coli* O157:H7 and *E. coli* non-O157:H7 serotypes (O26, O45, O103, O111, O121, and O145) are above a preset threshold, the test result is positive and additional testing is required.
5. If IC is invalid, the sample might contain PCR inhibitors. In this case the test needs to be repeated with diluted sample.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center http://b2b.qiagen.com/knowledge-and-support/troubleshooting-and-support/. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
No signal with positive control	
a) The selected fluorescence channel for PCR data analysis does not comply with the protocol	For data analysis, select the green, orange, and crimson channel for the samples and the yellow channel for the internal control. See the cycling protocols in Table 8, page 25.
b) Incorrect programming of the real-time PCR instrument	Compare the temperature profile with the protocol. See the cycling protocols in Table 8, page 25. Refer to the Rotor-Gene Q manual.
c) Incorrect configuration of the PCR	Ensure that reactions were set up according to Table 8, page 25. Repeat the PCR, if necessary.
d) The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page7)	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e) The *mericon* PCR Assay has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Comments and suggestions	
Weak or no signal in the internal amplification control	
a) The PCR conditions do not comply with the protocol	Check that PCR conditions match the cycling protocols in Table 8, page 25. Repeat PCR with corrected settings, if necessary.
b) The PCR was inhibited	Use the recommended DNA isolation method in this workflow. If there is inhibition, dilute DNA sample and repeat PCR
c) The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page 7)	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
d) The *mericon* PCR Assay has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Signals present for the negative controls	
a) Contamination occurred during PCR setup	Repeat the PCR with new reagents in replicates. If possible, close the PCR tubes directly after addition of the sample to be tested. Make sure to pipet the positive controls last. Make sure that the work space and instruments are decontaminated at regular intervals.
b) Contamination occurred during extraction	Repeat the extraction and PCR of the sample to be tested using new reagents. Make sure that the work space and instruments are decontaminated at regular intervals.

References

[1] QIAGEN GmbH: *mericon*® Automated Pathogen Detection Workflow Handbook, March 2012. http://www.qiagen.com/knowledge-and-support/resource-center/resource-download.aspx?id=c2df0606-cae7-4041-b618-541989a03de3&lang=en

[2] QIAGEN GmbH . *QIAsymphony*® SP/AS HandbookUser Manual May 2013. http://www.qiagen.com/knowledge-and-support/resource-center/resource-download.aspx?id=363d44fa-0560-4b0c-8aab-96c25b30cf39&lang=en

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AOAC OMA Method Safety Checklist

Submission Date	2016-12-06 12:24:03
First and Last Name	Marcia Armstrong
Organization	Qiagen
E-mail	LPhillips@aoac.org
METHOD TITLE	Evaluation of the mericon® E. coli O157 Screen Plus and merion E. coli STEC O-Type Pathogen Detection Assays in Select Foods
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?	YES
Are there any potential hazards in handling or storage of reagents, test samples, or standards?	NO
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

QIAGEN*mericon*® E. coliO157 Screen Plusand *mericon*® E.coli STEC O-Type Detection Workflows:

AOAC *Performance Tested Methods*SM101503 and 101504

Abstract

The QIAGEN*mericon*®E. coli O157 Screen Plus and *mericon*®E.coliSTEC O-TypePathogen Detection workflowsemploy Real-Time (RT) PCR technology incorporating a HotStarTaq DNA Polymerase for the rapid, accurate detection of *Escherichia coli*O157 and non-O157 Shiga toxin-producing*E. coli*STEC(O26, O45, O103, O111, O121, O145)from selected food products. In this study, both *mericon*workflows were evaluated according to current AOAC guidelines. The validation consisted of inclusivity, exclusivity, robustness, and a method comparison study of three food matrixes. The performance of the *mericon*pathogen detection workflows, using manual and automated DNA extraction procedures, was compared to Food and Drug Administration Bacteriological Analytical Manual (FDA/BAM) Chapter 4A: *Diarrheagenic Escherichia coli*for fresh spinach, the United States Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook (USDA/FSIS-MLG) 5.09: *Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges*for raw ground beef (70% lean), or the USDA/FSIS-MLG 5B.05:*Detection and Isolation of non-O157 Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products and Carcass and Environmental Sponges*for raw beef trim.

For the inclusivity and exclusivity evaluation, both *mericon*pathogen detection assays correctly identified all 50 target organism isolates and correctly excluded all 30 non-target organismsanalyzed.

In the method comparison study, both the *mericon*E.coliScreen PlusAssay and the *mericon*E. coliSTEC O-Type Assay with manual and automated DNA extraction demonstrated no significant differences between presumptive and confirmed results ($dPOD_{CP}$) or between candidate and reference method results ($dPOD_C$) for all 3 food matrixesafter 10 hours of enrichment.

Robustness testing demonstrated that minor variations in manual heat lysis time and sample master mix volume ratios for the *mericon*detection assays did not impact assay accuracy. The robustness testing did demonstrate that variations in incubation time did directly impact the assay. Based on the results obtained, it was determined that a minimum of 10 hours of incubation is needed.

The results of these studies demonstrate adequate sensitivity and selectivity of the *mericon*E.coliO157 Screen Plus and *mericon*E. coliSTEC O-Type Assays with manual and automated DNA extraction for the detection of *E. coli* O157:H7 and non-O157 STEC*(*O26, O45, O103, O111, O121, O145*)* in fresh spinach, raw ground beef, and raw beef trim.

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Scope of Method

(a) *Target organisms - E.coli O157 and E.coli non-O157 STECs(O26, O45,O103, O111, O121, O145).*The *mericon*E. coli O157 Screen Plus Assay screens for the presence of virulence factors *stx1/stx2, eae* andO157 while the *mericon*E.coliSTEC O-Type Assay detects the pathogenic STEC O-types (O26, O45, O103, O111, O121, O145) or O157:H7.

(b) *Matrixes*

1) Fresh spinach (25 g test portions)

2) Raw ground beef (70% lean) (325 g test portions)

3) Raw beef trim (325 g test portions)

(c) *Summary of validated performance claims*

The sensitivity of both*mericon*assays isequivalent to the FDA/BAM Chapter 4A [1], USDA/FSIS-MLG 5.09 [2], and USDA/FSIS-MLG 5B.05 [3]reference culture methods. The POD statistical analysis indicated no significant differences between the *mericon*E.coli O157Screen Plus and *mericon*E.coliSTEC O-TypePathogen Detection Assays, using either the manual *mericon*DNA extraction orthe automated QIAsymphonyDNA extraction procedures and the reference methods for all three foodmatrixes analyzed.Each*mericon*assay correctly identified all 50 target strains analyzed and correctly excluded all 30 non-target strains analyzed.

Definitions

(a) Probability of Detection

Probability of Detection (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. There are several POD measures that can be calculated, e.g., POD_{CP} (candidate method presumptive result POD), POD_{CC} (candidate method confirmation result POD), POD_C (confirmed candidate method POD), POD_R (reference method POD), and dPOD, the difference between any two POD values.

(b) General Definitions formericonE.coliO157Screen Plus and mericonE.coli STEC O-TypeAssays

- **Ct value:**The cycle threshold value is defined as the number of PCR cycles required for the fluorescent signal to become significantly different from the fluorescent background signal. It should be understood that Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e. the lower the Ct value the greater the amount of target nucleic acid in the sample).

- **Green Channel:**FAM™(detection at 495/520 nm wavelength), for the *mericon*E.coliO157Screen Plus Assay used for the detection of*stx1/stx2* and for the *mericon*E. coli STEC O-Type Assayused to detect the followingstrains of E. coli:: O26, O45, O103, O111, O121, O145.

- **Yellow Channel:**MAX™NHS Ester (detection at 524/557 nm wavelength), for both assays used for the detection of the Internal Control (IC).

- **Crimson Channel:**Cy®5.5 (detection at 685/707 nm wavelength),for the *mericon*Screen Plus Assay used for the detection of *eae.*

- **Orange Channel:**ROX™NHS Ester (detection at 588/608 nm wavelength), for the *mericonE. coli O157*Screen Plus Assay used for the detection of O157and for the *mericon*E. coliSTEC O-Type Assay used for the detection of O157:H7.

(c) Other Definitions

- **Inclusivity:**The strains or isolates of the target analyte(s) that the method can detect.

- **Exclusivity*:*** The non-target strains or isolates, which are potentially cross-reactive, that should not be detected by the method.

General Information

Most *E. coli* bacteria are harmless; however some produce a toxin (Shiga toxin) that can cause serious illness, including bloody diarrhea, hemolytic uremic syndrome, renal failure, and sometimes death. Like generic *E. coli*, toxin-producing Shiga-toxigenic *E. coli* (STEC) are Gram-negative, rod-shaped bacteria, but are characterized by the production of Shiga toxins (*stx1, stx2)*and *eae*. Depending on the reference cited, there are approximately 200 to 400 STEC serotypes, many of which have not been implicated in human illness; however, a subset of STEC called enterohemorrhagic *Escherichia coli* (EHEC) includes only those that cause serious illness. Serotype O157:H7 is the prototypic EHEC strain.

Although *E. coli*O157:H7 is currently the predominant strain and accounts for ~75% of the EHEC infections worldwide, other non-O157 EHEC serotypes are emerging as a cause of foodborne illnesses. In the United States the group known as the “Top 6” or “Big 6” (O111, O26, O121, O103, O145, and O45) accounts for the majority of non-O157:H7 serotypes isolated from clinical infections and, therefore, is currently a focus of concern.

EHEC outbreaks have been traced to many differenttypes of foods such asraw ground meats, unpasteurized (“raw”) milk, unpasteurized fruit juice, lettuce, spinach, sprouts, andmore recently, commercially manufactured frozen cookie dough. EHEC infections can lead to life-threatening complications with children and immuno-compromised individuals being at especially high risk. Thorough cooking of ground beef; washing raw fruits and vegetables under clean, running water; and not drinking unpasteurized (“raw”) milk or eating certain cheeses made from raw milk will decrease the chance of foodborne illness.[4]

Principle of the Method

QIAGEN’s*mericon*E.coliO157 Screen Plus and *mericon*E.coliSTEC O-TypePathogen Detection Assays are multiplex PCR assays that amplify both a specific DNA target and an internal control with high specificity. The internal control provides data regarding the presence of inhibitors in the tested samples and the overall quality of the PCR run. Each*mericon*PCR Assay includes a PCR primer set for a pathogen-specific target sequence, probes labeled with four distinct fluorescent dyes (*mericon*E. coliO157 Screen Plus) or three distinct fluorescent dyes (*mericon* E. coliSTEC O-Type), positive control DNA, and all of the reagents necessary to perform the analysis. The Multiplex PCR Master Mix included in each kit contains QIAGEN proprietary technology, including HotStarTaqplus DNApolymerase, patented multiplex PCR technology such as Factor MP, and fast cycling technology including Q-bond. [5]

The Rotor-Gene Q cycler produces raw data files that are further interpreted by the software using mathematical algorithms to characterize samples. The software guides the user through each step and provides simplicity for beginners as well as an experimental platform for advanced users. [6]

Materials and Methods

Test Kit Information

*(a) mericon*E. coliO157 Screen Plus Kit (24), CAT# 290403

*(b) mericon*E. coliSTEC O-TypeKit (24), CAT# 290233

*(c) mericon*DNA Bacteria Kit (100), CAT#69525

(d) QIAsymphony®*mericon*Bacteria Kit, CAT# 931156

Test Kit Components

*(a) mericon*E. coli O157 Screen Plus Kit

1) *mericon*Assay
2) RNase-free water
3) Multiplex PCR Master Mix

*(b) mericon*E. coliSTEC O-Type Kit

1) *mericon*Assay
2) RNase-free water
3) Multiplex PCR Master Mix

*(c) mericon*DNA Bacteria Kit

1) Fast Lysis Buffer
2) Product Insert
3) Quick-Start Protocol

(d) QIAsymphony *mericon*Bacteria Kit

1) Reagent Cartridge
2) Piercing Lid

3) Top Elute Fluid

4) Reuse Seal Set

5) Product Insert

6) Quick-Start Protocol

Ordering Information

QIAGEN, Inc.
27220 Turnberry Lane
Suite 200
Valencia, CA 91355
Tel: 800-426-8157
FAX: 800-718-2056
Website: www.qiagen.com/shop
Email: customercare-us@qiagen.com

Additional Supplies and Reagents

(a) Filter laboratory blender bags

(b) Modified tryptic soy broth, with the addition of casamino acids(USDA/MLG guidelines)

(c) Serological pipette tips – Aerosol resistant

(d) Micropipette tips - Aerosol resistant

(e) PCR grade water - Nuclease-free

(f) Microtubes with caps (2.0ml)

Apparatus

(a) Incubators- For maintaining bacterial enrichment media at specified temperatures.

(b) Top loading balance – capable of weighing and measuring 1 – 2000 grams.

(c) Laboratory paddle blender - Seward 3500 or equivalent: for sample homogenization

(d) Vortex – For sample mixing.

(e) Microcentrifuge – For 1.5 – 2.0 mL microcentrifuge tubes.

(f) Precision Pipettors – For sampling and delivering of 10 – 1000 µL

(g) Dry bath incubator – Capable of maintaining 100 ± 1°C

(h) Calibrated Thermometer – Capable of measuring 100 ± 1°C

(i) Rotor-Gene Q® - For automated detection of pathogens with a notebook computer containing Rotor-Gene Q Software.

(j) QIAsymphony® – For automated sample preparation, DNA extraction, and PCR preparation.

(k) 72-Well Rotor (cat. 9018903) - For holding 0.1 mL Strip Tubes and Caps.

(l) Locking Ring 72-Well Rotor (cat. 9018904) - For locking 0.1 mL Strip Tubes and Caps.

(m) Rotor-Disc 72 Rotor (cat. 9018899) - For holding Rotor-Disc 72 (cat. 981303 or 981301).

(n) Rotor-Disc 72 Locking Ring (cat.9018900) - For locking Rotor-Disc

(o) Reagent Cartridge Holder (2) (cat no 997008)

(p) Insert, 2.0 mL v2, samplecarrier. (24), Qsym (cat. 9242083)

(q) Cooling Adapter, EMT, v2, Qsym (cat. 920730)

(r) Sample Prep Cartridges, 8-well (cat. 997002)

(s) 8-Rod Covers (cat. 997004)

(t) Filter-Tips, 1500 µL (cat. 997024)

(u) Elution Microtubes CL with cap strips (cat. 19588)

(v) Cooling Adapter, Reagent Holder 1, (cat. 9018090)

(w) Adapter 2 x Rotor-Disc, Qsym (cat. 9242204)

(x) Rotor-Disc 72 Loading Block (cat. 9018910)

(y) Rotor-Disc Heat Sealing Film (cat. 981604 (600) / 981601 (60))

(z) Rotor-Disc Heat Sealer (cat. 9018898 (110V); *QIAGEN* cat. 9019725 (230V))

(aa) Filter-Tips, 200 µL (cat. 990332)

(bb) Filter-Tips, 50 µL (cat. 997120)

ReferenceMaterials

Organisms used in the inclusivity & exclusivity, robustness and method comparison studies were obtained from the following sources:

(a) American Type Culture Collection (ATCC; Manassas, VA)

(b) National Culture Type Collection (NCTC; Salisbury, UK)

(c) Michigan State University Culture Collection (The STEC Center, c/o Dr,Shannon Manning, MSU; East Lansing, MI)

(d) The Pennsylvania State University, E, coli Reference Center, Dr. Chitrita “Chobi” DebRoy, University Park, PA

(e) Q Laboratories Inc. Culture Collection (QL; Cincinnati, OH)

Organisms used in the lot-to-lot and product stability studies were obtained from:

(f) ifp, Institut für Produktqualität GmbH, Dr.WolfgangWeber,Berlin, Germany,

Standard Solutions

(a) 1N Hydrochloric Acid (1N HCl)

Safety Precautions

*mericon*E.coli O157 Screen Plus *and mericon*E.coli STEC O-Type Kits*and mericon*DNA Bacteria Kit

All chemicals should be considered potentially hazardous. When working with chemicals, always wear a suitable lab coat and disposable gloves. Product usage should follow good laboratory practices.

QIAsymphonymericonBacteria Kit

The buffers in the reagent cartridge contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite solution. Reagents in this kit are highly flammable and are harmful by inhalation, skin contact and swallowing.

Real-Time PCR System

Improper use of the Rotor-Gene Q may cause personal injuries or damage to the instrument. The Rotor-Gene Q must only be operated by qualified personnel who have been appropriately trained. Servicing of the Rotor-Gene Q must only be performed by QIAGEN Field Service Specialists.

Enrichment

Shiga-toxin producing *E. coli*is a Biosafety Level 2 organism. Biological samples such as enrichments have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes. Wear appropriate protective equipment which includes but is not limited to protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities utilizing the appropriate safety equipment (for example, physical containment devices). Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials. All enrichment broths should be sterilized following any culture-based confirmatory steps.

General Preparation

(a) Use of aseptic techniques.

(b) Use filter bags during enrichment to minimize particulates.

(c) Clean the work stations with a disinfectant of choice (e.g., sodium hypochlorite solution, phenol solution,Quaternary ammoniumsolution, etc.)before and after use.

(d) Separate work areas for the following: media preparation, sample preparation, DNA lysis, and DNA amplification and detection.

(e) Do not reuse kit disposables.

(f) Change pipette tips between samples.

(g) Wear personal protective equipment.

DNA Lysis

(a) Pre-warm heat block to 100 ± 1 °C.

(b) Change pipette tips between sample transfers.

(c) Do not disrupt the pellet when discarding the supernatant.

(d) Be sure to allow the samples to cool to room temperature (24 ± 2 °C) after the heat lysis procedure.

DNA Amplification

(a) PCR loading block should be stored at 2-5 °C.

(b) Use aseptic techniques.

(c) Change pipette tips between samples.

(d) Change pipette tips for the PCR Master Mix and the DNA samples.

(e) Use gloves and protective laboratory wear.

(f) Do not touch any PCR equipment or supplies without wearing gloves.

(g) In case of contamination, laboratory benches, apparatus, and pipettes can be decontaminated with a 1/10 dilution of commercial bleach solution.

General Preparation

Sample Preparation and DNA Amplification

Performing Enrichment

(a) Fresh Spinach (25 g test portions)
A 25 g test portion is added to 225 mL of pre-warmed modified tryptic soy broth containing casamino acids (mTSB+CAA), homogenized by stomaching for 1.5 min±10 sec, and incubated at 42 ± 1°C for 10 ± 1 hour.

(b) Raw Ground Beef (70% lean) (325 g test portions)
A 325 g test portion is added to 975 mL of pre-warmed mTSB+CAA, homogenized by stomaching for, and incubated at 42 ± 1°C for 10 ± 1 hour.

(c) Raw Beef Trim (325 g test portions)
A 325 g test portion is added to 975 mL ofpre-warmed mTSB+CAA, homogenized by stomaching for 2.0 min ±10 sec, and incubated at 42 ± 1°C for 10 ± 1 hour.

Preparing Samples

(a) Manual mericonDNA Extraction Method

1) Gently mix all enriched samples to disperse *E. coli* organisms throughout the sample. Transfer 1 mL of enriched sample to a sterile 2 mL screw cap tube and centrifuge for 5 minutes at 13,000 x*g*. Discard the supernatant with a pipettor, taking care not to disrupt the pellet. Add 200µLof Fast Lysis Buffer to the bacterial pellet, tightly cap tube and vortex to resuspend the pellet. Place the microcentrifuge tube into a heat block (100 ± 1°C) for 10 min ±10 sec. Remove the sample and allow it to cool to 15-24 °C for 2 min ± 10 sec. Centrifuge the tube at 13,000 x*g* for 5 minutes.

2) For all matrixes, dilute the extracted DNA 1:10 using sterile nuclease-free water.

3) Prepare the reconstituted assay by adding 1040 µL(96 sample kit) or 130 µl (24 sample kit) of the Multiplex PCR master mix to a vial of *mericon*Assay and mix by pipetting up and down 2-3 times. Prepare a positive control by re-suspending the lyophilized Positive Control DNA in 200µL of QuantiTectNucleic Acid Dilution Buffer and mix. Prepare the appropriate number of strip tubes by transferring 10µL of the reconstituted assay to the reaction tubes. Pipette 10µL of the extracted sample DNA into each sample strip tube and mix by pipetting up and down 2-3 times. Prepare the positive control tube by pipetting 10µL of the Positive Control DNA into a strip tube containing 10 µL of reconstituted assay. Prepare the negative control by pipetting 10 µL of RNase-free water into a strip tube containing 10 µL of thereconstituted assay. Seal the strip tubes with the strip tube caps. Load the samples into the Rotor-Gene-Q and initiate the analysis immediately.

(b) Automated QIAsymphonyDNA Extraction Method

1) Ensure the "Waste" drawer is prepared properly, and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag, if necessary.

2) Load an elution microtube rack (EMTR) into the "Eluate" drawer and perform an inventory scan of the "Eluate" drawer.

3) Load the *QIAsymphonymericon*bacteria kit and consumables into the "Reagents and Consumables" drawer.Select the "R+C" button on the touchscreen to open the screen that shows the consumables status ("Consumables/8-RodCovers/Tubes/ Filter-Tips/Reagent Cartridges").Select the "Scan Bottle" button to scan the bar code of the TopElute bottle with the handheld bar code scanner. Select the "OK" button.

4) Perform an inventory scan of the "Reagents andConsumables" drawer.

5) Transfer 500 µL of the enriched sample into a 2 mL screw cap tube.Place the samples into the appropriate tube carrier and load them into the "Sample" drawer.

6) Using the touchscreen, enter the required information for each batch of samples to be processed.

7) Choose elution volumes according to Table 1.

Table 1: Matrix Manual Dilution and Automated Elution Volumes

Matrix	Manual *mericon*Method DNA Dilution	Automated *QIAsymphony*Method DNA Elution Volume
Fresh Spinach	1:10	400 µL

Raw Ground Beef (70% Lean)	1:10	400 µL
Raw Beef Trim	1:10	400 µL

8) Select the “Run” button to start the purification procedure.

9) When sample processing is complete, directly transfer the elution rack to the QIAsymphony AS via the transfer module (integrated operation). Select “Transfer” to transfer the elution rack from slot 1 of the QIAsymphony SP to slot 2 of the QIAsymphony AS.

10) For independent transfer, open the eluatedrawer; select the “complete” button on the main QIAsymphony SP page to remove the eluate tray.

11) If a reagent cartridge is only partially used, seal it with the provided Reuse Seal Strips immediately after the end of the last protocol run to avoid evaporation.

12) Discard used sample tubes, plates, and waste according to your local safety regulations and replace the tip disposal bag.

13) Close the instrument drawers, and proceed with assay setup on the QIAsymphony AS.

14) Automated QIAsymphonyAssay Setup.

15) Insert the tip chute into its position on the right hand side in the front part of the QIAsymphonyAS module.

16) Install an empty tip disposal bag in the bag holder under the “Assays” drawer.

17) Switch user interface from sample preparation to assay setup by selecting the switch button.

18) Start the assay definition process.

19) For integrated operation (elution rack is automatically transferred from the QIAsymphony SP into the AS module) the “Sample Rack(s)” screen will appear directly.

20) All stored sample information (sample status, sample ID, sample volume, and rack ID) is transferred to the QIAsymphony AS module together with the elution rack and will automatically complete the required information in the “Sample Rack(s)” screen of the assay setup user interface.

21) If the assay setup is independent from a former QIAsymphony SP run, select the rack file of the corresponding QIAsymphony SP run or select the rack type of

your elution rack for the highlighted “Sample” position (slot 2) and then either manually enter the “Rack ID” of the elution rack or choose “Automatic ID” for a new ID.

22) In the “Assay Selection” screen, select the Assay Parameter Set(s) to use in the run.

23) In the “Assay Assignment” screen, assign the Assay Parameter Sets to samples.

24) In the “Assay Rack(s)” screen, define the assay rack ID. Either enter the assay rack ID manually or choose “Automatic ID” for a new ID.

25) Cooling of the samples and reagents will start automatically. Check the temperature of the cooling positions.

26) The “Loading Information” screen displays the working table of the QIAsymphony AS module with all previously defined sample and reagent rack types in the designated positions. The required position of the PCR reaction adapter is displayed as well as information on the required filter-tip types and number.

27) Place the reconstituted *mericon*E. coli O157Screen Plus or the *mericon*E.coliSTEC O-Type Pathogen Detection Assays, the reconstituted Positive Control(s) and the Negative Control(s), without lids, into the appropriate positions of the pre-cooled reagent adapters.

28) Open the “Eluate and Reagents” and “Assays” drawers.

29) Load the prepared reagent adapter into slot 3 of the “Eluate and Reagents” drawer according to the illustration in the “Loading Information” screen. Place the Rotor-Disc in the appropriate adapter and load the adapter into the designated slot of the “Assays” drawer.

30) Load the disposable filter-tips into the “Eluate and Reagents” and “Assays” drawers, according to the required number of each tip type.

31) Close the “Eluate and Reagents” and “Assays” drawers.

32) Upon closing each drawer, select “Yes” to start the inventory scan for each drawer.

33) Select “Queue”. Monitoring of the cooling starts.

34) Select “Run” to start the run.

35) After the run is finished, select “Remove” in the assaysetup “Overview” screen. Open the “Assays” drawer and unload the Rotor-Disc 72.

36) Place the Rotor-Disc sealing film over the Rotor-Disc72 and transfer to the Rotor-Disc heat sealer. Sealing is complete in about 2 minutes. Remove the excess film from the Rotor-Disc 72 and transfer to the Rotor-Disc 72 Rotor.In the “Sample Rack Layout” screen of the assay setup user interface, the elution rack in slot 2 is pictured.

Analysis

(a) Prepare Equipment

1) Turn on the Rotor-Gene Q instrument and attached computer. Load the Rotor-Gene Q Series Software and templates for the *mericon*E. coli O157 Screen Plus and *mericon* E.coli STEC O-type assays. Start an empty run to warm up the Rotor-Gene Q.

    i. Run a Disc

    ii. Load Samples

2) Manual mericonDNA Extraction Method

    i. Load the QIAGEN strip tubes into the 72-Well Rotor strip tube ring. Snap the locking ring in place over the strip tubes, and place into the Rotor-Gene Q. When running strip tubes, balance the ring using the empty strip tubes.

3) Automated QIAsymphonyDNA Extraction Method

    i. Load the QIAGEN 72 Rotor-Disk into the 72-Rotor Disk ring. Snap the locking ring in place over the Rotor Disk, and place into the Rotor-Gene Q.

4) Start Instrument Run

    i. Software Version 2.3.1 (Build 49)

    Follow the Rotor-Gene software instructions. Verify that the correct setting is selected for each preparation method: the Rotor-Disc 72 rotor is selected for the QIAsymphony (automated preparation method), and the 72 count strip tubes for the manual preparation method. Ensure that the locking ring is in place and select “Next”. Verify that the reaction volume is 20 µL and select “Next”. Select “Next” after verifying the temperature profile. Select “Start Run” to start the Rotor-Gene Q instrument.

5) When the run is complete, click “Save” and remove the samples from the instrument.

Interpretation and Test Result Report

(a) Viewing results

1) Software Version 2.3.1 (Build 49)

i. When the run is complete, select “Analysis” located on the top of the main page of the software. In the “Analysis” box, import the analysis settings into each channel by activating the “Quantitation Analysis” window for the channel and select the respective file from your directory with the “Import” function. Select “Ignore First” and ignore the first 10 cycles for all four channels. For all channels (Green, Crimson, Yellow, and Orange) select “Take Off Point Adjustment”. Adjust the settings so that if the take off point was calculated before cycle 15, then cycle 20 is used as the take of point, then select “OK”. Set the threshold for channels Green, Crimson, and Yellow to 0.035 and set the threshold for channel Orange to 0.08.

(b) Printing and Exporting Results

To export the results to Excel, go to the “File” menu, followed by “Save As” and then “Excel Analysis Sheet”. The results will be saved in a .csv format. The results may be saved to the notebook computer or an external flash drive. The results may be printed by opening the “Report Browser” window, choosing a report, and select “Show.” Choose “Print” on the report window.

(c) Interpreting the results

Determining the presence or absence of pathogen DNA is carried out based on the amplification of the target sequence and is visualized in real time on the amplification plot generated by the application software of the real-time PCR instrument used. A positive result is visible as a final point on the fluorescence curve that lies clearly above the threshold.

Partial inhibition of the PCR due to the presence of detectable but tolerable concentrations of inhibitors in the samples is typically indicated by a shift of the internal control to higher C_T values. As a guideline, the uninhibited internal control should give a C_T value ranging between 24 and 30. A cycle threshold above 30 indicates inhibition.

In the event of a PCR inhibited internal control channel and a positive target result, repeating the test is not necessary.

Confirmation

All samples were confirmed according to the appropriate reference methods specified for the matrix and analyte:

(a) USDA/FSIS-MLG 5.09: Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges

(b) USDA/FSIS-MLG 5B.05: Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat Products and Carcass and Environmental Sponges

(c) FDA/BAM Chapter 4A: Diarrheagenic *Escherichia coli*

Independent Validation Study

In accordance with an AOAC-RI Performance Tested Method program approved study design, the method developer study was conducted by an Independent laboratory, Q Laboratories, Inc., in Cincinnati, Ohio.

The study was conducted according to the procedures outlined in the AOAC Research Institute Performance Tested Methods[SM] protocol: *Independent Laboratory Study for the mericon*E. coli*O157 Screen Plus and mericon*E. coli *STEC O-Type Pathogen Detection Assay*(February 2015, Version 1) [7]. The *mericon*E. coli*O157 Screen Plus* and*mericon*E. coli *STEC O-Type Pathogen Detection Assay*were compared to the following reference methods: FDA/BAM Chapter 4A for fresh spinach, USDA/FSIS-MLG 5.09 for raw ground beef (70% lean), and to the USDA/FSIS-MLG 5B.05 for fresh raw beef trim. The study outline consisted of inclusivity, exclusivity, method comparison, and robustness evaluations. The inclusivity, exclusivity and robustness evaluations were conducted using the manual *mericon*DNA extraction procedure. The method comparison study used both the manual and automated *QIAsymphony*DNA extraction procedures. All data were analyzed using the Rotor-Gene Q series software.

Inclusivity and Exclusivity

For the inclusivity evaluation of the *mericon*E.coli O157Screen Plus and *mericon*E.coliSTEC O-Type Pathogen Detection Assay, 50 pathogenic *E.coli* strains (including the serovars: O157, O26, O45, O103, O111, and O145)were cultured in mTSB + CAA for 8-10 hours at 42 ± 1°C and then diluted to 100x the Limit of Detection (LOD =1 x 10^3).

For the exclusivity portion of the validation, 30 species/strains (including non-pathogenic *E.coli* and non-regulated STEC serovars) closely related to STECs were grown in Brain Heart Infusion (BHI) broth for 22 ± 2 hours at 37 ± 2ºC.

Inclusivity and exclusivity cultures were randomized, blind-coded and then analyzed with the appropriate *mericon*assay using the manual *mericon*DNA extraction procedure.

Matrix Study

Foodmatrixes were purchased from local grocery retailers and stored according to the manufacturers' guidelines. Prior to inoculation, the matrixes were screened for natural contamination of the target analytes using the specified reference method in the protocol. Aerobic plate counts were performed following FDA/BAM Chapter 3 [8] to determine the microbial load of each food matrix. Table2 below outlines the specific inoculation schemesfor each food evaluated in the study.

For the method comparison, a total of 30 samples for each of the 3 inoculated food matrixeswasas analyzed. Each sample set included 5 uninoculated control samples, 20 low-level samples, and 5 high-level samples. The target levels for each strain of *E. coli* used for challenging the food matrixes were as follows: 0 colony forming units (CFU)/test portion for the uninoculated control samples, 0.2-2 CFU/test portion for low-level inoculation and 2-5 CFU/test portion for the high-level inoculation. Fractionally positive results, those in which ether the reference or candidate method yields 5 to 15 positive results out of the 20 low-level inoculum replicates, were required for each matrix.

Table 2: Test Matrixes and Organisms for Inoculation

Matrix	Organism	Target Inoculum Levels	# of Replicates	Reference Method	Reference Method Test Portion Sizes	*mericon*Sample Prep Method
Fresh Spinach	*E. coli* O111 TW06315[1]	0 CFU/Test Portion	5	FDA/BAM Chapter 4A	200 g	Manual & QIAsymphony (Automated)
		0.2-2 CFU/Test Portion	20			
		2-5 CFU/Test Portion	5			
Raw Ground Beef (70% Lean)	*E. coli* O157:H7 ATCC 43895[2]	0 CFU/Test Portion	5	USDA/FSIS-MLG 5.09	325 g	
		0.2-2 CFU/Test Portion	20			
		2-5 CFU/Test Portion	5			
Raw Beef Trim	*E. coli* O26 ATCC BAA-1653[2]	0 CFU/Test Portion	5	USDA/FSIS-MLG 5B.05	325 g	
		0.2-2 CFU/Test Portion	20			
		2-5 CFU/Test Portion	5			

[1]Michigan State University Culture Collection
[2]American Type Culture Collection

Each matrix was inoculated with a different strain of *E. coli* as indicated in Table 2. Each inoculum was prepared by transferring a pure isolated colony of the specified organism from Trypticase Soy agar containing 5% sheep's blood (SBA) into BHIbroth followed by incubation at 35± 2°C for 24 ± 2 h. Post-incubation, the BHI broth culture was diluted to the target level using BHI broth as the diluent. Non-stressed cultures were used for all three matrixes.

For each matrix, bulk portions were inoculated and blended in a large stainless steel container using sterile spatulas to distributethe inoculum evenly.. All food matrixes were held for 48-72 hours post-inoculation at 2-8°C to allow for equilibration of the organism as

per AOAC guidelines. For the raw meat matrixes, 25 g of inoculated meat was mixed with 300 g of uninoculated meat to prepare the 325 g test portions.
The level of *E. coli* O157:H7 or non-O157H7 STECin the low level inoculum for raw ground beef and raw beef trim was determined by Most Probable Number (MPN) on the day of analysis by evaluating 5 x 650 g, 20 x 325 g (reference method test portions), and 5 x 130 g inoculated test samples. The high inoculation level for raw ground beef and raw beef trim was determined by evaluating 5 x 325 g (reference method test portions), 5 x 130 g, and 5 x 65 g. The level of *E. coli* O111 in the low level inoculum for fresh spinach was determined by MPN by evaluating 5 x 400 g, 20 x 200 g (reference method test portions),and 5 x 80 g inoculated test samples. The high level inoculum for fresh spinach test portions was determined by MPN evaluating 5 x 200 g (reference method test portions), 5 x 80 g, and 5 x 40 g inoculated test samples. Table 3 presents the MPN test portions for the low and the high inoculation levels. Each test portion was enriched using 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. [9] (http://www.lcfltd.com/customer/LCFMPNCalculator.exe)

Table 3:MPN Test Portion Sizes

Matrix	Inoculation Level	MPN Test Portions		
Raw Ground Beef and Raw Beef Trim	Low	5 x 650 g	20 x 325 g*	5 x130 g
	High	5 x 325 g*	5 x 130 g	5 x 65 g
Fresh Spinach	Low	5 x 400 g	20 x 200 g*	5 x 100 g
	High	5 x 200 g*	5 x 80 g	5 x 40 g

* - Test portions from matrix study or reference method

FDA/BAM Chapter 4A: Diarrheagenic Escherichia coli

For fresh spinach, 200 g test portions were enriched in 450 mL of 1 x modified Buffered Peptone water with pyruvate (mBPWp). All test portions were incubated statically for 5 hours at 37 ± 1 °C followed by the addition of Acriflavin, Cefsulodin, and Vancomycin supplements per the FDA/BAM method. Samples were re-incubated for an additional 18-24 hours at 42 ± 1 °C.

Following the primary enrichment of fresh spinach, an optional immunomagnetic separation (IMS) procedure was conducted due to the high background flora. A 1 mL aliquot was transferred into a 1.5 mL microcentrifuge tube containing 20 µL of immunomagnetic separation beads (E. coli O26 IMS Beads, Abraxis,Inc.).The microcentrifuge tubes were rotated on the Labquakerotator for 10 minutes, removed and placed onto a magnetic stand. The tubes were inverted three times and allowed to sit on the magnetic stand for 3 minutes. The contents of the microcentrifuge tube were decanted making sure that the pellet of beads was not disturbed. The microcentrifuge tubes were removed from the magnetic stand and1 mL of wash buffer was added to each tube ensuring the bead pellet

was fully reconstituted. The tubes were placed back onto the magnetic stand and inverted three times and allowed to sit for 3 minutes. This wash process was repeated three more times for a total of four washes. Following the fourth wash, the microcentrifuge tubes were removed from the magnetic stand and the bead pellet was reconstituted in 1.5 mL of wash buffer.

The leafy spinach IMS samples were serial diluted (1:10) in phosphate buffered water (PBW). A 100 µL aliquot of each serial dilution was plated in duplicate on Levine's eosin-methylene blue (L-EMB) agar and R&F non-O157 STEC Chromogenic plating mediain order to achieve isolated colonies. Both sets of plates were incubated for 18-24 hours at 37 ± 1°C. After incubation, plates containing typical colonies were screened for the O111 antigen by latex agglutination. Up to 10 isolated colonies that screened positive were streaked to SBA and tryptic soy agar containing 0.6% yeast extract (TSA/YE) to verify purity and incubated for 18-24 hours at 37 ± 1°C. Following incubation, a ColiComplete (CC) disc was placed on the densest growtharea on the TSA/YE plates and incubated for an additional 18-24 hours at 37 ± 1°C. The CC discs on the TSA/YE plates were observed for typical reactions (blue color change with no fluorescence under long wave UV) and a spot indole test was conducted. After incubation, SBA plates were observed for purity. Final biochemical confirmations were obtained by VITEK 2 GN Biochemical Identification following AOAC OMA 2011.17. [10]

USDA/FSIS-MLG 5.09: Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges

All raw ground beef test portions were prepared as outlined in the study protocol. Following equilibration of the inoculum in the matrix, 325 ± 32.5 g test portions were enriched in 975 ± 19.5 mL of pre-warmed mTSB+CAA, homogenized by stomaching for 2 minutes and incubated 15-24 hours at 42 ± 1°C. After incubation, the primary enrichment from each sample was screened using a USDA/FSIS-MLG 5.09 validated lateral flow device test system. Regardless of the screening result, all samples were subjected to isolation by immunomagnetic separation (IMS) by transferring 1.0 mL aliquots of the primary enrichment to a microcentrifuge tube containing a 50 µL suspension of *E. coli* O157 immunomagnetic (paramagnetic) beads(Dynal beads, ThermoFisher Scientific).. The microcentrifuge tube was then placed onto a Labquake™ agitator and rotated for 10 to 15 minutes at 18-30°C. After rotation the bead and sample solution was transferred to a MACSlarge cell separation (ferromagnetic) column and washed four times with E buffer (pre-warmed to 18-35°C) before the final elute was collected with 1 mL of E buffer in a sterile tube. Following the IMS procedure, 1:10 and 1:100 dilutions of each IMS suspension in E Buffer were spread-plated on modified Rainbowagar (mRBA).

A 450 µL aliquot of each remaining IMS suspension was transferred into a microcentrifuge tube and mixed with 25 µL of1 N HCl. The microcentrifuge tubes were vortexed and placed onto a Labquake agitator and rotated for 1 hour at 18 - 30°C. After rotating, 475 µL of E buffer was added to each sample tube. The acid-treated IMS suspension and a 1:10 dilution of this suspension in E Buffer were plated onto mRBA. All mRBA plates were incubated for 20 - 24 hours at 35 ± 2°C. After incubation, mRBA plates containing typical colonies were tested for O157 latex agglutination and up to 5 isolated colonies were streaked to SBA. The SBA plates were incubated for 16 - 24 hours at 35 ± 2°C. After incubation, SBA plates were observed for purity. Isolated colonies from SBA were confirmed positive by conducting a H7 latex agglutination test and testing for the presence

of Shiga-toxins using the USDA approved Real-Time PCR assay. Final biochemical confirmations were obtained by VITEK 2 GN Biochemical Identification following AOAC OMA 2011.17.

USDA/FSIS-MLG 5B.05: Detection and Isolation of non-O157 Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products and Carcass and Environmental Sponges

All raw beef trim test portions were prepared as outlined in the study protocol. Following equilibration of the inoculum in the matrix, 325 ± 32.5 g test portions were enriched in 975 ± 19.5 mL of pre-warmed mTSB + CAA, homogenized by stomaching for 2 minutes and incubated for 15-24 hours at 42 ± 1°C.

Following incubation, 30 µL of the primary enrichment from each sample was screened using a USDA/FSIS-MLG 5B.05 approved commercially available Real-Time PCR assay (DuPont Nutrition and Health, BAX System). Each sample was screened for the presence of STEC virulence factors Shiga-like toxin 1 and/or Shiga-like toxin 2 (*stx*1/*stx*2) and intimin (*eae*). If a sample produced positive results for *stx*1 or *stx*2 and *eae,*an additional screen for the targeted sixserogroups (O26, O45, O103, O111, O121 and O145) was conducted. Regardless of the screening result, all samples were subjected to isolation by immunomagnetic separation (IMS) in a ferromagnetic column with paramagnetic beads by transferring a 1.0 mL aliquot of the primary enrichment to microcentrifuge tubes containing a 50 µL suspension of *E. coli* O26 immunomagnetic beads (Abraxis, Inc.). The microcentrifuge tubes were placed on a Labquake agitator and rotated for 15 minutes. After rotation, the bead and sample solution was transferred to a MACS large cell separation column and was washed four times with 1 mL of E buffer before collecting 1 mL of the final elute in a sterile tube.

Following the IMS procedure, a 1:10 and 1:100 dilution of each IMS suspension in E Buffer was spread-plated on mRBA. A 450 µL aliquot of each remaining sample was transferred to a microcentrifuge tube and mixed with 25 µL of 1 N HCl. The microcentrifuge tubes were gently mixed and placed on a Labquake agitator and rotated for 1 hour. After rotating, 475 µL of E buffer was added to each sample tube. The acid-washed IMS suspension and a 1:10 dilution of the acid-washed IMS suspension in E Buffer were plated on mRBA. All mRBA plates were incubated for 20-24 hours at 35 ± 1°C. After incubation, any mRBA plates containing typical colonies were tested by serogroup specific latex agglutination. Up to 5 isolated colonies were streaked to SBA and incubated for 16-24 hours at 35 ± 1°C. After incubation, SBA plates were observed for purity. Isolated colonies from SBA were confirmed positive for the presence of *stx*1 or *stx*2 and *eae* by Real-Time PCR. The serogroup was confirmed by latex agglutination and by Real-Time PCR. Final biochemical confirmations were obtained by VITEK 2 GN Biochemical Identification following AOAC OMA 2011.17.

*mericon*E.coliO157Screen Plus and *mericon*E.coliSTEC O-Type Pathogen Detection Assay

For raw ground beef and raw beef trim, all test portionswere assessed concurrently with the reference methods. The paired325 g test portions were enriched in 975 mLof pre-warmed (42 ± 1°C) mTSB+CAA,homogenized by stomaching for 2 min± 10 sec, and incubated for 10± 1hr at 42 ± 1°C.

For fresh spinach, the 25 g test portions were enriched in 225 ml of pre-warmed mTSB+CAA, homogenized by stomaching for 1.5 min ± 10 sec, and incubated for 10±1hrat 42 ± 1°C.

Following 10 hours of incubation at 42 ± 1°C, the manual and automated DNA preparation methods were conducted as outlined in the “General Preparation”section.

Real-Time PCR

The 72 rotor disk or strip tubes containing samples were loaded into the Rotor-Gene Q instrument after which the prompts were followed using the Rotor-Gene Q series software to identify samples and controls. The *mericon*assayswere initiated and results were obtained within 90 minutes.

Interpretation of Results

Determining the presence or absence of pathogen DNA is carried out based on the amplification of the target sequence and is visualized in real time on the amplification plot generated by the application software of the real-time PCR instrument used. A positive result is visible as a final point on the fluorescence curve that lies clearly above the cycle threshold. Table 4 below shows a summary of possible outcomes analyzed by the software.

Table 4: Summary of Possible Outcomes

Amplification of Sample	Amplification of Internal Control	Result
$C_T$range <38	$C_T$range 24-30	Positive
$C_T$range 38.01 – 40	$C_T$range 24-30	Indeterminate; repeat test
No C_T	$C_T$range 24-30	Negative
No C_T	C_T ≥ 30.01 or No C_T	IC invalid, PCR inhibited; dilute sample and repeat test

Confirmation

All samples analyzed by the *mericon*assay, regardless of presumptive result, were confirmed by procedures outlined in the reference methods specified for each matrix. Final confirmation was achieved by VITEK 2 GN Biochemical Identification, AOAC OMA 2011.17.

Robustness Evaluation

Both the *mericon*E.coliO157 Screen Plus and *mericon*E.coliSTEC O-Type Pathogen Detection Assays were evaluated for robustness by examining the performance of the assay when small changes to the operating conditions were made. The following parameters were evaluated: (1) enrichment incubation time, (2) manual heat lysis time, and (3) sample and Master Mix volume ratio. Only DNA extracted with the manual extraction method was used in these studies.

All robustness parameters were evaluated using raw beef trim inoculated at a level expected to yield fractional positive results (2 to 8 positive samples out of 10 replicates). A bulk sample of beef trim was inoculated with *E.coli* O157, mixed thoroughly, and separated into 25g test portions. An additional 300 g of uninoculated trim was added to create the 325 g test portion. For each operational parameter, 10 inoculated replicates were analyzed along with 5 un-inoculated replicates. Lysed DNA samples were diluted 1:10 using nuclease free water.

The first parameter, incubation time, was evaluated by analyzing replicates after 8, 9 and 10 hours±5 minof incubation. The second parameter, manual heat lysis time, was evaluated by analyzing replicates heated to 100±1ºC in a dry bath for 8, 10 and 12 minutes (±15 sec). The third parameter, Master Mix to sample ratio, was evaluated by adding 8:12,10:10 or 12:8 µL of PCR Master Mix to DNA sample volume. Both *mericon*Pathogen Detection Assays were evaluated using nine different treatment combinations. The treatment parameters are presented in Table 6.

Table 5: Robustness Treatment Conditions

Treatment Combination	Enrichment Incubation Time (Hours)	Lysis Heat Time (Minutes)	Assay Composition (Assay Mix µL: DNA µL)
1	8	8	12 µL:8 µL
2	8	8	8 µL:12 µL
3	8	12	12 µL:8 µL
4	8	12	8 µL:12 µL
5	10	8	12 µL:8 µL
6	10	8	8 µL:12 µL
7	10	12	12 µL:8 µL
8	10	12	8 µL:12 µL
9	9	10	10 µL:10 µL

-All test portions were sampled from a single enrichment

Product Consistency (Lot to Lot) and Stability Evaluation

The Method Developer Study covering product consistency and stability was performed in the method developer's laboratory (QIAGEN R&D, Hilden Germany)

Product Consistency Study

This study examined three lots of each *mericon*kits for lot-to-lot variability and three Rotor-Gene Q instruments for variability. Included were the *E.coli* Screen Plus and *E. coli* STEC O-type PCR kits (3 lots of each primer/probe set and 3 lots of Master Mix), and the DNA

Bacteria Plus kit. First, each lot of primer/probe was combined with each lot of Master Mix and all combinations were tested with a single DNA Bacteria kit lot(see Table 6) on a single Rotor-Gene Q instrument.

Table 6. Evaluation of *mericon*E.coliO157 Screen Plus and *mericon*E.coliSTEC O-typeLot-to-lot Variability

Primer/Probes	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
Master Mix	Lot 1	Lot 1	Lot 1	Lot 2	Lot 2	Lot 2	Lot 3	Lot 3	Lot 3
DNA Bacteria	Lot 1	Lot 1	Lot 1	Lot 1	Lot 1	Lot 1	Lot 1	Lot 1	Lot 1

Second, each of 3 lots of DNA Bacteria kits was tested with a single lot of *mericon*E.coliO157Screen Plus or *mericon*E.coliSTEC O-type PCR kits primer/probe and master mix (see Table 7). At the same time, three Rotor-Gene Q instruments were tested side-by-side with the same kit lots.

Table 7. Evaluation of DNA Bacteria Plus and QIAsymphony Lot-to-lot Variability and Instrument Variability

Primer/Probes	Lot 1	Lot 1	Lot 1	Lot 1	Lot 1
Master Mix	Lot 1	Lot 1	Lot 1	Lot 1	Lot 1
DNA Bacteria	Lot 1	Lot 1	Lot 1	Lot 2	Lot 3
Rotor-Gene Q	RGQ #1	RGQ #2	RGQ #3	RGQ #1	RGQ #1

Bacterial strains used for the *mericon* E. coliO157 Screen Plus lot to lot variation studies wereE. coli strains O157:H7 (RM-HYG374), O157(RM-HYG381), O26:H (RM-HYG375), E. coli O111:H- (RM-YG376)and *Citrobacter freundii* (ifp culture collection)

Bacterial strains used for the *mericon*E.coliSTEC O-type lot to lot variation studies were E. coli strainsO157:H7 (RM-HYG374), O157(RM-HYG381),E.coli O26:H- (RM-HYG375), E. coli O111:H- (RM-YG376),E.coli (O145 RM-HYG390)and *Citrobacter freundii*.

Stability testing: Shelf life

Product stability data was submitted with the QIAGEN *mericon*Salmonella study (July 2012). For the purposes of this study, shelf life stability studies are being submitted for the *E. coli* Screen Plusand *E. coli* STEC O-typeprimer probe sets, since these are the only components of the workflow protocols that vary between the *mericon*protocols. The *Salmonella* summary data can be found in Appendix 2 of the current report. Bacterial strains used were*Salmonellaentericas*ubsp*entericas*erovar Abony*(NCTC* 6017)*, Salmonella entericas*ubsp*enterica* serovar Enteriditis, (Sm24/Rif12/Ssq), *Citrobacter freundii, (ifp culture collection).*

*mericon*E.coliO157 Screen Plus and *mericon*E.coliSTEC O-typepathogen kit stability studies

In this study, a single primer probe assay kit lot wasstored at 4 ± 1°C for 4 and 15 months. A second set of kits from the same lot was stored at 37 ± 1°C for 3 and 4.5 months. The twosets of primer probes stored at 37 ± 1°C extend the real time aging to approximately 18 and 24 months [9]. The same lot of Master Mix was used for all time points from 0 to 15 months. The 15 month time point kits were assayed once with the original Master Mix and in parallel with a fresh Master Mix. The study used purified reference DNA diluted from 10,000 to 10 copies per reaction.

For the *mericon*E. Coli O157 Screen Plus stability studies, the reference DNA was a synthetic E. coli Big 6 gBlock DNA (IDT) containing eae, stx1/stx2 and O157 components. Aliquots of the gBlock DNA were stored at -20°C, thawed and serially diluted prior to each test point.

For the *mericon* E. coli STEC O-type stability studies, the reference DNAwas prepared from E. coli strains O157:H7 (RM-HYG374) and O26 (RM-HYG375) (ifp culture collection)..The DNA concentration was adjusted to 10,000 copies/µl and stored in aliquots at -20°C. For testing, an aliquot was thawed and serially diluted prior to each test point.

Results

Inclusivity and Exclusivity Evaluation

Of the 50 inclusivity organisms assessed for both the *mericon*E.coliO157Screen Plus and *mericon*E.coliSTEC O-Type Pathogen Detection Assays, all were correctly identified..Similarly, all 30 exclusivity organisms examined using the twoE.coliSTEC O-Type Pathogen Detection Assays were correctly excluded.

Detailed results for the inclusivity and exclusivity validations are presented in Tables 1 - 4 of the Appendix.

Matrix Study

Results obtained from samples analyzed by the *mericon*methods were statistically comparable to those analyzed by the FDA/BAM or USDA/FSIS-MLG reference methods. The POD analysis between *mericon* methods and the reference methods for all matrixes indicated that there was no significant difference at the 5% level between the number of positive results by the two methods.

As per criteria outlined in Appendix J of the Official Methods of Analysis Manual, fractional positive results were obtained [11]. A detailed summary of results for each matrix is displayed below in Table 5 and Table 6.

The pre-evaluation pathogen screening and aerobic plate count (APC) results are presented in Table 5 of the Appendix. A summary of the MPN results is presented in Tables 6A-6C of the Appendix. A detailed summary of results for each matrix is presented in Tables 7-12 of the Appendix.

The probability of detection (POD) was calculated as the number of positive outcomes divided by the total number of trials [12]. The POD was calculated for the candidate presumptive results, $POD_{CP,}$ the candidate confirmatory results, POD_{CC}, the difference in the candidate presumptive and confirmatory results, $dPOD_{CP,}$ presumptive candidate results that confirmed positive, $POD_{C,}$ the reference method, POD_R, and the difference in the confirmed candidate and reference methods, $dPOD_C$. POD analyses were conducted for the *mericon* test points and compared to the appropriate reference method results. The POD analysis between the*mericon*methods presumptive and confirmed results for all matricies indicated that there was no significant difference at the 5% level. A summary of the POD analyses [13] is presented in Tables 13-20 of the Appendix. As per criteria outlined in Appendix J of the Official Methods of Analysis Manual, fractional positive results were obtained.[13].

Table 5: Summary of Results for *mericon*E. coliO157**Screen Plus**

Matrix	Inoculation Level	*mericon*E.coliO157 Screen Plus Pathogen Detection Assay		*mericon*E.coliO157 Screen Plus Pathogen Detection Assay		Reference Method Confirmed
		Manual	Automated	Manual	Automated	
		Presumptive	Presumptive	Confirmed	Confirmed	
Fresh Spinach	Un-inoculated	0/5	0/5	0/5	0/5	0/5
	Low	7/20	7/20	7/20	7/20	9/20
	High	5/5	5/5	5/5	5/5	5/5
Raw Ground Beef (70% Lean)	Un-inoculated	0/5	0/5	0/5	0/5	0/5
	Low	9/20	9/20	9/20	9/20	9/20
	High	5/5	5/5	5/5	5/5	5/5
Raw Beef Trim	Un-inoculated	0/5	0/5	0/5	0/5	0/5
	Low	13/20	13/20	13/20	13/20	13/20
	High	5/5	5/5	5/5	5/5	5/5

Table 6: Summary of Results for *mericon*E. coliSTEC O-Type Pathogen Detection

Matrix	Inoculation Level	*mericon*E.coli STEC O-Type Pathogen Detection Assay		*mericon*E.coli STEC O-Type Pathogen Detection Assay		Reference Method Confirmed
		Manual	Automated	Manual	Automated	
		Presumptive	Presumptive	Confirmed	Confirmed	
Fresh Spinach	Un-inoculated	0/5	0/5	0/5	0/5	0/5
	Low	7/20	7/20	7/20	7/20	9/20
	High	5/5	5/5	5/5	5/5	5/5
Raw Ground Beef (70% Lean)	Un-inoculated	0/5	0/5	0/5	0/5	0/5
	Low	9/20	9/20	9/20	9/20	9/20
	High	5/5	5/5	5/5	5/5	5/5
Raw Beef Trim	Un-inoculated	0/5	0/5	0/5	0/5	0/5
	Low	13/20	13/20	13/20	13/20	13/20
	High	5/5	5/5	5/5	5/5	5/5

Manual mericonDNA Extraction Procedure - E. coli Screen Plus

Fresh Spinach

For the low inoculation level of the*mericon* E. coli O157 Screen PlusPathogen Detection Assay, there were 7 presumptive positives and 7 confirmed positives following the FDA/BAM Chapter 4A 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 FDA/BAM Chapter 4A reference method confirmation procedure. There were 5 confirmed positives following the reference method.

For the low inoculation level, a $dPOD_C$ value of -0.10 was obtained with a 95% confidence interval of (-0.37, 0.19), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 13 and 17 of the Appendix.

Raw Ground Beef (70% Lean)

For the low inoculation level of the*mericon*E. coli O157 Screen PlusPathogen Detection Assay, there were 9 presumptive positives and 9 confirmed positives following the USDA/FSIS-MLG 5.09 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 USDA/FSIS-MLG 5.09 reference method confirmation procedure. There were 5 confirmed positives following the reference method.

For the low inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate

presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 13 and 17 of the Appendix.

Raw Beef Trim

For the low inoculation level of the*mericon* E. coli O157 Screen PlusPathogen Detection Assay, there were 13 presumptive positives and 13 confirmed positives following the USDA/FSIS-MLG 5B.05 reference method confirmation procedure. There were 13 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 5B.05 reference method confirmation procedure. There were 5 confirmed positives following the reference method.

For the low inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 13 and 17 of the Appendix.

Manual mericonDNA Extraction Procedure - E. coli STEC O-Type

Fresh Spinach

For the low inoculation level of the *mericon*E. coliSTEC O-Type Pathogen Detection Assay, there were 7 presumptive positives and 7 confirmed positives following the FDA/BAM Chapter 4A 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 FDA/BAM Chapter 4A reference method confirmation procedure. There were 5 confirmed positives following the reference method.

For the low inoculation level, a $dPOD_C$ value of -0.10 was obtained with a 95% confidence interval of (-0.37, 0.19), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 15 and 19 of the Appendix.

Raw Ground Beef (70% Lean)

For the low inoculation level of the *mericon*E. coli STEC O-Type Pathogen Detection Assay, there were 9 presumptive positives and 9 confirmed positives following the USDA/FSIS-MLG 5.09 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 USDA/FSIS-MLG 5.09 reference method confirmation procedure. There were 5 confirmed positives following the reference method.

For the low inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 15 and 19 of the Appendix.

Raw Beef Trim

For the low inoculation level of the *mericon*E. coliSTEC O-Type Pathogen Detection Assay, there were 13 presumptive positives and 13 confirmed positives following the USDA/FSIS-MLG 5B.05 reference method confirmation procedure. There were 13 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 5B.05 reference method confirmation procedure. There were 5 confirmed positives following the reference method.

For the low inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28)

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

For the high inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 15 and 19 of the Appendix.

QIAsymphonyDNA Extraction Procedure- E. coli Screen Plus

Fresh Spinach

For the low inoculation level of the*mericon* E. coli O157 Screen PlusPathogen Detection Assay, there were 7 presumptive positives and 7 confirmed positives following the FDA/BAM Chapter 4A 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 FDA/BAM Chapter 4A reference method confirmation procedure. There were 5 confirmed positives following the reference method.

For the low inoculation level, a $dPOD_C$ value of -0.10 was obtained with a 95% confidence interval of (-0.37, 0.19), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 14 and 18 of the Appendix.

Raw Ground Beef (70% Lean)

For the low inoculation level of the*mericon* E. coli O157 Screen PlusPathogen Detection Assay, there were 9 presumptive positives and 9 confirmed positives following the USDA/FSIS-MLG 5.09 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 USDA/FSIS-MLG 5.09 reference method confirmation procedure. There were 5 confirmed positives following the reference method.

For the low inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate presumptive and confirmed results.
For the high inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 14 and 18 of the Appendix.

Raw Beef Trim

For the low inoculation level of the*mericon*E. coli O157 Screen PlusPathogen Detection Assay, there were 13 presumptive positives and 13 confirmed positives following the USDA/FSIS-MLG 5B.05 reference method confirmation procedure. There were 13 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 5B.05 reference method confirmation procedure. There were 5 confirmed positives following the reference method.

For the low inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 14 and 18 of the Appendix.

QIAsymphonyDNA Extraction Procedure- E. coli STEC O-Type

Fresh Spinach

For the low inoculation level of the *mericon*E. coliSTEC O-TypePathogen Detection Assay, there were 7 presumptive positives and 7 confirmed positives following the FDA/BAM Chapter 4A 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 FDA/BAM Chapter 4A reference method confirmation procedure. There were 5 confirmed positives following the reference method.

For the low inoculation level, a $dPOD_C$ value of -0.10 was obtained with a 95% confidence interval of (-0.37, 0.19), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 16 and 20 of the Appendix.

Raw Ground Beef (70% Lean)

For the low inoculation level of the *mericon*E. coliSTEC O-Type Pathogen Detection Assay, there were 9 presumptive positives and 9 confirmed positives following the USDA/FSIS-MLG 5.09 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 USDA/FSIS-MLG 5.09 reference method confirmation procedure. There were 5 confirmed positives following the reference method.

For the low inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 16 and 20 of the Appendix.

Raw Beef Trim

For the low inoculation level of the *mericon*E. coliSTEC O-Type Pathogen Detection Assay, there were 13 presumptive positives and 13 confirmed

positives following the USDA/FSIS-MLG 5B.05 reference method confirmation procedure. There were 13 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 5B.05 reference method confirmation procedure. There were 5 confirmed positives following the reference method.

For the low inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.28, 0.28), indicating no significant difference between the candidate presumptive and confirmed results.

For the high inoculation level, a $dPOD_C$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate and reference methods. A $dPOD_{CP}$ value of 0.00 was obtained with a 95% confidence interval of (-0.43, 0.43), indicating no significant difference between the candidate presumptive and confirmed results. Detailed results of the POD analyses are presented in Tables 16 and 20 of the Appendix.

Robustness Evaluation

When changing the operational parameters of the enrichment incubation time,there was an observed effect on the results. For the 8 hour incubation, both assays failed to detect the target analyte. For the 9 hour incubation time, the Screen Plus Pathogen Detection Assay had no observed effect on the results but the STEC O-Type Pathogen Detection Assay produced two discrepant results when compared to the Screen Plus Pathogen Detection Assay.At the 10 hour time point, modification to the lysis heat time, and PCR Master Mix to DNA sample volume caused no observed discrepancies.

mericonE. coli O157 Screen Plus Pathogen Detection Assay

Treatment Combination 1

For treatment combination 1, there was 1 presumptive positive out of 10 replicates for the variations evaluated. For the 5 uninoculatedtest portions, there were 0 presumptive positives out of 5 replicates.

For the low inoculation level, a POD value of 0.10 was obtained with a 95% confidence interval of (0.00, 0.42). All 5 uninoculated test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.43).

Treatment Combination 2

For treatment combination 2, there were 4 presumptive positives out of 10 replicates for the variations evaluated. For the 5 uninoculated test portions, there were 0 presumptive positives out of 5 replicates.

For the low inoculation level, a POD value of 0.40 was obtained with a 95% confidence interval of (0.17, 0.69). All 5 uninoculated test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.43).

Treatment Combination 3 and 4

For both treatment combination 3 and 4, there were 2 presumptive positives out of 10 replicates for the variations evaluated. For the 5 uninoculated test portions, there were 0 presumptive positives out of 5 replicates.

For the low inoculation level, a POD value of 0.20 was obtained with a 95% confidence interval of (0.06, 0.51). All 5 uninoculated test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.43).

Treatment Combination 5, 6, 7, 8, and 9

For treatment combinations 5, 6, 7, 8, and 9 there were 5 presumptive positives out of 10 replicates for all of the variations evaluated. For the 5 uninoculated test portions, there were 0 presumptive positives out of 5 replicates.

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

mericonE. coli STEC O-Type Pathogen Detection Assay

Treatment Combination 1, 2, 3, and 4

For treatment combinations 1, 2, 3, and 4 there were 0 presumptive positives out of 10 replicates for all of the variations evaluated. For the 5 uninoculated test portions, there were 0 presumptive positives out of 5 replicates.

For the low inoculation level, a POD value of 0.00 was obtained with a 95% confidence interval of (0.00, 0.28). All 5 uninoculated test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.43).

Treatment Combination 5, 6, 7, and 8

For treatment combinations 5, 6, 7, and 8 there were 5 presumptive positives out of 10 replicates for all of the variations evaluated. For the 5 uninoculated test portions, there were 0 presumptive positives out of 5 replicates.

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

Treatment Combination 9

For treatment combination 9, there were 3 presumptive positives out 10 replicates for the variations evaluated. For the 5 uninoculated test portions, there were 0 presumptive positives out of 5 replicates.

For the low inoculation level, a POD value of 0.30 was obtained with a 95% confidence interval of (0.11, 0.60). All 5 uninoculated test portions were negative with a POD value of 0.00 with a 95% confidence interval of (0.00, 0.43).

Detailed results for the robustness tests are presented in Tables 21and 22ofthe Appendix.

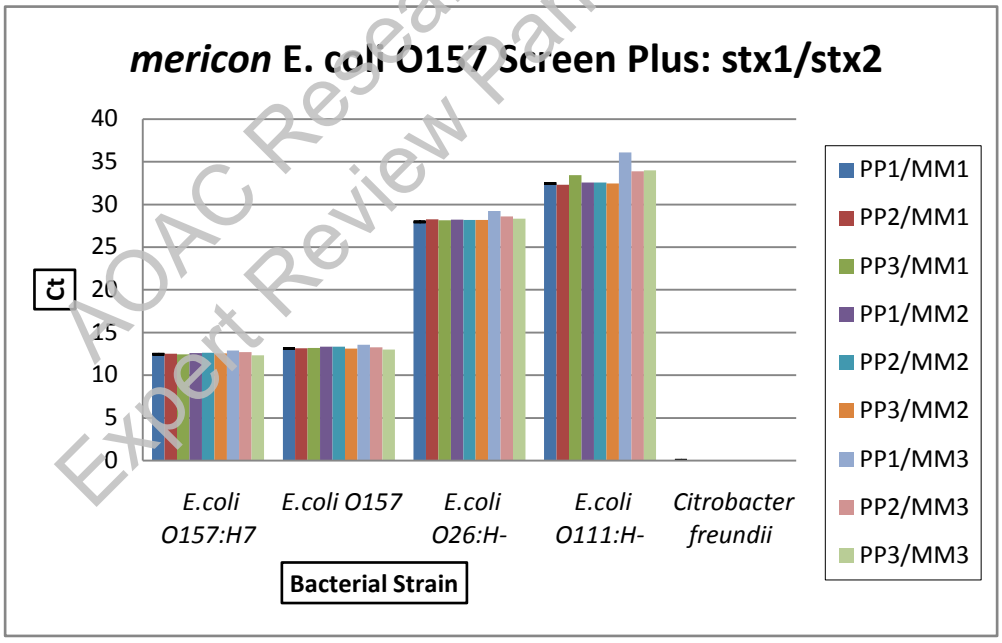
Product Consistency (Lot to Lot) and Stability Evaluation

Results Lot to lot consistency study

Primer probe: Master Mix lot variation
mericonE. coli O157 Screen Plus Pathogen Detection Assay

Each Pathogen detection assay was evaluated for detection consistency with each of the probes in the multiplex mixture (*stx1/stx2; eae,* and O157

Figure1. *mericon*E. coliO157 Screen Plus



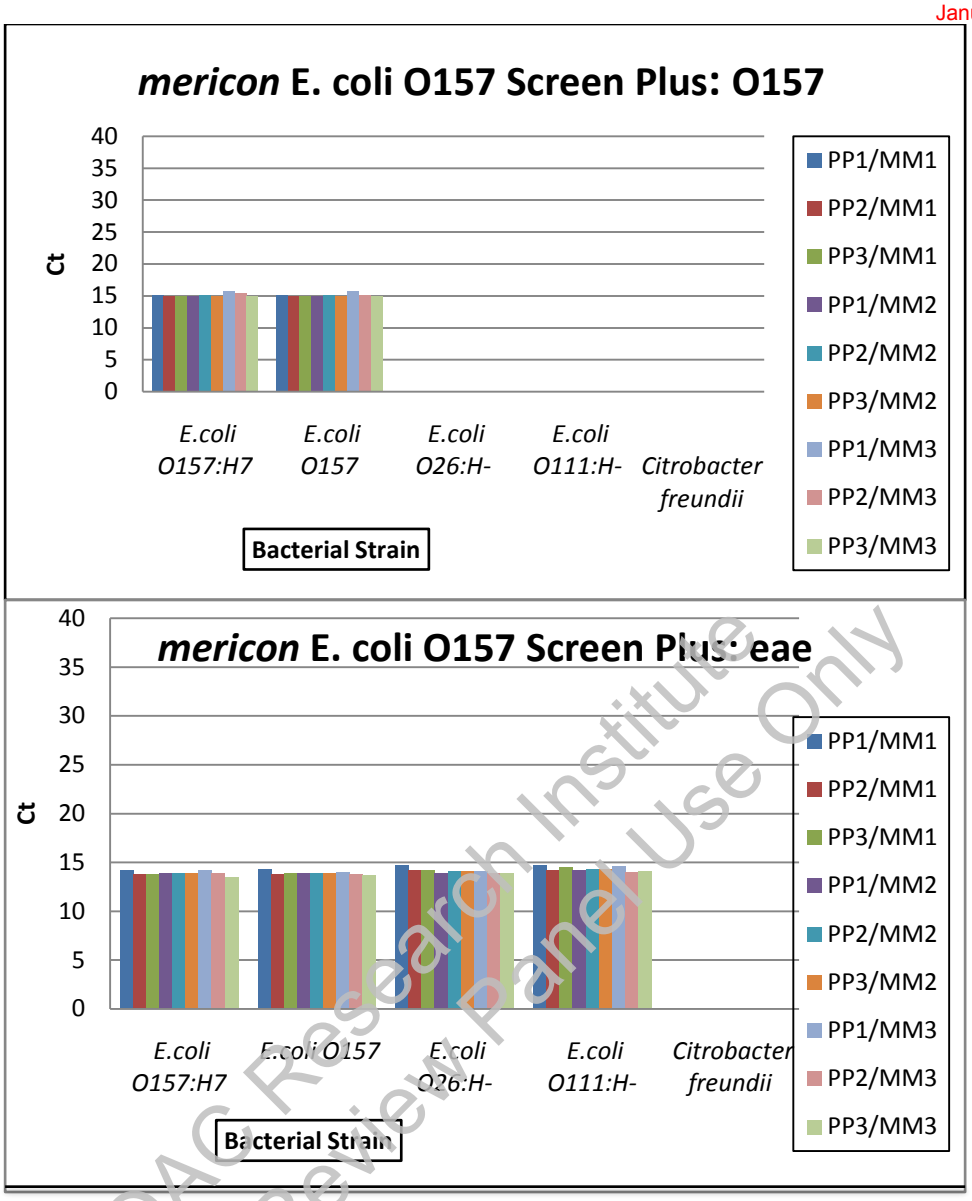


Figure 1: Primer-probe and master mix lot variation for *mericon*E. coliO157 Screen Plus assay.

Ct values for all primer/probe (PP) and Master Mix (MM) lot combinations for the*mericon*E. coliO157Screen Plus Pathogen Detection Assaywere not significantly different, indicating that there were no effects of individual lots of primer/probes and Master Mix on the detection results. The assay is a multiplex giving results in the green channel for *stx1/stx2,* in the orange channel for *O157¸* and in the crimson channel for *eae.* Results for *Citrobacter freundii* (a closely related but non-*E. coli* bacterial strain)are shown.

Primer probe: Master Mix lot variation

Each Pathogen detection assay was evaluated for detection consistency with each of the probes in the multiplex mixture (*stx1/stx2; eae,* and O157).

Figure 2.*mericon*E. coliSTEC O-type

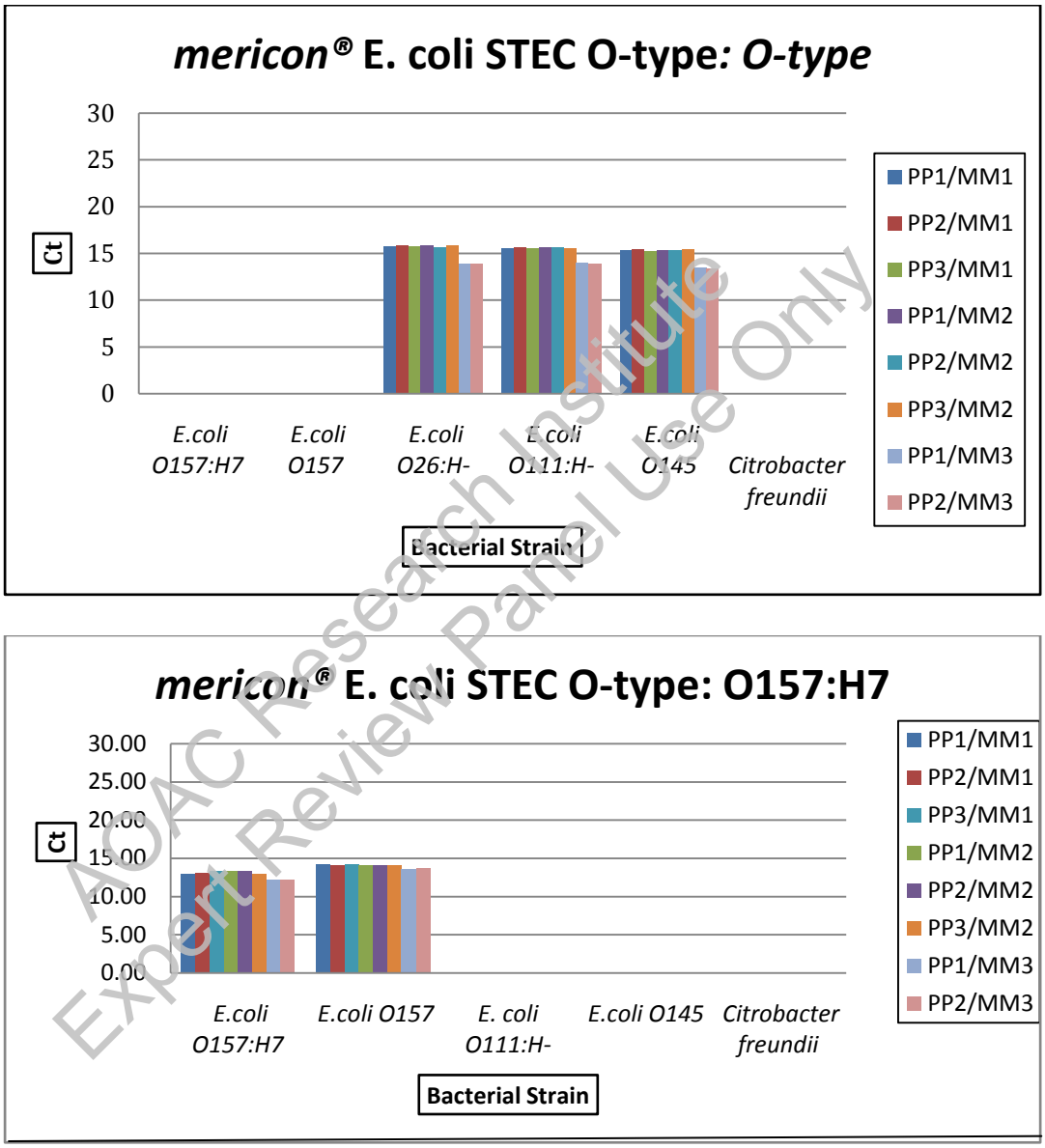


Figure 2: Primer-probe and master mix lot variation for *mericon*E. coliSTEC O-typeassay
Ct values for all primer/probe (PP) and Master Mix (MM) lot combinations for the*mericon*E. coli STEC O-type Pathogen Detection Assaywere not significantly different, indicating that there were no effects of individual lots of primer/probes and Master Mix on the detection results. The assay is a multiplex giving results in the green channel for the non-O157 Big 6 STEC O-

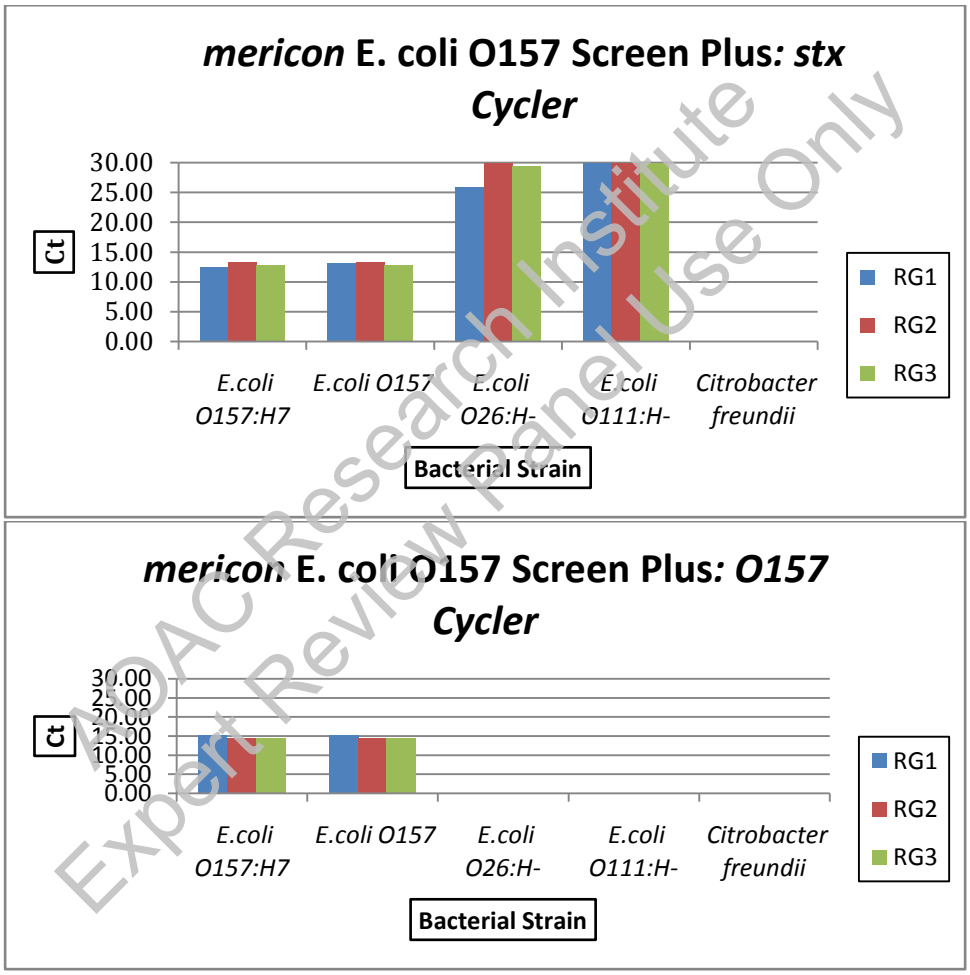
types and in the orange channel for *O157:H7*. Results for *Citrobacter freundii*(a closely related but non-*E. coli* bacterial strain), are shown.

Consistency study: Effect of Cycler

*mericon*E. coli O157 Screen Plus

Each Pathogen detection assay was evaluated for detection consistency with each of the probes in the multiplex mixture (*stx1/stx2; eae, and O157)* using 3 different Rotor-Gene Q cyclers.

Figure3. *E. coli* O157 Screen Plus assay using 3 Rotor-Gene Q cyclers



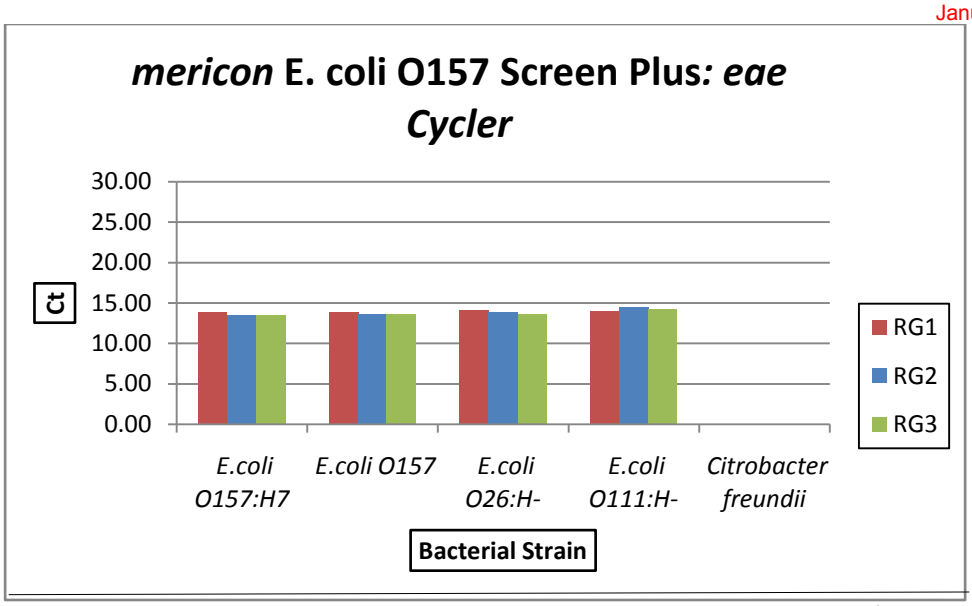


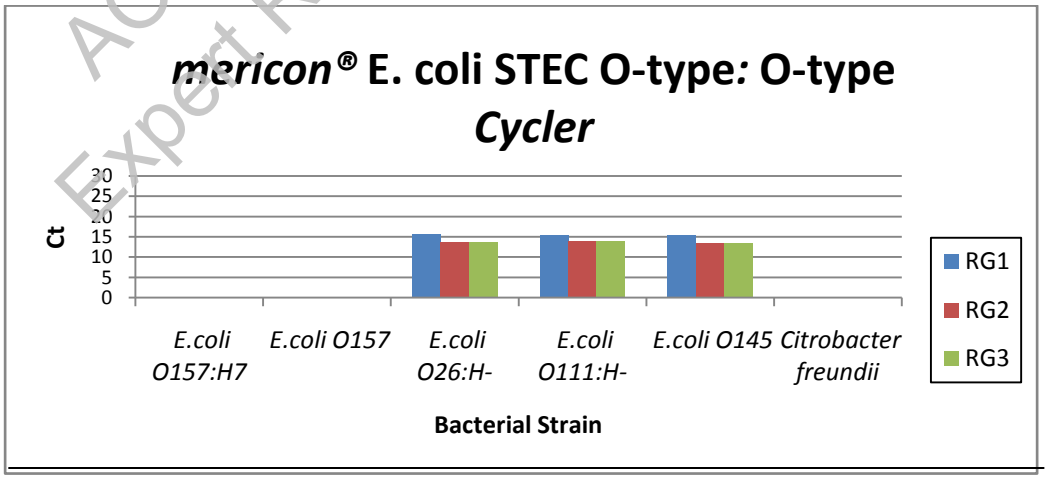
Figure 3: Cycler variation for the mericon E. coli O157 Screen Plus assay

Ct values for all Cycler combinations using consistent primer probe:master mix lots for the*mericon*E. coliO157 Screen Plus Pathogen Detection Assaywere not significantly different, indicating that there were no effects of different cyclers on the detection results.

*mericon*E. coli STEC O-type assay

Each Pathogen detection assay was evaluated for detection consistency with each of the probes in the multiplex mixture (*Big 6 STEC and O157:H7)* using 3 different Rotor-Gene Q cyclers.

Figure 4. *mericon*E. coli STEC O-type assayusing 3 Rotor-Gene Q cyclers



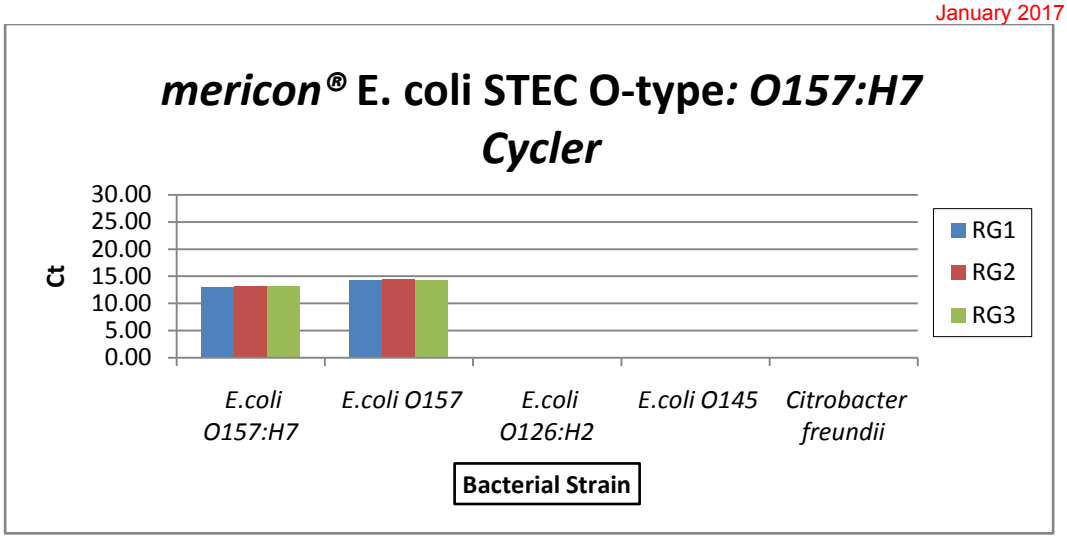


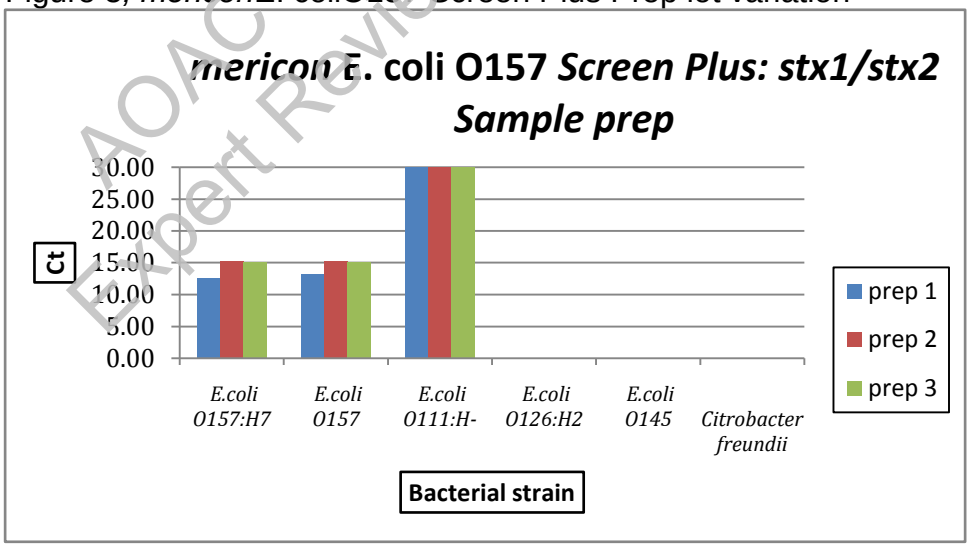
Figure4. Cycler variation for *mericon*E. coliSTEC O-type assay.

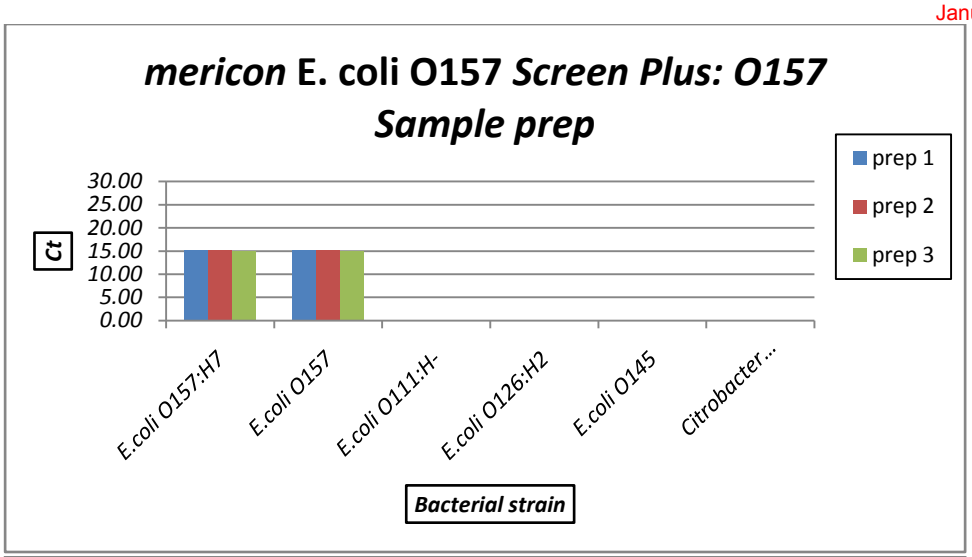
Ct values for all Cycler combinations using consistent primer probe:master mix lots for the*mericon*E. coliSTEC O-type Pathogen Detection Assaywere not significantly different, indicating that there were no effects of different cyclers on the detection results.

Consistency Study: Bacteria Plus Prep lot variation

Each Pathogen detection assay was evaluated for detection consistency with each of the probes in the multiplex mixture (*stx1/stx2; eae, and O157)* using 3 different lots of the *mericon* DNA Bacteria Plus kits.

Figure 5*; mericon*E. coliO157 Screen Plus Prep lot variation





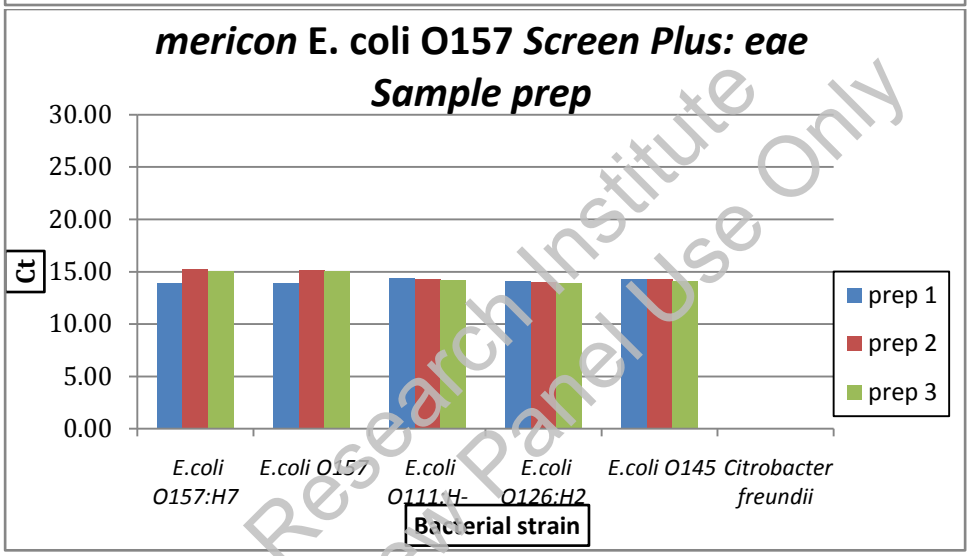


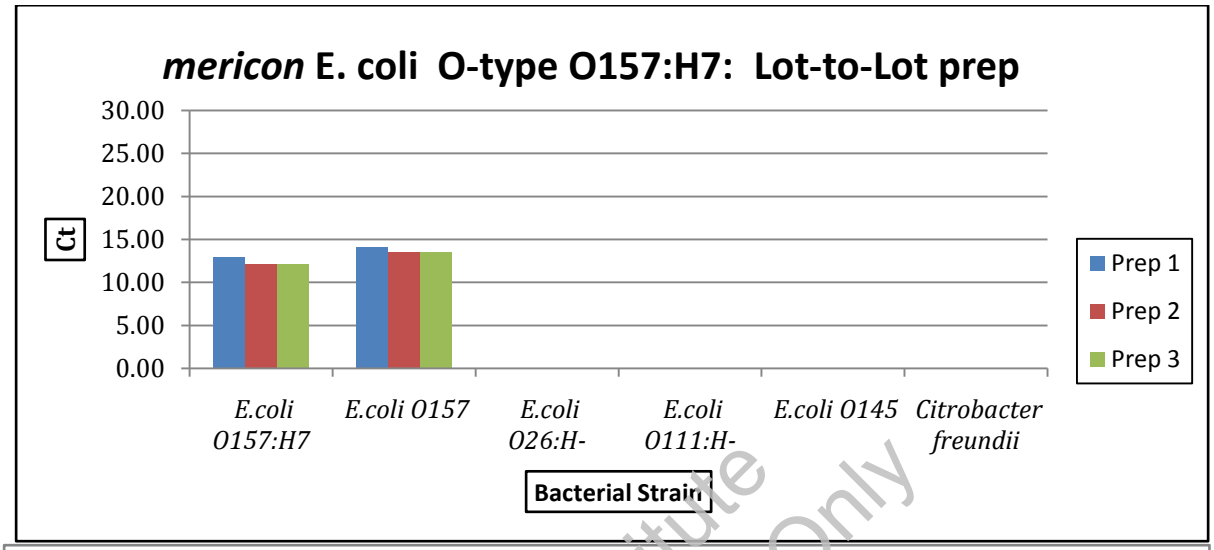
Figure 5. *mericonE. coli* O157 Screen Plus Prep lot variation

Ct values for DNA prep combinations using consistent primer probe:master mix lots for the *mericonE. coli* O157 Screen PlusPathogen Detection Assaywere not significantly different, indicating that there were no effects of different bacterial prep lots on the detection results

mericonE. coli STEC O-type assay

Each Pathogen detection assay was evaluated for detection consistency with each of the probes in the multiplex mixture (*Big 6 STEC and O157:H7)* using 3 different lots of the *mericon* DNA Bacteria Plus kits.

Figure 6.*mericonE. coli* STEC O-type assay: Prep lot variation



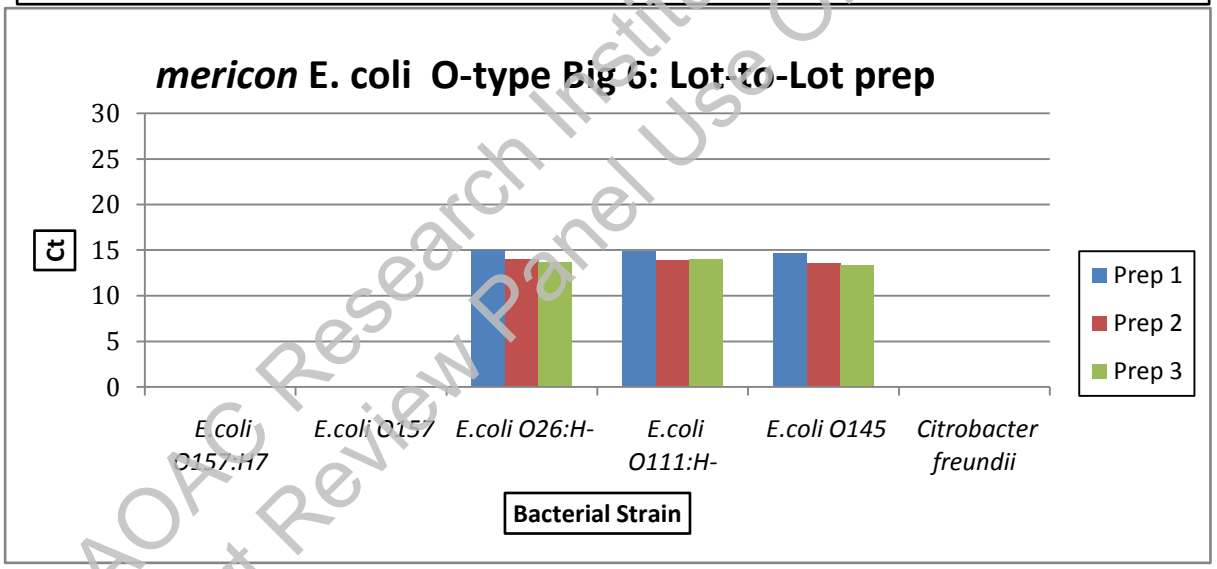


Figure 6.*mericon*E. coli STEC O-type Prep lot variation

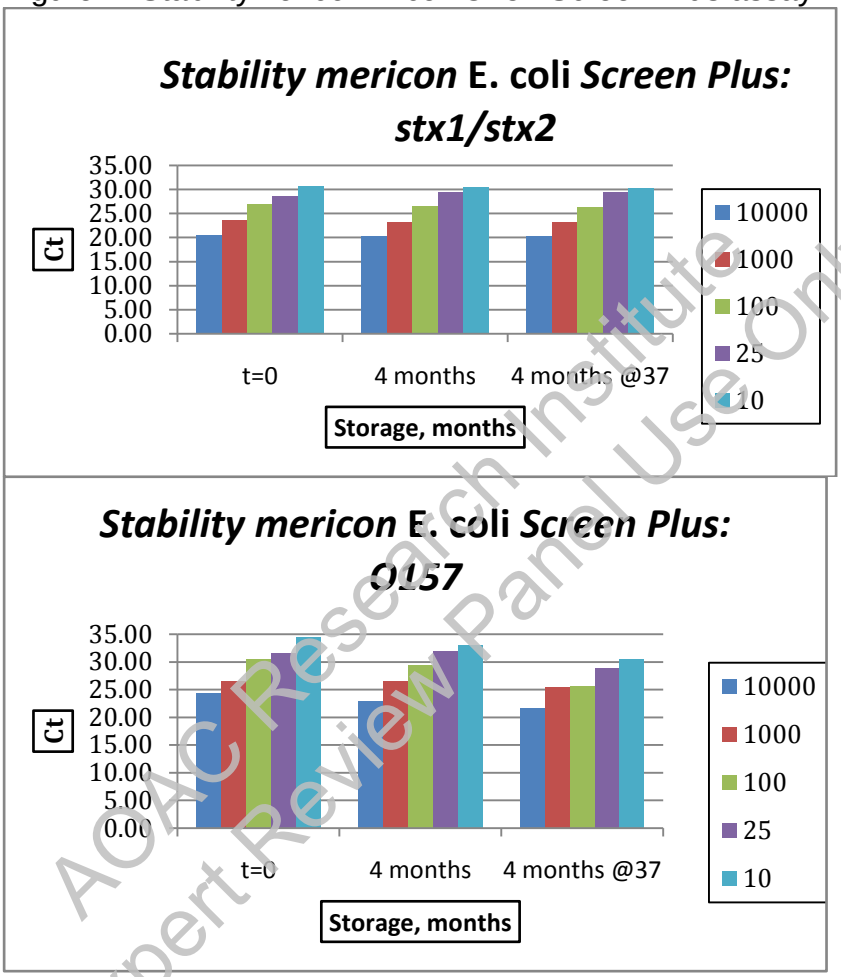
Ct values for DNA prep lots using consistent primer probe:master mix lots for the*mericonE. coli* STEC O-type Pathogen Detection Assaywere not significantly different, indicating that there were no effects of different bacterial prep lots on the detection results

Stability Study: Lot stability

In this study, a single lot of each of the primer probe assay kits (*E. coli* O157Screen Plusand *E. coil* STEC O-type)was stored at 4 ± 1°C for 4 and 15 months. A second set of kits from the same lot was stored at 37 ± 1°C for 3 and 4.5 months. The 2 sets of primer probes stored at 37 ± 1°C extends the

real time aging to approximately 18 and 24 months [9]. The same lot of Master Mix was used for all time points from time 0 to 15 months.To date, only the t=o and 4 month timepoints have been completed.Reference DNA was diluted from 10,000 copies/µl to 10 copies/µl

Figure 7. Stability*mericon*E. coli O157 Screen Plus assay



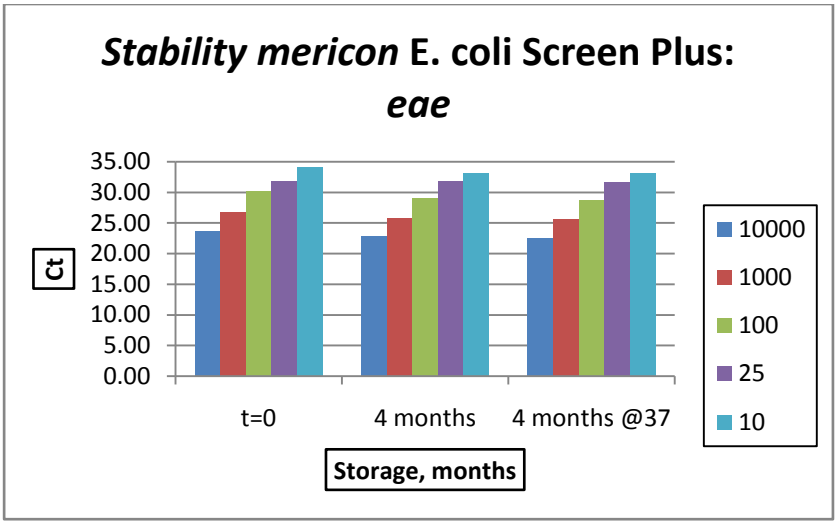
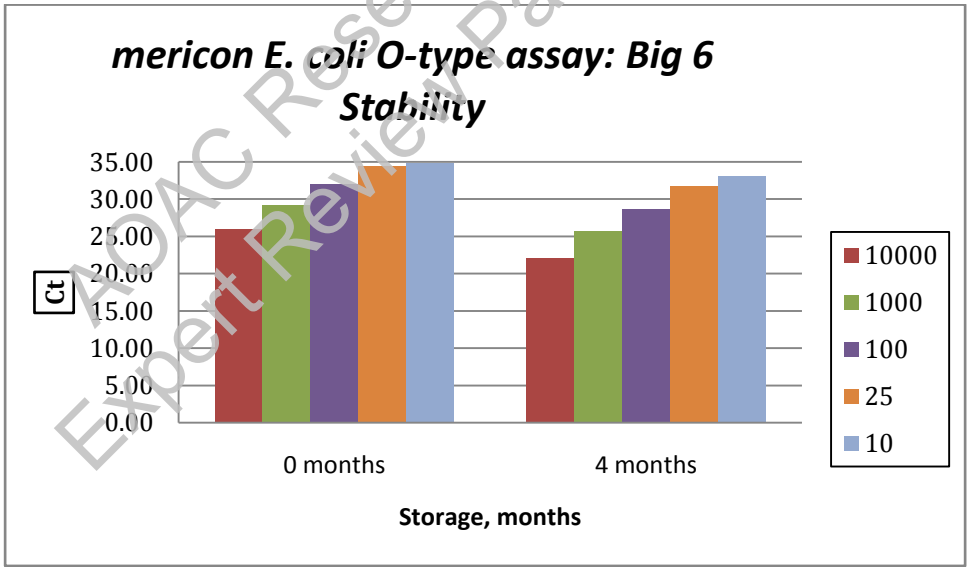


Figure 7. Stability *mericon*+E. coli O157 Screen Plus assay

There was no decrease (right shift)observed between the time points t=0 @ 4 ± 1°C, t=4 months @ 4 ± 1°C or t+4 months @ 37 ± 1°C (about 18 months equivalent storage). Storing the prime-probe lots had, therefore, no deleterious effect on the performance of the assay.

Figure 8. Stability *merion*E. coli STEC O-type assay



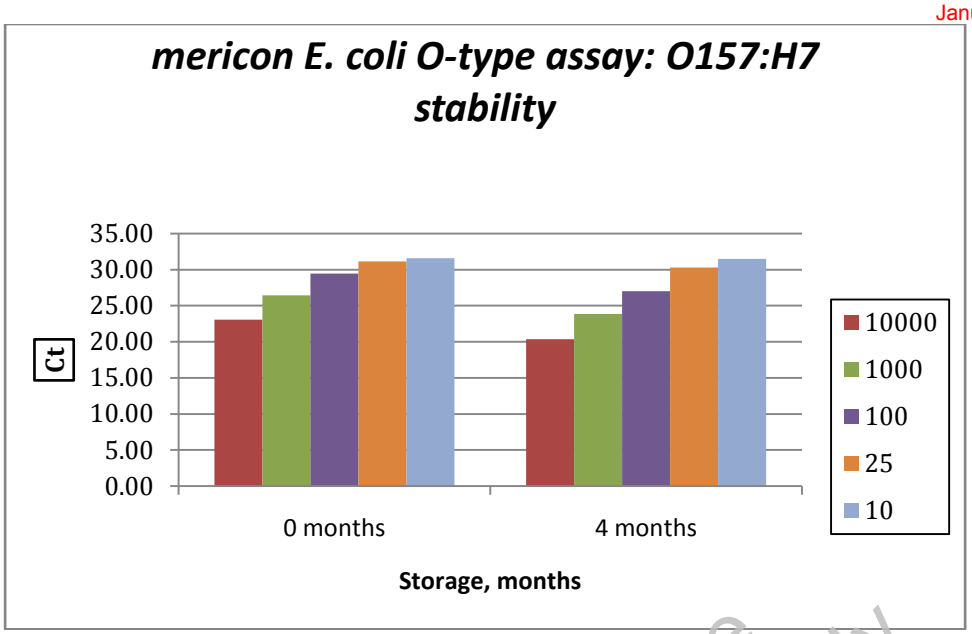


Figure 8. Stability *mericon*E. coli STEC O-type assay

There was no decrease (right shift)observed between the time points t=0 @ 4 ± 1°C, t=4 months @ 4 ± 1°C. Storing the prime-probe lots had, therefore, no deleterious effect on the performance of the assay in the first 4 months of storage. Stability studies are ongoing. Dilution series 10,000 copies/µl to 10 copies/µl

Discussion

The *mericon*E. coliO157 Screen Plus and *mericon*E. coliSTEC O-TypePathogen Detection Assays, using both the manual and automated DNA extraction procedures, successfully recovered *E. coli* non-O157:H7 STEC's and *E. coli* O157:H7fromfresh spinach, raw ground beef (70% lean), and raw beef trim. Using POD analysis, no statistically significant differences were seen between the number of positive samples detected by the candidate methods and the reference methods for all three matrixes tested.

The results of the inclusivity and exclusivity study demonstrated 100% agreement with expected results for the test panel.

The robustness study demonstrated that the *mericon*E. coliO157Screen Plus and *mericon*E. coliSTECO-Type Pathogen Detection Assays were sensitive to changes after 8 and 9 (*E. coli* STEC O-Type only) but not 10 hours of incubation.

The *mericon*E. coliO157Screen Plus and the*mericon*E. coliSTEC O-Type Pathogen Detection Assaysare quick and easy to perform, providing results within 15hoursfor 30 sample replicates for both the manual and the QIAsymphony (automated) preparation methods, including the pre-enrichment time. The QIAsymphony adds the benefit of

automated sample preparation and automated PCR preparation to minimize contamination. The *mericon*kitsallow the end user a choice of two DNA extraction methods. The automated *QIAsymphony*preparation method allows a high volume of samples to be processed with minimal supervision utilizing easy to use software with an option to follow step-by-step guidance through the sample prep workflow.

Conclusion

The data from these studies, within their statistical uncertainty, support the product claims of the *mericon*E. coli O157Screen Plus and*mericon*E. coliSTEC O-TypePathogen Detection Assaysfor the selected food matrixes tested, namely fresh spinach, raw ground beef, and raw beef trim.

Results obtained from POD analysis in the method comparison study demonstrated no statistically significant differences between the number of positive samples detected by the candidate method, using the manual or automated DNA extraction procedures, and the reference methods for fresh spinach, raw ground beef, and raw beef trim.

References

[1] FDA/BAM Chapter 4A: *Diarrheagenic Escherichia coli.* February, 2011.http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm064948.html (Accessed June 2015)

[2] USDA/FSIS-MLG 5.09: *Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges.* January, 2015.http://www.fsis.usda.gov/wps/wcm/connect/51507fdb-dded-47f7-862d-ad80c3ee1738/MLG-5.pdf?MOD=AJPERESAccessed June 2015)

[3] USDA/FSIS-MLG 5B.05: *Detection and Isolation of non-O157 Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products and Carcass and Environmental Sponges.* June, 2014.http://www.fsis.usda.gov/wps/wcm/connect/7ffc02b5-3d33-4a79-b50c-81f208893204/MLG-5B.pdf?MOD=AJPERES (Accessed June 2015)

[4] Feng, P. *Enterohemorrhagic Escherichia coli (EHEC).* Bad Bug Book – Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins, 2nd Ed. 2011

[5] QIAGEN GmbH: *mericon*Automated Pathogen Detection Workflow Handbook, March 2012. http://www.qiagen.com/knowledge-and-support/resource-center/resource-download.aspx?id=c2df0606-cae7-4041-b618-541989a03de3&lang=en

[6] QIAGENGmbH. *QIAsymphony*SP/AS Handbook User Manual May 2013.http://www.qiagen.com/knowledge-and-support/resource-center/resource-download.aspx?id=363d44fa-0560-4b0c-8aab-96c25b30cf39&lang=en

[7] AOAC Research Institute *Performance Tested Methods℠ Program* validation outline protocol: *Independent Laboratory Study for the mericonE. coli Screen Plus and mericonSTEC O-Type Detection kits:* (February 2015, Version 1).

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

[9] Least Cost Formulations, Ltd., MPN Calculator – Version 1.6 www.lcfltd.com/customer/LCFMPNCalculator.exe(Accessed June 2015)

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

[11] 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, www.eoma.aoac.org/app_j.pdf<http://www.eoma.aoac.org/app_j.pdf (Accessed June 2015)

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

[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. 94:335-347, (2011).

APPENDIX

Table 1: Inclusivity Results for the *mericon E. coli* Screen PlusPathogen Detection Assay

Organism	Source[1]	Origin	Result				Organism	Source[1]	Origin	Result			
			stx_1/ stx_2	*eae*	O157	Final				stx_1/ stx_2	*eae*	O157	Final
E. coli O157:H7	MSU[1]TW00116	Human	+	+	+	+	*E. coli* O157:H7	MSU DEC4D	Meat	+	+	+	+
E. coli O157:H7	MSU TW00975	Human	+	+	+	+	*E. coli* O157:H7	MSUDEC4E	Ground Beef	+	+	+	+
E. coli O157:H7	MSUTW02302	Hamburger	+	+	+	+	*E. coli* O157:H7	QL14077.7	Ground Beef	+	+	+	+
E. coli O157:H7	MSU TW04863	Human	+	+	+	+	*E. coli* O157:H7	QL14077.8	Beef Trim	+	+	+	+
E. coli O157:H7	MSU TW05356	Human	+	+	+	+	*E. coli* O157:H7	QL2-202	Ground Beef	+	+	+	+
E. coli O157:H7	MSU TW07587	Human	+	+	+	+	*E. coli* O157:H7	QL2-203	Ground Beef	+	+	+	+
E. coli O157:H7	ATCC[2]BAA-460	Human feces	+	+	+	+	*E. coli* O157:H7	QL2-204	Ground Beef	+	+	+	+
E. coli O157:H7	QL[3]14077.1	Raw Hamburger	+	+	+	+	*E. coli* O157:H7	QL 2-205	Ground Beef	+	+	+	+
E. coli O157:H7	QL14077.2	Raw Hamburger	+	+	+	+	*E. coli* O157:H7	QL2-206	Beef Trim	+	+	+	+
E. coli O157:H7	QL14077.3	Raw Hamburger	+	+	+	+	*E. coli* O157:H7	QL2-207	Beef Trim	+	+	+	+
E. coli O157:H7	QL14077.4	Raw Beef	+	+	+	+	*E. coli* O157:H7	QL2-214	Beef Trim	+	+	+	+
E. coli O157:H7	QL14077.5	Beef Trim	+	+	+	+	*E. coli* O157:H7	QL2-370	Beef Trim	+	+	+	+
E. coli O157:H7	QL14077.6	Beef Trim	+	+	+	+	*E. coli* O157:H7	QL2-701	Beef Trim	+	+	+	+
E. coli O157:H7	ATCC35150	Human feces	+	+	+	+	*E. coli* O157:H7	QL2-704	Beef Trim	+	+	+	+
E. coli O157:H7	ATCC43889	Human feces	+	+	+	+	*E. coli* O157:H7	QL2-705	Human Diarrhea	+	+	+	+
E. coli O157:H7	ATCC43890	Human feces	+	+	+	+	*E. coli* O157:H7	QL2-706	Human Feces	+	+	+	+
E. coli O157:H7	ATCC43894	Human feces	+	+	+	+	*E. coli* O157:H7	QL2-707	Beef Trim	+	+	+	+
E. coli O157:H7	ATCC43895	Hamburger	+	+	+	+	*E. coli* O157:H7	QL2-708	Ground Beef	+	+	+	+
E. coli O157:H7	ATCC51657	Clinical	+	+	+	+	*E. coli* O157:H7	QL2-709	Raw Milk	+	+	+	+
E. coli O157:H7	ATCC51658	Clinical	+	+	+	+	*E. coli* O157:H7	ATCC700376	Meat	+	+	+	+
E. coli O157:H7	ATCC51659	Clinical	+	+	+	+	*E. coli* O157:H7	MSU DEC3D	Human	+	+	+	+
E. coli O157:H7	ATCC 700531	Clinical	+	+	+	+	*E. coli* O157:H7	MSUDEC3E	Human	+	+	+	+
E. coli O157:H7	ATCC700599	Salami	+	+	+	+	*E. coli* O157:H7	MSU DEC4B	Human	+	+	+	+
E. coli O157:H7	ATCC 700729	Not Available	+	+	+	+	*E. coli* O157:H7	MSU DEC3B	Human	+	+	+	+
E. coli O157:H7	MSUDEC3A	Human	+	+	+	+	*E. coli* O157:H7	MSU DEC3C	Human	+	+	+	+

[1]MSU-Michigan State Culture Collection
[2]ATCC-American Type Culture Collection
[3]QL-Q Laboratories Inc. Culture Collection

Table 2: Exclusivity Results for the *mericon E. coli* Screen Plus PathogenDetection Assay

Organism	Source	Origin	Result			
			stx$_1$/*stx*$_2$	*eae*	O157	Final
Alcaligenes faecalis subsp faecalis	ATCC[1] 8750	Not Available	-	-	-	-
Citrobacter freundii	ATCC 8090	Not Available	-	-	-	-
Cronobactersakazakii	ATCC 29544	Child's Throat	-	-	-	-
Edwardsiellatarda	ATCC 15947	Human Feces	-	-	-	-
Enterobacteraerogenes	ATCC 13048	Sputum	-	-	-	-
E. blattae	ATCC 29907	Cockroach	-	-	-	-
E. coli O26:H11	MSU[2] DEC10E	Cow	+	+	-	-
E. coli O45	MSU TW10121	Human	+	-	-	-
E. coli O55:H6	MSU DEC1A	Human	-	-	-	-
E. coli O91	NCTC[3] 9091	Not Available	-	-	-	-
E. coli O103	NCTC 8196	Not Available	+	+	-	-
E. coli O111	MSU TW07926	Human	+	+	-	-
E. coli O113	NCTC 9113	Not Available	-	-	-	-
E. coli O115	NCTC 10444	Cat	-	-	-	-
E. coli O117	NCTC 9117	Not Available	-	-	-	-
E. coli O118	NCTC 9118	Not Available	-	-	-	-
E. coli O121	NCTC 9121	Not Available	+	+	-	-
E. coli O142	NCTC 10089	Not Available	-	-	-	-
E. coli O145	NCTC 10279	Not Available	+	+	-	-
E. coli O146	NCTC 10677	Not Available	-	-	-	-
E. fergusonii	ATCC 35469	Human Feces	-	-	-	-
E. hermannii	ATCC 33650	Human Toe	-	-	-	-
E. vulneris	ATCC 29943	Human Wound	-	-	-	-
Hafniaalvei	ATCC 51815	Milk	-	-	-	-
Klebsiella pneumoniae subsp. pneumonia	ATCC 4352	Cow's Milk	-	-	-	-
Microbacteriumtestaceum	ATCC 15329	Paddy	-	-	-	-
Pantoeaagglomerans	ATCC 19552	Sewage	-	-	-	-
Pseudomonas aeruginosa	ATCC9027	Outer Ear Infection	-	-	-	-
Rahnellaaquatilis	ATCC 55046	Not Available	-	-	-	-
Salmonella enterica subsp. enterica serovar Choleraesuis	ATCC10708	Not Available	-	-	-	-

[1]ATCC-American Type Culture Collection
[2]MSU-Michigan State University Culture Collection
[3]NCTC-National Culture Type Collection

Table 3: Inclusivity Results for the *mericon E. coli* STEC O-TypePathogen Detection Assay

Organism	Source[1]	Origin	Result			Organism	Source[1]	Origin	Result		
			[6]O Group	O157	Result				[6]O Group	O 157	Result
E. coli O26	MSU[1] TW07814	Human Isolate	+	-	+	*E. coli* O111	MSU TW14960	Human Isolate	+	-	+
E. coli O26	MSU TW07862	Human Feces	+	-	+	*E. coli* O111	MSU TW06296	Human Child	+	-	+
E. coli O26	MSU TW04270	Human Isolate	+	-	+	*E. coli* O111	MSU TW06315	Human Isolate	+	-	+
E. coli O26	MSU TW04284	Human Child	+	-	+	*E. coli* O111	PSU 12289-3A	Not Available	+	-	+
E. coli O26	MSU TW005992	Human Isolate	+	-	+	*E. coli* O121	NCTC[4] 9121	Not Available	+	-	+
E. coli O26	MSU TW08031	Human Isolate	+	-	+	*E. coli* O121	MSU TW07614	Human Isolate	+	-	+
E. coli O26	MSU DEC10B	Human Isolate	+	-	+	*E. coli* O121	MSU TW08023	Human Isolate	+	-	+
E. coli O45	MSU DEC11C	Human Isolate	+	-	+	*E. coli* O121	MSU TW08039	Human Isolate	+	-	+
E. coli O45	MSU TW09183	Human Isolate	+	-	+	*E. coli* O121	MSU TW07931	Human Isolate	+	-	+
E. coli O45	MSU TW10121	Human Isolate	+	-	+	*E. coli* O121	PSU 5.0959	Not Available	+	-	+
E. coli O45	MSU TW14003	Human Isolate	+	-	+	*E. coli* O121	PSU 7.1686	Not Available	+	-	+
E. coli O45	MSU TW07947	Human Isolate	+	-	+	*E. coli* O145	NCTC 10279	Not Available	+	-	+
E. coli O45	PSU[2] 1.2622	Not Available	+	-	+	*E. coli* O145	QL 15071.1	Human Isolate	+	-	+
E. coli O45	PSU 11.1079	Not Available	+	-	+	*E. coli* O145	MSU TW07596	Human Isolate	+	-	+
E. coli O103	QL[3] 15071.2	Not Available	+	-	+	*E. coli* O145	PSU 10.1438	Human Isolate	+	-	+
E. coli O103	MSU TW08101	Human Isolate	+	-	+	*E. coli* O145	MSU TW09356	Human Isolate	+	-	+
E. coli O103	MSU TW07971	Human Isolate	+	-	+	*E. coli* O145	PSU 7.1711	Not Available	+	-	+
E. coli O103	MSU TW11239	Human, Child	+	-	+	*E. coli* O145	PSU 10.0707	Not Available	+	-	+
E. coli O103	MSU TW07697	Human Isolate	+	-	+	*E. coli* O157:H7	ATCC[5] 35150	Human feces	-	+	+
E. coli O103	MSU TW05997	Human Isolate	+	-	+	*E. coli* O157:H7	ATCC 43888	Human feces	-	+	+
E. coli O103	MSU TW04162	Human Isolate	+	-	+	*E. coli* O157:H7	ATCC 43889	Human feces	-	+	+
E. coli O111	MSU DEC6A	Human, Infant	+	-	+	*E. coli* O157:H7	ATCC BAA-460	Human feces	-	+	+
E. coli O111	MSU TW06315	Human Isolate	+	-	+	*E. coli* O157:H7	ATCC 43894	Human feces	-	+	+
E. coli O111	MSU TW07926	Human Isolate	+	-	+	*E. coli* O157:H7	ATCC 43895	Raw Hamburger	-	+	+
E. coli O111	MSU DEC12A	Human, Infant	+	-	+	*E. coli* O157:H7	ATCC 51657	Clinical	-	+	+

[1]MSU-Michigan State University Culture Collection
[2] PSU-University of Pennsylvania Culture Collection
[3]QL-Q Laboratories Inc. Culture Collection
[4]NCTC-National Culture Type Collection
[5]ATCC-American Type Culture Collection
[6] O Group includes O26, O45, O103, O111, O121 and O145

Table 4: Exclusivity Results for the *mericon E. coli* STEC O-TypePathogen Detection Assay

Species	Source	Origin	Result		
			[6]O Group	O 157	Result
Alcaligenes faecalis subsp faecalis	ATCC[1] 8750	Not Available	-	-	-
Bacillus cereus	ATCC 11778	Not Available	-	-	-
Candida albicans	ATCC10231	Human	-	-	-
Citrobacter freundii	ATCC 8090	Not Available	-	-	-
Citrobacter farmerii	ATCC 51633	Human feces	-	-	-
Cronobactersakazakii	ATCC 29544	Child's Throat	-	-	-
Edwardsiellatarda	ATCC 15947	Human Feces	-	-	-
Enterobacteraerogenes	ATCC 13048	Sputum	-	-	-
Enterobacter cloacae	ATCC 13047	Spinal Fluid	-	-	-
E.blattae	ATCC 29907	Cockroach	-	-	-
E. coli O55:H6	MSU[2] DEC1A	Human	-	-	-
E.coli O91	NCTC[3]9091	Not Available	-	-	-
E. coli O113	NCTC 9113	Not Available	-	-	-
E. coli O115	NCTC 10444	Calf	-	-	-
E. coli O117	NCTC 9117	Not Available	-	-	-
E. coli O118	NCTC 9118	Not Available	-	-	-
E. coli O142	NCTC 10089	Not Available	-	-	-
E. coli O146	NCTC 10677	Feces	-	-	-
E.fergusonii	ATCC 35469	Human Toe	-	-	-
E.hermannii	ATCC 33650	Human Wound	-	-	-
E.vulneris	ATCC 29943	Milk, Minnesota	-	-	-
Hafniaalvei	ATCC 51815	Cow's Milk	-	-	-
Klebsiella pneumoniae subsp. pneumonia	ATCC 4352	Surface water	-	-	-
Kluyveraintermedia	ATCC 33110	Paddy	-	-	-
Microbacteriumtestaceum	ATCC15829	Sewage	-	-	-
Pantoeaagglomerans	ATCC19552	Outer Ear Infection	-	-	-
Pseudomonas aeruginosa	ATCC[1]# 9027	Soil, Wisconsin	-	-	-
Rahnellaaquatilis	ATCC 33046	Not Available	-	-	-
Salmonella enterica subsp. enterica serovar Choleraesuis	ATCC10708	Not Available	-	-	-
Vibrio vulnificus	QL[4] 021111-A	Shellfish	-	-	-

[1]ATCC-American Type Culture Collection
[2]MSU-Michigan State University Culture Collection
[3]NCTC-National Culture Type Collection
[4]QL-Q Laboratories Inc. Culture Collection
[6]O Group includes O26, O45, O103, O111, O121 and O145

Table 5: Aerobic Plate Count and Background Results (Prior to Inoculation)

Matrix	APC[1] (CFU/g)	*E. coli* STECPathogen Screen[1]				
		Replicate A	Replicate B	Replicate C	Replicate D	Replicate E
Fresh Spinach	1.4 x 10^6	None Detected	None Detected	None Detected	None Detected	None Detected
Matrix	**APC[1] (CFU/g)**	***E. coli* O157:H7 Pathogen Screen[2]**				
		Replicate A	Replicate B	Replicate C	Replicate D	Replicate E
Raw Ground Beef (70% Lean)	2.8 x 10^3	None Detected	None Detected	None Detected	None Detected	None Detected
Matrix	**APC[1] (CFU/g)**	***E. coli* STEC Pathogen Screen[1]**				
		Replicate A	Replicate B	Replicate C	Replicate D	Replicate E
Raw Beef Trim	4.9 x 10^4	None Detected	None Detected	None Detected	None Detected	None Detected

Screen[1]- Background screen conducted using BAX STEC Screen Kit
Screen[2]-Background screen conducted using AOAC approved LFD

Table 6A: MPN Summary Table for Fresh Spinach

Fresh Spinach (200 g) *E. coli* O111 MSUTW063[3]					
Low Level Inoculum (0.2-2 MPN/Test Portion)					
	A	**B**	**C**	**D**	**E**
400 g	-	+	+	-	+
20 x 200 g (Reference Samples)	**9/20**				
100 g	+	-	+	-	-
MPN/Test portion	**0.61**				
Low Conf. Limit MPN/Test Portion	**0.33**				
High Conf. Limit MPN/Test Portion	**1.02**				
High Level Inoculum (2-5 MPN/Test Portion)					
	A	**B**	**C**	**D**	**E**
5 x 200 g (Reference Samples)	**5/5**				
80 g	+	+	+	+	+
40 g	+	+	+	-	+
MPN/Test portion	**3.70**				
Low Conf. Limit MPN/Test Portion	**1.52**				
High Conf. Limit MPN/Test Portion	**9.02**				

[*]**MPN was calculated for the low level inoculation for fresh spinach using five 400 g, twenty 200 g (reference method test portions), five 100 g using the LCF MPN Calculator version 1.6 provided by AOAC-RI**
http://www.lcfltd.com/customer/LCFMPNCalculator
[*]**MPN was calculated for the high level inoculation for fresh spinach using five 200 g (reference method test portions), five 80 g and five 40 g using the LCF MPN Calculator version 1.6 provided by AOAC-RI**
http://www.lcfltd.com/customer/LCFMPNCalculator

Table 6B: MPN Summary Table for Raw Ground Beef (70% Lean)

Raw Ground Beef (70% Lean) (325 g) *E. coli* O157:H7 ATCC 43895					
Low Level Inoculum (0.2-2 MPN/Test Portion)					
	A	**B**	**C**	**D**	**E**
650 g	+	+	+	-	+
20 x 325 g (Reference Samples)	9/20				
130 g	-	+	-	+	+
MPN/Test portion	0.75				
Low Conf. Limit MPN/Test Portion	0.44				
High Conf. Limit MPN/Test Portion	1.23				
High Level Inoculum (2-5 MPN/Test Portion)					
	A	**B**	**C**	**D**	**E**
5 x 325 g (Reference Samples)	5/5				
130 g	+	+	+	+	+
65 g	-	+	+	+	+
MPN/Test portion	3.70				
Low Conf. Limit MPN/Test Portion	1.52				
High Conf. Limit MPN/Test Portion	9.02				

*MPN was calculated for the low level inoculation for raw ground beef using five 650 g, twenty 325 g (reference method test portions), and five 130 g using the LCF MPN Calculator version 1.6 provided by AOAC-RI http://www.lcfltd.com/customer/LCFMPNCalculator

*MPN was calculated for the high level inoculation for raw ground beef using five 325 g (reference method test portions), five 130 g and the five 65 g using the LCF MPN Calculator version 1.6 provided by AOAC-RI http://www.lcfltd.com/customer/LCFMPNCalculator

Table 6C: MPN Summary Table for Raw Beef Trim

Raw Beef Trim (325 g) *E. coli* O26 ATCC BAA-1653					
Low Level Inoculum (0.2-2 MPN/Test Portion)					
	A	**B**	**C**	**D**	**E**
650 g	-	+	+	-	+
20 x 325 g (Reference Samples)	13/20				
130 g	-	+	+	-	-
MPN/Test portion	0.89				
Low Conf. Limit MPN/Test Portion	0.52				
High Conf. Limit MPN/Test Portion	1.51				
High Level Inoculum (2-5 MPN/Test Portion)					
	A	**B**	**C**	**D**	**E**
5 x 325 g (Reference Samples)	5/5				
130 g	+	+	+	+	+
65 g	-	+	-	+	+
MPN/Test portion	2.58				
Low Conf. Limit MPN/Test Portion	1.15				
High Conf. Limit MPN/Test Portion	5.78				

*MPN was calculated for the low level inoculation for raw beef trim using five 650 g, twenty 325 g (reference method test portions), and five 130 g using the LCF MPN Calculator version 1.6 provided by AOAC-RI http://www.lcfltd.com/customer/LCFMPNCalculator

*MPN was calculated for the high level inoculation for raw beef trim using five 325 g (reference method test portions), five 130 g and the five 65 g using the LCF MPN Calculator version 1.6 provided by AOAC-RI http://www.lcfltd.com/customer/LCFMPNCalculator

Table 7: *mericon*E. coliO157Screen PlusPathogen Detection Assay for Fresh Spinach

Fresh Spinach (200 g) *E. coli* O111 MSU TW06315				
Low Level **0.61 (0.33, 1.02) MPN/Test Portion**				
Sample #	**manual *mericon*®** **Time Point** **10 Hour**	**QIASymphony*mericon*®** **Time Point** **10 Hour**	**Confirmation** **Time Point** **10 Hour**	**FDA/BAM Chapter 4A**
1	-	-	-	+
2	+	+	+	+
3	+	+	+	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	+
7	+	+	+	+
8	+	+	+	+
9	-	-	-	-
10	-	-	-	-
11	-	-	-	-
12	+	+	+	-
13	+	+	+	+
14	-	-	-	+
15	-	-	-	+
16	-	-	-	-
17	-	-	-	-
18	-	-	-	-
19	+	+	+	-
20	-	-	-	+
Total	**7/20**	**7/20**	**7/20**	**9/20**
High Level **3.70 (1.52, 9.02) MPN/Test Portion**				
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
Total	**5/5**	**5/5**	**5/5**	**5/5**
Uninoculated				
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	**0/5**	**0/5**	**0/5**	**0/5**

Table 8: *mericon*E. coliSTEC O-Type PathogenDetection Assay for Fresh Spinach

Fresh Spinach (200 g) *E. coli* O111 MSU TW06315				
Low Level **0.61 (0.33, 1.02) MPN/Test Portion**				
Sample #	**manual *mericon*®** **Time Point** **10 Hour**	**QIASymphony*mericon*®** **Time Point** **10 Hour**	**Confirmation** **Time Point** **10 Hour**	**FDA/BAM Chapter 4A**
1	-	-	-	+
2	+	+	+	+
3	+	+	+	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	+
7	+	+	+	+
8	+	+	+	+
9	-	-	-	-
10	-	-	-	-
11	-	-	-	-
12	+	+	+	-
13	+	+	+	+
14	-	-	-	+
15	-	-	-	+
16	-	-	-	-
17	-	-	-	-
18	-	-	-	-
19	+	+	+	-
20	-	-	-	+
Total	**7/20**	**7/20**	**7/20**	**9/20**
High Level **3.70 (1.52, 9.02) MPN/Test Portion**				
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
Total	**5/5**	**5/5**	**5/5**	**5/5**
Uninoculated				
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	**0/5**	**0/5**	**0/5**	**0/5**

Table 9: *mericon*E. coliO157Screen PlusPathogen Detection Assay for Raw Ground Beef (70% Lean)

Raw Ground Beef (70% Lean) (325 g) *E. coli* O157:H7 ATCC 43895				
Low Level **0.75 (0.44, 1.23) MPN/Test Portion**				
Sample #	**manual *mericon***	**QIASymphony*mericon*®**	**Confirmation**	**USDA/FSIS-MLG 5.09***
	Time Point	**Time Point**	**Time Point**	
	10 Hour	**10 Hour**	**10 Hour**	
1	+	+	+	+
2	-	-	-	-
3	+	+	+	+
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	+	+	+	+
8	-	-	-	-
9	-	-	-	-
10	+	+	+	+
11	-	-	-	-
12	+	+	+	+
13	+	+	+	+
14	-	-	-	-
15	+	+	+	+
16	+	+	+	+
17	-	-	-	-
18	+	+	+	+
19	-	-	-	-
20	-	-	-	-
Total	**9/20**	**9/20**	**9/20**	**9/20**
High Level **3.70 (1.52, 9.02) MPN/Test Portion**				
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
Total	**5/5**	**5/5**	**5/5**	**5/5**
Uninoculated				
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	**0/5**	**0/5**	**0/5**	**0/5**

***Paired test portions were used in accordance with the approved study protocol

Table 10: *mericon*E. coliSTEC O-Type PathogenDetection Assay for Raw Ground Beef (70% Lean)

Raw Ground Beef (70% Lean) (325 g) *E. coli* O157:H7 ATCC 43895				
Low Level **0.75 (0.44, 1.23) MPN/Test Portion**				
Sample #	**manual *mericon***	**QIASymphony*mericon*®**	**Confirmation**	**USDA/FSIS-MLG 5.09***
	Time Point	**Time Point**	**Time Point**	
	10 Hour	**10 Hour**	**10 Hour**	
1	+	+	+	+
2	-	-	-	-
3	+	+	+	+
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	+	+	+	+
8	-	-	-	-
9	-	-	-	-
10	+	+	+	+
11	-	-	-	-
12	+	+	+	+
13	+	+	+	+
14	-	-	-	-
15	+	+	+	+
16	+	+	+	+
17	-	-	-	-
18	+	+	+	+
19	-	-	-	-
20	-	-	-	-
Total	**9/20**	**9/20**	**9/20**	**9/20**
High Level **3.70 (1.52, 9.02) MPN/Test Portion**				
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
Total	**5/5**	**5/5**	**5/5**	**5/5**
Uninoculated				
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	**0/5**	**0/5**	**0/5**	**0/5**

***Paired test portions were used in accordance with the approved study protocol

Table 11: *mericon*E. coli O157Screen Plus PathogenDetection Assay for Raw Beef Trim

Raw Beef Trim (325 g) *E. coli* O26 ATCC BAA-1653				
Low Level **0.89 (0.52, 1.51) MPN/Test Portion**				
Sample #	**manual *mericon***	**QIASymphony*mericon*®**	**Confirmation**	**USDA/FSIS-MLG 5B.05***
	Time Point	**Time Point**	**Time Point**	
	10 Hour	**10 Hour**	**10 Hour**	
1	+	+	+	+
2	+	+	+	+
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	+	+	+	+
7	+	+	+	+
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+
11	-	-	-	-
12	+	+	+	+
13	-	-	-	-
14	+	+	+	+
15	-	-	-	-
16	+	+	+	+
17	-	-	-	-
18	+	+	+	+
19	+	+	+	+
20	+	+	+	+
Total	**13/20**	**13/20**	**13/20**	**13/20**
High Level **2.38 (1.15, 5.78) MPN/Test Portion**				
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
Total	**5/5**	**5/5**	**5/5**	**5/5**
Uninoculated				
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	**0/5**	**0/5**	**0/5**	**0/5**

***Paired test portions were used in accordance with the approved study protocol

Table 12: *mericon*E. coliSTEC O-Type PathogenDetection Assay for Raw Beef Trim

Raw Beef Trim (325 g) *E. coli* O26 ATCC BAA-1653				
Low Level **0.89 (0.52, 1.51) MPN/Test Portion**				
Sample #	**manual *mericon***	**QIASymphony*mericon*®**	**Confirmation**	**USDA/FSIS-MLG 5B.05***
	Time Point	**Time Point**	**Time Point**	
	10 Hour	**10 Hour**	**10 Hour**	
1	+	+	+	+
2	+	+	+	+
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	+	+	+	+
7	+	+	+	+
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+
11	-	-	-	-
12	+	+	+	+
13	-	-	-	-
14	+	+	+	+
15	-	-	-	-
16	+	+	+	+
17	-	-	-	-
18	+	+	+	+
19	+	+	+	+
20	+	+	+	+
Total	**13/20**	**13/20**	**13/20**	**13/20**
High Level **2.38 (1.15, 5.78) MPN/Test Portion**				
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
Total	**5/5**	**5/5**	**5/5**	**5/5**
Uninoculated				
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
Total	**0/5**	**0/5**	**0/5**	**0/5**

***Paired test portions were used in accordance with the approved study protocol

Table 13: Manual *mericon*E. coli O157Screen Plus PathogenDetection Assay, Candidate vs. Reference – POD Results

Matrix	Strain	Analysis Time Point	MPN[a]/ Test Portion	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Fresh Spinach	*E. coli* O111 MSU TW06315	10 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	7	0.35	0.18, 0.57	9	0.45	0.26, 0.66	-0.10	-0.37, 0.19
			3.70 (1.52, 9.02)	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]**	**Candidate**			**Reference***			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Raw Ground Beef (70% Lean)	*E. coli* O157:H7 ATCC 43895	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.75 (0.44, 1.23)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.28, 0.28
			3.70 (1.52, 9.02)	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]**	**Candidate**			**Reference***			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Raw Beef Trim	*E. coli* O26 ATCC BAA-1653	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.89 (0.52, 1.51)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

*****Reference-paired test portions used
[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 14: QIASymphony*mericon*E. coliScreen Plus PathogenDetection Assay, Candidate vs. Reference – POD Results

Matrix	Strain	Analysis Time Point	MPN[a]/ Test Portion	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Fresh Spinach	*E. coli* O111 MSU TW06315	10 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	7	0.35	0.18, 0.57	9	0.45	0.26, 0.66	-0.10	-0.37, 0.19
			3.70 (1.52, 9.02)	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]**	**Candidate**			**Reference***			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Raw Ground Beef (70% Lean)	*E. coli* O157:H7 ATCC 43895	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.75 (0.44, 1.23)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.28, 0.28
			3.70 (1.52, 9.02)	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]**	**Candidate**			**Reference***			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Raw Beef Trim	*E. coli* O26 ATCC BAA-1653	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.89 (0.52, 1.51)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

*****Reference-paired test portions used
[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 15: Manual *mericon*E. coliSTEC O-Type PathogenDetection Assay, Candidate vs. Reference – POD Results

Matrix	Strain	Analysis Time Point	MPN[a]/ Test Portion	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Fresh Spinach	*E. coli* O111 MSU TW06315	10 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	7	0.35	0.18, 0.57	9	0.45	0.26, 0.66	-0.10	-0.37, 0.19
			3.70 (1.52, 9.02)	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]**	**Candidate**			**Reference***			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Raw Ground Beef (70% Lean)	*E. coli* O157:H7 ATCC 43895	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.75 (0.44, 1.23)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.28, 0.28
			3.70 (1.52, 9.02)	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]**	**Candidate**			**Reference***			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Raw Beef Trim	*E. coli* O26 ATCC BAA-1653	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.89 (0.52, 1.51)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

*****Reference-paired test portions used
[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 16: QIASymphony*mericon*E. coliSTEC O-Type PathogenDetection Assay, Candidate vs. Reference – POD Results

Matrix	Strain	Analysis Time Point	MPN[a]/ Test Portion	N[b]	Candidate			Reference			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	X	POD$_R$[e]	95% CI		
Fresh Spinach	*E. coli* O111 MSU TW06315	10 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	7	0.35	0.18, 0.57	9	0.45	0.26, 0.66	-0.10	-0.37, 0.19
			3.70 (1.52, 9.02)	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]**	**Candidate**			**Reference***			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Raw Ground Beef (70% Lean)	*E. coli* O157:H7 ATCC 43895	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.75 (0.44, 1.23)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.28, 0.28
			3.70 (1.52, 9.02)	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]**	**Candidate**			**Reference***			**dPOD$_C$[f]**	**95% CI[g]**
					x[c]	**POD$_C$[d]**	**95% CI**	**X**	**POD$_R$[e]**	**95% CI**		
Raw Beef Trim	*E. coli* O26 ATCC BAA-1653	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.89 (0.52, 1.51)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

*****Reference-paired test portions used
[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 17: Manual *mericon*E. coliO157 Screen Plus PathogenDetection Assay, Presumptive vs. Confirmed – POD Results

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		
Fresh Spinach	*E. coli* O111 MSU TW06315	10 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	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.70 (1.52, 9.02)	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 Ground Beef (70% Lean)	*E. coli* O157:H7 ATCC 43895	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.75 (0.44, 1.23)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.28, 0.28
			3.70 (1.52, 9.02)	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 Beef Trim	*E. coli* O26 ATCC BAA-1653	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.89 (0.52, 1.51)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			2.58 (1.15, 5.78)	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 18: QIASymphony*mericon*E. coliO157Screen Plus PathogenDetection Assay, Presumptive vs. Confirmed – POD Results

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		
Fresh Spinach	*E. coli* O111 MSU TW06315	10 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	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.70 (1.52, 9.02)	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 Ground Beef (70% Lean)	*E. coli* O157:H7 ATCC 43895	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.75 (0.44, 1.23)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.28, 0.28
			3.70 (1.52, 9.02)	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 Beef Trim	*E. coli* O26 ATCC BAA-1653	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.89 (0.52, 1.51)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			2.58 (1.15, 5.78)	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 19: Manual *mericon*E. coliSTEC O-Type PathogenDetection Assay, Presumptive vs. Confirmed – POD Results

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		
Fresh Spinach	*E. coli* O111 MSU TW06315	10 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	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.70 (1.52, 9.02)	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 Ground Beef (70% Lean)	*E. coli* O157:H7 ATCC 43895	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.75 (0.44, 1.23)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.28, 0.28
			3.70 (1.52, 9.02)	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 Beef Trim	*E. coli* O26 ATCC BAA-1653	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.89 (0.52, 1.51)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			2.58 (1.15, 5.78)	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 20: QIASymphony*mericon*E. coliSTEC O-Type PathogenDetection Assay, Presumptive vs. Confirmed – POD Results

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		
Fresh Spinach	*E. coli* O111 MSU TW06315	10 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	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.70 (1.52, 9.02)	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 Ground Beef (70% Lean)	*E. coli* O157:H7 ATCC 43895	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.75 (0.44, 1.23)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.28, 0.28
			3.70 (1.52, 9.02)	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 Beef Trim	*E. coli* O26 ATCC BAA-1653	10 Hours	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.89 (0.52, 1.51)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			2.58 (1.15, 5.78)	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 21: Robustness Results for the *mericon*E. coli O157Screen Plus Pathogen Detection Assay

Treatment Combination	1	2	3	4	5	6	7	8	9
Incubation Time	8 Hours	8 Hours	8 Hours	8 Hours	10 Hours	10 Hours	10 Hours	10 Hours	9 Hours
Lysis Time	8 Minutes	8 Minutes	12 Minutes	12 Minutes	8 Minutes	8 Minutes	12 Minutes	12 Minutes	10 Minutes
[1]MM:DNA Ratio (µL)	12:8	8:12	12:8	8:12	12:8	8:12	12:8	8:12	10:10
Sample #	Inoculated								
1	-	+	-	+	+	+	+	+	+
2	-	-	+	-	+	+	+	+	+
3	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+
5	-	-	-	-	-	-	-	-	-
6	-	+	-	-	+	+	+	+	+
7	-	-	-	-	-	-	-	-	-
8	-	+	-	-	+	+	+	+	+
9	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-
Total Positives	1/10	4/10	2/10	2/10	5/10	5/10	5/10	5/10	5/10
Sample #	Uninoculated								
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-
Total Positives	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

[1]MM= Master Mix

Table 22: Robustness Results for the *mericon*E. coli STEC O-Type Pathogen Detection Assay

Treatment Combination	1	2	3	4	5	6	7	8	9
Incubation Time	8 Hours	8 Hours	8 Hours	8 Hours	10 Hours	10 Hours	10 Hours	10 Hours	9 Hours
Lysis Time	8 Minutes	8 Minutes	12 Minutes	12 Minutes	8 Minutes	8 Minutes	12 Minutes	12 Minutes	10 Minutes
[1]MM:DNA Ratio (µL)	12:8	8:12	12:8	8:12	12:8	8:12	12:8	8:12	10:10
Sample #	Inoculated								
1	-	-	-	-	+	+	+	+	+
2	-	-	-	-	+	+	+	+	+
3	-	-	-	-	-	-	-	-	-
4	-	-	-	-	+	+	+	+	+
5	-	-	-	-	-	-	-	-	-
6	-	-	-	-	+	+	+	+	-
7	-	-	-	-	-	-	-	-	-
8	-	-	-	-	+	+	+	+	-
9	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-
Total Positives	0/10	0/10	0/10	0/10	5/10	5/10	5/10	5/10	3/10
Sample #	Uninoculated								
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-
Total Positives	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

[1]MM= Master Mix

APPENDIX 2
*Salmonella spp*workflow
Stability and lot-to-lot variation

Figure 1. Primer/probe and Master Mix lot consistency study with Manual Bacteria DNA Preparation

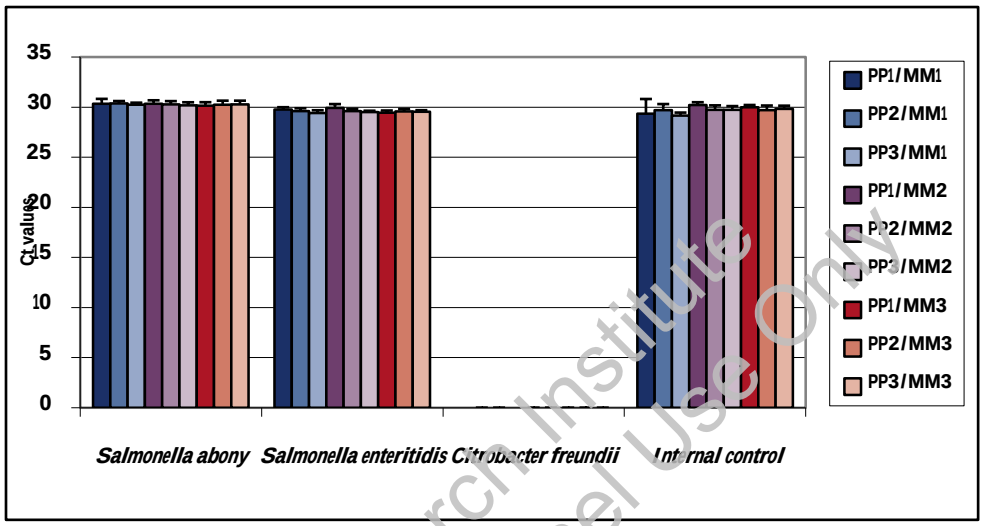


Figure1: Ct values for all primer/probe (PP) and master mix (MM) lot combinations for *Salmonella abony, Salmonella enteritidis, C. freundii* and the internal control were not significantly different, indicating that there were no effects of individual lots of primer/probes and master mix on the detection results

Figure 2. Summary: Lot consistency study with Manual Bacteria DNA Preparation

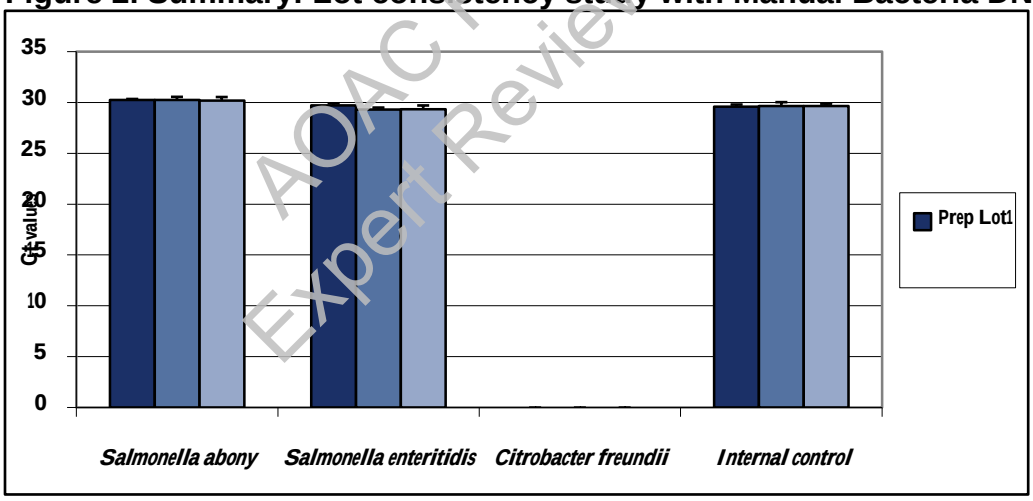


Figure 2: Ct values for *Salmonella abony, Salmonella enteritidis, C. freundii* and the internal control were not significantly different, indicating that there were no effects of the lot of the *mericon*Fast Lysis Buffer

Figure 3: Summary Rotor-Gene Q instrument variation

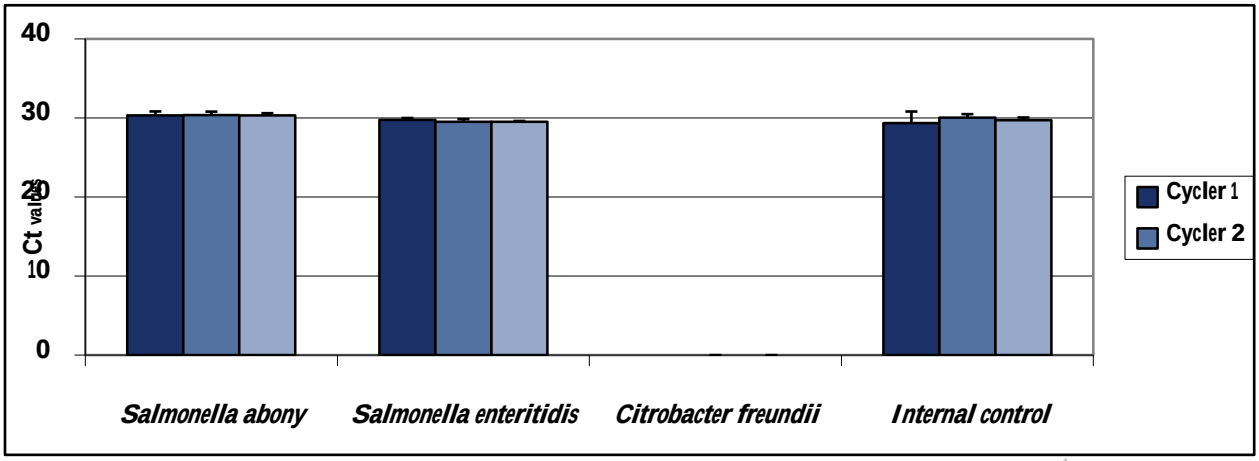


Figure 3: Ct values for *Salmonella abony, Salmonella enteritidis, C. freundii* and the internal control were not significantly different, indicating that there was no effect of the Rotor-Gene Q instruments on the results obtained

Figure 4: Primer/probe and Master Mix lot consistency study with Automated Bacteria DNA Preparation

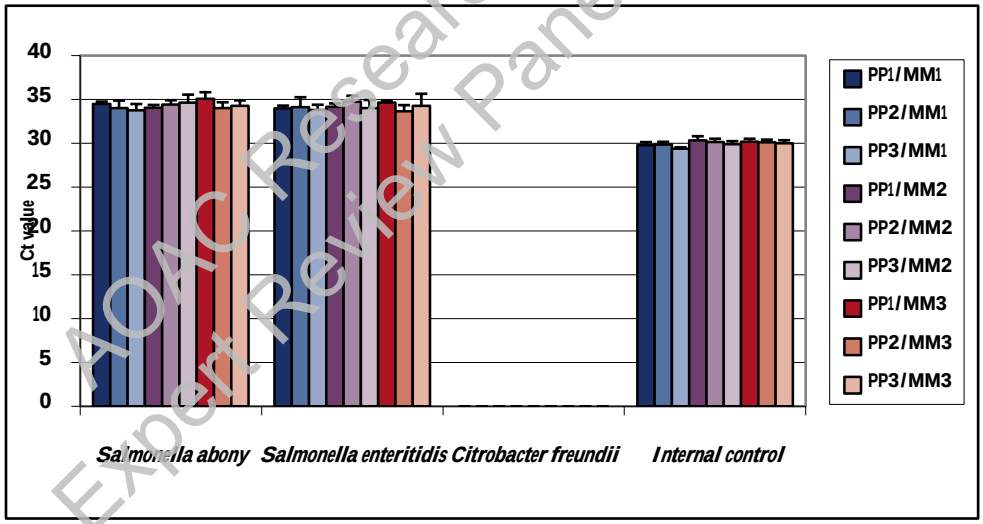


Figure 4: Ct values for all primer/probe (PP) and master mix (MM) lot combinations for *Salmonella abony, Salmonella enteritidis, and C. freundii* were not significantly different, indicating that there were no effects of individual lots of primer/probes and master mix on the detection results

Figure 5: Summary of sample prep lot variation Automated sample prep

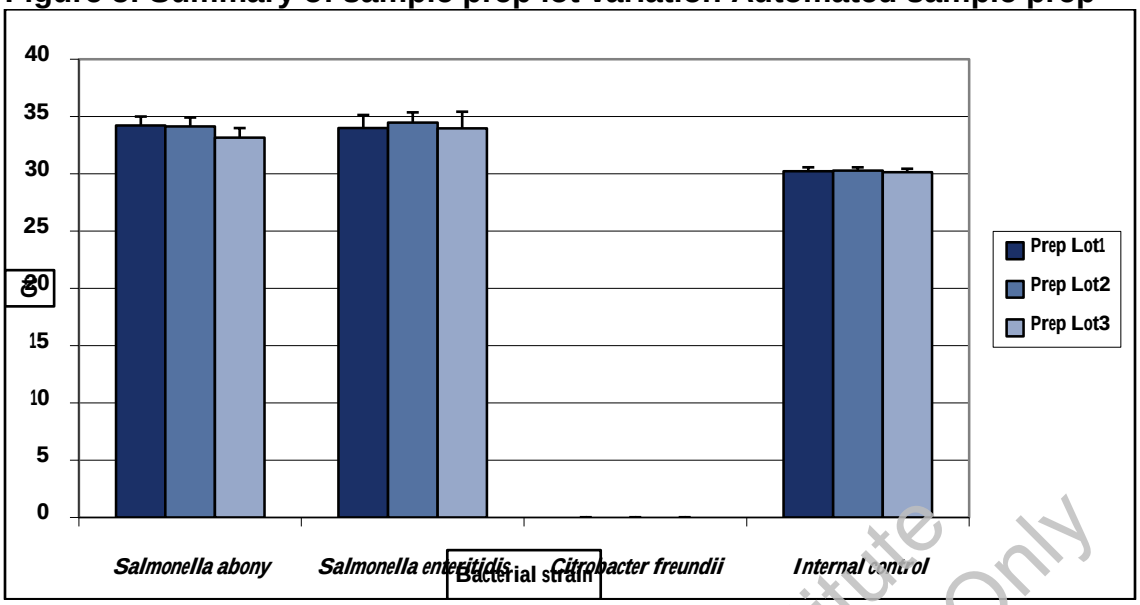


Figure 5: Ct values for *Salmonella abony, Salmonella enteritidis, C. freundii* and the internal control were not significantly different, indicating that there were no effects of the lot of the QIAsymphony Bacteria kit on the detection.

Figure 6: Rotor-Gene Q instrument variation with automated sample prep

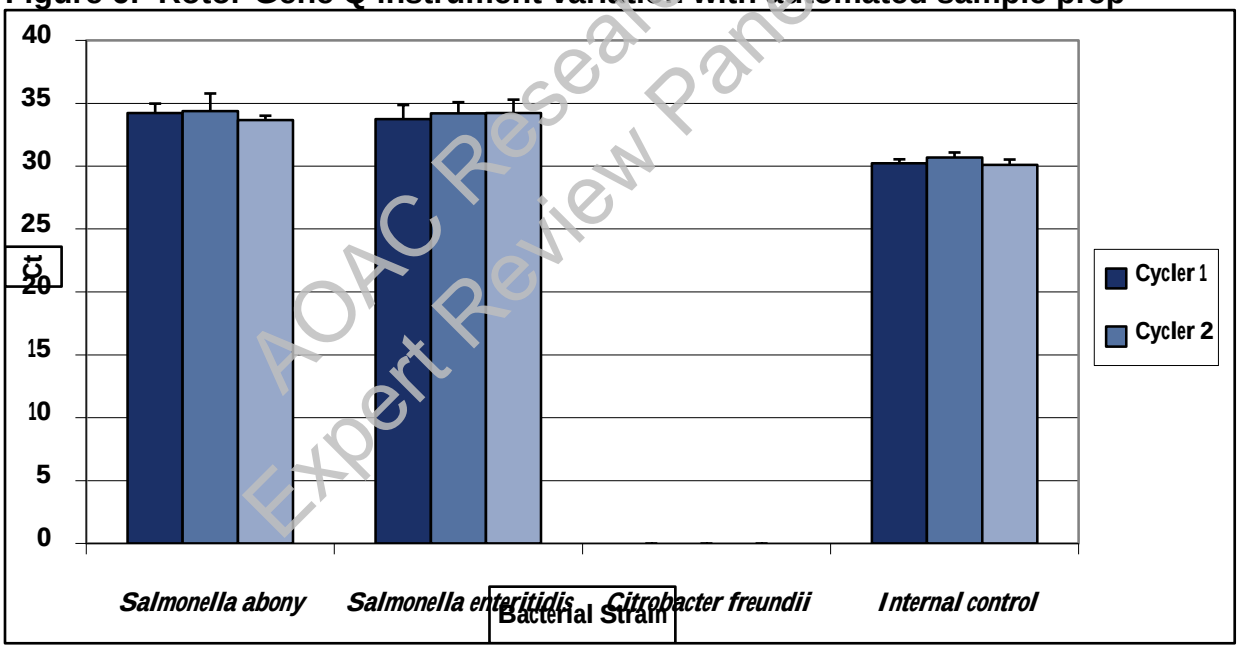


Figure 6: Ct values for *Salmonella abony, Salmonella enteritidis, C. freundii* and the internal control were not significantly different, indicating that there was no effect of the Rotor-Gene Q instruments on the results obtained

Figure7: Summary data: Top: Green channel (*Salmonella* detection) Lower: Yellow channel (internal control)

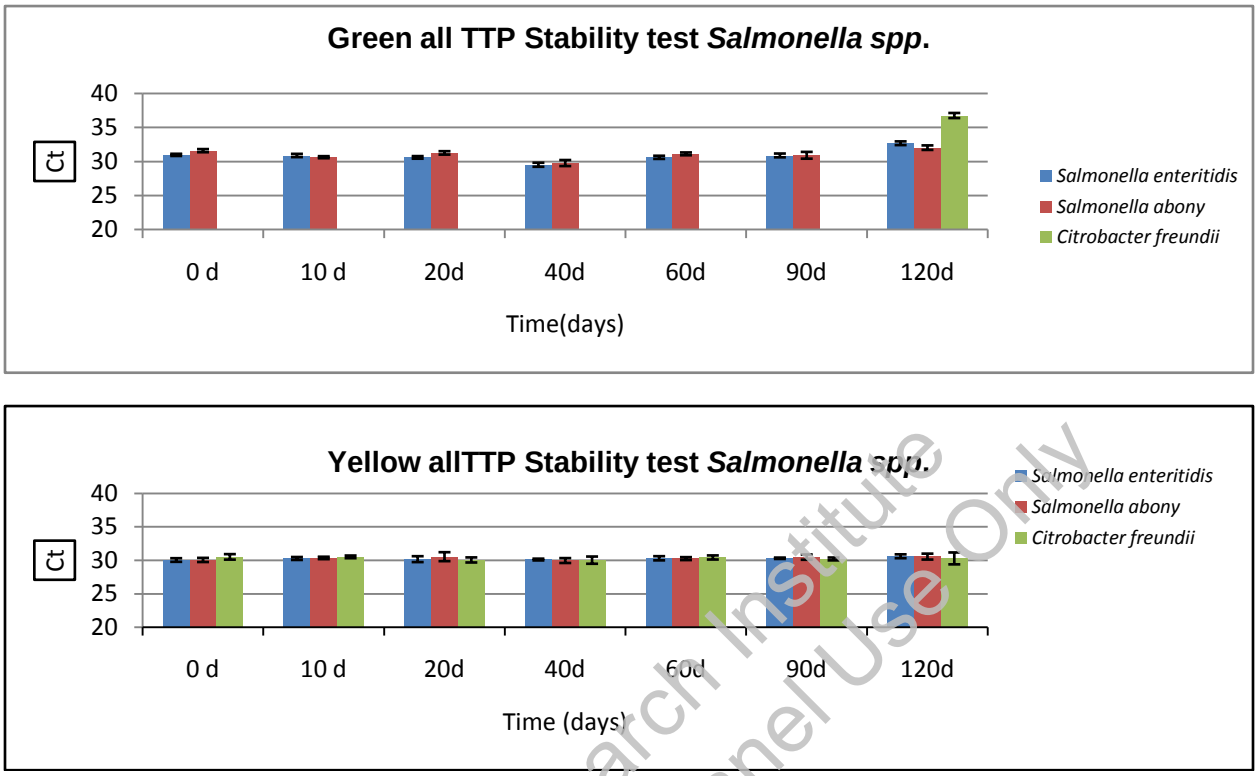


Figure7: Ct values from the accelerated aging studies showed no slope over the course of the 120 day accelerated aging study in either the *Salmonella* (green) detection channel or the internal control (yellow) channel. There was a weak green signal in the *C. freundii* channel at 120 days, attributed to cross contamination in the culture. (TTP: Test time point)

Method developer summary data shelf life QIAsymphony DNA Midi Kit

The QIAsymphony Bacteria Kit used in these studies is based on the QIAsymphony DNA Midi Kit. All cartridge chemicals are identical, except for the Proteinase K that is included in the QIAsymphony DNA Midi Kit but omitted from the QIAsymphony Bacteria Kit. Proteinase K is not required for the simple heated lysis implemented in the QIAsymphony Bacteria Kit. It is used in the reference DNA Midi Kit to disrupt the membranes of animal and human cells and tissues. The inclusion of the Proteinase K in the kits for functional testing did not show any changes over the storage of the DNA Midi Kit. One can reasonably assume that the other components of the parent kit (magnetic beads, lysis, binding and elution buffers) that remain constant between the kits would be stable for the same 24 month period. We are providing stability data on the QIAsymphony DNA

Midi Kit in support of the shelf life claims for the QIAsymphony Bacteria Kit. These studies were performed in the Method Developer's R&D laboratories in Hilden, Germany.

Table 4. Shelf life QIAsymphony DNA Midi Kit

Data shown covers functional testing of kits stored over a 24 month span at 25°C; data for accelerated aging at 45°C is shown for the first 4 months of the study.

Summary of functional testing of the QIAsymphony DNA Midi Kit

TTP [month]	Temp	Date of Extraction	µg Yield (Mean of replicates)	Theoret. Yield	CV (%)	Ratio 260nm / 280nm	Hugl PCR (positives)	DNA Integrity		Assessment	
								OK	Not OK	OK	Not OK
0	-	27.03.2008	30,63	91,66%	2,86	1,86	24/24	24/24	-	√	-
2	5°C	20.05.2008	26,56	64,14%	3,4	1,87	24/24	24/24	-	√	-
	25°C	20.05.2008	25,1	60,62%	4,28	1,86	24/24	24/24	-	√	-
	45°C	20.05.2008	25,16	60,76%	3,75	1,86	24/24	24/24	-	√	-
4	45°C	24.07.2008	31,89	67,93%	5,74	1,83	24/24	24/24	-	√	-
8	25°C	19.11.2008	30,14	70,69%	4,8	1,85	24/24	24/24	-	√	-
10	25°C	20.01.2009	31,44	93,40%	5,53	1,84	23/24	24/24	-	√	-
13	5°C	22.04.2009	30,89	72,01%	5,7	1,83	23/24	24/24	-	√	-
	25°C	22.04.2009	30,11	70,19%	3,36	1,83	24/24	24/24	-	√	-
16	25°C	04.08.2009	24,87	64,98%	4,64	1,82	24/24	24/24	-	√	-
19	25°C	13.10.2009	28,51	59,99%	6,86	1,86	24/24	24/24	-	√	-
24	25°C	23.03.2010	30,48	63,25%	6,78	1,83	24/24	24/24	-	√	-
25	5°C	28.04.2010	25,42	75,52%	5,18	1,74	24/24	24/24	-	-	X*
	25°C	28.04.2010	25,11	74,60%	5,25	1,75	24/24	24/24	-	√	-
WDH	25°C	01.06.2010	38,19	73,24	4,55	1,84	40524	12/12	-	√	-

X out of specification but accepted

Acceptance criteria for yield, purity, integrity and inhibition were met for cartridges stored up to 4 month at 45°C and up to 25 month at 5 and 25°C for all tested TTPs (besides for TTP 25M, 5°C). All deviations observed could be resolved.

* = accepted as real time testing for this final TTP was within specification.

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TTP: test time point
WDH: repetition data point