AOAC Official Methods of Analysis℠ (OMA)

AOAC EXPERT REVIEW PANEL

FOR MICROBIOLOGY FOR FOODS AND ENVIRONMENTAL SURFACES

TUESDAY, SEPTEMBER 20, 2016
8:30AM – 12:00PM
MEETING ROOM: STATE 4

2016 AOAC ANNUAL MEETING & EXHIBITION
SHERATON DALLAS HOTEL
400 N. OLIVE STREETDALLAS, TEXAS 75201 USA

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

TABLE OF CONTENTS

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

AOAC ANNUAL MEETING & EXPOSITION
Sheraton Dallas Hotel
400 N. Olive Street, Dallas, TX 75201 USA

Tuesday, September 20, 2016
Meeting Room: State 4
8:30AM – 12:00PM

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. 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.

1) **2014.01** *Salmonella* in Selected Foods, 3M™ Petrifilm™ Salmonella Express System
2) **2014.05** Enumeration of Yeast and Mold in Food, 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate
3) **2014.06*** *Listeria species* in Selected Foods and Environmental Surfaces, 3M™ Molecular Detection Assay (MDA) *Listeria*
4) **2014.07*** *Listeria monocytogene*s in Selected Foods and Environmental Surfaces, 3M™ Molecular Detection Assay (MDA) *Listeria monocytogenes*
These methods were modified in March, 2016 to revise the applicability statements. These methods are now AOAC First Action methods until 2018.

IV. Next Steps and Upcoming Meetings

V. Adjournment

**Agenda is subject to change. V1
WI-FI Name: AOAC Annual Meeting
Password: AOAC2016

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.

* * * * * *

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

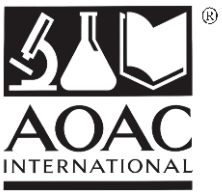
AOAC INTERNATIONAL
POLICY ON THE USE OF THE ASSOCIATION NAME, INITIALS, IDENTIFYING INSIGNIA, LETTERHEAD, AND BUSINESS CARDS

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.

First Action Method Updates

Expert Review Panel Tracking and Recommendations of First Action Methods

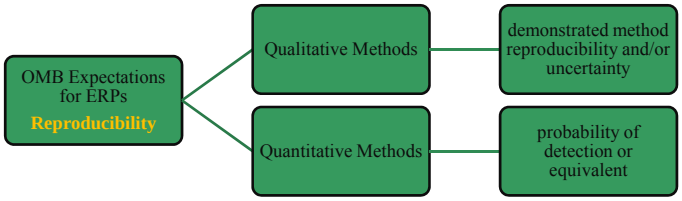
AOAC Policies & Procedures

- 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

OMA, Appendix G

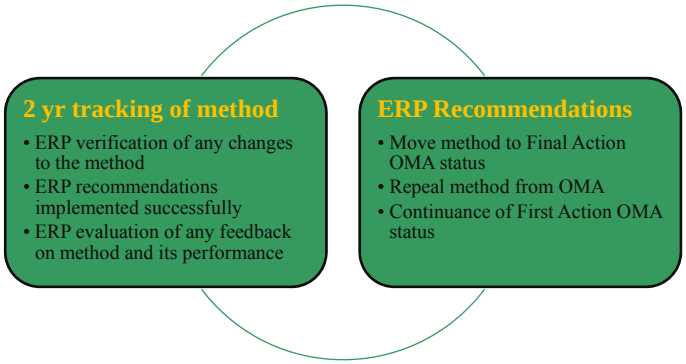
Further data indicative of adequate method reproducibility (between laboratory) performance to be collected. Data may be collected via a collaborative study or by proficiency or other testing data of similar magnitude.

- 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).



AOAC RESEARCH INSTITUTE

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

AOAC RESEARCH INSTITUTE

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

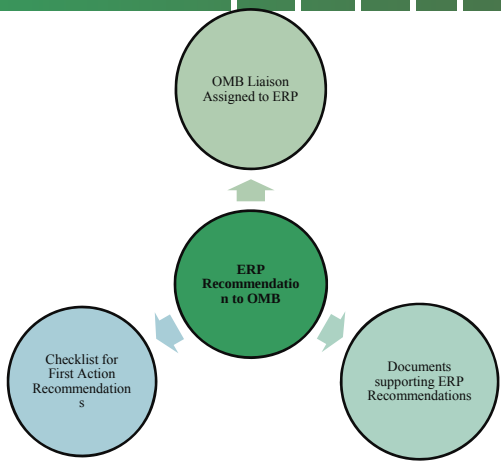
- Tracking period is ≤ 2 years and begins on the date of the ERP's decision to adopt a method for OMA First Action status.

No Demonstration of Method Reproducibility in ≤ 2 Years

- Repeal from OMA

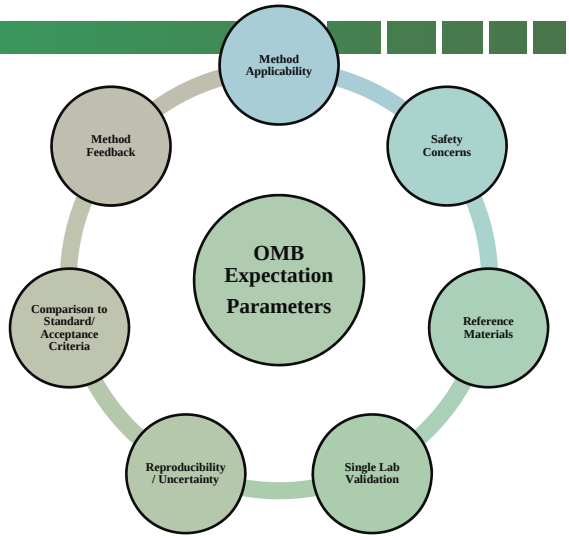
OMA, Appendix G

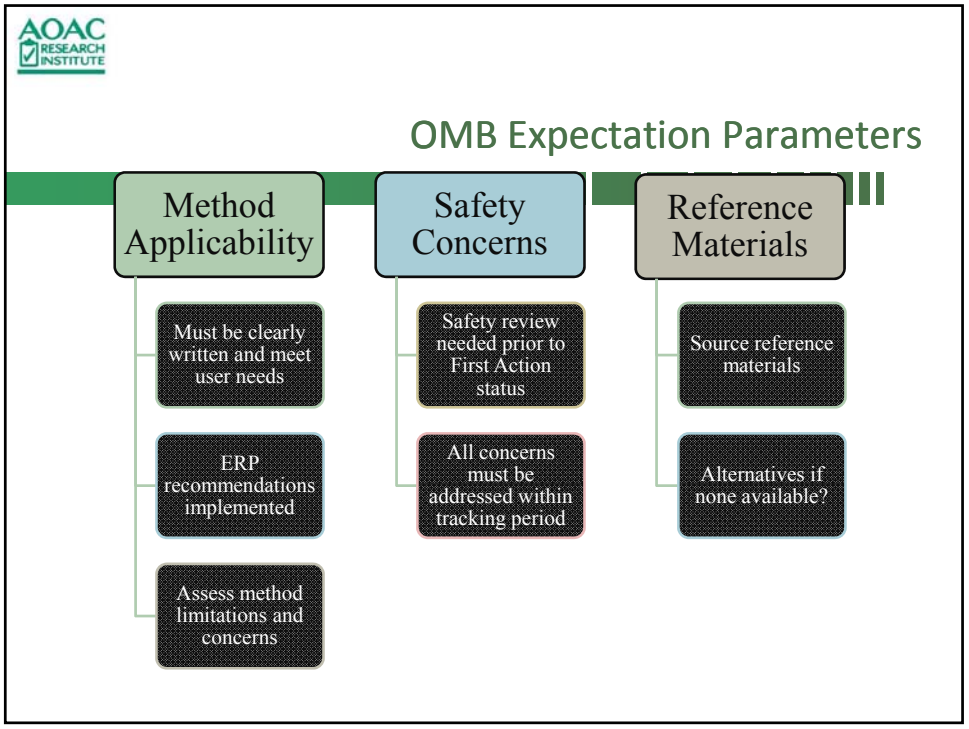
ERP to recommend Method to Official Final Action Status to the OMB.

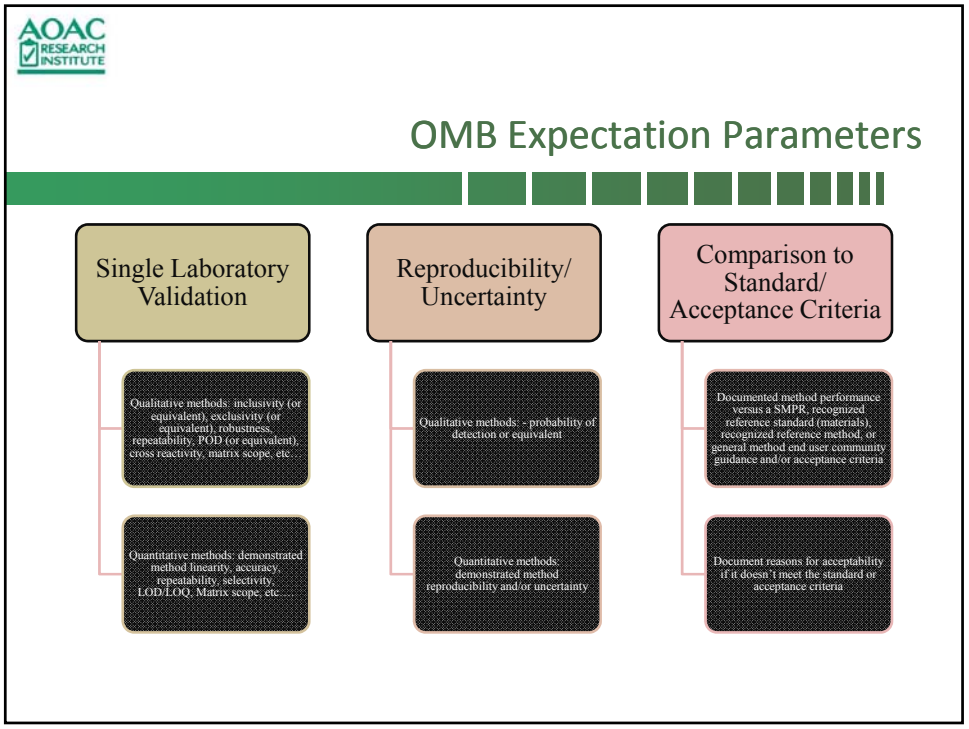


OMA, Appendix G

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




AOAC RESEARCH INSTITUTE
OMB Expectation Parameters
Method Applicability
Must be clearly written and meet user needs
ERP recommendations implemented
Assess method limitations and concerns
Safety Concerns
Safety review needed prior to First Action status
All concerns must be addressed within tracking period
Reference Materials
Source reference materials
Alternatives if none available?


AOAC RESEARCH INSTITUTE
OMB Expectation Parameters
Single Laboratory Validation
Qualitative methods: inclusivity (or equivalent), exclusivity (or equivalent), robustness, repeatability, POD (or equivalent), cross reactivity, matrix scope, etc…
Quantitative methods: demonstrated method linearity, accuracy, repeatability, selectivity, LOD/LOQ, Matrix scope, etc…
Reproducibility/ Uncertainty
Qualitative methods: - probability of detection or equivalent
Quantitative methods: demonstrated method reproducibility and/or uncertainty
Comparison to Standard/ Acceptance Criteria
Documented method performance versus a SMPR, recognized reference standard (materials), recognized reference method, or general method end user community guidance and/or acceptance criteria
Document reasons for acceptability if it doesn't meet the standard or acceptance criteria

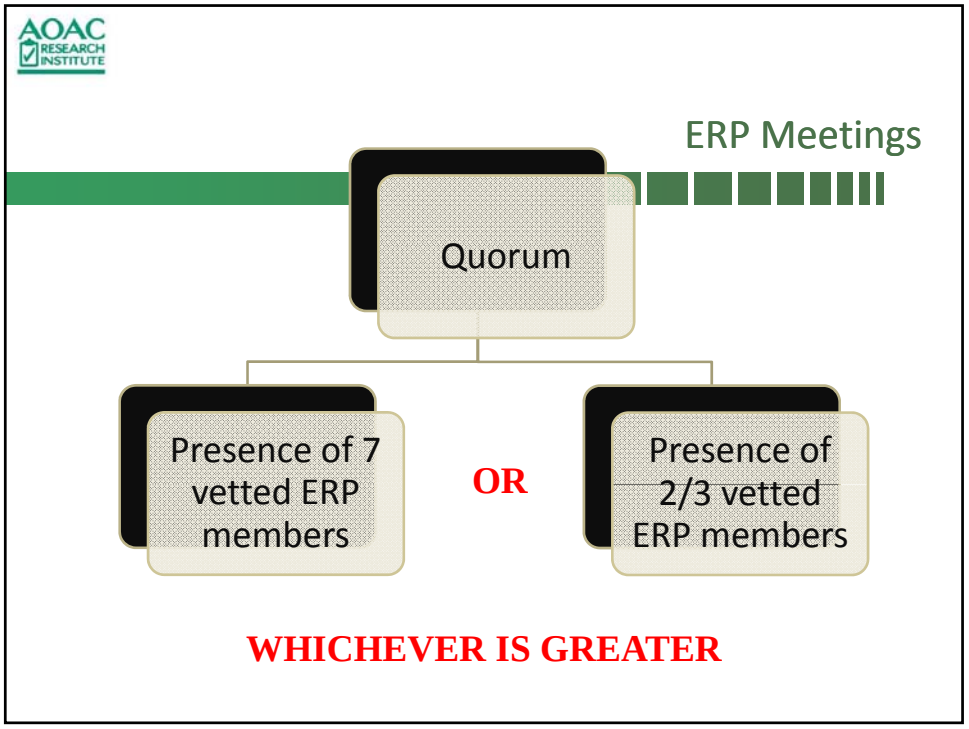
OMB Expectation Parameters

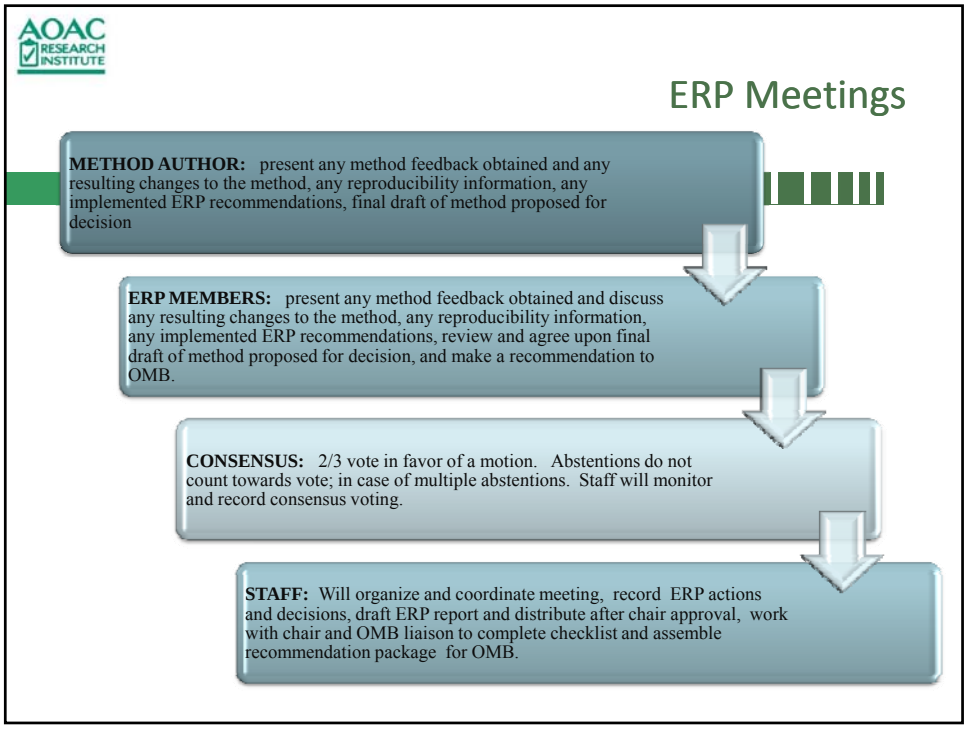
Method Feedback from End Users

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


AOAC RESEARCH INSTITUTE
ERP Meetings
Quorum
Presence of 7 vetted ERP members
OR
Presence of 2/3 vetted ERP members
WHICHEVER IS GREATER


AOAC RESEARCH INSTITUTE
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.

AOAC Official Method 2014.01
Salmonella in Selected Foods
3M™ Petrifilm™ *Salmonella* Express System
First Action 2014

[Applicable to detection of *Salmonella* spp. in raw ground beef (25 g), raw ground chicken (25 g), pasteurized liquid whole egg (100 g), raw ground pork (25 g), cooked chicken nuggets (325 g), frozen uncooked shrimp (25 g), fresh bunched spinach (25 g), dry dog food (375 g), and stainless steel. Not applicable to some lactose-positive *Salmonella* species.]

See Tables **2014.01A** and **B** for results of the interlaboratory study supporting acceptance of the method. *See* Appendix available on the *J. AOAC Int*. website for detailed tables of results of the collaborative study (http://aoac.publisher.ingentaconnect.com/content/aoac/jaoac).

Caution: Do not use the 3M Petrifilm SALX System method in the diagnosis of conditions in humans or animals. To reduce the risks associated with exposure to chemicals and biohazards, perform pathogen testing in a properly equipped laboratory under the control of trained personnel. Always follow standard good laboratory safety practices (GLP), including proper containment procedures, and wearing appropriate protective apparel and eye protection while handling testing materials and test samples. Avoid direct contact with the contents of the enrichment medium and inoculated plates. Dispose of enrichment media and inoculated plates according to all applicable government regulatory regulations and applicable laboratory procedures. Wear appropriate protective apparel while handling the 3M Petrifilm SALX Plate as some of the components may be considered allergenic and irritants to some individuals.

Table 2014.01A. Summary of results for detection of *Salmonella* in raw ground beef (25 g)

Method[a]	3M Petrifilm *Salmonella* Express System with alternative confirmation			3M Petrifilm *Salmonella* Express System with traditional confirmation		
Inoculation level	Uninoculated	Low	High	Uninoculated	Low	High
Candidate presumptive positive/ total No. of samples analyzed	2/168	85/168	168/168	2/168	85/168	168/168
Candidate presumptive POD (CP)	0.01 (0.00, 0.04)	0.51 (0.43, 0.58)	1.00 (0.98, 1.00)	0.01 (0.00, 0.04)	0.51 (0.43, 0.58)	1.00 (0.98, 1.00)
s_r[b]	0.11 (0.10, 0.15)	0.51 (0.46, 0.52)	0.00 (0.00, 0.15)	0.11 (0.10, 0.15)	0.51 (0.46, 0.52)	0.00 (0.00, 0.15)
s_L[c]	0.00 (0.00, 0.04)	0.00 (0.00, 0.13)	0.00 (0.00, 0.15)	0.00 (0.00, 0.04)	0.00 (0.00, 0.13)	0.00 (0.00, 0.15)
s_R[d]	0.11 (0.10, 0.12)	0.51 (0.47, 0.52)	0.00 (0.00, 0.21)	0.11 (0.10, 0.12)	0.51 (0.47, 0.52)	0.00 (0.00, 0.21)
P-value[e]	0.5158	0.9341	1.0000	0.5158	0.9341	1.0000
Candidate confirmed positive/ total No. of samples analyzed	0/168	83/168	168/168	1/168	83/168	168/168
Candidate confirmed POD (CC)	0.00 (0.00, 0.02)	0.49 (0.42, 0.57)	1.00 (0.98, 1.00)	0.01 (0.00, 0.03)	0.49 (0.42, 0.57)	1.00 (0.98, 1.00)
s_r	0.00 (0.00, 0.15)	0.51 (0.46, 0.52)	0.00 (0.00, 0.15)	0.08 (0.07, 0.15)	0.51 (0.46, 0.52)	0.00 (0.00, 0.15)
s_L	0.00 (0.00, 0.15)	0.00 (0.00, 0.11)	0.00 (0.00, 0.15)	0.00 (0.00, 0.03)	0.00 (0.00, 0.11)	0.00 (0.00, 0.15)
s_R	0.00 (0.00, 0.21)	0.51 (0.47, 0.52)	0.00 (0.00, 0.21)	0.08 (0.07, 0.09)	0.51 (0.47, 0.52)	0.00 (0.00, 0.21)
P-value	1.0000	0.9757	1.0000	0.4418	0.9757	1.0000
Positive reference samples/ total No. of samples analyzed	0/168	86/168	167/168	0/168	86/168	167/168
Reference POD	0.00 (0.00, 0.02)	0.51 (0.43, 0.59)	0.99 (0.97, 1.00)	0.00 (0.00, 0.02)	0.51 (0.43, 0.59)	0.99 (0.97, 1.00)
s_r	0.00 (0.00, 0.15)	0.51 (0.46, 0.52)	0.08 (0.07, 0.15)	0.00 (0.00, 0.15)	0.51 (0.46, 0.52)	0.08 (0.07, 0.15)
s_L	0.00 (0.00, 0.15)	0.00 (0.00, 0.12)	0.00 (0.00, 0.03)	0.00 (0.00, 0.15)	0.00 (0.00, 0.12)	0.00 (0.00, 0.03)
s_R	0.00 (0.00, 0.21)	0.51 (0.47, 0.52)	0.08 (0.07, 0.09)	0.00 (0.00, 0.21)	0.51 (0.47, 0.52)	0.08 (0.07, 0.09)
P-value	1.0000	0.9695	0.4418	1.0000	0.9695	0.4418
dLPOD (candidate vs reference)[f]	0.00 (–0.02, 0.02)	–0.02 (–0.13, 0.09)	0.01 (–0.02, 0.03)	0.01 (–0.02, 0.03)	–0.02 (–0.13, 0.09)	0.01 (–0.02, 0.03)
dLPOD (candidate presumptive vs candidate confirmed)[f]	0.01 (–0.01, 0.04)	0.01 (–0.10, 0.12)	0.00 (–0.02, 0.02)	0.01 (–0.02, 0.04)	0.01 (–0.10, 0.12)	0.00 (–0.02, 0.02)

[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.

To reduce the risks associated with environmental contamination, follow current industry standards and local regulations for disposal of contaminated waste. Consult the Material Safety Data Sheet for additional information. For questions about specific applications or procedures, visit www.3M.com/foodsafety or contact your local 3M representative or distributor. Review the policies recommend by the Centers for Disease Control and Prevention on dealing with pathogens (http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf).

A. Principle

The 3M Petrifilm SALX System is a chromogenic culture medium system that is intended for the rapid and specific detection and biochemical confirmation of *Salmonella* spp. from food and food process environmental samples. After enrichment in prewarmed 3M *Salmonella* Enrichment Base with 3M *Salmonella* Enrichment Supplement, the 3M Petrifilm SALX System provides presumptive positive results in as little as 40 h from low microbial background foods ($<10^4$ CFU/g) and 48 h from high microbial foods ($\geq 10^4$ CFU/g). The 3M Petrifilm SALX System does not specifically differentiate some lactose-positive *Salmonella* species (primarily *S. arizonae* and *S. diarizonae*) from other lactose-positive organisms. Refer to the 3M Petrifilm *Salmonella* Express System Instructions for Use for additional information.

B. Apparatus and Reagents

(**a**) *3M Petrifilm Salmonella Express Plate.*—Twenty-five plates/pouch (3M Food Safety, St. Paul, MN, USA).

(**b**) *3M Petrifilm Salmonella Express Confirmation Disk.*—Five disks/pouch (3M Food Safety).

(**c**) *3M Salmonella Enrichment Base.*—500 g or 2.5 kg/bottle (3M Food Safety).

(**d**) *3M Salmonella Enrichment Supplement.*—1 g/vial (3M Food Safety).

(**e**) *3M Petrifilm Flat Spreader.*—Two spreaders/box (3M Food Safety).

Table 2014.01B. Summary of results for detection of *Salmonella* in dry dog food (375 g)

Method[a]	3M Petrifilm *Salmonella* Express System with alternative confirmation			3M Petrifilm *Salmonella* Express System with traditional confirmation		
Inoculation level	Uninoculated	Low	High	Uninoculated	Low	High
Candidate presumptive positive/ total No. of samples analyzed	0/144	82/144	142/144	0/144	82/144	142/144
Candidate presumptive POD (CP)	0.00 (0.00, 0.03)	0.57 (0.48, 0.66)	0.99 (0.95, 1.00)	0.00 (0.00, 0.03)	0.57 (0.48, 0.66)	0.99 (0.95, 1.00)
s_r[b]	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.12 (0.11, 0.16)	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.12 (0.11, 0.16)
s_L[c]	0.00 (0.00, 0.16)	0.08 (0.00, 0.24)	0.00 (0.00, 0.04)	0.00 (0.00, 0.16)	0.08 (0.00, 0.24)	0.00 (0.00, 0.04)
s_R[d]	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.12 (0.11, 0.13)	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.12 (0.11, 0.13)
P-value[e]	1.0000	0.2242	0.9861	1.0000	0.2242	0.9861
Candidate confirmed positive/ total No. of samples analyzed	0/144	81/144	141/144	0/144	82/144	141/144
Candidate confirmed POD (CC)	0.00 (0.00, 0.03)	0.56 (0.46, 0.66)	0.98 (0.94, 0.99)	0.00 (0.00, 0.03)	0.57 (0.48, 0.67)	0.98 (0.94, 0.99)
s_r	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.14 (0.12, 0.16)	0.00 (0.00, 0.16)	0.49 (0.43, 0.52)	0.14 (0.12, 0.16)
s_L	0.00 (0.00, 0.16)	0.10 (0.00, 0.26)	0.03 (0.00, 0.08)	0.00 (0.00, 0.16)	0.11 (0.00, 0.27)	0.03 (0.00, 0.08)
s_R	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.14 (0.13, 0.17)	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.14 (0.13, 0.17)
P-value	1.0000	0.1290	0.0976	1.0000	0.1114	0.0976
Positive reference samples/ total No. of samples analyzed	0/144	71/144	144/144	0/144	71/144	144/144
Reference POD	0.00 (0.00, 0.03)	0.49 (0.39, 0.59)	1.00 (0.97, 1.00)	0.00 (0.00, 0.03)	0.49 (0.39, 0.59)	1.00 (0.97, 1.00)
s_r	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.00 (0.00, 0.16)	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.10 (0.00, 0.26)	0.00 (0.00, 0.16)	0.00 (0.00, 0.16)	0.10 (0.00, 0.26)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.00 (0.00, 0.22)	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.00 (0.00, 0.22)
P-value	1.0000	0.1550	1.0000	1.0000	0.1550	1.0000
dLPOD (C vs R)[f]	0.00 (–0.03, 0.03)	0.07 (–0.07, 0.21)	–0.02 (–0.06, 0.01)	0.00 (–0.03, 0.03)	0.08 (–0.07, 0.22)	–0.02 (–0.06, 0.01)
dLPOD (CP vs CC)[f]	0.00 (–0.03, 0.03)	0.01 (–0.18, 0.22)	0.01 (–0.03, 0.05)	0.00 (–0.03, 0.03)	0.00 (–0.14, 0.14)	0.01 (–0.03, 0.05)

[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 2014.01C. Sample matrix and enrichment scheme[a]

Sample matrix	Sample size, g	Enrichment broth volume, mL	Enrichment time, h	Secondary enrichment time, h
Raw ground beef (80% lean)	25	225	18–24	8–24
Raw ground chicken	25	225	18–24	8–24
Raw ground pork	25	225	18–24	8–24
Frozen uncooked shrimp	25	225	18–24	8–24
Fresh bunched spinach	25	225	18–24	24
Stainless steel; environmental sponges	1 Sponge (4 × 4 in.)	225	18–24	
Pasteurized liquid whole egg	100	900	18–24	
Cooked breaded chicken	325	2925	18–24	
Dry dog food	375	3375	18–24	

[a] AOAC RI Certificate No. 061301.

(**f**) *3M Rappaport-Vassiliadis R10 (R-V R10) Broth.*—500 g/bottle (3M Food Safety).

(**g**) *Sterile diluents.*—Butterfield's Phosphate Diluent, distilled water, or reverse osmosis water.

(**h**) *Sterile 10 µL inoculation loop.*

(**i**) *Pipet.*—Capable of dispensing 2 mL.

(**j**) *Pipettor.*—Capable of dispensing 100 µL.

(**k**) *Sterile pipet tips.*—Capable of 100 µL.

(**l**) *Filter stomacher bags.*—Seward Laboratory Systems Inc. (Bohemia, NY, USA), or equivalent.

(**m**) *Stomacher.*—Seward Laboratory Systems Inc., or equivalent.

(**n**) *Permanent ultra-fine tipped marker.*—For circling presumptive positive colonies on the 3M Petrifilm *Salmonella* Express Plate.

(**o**) *Incubators.*—Capable of maintaining 41.5 ± 1°C.

(**p**) *Freezer.*—Capable of maintaining –10 to –20°C, for storing opened 3M Petrifilm *Salmonella* Express Plate pouches, hydrated 3M Petrifilm SALX Plates, and 3M Petrifilm SALX Plates after incubation.

(**q**) *Refrigerator.*—Capable of maintaining 2–8°C for storing unopened 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disk.

C. General Instructions

(**a**) Store 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disks at 2–8°C. After opening the 3M Petrifilm SALX Plate pouches, seal the pouch and store at ambient temperature, less than 60% relative humidity (RH). Hydrated 3M Petrifilm SALX Plates can be stored up to 7 days at 2–8°C. Post-incubation 3M Petrifilm SALX Plates can be stored at –10 to –20°C for up to 3 days. Hydrate the 3M Petrifilm SALX Plates with 2.0 ± 0.1 mL sterile diluent. Do not allow the top film to close before dispensing the entire 2.0 mL volume. Gently roll down the top film onto the diluent to prevent trapping air bubbles. Place the 3M Petrifilm Flat Spreader on the center of the plate. Press gently on the center of the spreader to distribute the diluent evenly. Spread the diluent over the entire 3M Petrifilm SALX Plate. Remove the spreader and leave the 3M Petrifilm SALX Plate undisturbed for 1 min. Prior to use, place the plates on a flat surface for 1 h at room temperature (20–25°C/<60% RH) and protected from light to allow the gel to form. Hydrated plates can be stored at room temperature (20–25°C/<60% RH) protected from light for up to 8 h before use.

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

(**c**) After use, the enrichment medium and the 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disks can potentially contain pathogenic materials. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Material Safety Data Sheet for additional information and local regulations for disposal.

D. Sample Enrichment

(*1*) Prewarm 3M *Salmonella* Enrichment Base with 3M *Salmonella* Enrichment Supplement (50 mg/L) to 41.5 ± 1°C.

(*2*) Aseptically combine the enrichment medium and sample following Table **2014.01C**. For all meat and highly particulate samples, the use of filter bags is recommended. Homogenize thoroughly for 2 min and incubate at 41.5 ± 1°C for 18–24 h.

(*a*) *Foods with high microbial backgrounds (≥10^4 CFU/g).*—Transfer 0.1 mL of the primary enrichment into 10.0 mL R-V R10 broth. Incubate for 8–24 h at 41.5 ± 1°C.

(*b*) *Foods with low microbial backgrounds (<10^4 CFU/g).*—Proceed to 3M Petrifilm SALX Plate preparation as described in **E**.

E. Preparation of the 3M Petrifilm Salmonella Express Plates

(*1*) Place the 3M Petrifilm SALX Plate on a flat, level surface.

(*2*) Use prescribed diluents to hydrate the 3M Petrifilm SALX Plates: Butterfield's Phosphate Diluent, distilled water, or reverse osmosis water.

(*3*) Lift the top film and with the pipet perpendicular dispense 2.0±0.1 mL sterile diluent onto the center of bottom film. Do not close the top film before dispensing the entire 2.0 mL volume.

(*4*) Gently roll down the top film onto the diluent to prevent trapping air bubbles.

(*5*) Place the 3M Petrifilm Flat Spreader on the center of the plate. Press gently on the center of the spreader to distribute the diluent evenly. Spread the diluent over the entire 3M Petrifilm SALX Plate growth area before the gel is formed. Do not slide the spreader across the film.

(*6*) Remove the spreader and leave the 3M Petrifilm SALX Plate undisturbed for at least 1 min.

(*7*) Place 3M Petrifilm SALX Plate on a flat surface for at least 1 h at room temperature (20–25°C/<60% RH), protected from light to allow the gel to form prior to use. Hydrated 3M Petrifilm SALX

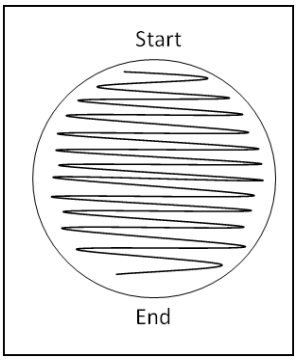


Figure 2014.01. Streaking pattern on the 3M Petrifilm SALX Plate.

Plates can be stored at room temperature (20–25°C/<60% RH) for up to 8 h before use if protected from light.

(*8*) If hydrated plates are not used within 8 h, store in a sealed plastic bag, protected from light, and store at –20 to –10°C for up to 5 days.

F. 3M Petrifilm Salmonella Express Plate Inoculation

(*1*) Remove the enrichment medium from the incubator and agitate contents by hand.

(*2*) Use a sterile 10 µL loop (3 mm diameter) to withdraw each sample. Use a smooth loop (one that does not have jagged edges and is not distorted) to prevent the gel surface from breaking.

(*3*) Open the 3M Petrifilm SALX Plate and streak onto the gel. Perform a single streak to obtain isolated colonies (Figure **2014.01**).

(*4*) Roll down the top film to close the 3M Petrifilm SALX Plate.

(*5*) Using a gloved hand (while practicing GLP to avoid cross-contamination and/or direct contact with the plate), gently apply a sweeping motion with even pressure onto the top film to remove any air bubbles in the inoculation area.

(*6*) Streak each enriched test portion onto a 3M Petrifilm SALX Plate and incubate at 41.5 ± 1°C for 24 ± 2 h in a horizontal position with the colored side up in stacks of no more than 20 plates.

Table 2014.01D. Interpretation for presumptive positive *Salmonella* species

Colony color			Colony metabolism		
Red	Dark red	Brown	Yellow zone	Gas bubble	Result
√			√		Presumptive +
√				√	Presumptive +
√			√	√	Presumptive +
	√		√		Presumptive +
	√			√	Presumptive +
	√		√	√	Presumptive +
		√	√		Presumptive +
		√		√	Presumptive +
		√	√	√	Presumptive +

G. Confirmation of 3M Petrifilm Salmonella Express Plates

(*1*) Using a permanent ultra-fine tip marker, circle at least five presumptive positive colonies (red to brown colonies with a yellow zone or associated gas bubble, or both) on the plate top film (*see* Table **2014.01D**).

(*2*) Lift the top film of the 3M Petrifilm SALX Plate and insert the 3M Petrifilm SALX Confirmation Disk by rolling it onto the gel to avoid entrapping air bubbles. Close the 3M Petrifilm SALX Plate. Using a gloved hand, gently apply a sweeping motion with even pressure onto the top film to remove any air bubbles in the inoculation area and ensure good contact between the gel and the 3M Petrifilm SALX Confirmation Disk.

(*3*) Incubate the 3M Petrifilm SALX System (plate and disk) at 41.5 ± 1°C for 4–5 h in a horizontal position, right side up, in stacks of no more than 20 plates.

(*4*) Observe circled colonies for color change. Red/brown to green blue, blue, dark blue, or black confirms the colony as *Salmonella* spp. No color change indicates the colony is negative. If presumptive positive *Salmonella* colonies are not present, then report the results as *Salmonella* not detected in the matrix.

Reference: *J. AOAC Int*. **97**, 1563(2014)
DOI: 10.5740/jaoacint.14-120

Posted: June 11, 2015

AOAC Official Methods of Analysis (OMA) Method Use & Performance Feedback (for NON-SMPR approved O...

Submission Date	2015-08-11 16:11:05
Please indicate your perspective	Expert Review Panel Member
AOAC Official Methods Number (XXXX.XX)	2013.09
Method Name or Manuscript Title	Salmonella in Selected Foods 3M Molecular Detection Assay (MDA) Salmonella Method. First Action 2013. Evaluation of Modification of the 3M Molecular Detection Assay (MDA) Salmonella Method (2013.09) for the Detection of Salmonella in Selected Foods: Collaborative Study. Revised First Action 2014.
Please include the manuscript publication reference, if available. (OMA methods have manuscripts unless otherwise noted in the OMA)	Patrick Bird et al. and DeAnn Benesh & John David. J AOAC Int. 96, 1325-1335(2013) Patrick Bird et al. and DeAnn Benesh & John David. J AOAC Int. 97, 1329-1342 (2014)
5. Do you have any information that supports the reproducibility of this method as written? If so, please specify and submit information.	No
6. Based on your experience with the method, are there any recommended changes to the AOAC First Action method as written?	No
Please attach any documentation to support your feedback, if available. (The maximum file size is 20MB. Multiple files may be attached.)	2013.09.docx
Would you like to provide comment(s) or feedback on additional methods? This form contains feedback for three (3) methods. You may now provide feedback on two (2) additional methods.	Additional feedback
AOAC Official Methods Number (XXXX.XX)	2013.10
Method Name or Manuscript Title	Listeria Species in a Variety of Foods and Environmental Surfaces VIDAS UP Listeria (LPT) Method.

Please include the manuscript publication reference, if available. (OMA methods have manuscripts unless otherwise noted in the OMA)	Erin Crowley et al. and Ronald L. Johnson J AOAC Int. 97, 431-441 (2014)
5. Do you have any information that supports the reproducibility of this method as written? If so, please specify and submit information.	No
Please attach any documentation to support your feedback, if available. (The maximum file size is 20MB. Multiple files may be attached.)	2013.10.docx
Would you like to provide comment(s) or feedback on additional methods? This form contains feedback for three (3) methods. You may now provide feedback on one (1) additional method.	Additional feedback
AOAC Official Methods Number (XXXX.XX)	2013.14
Method Name or Manuscript Title	Identification of Salmonella spp. from colony picks. First Action 2013.14
Please include the manuscript publication reference, if available. (OMA methods have manuscripts unless otherwise noted in the OMA)	Mark Mozola et al. J AOAC Int 97, 829-836 (2014)
6. Based on your experience with the method, are there any recommended changes to the AOAC First Action method as written?	No
Name	Anthony D. Hitchins
Organization	FDA (ret.)
E-mail Address	ahitchins@verizon.net

AOAC Official Methods of Analysis (OMA) Method Use & Performance Feedback (for NON-SMPR approved O...

Submission Date	2016-08-23 15:01:16
Please indicate your perspective	Expert Review Panel Member
AOAC Official Methods Number (XXXX.XX)	2014.01
Method Name or Manuscript Title	3M Petrifilm Salmonella Express
Please include the manuscript publication reference, if available. (OMA methods have manuscripts unless otherwise noted in the OMA)	JAOC Int. 97 (6) 1563-1575 (2014)
1. In your experience using the method, does the method perform according to the method''s applicability as written?	Yes
2. In your experience with the method, are there any safety concerns identified while using or regarding use of the method?	No
3. In your experience with the method, are there available reference materials? Were there any concerns identified while using or regarding use of the method?	No
4. In your experience with the method, do you have data demonstrating linearity, accuracy, repeatability, LOD/LOQ?	No
If No, please explain.	ERP member
5. Do you have any information that supports the reproducibility of this method as written? If so, please specify and submit information.	Yes
Please specify the information to be provided to support the reproducibility of this method as written.	Abirami, N et al. Food Control 76: 74-78 (2016)

6. Based on your experience with the method, are there any recommended changes to the AOAC First Action method as written?	No
Would you like to provide comment(s) or feedback on additional methods? This form contains feedback for three (3) methods. You may now provide feedback on two (2) additional methods.	Additional feedback
AOAC Official Methods Number (XXXX.XX)	2014.05
Method Name or Manuscript Title	3M Petrifilm Rapid Yeast & Mold Count Plate for Enumeration
Please include the manuscript publication reference, if available. (OMA methods have manuscripts unless otherwise noted in the OMA)	JAOAC Int. 98 (3) 767-783 (2015)
1. In your experience using the method, does the method perform according to the method''s applicability as written?	Yes
2. In your experience with the method, are there any safety concerns identified while using or regarding use of the method?	No
3. In your experience with the method, are there available reference materials? Were there any concerns identified while using or regarding use of the method?	No
4. In your experience with the method, do you have data demonstrating linearity, accuracy, repeatability, LOD/LOQ?	No
If No, please explain.	ERP member w/o laboratory

AOAC Official Methods of Analysis (OMA) Method Use & Performance Feedback (for NON-SMPR approved O...

Submission Date	2016-07-14 13:36:53
Please indicate your perspective	Method Developer
AOAC Official Methods Number (XXXX.XX)	2014.01
Method Name or Manuscript Title	Evaluation of the 3M™ Petrifilm™ Salmonella Express System for the Detection of Salmonella Species in Selected Foods: Collaborative Study
Please include the manuscript publication reference, if available. (OMA methods have manuscripts unless otherwise noted in the OMA)	Journal of AOAC International Vol. 97, No. 6, 2014
Upload documentation to support the Safety Concerns	MethodSafetyandRiskAssessmentv2.doc
Please attach any documentation to support your feedback, if available. (The maximum file size is 20MB. Multiple files may be attached.)	3M™ Petrifilm™ Salmonella Express Final Action 9-2016.pdf
Would you like to provide comment(s) or feedback on additional methods? This form contains feedback for three (3) methods. You may now provide feedback on two (2) additional methods.	Additional feedback
AOAC Official Methods Number (XXXX.XX)	2014.05
Method Name or Manuscript Title	Evaluation of the 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate for the Enumeration of Yeast and Mold in Food: Collaborative Study, First Action 2014.05
Please include the manuscript publication reference, if available. (OMA methods have manuscripts unless otherwise noted in the OMA)	Journal of AOAC International Vol. 98, No. 3, 2015
6. Based on your experience with the method, are there any recommended changes to the AOAC First Action method as written?	No

Please attach any documentation to support your feedback, if available. (The maximum file size is 20MB. Multiple files may be attached.)	3M™ Petrifilm™ Rapid Yeast and Mold Final Action 9-2016.pdf
Would you like to provide comment(s) or feedback on additional methods? This form contains feedback for three (3) methods. You may now provide feedback on one (1) additional method.	No, thank you!
AOAC Official Methods Number (XXXX.XX)	q
Method Name or Manuscript Title	q
1. In your experience using the method, does the method perform according to the method"s applicability as written?	Yes
2. In your experience with the method, are there any safety concerns identified while using or regarding use of the method?	No
3. In your experience with the method, are there available reference materials? Were there any concerns identified while using or regarding use of the method?	No
4. In your experience with the method, do you have data demonstrating linearity, accuracy, repeatability, LOD/LOQ?	Yes
5. Do you have any information that supports the reproducibility of this method as written? If so, please specify and submit information.	No
Name	Bob Jechorek
Organization	3M Food Safety
E-mail Address	rpjechorek@mmm.com

FOOD BIOLOGICAL CONTAMINANTS

Evaluation of the 3M™ Petrifilm™ *Salmonella* Express System for the Detection of *Salmonella* Species in Selected Foods: Collaborative Study

PATRICK BIRD, JONATHAN FLANNERY, ERIN CROWLEY, JAMES AGIN, and DAVID GOINS
Q Laboratories, Inc., 1400 Harrison Ave, Cincinnati, OH 45214
ROBERT JECHOREK[1]
3M Food Safety Department, 3M Center–Bldg 260-6B-01, St. Paul, MN 55144

Collaborators: J. Adams, K. Beers, K. Blanchard, R. Brooks, M. Boyle, R. Colvin, H. Elgaali, W. Fedio, D. Lewis, A. Martin, K. McCallum, J. Meyer, A. Morey, A. Morris, K. Newman, J. Pickett, K. Rajkowski, D. Rodgers, B. Stawick, J. Marchant-Tambone

The 3M™ Petrifilm™ *Salmonella* Express (SALX) System is a simple, ready-to-use chromogenic culture medium system for the rapid qualitative detection and biochemical confirmation of *Salmonella* spp. in food and food process environmental samples. The 3M Petrifilm SALX System was compared using an unpaired study design in a multilaboratory collaborative study to the U.S. Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) *Microbiology Laboratory Guidebook* (MLG) 4.07 (2013) *Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg and Catfish Products and Carcass and Environmental Sponges* for raw ground beef and the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM) Chapter 5, *Salmonella* (2011) reference method for dry dog food following the current AOAC validation guidelines. For this study, a total of 17 laboratories located throughout the continental United States evaluated 1872 test portions. For the 3M Petrifilm SALX System, raw ground beef was analyzed using 25 g test portions, and dry dog food was analyzed using 375 g test portions. For the reference methods, 25 g test portions of each matrix were analyzed. The two matrices were artificially contaminated with *Salmonella* at three inoculation levels: an uninoculated control level (0 CFU/test portion), a low inoculum level (0.2–2 CFU/test portion), and a high inoculum level (2–5 CFU/test portion). Each inoculation level was statistically analyzed using the probability of detection statistical model. For the raw ground beef and dry dog food test portions, no significant differences at the 95% confidence interval were observed in the number of positive samples detected by the 3M Petrifilm SALX System versus either the USDA/FSIS-MLG or FDA/BAM methods.

In the last quarter century, significant efforts have been made to reduce the occurrence of *Salmonella* in food products, yet *Salmonella* spp. continues to be the most frequently reported cause of foodborne illness in the United States (1). Over 2500 different serotypes of *Salmonella* spp. have been isolated from a wide range of food products including raw meats and poultry, shell eggs, chocolate, fresh fruit and vegetables, and low-moisture ingredients such as spices and peanut butter (2). This broad range of implicated products further illustrates why testing for and confirming the presence of *Salmonella* as rapidly as possible is so critical to food safety. The 3M™ Petrifilm™ *Salmonella* Express (SALX) System, a chromogenic culture medium system, uses a cold-water-soluble gelling agent to selectively differentiate *Salmonella* from background flora in enriched food and food process environmental samples.

The 3M Petrifilm SALX System allows for the rapid and specific detection and biochemical confirmation of *Salmonella* species from food and environmental samples. Following enrichment in 3M™ *Salmonella* Enrichment Base containing 3M™ *Salmonella* Enrichment Supplement, the 3M Petrifilm SALX System can provide presumptive results in as little as 40 h from low microbial background foods ($<10^4$ CFU/g) and 48 h from high-microbial background foods ($\geq 10^4$ CFU/g). Confirmation of multiple presumptive *Salmonella* colonies at once is accomplished using the 3M™ Petrifilm™ *Salmonella* Express (SALX) Confirmation Disk which uses biochemical enzymes to facilitate the reaction. The method developer studies demonstrated that the 3M Petrifilm SALX System did not specifically differentiate some lactose-positive *Salmonella* species (primarily *S. arizonae* and *S. diarizonae*) from other lactose-positive organisms.

Prior to the collaborative study, the 3M Petrifilm SALX System was validated according to AOAC Validation Guidelines (3) in a harmonized AOAC *Performance Tested Method*SM (PTM) study. The objective of the PTM study

Received May 29, 2014.
The method was approved by the Expert Review Panel for Microbiology Methods for Food and Environmental Surfaces as First Action.
The Expert Review Panel for Microbiology Methods for Food and Environmental Surfaces invites method users to provide feedback on the First Action methods. Feedback from method users will help verify that the methods are fit for purpose and are critical to gaining global recognition and acceptance of the methods. Comments can be sent directly to the corresponding author or methodfeedback@aoac.org.
[1] Corresponding author's e-mail: rpjechorek@mmm.com
An appendix is available on the *J. AOAC Int.* website, http://aoac.publisher.ingentaconnect.com/content/aoac/jaoac
DOI: 10.5740/jaoacint.14-120

was to demonstrate that the 3M Petrifilm SALX System detects *Salmonella* spp. in selected foods as claimed by the manufacturer. For the 3M Petrifilm SALX System evaluation, nine matrices were evaluated: raw ground beef (25 g), raw ground chicken (25 g), pasteurized liquid whole egg (100 g), raw ground pork (25 g), cooked chicken nuggets (325 g), frozen uncooked shrimp (25 g), fresh bunched spinach (25 g), dry dog food (375 g), and stainless steel.

All other PTM parameters (inclusivity, exclusivity, ruggedness, stability, and lot-to-lot variability) tested in the PTM studies satisfied the performance requirements for PTM approval. The method was awarded PTM certification No. 061301 on June 5, 2013.

The aim of this collaborative study was to compare the 3M Petrifilm SALX System to the U.S. Department of Agriculture (USDA) Food Safety Inspection Service (FSIS)/*Microbiology Laboratory Guidebook* (MLG) 4.07 (4) for raw ground beef, and the U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual* (BAM) Chapter 5 (5) method for dry dog food.

Collaborative Study

Study Design

For this collaborative study, two matrices, raw ground beef (80% lean) and dry dog food, were evaluated. The matrices were obtained from local retailers and screened for the presence of *Salmonella* spp. by either the MLG or BAM reference methods. The raw ground beef was artificially contaminated with *Salmonella* Ohio Sequence Types 81 (University of Pennsylvania Culture Collection) and the dry dog food with *Salmonella* Poona National Collection of Type Cultures (NCTC) 4840. There were three inoculation levels for each matrix: a high inoculation level of approximately 2–5 CFU/test portion, a low inoculation level of approximately 0.2–2 CFU/test portion, and an uninoculated control level at 0 CFU/test portion. Twelve replicate samples from each of the three inoculation levels of product were analyzed by both the candidate and reference method. Two sets of unpaired samples (72 total) were sent to each laboratory for analysis by the 3M Petrifilm SALX System and either the MLG (raw ground beef) or BAM (dry dog food) reference method due to differences in enrichment protocols. For both matrices, collaborators were sent an additional 60 g test portion and instructed to conduct a total aerobic plate count (APC) using 3M™ Petrifilm™ Aerobic Count Plate (AOAC *Official Method* **990.12**) on the day samples were received. Foods with an APC count of greater than or equal to 1.0×10^4 CFU/g were categorized as high microbial load foods, and those foods lower than 1.0×10^4 CFU/g were categorized as low microbial load. A detailed collaborative study packet outlining all necessary information related to the study including media preparation, method-specific test portion preparation, and documentation of results was sent to each collaborating laboratory prior to the initiation of the study.

Preparation of Inocula and Test Portions

The *Salmonella* cultures used in this evaluation were propagated in 10 mL Brain Heart Infusion (BHI) broth from a frozen stock culture stored at –70°C at Q Laboratories, Inc. The broth was incubated for 18–24 h at 35 ± 1°C. For both matrices, a bulk lot of each matrix was inoculated with a liquid inoculum and mixed thoroughly by hand-kneading to ensure an even distribution of microorganisms. Appropriate dilutions of the cultures were prepared based on previously established growth curves for both low and high inoculation levels, resulting in fractional positive outcomes for at least one level. For the dry pet food, prior to inoculation, the inoculum was heat-stressed in a 50°C water bath for 10 min to obtain a percent injury of 50–80% (as determined by plating onto selective xylose deoxycholate agar and nonselective tryptic soy agar). The degree of injury was estimated as:

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

where n_{select} = number of colonies on selective agar, and $n_{nonselect}$ = number of colonies on nonselective agar. The raw ground beef was inoculated on the day of shipment so that the organism had equilibrated within the matrix for 96 h before testing was initiated. Dry dog food was inoculated and held at room temperature (24 ± 2°C) so that the organism would have equilibrated for a minimum of 2 weeks prior to initiation of testing. The shipment and hold times of the inoculated test material were verified as a QC measure prior to study initiation. For the evaluation of the raw ground beef, the bulk lot of inoculated test material was divided into 30 g portions for shipment to the collaborators. For the evaluation of the dry dog food, 25 g of inoculated test product was mixed with 350 g of uninoculated test product for shipment to the collaborators for the analysis by the 3M Petrifilm SALX System. For analysis by the reference methods, collaborators received 30 g portions. Validation criterion were satisfied when inoculated test portions produced fractional recovery of the spiked organism, defined as either the reference or candidate method yielding 25–75% positive results.

To determine the level of *Salmonella* spp. in the matrices, a five-tube most probable number (MPN) was conducted at Q Laboratories, Inc. on the day of initiation of analysis using the BAM Chapter 5 reference method for dry dog food or the MLG 4.07 reference method for raw ground beef. From both the high and low inoculated levels, five 100 g test portions, the reference method test portions from the collaborating laboratories, and five 10 g test portions were analyzed following the appropriate reference method. The MPN and 95% confidence intervals were calculated from the high, medium, and low levels using the Least Cost Formulations (LCF) MPN Calculator , Version 1.6, provided by AOAC (www.lcfltd.com/customer/LCFMPNCalculator.exe; 6). Confirmation of the samples was conducted according to the appropriate reference method, dependent on the matrix.

Test Portion Distribution

All samples were labeled with a randomized, blind-coded three 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 International Air Transport Association. Raw ground beef samples were packed with cold packs to target a temperature of <7°C during shipment. Upon receipt, samples were held by the collaborating laboratory at refrigerated temperature (3–5°C) until the following Monday when analysis was initiated. Dry dog

food samples were packed and shipped at ambient temperature. Upon receipt, samples were held by the collaborating laboratory at room temperature (24 ± 2°C). In addition to each of the test portions and the total plate count replicate, collaborators also received a test portion for each matrix labeled as "temperature control." Participants were instructed to record the temperature of this portion upon receipt of the shipment, document results on the Sample Receipt Confirmation form provided, and fax to the Study Director. For both matrices, several shipments were delayed from getting to the testing facilities on time due to inclement weather. Upon receiving their packages on either Saturday or Monday, the testing laboratories were instructed to document the temperature of the samples and to continue testing. No laboratories were solely excluded from testing due to the delay in package receipt.

Test Portion Analysis

Collaborators followed the appropriate preparation and analysis protocol according to the method specified for each matrix. For both matrices, each collaborator received 72 test portions of each food product (12 high, 12 low, and 12 controls for each method). For the analysis of the raw ground beef test portions by the 3M Petrifilm SALX System, a 25 g portion was enriched with 225 mL of prewarmed (41.5 ± 1°C) 3M *Salmonella* Enrichment Base containing 3M *Salmonella* Enrichment Supplement (50 mg/L), homogenized for 2 min, and incubated for 18–24 h at 41.5 ± 1°C. For the dry dog food test portions analyzed by the 3M Petrifilm SALX System, a 375 g portion was enriched with 3375 mL prewarmed (41.5 ± 1°C) 3M *Salmonella* Enrichment Base containing 3M *Salmonella* Enrichment Supplement (50 mg/L), homogenized for 2 min, and incubated for 18–24 h at 41.5 ± 1°C.

Following enrichment of raw ground beef samples, the enrichment protocol for high microbial load foods was followed where a 0.1 mL aliquot of each test portion was transferred into 10.0 mL Rappaport-Vassiliadis R10 (R-V R10) broth and incubated for 8–24 h at 41.5 ± 1°C. After incubation, a loopful of the secondary enrichment was streaked directly onto hydrated 3M Petrifilm SALX Plates and incubated for 24 ± 2 h at 41.5 ± 1°C. For dry dog food samples, the enrichment protocol for low microbial load foods was followed where a loopful of the primary enrichment was streaked directly onto hydrated 3M Petrifilm SALX Plates and incubated for 24 ± 2 h at 41.5 ± 1°C. For both matrices, the 3M Petrifilm SALX Plates were examined for typical colonies (red to brown colony with a yellow zone or associated gas bubble, or both).

Typical colonies were circled on the plate top film using a fine tip permanent black marker. The top of the 3M Petrifilm SALX Plate was lifted and a 3M Petrifilm SALX Confirmation Disk was placed onto the gel. The film was lowered and air bubbles were removed using a sweeping motion. The plates were incubated for 4–5 h at 41.5 ± 1°C. After incubation, the circled colonies were observed for color change: red/brown to green blue, blue, dark blue, or black. Typical colonies were transferred to triple sugar iron/lysine iron agar (TSI/LIA) slants and confirmed following the standard reference methods. Additionally, for each matrix analyzed by the 3M Petrifilm SALX System, aliquots of the primary enrichment were transferred to the secondary enrichments and confirmed following procedures outlined in the MLG or BAM.

Both test portion sizes analyzed by the 3M Petrifilm SALX System were compared to samples (25 g) analyzed using either the MLG or BAM reference method in an unpaired study design. All positive test portions were biochemically confirmed by the API 20E biochemical test, AOAC *Official Method* **978.24** or by the VITEK 2 GN identification test, AOAC *Official Method* **2011.17**. The biochemical method was determined by each individual participating laboratory based on their current method used for confirmation of routine samples. Serological testing, Group Poly O A-I & Vi and Poly H latex agglutination, was also performed.

Statistical Analysis

Each collaborating laboratory recorded results for the reference method and the 3M Petrifilm SALX System on the data sheets provided in the collaborative study outline or the electronic spreadsheet created as a result of multiple requests for electronic data entry. The data sheets were submitted to the Study Director at the end of each week of testing for analysis. The results of each test portion for each sample were compiled by the Study Director and the qualitative 3M Petrifilm SALX System results were compared to the reference methods for statistical analysis. Data for each test portion size were analyzed using the probability of detection (POD) statistical model (3, 7). A confidence interval of a dLPOD (difference between the POD of the reference and candidate method) not containing the point zero would indicate a statistically significant difference between the 3M Petrifilm SALX System and the MLG or BAM reference methods at the 5% probability level (8). In addition to calculating the POD for each inoculation level, the repeatability standard deviation, among-laboratory standard deviation, reproducibility standard deviation, and a *P*-value for homogeneity were calculated. For the collaborative study, the 3M Petrifilm SALX System produced 479 presumptive positive results with 475 confirming positive by the traditional confirmation and 473 confirming positive by the alternative confirmation. There were 468 confirmed positives by the reference method.

AOAC Official Method 2014.01
Salmonella in Selected Foods
3M™ Petrifilm™ *Salmonella* Express System
First Action 2014

[Applicable to detection of *Salmonella* spp. in raw ground beef (25 g), raw ground chicken (25 g), pasteurized liquid whole egg (100 g), raw ground pork (25 g), cooked chicken nuggets (325 g), frozen uncooked shrimp (25 g), fresh bunched spinach (25 g), dry dog food (375 g), and stainless steel. Not applicable to some lactose-positive *Salmonella* species.]

See Tables **2014.01A** and **B** for results of the interlaboratory study supporting acceptance of the method. *See* Appendix available on the *J. AOAC Int*. website for detailed tables of results of the collaborative study.

Caution: Do not use the 3M Petrifilm SALX System method in the diagnosis of conditions in humans or animals. To reduce the risks associated with exposure to chemicals and biohazards, perform pathogen testing in a properly equipped laboratory under the control of trained personnel. Always follow

Table 2014.01A. Summary of results for detection of *Salmonella* in raw ground beef (25 g)

Method[a]	3M Petrifilm *Salmonella* Express System with alternative confirmation			3M Petrifilm *Salmonella* Express System with traditional confirmation		
Inoculation level	Uninoculated	Low	High	Uninoculated	Low	High
Candidate presumptive positive/ total No. of samples analyzed	2/168	85/168	168/168	2/168	85/168	168/168
Candidate presumptive POD (CP)	0.01 (0.00, 0.04)	0.51 (0.43, 0.58)	1.00 (0.98, 1.00)	0.01 (0.00, 0.04)	0.51 (0.43, 0.58)	1.00 (0.98, 1.00)
s_r[b]	0.11 (0.10, 0.15)	0.51 (0.46, 0.52)	0.00 (0.00, 0.15)	0.11 (0.10, 0.15)	0.51 (0.46, 0.52)	0.00 (0.00, 0.15)
s_L[c]	0.00 (0.00, 0.04)	0.00 (0.00, 0.13)	0.00 (0.00, 0.15)	0.00 (0.00, 0.04)	0.00 (0.00, 0.13)	0.00 (0.00, 0.15)
s_R[d]	0.11 (0.10, 0.12)	0.51 (0.47, 0.52)	0.00 (0.00, 0.21)	0.11 (0.10, 0.12)	0.51 (0.47, 0.52)	0.00 (0.00, 0.21)
P-value[e]	0.5158	0.9341	1.0000	0.5158	0.9341	1.0000
Candidate confirmed positive/ total No. of samples analyzed	0/168	83/168	168/168	1/168	83/168	168/168
Candidate confirmed POD (CC)	0.00 (0.00, 0.02)	0.49 (0.42, 0.57)	1.00 (0.98, 1.00)	0.01 (0.00, 0.03)	0.49 (0.42, 0.57)	1.00 (0.98, 1.00)
s_r	0.00 (0.00, 0.15)	0.51 (0.46, 0.52)	0.00 (0.00, 0.15)	0.08 (0.07, 0.15)	0.51 (0.46, 0.52)	0.00 (0.00, 0.15)
s_L	0.00 (0.00, 0.15)	0.00 (0.00, 0.11)	0.00 (0.00, 0.15)	0.00 (0.00, 0.03)	0.00 (0.00, 0.11)	0.00 (0.00, 0.15)
s_R	0.00 (0.00, 0.21)	0.51 (0.47, 0.52)	0.00 (0.00, 0.21)	0.08 (0.07, 0.09)	0.51 (0.47, 0.52)	0.00 (0.00, 0.21)
P-value	1.0000	0.9757	1.0000	0.4418	0.9757	1.0000
Positive reference samples/ total No. of samples analyzed	0/168	86/168	167/168	0/168	86/168	167/168
Reference POD	0.00 (0.00, 0.02)	0.51 (0.43, 0.59)	0.99 (0.97, 1.00)	0.00 (0.00, 0.02)	0.51 (0.43, 0.59)	0.99 (0.97, 1.00)
s_r	0.00 (0.00, 0.15)	0.51 (0.46, 0.52)	0.08 (0.07, 0.15)	0.00 (0.00, 0.15)	0.51 (0.46, 0.52)	0.08 (0.07, 0.15)
s_L	0.00 (0.00, 0.15)	0.00 (0.00, 0.12)	0.00 (0.00, 0.03)	0.00 (0.00, 0.15)	0.00 (0.00, 0.12)	0.00 (0.00, 0.03)
s_R	0.00 (0.00, 0.21)	0.51 (0.47, 0.52)	0.08 (0.07, 0.09)	0.00 (0.00, 0.21)	0.51 (0.47, 0.52)	0.08 (0.07, 0.09)
P-value	1.0000	0.9695	0.4418	1.0000	0.9695	0.4418
dLPOD (candidate vs reference)[f]	0.00 (–0.02, 0.02)	–0.02 (–0.13, 0.09)	0.01 (–0.02, 0.03)	0.01 (–0.02, 0.03)	–0.02 (–0.13, 0.09)	0.01 (–0.02, 0.03)
dLPOD (candidate presumptive vs candidate confirmed)[f]	0.01 (–0.01, 0.04)	0.01 (–0.10, 0.12)	0.00 (–0.02, 0.02)	0.01 (–0.02, 0.04)	0.01 (–0.10, 0.12)	0.00 (–0.02, 0.02)

[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.

standard good laboratory safety practices (GLP), including proper containment procedures, and wearing appropriate protective apparel and eye protection while handling testing materials and test samples. Avoid direct contact with the contents of the enrichment medium and inoculated plates. Dispose of enrichment media and inoculated plates according to all applicable government regulatory regulations and applicable laboratory procedures. Wear appropriate protective apparel while handling the 3M Petrifilm SALX Plate as some of the components may be considered allergenic and irritants to some individuals.

To reduce the risks associated with environmental contamination, follow current industry standards and local regulations for disposal of contaminated waste. Consult the Material Safety Data Sheet for additional information. For questions about specific applications or procedures, visit www.3M.com/foodsafety or contact your local 3M representative or distributor. Review the policies recommend by the Centers for Disease Control and Prevention on dealing with pathogens (http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf).

A. Principle

The 3M Petrifilm SALX System is a chromogenic culture medium system that is intended for the rapid and specific detection and biochemical confirmation of *Salmonella* spp. from food and food process environmental samples. After enrichment in prewarmed 3M *Salmonella* Enrichment Base with 3M *Salmonella* Enrichment Supplement, the 3M Petrifilm SALX System provides presumptive positive results in as little as 40 h from low microbial background foods ($<10^4$ CFU/g) and 48 h from high microbial foods ($\geq 10^4$ CFU/g). The 3M

Table 2014.01B. Summary of results for detection of *Salmonella* in dry dog food (375 g)

Method[a]	3M Petrifilm *Salmonella* Express System with alternative confirmation			3M Petrifilm *Salmonella* Express System with traditional confirmation		
Inoculation level	Uninoculated	Low	High	Uninoculated	Low	High
Candidate presumptive positive/ total No. of samples analyzed	0/144	82/144	142/144	0/144	82/144	142/144
Candidate presumptive POD (CP)	0.00 (0.00, 0.03)	0.57 (0.48, 0.66)	0.99 (0.95, 1.00)	0.00 (0.00, 0.03)	0.57 (0.48, 0.66)	0.99 (0.95, 1.00)
s_r[b]	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.12 (0.11, 0.16)	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.12 (0.11, 0.16)
s_L[c]	0.00 (0.00, 0.16)	0.08 (0.00, 0.24)	0.00 (0.00, 0.04)	0.00 (0.00, 0.16)	0.08 (0.00, 0.24)	0.00 (0.00, 0.04)
s_R[d]	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.12 (0.11, 0.13)	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.12 (0.11, 0.13)
P-value[e]	1.0000	0.2242	0.9861	1.0000	0.2242	0.9861
Candidate confirmed positive/ total No. of samples analyzed	0/144	81/144	141/144	0/144	82/144	141/144
Candidate confirmed POD (CC)	0.00 (0.00, 0.03)	0.56 (0.46, 0.66)	0.98 (0.94, 0.99)	0.00 (0.00, 0.03)	0.57 (0.48, 0.67)	0.98 (0.94, 0.99)
s_r	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.14 (0.12, 0.16)	0.00 (0.00, 0.16)	0.49 (0.43, 0.52)	0.14 (0.12, 0.16)
s_L	0.00 (0.00, 0.16)	0.10 (0.00, 0.26)	0.03 (0.00, 0.08)	0.00 (0.00, 0.16)	0.11 (0.00, 0.27)	0.03 (0.00, 0.08)
s_R	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.14 (0.13, 0.17)	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.14 (0.13, 0.17)
P-value	1.0000	0.1290	0.0976	1.0000	0.1114	0.0976
Positive reference samples/ total No. of samples analyzed	0/144	71/144	144/144	0/144	71/144	144/144
Reference POD	0.00 (0.00, 0.03)	0.49 (0.39, 0.59)	1.00 (0.97, 1.00)	0.00 (0.00, 0.03)	0.49 (0.39, 0.59)	1.00 (0.97, 1.00)
s_r	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.00 (0.00, 0.16)	0.00 (0.00, 0.16)	0.49 (0.44, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.10 (0.00, 0.26)	0.00 (0.00, 0.16)	0.00 (0.00, 0.16)	0.10 (0.00, 0.26)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.00 (0.00, 0.22)	0.00 (0.00, 0.22)	0.50 (0.45, 0.52)	0.00 (0.00, 0.22)
P-value	1.0000	0.1550	1.0000	1.0000	0.1550	1.0000
dLPOD (C vs R)[f]	0.00 (–0.03, 0.03)	0.07 (–0.07, 0.21)	–0.02 (–0.06, 0.01)	0.00 (–0.03, 0.03)	0.08 (–0.07, 0.22)	–0.02 (–0.06, 0.01)
dLPOD (CP vs CC)[f]	0.00 (–0.03, 0.03)	0.01 (–0.18, 0.22)	0.01 (–0.03, 0.05)	0.00 (–0.03, 0.03)	0.00 (–0.14, 0.14)	0.01 (–0.03, 0.05)

[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.

Petrifilm SALX System does not specifically differentiate some lactose-positive *Salmonella* species (primarily *S. arizonae* and *S. diarizonae*) from other lactose-positive organisms. Refer to the 3M Petrifilm *Salmonella* Express System Instructions for Use for additional information.

B. Apparatus and Reagents

(**a**) *3M Petrifilm Salmonella Express Plate.*—Twenty-five plates/pouch (3M Food Safety, St. Paul, MN).

(**b**) *3M Petrifilm Salmonella Express Confirmation Disk.*—Five disks/pouch (3M Food Safety).

(**c**) *3M Salmonella Enrichment Base.*—500 g or 2.5 kg/bottle (3M Food Safety).

(**d**) *3M Salmonella Enrichment Supplement.*—1 g/vial (3M Food Safety).

(**e**) *3M™ Petrifilm™ Flat Spreader.*—Two spreaders/box (3M Food Safety).

(**f**) *3M Rappaport-Vassiliadis R10 (R-VR10) Broth.*—500 g/bottle (3M Food Safety).

(**g**) *Sterile diluents.*—Butterfield's Phosphate Diluent, distilled water, or reverse osmosis water.

(**h**) *Sterile 10 µL inoculation loop.*

(**i**) *Pipet.*—Capable of dispensing 2 mL.

(**j**) *Pipettor.*—Capable of dispensing 100 µL.

(**k**) *Sterile pipet tips.*—Capable of 100 µL.

(**l**) *Filter stomacher bags.*—Seward Laboratory Systems Inc., Bohemia, NY, or equivalent.

(**m**) *Stomacher.*—Seward Laboratory Systems Inc., or equivalent.

(**n**) *Permanent ultra-fine tipped marker.*—For circling presumptive positive colonies on the 3M Petrifilm *Salmonella* Express Plate.

(**o**) *Incubators.*—Capable of maintaining 41.5 ± 1°C.

(**p**) *Freezer.*—Capable of maintaining –10 to –20°C, for storing opened 3M Petrifilm *Salmonella* Express Plate pouches,

Table 2014.01C. Sample matrix and enrichment scheme[a]

Sample matrix	Sample size, g	Enrichment broth volume, mL	Enrichment time, h	Secondary enrichment time, h
Raw ground beef (80% lean)	25	225	18–24	8–24
Raw ground chicken	25	225	18–24	8–24
Raw ground pork	25	225	18–24	8–24
Frozen uncooked shrimp	25	225	18–24	8–24
Fresh bunched spinach	25	225	18–24	24
Stainless steel; environmental sponges	1 Sponge (4 × 4 in.)	225	18–24	
Pasteurized liquid whole egg	100	900	18–24	
Cooked breaded chicken	325	2925	18–24	
Dry dog food	375	3375	18–24	

[a] AOAC RI Certificate No. 061301.

hydrated 3M Petrifilm SALX Plates, and 3M Petrifilm SALX Plates after incubation.

(**q**) *Refrigerator.*—Capable of maintaining 2–8°C for storing unopened 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disk.

C. General Instructions

(**a**) Store 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disks at 2–8°C. After opening the 3M Petrifilm SALX Plate pouches, seal the pouch and store at ambient temperature, less than 60% relative humidity (RH). Hydrated 3M Petrifilm SALX Plates can be stored up to 7 days at 2–8°C. Post-incubation 3M Petrifilm SALX Plates can be stored at –10 to –20°C for up to 3 days. Hydrate the 3M Petrifilm SALX Plates with 2.0 ± 0.1 mL sterile diluent. Do not allow the top film to close before dispensing the entire 2.0 mL volume. Gently roll down the top film onto the diluent to prevent trapping air bubbles. Place the 3M Petrifilm Flat Spreader on the center of the plate. Press gently on the center of the spreader to distribute the diluent evenly. Spread the diluent over the entire 3M Petrifilm SALX Plate. Remove the spreader and leave the 3M Petrifilm SALX Plate undisturbed for 1 min. Prior to use, place the plates on a flat surface for 1 h at room temperature (20–25°C/<60% RH) and protected from light to allow the gel to form. Hydrated plates can be stored at room temperature (20–25°C/<60% RH) protected from light for up to 8 h before use.

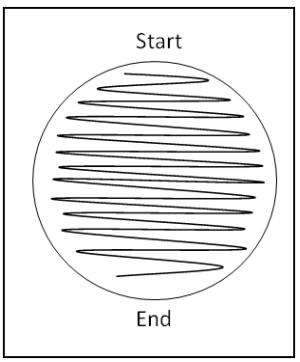


Figure 2014.01. Streaking pattern on the 3M Petrifilm SALX Plate.

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

(**c**) After use, the enrichment medium and the 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disks can potentially contain pathogenic materials. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Material Safety Data Sheet for additional information and local regulations for disposal.

D. Sample Enrichment

(*1*) Prewarm 3M *Salmonella* Enrichment Base with 3M *Salmonella* Enrichment Supplement (50 mg/L) to 41.5 ± 1°C.

(*2*) Aseptically combine the enrichment medium and sample following Table **2014.01C**. For all meat and highly particulate samples, the use of filter bags is recommended. Homogenize thoroughly for 2 min and incubate at 41.5 ± 1°C for 18–24h.

(*a*) *Foods with high microbial backgrounds (≥10^4 CFU/g).*—Transfer 0.1 mL of the primary enrichment into 10.0 mL R-V R10 broth. Incubate for 8–24 h at 41.5 ± 1°C.

(*b*) *Foods with low microbial backgrounds (<10^4 CFU/g).*—Proceed to 3M Petrifilm SALX Plate preparation as described in **E**.

E. Preparation of the 3M Petrifilm Salmonella Express Plates

(*1*) Place the 3M Petrifilm SALX Plate on a flat, level surface.

(*2*) Use prescribed diluents to hydrate the 3M Petrifilm SALX Plates: Butterfield's Phosphate Diluent, distilled water, or reverse osmosis water.

(*3*) Lift the top film and with the pipet perpendicular dispense 2.0 ± 0.1 mL sterile diluent onto the center of bottom film. Do not close the top film before dispensing the entire 2.0 mL volume.

(*4*) Gently roll down the top film onto the diluent to prevent trapping air bubbles.

(*5*) Place the 3M Petrifilm Flat Spreader on the center of the plate. Press gently on the center of the spreader to distribute the diluent evenly. Spread the diluent over the entire 3M Petrifilm SALX Plate growth area before the gel is formed. Do not slide the spreader across the film.

Table 2014.01D. Interpretation for presumptive positive *Salmonella* species

Colony color			Colony metabolism		
Red	Dark red	Brown	Yellow zone	Gas bubble	Result
√			√		Presumptive +
√				√	Presumptive +
√			√	√	Presumptive +
	√		√		Presumptive +
	√			√	Presumptive +
	√		√	√	Presumptive +
		√	√		Presumptive +
		√		√	Presumptive +
		√	√	√	Presumptive +

(*6*) Remove the spreader and leave the 3M Petrifilm SALX Plate undisturbed for at least 1 min.

(*7*) Place 3M Petrifilm SALX Plate on a flat surface for at least 1 h at room temperature (20–25°C/<60% RH), protected from light to allow the gel to form prior to use. Hydrated 3M Petrifilm SALX Plates can be stored at room temperature (20–25°C/<60% RH) for up to 8 h before use if protected from light.

(*8*) If hydrated plates are not used within 8 h, store in a sealed plastic bag, protected from light, and store at –20 to –10°C for up to 5 days.

F. 3M Petrifilm Salmonella Express Plate Inoculation

(*1*) Remove the enrichment medium from the incubator and agitate contents by hand.

(*2*) Use a sterile 10 µL loop (3 mm diameter) to withdraw each sample. Use a smooth loop (one that does not have jagged edges and is not distorted) to prevent the gel surface from breaking.

(*3*) Open the 3M Petrifilm SALX Plate and streak onto the gel. Perform a single streak to obtain isolated colonies (Figure **2014.01**).

(*4*) Roll down the top film to close the 3M Petrifilm SALX Plate.

(*5*) Using a gloved hand (while practicing GLP to avoid cross-contamination and/or direct contact with the plate), gently apply a sweeping motion with even pressure onto the top film to remove any air bubbles in the inoculation area.

(*6*) Streak each enriched test portion onto a 3M Petrifilm SALX Plate and incubate at 41.5±1°C for 24±2 h in a horizontal position with the colored side up in stacks of no more than 20 plates.

G. Confirmation of 3M Petrifilm Salmonella Express Plates

(*1*) Using a permanent ultra-fine tip marker, circle at least five presumptive positive colonies (red to brown colonies with a yellow zone or associated gas bubble, or both) on the plate top film (*see* Table **2014.01D**).

(*2*) Lift the top film of the 3M Petrifilm SALX Plate and insert the 3M Petrifilm SALX Confirmation Disk by rolling it onto the gel to avoid entrapping air bubbles. Close the 3M Petrifilm SALX Plate. Using a gloved hand, gently apply a sweeping motion with even pressure onto the top film to remove any air bubbles in the inoculation area and ensure good contact between the gel and the 3M Petrifilm SALX Confirmation Disk.

(*3*) Incubate the 3M Petrifilm SALX System (plate and disk) at 41.5±1°C for 4–5 h in a horizontal position, right side up, in stacks of no more than 20 plates.

(*4*) Observe circled colonies for color change. Red/brown to green blue, blue, dark blue, or black confirms the colony as *Salmonella* spp. No color change indicates the colony is negative. If presumptive positive *Salmonella* colonies are not present, then report the results as *Salmonella* not detected in the matrix.

(*5*) Transfer typical colonies from 3M Petrifilm SALX Plate to TSI/LIA slants. Incubate 35±1°C for 24±2 h.

(*6*) Confirm a minimum of one typical colony per test portion with biochemical/serological procedures prescribed by the current versions of the USDA/FSIS-MLG or FDA/BAM reference methods.

Results of Collaborative Study

In this collaborative study, the 3M Petrifilm SALX System was compared to the USDA/FSIS-MLG 4.07 reference method for raw ground beef and to the FDA/BAM Chapter 5 reference method for dry dog food. A total of 17 laboratories throughout the United States participated in this study, with 15 laboratories submitting data for the raw ground beef and 14 laboratories submitting data for the dry dog food as presented in Table 1. Results of the heat stress analysis for the dry dog food inocula are presented in Table 2. Each laboratory analyzed 36 test portions for each method: 12 inoculated with a high level of *Salmonella*, 12 inoculated with a low level of *Salmonella*, and 12 uninoculated controls. A background screen of the matrix indicated an absence of indigenous *Salmonella* species. As per criteria outlined in Appendix J of the AOAC Validation Guidelines, fractional positive results were obtained for both matrices. For each matrix, the actual level of *Salmonella* was determined by MPN determination on the day of initiation of analysis by the coordinating laboratory. The individual laboratory and sample results are presented in Tables 3–4. Tables **2014.01A** and **B** summarize the collaborative study results for each matrix tested, including POD statistical analysis (7). Detailed results for each laboratory are presented in Appendix Tables 1–4 and Appendix Figures 1–8. The result for each collaborating laboratory's APC analysis for each matrix is presented in Appendix Table 5.

Raw Ground Beef (25 g Test Portions)

Raw ground beef test portions were inoculated at low and high levels and were analyzed (Table 3) for the detection of *Salmonella* spp. Uninoculated controls were included in each analysis. Seventeen laboratories participated in the analysis of this matrix and the results of 14 laboratories were included in the statistical analysis. Laboratories 4, 6, and 9 reported that there were specific protocol deviations and therefore results from these laboratories were excluded from statistical analysis. The MPNs obtained for this matrix, with 95% confidence intervals, were 0.77 MPN/test portion (0.57, 0.88) for the low level and 4.67 MPN/test portion (3.38, 6.44) for the high

Table 1. Participation of each collaborating laboratory[a]

Lab	Raw ground beef (25 g test portions)	Dry dog food (375 g test portions)
1	Y	Y
2	Y	Y[b]
3	Y	Y
4	Y[b]	Y[b]
5	Y	Y
6	Y[b]	Y[b]
7	Y	Y
8	Y	Y
9	Y[b]	Y
10	Y	Y
11	Y	Y
12	Y	Y
13	Y	Y
14	Y	Y[b]
15	Y	Y
16	Y	Y
17	Y	N

[a] Y = Collaborator analyzed the food type and N = collaborator did not analyze the food type.

[b] Results were not used in statistical analysis due to deviation of testing protocol or laboratory error.

level. For the 3M Petrifilm SALX System, one test portion was confirmed positive by the traditional confirmation that was confirmed negative by the alternative confirmation. For all other test portions, no difference was observed between confirmation of samples using the alternative confirmation procedure and the traditional reference method confirmation procedure.

For the high level, 168 out of 168 test portions were reported as presumptive positive by the 3M Petrifilm SALX System with all test portions confirming positive by both the traditional and alternative confirmation methods. For the low level, 85 out of 168 test portions were reported as presumptive positive by the 3M Petrifilm SALX System, with 83 test portions confirming positive by both the traditional and alternative confirmation procedures. For the uninoculated controls, 2 out of 168 samples produced a presumptive positive result by the 3M Petrifilm SALX System method with one of the two presumptive positive samples confirming positive by the traditional reference method. All other test portions were negative. For test portions analyzed by the USDA/FSIS-MLG method, 167 out of 168 high inoculum and 86 out of 168 low inoculum test portions confirmed positive. For the uninoculated controls, 0 out of 168 test portions confirmed positive.

Table 2. Heat-stress injury results

Matrix	Test organism[a]	CFU/XLD (selective agar)	CFU/TSA (Non-selective agar)	Degree injury
Dry dog food	*Salmonella* Poona NCTC 4840	3.0×10^8	9.0×10^8	77.7%

[a] NCTC = National Collection of Type Cultures.

For the low-level inoculum, a $dLPOD_C$ value of –0.02 (–0.13, 0.09) was obtained between the 3M Petrifilm SALX System using both confirmatory procedures and the USDA/FSIS-MLG method. The confidence intervals obtained for $dLPOD_C$ indicated no significant difference between the two methods. A $dLPOD_{CP}$ of 0.01 (–0.10, 0.12) was obtained between presumptive and confirmed 3M Petrifilm SALX System results for both confirmation procedures. The confidence intervals obtained for $dLPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results.

For the high-level inoculum, a $dLPOD_C$ value of 0.01 (–0.02, 0.03) was obtained between the 3M Petrifilm SALX System using both confirmatory procedures and the USDA/FSIS-MLG method. The confidence intervals obtained for $dLPOD_C$ indicated no significant difference between the two methods. A $dLPOD_{CP}$ of 0.00 (–0.02, 0.02) was obtained between presumptive and confirmed 3M Petrifilm SALX System results for both confirmation procedures. The confidence intervals obtained for $dLPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results.

For the uninoculated control level, $dLPOD_C$ values of 0.01 (–0.02, 0.03) and 0.00 (–0.02, 0.02) were obtained between the 3M Petrifilm SALX System using the traditional and alternative confirmation procedures, respectively, and the USDA/FSIS-MLG method. The confidence intervals obtained for $dLPOD_C$ indicated no significant difference between the two methods. A $dLPOD_{CP}$ of 0.01 (–0.02, 0.04) and 0.01 (–0.01, 0.04) was obtained between presumptive and confirmed 3M Petrifilm SALX System results using the traditional and alternative confirmation procedures, respectively. The confidence intervals obtained for $dLPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results. Results of the POD statistical analysis are presented in Table **2014.01A** and Appendix Tables 1–2 and Appendix Figures 1–4.

Dry Dog Food (375 g Test Portions)

Dry dog food test portions were inoculated at low and high levels and were analyzed (Table 4) for the detection of *Salmonella* spp. Uninoculated controls were included in each analysis. Sixteen laboratories participated in the analysis of this matrix and the results of 12 of the laboratories were included in the statistical analysis. Two laboratories, 4 and 6, were unable to initiate sample testing at the start of the evaluation due to equipment malfunction or a delay in receiving their samples and therefore did not analyze any test portions. Two additional laboratories, 2 and 14, reported deviations from the testing protocol and therefore results from these laboratories were excluded from statistical analysis. The MPN obtained for this matrix, with 95% confidence intervals, were 0.69 MPN/test portion (0.54, 0.86) for the low level and 5.42 MPN/test portion (3.53, 8.30) for the high level. For the 3M Petrifilm SALX System, one test portion was confirmed positive by the traditional confirmation that was confirmed negative by the alternative confirmation. For all other test portions, no difference was observed between confirmation of samples using the alternative confirmation procedure and the traditional reference method confirmation procedure.

For the high level, 142 out of 144 test portions were reported as presumptive positive by the 3M Petrifilm SALX System with

Table 3. Individual collaborator results for raw ground beef (25 g test portions)[a]

	High-level test portions												Low-level test portions												Uninoculated test portions											
Lab	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 Petrifilm SALX System[b]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
2	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	–	+	+	+	+	–	+	–[d]	–	–	–	–	–	–	–	–	–	–	–
3	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
4[e]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6[e]	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
7	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	–	–	–	-	+	–	–	–	–	–	–	–	–	–	–	–	–	–
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	-	+	–	–	–	–	–	–	–	–	–	–	–	–	–
9[e]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
10	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	+	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
12	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
13	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	–[d]	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
14	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
15	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	–	+	+	+	–	+	–	–	–	–	+[c]	–	–	–	–	–	–	–	–
16	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–[d]	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
USDA/FSIS-MLG[d]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
2	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
4[e]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6[e]	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
8	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9[e]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
10	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	–	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–

Table 3. (*continued*)

Lab	High-level test portions												Low-level test portions												Uninoculated 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
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
13	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
17	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] + = *Salmonella* spp. were detected in samples and – = *Salmonella* spp. were not detected in sample.

[b] Confirmed results from alternative and traditional confirmation were identical for each test portion except where noted.

[c] Sample confirmed positive by traditional method was negative by alternative confirmation.

[d] Sample was presumptive positive but confirmed negative.

[e] Results were not used in statistical analysis due to deviation of testing protocol laboratory error.

141 test portions confirming positive by both the traditional and alternative confirmation methods. For the low level, 82 out of 144 test portions were reported as presumptive positive by the 3M Petrifilm SALX System, with 82 test portions confirming positive by the traditional confirmation procedure and 81 test portions confirming positive by the alternative confirmation procedure. For the uninoculated controls, 0 out of 144 samples produced a presumptive positive result by the 3M Petrifilm SALX System method with 0 samples confirming positive by the traditional reference method. All other test portions were negative. For test portions analyzed by the FDA/BAM Chapter 5 Method, 144 out of 144 high inoculum and 71 out of 144 low inoculum test portions confirmed positive. For the uninoculated controls, 0 out of 144 test portions confirmed positive.

For the low-level inoculum, a $dLPOD_C$ value of 0.08 (–0.07, 0.22) and 0.07 (–0.07, 0.21) was obtained between the 3M Petrifilm SALX System with the traditional confirmation and alternative confirmation procedures, respectively, and the FDA/BAM method. The confidence intervals obtained for $dLPOD_C$ indicated no significant difference between the two methods.

A $dLPOD_{CP}$ of 0.00 (–0.14, 0.14) and 0.01 (–0.18, 0.22) was obtained between presumptive and confirmed 3M Petrifilm SALX System results for the traditional and alternative confirmation procedures, respectively. The confidence intervals obtained for $dLPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results.

For the high-level inoculum, a $dLPOD_C$ value of –0.02 (–0.06, 0.01) was obtained between the 3M Petrifilm SALX System with the traditional and alternative confirmation procedures and the FDA/BAM method. The confidence intervals obtained for $dLPOD_C$ indicated no significant difference between the two methods. A $dLPOD_{CP}$ of 0.01 (–0.03, 0.05) was obtained between presumptive and confirmed 3M Petrifilm SALX System results for the traditional and alternative confirmation procedures. The confidence intervals obtained for $dLPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results.

For the uninoculated control level, $dLPOD_C$ values of 0.00 (–0.03, 0.03) were obtained between the 3M Petrifilm SALX System using the traditional and alternative confirmation procedures and the FDA/BAM method. The confidence intervals obtained for $dLPOD_C$ indicated no significant difference between the two methods. A $dLPOD_{CP}$ of 0.03 (–0.03, 0.03) was obtained between presumptive and confirmed 3M Petrifilm SALX System results using the traditional and alternative confirmation procedures. The confidence intervals obtained for $dLPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results. Results of the POD statistical analysis are presented in Table **2014.01A**, and Appendix Tables 3–4 and Appendix Figures 5–8.

Discussion

No negative feedback was reported to the Study Directors from the collaborating laboratories in regard to the performance of the 3M Petrifilm SALX System. For the analysis of the raw ground beef test portions by the 3M Petrifilm SALX System, three false-positive samples were obtained. For the analysis of the dry dog food, two false-positive samples and two false-negative samples were obtained.

Table 4. Individual collaborator results for dry dog food (375 g test portions)[a]

	High-level test portions												Low-level test portions												Uninoculated test portions											
Lab	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 Petrifilm SALX System[b]																																				
1	+	+	+	–	+	+	+	+	+	+	+	–[d]	+	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
2[f]	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–[d]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
4[f]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
5	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
6[f]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
7	+	+	+	+	+	+	+	+[e]	+	+	+	+	–	+	–	–	+[e]	+	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
8	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
10	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	–	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14[f]	+	+	+	+	+	+	+	+	+	+	–[d]	+	–[d]	+	–[d]	–[d]	–[d]	–	–[c]	+	–	+	+	–	–	+	–	–[d]	–	–	–	+	–	–[d]	–	–[d]
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–[d]	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
17	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
FDA/BAM																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
2[f]	+	+	+	+	+	+	+	+	+	–	+	+	–	–	+	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
4[f]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
5	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
6[f]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
10	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 4. (*continued*)

	High-level test portions												Low-level test portions												Uninoculated test portions											
Lab	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
12	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
14[f]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
15	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
16	–	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
17	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	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] + = *Salmonella* spp. were detected in samples, – = *Salmonella* spp. were not detected in sample, and n/a = laboratory did not participate in this matrix or results were not received.

[b] Confirmed results from alternative and traditional confirmation were identical for each test portion unless noted.

[c] Sample was presumptive positive and confirmed negative by traditional confirmation but confirmed positive by alternative.

[d] Sample was presumptive positive but confirmed negative.

[e] Sample was presumptive negative but confirmed positive using the traditional confirmation.

[f] Results were not used in statistical analysis due to deviation of testing protocol laboratory error.

For the dry dog food, some laboratories reported high amounts of atypical growth on varying test portions and no background growth on other test portions. Because over 600 lbs of pet food was used in the evaluation, variability in the level of competing microflora among individual samples may have led to these discrepancies. Laboratory 7, which reported the two false-negative results for the dry dog food, indicated that a significant amount of atypical growth was observed on the 3M Petrifilm SALX Plate and believed it may have contributed to the difficulty in isolating *Salmonella* from those two samples.

The false-positive results observed for both matrices may have been the result of misidentification of typical colonies due to the misinterpretation of colony color (only five false positives out of 972 test portions) on the 3M Petrifilm SALX Plate. Additional experience with the method may eliminate some analyst uncertainty when selecting colonies believed to be presumptive positive for *Salmonella*. Because presumptive colonies are verified by placing the 3M Petrifilm SALX Confirmation Disk onto the 3M Petrifilm SALX Plate, no additional follow-up to verify if the correct colony was selected was possible by testing at the coordinating laboratory. For the raw ground beef, Laboratory 15 identified a presumptive positive colony in its uninoculated test portions, which was confirmed positive by the reference method. The data from this laboratory were included in the statistical analysis. Using the POD statistical model, no significant difference in the number of positive results obtained between the two methods being compared was observed at both the low and high inoculum levels for both matrices. Additionally, no significant difference was observed between presumptive and confirmed results for the candidate method.

Recommendations

It is recommended that the 3M Petrifilm *Salmonella* Express (SALX) System be adopted as Official First Action status for the detection of *Salmonella* in raw ground beef (25 g), raw ground chicken (25 g), pasteurized liquid whole egg (100 g), raw ground pork (25 g), cooked chicken nuggets (325 g), frozen uncooked shrimp (25 g), fresh bunched spinach (25 g), dry dog food (375 g), and stainless steel.

Acknowledgments

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

Brad Stawick and Keith Blanchard, Microbac Laboratories, Inc., Warrendale, PA

Delando Lewis and Robert Colvin, Microbac Laboratories, Inc., Baltimore, MD

Ashley Morris, Microbac Laboratories, Inc., Maryville, TN

Joe Meyer, Covance Laboratories, Monona, WI

Amit Morey, Food Safety Net Services, San Antonio, TX

Kyle Newman, Venture Laboratories, Inc., Lexington, KY

Robert Brooks, ATC Microbiology, LLC, North Little Rock, AR

Christine Gwinn and Scott Moosekian, Covance Laboratories, Inc., Battle Creek, MI

Joey Marchant-Tambone, U.S. Food and Drug Administration, Gulf Coast Seafood Laboratory, Dauphin Island, AL

Kathleen T. Rajkowski, U.S. Department of Agriculture,

Agricultural Research Services, Eastern Regional Research Center, Food Safety and Technologies Initiative, Glenside, PA

Hesham Elgaali, Indiana State Department of Health, Food and Dairy Microbiology Division, Indianapolis, IN

Kristi McCallum, Colorado Department of Agriculture, Biochemistry Laboratory, Denver, CO

Dora Rodgers, Wisconsin Department of Agriculture, Madison, WI

Karen Beers, Safe Foods Corp., Rogers, AR

Jerry Lynn Picket and Jacquelyn Adams, Tyson WBA Analytical, Springdale, AR

Allie Martin, Alliance Analytical Laboratories, Coopersville, MI

Willis Fedio, New Mexico State University, Center for Animal Health, Food Safety and Biosecurity, Las Cruces, NM

Megan Boyle, Q Laboratories Inc., Cincinnati, OH

We extend special thanks to the following team members at Q Laboratories, Inc. for their efforts during the collaborative study: Kiel Fisher, Ben Bastin, Paige Bedinghaus, Kateland Koch, Will Judd, and Nicole Klass.

We would also like to extend a special thanks to Cathy Hill and John David of 3M Technical Services for their efforts in training the collaborators.

References

(1) USDA/FSIS (August 7, 2013) *Salmonella Questions and Answers.* http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/foodborne-illness-and-disease/salmonella-questions-and-answers/ct_index (accessed January, 2014)

(2) Hammack, T. (2011) *Salmonella species* in *Bad Bug Book–Foodborne Pathogenic Microorganisms and Natural Toxins*, 2nd Ed., U.S. Food and Drug Administration, College Park, MD

(3) *Official Methods of Analysis* (2012) 19th Ed., Appendix J: AOAC INTERNATIONAL, Gaithersburg, MD, http://www.eoma.aoac.org/app_j.pdf (accessed November, 2013)

(4) *Microbiology Laboratory Guidebook, Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg and Catfish Products and Carcass and Environmental Sponges* (2013) Revision 4.07, U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC. http://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/MLG-4.pdf?MOD=AJPERES (accessed December, 2013)

(5) Andrews, W.H., & Hammack, T. (2011) FDA/*Bacteriological Analytical Manual*, Chapter 5 *Salmonella.* http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm (accessed December, 2013)

(6) Least Cost Formulations, Ltd (2011) *MPN Calculator–Version 1.6.* www.lcfltd.com/customer/LCFMPNCalculator.exe (accessed January, 2014)

(7) Least Cost Formulations, Ltd (2011) *AOAC Binary Data Interlaboratory Study Workbook Version 2.2* (2011) http://lcfltd.com/aoac/aoac-binary-v2-2.xls (accessed January, 2014)

(8) Wehling, P., LaBudde, R., Brunelle, S., & Nelson, M. (2011) *J. AOAC Int.* **94**, 335–347

AOAC RESEARCH INSTITUTE

Official Methods of Analysis℠ (OMA)

Expert Review Panel on Microbiology for Food and Environmental Surfaces

OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final revised version of the Official Chair's Report for the Expert Review Panel on Microbiology for Food and Environmental Surfaces held on March 20, 2014.

Wendy McMahon

Wendy McMahon, Expert Review Panel Chair

April 10, 2014

Date

Please sign, date and fax this document to La'Kia Phillips at 301-924-7089.

AOAC RESEARCH INSTITUTE

Official Methods of Analysis[SM] (OMA)

Expert Review Panel on Microbiology for Food and Environmental Surfaces

METHODS FOR CONSIDERATION

Conclusion: The Expert Review Panel reviewed the collaborative study for OMAMAN-08: Evaluation of the 3M™ Petrifilm™ Salmonella Express System for the Detection of Salmonella in Selected Foods: Collaborative Study and Review the OMA Modification for 2013.09: 3M™ Molecular Detection Assay (MDA) Salmonella. **Methods Reviewed:** Each method collected by AOAC for consideration by this ERP is reviewed by all members. The decisions of this ERP are reflective of both the submitted method review forms and the in person meeting held on Thursday, March 20, 2014.

METHOD NO.	MANUSCRIPT TITLE
OMA Modification for 2013.09	**EVALUATION OF MODIFICATION OF THE 3M™ MOLECULAR DETECTION ASSAY (MDA) SALMONELLA FOR THE DETECTION OF SALMONELLA IN SELECTED FOODS: COLLABORATIVE STUDY** *AUTHORS* Patrick Bird, Kiel Fisher, Megan Boyle, Travis Huffman, M. Joseph Benzinger, Jr., Paige Bedinghaus, Jonathon Flannery, Erin Crowley, James Agin, David Goins, Q Laboratories, Inc., 1400 Harrison Ave, Cincinnati, OH 45214, DeAnn Benesh, John David, 3M Food Safety Department, 3MCenter – Bldg. 260-6B-01, St. Paul, MN 55144 *COLLABORATORS* K. Newman, V. Gill, I. Mello, M. Ontiberos, C. Gwinn, S. Moosekian, J. Marchent, J. Dyszel, M. Vross, K. Blanchard, D. Lewis, M. Horan, B. Stawick, J. Ruebl, K. Rajkowski, A. Morey, S. Montez, J. Jurgens, L. Thompson, M. Bandu, M. Oltman, D. Bosco, R. Brooks, S. Luce, H. Dammann, D. Clark Jr., W. McMahon, D. Awad, M. Kelly, M. Greenwell

ERP DECISION(S)	ERP ACTIONS FOR OTHER & FINAL ACTION REQUIREMENTS	VOTE	DECISION DATE
Motion to move forward to First Action Official Methods status.	*N/A*	MOTION PASSED ***UNANIMOUS*** *Salfinger, Hammack*	March 20, 2014

AOAC RESEARCH INSTITUTE

Official Methods of Analysis[SM] (OMA)

Expert Review Panel on Microbiology for Food and Environmental Surfaces

METHOD NO.	MANUSCRIPT TITLE
OMAMAN-08	**EVALUATION OF THE 3M™PETRIFILM™SALMONELLA EXPRESS SYSTEM FOR THE DETECTION OF SALMONELLA SPECIES IN SELECTED FOODS: COLLABORATIVE STUDY** *AUTHORS* Patrick Bird, Jonathan Flannery, Erin Crowley, James Agin, David Goins, Q Laboratories, Inc., 1400 Harrison Ave, Cincinnati, OH45214, Robert Jechorek, 3M Food Safety Department, 3MCenter – Bldg. 260-6B-01, St. Paul, MN 55144 *COLLABORATORS* K. Newman, J. Pickett, J. Adams, A. Martin, J. Meyer, H. Elgaali, J. Marchant-Tambone, K. Blanchard, D. Lewis, R. Colvin, B. Stawick, K. Rajkowski, D. Rodgers, K. Beers, A. Morris, K. McCallum, A. Morey, W. Fedio, R. Brooks, M. Boyle

ERP DECISION(S)	ERP ACTIONS FOR OTHER & FINAL ACTION REQUIREMENTS	VOTE	DECISION DATE
Motion to move forward to First Action Official Methods status.	*N/A*	MOTION PASSED ***UNANIMOUS*** *Brodsky, Douey*	March 20, 2014

AOAC RESEARCH INSTITUTE

Official Methods of Analysis[SM] (OMA)

Expert Review Panel on Microbiology for Food and Environmental Surfaces

EXPERT REVIEW PANEL MEMBERS

Wendy McMahon, Silliker, Inc. (CHAIR)
Michael Brodsky, Brodsky Consultants
Maya Achen, Ohio Dept. of Agriculture
Donna Douey, CFIA
Maria C. Fernandez, ANMAT/Ministry of Health Univ. of Buenos Aires
Thomas Hammack, US FDA-CFSAN
Tony Hitchins, US FDA-CFSAN (retired)
Yvonne Salfinger

Not Present

Yi Chen, US FDA-CFSAN (Alternate)

AOAC STAFF

Jim Bradford, Executive Director
Delia Boyd
Deborah McKenzie
La'Kia Phillips

OBSERVERS

DeAnn Benesh, 3M
Patrick Bird, Q Laboratories
Farpan Bower, Sample 6
Erin Crowley, Q Laboratories
Steven Hoelzer, DuPont Nutrition & Health
Robert Jechorek, 3M
Mike Koeris, Sample 6
Todd Marrow, University of Guelph
Sam Mohajer, CFIA
Karen Silbernagel, AOAC Technical Consultant

OMA Modification of 2013.09 Reviewers

Michael Brodsky	Primary Reviewer
Yvonne Salfinger	Secondary Reviewer
Yvonne Salfinger	Safety Reviewer
Hilde Skaar-Norli	Statistical Reviewer

OMAMAN-08 Reviewers

Maria Christina Fernandez	Primary Reviewer
Maya Achen	Secondary Reviewer
Yvonne Salfinger	Safety Reviewer
Caryn Thompson	Statistical Reviewer

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

Detection of *Salmonella* in Selected Foods by the 3M™ Petrifilm™ Salmonella Express System (SALX): Collaborative Study Protocol

OMA 2013-July-25

DRAFT DOCUMENT

Table of Contents

1.0 INTRODUCTION

The goal of this collaborative study is to estimate the following performance parameters of the 3M™ Petrifilm™ Salmonella Express (SALX) System: 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 Petrifilm SALX method along with the corresponding reference method. Raw fresh ground beef will be tested at 25g and dry dog food will be tested at 375g.

1.1 Description of the 3M Petrifilm SALX Method

The 3M Petrifilm SALX method is intended for the rapid and specific detection and biochemical confirmation of *Salmonella* from food and food process environmental samples after enrichment in 3M™ *Salmonella* Enrichment Base with the added 3M™ *Salmonella* Enrichment Supplement. The 3M™ Petrifilm™ Salmonella Express Plate is a simple ready-to-use chromogenic culture medium system that contains a cold-water-soluble gelling agent and is selective and differential for *Salmonella*, providing a presumptive result in as little as 44 hours from low microbial background foods (≤10^4 cfu/g), and 52 hours from high microbial background foods (≥10^4 cfu/g). The 3M™ Petrifilm™ Salmonella Express Confirmation Disk contains a biochemical substrate that facilitates the biochemical confirmation of *Salmonella* organisms

1.2 Summary of PTM/Precollaborative Study

The 3M Petrifilm SALX method was validated according to AOAC Guidelines (2002) in a harmonized PTM/OMA study. The aim of this study was to demonstrate that the 3M Petrifilm SALX method could detect *Salmonella* in select foods as claimed by the manufacturer.

The 3M Petrifilm Salmonella Express System was evaluated following the AOAC *Performance Tested Methods* requirements: inclusivity/exclusivity, ruggedness, stability/lot to lot consistency and method comparison. The 3M Petrifilm SALX method was compared to the USDA/FSIS-MLG 4.05 *Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg and Catfish Products* for raw ground chicken, pasteurized liquid whole egg, raw ground beef, raw ground pork, and cooked chicken nuggets and to FDA/BAM chapter 5 *Salmonella* for frozen uncooked shrimp, fresh bunched spinach, dry dog food, and stainless steel.

Overall the assay was equivalent to the reference methods based on the Chi square test for significant difference and the Probability of Detection (POD). There were no unexpected results in all of the other PTM requirements.

The 3M Petrifilm Salmonella Express System is sufficiently rugged to withstand small variations in the experimental parameters of enrichment incubation time and temperature, plate incubation time and temperature, disk incubation time and temperature and various plate hydration options. Each parameter was tested using both the high and low microbial load methods as described in the Instructions

for Use.

The method was awarded PTM certification number 061301 on June 5, 2013.

1.3 Study Director for Collaborative Study

The co-Study Directors for this collaborative study:

Robert P. Jechorek
3M Food Safety Department
3M Center - Bldg 260-6B-01
St. Paul, MN 55144-1000
Email: rpjechorek@mmm.com
Phone: 651-733-9764

Co-SD: Erin Crowley and Patrick Bird
Q Laboratories, Inc.
Cincinnati, OH 45214
Email: ecrowley@qlaboratories.com
pbird@qlaboratories.com
Phone: 513-471-1300

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 Petrifilm, will act as collaborators. All collaborators have been, or will be, thoroughly trained by a 3M representative on the use of Petrifilm.

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

3.1 The laboratory conducting the study will prepare the test portions. In this study, raw fresh ground beef (25g) and dry dog food (375g) will be analyzed for *Salmonella* following the 3M Petrifilm SALX protocol and appropriate reference method. The USDA-FSIS MLG Ch. 4.06 method will be used as the reference method for ground beef and FDA/BAM Chapter 5 will be used as the reference method for dry dog food.

3.2 Each week, collaborators will receive 72 samples of a test matrix for analysis: 12 uninoculated 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 - SALX and reference method. See flow diagrams 8.5 and 8.6 for the study details. Each laboratory will test all test portions. Each test matrix will be inoculated with a different *Salmonella* spp. as listed in Table 1, randomized and blind-coded for analysis.

Table 1. Overview of Study Design

MATRIX	Inoculating organism	Target *Salmonella* Levels	# Test Portions (per method)	Test Portion Codes	Reference Method
Raw ground beef (80/20) *25g-SALX 25g-MLG*	*S. enterica* subsp. Ohio STS 81 (Fresh)	0 cfu/test portion	12	Random number between 100-199	USDA/FSIS MLG Ch. 4.06
		0.2-2 cfu/test portion	12		
		2-5 cfu/test portion	12		
Dry dog food *375g-SALX 25g-BAM*	*Salmonella enterica* ser. *Poona* ATCC 4840	0 cfu/test portion	12	Random number between 200-299	FDA/BAM Chapter 5
		0.2-2 cfu/test portion	12		
		2-5 cfu/test portion	12		

3.3 Study Schedule

3.3.1 One matrix will be tested each week of the study. All data should be sent to the SD by the 4th week of study.

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

4.0 Test Portion Preparation

4.1 Fresh raw ground beef and dry dog food will be obtained from local retail outlets and screened for the presence of *Salmonella*. 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 for raw ground beef and a lyophilized inoculum for dry dog food.

4.2 **Preparation of test portions**

4.2.1 *Ground beef:* 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. The test portions will be stored at 2-8°C for 72-96 h prior to testing.

4.2.2 *Dry dog food*: A wet inoculums in pellet form will be added to a bulk quantity of dry dog food 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. The test portions will be stored at room temperature for 2 weeks prior to testing. Samples should be checked during the stabilization period to ensure

the correct contamination levels are achieved. Test portions (25g) will be pulled from this bulk lot and randomly assigned to the reference method or the 3M SALX method. An additional 350g uncontaminated dry dog food will be added to the 25g test portion assigned to the 3M SALX method resulting in a 375g test portion.

4.3 **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. All dry test portions will be shipped at ambient temperature (22-25°C). 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 and the dry dog food test portions will be stored at room temperature until they are analyzed.

4.4 **Microbiological analysis.** The test portion preparation is different for the 3M Petrifilm SALX and reference methods. Therefore, each collaborator will receive a separate test portion for each method. They will be instructed to test 25g ground beef using the 3M Petrifilm SALX method and another 25g ground beef using the MLG reference method. They will be instructed to test 375g dry dog food using the 3M Petrifilm SALX method and 25g dry dog food using the BAM reference method. All test portions assayed by the 3M method will be confirmed following the reference method for confirmation of *Salmonella* species as described in the Alternative Confirmation section. 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.

4.5 **Most Probable Number (MPN) Analysis.** To determine the level of *Salmonella* spp. in the inoculated food test portions on the day of initiation of analysis, a 5-tube MPN determination is performed by the coordinating laboratory following the appropriate reference method. From each of the inoculated food batches, prepare 5 test portions of 100 g, 5 test portions of 25 g and 5 test portions of 10 g. To each test portion, add BPW to make a 1:4 dilution. Follow the reference method through to confirmations. Use the number of positives from the high, medium and low levels to calculate the MPN for each inoculated level of each matrix. Access the MPN calculator (http://www.lcfltd.com/customer/LCFMPNCalculator.exe) to determine the MPN values and 95% confidence intervals. Data should be entered from high level to low. Report MPN as MPN/test portion.

5.0 3M™ Petrifilm™ Salmonella Express (SALX) System Method

5.1 *Applicability*

For the detection of *Salmonella* species in a Select Foods, Dry Dog Food, and Stainless Steel.

The 3M Petrifilm SALX Plate is a sample ready-to-use chromogenic culture medium system that contains a cold-water-soluble gelling agent and is selective and differential for *Salmonella*, providing a presumptive result. The 3M Petrifilm SALX Confirmation Disk contains a biochemical substrate that facilitates the biochemical confirmation of *Salmonella* organisms.

5.2 *Test Kit information*

5.2.1 **3M Petrifilm Salmonella Express Plate**:

5.2.1.1 6536 - Petrifilm Salmonella Express Plate 25 plates/pouch; 2 pouches/box

5.2.1.2 6537 - Petrifilm Salmonella Express Plate 25 plates/pouch; 8 pouches/case

5.2.2 Typical Formula

5.2.2.1	Magnesium chloride (anhydrous)	13.4g
5.2.2.2	Sodium chloride	7.2g
5.2.2.3	Casein peptone	4.54g
5.2.2.4	Monopotassium phosphate	1.45g
5.2.2.5	Malachite green	0.056g
5.2.2.6	Demineralized water	1000 mL
5.2.2.7	pH 5.1 ± 0.2 at 25°C	
5.2.2.8	Adjust pH as required to meet performance standards.	

5.2.3 **3M Petrifilm Salmonella Confirmation Disk:**

5.2.3.1 6538 - Petrifilm Salmonella Confirmation Disk - 5 disks/pouch; 1 pouch/box

5.2.3.2 6539 - Petrifilm Salmonella Confirmation Disk - 5 disks/pouch; 5 pouches /case

5.3 *Reagents*

5.3.1 3M Salmonella Enrichment Broth: SEB500 - 500g/bottle, SEB025 - 2.5kg/bottle

5.3.2 3M Salmonella Enrichment Supplement: SESUP001 - 1.0g/vial

5.3.3 3M Rappaport-Vassiliadis R10 (R-VR10) broth (BP0288500)

5.4 *Selective agar*

5.4.1 Hektoen Agar

5.4.2 XLD Agar

5.4.3 XLT4 Agar

5.4.4 Bismuth Sulfite (Wilson-Blair Agar)

5.4.5 Brilliant Green Sulfa Agar

Formatted: Font: Italic

5.5 *Apparatus*

5.5.1 *Sterile 10µL inoculation loop.* - for streaking enrichments onto the SALX plate

5.5.2 *Permanent ultra fine tip marker.* – for circling presumptive positive colonies on the SALX plate

5.5.3 *Sterile serological pipettes.* – capable of 1mL
5.5.4 *Pipettes*. – capable of 100µL
5.5.5 *Sterile pipette tips*. – capable of 100µL
5.5.6 *Stomacher®*. – Seward or equivalent
5.5.7 *Filter Stomacher® bags*. - Seward or equivalent
5.5.8 *Incubators*. – capable of maintaining 41.5° ± 1°C
5.5.9 Incubator -capable of maintaining 35°C± 1°C
5.5.10 5.5.10Waterbath- capable of maintaining 42°C± 0.2°C(circulating, thermostatically controlled)
5.5.11 *Freezer*. – capable of maintaining -10° to -20°C, for storing opened SALX pouches , hydrated SALX plates, SALX plates after incubation
5.5.12 *5.5.11Refrigerator*. – capable of maintaining 2° - 8°C, for storing unopened 3M SALX plates and SALX disks
5.5.13 *Sterile distilled water*
5.5.14 Sterile reverse osmosis water
5.5.15 Sterile Butterfield's Phosphate Diluent
5.5.16 3M Petrifilm Flat Spreader

5.6 *Enrichment*

5.6.1.1 **Raw ground beef (25g, high microbial load)**
5.6.1.1.1 Aseptically add the 25g test portion to 225 mL pre-warmed (41.5°C) 3M *Salmonella* Enrichment Base with supplement.
5.6.1.1.2 Mix in a Stomacher® bag with filter for 2 min.
5.6.1.1.3 Incubate 18-24 hr at 41.5 ± 1°C.
5.6.1.1.4 After primary enrichment incubation, transfer 0.1 mL of the primary enrichment into 10.0 mL Rappaport-Vassiliadis R10 (R-V R10) Broth. Incubate at 41.5 ± 1.0°C for 8-24 hours.

5.6.1.2 **Dry dog food (375g, low microbial load)**
5.6.1.2.1 Aseptically add the 375g test portion to 3375 mL pre-warmed (41.5°C) 3M *Salmonella* Enrichment Base with supplement.
5.6.1.2.2 Mix in a Stomacher® bag with filter for 2 min.
5.6.1.2.3 Incubate 18-24 hr at 41.5 ± 1°C.

5.7 *Plate Hydration*

5.7.1 Use prescribed sterile diluents to hydrate the 3M Petrifilm SALX Plates: Butterfield's Phosphate Diluent, distilled water, or reverse osmosis water.
5.7.2 Place the 3M Petrifilm SALX Plate on a flat, level surface (figure A).
5.7.3 Lift the top film and with the pipette perpendicular dispense 2.0 mL ± 0.1 mL of sterile diluent onto the center of bottom film (figure B). Do not close the top film before dispensing the entire 2.0 mL volume.
5.7.4 Gently roll down the top film onto the diluent to prevent trapping air bubbles (figure C).
5.7.5 Place the 3M™ Petrifilm™ Flat Spreader (Catalog #6425) on the center of the plate. Press gently on the center of the spreader to distribute the diluent evenly.

Spread the diluent over the entire 3M Petrifilm SALX Plate growth area before the gel is formed. Do not slide the spreader across the film (figure D).

5.7.6 Remove the spreader and leave the 3M Petrifilm SALX Plate undisturbed for at least 1 minute.

5.7.7 Place 3M Petrifilm SALX Plate on a flat surface for at least 1 hour at room temperature (20-25°C / <60% RH), protected from light, to allow the gel to form. Hydrated 3M Petrifilm SALX Plates can be stored at room temperature (20-25°C / <60% RH) for up to 8 hours before use if protected from light,. If hydrated 3M Petrifilm SALX Plates will not be used within 8 hours, seal them in a plastic bag,. protected from light and store at -20 to -10°C for up to 5 days.

5.7.8 After 3M Petrifilm SALX Plates are removed from storage, allow them to warm to room temperature before use.

5.8 *Plate Inoculation*

5.8.1 Remove the enrichment medium from the incubator and agitate contents by gentle hand massage.

5.8.2 Use a sterile 10 µL loop (3 mm diameter) to withdraw each sample. Use a smooth loop (one that does not have jagged edges and is not distorted) to prevent the gel surface from breaking.

5.8.3 Open the 3M Petrifilm SALX Plate and streak onto the gel (figure E). Perform a single streak to obtain isolated colonies (Figure 1).

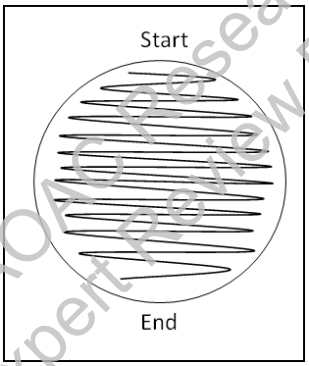


Figure 1: Streaking pattern on the 3M Petrifilm SALX Plate

5.8.4 Roll down the top film to close the 3M Petrifilm SALX Plate.

5.8.5 Using a gloved hand (while practicing good laboratory practices to avoid cross contamination and/or direct contact with the plate), gently apply a sweeping motion with even pressure onto the top film to remove any air bubbles in the inoculation area (figure F).

5.9 *Plate Incubation*

5.9.1 Incubate plates at 41.5 ± 1.0°C for 24 hours ± 2 hours in a horizontal position with the colored side up in stacks of no more than 20 plates.

5.10 *Interpretation*

5.10.1 Remove the 3M Petrifilm SALX Plates from the incubator and visually examine the isolated colonies. See Table 2. Do not count artifact bubbles that may be present.

5.10.2 Note: Using indirect back lighting may enhance reading of colony color, discrete yellow zones, and gas bubbles associated with a colony.

5.10.3 Presumptive positive *Salmonella* species are red to brown colonies with a yellow zone or associated gas bubble, or both (figure G). An associated gas bubble is defined as being located within one colony diameter distance from the colony (see Table 2 below).

Table 2: Interpretation for Presumptive Positive *Salmonella* species

Colony Color			Colony Metabolism		Result
Red	Dark Red	Brown	Yellow zone	Gas bubble	
✓			✓		Presumptive +
✓				✓	Presumptive +
✓			✓	✓	Presumptive +
	✓		✓		Presumptive +
	✓			✓	Presumptive +
	✓		✓	✓	Presumptive +
		✓	✓		Presumptive +
		✓		✓	Presumptive +
		✓	✓	✓	Presumptive +

Non-*Salmonella* species (figure H):

- Blue colonies, green colonies, blue to green colonies, and/or black colonies with or without a yellow zone and/or associated gas bubble are non-*Salmonella* organisms.
- Red, dark red, and brown colonies with no yellow zone and no associated gas are non-*Salmonella* organisms.
- Red, dark red, and brown colonies with a magenta zone are non-*Salmonella* organisms.

If presumptive positive *Salmonella* colonies are not present, then *report the results as Salmonella* not detected in the matrix.

If presumptive positive *Salmonella* colonies are present, then continue with the following Biochemical Confirmation steps:

a. On the 3M Petrifilm SALX Plate top film, **circle up to five isolated presumptive positive *Salmonella* colonies using a permanent, ultra fine tip marker** (figure I).
b. Biochemically confirm all *Salmonella* presumptive positive results using the 3M Petrifilm SALX Confirmation Disk. See the Biochemical Confirmation section.

Note: If the 3M Petrifilm SALX Plates cannot be analyzed within 1 hour of removal from the incubator**, first circle the presumptive *Salmonella* colonies on the top film by using a permanent, ultra fine tip marker** and then place the plates in a sealed plastic bag for later analysis. Protect 3M Petrifilm SALX Plates from light and store at -20 to -10°C for no longer than 72 hours. Allow plates to warm to room temperature (20-25°C / <60% RH) before adding the disk to the plate.

⚠ **WARNING:** To reduce the risks associated with a false-negative result leading to the release of contaminated product and the possibility of false positive results requiring a retest, always use a permanent, ultra fine tip marker to circle the characteristic presumptive *Salmonella* colonies on the top film of the 3M Petrifilm SALX Plate before appropriate storage of the plate and/or before placing the 3M Petrifilm SALX Confirmation Disk onto the gel.

5.11 **BIOCHEMICAL CONFIRMATION – 3M Petrifilm SALX Confirmation Disk**

5.11.1 **NOTE:** Use good laboratory practices to avoid cross contamination and/or direct contact with the 3M Petrifilm SALX Plate and/or 3M Petrifilm SALX Confirmation Disk.

5.11.2 Remove an individually packaged 3M Petrifilm SALX Confirmation Disk from its pouch and allow it to come to room temperature (20-25°C / <60% RH). Then remove the 3M Petrifilm SALX Confirmation Disk from its individual package by peeling the package to expose the 3M Petrifilm SALX Confirmation Disk's tab, grasping the tab with a gloved hand, and removing the 3M Petrifilm SALX Confirmation Disk.

5.11.3 Lift the top film (with the already circled presumptive *Salmonella* colonies) of the 3M Petrifilm SALX Plate and insert the 3M Petrifilm SALX Confirmation Disk by rolling it onto the gel to avoid entrapping air bubbles (figure J). Close the 3M Petrifilm SALX Plate.

5.11.4 Using a gloved hand, gently apply a sweeping motion with even pressure onto the top film to remove any air bubbles in the inoculation area and assure good contact between the gel and the 3M Petrifilm SALX Confirmation Disk (figure K).

5.11.5 Incubate the 3M Petrifilm SALX System (plate and disk) at 41.5 ± 1.0°C for 4 - 5 hours in a horizontal position, colored side up, no higher than 20 plates.

5.11.6 Remove the 3M Petrifilm SALX System from the incubator and proceed with reading the results. **Only interpret the results for the circled presumptive *Salmonella* colonies:**

5.11.6.1 Biochemically confirmed positive results: Colonies that are green to blue, blue to dark blue, or black or have a blue precipitate around them are biochemically confirmed positive for *Salmonella* species.

5.11.6.2 Biochemically confirmed negative results: Colonies that remain the same red, dark red or brown color without a blue precipitate are negative for *Salmonella* species.

5.11.7 Colonies may be subcultured for further identification. When subculturing, wear appropriate protective apparel and follow standard good laboratory safety practices (GLP).

5.11.8 With a gloved hand, lift the top film and aseptically remove the colony either from the gel or the 3M Petrifilm SALX Confirmation Disk. If a 3M Petrifilm SALX Confirmation Disk is covering the gel, aseptically peel the disk away and then aseptically remove the colony from the gel.

5.11.9 Streak the colony onto/into media per appropriate reference method.

5.11.10 **NOTE:** If the colonies cannot be subcultured within 1 hour of plate removal from the incubator, then store the 3M Petrifilm SALX Plates for later analysis by placing in a sealed plastic bag at -20 to -10°C for no longer than 72 hours in the dark. Allow 3M Petrifilm SALX Plates to warm to room temperature (20-25°C / <60% RH) before continuing subculturing for identification.

5.11.11 After the test is complete, dispose of the 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disks in accordance with current industry standards and/or local regulations.

6.0 Reporting Raw Data

6.1 Report data using the data report form in Appendix 8.2.

6.2 Upon completion of each food type, the laboratory will fax or email the completed data form to the Study Director.

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

7.0 Analyzing Raw Data

The resulting data will be analyzed by two different calculations: probability of detection (POD) and relative limit of detection (RLOD).

7.1 **Probability of Detection (POD) -** POD is the proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent. Please see the revised microbiology guidelines[1] for complete analysis information on POD calculations.

7.1.1 Calculate:

7.1.1.1 Estimate of repeatability - standard deviation (s_r)

7.1.1.2 Determine POD values for each matrix and concentration - the number of positive outcomes divided by the total number of trials.

7.1.1.3 Estimate of reproducibility – standard deviation of the laboratory POD values (s_{POD}) and confidence intervals.

[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

7.1.1.4 Cross laboratory probability of detection (LPOD) – LPODs by matrix and concentration with 95% confidence intervals for candidate method (presumptive and confirmed) results.

7.1.1.5 Difference of cross-laboratory probability of detection (dLPOD) – difference probability of detection is the difference between any two LPOD values.

7.1.1.5.1 Estimate $dLPOD_C$ as the difference between the candidate and reference LPOD values, include CIs.

7.1.1.5.2 Estimate $dLPOD_{CP}$ as the difference between the presumptive and confirmed LPOD values, include CIs.

7.1.1.5.3 **If the CI of a dLPOD does not contain zero then the difference is statistically significant**.

7.1.1.6 For each matrix, graph POD_R, $LPOD_C$ and $dLPOD_C$ by level with 95% confidence intervals.

8.0 Appendices

8.1 Instructions to Collaborators
8.2 Test Portion Data Report Forms
8.3 Collaborator Comment Form
8.4 Study Materials
8.5 Flow Diagram – raw ground beef
8.6 Flow Diagram – dry dog food
8.7 Collaborator Information Sheet

Appendix 8.1

Instructions to Collaborators

General Instructions about Collaborative Studies

Introduction

The purpose of this document is to provide detailed instructions for performing the collaborative study for the 3M™ Petrifilm™ Salmonella Express System.

The trial will be conducted by co-Study Directors, Bob Jechorek 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:

Robert P. Jechorek
3M Food Safety Department
3M Center - Bldg 260-6B-01
St. Paul, MN 55144-1000
Email: rpjechorek@mmm.com
Phone: 651-733-9764

Co-SD: TBD

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 Petrifilm SALX 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 (80/20) *25g-SALX* *25g-MLG*	Thursday	Monday
Dry dog food *375g-SALX* *25g-BAM*	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 refrigerated products should arrive cold to the touch and should be stored at 4°C (refrigerated) until the day of analysis. Dry products should be stored at room temperature until the day of analysis.

Test Portion Analysis

Collaborators will be provided with enough test portion to test each matrix using the 3M Petrifilm SALX method and the appropriate reference method. For the week of raw ground beef testing, collaborators will receive two sets of test portions: 36 x 25g (3M) and 36 x 25g (MLG). For the week of dry dog food testing, collaborators will receive: 36 x 375g (3M) and 36 x 25g (BAM). See Appendices 8.5-8.6 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.

Food Type	Reference	Reference Pre-enrichment	3M Petrifilm SALX 1° Enrichment	3M Petrifilm SALX 2° Enrichment
Raw ground beef (80/20) *25g-SALX* *25g-MLG*	USDA-MLG[2]	BPW	3M Salmonella enrichment + supplement	Rappaport-Vassiliadis R10 (R-V R10) Broth
Dry dog food *375g-SALX* *25g-BAM*	FDA-BAM[3]	Lactose broth	3M Salmonella enrichment + supplement	None

1.0 **Ground beef - 3M Petrifilm SALX**

1.1 Aseptically add the 25g test portion to 225 mL pre-warmed (41.5°C) 3M *Salmonella* Enrichment Base with supplement.

1.2 Mix in a Stomacher® bag with filter for 2 min.

1.3 Incubate 18-24 hr at 41.5 ± 1°C.

1.4 After primary enrichment incubation, transfer 0.1 mL of the primary enrichment into 10 mL Rappaport-Vassiliadis R10 (R-V R10) Broth. Incubate at 41.5 ± 1.0°C for 8-24 hours.

1.5 Prior to analysis, hydrate the 3M Petrifilm SALX Plates using 20 mL of sterile distilled water. After hydration, the liquid was spread across of the surface of the plate using a plastic spreader to evenly distribute the diluent. The plates were left undisturbed and protected from light for a minimum of 1 hour prior to use.

1.6 Streak each enriched test portion onto 3M Petrifilm SALX plate and incubate at 41.5° ± 1°C for 24 ± 2 hours.

1.7 **Alternative 3M SALX Confirmation Method**

1.7.1 Using a fine tip marker, circle presumptive positive colonies (red to brown colonies with discrete yellow zones and/or gas bubbles) on the plate top film.

1.7.2 Lift the top film and place a 3M Petrifilm *Salmonella* Express Confirmation Disk onto the gel. Close the film using a gloved hand (while practicing good laboratory technique) and remove air bubbles by gently applying a sweeping motion with even pressure onto the top of the film with the analyst's fingers.

1.7.3 Incubate the plates with confirmation disks at 41.5° ± 1°C for 4 to 5 hours.

1.7.4 Observe circled colonies for color change - red/brown to green blue, blue, dark blue or black confirm the colony as *Salmonella* species. No color change, colony is negative.

1.7.5 Transfer typical colonies from SALX plate to TSI/LIA slants. Incubate 35 ± 1°C for 24 ± 2h.

1.7.6 Confirm a minimum of one typical colony per test portion with biochemical/serological procedures prescribed by the USDA/FSIS method. Either the API20E (Official Method 978.24) or the VITEK GN (Official Method 2011.17) will be used as an alternative to the conventional biochemical tests. The somatic (O) and flagellar (H) tests will also be performed.

[2] http://www.fsis.usda.gov/PDF/MLG_4_05.pdf
[3] http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm

1.8 **Reference Method Confirmation**

1.8.1 Transfer 0.5 ± 0.05 mL 3M SALX enriched sample into 10 mL tetrathionate (TT-Hajna) broth and 0.1 ± 0.02 mL into 10 mL modified Rappaport-Vassiliadis (mRV) broth. Incubate at 42 ± 0.5°C for 22-24 h.

1.8.2 Streak both secondary enrichments onto double modified lysine iron agar (DMLIA) or xylose lysine Tergitol (XLT4) agar and brilliant green sulfa (BGS) agar. Use one loopful of inoculum for each plate. Incubate 35 ± 2°C for 18-24h. Select colonies. Re-incubate all plates for an additional 18-24 h. Reexamine initially negative plates and pick colonies as above.

1.8.3 Transfer typical colonies to TSI/LIA slants. Incubate 35 ± 1°C for 24 ± 2h.

1.8.4 Confirm a minimum of one typical colony per sample with biochemical/serological procedures prescribed by the USDA/FSIS method. Either the API20E (Official Method 978.24) or the VITEK GN (Official Method 2011.17) will be used as an alternative to the conventional biochemical tests. The somatic (O) and flagellar (H) tests will also be performed.

2.0 **USDA-FSIS MLG Ch. 4.06 – Ground beef**

2.1 To each 25g test portion, add 225 mL buffered peptone water. Stomach each sample for approximately two minutes. Incubate at 35±1°C for 22±2h.

2.2 Transfer 0.5 ± 0.05 mL enriched sample into 10 mL tetrathionate (TT-Hajna) broth and 0.1 ± 0.02 mL into 10 mL modified Rappaport-Vassiliadis (mRV) broth. Incubate at 42 ± 0.5°C for 22-24 h.

2.3 Streak both secondary enrichments onto double modified lysine iron agar (DMLIA) or xylose lysine Tergitol (XLT4) agar and brilliant green sulfa (BGS) agar. Use one loopful of inoculum for each plate. Incubate 35 ± 2°C for 18-24h. Select colonies. Re-incubate all plates for an additional 18-24 h. Reexamine initially negative plates and pick colonies as above.

2.4 Transfer typical colonies to TSI/LIA slants. Incubate 35 ± 1°C for 24 ± 2h.

2.5 Confirm a minimum of one typical colony per sample with biochemical/serological procedures prescribed by the USDA/FSIS method. Either the API20E (Official Method 978.24) or the VITEK GN (Official Method 2011.17) will be used as an alternative to the conventional biochemical tests. The somatic (O) and flagellar (H) tests will also be performed.

3.0 **Dry dog food - 3M Petrifilm SALX:** Aseptically add the 375g test portion to 3375 mL pre-warmed (41.5°C) 3M *Salmonella* Enrichment Base with supplement.

3.1 Mix in a Stomacher® bag with filter for 2 min.

3.2 Incubate 18-24 hr at 41.5 ± 1°C.

3.3 Prior to analysis, hydrate the 3M Petrifilm SALX Plates using 2.0 mL of sterile distilled water. After hydration, the liquid was spread across of the surface of the plate using a plastic spreader to evenly distribute the diluent. The plates were left undisturbed and protected from light for a minimum of 1 hour prior to use.

3.4 Streak each enriched test portion onto 3M Petrifilm SALX plate and incubate at 41.5° ± 1°C for 24 ± 2 hours.

3.5 After incubation, record presumptive positive or negative result.

3.5.1 Positive – red to brown with yellow zone or gas bubble

3.5.2 Negative – no typical colonies present

3.6 **Alternative 3M SALX Confirmation Method**

3.6.1 Using a fine tip marker, circle presumptive positive colonies (red to brown colonies with discrete yellow zones and/or gas bubbles) on the plate top film.

3.6.2 Lift the top film and place a 3M Petrifilm *Salmonella* Express Confirmation Disk onto the gel. Close the film using a gloved hand (while practicing good laboratory technique) and remove air bubbles by gently applying a sweeping motion with even pressure onto the top of the film with the analyst's fingers.

3.6.3 Incubate the plates with confirmation disks at 41.5° ± 1°C for 4 to 5 hours.

3.6.4 Observe circled colonies for color change - red/brown to green blue, blue, dark blue or black confirm the colony as *Salmonella* species. No color change, colony is negative.

3.6.5 Transfer typical colonies from SALX plate to TSI/LIA slants. Incubate 35 ± 1°C for 24 ± 2h.

3.6.6 Confirm a minimum of one typical colony per test portion with biochemical/serological procedures prescribed by the USDA/FSIS method. Either the API20E (Official Method 978.24) or the VITEK GN (Official Method 2011.17) will be used as an alternative to the conventional biochemical tests. The somatic (O) and flagellar (H) tests will also be performed.

3.7 **Reference Method Confirmation**

3.7.1 Transfer 0.5 ± 0.05 mL 3M SALX enriched sample into 10 mL tetrathionate (TT-Hajna) broth and 0.1 ± 0.02 mL into 10 mL modified Rappaport-Vassiliadis (mRV) broth. Incubate at 42 ± 0.5°C for 22-24 h.

3.7.2 Streak both secondary enrichments onto double modified lysine iron agar (DMLIA) or xylose lysine Tergitol (XLT4) agar and brilliant green sulfa (BGS) agar. Use one loopful of inoculum for each plate. Incubate 35 ± 2°C for 18-24h. Select colonies. Re-incubate all plates for an additional 18-24 h. Reexamine initially negative plates and pick colonies as above.

3.7.3 Transfer typical colonies to TSI/LIA slants. Incubate 35 ± 1°C for 24 ± 2h.

3.7.4 Confirm a minimum of one typical colony per sample with biochemical/serological procedures prescribed by the USDA/FSIS method. Either the API20E (Official Method 978.24) or the VITEK GN (Official Method 2011.17) will be used as an alternative to the conventional biochemical tests. The somatic (O) and flagellar (H) tests will also be performed.

4.0 **FDA-BAM Chapter 5 – Dry dog food**

1.1.1. Add twenty 25g contaminated test portions and five 25g uncontaminated test portions to 225ml lactose broth. Blend 2 min. Let stand 60±5 min at room temperature. Mix and adjust pH to 6.8±0.2 if necessary. Incubate 24 ± 2h at 35°C.

1.1.2. Transfer 0.1 mL to 10 mL RV broth (prepared from individual ingredients) and 1 mL to 10 mL TT broth. Incubate the RV at 42 ± 0.2°C for 24 ± 2 h. Incubate the TT broth at 35 ± 2.0°C for 24 ± 2 h in a forced air incubator.

1.1.3. Mix and streak 3 mm loopful (10 µL) RV broth onto BS, XLD and HE agars. Repeat from TT broth. Incubate at 35°C for 24 ± 2 h. Follow isolation procedure according to FDA-BAM.

1.1.4. Transfer typical colonies to TSI and LIA. Incubate at 35°C for 24 ± 2 h.

1.1.5. Confirm 1 typical colony per test portion with biochemical/serological procedures prescribed by the FDA-BAM method. Either the API20E® Enteric Identification System (Official Method 978.24), the MICRO-ID® (Official Method 989.12) or the VITEK® GNI (Official Method 991.13) will be used as an alternative to the conventional biochemical tests. The somatic (O) and flagellar (H) tests will also be performed.

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 Petrifilm SALX and reference methods. Immediately upon completion of each food type, the data sheet should be faxed or emailed to TBD, co-Study Director.

Appendix 8.2 - 3M Petrifilm SALX Collaborative Study Data Report Form, Raw Ground Beef 25g (3M Petrifilm SALX**)**

Lab:
Analyst:
Biochemical ID method:
APC:
Analyst start date:
Analyst completion date:
3M SALX plate lot number:
3M SALX Confirmation Disk lot:

3M Petrifilm SALX Method

Sample#	Petrifilm Result	Reference Method Confirmation										3M SALX Confirmation						
		TT		mRV		ID												
		BGS	XLT4	BGS	XLT4	TSI	LIA	biochemical ID	Somatic O	Flagellar H	Final result	Result from 3M SALX disk	TSI	LIA	biochemical ID	Somatic O	Flagellar H	Final result

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

3M Petrifilm SALX Collaborative Study Data Report Form, Raw Ground Beef (USDA/MLG)

Lab:
Analyst:
Biochemical ID method:
APC:

Analyst start date:
Analyst completion date:

USDA/FSIS Method										
Sample#	Reference Method Confirmation									
	TT		mRV		ID					
	BGS	XLT4	BGS	XLT4	TSI	LIA	biochemical ID	Somatic O	Flagellar H	Final result

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

3M Petrifilm SALX Collaborative Study Data Report Form, Dry Dog Food 375g (3M Petrifilm SALX)

Lab:
Analyst:
Biochemical ID method:

Analyst start date:
Analyst completion date:
3M SALX plate lot number:
3M SALX Confirmation Disk lot:

3M Method																			
		FDA-BAM Reference Method Confirmation											3M SALX Confirmation						
		TT			mRV			ID											
Sample#	3M SALX Presumptive Result	BS	XLD	HE	BS	XLD	HE	TSI	LIA	biochemical ID	Somatic O	Flagellar H	Final result	Result from 3M SALX disk	TSI	LIA	biochemical ID	Somatic O	Flagellar H	Final result

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

3M Petrifilm SALX Collaborative Study Data Report Form, Dry Dog Food 375g (FDA-BAM)

Lab:
Analyst:
Biochemical ID method:

Analyst start date:
Analyst completion date:
APC:

FDA-BAM Method												
Sample#	TT			mRV			ID					Final result
	BS	XLD	HE	BS	XLD	HE	TSI	LIA	biochemical ID	Somatic O	Flagellar H	

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

Appendix 8.3

3M Petrifilm SALX Collaborative Study
Collaborator Comments Form

Collaborating Laboratory:

Date:

General Comments about Method:

Appendix 8.4

Study Materials

Materials provided by collaborator

Sterile 10µL inoculation loop. - for streaking enrichments onto the SALX plate
Permanent ultra fine tip marker. – for circling presumptive positive colonies on the SALX plate
Sterile serological pipettes. – capable of 1mL
Pipettes. – capable of 100µL
Sterile pipette tips. – capable of 100µL
Stomacher®. – Seward or equivalent
Filter Stomacher® bags. - Seward or equivalent
Incubators. – capable of maintaining 41.5° ± 1°C
Incubator -capable of maintaining 35°C± 1°C
Waterbath- capable of maintaining 42°C± 0.2°C(circulating, thermostatically controlled)
Freezer. – capable of maintaining -10° to -20°C, for storing opened SALX pouches , hydrated SALX plates, SALX plates after incubation
Refrigerator. – capable of maintaining 2° - 8°C, for storing unopened 3M SALX plates and SALX disks
Sterile distilled water

Materials and reagents provided by the study sponsor

3M Petrifilm Salmonella Express Plate
3M Petrifilm Salmonella Confirmation Disk
3M Salmonella Enrichment Broth
3M Salmonella Enrichment Supplement
3M Rappaport-Vassiliadis R10 (R-VR10) broth
3M Petrifilm Flat Spreader

Enrichment

Buffered Peptone Water
Lactose Broth
mRV
TT broth (Hajna formulation)

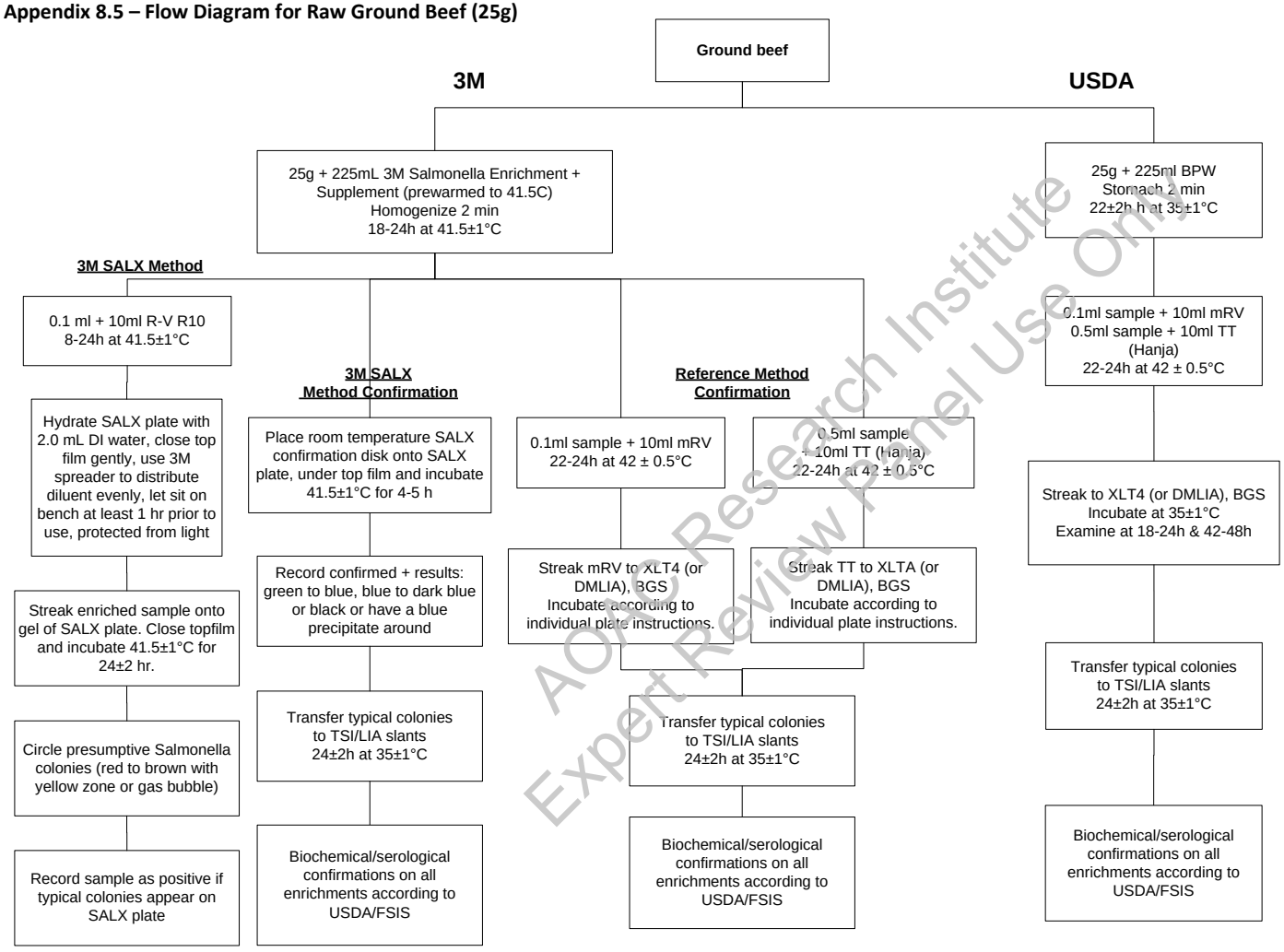
Selective agar

Hektoen Agar - BAM
XLD Agar - BAM
BS - BAM
XLT4 Agar - MLG
BGS - MLG

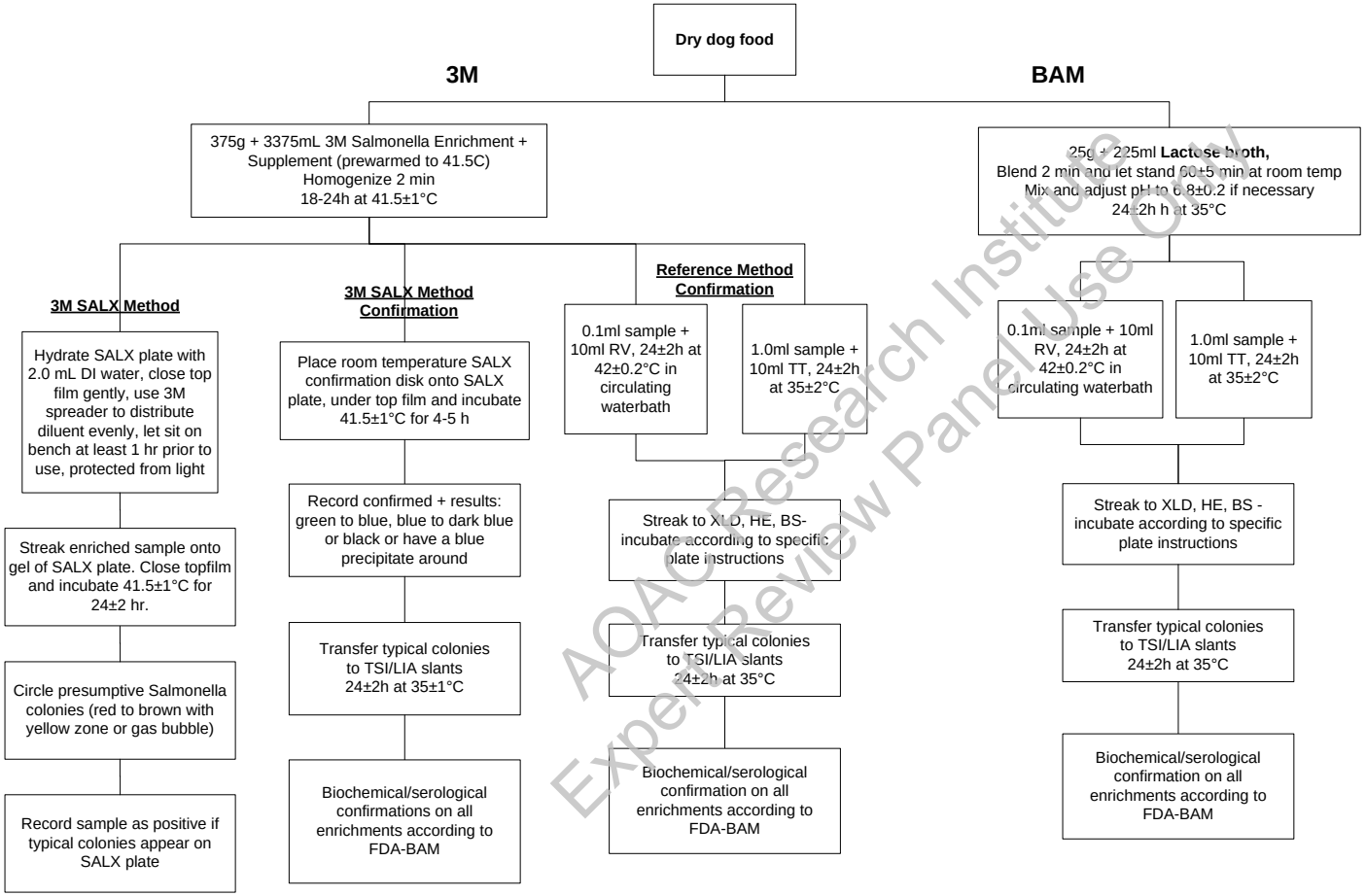
Confirmation

TSI
LIA
API 20E or VITEK
O and H serotyping supplies

Appendix 8.5 – Flow Diagram for Raw Ground Beef (25g)



Appendix 8.6 – Flow Diagram for Dry Dog Food
(37



Appendix 8.7

3M Petrifilm SALX Collaborative Study
Collaborator Information Sheet

Please complete the information below and return to TBD.

Contact name:

Laboratory name:

Fax number:

Phone number:

Email address:

Mailing address:

Shipping address:

Appendix 8.8

USDA United States Department of Agriculture | Food Safety and Inspection Service | Office of Public Health Science | Laboratory QA/QC Division 950 College Station Road Athens, GA 30605

Laboratory Guidebook
Notice of Change

Chapter new, **revised**, or archived: MLG 4.06

Title: Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg and Catfish Products

Effective Date: 05/01/2013

Description and purpose of change(s):

Section 4.3.1 Method Controls includes an option of starting the H_2S-negative *Salmonella* spp. positive culture control from streaking the plating media.

Section 4.5 Sample Preparation includes specific instructions on disinfecting packaging and weighing samples of raw, RTE and multi-component food products.

Section 4.5 Sample Preparation Table 1 includes a 325 g analytical portion option for raw meat and poultry product.

The methods described in this guidebook are for use by the FSIS laboratories. FSIS does not specifically endorse any of the mentioned test products and acknowledges that equivalent products may be available for laboratory use. Method validation is necessary to demonstrate the equivalence of alternative tests. FSIS provides guidance at:
http://www.fsis.usda.gov/PDF/Validation_Studies_Pathogen_Detection_Methods.pdf

QD-F-Micro-0004.05
Page 1 of 1
Effective: 5/10/12

Issuing Authority: Director, Laboratory Quality Assurance Division (LQAD)

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 1 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

Procedure Outline

Issuing Authority: Director, Laboratory Quality Assurance Division (LQAD)

1
2

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 2 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

4.1 Introduction

This method describes the analysis of various meat, poultry and catfish products, sponge and rinse samples, and egg products for *Salmonella*. It is not intended for the isolation and identification of *Salmonella* Typhi.

Success in isolating *Salmonella* from any food can be related to a number of factors including food preparation procedures, the number of organisms present, sample handling after collection, etc. With raw samples, the competitive flora may be the most important factor. It varies from sample to sample and from one kind of matrix to another.

Another consideration is whether the examination is for routine monitoring or epidemiological purposes. The analyst may choose to augment the method for epidemiological purposes with additional enrichment procedures and culture media, two temperatures of incubation, intensified picking of colonies from plates, and/or rapid screening methods.

All isolates must be identified as *Salmonella* biochemically and serologically.

Unless otherwise stated all measurements cited in this method have a tolerance range of ± 2%.

4.2 Safety Precautions

Salmonella are generally categorized as Biosafety Level 2 pathogens. CDC guidelines for manipulating Biosafety Level 2 pathogens should be followed whenever live cultures of *Salmonella* are used. A Class II laminar flow biosafety cabinet is recommended for procedures in which infectious aerosols or splashes may be created. The Material Safety Data Sheet (MSDS) must be obtained from the manufacturer for the media, chemicals, reagents and microorganisms used in the analysis. The personnel who will handle the material should read the MSDS prior to startup.

4.3 Quality Control Procedures

4.3.1 Method Controls

A *Salmonella* spp. H_2S positive culture and an uninoculated media control must be used from the start of the analysis. A H_2S-negative *Salmonella* spp. positive culture may be included either from the start of the analysis or limited to sets containing

1
2

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 3 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

screen positive samples, from the differentiation and confirmation steps starting with streaking to BGS and either DMLIA or XLT4 agar plates. To facilitate identification of control isolates, the laboratory may use strains of uncommonly found serogroups. *S. Abaetetuba* is suggested as a readily available, H_2S-positive culture that is not commonly found in meats or meat products. *S. Choleraesuis* is typically negative for H_2S production. These cultures may be obtained from ATCC. Other serotypes may be found that have aberrant H_2S-negative strains. The control cultures should be inoculated into either a meat matrix or the matrix that is being analyzed. Once the control culture is started, incubate the controls along with the samples, and analyze them in the same manner as the samples. Confirm at least one isolate from the H_2S positive control sample. Confirmation of at least one colony from the H_2S negative control is required when confirming H_2S negative samples. In the absence of a positive test sample, control cultures may be terminated at the same point as the sample analyses.

4.3.2 Specific Procedure Controls

The biochemical and serological tests used for confirmation of the sample isolates require the use of appropriate controls to verify that the results are valid. *Salmonella* 'O' antisera should be tested with QC control cultures or sera before initial use, and with a saline control for each test. Biochemical kit and rapid test manufacturers may specify control cultures for use with their products. If not specified, quality control procedures for biochemical tests and test media should include cultures that will demonstrate pertinent characteristics of the product.

4.4 Equipment, Reagents, Media and Test Kits

Not all of the materials listed below may be needed. Media and reagents specific to the selected biochemical test method may be needed in addition to the materials listed below.

4.4.1 Equipment

a. Sterile tablespoons, scissors, forceps, knives, glass stirring rods, pipettes, petri dishes, test tubes, bent glass rods ("hockey sticks") as needed
b. Blending/mixing equipment: Paddle blender, Sterile Osterizer-type blender with sterilized cutting assemblies, and blender jars or equivalent and adapters for use with Mason jars;
c. Sterile plain, clear polypropylene bags (ca. 24" x 30 - 36"), or Whirl-Pak™ type bags (or equivalent)

1

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 4 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

d. Incubator, 35 ± 2°C
e. Incubator or water bath, 42 ± 0.5°C
f. Water bath, 48-50°C
g. Glass slides, glass plate marked off in one inch squares or agglutination ring slides
h. Balance, 2000 g capacity, sensitivity of 0.1 g
i. Inoculating needles and loops
j. Vortex mixer
k. VITEK® system, VITEK® 2 Compact System, or equivalent

4.4.2 Reagents

a. Crystal violet dye, 1% aqueous solution
b. Butterfield's phosphate diluent
c. Saline, 0.85%
d. Saline, 0.85% with 0.6% formalin for flagellar antigen tests
e. Calcium carbonate, sterile
f. *Salmonella* polyvalent O antiserum and *Salmonella* individual O grouping sera for groups A-I (antisera for further O groups are optional)
g. *Salmonella* polyvalent H antiserum, Slide Agglutination H Antisera from Statens Serum Institut (SSI), or Oxoid *Salmonella* Latex Test (Unipath Company, Oxoid Division, Ogdensburg, NY) or equivalent
h. Additional reagents as needed for biochemical tests: e.g. GNI cards for VITEK® or GN cards for VITEK® 2 Compact System

4.4.3 Media

a. Buffered peptone water (BPW)
b. TT broth (Hajna)
c. Modified Rappaport Vassiliadis (mRV) broth, Rappaport-Vassiliadis R10 broth, or Rappaport-Vassiliadis Soya Peptone Broth (RVS)
d. Brilliant green sulfa agar (BGS; contains 0.1% sodium sulfapyridine)
e. Xylose lysine Tergitol™ 4 agar (XLT4) or Double modified lysine iron agar (DMLIA)
f. Triple sugar iron agar (TSI)
g. Lysine iron agar (LIA)
h. Trypticase soy broth (TSB) or Tryptose broth
i. Trypticase soy agar (TSA)
j. Nutrient agar slants

Issuing Authority: Director, Laboratory Quality Assurance Division (LQAD)

1
2

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 5 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

k. Nutrient broth, semi-solid
l. Tryptic soy agar with 5% sheep blood agar
m. Additional media as needed for biochemical tests

4.5 Sample Preparation

Intact retail packages must be disinfected at the incision sites immediately prior to incision for sampling using an appropriate disinfectant, e.g., 3% hydrogen peroxide, ca. 70% ethanol or ca. 70% isopropanol. If the package does not appear to be clean, scrub gently using soapy water and rinse thoroughly prior to disinfection. A sterile scalpel may be helpful for cutting the packaging. Aseptically pull the packaging away to expose the product for sampling.

Note: For Ready-to-Eat (RTE) sausages in casing, the shell/casing is an integral part of the sample and should be free of pathogens and toxins. The casing is not to be disinfected since some casings are permeable and the disinfectant may be introduced into the core of the product. In addition consumers often slice through an inedible casing and then remove it thus any contamination on the surface of the casing could be transferred to the edible core of the product.

Sample preparation and enrichment incubation times may vary by matrix and program. Refer to Table 1 and the following sample preparation sections for additional details.

Issuing Authority: Director, Laboratory Quality Assurance Division (LQAD)

1
2

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 6 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

Table 1. Sample Preparation and Enrichment Guide

Product	Sample Preparation		Incubation Time
	Portion Size	BPW Amount *	Cultural or PCR rapid screen
Breading Mixes, Dehydrated Sauces and Dried Milk	325 ± 6.5 g	2925 ± 58.5 ml	18-24 h
Ready-to-Eat Foods	325 ± 6.5 g	2925 ± 58.5 ml	18-24 h
Fermented Products	325 ± 6.5 g + 10 g of sterilized calcium carbonate	2925 ± 58.5 ml of BPW with 1 ml of a 1% aqueous solution of crystal violet per liter	18-24 h
Raw Meat and Poultry Products	325 ± 32.5 g or 25 ± 2.5 g	1625 ± 32.5ml or 225 ± 4.5 ml	20-24 h
Carcass Sponge and Environmental Swabs	1 sponge premoistened with 10ml diluent	50 ± 1 ml (brings total volume to 60 ml)	20-24 h
Whole Bird Rinses	30 ± 0.6 ml sample rinse fluid	30 ± 0.6 ml	20-24 h
Pasteurized Liquid, Frozen or Dried Egg Products	100 ± 2 g	900 ± 18 ml	18-24 h
Raw Catfish Products	25 ± 2.5 g	225 ± 4.5 ml	22-26 h

* BPW Amount may be determined by volume or weight.

4.5.1 Ready-to-Eat Foods

Follow additional program requirements for preparing sample and sub-sample composites. Outbreak samples may require a different sample preparation. Follow customer specifications.

Include representative portions from each submitted package to achieve the analytical sample portion. Using a sterile scalpel, knife, spoon, chisel or other tool cut small pieces from representative sites of submitted product to prepare a

Issuing Authority: Director, Laboratory Quality Assurance Division (LQAD)

1

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 7 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

composite sample portion. While multiple packages of a product are usually submitted, for large products a single package may be submitted.

For multi-component RTE products, follow the appropriate sample preparation instructions listed below:

If the meat or poultry component is separate and distinct from other non-meat ingredients, analyze only the representative meat/poultry portion of the RTE product. Examples include products with the meat/poultry portion separate from any vegetable/dessert component, or fajita kits with meat/poultry, onions/peppers, and tortillas in three separate internal packages/bags within an outer package.

When meat/poultry is combined with other ingredients to form the product (e.g., beef stew containing vegetables, potatoes, etc.), analyze representative meat/poultry portions in combination with other ingredients.

a. Weigh the composite sample into a large sterile bag (or sterile blender jar if required by the customer or sample type).

b. Add approximately one third to one-half of the ambient temperature sterile BPW. Blend or stomach approximately two minutes then add the remainder of the BPW.

c. Incubate at 35 ± 2°C for 18-24 h.

d. Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

4.5.2 Fermented Products

Follow the procedure for RTE foods in Section 4.5.1 except:

a. Blend/stomach the sample with 10 ± 0.2 g of sterilized calcium carbonate.

b. Use buffered peptone water that contains 1 ml of a 1% aqueous solution of crystal violet per liter.

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 8 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

4.5.3 Raw Meat and Poultry Products

a. Unless the exact sample portion is submitted, weigh the meat into a sterile polypropylene bag, sterile blender jar, or other sterile jar. Note: If the sample is not already ground, in some cases it may be best to mince it with sterile scissors or leave it whole (e.g. chicken wings) to avoid jamming blender blades with skin or connective tissue.

b. Add the BPW. Stomach, blend or hand massage until clumps are dispersed.

c. Incubate at 35 ± 2°C for 20-24 h.

d. Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

4.5.4 Carcass Sponges and Environmental Swabs

a. Add the BPW to the sample bag containing the moistened sponge to bring the total volume to 60 ml. Mix well.

b. Incubate at 35 ± 2°C for 20-24 h.

c. Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

4.5.5 Whole Bird Rinses

Due to differences between sample types/sizes (e.g. chicken vs. turkey carcasses), follow instructions given in the specific program protocol.

a. For chicken carcasses, aseptically drain excess fluid from the carcass and transfer the carcass to a large sterile bag.

b. Pour 400 ml (or other volume specified in program protocol) of BPW into the cavity of the carcass contained in the bag.

c. Rinse the bird inside and out with a rocking motion for one minute (ca. 35 RPM). This is done by grasping the broiler carcass in the bag with one hand and the closed top of the bag with the other. Rock with a reciprocal motion in about

1
2

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 9 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

an 18-24 inch arc, assuring that all surfaces (interior and exterior of the carcass) are rinsed.

d. Transfer the sample rinse fluid to a sterile container.

e. Use 30 ± 0.6 ml of the sample rinse fluid obtained above for *Salmonella* analysis. Add 30 ± 0.6 ml of sterile BPW and mix well.

f. Incubate at 35 ± 2°C for 20-24 h.

g. Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

NOTE: If analyses other than *Salmonella* are to be performed, the carcass may be rinsed in BPW and dilutions made directly from the BPW rinse. Alternatively, the carcass may be rinsed in Butterfield's Phosphate Diluent instead of BPW. In this case, add 30 ml of 2X BPW to 30 ml of carcass-rinse fluid, mix well, and continue as above.

4.5.6 Pasteurized Liquid, Frozen, or Dried Egg Products

a. Mix the liquid sample with a sterile spoon, spatula, or by shaking.

b. Weigh the liquid egg product into a sterile polypropylene bag, sterile blender jar, or other sterile jar.

c. Mix the inoculated BPW well by shaking, stomaching, or blending.

Note: If a special sample or specification requires a sample size other than 100 g, the ratio of egg sample to BPW is to be maintained at 1:10.

d. With dried egg samples, gradually add BPW to the sample. Add a small portion of sterile BPW and mix to obtain a homogeneous suspension. Add the remainder of the BPW. Mix until a lump-free suspension is obtained.

e. Incubate at 35 ± 2°C for 18-24 h.

f. Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

Issuing Authority: Director, Laboratory Quality Assurance Division (LQAD)

1
2

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 11 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

c. Carefully mix contents of tube by vortexing or equivalent means. Streak to BGS and either DMLIA or XLT4 agar plates using a 10 uL loopful of inoculum for each plate. Streak the entire agar plate with a single sample enrichment.

d. Incubate at 35 ± 2°C for 18-24 h.

e. Select typical colonies.

4.7 Examination of and Picking Colonies from Plating Media

4.7.1 Picking Colonies

a. After the recommended incubation interval, examine the selective-differential agar plates and controls for the presence of colonies meeting the description for suspect *Salmonella* colonies. Pick well-isolated colonies.

- BGS. Select colonies that are pink and opaque with a smooth appearance and entire edge surrounded by a red color in the medium. On very crowded plates, look for colonies that give a tan appearance against a green background.
- XLT4. Select black colonies (H_2S-positive) or red colonies with (H_2S-positive) or without (H_2S-negative) black centers. The rim of the colony may still be yellow in 24 h; later it should turn red.
- DMLIA. Select purple colonies with (H_2S-positive) or without (H_2S-negative) black centers. Since *Salmonella* typically decarboxylate lysine and ferment neither lactose nor sucrose, the color of the medium reverts to purple.

b. Pick up to three colonies from each plate, if available. (NOTE: Before any sample is reported as *Salmonella*-negative, a total of three typical colonies, if available, from each selective agar plate must be examined). Pick only from the surface and center of the colony. Avoid touching the agar because these highly selective media suppress growth of many organisms that may be viable.

If there are typical colonies on a plate that are not well isolated, pick from the typical colonies and streak directly to a new set of selective agar plates. Alternatively, transfer typical colonies into a tube of TT or mRV broth and incubate overnight, then streak to selective agars.

1

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 11 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

c. Carefully mix contents of tube by vortexing or equivalent means. Streak to BGS and either DMLIA or XLT4 agar plates using a 10 uL loopful of inoculum for each plate. Streak the entire agar plate with a single sample enrichment.

d. Incubate at 35 ± 2°C for 18-24 h.

e. Select typical colonies.

4.7 Examination of and Picking Colonies from Plating Media

4.7.1 Picking Colonies

a. After the recommended incubation interval, examine the selective-differential agar plates and controls for the presence of colonies meeting the description for suspect *Salmonella* colonies. Pick well-isolated colonies.

- BGS. Select colonies that are pink and opaque with a smooth appearance and entire edge surrounded by a red color in the medium. On very crowded plates, look for colonies that give a tan appearance against a green background.
- XLT4. Select black colonies (H_2S-positive) or red colonies with (H_2S-positive) or without (H_2S-negative) black centers. The rim of the colony may still be yellow in 24 h; later it should turn red.
- DMLIA. Select purple colonies with (H_2S-positive) or without (H_2S-negative) black centers. Since *Salmonella* typically decarboxylate lysine and ferment neither lactose nor sucrose, the color of the medium reverts to purple.

b. Pick up to three colonies from each plate, if available. (NOTE: Before any sample is reported as *Salmonella*-negative, a total of three typical colonies, if available, from each selective agar plate must be examined). Pick only from the surface and center of the colony. Avoid touching the agar because these highly selective media suppress growth of many organisms that may be viable.

If there are typical colonies on a plate that are not well isolated, pick from the typical colonies and streak directly to a new set of selective agar plates. Alternatively, transfer typical colonies into a tube of TT or mRV broth and incubate overnight, then streak to selective agars.

Issuing Authority: Director, Laboratory Quality Assurance Division (LQAD)

1
2
3

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 12 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

c. Incubate all plates for an additional 18-24 h at 35 ± 2°C.

d. Reexamine initially negative plates and pick colonies as above. After 48-hour incubation, plates with no typical colonies may be discarded as negative. Plates with colonies undergoing confirmation testing should be stored at 2-8°C until testing is complete. If suspect *Salmonella* colonies do not confirm, reexamine the plates from which they were picked, and if appropriate, re-pick colonies for confirmation following Section 4.7.1.b.

4.7.2 Screening Media

a. Inoculate TSI and LIA slants in tandem with a single pick from a colony by stabbing the butts and streaking the slants in one operation. If screw cap tubes are used, the caps must be loosened. Incubate at 35 ± 2°C for 24 ± 2 h.

Note: the same colony may be used to streak a plate used for subsequent biochemical testing described in Section 4.9.

Examine TSI and LIA slants as sets. Note the colors of butts and slants, blackening of the media, and for TSI slants presence of gas as indicated by gas pockets or cracking of the agar. Note also the appearance of the growth on the slants along the line of streak. A typical control on LIA should produce a purple butt with (H_2S-positive) or without (H_2S-negative) blackening of the media. A typical control on TSI should produce a yellow butt and red slant, with (H_2S-positive) or without (H_2S-negative) blackening of the media.

Discard, or re-streak for isolation, any sets that show "swarming" from the original site of inoculation. Discard sets that show a reddish slant in lysine iron agar. Isolates giving typical *Salmonella* spp. reactions and isolates that are suggestive but not typical of *Salmonella* spp. should be confirmed by a combination of biochemical and serological procedures. Refer to Table 2 for a summary of TSI-LIA reactions.

Note: TSI and LIA slants may be held at 2-8°C for up to 96 hours. Prior to any subsequent testing, fresh TSI and LIA slant(s) must be inoculated and incubated per instructions in a (above).

Issuing Authority: Director, Laboratory Quality Assurance Division (LQAD)

1

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 13 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

b. The motility testing in the last column of the table is optional. Refer to "Edwards and Ewing's Identification of Enterobacteriaceae" (Ewing, 1986) for additional information.

4.8 Serological Tests

4.8.1 Somatic (O) Antigen Agglutination Tests

At a minimum, isolates should be tested with polyvalent O antiserum reactive with serogroups A through I. Following a positive reaction with polyvalent O antiserum, test the isolate using individual *Salmonella* antisera for O groups A through I. Additional individual O groups may be tested. Testing for O groups A through I should encompass the majority of the *Salmonella* serotypes commonly recovered from meat and poultry products.

Use growth from either the TSI or LIA slant. Test first with polyvalent O antiserum. Include a saline control with each isolate. If there is agglutination with the saline control alone (autoagglutination), identify such a culture by biochemical reactions only. If the saline control does not agglutinate and the polyvalent serum does, test the culture with *Salmonella* O grouping antisera.

Typically, a positively identified isolate and/or control is identified by the individual O group result, but an isolate can be identified by the poly group if it tests as positive for multiple individual O groups. The isolate is noted as an auto agglutinator if it reacts with saline.

Occasionally, an isolate will be recovered which is typical of *Salmonella* biochemically and is serologically poly H-positive, but is non-reactive with any of the available O group antisera. Report these isolates as "*Salmonella* non A-I" or "*Salmonella* O group beyond I" if no further testing is performed. Further tested isolates are reported by their specific serotype.

4.8.2 Flagellar (H) Antigen Agglutination Tests

The Oxoid *Salmonella* Latex Test, SSI H Antisera for Slide Agglutination, or equivalent, may be used for H antigen agglutination testing. Follow the manufacturer's instructions for performing the test.

1
2

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 14 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

Alternatively, use growth from either the TSI or LIA slant to inoculate a tube of trypticase soy broth or tryptose broth. Incubate at 35 ± 2°C overnight. Add an equal amount of saline containing 0.6% formalin and let sit one hour. Remove one ml to each of two 13 x 100 mm test tubes. To one of the tubes, add *Salmonella* polyvalent H serum in an amount indicated by the serum titer or according to the manufacturer's instructions. The other tube serves as an autoagglutination control. Incubate both tubes at 48-50°C in a water bath for up to 1 hr without mixing or shaking during incubation. Record the presence or absence of agglutination.
If desired, use Spicer-Edwards pooled serum or H typing serum. The specific procedure may be found in "Edwards and Ewing's Identification of Enterobacteriaceae" (Ewing, 1986).

4.9 Biochemical Procedures

Commercially available biochemical test kits, including automated systems may be used for biochemical identification. If a VITEK® system is used, the cytochrome oxidase and Gram stain tests are optional. Alternatively, use traditional methods of biochemical identification. Refer to AOAC Official Method 967.27 or "Edwards and Ewing's Identification of Enterobacteriaceae", 4th Edition, for biochemical reactions of *Enterobacteriaceae* and for fermentation media and test procedures.

For some biochemical test kits, i.e. the VITEK system, streak a TSA + 5% sheep blood agar plate from either the TSI or LIA slant. Incubate 18-24 h at 35 ± 2°C. Some commercial biochemical test systems may require streaking to other non-selective media prior to inoculation of their test kit. Follow manufacturer's instructions.

Note: Plates may be stored up to 96 hours at 2-8C. Stored plates must be streaked to a new plate and incubated per instruction prior to use.

Table 2 is a list of potential reactions requiring biochemical analysis. Additional culture work may be required and other factors should be considered before discarding any sample.

1

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 15 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

Table 2. Potential *Salmonella* Reactions

Triple Sugar Iron Agar			Lysine Iron Agar		Polyvalent Sera		Further Testing/ Disposal
Butt	Slant	H_2S	Butt	H_2S	O	H	
Y	R	+	P	+	+	+	B. & M. T.
Y	R	+	P	+	+	-	B. & M. T.
Y	R	-	P	-			B. & M. T.
Y	R	-	Y	-	+	-	B. & M. T.
Y	R	-	Y	-	-	-	B. & M. T.
Y	R	+	Y	+/-			B. & M. T.
Y	Y	-	Y or P	-			Discard
Y	Y	+	P	+			B. & M. T.
NC	NC						Discard

Y = Yellow; R = Red; P = Purple; B. & M. T. = Perform biochemical testing, e.g., VITEK® system, and optionally motility tests;
NC = No change in color from uninoculated medium.

4.10 Culture Storage and Maintenance

For short-term (no longer than 3 months) storage, inoculate a nutrient agar slant, incubate at 35 ± 2°C overnight and then store at 2-8°C. For long-term storage, lyophilize cultures or freeze using cryo-beads, i.e. Cryostor™ or equivalent.

Maintain "working" *Salmonella* stock cultures on nutrient agar slants or equivalent. Transfer stocks monthly onto duplicate nutrient agar slants, incubate overnight at 35 ± 2°C,

Issuing Authority: Director, Laboratory Quality Assurance Division (LQAD)

United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science

MLG 4.06		Page 16 of 16
Title: Isolation And Identification of *Salmonella* From Meat, Poultry, Pasteurized Egg and Catfish Products		
Revision:06	Replaces: MLG 4.05	Effective: 05/01/2013

and then maintain them at 2-8°C. Use one of the slants as the working culture. Use the other slant for sub-culturing to reduce the opportunity for contamination. Cultures may be subcultured up to 5 times. After this period, the culture must be re-confirmed biochemically or a new culture initiated.

4.11 Selected References

Bailey, J. S., J. Y. Chiu, N. A. Cox, and R. W. Johnston. 1988. Improved selective procedure for detection of salmonellae from poultry and sausage products. J. Food Prot. **51**:391-396.

Centers for Disease Control and Prevention and National Institutes of Health (CDC/NIH). 2007. BioSafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Government Printing Office, Washington, D.C. (internet site: http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm)

Horowitz, William. (ed.). 2000. Official methods of analysis of AOAC International, 17th Edition. AOAC International Inc., Gaithersburg, MD 20877.

Ewing, W. H. 1986. Edwards and Ewing's Identification of Enterobacteriaceae, 4th Edition. Elsevier Science Publishing Co., Inc., New York.

Federal Register, Vol. 61, No. 144, Thursday, July 25, 1996, Appendix E, pp. 38917 – 38925.

Miller, R. G., C. R. Tate, and E. T. Mallinson. 1994. Improved XLT4 agar: small addition of peptone to promote stronger production of hydrogen-sulfide by Salmonellae. J. Food Prot. 57:854-858.

Vassiliadis, P., D. Trichopoulos, A. Kalandidi, and E. Xirouchaki. 1978. Isolation of salmonellae from sewage with a new procedure of enrichment. J. Appl. Bacteriol. 66:523-528.

Insert No. 15983: *Salmonella* Antisera for *in vitro* diagnostic use, Statens Serum Institut, Denmark, December 2005

Vassiliadis, P. 1983. The Rappaport-Vassiliadis (RV) enrichment medium for the isolation of *Salmonellas*: an overview. J. Appl. Bacteriol. **54**:69-76.

Appendix 8.9

3

Home Food Science & Research (Food) Laboratory Methods

Food

BAM: *Salmonella*

NOTICE:

If you are looking for BAM Chapter 5: *Salmonella* (December 2007 Edition) that is incorporated by reference in 21 CFR Parts 16 and 118: Federal Register Final Rule[1] (July 9, 2009, 74 FR 33030): Prevention of Salmonella Enteritidis in Shell Eggs During Production, Storage, and Transportation, please use these versions of the BAM *Salmonella* Chapter[2] (PDF, 189 Kb) and Appendix 1: Rapid Methods for Detecting Foodborne Pathogens[3] (PDF, 195 Kb). These 2 documents are also available as a combined file[4] (PDF, 382 Kb).

The most recent Edition of BAM Chapter 5: *Salmonella* is available below this notice.

November 2011 Version

Bacteriological Analytical Manual
Chapter 5
Salmonella

Authors: Wallace H. Andrews, Andrew Jacobson, and Thomas Hammack

Revision History:

- August 2012, November 2011 - Made available in PDF format versions of Chapter 5: *Salmonella* and Appendix 1 (archived) from 2009 which were incorporated by reference in 21 CFR Parts 16 and 118: Federal Register Final Rule (July 9, 2009, 74 FR 33030): Prevention of Salmonella Enteritidis in Shell Eggs During Production, Storage, and Transportation.
- November 2011 - Addition to Section C: Preparation of foods for isolation of *Salmonella*: Leafy green vegetables and herbs.
- February 2011 – Removed link to Appendix 1: Rapid Methods for Detecting Foodborne Pathogens (now archived).
- December 2007 – Mamey pulp method added, and Section D revised.
- June 2006 – Eggs method revised for shell eggs and liquid whole eggs.
- April 2003 – Frog legs method, Lactic casein, Rennet casein, Sodium caseinate and Rabbit carcass methods revised, top ears and other dog chew toys added. Removed section A.25, Mechanical shaker.
- October 25, 2001 – Extension of the applicability of the orange juice method in section C.19 to apple juice and apple cider.
- 1999-DEC, 2000-MAR, and 2000-AUG Final revision on 2000-NOV-14 (see the Introduction for a summary of changes).

To obtain a copy of a prior version not currently posted, please contact Thomas Hammack

Chapter Contents

http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm 6/30/2013

Introduction

Several changes are being introduced in this edition of BAM (8th Edition). The first change involves the expanded use of Rappaport-Vassiliadis (RV) medium[5] for foods with both high and low levels of competitive microflora. In the previous edition, RV medium was recommended only for the analysis of shrimp. Based on the completion of AOAC precollaborative (5, 6) and collaborative (7, 8) studies, RV medium is now being recommended for the analysis of high microbial and low microbial load foods. RV medium replaces selenite cystine (SC) broth for the analysis of all foods, except guar gum. In addition, RV medium replaces lauryl tryptose broth for use with dry active yeast.

Tetrathionate (TT)[6] broth continues to be used as the second selective enrichment broth. However, TT broth is to be incubated at 43°C for the analysis of high microbial load foods and at 35°C for the analysis of low microbial load foods, including guar gum.

The second change involves the option of refrigerating incubated preenrichments and selective enrichments of low-moisture foods for up to 72 h. With this option, sample analyses can be initiated as late as Wednesday or Thursday without weekend work being involved.

The third change involves reducing the period of incubation of the lysine iron agar (LIA)[7] slants. In the former edition (BAM-7), triple sugar iron agar (TSI)[8] and LIA slants were incubated at 35°C for 24 ± 2 h and 48 ± 2 h, respectively. Unpublished data have demonstrated that the 48 h reading of LIA slants is without diagnostic value. Of 193 LIA slants examined, all gave definitive results within 24 ± 2 h of incubation. No significant changes altered the final test result when the slants were incubated an additional 24 h. Thus, both the TSI and LIA slants are now incubated for 24 ± 2 h.

The fourth change involves the procedure for surface disinfection of shell eggs. In the previous edition (BAM-7) egg shells were surface-disinfected by soaking in 0.1% mercuric chloride solution for 1 h followed by soaking in 70% ethanol for 30 min. Mercuric chloride is classified as a hazardous waste, and is expensive to dispose of according to Environmental Protection Agency guidelines. In this edition (BAM-8) egg shells are now surface-disinfected by soaking for at least 10 sec in a 3:1 solution consisting of 3 parts of 70% alcohol (ethyl or isopropyl) to 1 part of iodine/potassium iodide solution.

The fifth change involves the sample preparation of eggs. Egg contents (yolk and albumen) are thoroughly mixed before analysis. After mixing the egg contents, 25 g (ml) are added to 225 ml trypticase (tryptic) soy broth supplemented with ferrous sulfate.

A method for the analysis of guar gum has been included. When guar gum is preenriched at a 1:9 sample/broth ratio, a highly viscous, nonpipettable mixture results. Addition of the enzyme cellulase to the preenrichment medium, however, results in a readily pipettable mixture.

A method for orange juice (pasteurized and unpasteurized) has been included due to recent orange juice-related outbreaks.

The directions for picking colonies from the selective plating agars have been made more explicit to reflect the intent of the method. In the absence of typical or suspect colonies on the selective plating agars, it is recommended that atypical colonies be picked to TSI and LIA slants. This recommendation is based on the fact that up to 4% of all *Salmonella* cultures isolated by FDA analysts from certain foods, especially seafoods, during the past several years have been atypical.

Finally, since the publication of BAM-7, a 6-way comparison was conducted of the relative effectiveness of the three selective plating agars recommended in the BAM (bismuth sulfite[9], Hektoen enteric[10], and xylose lysine desoxycholate agars[11]) and three relatively new agars (ES-18, xylose lysine Tergitol 4, and Rambach agars). Our results (9) indicated no advantage in replacing any of the BAM recommended agars with one or more of the newer agars. Thus, the combination of selective plating agars recommended in BAM-7 remains unchanged.

Return to Chapter Contents

A. Equipment and Materials

1. Blender and sterile blender jars (*see* Chapter 1[12])
2. Sterile, 16 oz (500 ml) wide-mouth, screw-cap jars, sterile 500 ml Erlenmeyer flasks, sterile 250 ml beakers, sterile glass or paper funnels of appropriate size, and, optionally, containers of appropriate capacity to accommodate composited samples
3. Sterile, bent glass or plastic spreader rods
4. Balance, with weights; 2000 g capacity, sensitivity of 0.1 g
5. Balance, with weights; 120 g capacity, sensitivity of 5 mg
6. Incubator, 35 ± 2 °C
7. Refrigerated incubator or laboratory refrigerator, 4 ± 2°C
8. Water bath, 49 ± 1°C

1

9. Water bath, circulating, thermostatically-controlled, 43 ± 0.2°C
10. Water bath, circulating, thermostatically-controlled,42 ± 0.2°C
11. Sterile spoons or other appropriate instruments for transferring food samples
12. Sterile culture dishes, 15 x 100 mm, glass or plastic
13. Sterile pipets, 1 ml, with 0.01 ml graduations; 5 and 10 ml, with 0.1 ml graduations
14. Inoculating needle and inoculating loop (about 3 mm id or 10 5l), nichrome, platinum-iridium, chromel wire, or sterile plastic
15. Sterile test or culture tubes, 16 x 150 mm and 20 x 150 mm; serological tubes, 10 x 75 mm or 13 x 100 mm
16. Test or culture tube racks
17. Vortex mixer
18. Sterile shears, large scissors, scalpel, and forceps
19. Lamp (for observing serological reactions)
20. Fisher or Bunsen burner
21. pH test paper (pH range 6-8) with maximum graduations of 0.4 pH units per color change
22. pH meter
23. Plastic bags, 28 x 37 cm, sterile, with resealable tape. (Items 23-24 are needed in the analysis of frog legs and rabbit carcasses.)
24. Plastic beakers, 4 liter, autoclavable, for holding plastic bag during shaking and incubation.
25. Sponges, non-bactericidal (Nasco cat # B01299WA), or equivalent.
26. Swabs, non-bactericidal, cotton-tipped.

B. **Media[13] and Reagents[14]**

For preparation of media and reagents, refer to Methods 967.25-967.28 in *Official Methods of Analysis* (1).

1. Lactose broth (M74[15])
2. Nonfat dry milk (reconstituted) (M111[16])
3. Selenite cystine (SC) broth (M134[17])
4. Tetrathionate (TT) broth (M145[18])
5. Rappaport-Vassiliadis (RV) medium (M132[19]). NOTE: RV medium must be made from its individual ingredients. Commercial formulations are not acceptable.
6. Xylose lysine desoxycholate (XLD) agar (M179[20])
7. Hektoen enteric (HE) agar (M61[21])
8. Bismuth sulfite (BS) agar (M19[22])
9. Triple sugar iron agar (TSI) (M149[23])
10. Tryptone (tryptophane) broth (M164[24])
11. Trypticase (tryptic) soy broth (M154[25])
12. Trypticase soy broth with ferrous sulfate (M186[26])
13. Trypticase soy-tryptose broth (M160[27])
14. MR-VP broth (M104[28])
15. Simmons citrate agar (M138[29])
16. Urea broth (M171[30])
17. Urea broth (rapid) (M172[31])
18. Malonate broth (M92[32])
19. Lysine iron agar (LIA) (Edwards and Fife) (M89[33])
20. Lysine decarboxylase broth (M87[34])
21. Motility test medium (semisolid) (M103[35])
22. Potassium cyanide (KCN) broth (M126[36])
23. Phenol red carbohydrate broth (M121[37])
24. Purple carbohydrate broth (M130[38])
25. MacConkey agar (M91[39])
26. Nutrient broth (M114[40])
27. Brain heart infusion (BHI) broth (M24[41])

1

28. Papain solution, 5% (M56a[42])
29. Cellulase solution, 1% (M187[43])
30. Tryptose blood agar base (M166[44])
31. Universal preenrichment broth (M188[45])
32. Universal preenrichment broth (without ferric ammonium citrate) (M188a[46])
33. Buffered peptone water (M192[47])
34. Dey-Engley broth (M193[48])
35. Potassium sulfite powder, anhydrous
36. Chlorine solution, 200 ppm, containing 0.1% sodium dodecyl sulfate (R12a[49])
37. Ethanol, 70% (R23[50])
38. Kovacs' reagent (R38[51])
39. Voges-Proskauer (VP) test reagents (R89[52])
40. Creatine phosphate crystals
41. Potassium hydroxide solution, 40% (R65[53])
42. 1 N Sodium hydroxide solution (R73[54])
43. 1 N Hydrochloric acid (R36[55])
44. Brilliant green dye solution, 1% (R8[56])
45. Bromcresol purple dye solution, 0.2% (R9[57])
46. Methyl red indicator (R44[58])
47. Sterile distilled water
48. Tergitol Anionic 7 (R78[59])
49. Triton X-100 (R86[60])
50. Physiological saline solution, 0.85% (sterile) (R63[61])
51. Formalinized physiological saline solution (R27[62])
52. *Salmonella* polyvalent somatic (O) antiserum
53. *Salmonella* polyvalent flagellar (H) antiserum
54. *Salmonella* somatic group (O) antisera: A, B, C_1, C_2, C_3, D_1, D_2, E_1, E_2, E_3, E_4, F, G, H, I, Vi, and other groups, as appropriate
55. *Salmonella* Spicer-Edwards flagellar (H) antisera

Return to Chapter Contents

C. Preparation of foods for isolation of *Salmonella*

NOTICE:

If you are looking for BAM Chapter 5: *Salmonella* (December 2007 Edition) that is incorporated by reference in 21 CFR Parts 16 and 118: Federal Register Final Rule[63] (July 9, 2009, 74 FR 33030): Prevention of Salmonella Enteritidis in Shell Eggs During Production, Storage, and Transportation, please use these versions of the BAM *Salmonella* Chapter[64] (PDF, 189 Kb) and Appendix 1: Rapid Methods for Detecting Foodborne Pathogens[65] (PDF, 195 Kb). These 2 documents are also available as a combined file[66] (PDF, 382 Kb).

The most recent Edition of BAM Chapter 5: *Salmonella* continues below this notice.

The following methods are based on the analysis of a 25 g analytical unit at a 1:9 sample/broth ratio. Depending on the extent of compositing, add enough broth to maintain this 1:9 ratio unless otherwise indicated. For samples not analyzed on an exact weight basis, e.g., frog legs, refer to the specific method for instructions.

1

Dried egg yolk, dried egg whites, dried whole eggs, liquid milk (skim milk, 2% fat milk, whole, and buttermilk), and prepared powdered mixes (cake, cookie, doughnut, biscuit, and bread), infant formula, and oral or tube feedings containing egg. Preferably, do not thaw frozen samples before analysis. If frozen sample must be tempered to obtain analytical portion, thaw suitable portion as rapidly as possible to minimize increase in number of competing organisms or to reduce potential of injuring *Salmonella* organisms. Thaw below 45°C for 15 min with continuous agitation in thermostatically controlled water bath or thaw within 18 h at 2-5°C. Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. For nonpowdered samples, add 225 ml sterile lactose broth[67]. If product is powdered, add about 15 ml sterile lactose broth and stir with sterile glass rod, spoon, or tongue depressor to smooth suspension. Add 3 additional portions of lactose broth, 10, 10, and 190 ml, for total of 225 ml. Stir thoroughly until sample is suspended without lumps. Cap jar securely and let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2 with sterile 1 N NaOH or 1 N HCl. Cap jar securely and mix well before determining final pH. Loosen jar cap about 1/4 turn and incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.

2. **Eggs**
   a. **Shell eggs.** Remove any adherent material from the shell surface. Disinfect eggs with 3:1 solution consisting of 3 parts of 70% alcohol (ethyl or isopropyl) to 1 part iodine/potassium iodide solution. Prepare 70% alcohol solution either by diluting 700 ml 100% alcohol with sterile distilled water for a final volume of 1,000 ml or by diluting 700 ml 95% alcohol with sterile distilled water for a final volume of 950 ml. Prepare iodine/potassium iodide solution by dissolving 100 g potassium iodide in 200-300 ml sterile distilled water. Add 50 g iodine and heat gently with constant mixing until the iodine is dissolved. Dilute the iodine/potassium iodide solution to 1,000 ml with sterile distilled water. Store iodine/potassium iodide solution in amber glass-stoppered bottle in the dark. Prepare the disinfection solution by adding 250 ml iodine/potassium iodide solution to 750 ml 70% alcohol solution and mix well. Submerge eggs in disinfection solution for at least 10 seconds. Remove eggs and allow to air dry. Eggs with chipped, cracked, or broken shells are not included in the sample. Each sample shall consist of twenty (20) eggs cracked aseptically into a Whirl-Pak bag, for a total of fifty (50) samples per poultry house. Eggs are cracked aseptically by gloved hands, with a change of gloves between samples. Mix samples thoroughly by gloved hands, with a change of gloves between samples. Mix samples thoroughly by hand until yolks are completely mixed with the albumen. Samples are held at room temperature (20-24°C) for 96 ± 2 h. After 96 ± 2 h, remove 25 ml portion from each sample of pooled eggs, and preenrich 25 ml test portion in 225 ml sterile trypticase soy broth (TSB) supplemented with ferrous sulfate[68] (35 mg ferrous sulfate added to 1000 ml TSB) and mix well by swirling. Let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.
   b. **Liquid whole eggs (homogenized).** Combine fifteen (15) 25 ml test portions into a 375 ml composite contained in a 6-liter Erlenmeyer flask. Composites are held at room temperature (20-24°C) for 96 ± 2 h. After 96 ± 2 h, add 3,375 ml sterile TSB supplemented with ferrous sulfate[69], as described above, and mix well by swirling. Let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.
   c. **Hard-boiled eggs (chicken, duck, and other).** If the egg shells are still intact, disinfect the shells as described above and aseptically separate the shells from the eggs. Pulverize the eggs (egg yolk solids and egg white solids) aseptically and weigh 25 g into a sterile 500 ml Erlenmeyer flask or other appropriate container. Add 225 ml TSB[70] (without ferrous sulfate) and mix well by swirling. Continue as described above.
3. **Nonfat dry milk**
   a. **Instant.** Aseptically weigh 25 g sample into sterile beaker (250 ml) or other appropriate container. Using sterile glass or paper funnel (made with tape to withstand autoclaving), pour 25 g analytical unit gently and slowly over surface of 225 ml brilliant green water contained in sterile 500 ml Erlenmeyer flask or other appropriate container. Alternatively, 25 g analytical units may be composited and poured over the surface of proportionately larger volumes of brilliant green water. Prepare brilliant green water by adding 2 ml 1% brilliant green dye solution[71] per 1000 ml sterile distilled water. Let container stand undisturbed for 60 ± 5 min. Incubate loosely capped container, without mixing or pH adjustment, for 24 ± 2 h at 35°C. Continue as in D, 1-11, below.
   b. **Non-Instant.** Examine as described for instant nonfat dry milk, except that the 25 g analytical units may not be composited.
4. **Dry whole milk.** Examine as described for instant nonfat dry milk, except that the 25 g analytical units

1

may not be composited.

5. **Casein**
   a. **Lactic casein.** Aseptically weigh 25 g sample into sterile beaker (250 ml) or other appropriate container. Using sterile glass or paper funnel (made with tape to withstand autoclaving), pour 25 g analytical unit gently and slowly over the surface of 225 ml Universal Preenrichment broth contained in sterile 500 ml Erlenmeyer flask or other appropriate container. Analytical units (25 g) may be composited. Let container stand undisturbed 60 ± 5 min. Incubate loosely capped container, without mixing or pH adjustment, for 24 ± 2 h at 35°C. Continue as in D, 1-11, below.
   b. **Rennet casein.** Aseptically weigh 25 g sample into sterile beaker (250 ml) or other appropriate container. Using sterile glass or paper funnel (made with tape to withstand autoclaving), pour 25 g analytical unit gently and slowly over the surface of 225 ml lactose broth contained in sterile 500 ml Erlenmeyer flask or other appropriate container. Analytical units (25 g) may be composited. Let container stand undisturbed 60 ± 5 min. Incubate loosely capped container, without mixing or pH adjustment, for 24 ± 2 h at 35°C. Continue as in D, 1-11 , below.
   c. **Sodium caseinate.** Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile lactose broth and mix well. Analytical units may be composited. Let stand 60 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Loosen jar about 1/4 turn and incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.
6. **Soy flour.** Examine as described for rennet casein, except 25 g analytical units (25 g) may not be composited.
7. **Egg-containing products (noodles, egg rolls, macaroni, spaghetti), cheese, dough, prepared salads (ham, egg, chicken, tuna, turkey), fresh, frozen, or dried fruits and vegetables, nut meats, crustaceans (shrimp, crab, crayfish, langostinos, lobster), and fish.** Preferably, do not thaw frozen samples before analysis. If frozen sample must be tempered to obtain analytical portion, thaw below 45°C for <15 min with continuous agitation in thermostatically controlled water bath or thaw within 18 h at 2-5°C.

   Aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile lactose broth[72] and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Mix well and loosen jar cap about 1/4 turn. Incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.
8. **Dried yeast (active and inactive yeast).** Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile trypticase soy broth[73]. Mix well to form smooth suspension. Let stand 60 ± 5 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2, mixing well before determining final pH. Loosen jar cap 1/4 turn and incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.
9. **Frosting and topping mixes.** Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml nutrient broth[74] and mix well. Cap jar securely and let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Loosen jar cap about 1/4 turn and incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.
10. **Spices**
    a. **Black pepper, white pepper, celery seed or flakes, chili powder, cumin, paprika, parsley flakes, rosemary, sesame seed, thyme, and vegetable flakes.** Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile trypticase soy broth (TSB)[75] and mix well. Cap jar securely and let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Loosen jar cap about 1/4 turn and incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.
    b. **Onion flakes, onion powder, garlic flakes.** Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Preenrich sample in TSB[76] with added K_2SO_3 (5 g K_2SO_3 per 1000 ml TSB, resulting in final 0.5% K_2SO_3 concentration). Add K_2SO_3 to broth before autoclaving 225 ml volumes in 500 ml Erlenmeyer flasks at 121°C for 15 min. After autoclaving, aseptically determine and, if necessary, adjust final volume to 225 ml. Add 225 ml sterile TSB with added K_2SO_3 to sample and mix well. Continue as in C-10a.
    c. **Allspice, cinnamon, cloves, and oregano.** At this time there are no known methods for

1

neutralizing the toxicity of these 4 spices. Dilute them beyond their toxic levels to examine them. Examine allspice, cinnamon, and oregano at 1:100 sample/broth ratio, and cloves at 1:1000 sample/broth ratio. Examine leafy condiments at sample/broth ratio greater than 1:10 because of physical difficulties encountered by absorption of broth by dehydrated product. Examine these spices as described in C-10a, above, maintaining recommended sample/broth ratios.

11. **Candy and candy coating (including chocolate).** Aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile, reconstituted nonfat dry milk[77] and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Add 0.45 ml 1% aqueous brilliant green dye solution and mix well. Loosen jar caps 1/4 turn and incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.

12. **Coconut.** Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile lactose broth[78], shake well, and let stand 60 ± 5 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Add up to 2.25 ml steamed (15 min) Tergitol Anionic 7[79] and mix well. Alternatively, use steamed (15 min) Triton X-100[80]. Limit use of these surfactants to minimum quantity needed to initiate foaming. For Triton X-100 this quantity may be as little as 2 or 3 drops. Loosen jar cap about l/4 turn and incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.

13. **Food dyes and food coloring substances.** For dyes with pH 6.0 or above (10% aqueous suspension), use method described for dried whole eggs (C-l, above). For laked dyes or dyes with pH below 6.0, aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml tetrathionate broth[81] without brilliant green dye. Mix well and let stand 60 ± 5 min at room temperature with jar securely capped. Using pH meter, adjust pH to 6.8 ± 0.2. Add 2.25 ml 0.1% brilliant green dye solution[82] and mix thoroughly by swirling. Loosen jar cap about 1/4 turn and incubate 24 ± 2 h at 35°C. Continue as in D, 3-11, below.

14. **Gelatin.** Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile lactose broth[83] and 5 ml 5% aqueous papain solution[84] and mix well. Cap jar securely and incubate at 35°C for 60 ± 5 min. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Loosen jar cap about 1/4 turn and incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.

15. **Meats, meat substitutes, meat by-products, animal substances, glandular products, and meals (fish, meat, bone).** Aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile lactose broth[85] and blend 2 min. Aseptically transfer homogenized mixture to sterile wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. If mixture is powder or is ground or comminuted, blending may be omitted. For samples that do not require blending, add lactose broth and mix thoroughly; let stand for 60 ± 5 min at room temperature with jar securely capped.

    Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Add up to 2.25 ml steamed (15 min) Tergitol Anionic 7 and mix well. Alternatively, use steamed (15 min) Triton X-100. Limit use of these surfactants to minimum quantity needed to initiate foaming. Actual quantity will depend on composition of test material. Surfactants will not be needed in analysis of powdered glandular products. Loosen jar caps 1/4 turn and incubate sample mixtures 24 ± 2 h at 35°C. Continue as in D, 1-11, below.

16. **Frog legs.** (This method is used for all domestic and imported frog legs.) Place 15 pairs of frog legs into sterile plastic bag and cover with sterile lactose broth at a 1:9 sample-to-broth (g/ml) ratio (**see** A, 23-24, above). If single legs are estimated to average 25 g or more, examine only one leg of each of 15 pairs. Place bag in large plastic beaker or other suitable container. Mix well and let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Place plastic bag containing the frog legs and lactose broth into plastic beaker or other suitable container. Incubate 24 ± 2 h at 35°C. Continue examination as in D, 1-11, below.

17. **Rabbit carcasses.** (This method is used for all domestic and imported rabbit carcasses.) Place rabbit carcass into sterile plastic bag. Place bag in beaker or other suitable container. Add sterile lactose broth at a 1:9 sample-to-broth (g/ml) ratio to cover carcass (see A, 23-24, above). Mix well by swirling and let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Incubate 24 ± 2 h at 35° C . Continue examination as in D, 1-11, below.

18. **Guar gum.** Aseptically weigh 25 g sample into sterile beaker (250 ml) or other appropriate container. Prepare a 1.0% cellulase solution (add 1 g cellulase to 99 ml sterile distilled water). Dispense into 150 ml bottles. (Cellulase solution may be stored at 2-5°C for up to 2 weeks). Add 225 ml sterile lactose

1

broth[86] and 2.25 ml sterile 1% cellulase solution to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. While vigorously stirring the cellulase/lactose broth with magnetic stirrer, pour 25 g analytical unit quickly through sterile glass funnel into the cellulase/lactose broth. Cap jar securely and let stand 60 ± 5 min at room temperature. Incubate loosely capped container without pH adjustment, for 24 ± 2 h at 35°C. Continue as in D, 1-11, below.

19. **Orange juice (pasteurized and unpasteurized), apple cider (pasteurized and unpasteurized), and apple juice (pasteurized).** Aseptically add 25 ml sample to 225 ml Universal preenrichment broth[87] in a sterile, wide mouth, screw-capped jar (500 ml) or other appropriate container. Swirl the flask contents thoroughly. Cap jar securely and let stand 60 ± 5 min at room temperature. Do not adjust pH. Incubate loosely capped container for 24 ± 2 h at 35°C. Continue as in D, 1-11, below (treat as a low microbial load food).

20. **Pig ears and other types of dog chew pieces.** Place 1 piece (or 2-3 pieces if smaller sizes) from each sample unit into sterile plastic bag. Place bag into large beaker or other suitable container. Add sterile lactose broth at a 1:9 sample-to-broth (g/ml) ratio to cover pieces (see A, 23-24, above). Mix well by swirling and let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Add either steamed (15 min) Tergitol Anionic 7 or steamed (15 min) Triton X-100 up to a 1% concentration. For example, if 225 ml lactose broth is added, the maximum volume of added surfactant is 2.25 ml. Limit use of these surfactants to minimum quantity to initiate foaming. Incubate 24 ± 2 h at 35° C. Continue examination as in D, 1-11, below.

21. **Cantaloupes.** Preferably, do not thaw frozen samples before analysis. If frozen sample must be tempered to obtain analytical portion, thaw below 45°C for <15 min with continuous agitation in thermostatically controlled water bath or thaw within 18 h at 2-5°C.

    For comminuted or cut fruit, aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile Universal preenrichment broth[88] (UP) and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Do not adjust pH. Mix well and loosen jar cap about 1/4 turn. Incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.

    For whole cantaloupes, do not rinse even if there is visible dirt. Examine the cantaloupes "as is".

    Place the cantaloupe into a sterile plastic bag. Add enough UP[89] broth to allow the cantaloupe to float. The volume of UP[90] broth may be 1.5 times the weight of the cantaloupes. For example, cantaloupes weighing 1500 g will probably need a volume of approximately 2250 ml UP[91] broth to float. Add more broth, if necessary. Place the plastic bag, with cantaloupes and UP[92] broth, into a 5 liter beaker, or other appropriate container, for support during incubation. Allow the open-end flap of the plastic bag to "fold over" so as to form a secure, but not air-tight, closure during incubation.

    Let stand for 60 ± 5 min at room temperature. Do not adjust pH. Incubate slightly opened bag, containing cantaloupe, for 24 ± 2 h at 35°C. Continue as in D, 1-11, below.

22. **Mangoes.** Preferably, do not thaw frozen samples before analysis. If frozen sample must be tempered to obtain analytical portion, thaw below 45°C for <15 min with continuous agitation in thermostatically controlled water bath or thaw within 18 h at 2-5°C.

    For comminuted or cut fruit, aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile buffered peptone water (BPW)[93] and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Mix well and loosen jar cap about 1/4 turn. Incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.

    For whole mangoes, do not rinse even if there is visible dirt. Examine the mangoes "as is".

    Place the mango into a sterile plastic bag. Add enough BPW[94] to allow the mango to float. The volume of BPW[95] may be 1.0 times the weight of the mangoes. For example, mangoes weighing 500 g will probably need a volume of approximately 500 ml BPW[96] broth to float. Add more broth, if necessary. Place the plastic bag, with mangoes and BPW[97] broth, into a 5 liter beaker, or other appropriate container, for support during incubation.

    Let stand for 60 ± 5 min at room temperature. Adjust pH to 6.8 ± 0.2, if necessary. Incubate slightly opened bag for 24 ± 2 h at 35°C. Continue as in D, 1-11, below.

23. **Tomatoes.** For comminuted or cut fruit, aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile buffered peptone water and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Mix well and loosen jar cap about 1/4 turn. Incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.

1

For whole tomatoes, do not rinse even if there is visible dirt. Examine the tomatoes "as is".

Place the tomato into a sterile plastic bag or other suitable container (sterile foil covered beaker can be used). Add enough UP[98] broth to allow the tomato to float. The volume of UP[99] broth may be 1.0 times the weight of the tomato. For example, tomatoes weighing 300 g will probably need a volume of approximately 300 ml UP[100] broth to float. Add more, if necessary. Place the plastic bag (if used), with tomato and UP[101] broth, into a sterile beaker (beaker size is dependent on the size of the tomato), or other appropriate container, for support during incubation. Allow the open-end flap of the plastic bag to "fold over" so as to form a secure, but not air-tight, closure during incubation.

Let stand for 60 ± 5 min at room temperature. Do not adjust pH. Incubate slightly opened bag for 24 ± 2 h at 35°C. Continue as in D, 1-11, below.

24. **Environmental testing.** Sample environmental surfaces with sterile swabs or sponges. Place the swab/sponge in a sterile Whirl-pak bag, or equivalent, that contains enough Dey-Engley (DE) broth[102] to cover the swab/sponge.

    Transport swabs/sponges in an insulated transport container with frozen gel packs to keep the samples cold, but not frozen. If samples cannot be processed immediately, refrigerate at 4 ± 2°C. Start sample analysis within 48 ± 2 h of collection.

    Add swab/sponge to 225 ml lactose broth in a sterile, wide mouth, screw-capped jar (500 ml) or other appropriate container. Swirl the flask contents thoroughly. Cap jar securely and let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Incubate 24 ± 2 h at 35°C. Continue examination as in D, 1-11, below.

25. **Alfalfa seeds and mung beans.** Aseptically weigh 25g alfalfa seeds or mung beans into a sterile 500 mL Erlenmeyer flask. Aseptically add 225 mL lactose broth to the test portion and swirl the Erlenmeyer flask. Cover the mouth of the Erlenmeyer flask with sterile aluminum foil and allow content to stand at room temperature for 60 ± 5 min. Adjust the pH of the culture to 6.8 ± 0.2, if necessary. Incubate for 24 ± 2h at 35 ± 2°C. Continue as in D, 1-11 , below (treat as high microbial load food).

26. **Mamey pulp.** If frozen, sample must be tempered to obtain analytical portion. Thaw below 45°C for <15 min with continuous agitation in thermostatically controlled water bath or thaw within 18 h at 2-5° C.

    For mamey pulp, suspected to be contaminated with *S.* Typhi, aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile Universal Preenrichment broth without ferric ammonium citrate[103], mix by swirling, and let stand 60 ± 5 min at room temperature with jar securely capped. Do not adjust pH. Mix well and loosen jar cap about 1/4 turn. Incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below. Treat as a low microbial load food.

    For mamey pulp, NOT suspected to be contaminated with *S.* Typhi, aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile Universal Preenrichment broth, mix by swirling, and let stand 60 ± 5 min at room temperature with jar securely capped. Do not adjust pH. Mix well and loosen jar cap about 1/4 turn. Incubate 24 ± 2 h at 35°C. Continue as in D, 1-11, below.

27. **Leafy green vegetables and herbs (baby spinach, Romaine lettuce, cilantro, curly parsley, Italian parsley, culantro, cabbage, and basil).** Aseptically weigh 25 g into a sterile wide mouth Erlenmeyer flask or other appropriate container. Add 225 mL lactose broth and manually mix contents by vigorously swirling the flask 25 times clockwise and 25 times counterclockwise. Allow the flask to stand at room temperature for 60 ± 5.0 minutes, measure the pH and adjust it to 6.8 ± 0.2 with 1N NaOH or 1N HCl, if necessary. Incubate at 35° ± 2.0° C for 24 ± 2.0 hours and continue as in D, 1-11, below.

Return to Chapter Contents

D. Isolation of *Salmonella*

1. Tighten lid and gently shake incubated sample.

   Guar gum and foods suspected to be contaminated with *S.* Typhi. Transfer 1 ml mixture to 10 ml selenite cystine (SC) broth [104] and another 1 ml mixture to 10 ml TT broth [105]. Vortex.

   All other foods. Transfer **0.1** ml mixture to 10 ml Rappaport-Vassiliadis (RV) medium[106] and another 1 ml mixture to 10 ml tetrathionate (TT) broth[107]. Vortex.

2. Incubate selective enrichment media as follows:

   Foods with a high microbial load. Incubate RV medium 24 ± 2 h at 42 ± 0.2°C (circulating, thermostatically-controlled, water bath). Incubate TT broth 24 ± 2 h at 43 ± 0.2°C (circulating, thermostatically-controlled, water bath).

1

Foods with a low microbial load (except guar gum and foods suspected to be contaminated with *S.* Typhi). Incubate RV medium 24 ± 2 h at 42 ± 0.2°C (circulating, thermostatically controlled, water bath). Incubate TT broth 24 ± 2 h at 35 ± 2.0°C.

Guar gum and foods suspected to be contaminated with *S.* Typhi. Incubate SC and TT broths 24 ± 2 h at 35°C.

3. Mix (vortex, if tube) and streak 3 mm loopful (10 µl) incubated TT broth on bismuth sulfite (BS) agar[108], xylose lysine desoxycholate (XLD) agar[109], and Hektoen enteric (HE) agar[110]. **Prepare BS plates the day before streaking and store in dark at room temperature until streaked.**
4. Repeat with 3 mm loopful (10 µl) of RV medium (for samples of high and low microbial load foods) and of SC broth (for guar gum).
5. Refer to 994.04 in *Official Methods of Analysis* (1) for option of refrigerating incubated sample preenrichments and incubated sample selective enrichments (SC and TT broths only) of low moisture foods. This option allows sample analyses to be initiated as late as Thursday while still avoiding weekend work.
6. Incubate plates 24 ± 2 h at 35°C.
7. Examine plates for presence of colonies that may be *Salmonella*.

TYPICAL *Salmonella* COLONY MORPHOLOGY

Pick 2 or more colonies of *Salmonella* from each selective agar after 24 ± 2 h incubation. Typical *Salmonella* colonies are as follows:

a. **Hektoen enteric (HE) agar.** Blue-green to blue colonies with or without black centers. Many cultures of *Salmonella* may produce colonies with large, glossy black centers or may appear as almost completely black colonies.

b. **Xylose lysine desoxycholate (XLD) agar.** Pink colonies with or without black centers. Many cultures of *Salmonella* may produce colonies with large, glossy black centers or may appear as almost completely black colonies.

c. **Bismuth sulfite (BS) agar.** Brown, gray, or black colonies; sometimes they have a metallic sheen. Surrounding medium is usually brown at first, but may turn black in time with increased incubation, producing the so-called halo effect.

If typical colonies are present on the BS agar after 24 ± 2 h incubation, then pick 2 or more colonies. Irrespective of whether or not BS agar plates are picked at 24 ± 2 h, reincubate BS agar plates an additional 24 ± 2 h. After 48 ± 2 h incubation, pick 2 or more typical colonies, if present, from the BS agar plates, only if colonies picked from the BS agar plates incubated for 24 ± 2 h give atypical reactions in triple sugar iron agar (TSI) and lysine iron agar (LIA) that result in culture being discarded as not being *Salmonella* . **See** sections D.9 and D.10, below, for details in interpreting TSI and LIA reactions.

ATYPICAL *Salmonella* COLONY MORPHOLOGY

In the absence of typical or suspicious *Salmonella* colonies, search for atypical *Salmonella* colonies as follows:

a. **HE and XLD agars.** Atypically a few *Salmonella* cultures produce yellow colonies with or without black centers on HE and XLD agars. In the absence of typical *Salmonella* colonies on HE or XLD agars after 24 ± 2 h incubation, then pick 2 or more atypical *Salmonella*colonies.

b. **BS agar.** Atypically some strains produce green colonies with little or no darkening of the surrounding medium. If typical or suspicious colonies are not present on BS agar after 24 ± 2 h, then do not pick any colonies but reincubate an additional 24 ± 2 h. If typical or suspicious colonies are not present after 48 ± 2 h incubation, then pick 2 or more atypical colonies.

SUGGESTED CONTROL CULTURES

In addition to the positive control cultures (typical *Salmonella*), 3 additional *Salmonella* cultures are recommended to assist in the selection of atypical *Salmonella* colony morphology on selective agars. These cultures are a lactose-positive, H_2S-positive *S. diarizonae* (ATCC 12325) and a lactose-negative, H_2S-negative *S. abortus equi* (ATCC 9842); **OR** a lactose-positive, H_2S-negative *S. diarizonae* (ATCC 29934). These cultures may be obtained from the American Type Culture Collection[111], 10801 University Boulevard, Manassas, VA 20110-2209.

8. Lightly touch the very center of the colony to be picked with sterile inoculating needle and inoculate TSI slant by streaking slant and stabbing butt. Without flaming, inoculate LIA slant by stabbing butt twice and then streaking slant. Since lysine decarboxylation reaction is strictly anaerobic, the LIA slants must

have deep butt (4 cm). Store picked selective agar plates at 5-8°C.

9. Incubate TSI and LIA slants at 35°C for 24 ± 2 h. Cap tubes loosely to maintain aerobic conditions while incubating slants to prevent excessive H_2S production. *Salmonella* in culture typically produces alkaline (red) slant and acid (yellow) butt, with or without production of H_2S (blackening of agar) in TSI. In LIA, *Salmonella* typically produces alkaline (purple) reaction in butt of tube. Consider only distinct yellow in butt of tube as acidic (negative) reaction. Do not eliminate cultures that produce discoloration in butt of tube solely on this basis. Most *Salmonella* cultures produce H_2S in LIA. Some non- *Salmonella* cultures produce a brick-red reaction in LIA slants.

10. All cultures that give an alkaline butt in LIA, regardless of TSI reaction, should be retained as potential *Salmonella* isolates and submitted for biochemical and serological tests. Cultures that give an acid butt in LIA and an alkaline slant and acid butt in TSI should also be considered potential *Salmonella* isolates and should be submitted for biochemical and serological tests. Cultures that give an acid butt in LIA and an acid slant and acid butt in TSI may be discarded as not being *Salmonella* . Test retained, presumed-positive TSI cultures as directed in D-11, below, to determine if they are *Salmonella* species, including *S. arizonae*. If TSI cultures fail to give typical reactions for *Salmonella* (alkaline slant and acid butt) pick additional suspicious colonies from selective medium plate not giving presumed-positive culture and inoculate TSI and LIA slants as described in D-8, above.

11. Apply biochemical and serological identification tests to:

    a. Three presumptive TSI cultures recovered from set of plates streaked from RV medium (or SC broth for guar gum), if present, and 3 presumptive TSI agar cultures recovered from plates streaked from TT broth, if present.

    b. If 3 presumptive-positive TSI cultures are not isolated from one set of agar plates, test other presumptive-positive TSI agar cultures, if isolated, by bioche mical and serological tests. Examine a minimum of 6 TSI cultures for each 25 g analytical unit or each 375 g composite.

Return to Chapter Contents

E. Identification of *Salmonella*

1. **Mixed cultures.** Streak TSI agar cultures that appear to be mixed on MacConkey agar[112], HE agar[113], or XLD agar[114]. Incubate plates 24 ± 2 h at 35°C. Examine plates for presence of colonies suspected to be *Salmonella*.

    a. **MacConkey agar.** Typical colonies appear transparent and colorless, sometimes with dark center. Colonies of *Salmonella* will clear areas of precipitated bile caused by other organisms sometimes present.

    b. **Hektoen enteric (HE) agar. See** D-7a, above.

    c. **Xylose lysine desoxycholate (XLD) agar. See** D-7b, above. Transfer at least 2 colonies suspected to be *Salmonella* to TSI and LIA slants as described in D-7, above, and continue as in D-9, above.

2. **Pure cultures**

    a. **Urease test (conventional).** With sterile needle, inoculate growth from each presumed-positive TSI slant culture into tubes of urea broth[115]. Since occasional, uninoculated tubes of urea broth turn purple-red (positive test) on standing, include uninoculated tube of this broth as control. Incubate 24 ± 2 h at 35°C.

    b. **Optional urease test (rapid).** Transfer two 3-mm loopfuls of growth from each presumed-positive TSI slant culture into tubes of rapid urea broth[116]. Incubate 2 h in 37 ± 0.5°C water bath. Discard all cultures giving positive test. Retain for further study all cultures that give negative test (no change in color of medium).

3. **Serological polyvalent flagellar (H) test**

    a. Perform the polyvalent flagellar (H) test at this point, or later, as described in E-5, below. Inoculate growth from each urease-negative TSI agar slant into either 1) BHI broth[117] and incubate 4-6 h at 35°C until visible growth occurs (to test on same day); or 2) trypticase soy-tryptose broth[118] and incubate 24 ± 2 h at 35°C (to test on following day). Add 2.5 ml formalinized physiological saline solution to 5 ml of either broth culture.

    b. Select 2 formalinized broth cultures and test with *Salmonella* polyvalent flagellar (H) antisera. Place 0.5 ml of appropriately diluted *Salmonella* polyvalent flagellar (H) antiserum in 10 x 75 mm or 13 x 100 mm serological test tube. Add 0.5 ml antigen to be tested. Prepare saline control by

1
2

mixing 0.5 ml formalinized physiological saline solution with 0.5 ml formalinized antigen. Incubate mixtures in 48-50°C water bath. Observe at 15 min intervals and read final results in 1 h.

Positive — agglutination in test mixture and no agglutination in control.

Negative — no agglutination in test mixture and no agglutination in control.

Nonspecific — agglutination in both test mixture and control. Test the cultures giving such results with Spicer-Edwards antisera.

4. **Spicer-Edwards serological test.** Use this test as an alternative to the polyvalent flagellar (H) test. It may also be used with cultures giving nonspecific agglutination in polyvalent flagellar (H) test. Perform Spicer-Edwards flagellar (H) antisera test as described in E, 3b, above. Perform additional biochemical tests (E, 5a-c, below) on cultures giving positive flagellar test results. If both formalinized broth cultures are negative, perform serological tests on 4 additional broth cultures (E, 3a, above). If possible, obtain 2 positive cultures for additional biochemical testing E, 5a-c, below). If all urease-negative TSI cultures from sample give negative serological flagellar (H) test results, perform additional biochemical tests E, 5a-c, below).

5. **Testing of urease-negative cultures**

   a. **Lysine decarboxylase broth**[119]. If LIA test was satisfactory, it need not be repeated. Use lysine decarboxylase broth for final determination of lysine decarboxylase if culture gives doubtful LIA reaction. Inoculate broth with small amount of growth from TSI slant suspicious for *Salmonella*. Replace cap tightly and incubate 48 ± 2 h at 35°C but examine at 24 h intervals. *Salmonella* species cause alkaline reaction indicated by purple color throughout medium. Negative test is indicated by yellow color throughout medium. If medium appears discolored (neither purple nor yellow) add a few drops of 0.2% bromcresol purple dye and re-read tube reactions.

   b. **Phenol red dulcitol broth**[120] **or purple broth base**[121] **with 0.5% dulcitol.** Inoculate broth with small amount of growth from TSI culture. Replace cap loosely and incubate 48 ± 2 h at 35°C, but examine after 24 h. Most *Salmonella* species give positive test, indicated by gas formation in inner fermentation vial and acid pH (yellow) of medium. Production of acid should be interpreted as a positive reaction. Negative test is indicated by no gas formation in inner fermentation vial and red (with phenol red as indicator) or purple (with bromcresol purple as indicator) color throughout medium.

   c. **Tryptone (or tryptophane) broth**[122]. Inoculate broth with small growth from TSI agar culture. Incubate 24 ± 2 h at 35°C and proceed as follows:

      1. **Potassium cyanide (KCN) broth.** [123]Transfer 3 mm loopful of 24 h tryptophane broth culture to KCN broth. Heat rim of tube so that good seal is formed when tube is stoppered with wax-coated cork. Incubate 48 ± 2 h at 35°C but examine after 24 h. Interpret growth (indicated by turbidity) as positive. Most *Salmonella* species do not grow in this medium, as indicated by lack of turbidity.

      2. **Malonate broth**[124]. Transfer 3 mm loopful of 24 h tryptone broth culture to malonate broth. Since occasional uninoculated tubes of malonate broth turn blue (positive test) on standing, include uninoculated tube of this broth as control. Incubate 48 ± 2 h at 35°C, but examine after 24 h. Most *Salmonella* species cultures give negative test (green or unchanged color) in this broth.

      3. **Indole test.** Transfer 5 ml of 24 h tryptophane broth culture to empty test tube. Add 0.2-0.3 ml Kovacs' reagent[125]. Most *Salmonella* cultures give negative test (lack of deep red color at surface of broth). Record intermediate shades of orange and pink as ±.

      4. **Serological flagellar (H) tests for *Salmonella*.** If either polyvalent flagellar (H) test (E-3, above) or the Spicer-Edwards flagellar (H) test tube test (E-4, above) has not already been performed, either test may be performed here.

      5. Discard as not *Salmonella* any culture that shows either positive indole test and negative serological flagellar (H) test, or positive KCN test and negative lysine decarboxylase test.

6. **Serological somatic (O) tests for *Salmonella*.**
(Pre-test all antisera to *Salmonella* with known cultures.)

   a. **Polyvalent somatic (O) test.** Using wax pencil, mark off 2 sections about 1 x 2 cm each on inside of glass or plastic petri dish (15 x 100 mm). Commercially available sectioned slides may be used. Emulsify 3 mm loopful of culture from 24-48 h TSI slant or, preferably, tryptose blood agar base (without blood) with 2 ml 0.85% saline. Add 1 drop of culture suspension to upper portion of each rectangular crayon-marked section. Add 1 drop of saline solution to lower part of one section only. Add 1 drop of *Salmonella* polyvalent somatic (O) antiserum to other section

1
2

only. With clean sterile transfer loop or needle, mix culture suspension with saline solution for one section and repeat for other section containing antiserum. Tilt mixtures in back-and-forth motion for 1 min and observe against dark background in good illumination. Consider any degree of agglutination a positive reaction. Classify polyvalent somatic (O) test results as follows:

Positive — agglutination in test mixture; no agglutination in saline control.

Negative — no agglutination in test mixture; no agglutination in saline control.

Nonspecific — agglutination in test and in control mixtures. Perform further biochemical and serological tests as described in *Edwards and Ewing's Identification of Enterobacteriaceae* (2).

b. **Somatic (O) group tests.** Test as in E-6a, above, using individual group somatic (O) antisera including Vi, if available, in place of *Salmonella* polyvalent somatic (O) antiserum. For special treatment of cultures giving positive Vi agglutination reaction, refer to sec. 967.28B in *Official Methods of Analysis* (1). Record cultures that give positive agglutination with individual somatic (O) antiserum as positive for that group. Record cultures that do not react with individual somatic (O) antiserum as negative for that group.

7. **Additional biochemical tests.** Classify as *Salmonella* those cultures which exhibit typical *Salmonella* reactions for tests 1-11, shown in Table 1. If one TSI culture from 25 g analytical unit is classified as *Salmonella*, further testing of other TSI cultures from the same 25 g analytical unit is unnecessary. Cultures that contain demonstrable *Salmonella* antigens as shown by positive *Salmonella* flagellar (H) test but do not have biochemical characteristics of *Salmonella* should be purified (E-l, above) and retested, beginning with E-2, above.

   Perform the following additional tests on cultures that do not give typical *Salmonella* reactions for tests 1-11 in Table 1 and that consequently do not classify as *Salmonella*.

   a. **Phenol red lactose broth[126] or purple lactose broth[127].**

      1. Inoculate broth with small amount of growth from unclassified 24-48 h TSI slant. Incubate 48 ± 2 h at 35°C, but examine after 24 h.

         Positive — acid production (yellow) and gas production in inner fermentation vial. Consider production of acid only as positive reaction. Most cultures of *Salmonella* give negative test result, indicated by no gas formation in inner fermentation vial and red (with phenol red as indicator) or purple (with bromcresol purple as indicator) throughout medium.

      2. Discard as not *Salmonella*, cultures that give positive lactose tests, except cultures that give acid slants in TSI and positive reactions in LIA, or cultures that give positive malonate broth reactions. Perform further tests on these cultures to determine if they are *S. arizonae*.

   b. **Phenol red sucrose broth[128] or purple sucrose broth[129].** Follow procedure described in E,7a-1, above. Discard as not *Salmonella*, cultures that give positive sucrose tests, except those that give acid slants in TSI and positive reactions in LIA.

   c. **MR-VP broth[130].** Inoculate medium with small amount of growth from each unclassified TSI slant suspected to contain *Salmonella*. Incubate 48 ± 2 h at 35°C.

      1. Perform Voges-Proskauer (VP) test at room temperature as follows: Transfer 1 ml 48 h culture to test tube and incubate remainder of MR-VP broth an additional 48 h at 35°C. Add 0.6 ml α-naphthol and shake well. Add 0.2 ml 40% KOH solution and shake. To intensify and speed reaction, add a few crystals of creatine. Read results after 4 h; development of pink-to-ruby red color throughout medium is positive test. Most cultures of *Salmonella* are VP-negative, indicated by absence of development of pink-to-red color throughout broth.

      2. Perform methyl red test as follows: To 5 ml of 96 h MR-VP broth, add 5-6 drops of methyl red indicator. Read results immediately. Most *Salmonella* cultures give positive test, indicated by diffuse red color in medium. A distinct yellow color is negative test. Discard, as not *Salmonella*, cultures that give positive KCN and VP tests and negative methyl red test.

   d. **Simmons citrate agar.** Inoculate this agar, using needle containing growth from unclassified TSI agar slant. Inoculate by streaking slant and stabbing butt. Incubate 96 ± 2 h at 35°C. Read results as follows:

      Positive — presence of growth, usually accompanied by color change from green to blue. Most cultures of *Salmonella* are citrate-positive.

      Negative — no growth or very little growth and no color change.

8. **Classification of cultures.** Classify, as *Salmonella*, cultures that have reaction patterns of Table I. Discard, as not *Salmonella*, cultures that give results listed in any subdivision of Table 2. Perform additional tests described in *Edwards and Ewing's Identification of Enterobacteriaceae* (2) to classify any

1
2

culture that is not clearly identified as *Salmonella* by classification scheme in Table I or not eliminated as not being *Salmonella* by test reactions in Table 2. If neither of 2 TSI cultures carried through biochemical tests confirms the isolate as *Salmonella*, perform biochemical tests, beginning with E-5, on remaining urease-negative TSI cultures from same 25 g analytical unit.

Table 1. Biochemical and serological reactions of *Salmonella*

#	Test or substrate	Result		*Salmonella* species reaction[a]
		Positive	Negative	
1.	Glucose (TSI)	yellow butt	red butt	+
2.	Lysine decarboxylase (LIA)	purple butt	yellow butt	+
3.	H_2S (TSI and LIA)	blackening	no blackening	+
4.	Urease	purple-red color	no color change	−
5.	Lysine decarboxylase broth	purple color	yellow color	+
6.	Phenol red dulcitol broth	yellow color and/or gas	no gas; no color change	+[b]
7.	KCN broth	growth	no growth	−
8.	Malonate broth	blue color	no color change	−[c]
9.	Indole test	violet color at surface	yellow color at surface	−
10.	Polyvalent flagellar test	agglutination	no agglutination	+
11.	Polyvalent somatic test	agglutination	no agglutination	+
12.	Phenol red lactose broth	yellow color and/or gas	no gas; no color change	−[c]
13.	Phenol red sucrose broth	yellow color and/or gas	no gas; no color change	−
14.	Voges-Proskauer test	pink-to-red color	no color change	−
15.	Methyl red test	diffuse red color	diffuse yellow color	+
16.	Simmons citrate	growth; blue color	no growth; no color change	v

[a] +: 90% or more positive in 1 or 2 days; −: 90% or more negative in 1 or 2 days; v: variable.
[b] Majority of *S. arizonae* cultures are negative.
[c] Majority of *S. arizonae* cultures are positive.

Table 2. Criteria for discarding non-*Salmonella* cultures

#	Test or substrate	Results
1.	Urease	positive (purple-red color)
2.	Indole test and	positive (violet color at surface)
	Polyvalent flagellar (H) test;	negative (no agglutination)
	or	
	Indole test and	positive (violet color at surface)
	Spicer-Edwards flagellar test	negative (no agglutination)
3.	Lysine decarboxylase and	negative (yellow color)
	KCN broth	positive (growth)
4.	Phenol red lactose broth	positive (yellow color and/or gas)[a], [b]
5.	Phenol red sucrose broth	positive (yellow color and/or gas)[b]
6.	KCN broth,	positive (growth)
	Voges-Proskauer test, and	positive (pink-to-red color)
	Methyl red test	negative (diffuse yellow color)

[a] Test malonate broth positive cultures further to determine if they are *S. arizonae*.
[b] Do not discard positive broth cultures if corresponding LIA cultures give typical *Salmonella* reactions; test further to determine if they are *Salmonella* species.

9. **Presumptive generic identification of *Salmonella*.** As alternative to conventional biochemical tube system, use any of 5 commercial biochemical systems (API 20E, Enterotube II, *Enterobacteriaceae* II, MICRO-ID, or Vitek GNI) for presumptive generic identification of foodborne *Salmonella*. Choose a commercial system based on a demonstration in analyst's own laboratory of adequate correlation between commercial system and biochemical tube system delineated in this identification section. Commercial biochemical kits should not be used as a substitute for serological tests (I). Assemble supplies and prepare reagents required for the kit. Inoculate each unit according to Method 978.24 (API 20E, Enterotube II, and *Enterobacteriaceae* II), sec. 989.12 (MICRO-ID), and Method 991.13 (Vitek GNI) in *Official Methods of Analysis* (1), incubating for time and temperature specified. Add reagents, observe, and record results. For presumptive identification, classify cultures, according to ref. 1, above, as *Salmonella* or not *Salmonella*.

For confirmation of cultures presumptively identified as *Salmonella*, perform the *Salmonella* serological somatic (O) test (E-6, above) and the *Salmonella* serological flagellar (H) test (E-3, above) or the Spicer-Edwards flagellar (H) test (E-4, above), and classify cultures according to the following

guidelines:

a. Report as *Salmonella* those cultures classified as presumptive *Salmonella* with commercial biochemical kits when the culture demonstrates positive *Salmonella* somatic (O) test and positive *Salmonella* (H) test.

b. Discard cultures presumptively classified as not *Salmonella* with commercial biochemical kits when cultures conform to AOAC criteria (1) for classifying cultures as not *Salmonella*.

c. For cultures that do not conform to a or b, classify according to additional tests specified in E, 2-7, above, or additional tests as specified by Ewing (2), or send to reference typing laboratory for definitive serotyping and identification.

10. **Treatment of cultures giving negative flagellar (H) test.** If biochemical reactions of certain flagellar (H)-negative culture strongly suggest that it is *Salmonella*, the negative flagellar agglutination may be the result of nonmotile organisms or insufficient development of flagellar antigen. Proceed as follows: Inoculate motility test medium in petri dish, using small amount of growth from TSI slant. Inoculate by stabbing medium once about 10 mm from edge of plate to depth of 2-3 mm. Do not stab to bottom of plate or inoculate any other portion. Incubate 24 h at 35°C. If organisms have migrated 40 mm or more, retest as follows: Transfer 3 mm loopful of growth that migrated farthest to trypticase soy-tryptose broth. Repeat either polyvalent flagellar (H) (E-3, above) or Spicer-Edwards (E-4 , above) serological tests. If cultures are not motile after the first 24 h, incubate an additional 24 h at 35°C. If still not motile, incubate up to 5 days at 25°C. Classify culture as nonmotile if above tests are still negative. If flagellar (H)-negative culture is suspected of being a species of *Salmonella* on the basis of its biochemical reactions, FDA laboratories should submit the culture to

FDA Denver Laboratory
Attention Sample Custodian
Denver Federal Center, Building 20
6th Avenue & Kipling Streets
Denver, CO 80225-0087

(Above address effective October 1, 2004)

for further identification and/or serotyping. Laboratories other than FDA should make arrangements with a reference laboratory for the serotyping of *Salmonella* cultures.

11. **Submission of cultures for serotyping.** Submit 1 isolate of each somatic group recovered from each analytical unit, unless otherwise instructed. Submit cultures on PRT agar slants in screw-cap tubes (13 x 100 mm or 16 x 125 mm) with caps secured tightly. Label each tube with sample number, subsample (analytical unit) number, and code, if applicable. Submit a copy of the Collection Report, FD-464, or Import Sample Report, FD-784 for each sample. Place cultures in culture container with official FDA seal. Place accompanying records (E-11, above) inside shipping carton but not within officially sealed culture container. Submit memo or cover letter for each sample number to expedite reporting of results. Prepare cultures for shipment according to requirements for shipment of etiological agents (3). Label secondary shipping container according to ref. 4. Send container by most rapid mail service available. Maintain duplicate cultures of those submitted for serotyping only on those samples under consideration for legal action.

Microbiology Field laboratories should follow the following guidance in sending Salmonella isolates for serotyping:

Isolates from NRL, WEAC, SRL and ARL will be serotyped in ARL:

Arkansas Regional Laboratory
3900 NCTR Road Building 26
Jefferson, AR 72079
Attention: Gwendolyn Anderson
Tel # 870-543-4621
Fax# 870-543-4041

Isolates from SAN, PRL-NW, PRL-SW and DEN will be serotyped in DEN

Denver District Laboratory
6th Avenue & Kipling Street
DFC Building 20
Denver, CO 80225-0087
Attention: Doris Farmer
Tel # 303-236-9604
Fax # 303-236-9675

Return to Chapter Contents

1

References

1. AOAC INTERNATIONAL. 2000. Official Methods of Analysis, 17th ed., Methods 967.25-967.28, 978.24, 989.12, 991.13, 994.04, and 995.20. AOAC INTERNATIONAL, Gaithersburg, MD.
2. Ewing, W.H. 1986. Edwards and Ewing's Identification of Enterobacteriacae, 4th ed. Elsevier, New York.
3. Federal Register. 1971. **36**(93):8815 (secs d, e, and f).
4. Federal Register. 1972. **37**(191):20556 (sec. 173.388(a)).
5. June, G.A., P.S. Sherrod, T.S. Hammack, R.M. Amaguana, and W.H. Andrews. 1995. Relative effectiveness of selenite cystine broth, tetrathionate broth, and Rappaport-Vassiliadis medium for the recovery of *Salmonella* from raw flesh and other highly contaminated foods: Precollaborative study. *J. AOAC Int.* **78**:375-380.
6. Hammack, T.S., R.M. Amaguana, G.A. June, P.S. Sherrod, and W.H. Andrews. 1999. Relative effectiveness of selenite cystine broth, tetrathionate broth, and Rappaport-Vassiliadis medium for the recovery of *Salmonella* from foods with a low microbial load. *J. Food Prot.* **62**:16-21.
7. June, G.A., P.S. Sherrod, T.S. Hammack, R.M. Amaguana, and W.H. Andrews. 1996. Relative effectiveness of selenite cystine broth, tetrathionate broth, and Rappaport-Vassiliadis medium for the recovery of *Salmonella* from raw flesh, highly contaminated foods, and poultry feed: Collaborative study. *J. AOAC Int.* **79**:1307-1323.
8. Hammack, T.S., R.M. Amaguana, and W.H. Andrews. 2001. Rappaport-Vassiliadis medium for the recovery of *Salmonella* from foods with a low microbial load: Collaborative study. *J. AOAC Int.* **84**:(1) 65-83.
9. Sherrod, P.S., R.M. Amaguana, W.H. Andrews, G.A. June, and T.S. Hammack. 1995. Relative effectiveness of selective plating agars for the recovery of *Salmonella* species from selected high-moisture foods. *J. AOAC Int.* **78**:679-690.

Return to Chapter Contents

Hypertext Source: Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. Chapter 4.

Evaluation of the 3M™ Petrifilm™ Salmonella Express System for the Detection of Salmonella in Select Foods

AOAC Research Institute
Performance Tested Method

Abstract

The 3M™ Petrifilm™ Salmonella Express SALX System is used for the rapid and specific detection and l biochemical confirmation of *Salmonella* spp. from enriched food matrices. The 3M Petrifilm SALX System was compared to the USDA/FSIS-MLG 4.05 *Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg and Catfish Products* for raw ground chicken, pasteurized liquid whole egg, raw ground beef, raw ground pork, and cooked chicken nuggets and to FDA/BAM Chapter 5 *Salmonella* for frozen uncooked shrimp, fresh bunched spinach, dry dog food, and stainless steel. Twenty (20) replicates of each matrix were analyzed at one inoculum level: 0.2-2 CFU/test portion. Five (5) control replicates were also analyzed at 0 CFU/test portion (un-inoculated). Results were analyzed by both the Mantel-Haenszel chi square for unpaired test portions and by the Probability of Detection (POD). There were no statistically significant differences in the number of positive samples detected and confirmed by the 3M Petrifilm SALX System and the reference methods for the nine matrices analyzed. Lot-to-lot assay variability, shelf-life stability, and assay ruggedness parameters (primary enrichment incubation time, incubation time of the 3M™ Petrifilm™ Salmonella Plate, incubation temperature of the 3M™ Petrifilm™ Salmonella Plate, incubation temperature of the 3M™ Petrifilm™ Salmonella Disk, incubation time of the 3M™ Petrifilm™ Salmonella Disk, and various plate rehydration options were evaluated and found no unexpected results. The 3M™ Petrifilm™ SALX System demonstrated reliability as a rapid qualitative method for the detection and biochemical confirmation of *Salmonella* in a wide variety of foods.

Method Author

Robert P. Jechorek
3M Food Safety Department
3M Center - Bldg 260-6B-01
St. Paul, MN 55144-1000

Submitting Company

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

St. Paul, MN 55144-1000

Independent Laboratory
Aegis Food Testing Laboratories
224 Derby Lane
North Sioux City, SD 57049

Reviewers of Final Submission
Yi Chen
Microbial Methods Development Branch
Division of Microbiology
Office of Regulatory Science
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway, Rm 3E-021
College Park, MD 20740

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

Wayne Ziemer
Consultant
1301 Kristen Lane
Loganville, GA 30052

General Information
Salmonella is widely dispersed in nature and is spread via the fecal/oral route[1]. According to the CDC[2], *Salmonella* species are responsible for about 48 million cases of food borne illness each year. *Salmonella* is found in a wide variety of foods including: Meats, poultry, eggs, milk and dairy products, fish, shrimp, spices, yeast, coconut, sauces, cream-filled desserts, peanut butter, cocoa, produce (fruits and vegetables, such as tomatoes, peppers, and cantaloupes), and chocolate[1]. Traditional culture and confirmation methods for

Salmonella can take as long as 5-7 days to provide confirmed results. The 3M™ Petrifilm™ Salmonella Express System was developed to provide rapid and specific detection and biochemical confirmation of *Salmonella* species in as little as 44 hours from enriched food samples.

Principle

The 3M PetrifilmSALX method is intended for the rapid and specific detection and biochemical confirmation of *Salmonella* from food and food process environmental samples after enrichment in 3M™ *Salmonella* Enrichment Base with the added 3M™ *Salmonella* Enrichment Supplement. The 3M™ Petrifilm™ Salmonella Express Plate is a sample ready-to-use chromogenic culture medium system that contains a cold-water-soluble gelling agent and is selective and differential for *Salmonella,* providing a presumptive result in as little as 44 hours from low microbial background foods ($\leq 10^4$ cfu/g), and 52 hours from high microbial background foods ($\geq 10^4$ cfu/g). The 3M™ Petrifilm™ Salmonella Express Confirmation Disk contains a biochemical substrate that facilitates the biochemical confirmation of *Salmonella* organisms.

The limit of detection of a method is defined as the lowest concentration point where reliable analytical results can be obtained. This can vary with different serotypes. For the 3M Petrifilm SALX System this has been demonstrated to be 1-5 CFU/ 25 g of sample.

The 3M Petrifilm SALX System has been validated using representative samples of the following matrices: raw ground chicken, pasteurized liquid whole egg, raw ground beef, raw ground pork, and cooked chicken nuggets, frozen uncooked shrimp, fresh bunched spinach, dry dog food, and stainless steel.

As with all test methods, the enrichment medium formulation can influence the results. The 3M Petrifilm SALX Plate has only been evaluated for use with the 3M *Salmonella* Enrichment Base, the 3M *Salmonella* Enrichment Supplement, and Rappaport-Vassiliadis R10 (R-V R10) Broth (typical formulation of R-V R10 broth follows below):

Typical Formula

Magnesium chloride (anhydrous)	13.4 grams
Sodium chloride	7.2 grams
Casein peptone	4.54 grams
Monopotassium phosphate	1.45 grams
Malachite green	0.036 grams
Demineralized water	1000.0 mL
pH 5.1 ± 0.2 @ 25°C	

Adjust pH as required to meet performance standards.
Plate presumptive positive results are confirmed by using the 3M Petrifilm Salmonella Express Disk.

Materials and Methods

Test Kit Information
Kit Name: 3M™ Petrifilm™ Salmonella Express System
Catalog Numbers:
3M Salmonella Enrichment Broth: SEB500 - 500g/bottle, SEB025 - 2.5kg/bottle
3M Salmonella Enrichment Supplement: SESUP001 - 1.0g/vial
3M Petrifilm Salmonella Express Plate:
6536 - Petrifilm Salmonella Express Plate 25 plates/pouch; 2 pouches/box
6537 - Petrifilm Salmonella Express Plate 25 plates/pouch; 8 pouches/case
3M Petrifilm Salmonella Confirmation Disk:
6538 Petrifilm Salmonella Confirmation Disk - 5 disks/pouch; 1 pouch/box
6539 - Petrifilm Salmonella Confirmation Disk - 5 disks/pouch; 5 pouches /case

Ordering Information
3M United States
3M Center
Food Safety Department
Bldg. 275-5W-05
St. Paul, MN 55144-1000
USA
1-800-328-6553

Non-US information
3M Canada
Post Office Box 5757
London, Ontario N6A 4T1
Canada
1-800-563-2921

3M Europe and MEA
3M Deutschland GmbH
Carl-Schurz - Strasse 1
D41453 Neuss/Germany
+49-2131-14-3000

3M Latin America
3M Center
Bldg. 275-5W-05
St. Paul, MN 55144-1000
USA
1-954-340-8263

3M Asia Pacific
No 1, Yishun Avenue 7
Singapore, 768923
65-64508869

3M Japan
3M Health Care Limited
33-1, Tamagawadai 2-chrome
Setagaya-ku, Tokyo
158-8583, Japan
81-570-011-321

3M Australia
Bldg A, 1 Rivett Road
North Ryde, NSW 2113
Australia
61 1300 363 878

3M Petrifilm *Salmonella* Express (SALX) Method Components – all packaged separately
1. 3M *Salmonella* Enrichment Base

2. 3M *Salmonella* Enrichment Supplement
3. 3M Petrifilm *Salmonella* Express (SALX) Plate
4. 3M Petrifilm *Salmonella* Express (SALX) Confirmation Disk

Additional Supplies and Reagents available from 3M Food Safety, St. Paul, MN

1. *3M Petrifilm Flat Spreader*
2. *3M Rappaport-Vassiliadis R10 (R-VR10) Broth*

Additional Supplies and Reagents required but not provided

1. *Sterile 10µL inoculation loop.* - for streaking enrichments onto the SALX plate
2. *Permanent ultra fine tip marker.* – for circling presumptive positive colonies on the SALX plate
3. *Sterile serological pipettes.* – capable of 1mL
4. *Pipettes*. – capable of 100µL
5. *Sterile pipette tips.* – capable of 100µL
6. *Stomacher®*. – Seward or equivalent
7. *Filter Stomacher® bags*. - Seward or equivalent
8. *Incubators*. – capable of maintaining 41.5° ± 1°C
9. *Freezer*. – capable of maintaining -10° to -20°C, for storing opened SALX pouches , hydrated SALX plates, SALX plates after incubation
10. *Refrigerator*. – capable of maintaining 2° - 8°C, for storing unopened 3M SALX plates and SALX disks
11. *Sterile distilled water*
12. Sterile reverse osmosis water
13. Sterile Butterfield's Phosphate Diluent

Safety Precautions

The 3M Petrifilm SALX System 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. For example, 3M has not documented this product for testing water, pharmaceutical, cosmetic, clinical or veterinary samples. The 3M Petrifilm SALX System has not been evaluated with all possible food products, food processes and food processing environments, testing protocols or with all possible strains of bacteria and may not detect all *Salmonella* strains. 3M has not validated the 3M Petrifilm SALX System using composite samples.

Do not use the 3M Petrifilm SALX System in the diagnosis of conditions in humans or animals.

3M Petrifilm SALX System will not specifically differentiate some lactose positive *Salmonella* sp. (primarily *S. arizonae* and *S. diarizonae*) from other lactose positive organisms. Lactose positive *Salmonella* strains will appear as non-*Salmonella* (blue colonies, green colonies, blue to green colonies, and/or black colonies with or without a yellow zone and/or associated gas bubble). It has been stated that these strains account for less than 1% of the total *Salmonella* serotypes[2].
The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices or ISO 17025[3,4].

~~After use, the enrichment medium and the 3M Molecular Detection Assay *E. coli* O157 tubes can potentially contain pathogenic materials. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Material Safety Data Sheet for additional information and local regulations for disposal.~~

SAMPLE ENRICHMENT

Foods

Table 1 presents guidance for test matrix samples. It is the user's responsibility to validate alternate sampling protocols (e.g., compositing) or dilution ratios to ensure this test method meets the user's criteria.
For Low Microbial Load Foods:

Low microbial load foods have a total aerobic colony count of ≤ 10^4 colony forming units/gram. Examples include: pasteurized, cooked, or processed foods.

1. Incubate at 41.5 ± 1.0°C for 18-24 hours (see Table 1).

For High Microbial Load Foods:

High microbial load foods have a total aerobic colony count of > 10^4 colony forming units/gram and require the use of Rappaport-Vassiliadis R10 (R-V R10) Broth. Examples include: raw, unprocessed foods.

2. Incubate at 41.5 ± 1.0°C for 18-24 hours (see Table 1).
2. After primary enrichment incubation, transfer 0.1 mL of the primary enrichment into 10.0 mL Rappaport-Vassiliadis R10 (R-V R10) Broth. Incubate at 41.5 ± 1.0°C for 8-24 hours.

Environmental samples

For sample collection, use a biocide-free cellulose sponge hydrated with Dey-Engley (D/E) Neutralizing Broth. Following user established procedures, remove any remaining D/E Neutralizing Broth residue from the sampled surface.

The recommended size of the sampling area to verify the presence or absence of the pathogen on the surface is 100 cm^2 (10 cm x 10 cm or 4 in. x 4 in.)[4]. When sampling with a sponge, cover the entire area going in two directions (left to right then up and down).

1. .
2. If the environmental sample has a high microbial load (total aerobic colony count of > 10^4 colony forming units/sample), then after primary enrichment incubation, transfer 0.1 mL of the primary enrichment into 10.0 mL Rappaport-Vassiliadis R10 (R-V R10) Broth. Incubate at 41.5 ± 1.0°C for 8-24 hours.

Table 1: Sample Enrichment Protocols

TEST MATRIX	HIGH MICROBIAL LOAD (≥10^4CFU/G)	SAMPLE SIZE	PRIMARY ENRICHMENT			SECONDARY ENRICHMENT		
			ENRICHMENT VOLUME (mL)	Temperature (±1.0°C)	Enrichment Time (hr)	ENRICHMENT MEDIUM	Temperature (±1.0°C)	Enrichment Time (hr)
Foods: Raw Meat, Poultry	✓	25 g	225	41.5	18-24	R-V R10 Broth : 0.1 mL into 10.0 mL	41.5	8-24
Foods: Seafood and Fish	✓	25 g	225			R-V R10 Broth : 0.1 mL into 10.0 mL	41.5	8-24
Foods: Pasteurized Eggs		100 g	900			Not required for low microbial load foods.		
Animal Feed		375 g	3375			Not required for low microbial load foods.		

Foods: Produce	✓	25 g	225			R-V R10 Broth : 0.1 mL into 10.0 mL	41.5	8-24
Environmental	*	1 Sponge	225			Not required for low microbial load foods.		
Other Foods	*	Follow appropriate reference method for sample size and enrichment volume						

*** SOME ENVIRONMENTAL AND OTHER FOOD SAMPLES MAY HAVE A HIGH MICROBIAL LOAD AND REQUIRE THE USE OF R-V R10 SECONDARY ENRICHMENT.**

PLATE HYDRATION

1. Use prescribed sterile diluents to hydrate the 3M Petrifilm SALX Plates: Butterfield's Phosphate Diluent, distilled water, or reverse osmosis water.

2. Place the 3M Petrifilm SALX Plate on a flat, level surface (figure A).
3. Lift the top film and with the pipette perpendicular dispense 2.0 mL ± 0.1 mL of sterile diluent onto the center of bottom film (figure B). Do not close the top film before dispensing the entire 2.0 mL volume.
4. Gently roll down the top film onto the diluent to prevent trapping air bubbles (figure C).
5. Place the 3M™ Petrifilm™ Flat Spreader (Catalog #6425) on the center of the plate. Press gently on the center of the spreader to distribute the diluent evenly. Spread the diluent over the entire 3M Petrifilm SALX Plate growth area before the gel is formed. Do not slide the spreader across the film (figure D).
6. Remove the spreader and leave the 3M Petrifilm SALX Plate undisturbed for at least 1 minute.
7. Place 3M Petrifilm SALX Plate on a flat surface for at least 1 hour at room temperature (20-25°C / <60% RH), protected from light, to allow the gel to form. Hydrated 3M Petrifilm SALX Plates can be stored at room temperature (20-25°C / <60% RH) for up to 8 hours before use if protected from light,. If hydrated 3M Petrifilm SALX Plates will not be used within 8 hours, seal them in a plastic bag,. protected from light and store at -20 to -10°C for up to 5 days.
8. After 3M Petrifilm SALX Plates are removed from storage, allow them to warm to room temperature before use.

PLATE INOCULATION

1. Remove the enrichment medium from the incubator and agitate contents by gentle hand massage.
2. Use a sterile 10 µL loop (3 mm diameter) to withdraw each sample. Use a smooth loop (one that does not have jagged edges and is not distorted) to prevent the gel surface from breaking.
3. Open the 3M Petrifilm SALX Plate and streak onto the gel (figure E). Perform a single streak to obtain isolated colonies (Figure 1).

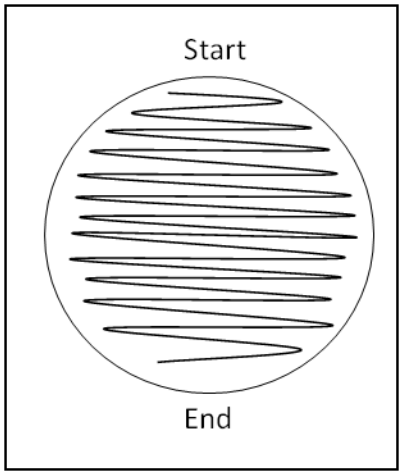


Figure 1: Streaking pattern on the 3M Petrifilm SALX Plate

4. Roll down the top film to close the 3M Petrifilm SALX Plate.
5. Using a gloved hand (while practicing good laboratory practices to avoid cross contamination and/or direct contact with the plate), gently apply a sweeping motion with even pressure onto the top film to remove any air bubbles in the inoculation area (figure F).

PLATE INCUBATION

Incubate plates at 41.5 ± 1.0°C for 24 hours ± 2 hours in a horizontal position with the colored side up in stacks of no more than 20 plates.

INTERPRETATION

1. Remove the 3M Petrifilm SALX Plates from the incubator andVisually examine the isolated colonies. See Table 2. Do not count artifact bubbles that may be present. **Note: Using indirect back lighting may enhance reading of colony color, discrete yellow zones, and gas bubbles associated with a colony**.

2. Presumptive positive *Salmonella* species are red to brown colonies with a yellow zone or associated gas bubble, or both (figure G). An associated gas bubble is defined as being located within one colony diameter distance from the colony (see Table 2 below).

Table 2: Interpretation for Presumptive Positive *Salmonella* species

Colony Color			**Colony Metabolism**		**Result**
Red	Dark Red	Brown	Yellow zone	Gas bubble	
✓			✓		Presumptive +
✓				✓	Presumptive +
✓			✓	✓	Presumptive +
	✓		✓		Presumptive +
	✓			✓	Presumptive +
	✓		✓	✓	Presumptive +
		✓	✓		Presumptive +
		✓		✓	Presumptive +
		✓	✓	✓	Presumptive +

Non-*Salmonella* species (figure H):

- Blue colonies, green colonies, blue to green colonies, and/or black colonies with or without a yellow zone and/or associated gas bubble are non-*Salmonella* organisms.
- Red, dark red, and brown colonies with no yellow zone and no associated gas are non-*Salmonella* organisms.
- Red, dark red, and brown colonies with a magenta zone are non-*Salmonella* organisms.

If presumptive positive *Salmonella* colonies are not present, then *report the results as Salmonella* not detected in the matrix.

If presumptive positive *Salmonella* colonies are present, then continue with the following Biochemical Confirmation steps:

a. On the 3M Petrifilm SALX Plate top film, **circle up to five isolated presumptive positive *Salmonella* colonies using a permanent, ultra fine tip marker** (figure I).

b. Biochemically confirm all *Salmonella* presumptive positive results using the 3M Petrifilm SALX Confirmation Disk. See the Biochemical Confirmation section.
Note: If the 3M Petrifilm SALX Plates cannot be analyzed within 1 hour of removal from the incubator**, first circle the presumptive *Salmonella* colonies on the top film by using a permanent, ultra fine tip marker** and then place the plates in a sealed plastic bag for later analysis. Protect 3M Petrifilm SALX Plates from light and store at -20 to -10°C for no longer than 72 hours. Allow plates to warm to room temperature (20-25°C / <60% RH) before adding the disk to the plate.

⚠ **WARNING:** To reduce the risks associated with a false-negative result leading to the release of contaminated product and the possibility of false positive results requiring a retest, always use a permanent, ultra fine tip marker to circle the characteristic presumptive *Salmonella* colonies on the top film of the 3M Petrifilm SALX Plate before appropriate storage of the plate and/or before placing the 3M Petrifilm SALX Confirmation Disk onto the gel.

BIOCHEMICAL CONFIRMATION

NOTE: Use good laboratory practices to avoid cross contamination and/or direct contact with the 3M Petrifilm SALX Plate and/or 3M Petrifilm SALX Confirmation Disk.

1. Remove an individually packaged 3M Petrifilm SALX Confirmation Disk from its pouch and allow it to come to room temperature (20-25°C / <60% RH). Then remove the 3M Petrifilm SALX Confirmation Disk from its individual package by peeling the package to expose the 3M Petrifilm SALX Confirmation Disk's tab, grasping the tab with a gloved hand, and removing the 3M Petrifilm SALX Confirmation Disk.
2. Lift the top film (with the already circled presumptive *Salmonella* colonies) of the 3M Petrifilm SALX Plate and insert the 3M Petrifilm SALX Confirmation Disk by rolling it onto the gel to avoid entrapping air bubbles (figure J). Close the 3M Petrifilm SALX Plate.
3. Using a gloved hand, gently apply a sweeping motion with even pressure onto the top film to remove any air bubbles in the inoculation area and assure good contact between the gel and the 3M Petrifilm SALX Confirmation Disk (figure K).
4. Incubate the 3M Petrifilm SALX System (plate and disk) at 41.5 ± 1.0°C for 4 - 5 hours in a horizontal position, colored side up, no higher than 20 plates.
5. Remove the 3M Petrifilm SALX System from the incubator and proceed with reading the results. **Only interpret the results for the circled presumptive *Salmonella* colonies. :**

✓			
	✓		
		✓	

- Biochemically confirmed positive results:
  Colonies that are green to blue, blue to dark blue, or black or have a blue precipitate around them are biochemically confirmed positive for *Salmonella* species.

- Biochemically confirmed negative results:
  Colonies that remain the same red, dark red or brown color without a blue precipitate are negative for *Salmonella* species.

6. Colonies may be subcultured for further identification. When subculturing, wear appropriate protective apparel and follow standard good laboratory safety practices (GLP)[2].

a. With a gloved hand, lift the top film and aseptically remove the colony either from the gel or the 3M Petrifilm SALX Confirmation Disk. If a 3M Petrifilm SALX Confirmation Disk is covering the gel, aseptically peel the disk away and then aseptically remove the colony from the gel.

b. Streak the colony onto/into media per appropriate reference method [5,6,7].
   NOTE: If the colonies cannot be subcultured within 1 hour of plate removal from the incubator, then store the 3M Petrifilm SALX Plates for later analysis by placing in a sealed plastic bag at -20 to -10°C for no longer than 72 hours in the dark. Allow 3M Petrifilm SALX Plates to warm to room temperature (20-25°C / <60% RH) before continuing subculturing for identification.

7. After the test is complete, dispose of the 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disks in accordance with current industry standards and/or local regulations.

Validation Study

This study was conducted according to AOAC guidelines outlined in the AOAC General Referee approved harmonized PTM/OMA validation protocol, "Validation Outline for 3M™ Petrifilm™ *Salmonella* Express System."

Inclusivity Testing Methodology

Inclusivity testing was conducted to ensure that the 3M Petrifilm SALX System can detect many different serovars of *Salmonella*. One hundred four (104) pure cultures (Table 2 in Appendix) were

used to confirm the inclusivity of the test, post complete low and high microbial load enrichment of the organisms. Organisms were cultured overnight in 3M Salmonella Enrichment Broth with 3M Salmonella Enrichment Supplement at 41.5 ± 1°C for 18 hours then diluted via ten-fold serial dilutions in the enrichment medium and supplement to a level of less than 10^5 CFU/mL (Colony Forming Unit/mL) prior to evaluation following the 3M Petrifilm SALX System.

Exclusivity Testing Methodology

Exclusivity testing was conducted to ensure that the 3M Petrifilm SALX System is specific for the detection of the target organisms. Thirty-two (32) different non- *Salmonella* pure cultures (Table 3 in Appendix) were used to confirm the exclusivity of the test. Organisms were cultured overnight in Brain Heart Infusion Broth (BHI) at 37° ± 1 °C for 18 hours then evaluated following the 3M SALX System.

Inclusivity/Exclusivity Results

All 104 cultures tested in the inclusivity study were correctly detected and confirmed as *Salmonella* and all of the 32 non- *Salmonella* cultures tested in the exclusivity study were negative for *Salmonella* by the 3M Petrifilm SALX System.

Ruggedness Testing Methodology

This study evaluated the ability of the 3M Petrifilm SALX System to remain unaffected by variations in method parameters that might be expected to occur when the method is performed by an end user. The effects of perturbations in six method parameters were investigated:

1. Primary enrichment incubation time: Following the Sample Enrichment instructions, organism enrichment was carried out for 16, 18, 24, and 26 hours.

2. Incubation time of the 3M Petrifilm SALX Plate: Following the Plate Incubation instructions, the inoculated the 3M Petrifilm SALX Plates were incubated for 20, 22, 24, 26 and 28 hours.

3. Incubation temperature of the 3M SALX Plates: Following the Plate Incubation instructions, inoculated 3M Petrifilm SALX Plates were incubated at the following temperatures: 40, 41.5, and 43°C.

4. Incubation time of the 3M Petrifilm SALX Confirmation Disk: Following the instructions for Biochemical Confirmation, the 3M Petrifilm SALX Confirmation Disk was added to inoculated 3M Petrifilm SALX Plates for 2, 3, 4, 5, and 6 hours.

5. Incubation temperature of the 3M SALX Confirmation Disk: Following the instructions for Biochemical Confirmation, the 3M Petrifilm SALX Confirmation Disk was added to inoculated 3M Petrifilm SALX Plates and incubated at 40, 41.5 and 43°C.

6. Various plate hydration options: Following the Plate Hydration instructions, 3M Petrifilm SALX Plates were hydrated with sterile reverse osmosis water (RO), Butterfield Phosphate Diluent, and sterile distilled water before inoculation.

Two serovars of *Salmonella* (S. Typhimurium ATCC 14028 and S. Enteritidis ATCC 13076) were analyzed within one log of the limit of detection of the kit. Each serovar was cultured in 3M Salmonella Enrichment Broth with 3M Salmonella Enrichment Supplement using both the low and high microbial load protocols and then diluted in the same broth to within one log limit of detection of the kit. One non-Salmonella organism (*Citrobacter braakii* ATCC 43162) was analyzed at the overnight growth level achieved in a nonselective broth (BHI). Ten (10) replicates of each organism were analyzed using the 3M Petrifilm SALX System for each ruggedness parameter with the exception of the hydration fluid. For this parameter, thirty (30) replicates of each organism were tested.

Ruggedness Testing Results

Results for the 3M Petrifilm SALX System ruggedness study are summarized in Tables 4-7 in the Appendix. Both *Salmonella* strains were positive for all replicates and method variations. The *Citrobacter braakii* strain was negative for all replicates and method variations.

Shelf Life and Lot-to-Lot Consistency Methodology

For the shelf life study, one lot of plates and disks was evaluated. Two more lots are being produced. Results from the two new lots will be submitted with the yearly PTM renewal. The target shelf-life for the 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disks is 18 months.

Two serovars of *Salmonella* (*Salmonella* Typhimurium ATCC 14028 and *Salmonella* Poona NCTC 4840) and one non-*Salmonella* strain (*Citrobacter braakii* ATCC 13048) were analyzed. Each organism was cultured in 3M Salmonella Enrichment with 3M Salmonella Enrichment Supplement and then diluted in the same broth to within one log limit of detection of the method. For each organism, five replicates of were tested (10 positive and 5 negative) using the 3M Petrifilm Salmonella Express System Method.

Shelf Life and Lot-to-Lot Consistency Results

Results presented are for shelf life of the one lot of 3M Petrifilm SALX plates and disks. Results are summarized in Table 8 in the Appendix. Both *Salmonella* serovars were positive for all replicates tested. The *Citrobacter braakii* strain was negative for all replicates.

3M Petrifilm Salmonella Express System Method-Reference Method Comparison The 3M Petrifilm Salmonella Express System was compared to the FDA/BAM Chapter 5 *Salmonella* reference method (3) for shrimp, spinach, dry dog food, and stainless steel and to the USDA/FSIS MLG 4.05 Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg and Catfish Products reference method (4) for raw ground chicken, raw ground beef, raw ground pork, pasteurized liquid whole eggs, and cooked chicken nuggets.

Sample Preparation

The *Salmonella* cultures were prepared to deliver a target level of 0.2 - 2 CFU / test portion. Each matrix was inoculated with a different strain of *Salmonella* species. A diluted 24 hour broth culture inoculum was added to a bulk sample of each food matrix and mixed thoroughly. The cooked chicken nuggets and pasteurized liquid whole eggs were inoculated with an organism that had been heat stressed for 10 minutes at 50°C in a water bath. The degree of injury of the culture was estimated by plating an aliquot of diluted culture onto Xylose Lysine Desoxycholate (XLD) and Tryptic Soy agar (TSA). The agars were incubated at 37° ± 1°C for 24 ± 2 hours and the colonies were counted. The degree of injury was estimated as $(1-\frac{n_{select}}{n_{nonselect}})x100$, where n_{select} = number of colonies on selective agar and $n_{nonselect}$ = number of colonies on nonselective agar. The matrix was well mixed and stored at 4°C for 48-72 hr to allow the inoculum to equilibrate with the food environment and to cold stress the cells. The inoculated bulk samples were then used to generate all inoculated test portions. Ground beef test samples of 325g and 375g were made with 25g of inoculated bulk and 300 grams or 350 grams, respectively, of uninoculated bulk (3M and MLG samples). Inoculated and uninoculated portions were well mixed to ensure homogeneity. At the time of enrichment, the inoculated products were randomly split into test groups.

For the analysis of the dry dog food test portions, a bulk lot of test product was inoculated with a lyophilized culture and held at 25° ± 2°C for two weeks to allow time for the organism to equilibrate. Test portions analyzed by the 3M Petrifilm SALX System were prepared by mixing 25 g of inoculated test product with 350g of un-inoculated test product. Twenty-five grams of inoculated test product were analyzed by the FDA/BAM method. For the analysis of the cooked chicken nuggets, 25 g of inoculated test product was mixed with 300 g of un-inoculated test product and analyzed by both the 3M Petrifilm SALX System and USDA reference method.

Environmental surfaces were inoculated at a level expected to yield fractional recovery. Replicate 4"x 4" stainless steel surfaces areas were inoculated with 0.10 mL of diluted culture and dried for 16-24 hours at room temperature (24° ± 2°C). The inoculation level for the stainless steel test portions was determined by plating the inoculum onto Tryptic Soy agar (TSA) in triplicate. Sampling sponges, pre-moistened with Dey-Engley neutralizing broth, were used to sample each test area using horizontal and vertical motions. The sponges were held at room temperature for at least 2 hours prior to analysis.

A background screen of each food matrix was conducted prior to inoculation and no natural contamination of the target organisms was detected in the test matrices.

Most Probable Number (MPN) Determination - The level of *Salmonella* was determined by Most Probable Number (MPN) on the day of analysis by analyzing 3 x 100 g, 20 x 25 g (reference method test portions) and 3 x 5.0 g inoculated test samples for the raw ground chicken, raw ground beef, raw ground pork, frozen uncooked shrimp and fresh spinach. For the cooked chicken nuggets and dry dog food, 5 x 75 g, 5 x 25 g (from the bulk lot of inoculated test product) and 5 x 10.0 g test portions were analyzed. For the pasteurized liquid whole eggs, 5 x 200 g, the 20 reference method test portions and 5 x 25 g test portions were analyzed. Each test portion was enriched with the appropriate reference method enrichment broth at a 1:10 dilution and analyzed by the reference method procedure. The number of positives from the 3 test levels was used to calculate the MPN using the AOAC MPN calculator. (http://www.lcfltd.com/customer/LCFMPNCalculator.exe)

Anaytical Protocols

USDA/FSIS-MLG 4.05 Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg and Catfish.

For the USDA/FSIS method, all test portions for each food product, raw ground chicken, pasteurized liquid whole eggs, raw ground beef, raw ground pork and cooked chicken nuggets were prepared as outlined in the study protocol. Following equilibration of the microorganism in the matrix, 25 test portions consisting of 25 g for raw ground chicken, raw ground beef and raw ground pork were enriched with 225 mL of Buffered Peptone Water (BPW) and homogenized for 2 minutes. For cooked chicken nuggets, 25 test portions consisting of 325 g were enriched with 1500 mL of BPW and homogenized for 2 minutes. After mixing, an additional 1425 mL of BPW was added to the sample. For pasteurized liquid whole eggs, 25 test portions of 100 g were enriched with 900 mL of BPW and homogenized for 2 minutes. All test portions were incubated at 35° ± 2°C for 20-24 hours.

After incubation, 0.1 ml of primary enrichment for each sample was transferred to 10 ml of modified Rappaport-Vassiliadis broth (mRV) and 0.5 ml to 10 ml of Tetrathionate Hanja (TTH) broth. The mRV and TTH tubes were incubated at 42° ± 0.5°C for 22-24 hours. Following incubation, a loopful of each secondary enrichment was streaked to Brilliant Green Sulfa (BGS) agar and Xylose Lysine Tergitol (XLT4) agar and incubated at 35° ± 2°C for 18-24 hours. If no visible colonies were present, both the BGS and XLT4 plates were re-incubated for an additional 18-24 hours at 35° ± 2°C. Up to 3 suspect colonies from each selective agar were transferred to Triple Sugar Iron agar (TSI) and Lysine Iron agar (LIA) and incubated at 35° ± 2°C for 22-26 hours. Growth from samples producing typical biochemical reactions in TSI and LIA, were streaked to TSA slants and incubated for 18-24 hours at 35° ± 2°C. Growth from the TSA slant was used to conduct the flagellar H serological test and polyvalent somatic O serological test and for biochemical confirmation. Growth from the TSA slants was used for final confirmation of *Salmonella* by VITEK® 2 GN following AOAC Official Method 2011.17.

FDA-BAM Chapter 5 Salmonella

For the FDA/BAM method, all test portions for each food product, frozen uncooked shrimp, fresh spinach, dry dog food and stainless steel environmental surfaces were prepared as outlined in the study protocol. Following equilibration of the microorganism in the matrix, 25 test portions consisting of 25 g for each food matrix were enriched with 225 mL of Lactose broth and homogenized for 2 minutes. For sponges used to analyze the stainless steel environmental surfaces, each sponge was enriched with 225 mL of Lactose broth and mixed by hand massaging. All test portions were allowed to stand for 60 minutes to adjust pH to 6.8 ± 0.2 if necessary. Test portions for each matrix were incubated for 22-26 hours at 35° ± 2°C.

After incubation of the samples, 0.1 ml aliquot of primary enrichment for each sample was transferred to 10 mL of Rappaport-Vassiliadis medium (RV) and 1.0 ml to 10 mL of Tetrathionate (TT) broth. RV tubes were incubated for 22-26 hours at 42° ± 2°C. For high microbial load foods (fresh spinach and frozen uncooked shrimp), TT tubes were incubated for 22-26 hours at 43° ± 0.2°C in a circulating water bath. For low microbial load foods (dry dog food and stainless steel environmental surfaces), TT tubes were incubated 22-26 hours at 35° ± 2°C. Following incubation, a loopful of each secondary enrichment was streaked to Bismuth Sulfite (BS) agar, Hektoen Enteric (HE) agar, and XLD agar and incubated at 35° ± 2°C for 22-26 hours. If no visible colonies were present after 24 hours of incubation, BS plates were re-incubated for an additional 22-26 hours at 35° ± 2°C. Up to 2 or more suspect colonies from each selective agar were transferred to TSI and LIA and incubated at 35° ± 2°C for 22-26 hours. Growth from samples producing typical biochemical reactions in TSI and LIA, were streaked to TSA slants and incubated for 18-24 hours at 35° ± 2°C. Growth from the TSA slant was used to conduct the flagellar H serological test and polyvalent and individual somatic O serological test and for biochemical confirmation. Growth from the TSA slants was used for final confirmation of *Salmonella* by VITEK® 2 GN following AOAC Official Method 2011.17.

3M Petrifilm Salmonella Express System

After equilibration of the inoculum, 25 test portions for each matrix were enriched using pre-warmed (41.5° ± 1°C) 3M *Salmonella* Enrichment Base containing 3M *Salmonella* Enrichment Supplement. For the analysis of raw ground beef, raw ground pork, frozen uncooked shrimp, and fresh spinach, 25 g test portions were enriched in 225 mL of 3M *Salmonella* Enrichment Base. For the analysis of cooked chicken nuggets, 325 g test portions were enriched with 2925 mL of 3M *Salmonella* Enrichment Base. For the analysis of pasteurized liquid whole eggs, 100 g test portions were enriched with 900 mL of 3M *Salmonella* Enrichment Base. For the analysis of dry dog food, 375 g samples were enriched with 3375 mL of 3M *Salmonella* Enrichment Base. Sponges used to sample stainless steel environmental surfaces were

enriched with 225 mL of 3M *Salmonella* Enrichment Base. All matrices were homogenized for 2 minutes and incubated at 41.5° ± 1°C for 18-24 hours.

Prior to analysis, the 3M Petrifilm SALX Plates were hydrated using 2.0 mL of sterile distilled water. After hydration, the liquid was spread across of the surface of the plate using a plastic spreader to evenly distribute the diluent. The plates were left undisturbed and protected from light for a minimum of 1 hour prior to use. For foods with low microbial loads (pasteurized liquid whole egg, dry dog food, cooked chicken nuggets and stainless steel environmental surfaces), test portions were streaked in duplicate after 18 and 24 hours of primary enrichment. For foods that contained a high microbial load (raw ground chicken, frozen uncooked shrimp, fresh spinach, raw ground beef and raw ground pork), a 0.1 mL aliquot of the primary enrichment was transferred into 9.9 mL of Rappaport-Vassiliadis R10 (RV [R10]) broth at both 18 and 24 hours of primary enrichment and incubated at 41.5° ± 1°C for 8 and 24 hours. At 8 and 24 hours, a loopful of each test portion was streaked in duplicate to 3M Petrifilm SALX Plates and the plates were incubated at 41.5° ± 1°C for 24 ± 2 hours. Test portions from both low and high microbial foods were confirmed following two procedures, traditional confirmation using secondary selective enrichments and an alternative confirmation directly from the 3M Petrifilm SALX Plates.

Confirmation

Reference Methods

Aliquots from the primary enrichment of each test portion were also transferred to the secondary selective enrichments specified by each reference method, TT and RV for samples confirmed following the FDA/BAM method and TTH and RVS for samples confirmed following the USDA/FSIS-MLG method. After incubation, test portions from the secondary selective enrichments were streaked to selective agars specified by each reference method and positive samples were confirmed following traditional biochemical tests for *Salmonella* species by each reference method including somatic O and flagellar H tests. Final confirmation at each time point was achieved by VITEK 2 GN conducted per AOAC Official Method 2011.17.

3M Petrifilm Salmonella Express System

After incubation of the 3M Petrifilm SALX Plates, the plates were observed for growth of presumptive *Salmonella* colonies, red to brown colonies with discrete yellow zones and/or gas bubbles. Using a fine tip marker, presumptive positive colonies were circled on the plate top film. The film was lifted and a 3M Petrifilm *Salmonella* Express Confirmation Disk was placed onto the gel. The film was closed and using a gloved hand (while practicing good laboratory technique) air bubbles were removed by gently applying a sweeping motion with even pressure onto the top of the film with the analyst's fingers. The plates with confirmation disks were incubated at 41.5° ± 1°C for 4 to 5 hours. After incubation, the circled presumptive colonies were observed. A color change from red/brown to green blue, blue, dark blue or black confirmed the colony as *Salmonella* species. If the circled colony remained red or brown it was marked as negative. Typical colonies were picked to TSI and LIA and samples were confirmed following traditional biochemical tests for *Salmonella* species by each reference method including somatic O and flagellar H tests. Final confirmation was achieved by VITEK 2 GN conducted per AOAC Official Method 2011.17.

Statistical Analysis

For the method comparison, results obtained from samples analyzed by the 3M Petrifilm SALX System were comparable to those analyzed by the FDA/BAM Chapter 5, USDA/FSIS-MLG 4.05 and ISO 6579 reference methods. A Mantel-Haenszel chi-square analysis (for unmatched test portions) was used to compare the 3MPetrifilm SALX System samples and the reference method samples.

For the low inoculum level and the un-inoculated control level, 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, POD_{CC}, the difference in the candidate presumptive and confirmatory results, $dPOD_{CP,}$ the reference method, POD_R, and the difference in the confirmed candidate and reference methods, $dPOD_C$. POD analyses were conducted for each time point and of the 3M Petrifilm SALX System and the reference methods.

Based on the results obtained from the MPN analyses, the target levels of 0.2-2 CFU/ test portion for the low inoculum level were achieved for each matrix tested. In addition, the requirement of obtaining fractionally positive results (5-15 positives out of 20 replicates) was achieved by either the candidate or reference methods or both for each of the matrices analyzed in this study.

RESULTS AND DISCUSSION

High Microbial Foods

Raw Ground Chicken

For raw ground chicken test portions, there were 15 confirmed positives by the 3M Petrifilm SALX System at all four time points (18 hours and 24 hours of primary enrichment transferred into RV[R10] secondary enrichment and streaked at 8 and 24 hours) and 14 confirmed positives by the USDA/FSIS-MLG method. All un-inoculated control samples were negative by both methods. A χ^2 value of 0.12 was obtained between the reference methods and the candidate method when using the alternative confirmation indicating no significant difference between the candidate and reference method at all four time points. A χ^2 value of 0.0 was obtained between the reference method and the candidate method when using the traditional confirmation, indicating no significant difference between the candidate and reference method at any of the time points.

For the low level inoculum, a $dPOD_C$ value of 0.05 with 95% confidence intervals of (-0.22, 0.31) was obtained between the USDA/FSIS-MLG reference method and the candidate method samples confirmed using alternative confirmation at all four time points. A $dPOD_C$ value of 0.00 with 95% confidence intervals of (-0.26, 0.26) was obtained between the USDA/FSIS-MLG reference method and the candidate method samples confirmed using traditional confirmation at all four time points. The confidence intervals obtained for the $dPOD_C$ values indicated no significant difference between the two methods using either the traditional or alternative confirmatory procedure. A $dPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.26, 0.26) were obtained between presumptive and confirmed results using either the traditional or alternative confirmatory procedure at all four time points. The confidence intervals obtained for $dPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results.

Frozen Uncooked Shrimp

For frozen uncooked shrimp test portions, there were 12 confirmed positives by the 3M Petrifilm SALX System at all four time points and 10 confirmed positives by the FDA/BAM method. All un-inoculated control samples were negative for both methods. A χ^2 value of 0.39 was obtained between the reference

and candidate method using both the traditional and alternative confirmation procedures, indicating no significant difference between the candidate and reference methods at any of the time points.

For the low level inoculum, a $dPOD_C$ value of 0.10 with 95% confidence intervals of (-0.19, 0.37) was obtained between the reference and candidate method samples confirmed using either the traditional or alternative confirmation procedure at all four time points. The confidence intervals obtained for $dPOD_C$ indicated no significant difference between the two methods. A $dPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.28, 0.28) was obtained between presumptive and confirmed results using either the traditional or alternative confirmation procedure for all four time points. The confidence intervals obtained for $dPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results at all four time points.

Fresh Spinach

For fresh spinach test portions, there were 11 confirmed positives the 3M Petrifilm SALX System for both 18 and 24 hour primary enrichment samples transferred to RV-R10 and incubated for 24 hours and confirmed following both the traditional and alternative confirmation procedures. For test portions enriched for 18 and 24 hours and transferred to RV-R10 and incubated for 8 hours there were 7 confirmed positives by the alternative confirmation and 11 confirmed positives by the traditional confirmation indicating the presence of 4 false negative results. There were 10 confirmed positives for samples analyzed by the FDA/BAM method. All un-inoculated control samples were negative by both methods. A χ^2 value of 0.10 was obtained between the reference method and the candidate method using the traditional confirmation for all four time points. For candidate samples confirmed following the alternative confirmation procedure, a χ^2 value of 0.10 was obtained for samples enriched for either 18 hours or 24 hours in the primary enrichment, transferred to RV-R10 and incubated for 24 hours. For candidate samples confirmed following the alternative confirmation procedure, a χ^2 value of 0.90 was obtained for samples enriched for either 18 hours or 24 hours in the primary enrichment, transferred to RV-R10 and incubated for 8 hours. The chi-square values indicate no significant difference between the candidate and reference method at any of the time points. Although there was no statistical significant difference between the number of positive test portions at 8 or 24 hours in RV-R10 (7 positive at 8 hours and 11 positive at 24 hours), it is recommended that only 24 hours of secondary enrichment in RV-R10 be used for fresh spinach. Secondary enrichment for 24 hours in RV-R10 significantly reduced the normal background microbial load, allowing the target organism to reach a higher titer and thus making it easier to identify presumptive positive colonies on the 3M Petrifilm SALX Plate.

For the low level inoculum, a $dPOD_C$ value of 0. 05 with 95% confidence intervals of (-0.24, 0.33) was obtained between the reference and candidate method samples confirmed using either the traditional or alternative confirmation procedures at all four time points. The confidence intervals obtained for dPODc indicate no significant difference between the two methods. A $dPOD_{CP}$ values of 0.00 with 95% confidence intervals of (-0.28, 0.28) was obtained between the presumptive and confirmed results using the alternative confirmation for all four time points and for test portions incubated in the primary enrichment for either 18 or 24 hours and then incubated for a full 24 hours in RV-R10 and confirmed using the traditional method. A $dPOD_{CP}$ value of -0.20 with 95% confidence intervals of (-0.28, 0.28) were obtained between the presumptive and confirmed results for test portions incubated in the primary enrichment for either 18 or 24 hours and then incubated for 8 hours in RV-R10. The confidence intervals obtained for all $dPOD_{CP}$ values indicated no significant difference between the presumptive and confirmed results at all four time points.

Raw Ground Beef

For raw ground beef test portions, there were 13 confirmed positives the 3M Petrifilm SALX method at all four time points and 13 confirmed positives for the USDA/FSIS-MLG method. All un-inoculated control samples were negative by both methods. A χ^2 value of 0.0 was obtained between the reference and candidate method using both the traditional and alternative confirmation procedures, indicating no significant difference between the candidate and reference methods at any of the four time points.

For the low level inoculum, a $dPOD_C$ value of 0.00 with 95% confidence intervals of (-0.28, 0.28) was obtained between the reference and candidate method samples confirmed using either the traditional or alternative confirmation procedures at all four time points. The confidence intervals obtained for $dPOD_C$ indicated no significant difference between the two methods. A $dPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.28, 0.28) was obtained between presumptive and confirmed results using either confirmation procedure for all four time points. The confidence intervals obtained for $dPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results at all four time points.

Raw Ground Pork

For raw ground pork test portions, there were 9 confirmed positives by the 3M Petrifilm SALX System at all four time points and 9 confirmed positives by the USDA/FSIS-MLG method. All un-inoculated control samples were negative by both methods. A χ^2 value of 0.0 was obtained between the reference and candidate method samples using both the traditional and alternative confirmation procedures, indicating no significant difference between the candidate and reference methods at any of the four time points.

For the low level inoculum, a $dPOD_C$ value of 0.00 with 95% confidence intervals of (-0.28, 0.28) was obtained between the reference method and candidate method samples confirmed using either the traditional or alternative confirmation procedure at all four time points. The confidence intervals obtained for $dPOD_C$ indicated no significant difference between the two methods. A $dPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.28, 0.28) was obtained between presumptive and confirmed results using either the traditional or alternative confirmation procedure for all four time points. The confidence intervals obtained for $dPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results at all four time points.

<u>Low Microbial Foods</u>

Pasteurized Liquid Whole Eggs

For pasteurized liquid whole egg test portions, there were 6 confirmed positives by the 3M Petrifilm SALX method at both time points and 4 confirmed positives by the USDA/FSIS-MLG method. All un-inoculated control samples were negative for both methods. A χ^2 value of 0.98 was obtained between the USDA/FSIS-MLG and candidate method using both traditional and alternative confirmation procedures, indicating no significant difference between the candidate and reference method at any of the time points.

For the low level inoculum, a $dPOD_C$ value of 0.10 with 95% confidence intervals of (-0.17, 0.35) was obtained between the USDA/FSIS-MLG method and the candidate method samples confirmed using either the traditional or alternative confirmation procedure at both the 18 hour and 24 hour primary enrichment time points. A $dPOD_C$ value of 0.05 with 95% confidence intervals of (-0.22, 0.31) was obtained between the ISO 6579 method and the candidate method samples confirmed using either the traditional or alternative confirmation procedure at both the 18 hour and 24 hour primary enrichment time points. The confidence

intervals obtained for the $dPOD_C$ values indicated no significant difference between the candidate and reference method. A $dPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.27, 0.27) were obtained between presumptive and confirmed results using either the traditional or alternative confirmatory procedure at both the 18 hour and 24 hour primary enrichment time points. The confidence intervals obtained for $dPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results.

Dry Dog Food

For dry dog food test portions, there were 16 confirmed positives by the 3M Petrifilm SALX System at both the 18 hour and 24 hour primary enrichment time points and 14 confirmed positives the FDA/BAM method. All un-inoculated control samples were negative for both methods. A χ^2 value of 0.52 was obtained between the reference and candidate method using both the alternative and traditional confirmation procedures, indicating no significant difference between the candidate and reference methods at either of the time points.

For the low level inoculum, a $dPOD_C$ value of 0.10 with 95% confidence intervals of (-0.17, 0.35) was obtained between the reference method and candidate method samples confirmed following either the traditional or alternative confirmation procedure at both the 18 hour and 24 hour primary enrichment time points. The confidence intervals obtained for $dPOD_C$ indicated no significant difference between the two methods. A $dPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.25, 0.25) was obtained between presumptive and confirmed results using either the traditional or alternative confirmation procedure for both the 18 hour and 24 hour primary enrichment time points. The confidence intervals obtained for $dPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results.

Stainless Steel Environmental Surface

For stainless steel environmental surfaces, there were 6 confirmed positives for the 3M Petrifilm SALX System at both the 18 hour and 24 hour primary enrichment time points and 7 confirmed positives for the FDA/BAM method. All un-inoculated control samples were negative for both methods. A χ^2 value of 0.11 was obtained between the reference and candidate method using both the traditional and alternative confirmation procedures, indicating no significant difference between the candidate and reference methods at any of the time points.

For the low level inoculum, a $dPOD_C$ value of -0.05 with 95% confidence intervals of (-0.32, 0.23) was obtained between the reference method and candidate method samples confirmed using either the traditional or alternative confirmation at both 18 hour and 24 hour primary enrichment time points. The confidence intervals obtained for $dPOD_C$ indicated no significant difference between the two methods. A $dPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.27, 0.27) was obtained between presumptive and confirmed results using either the traditional or alternative confirmation procedure for both the 18 hour and 24 hour primary enrichment time points. The confidence intervals obtained for $dPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results.

Cooked Chicken Nuggets

For cooked chicken nugget test portions, there were 6 confirmed positives by the 3M Petrifilm SALX method at both the 18 hour and 24 hour primary enrichment time points and 6 confirmed positives the USDA/FSIS-MLG method. All un-inoculated control samples were negative for both methods. A χ^2 value of 0.0 was obtained between the reference and candidate method using both the traditional and alternative confirmation procedures, indicating no significant difference between the candidate and reference methods at any of the time points.

For the low level inoculum, a $dPOD_C$ value of 0.00 with 95% confidence intervals of (-0.27, 0.27) was obtained between the reference and candidate method samples confirmed following both the traditional and alternative confirmation procedures at both the 18 hour and 24 hour primary enrichment time points. The confidence intervals obtained for $dPOD_C$ indicated no significant difference between the two methods. A $dPOD_{CP}$ value of 0.00 with 95% confidence intervals of (-0.27, 0.27) was obtained between presumptive and confirmed results using either the traditional and alternative confirmation procedures for both the 18 and 24 hour primary enrichment time points. The confidence intervals obtained for $dPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results.

INDEPENDENT LABORATORY STUDY

The independent laboratory study was conducted at Aegis Food Testing Laboratories in North Sioux City, SD. The study was conducted according to the methods described above for raw ground chicken and pasteurized liquid whole eggs.

Raw Ground Chicken

All uninoculated control samples of ground chicken were confirmed negative for *Salmonella*. The samples were inoculated *S. enterica* subsp. Enteritidis (NCTC 5717), with an estimated 0.031 cells/g as determined by MPN. The 3M Petrifilm SALXSystem detected 13 suspect colonies of which12 samples were presumptive when the confirmation disk was added. All 12 of the presumptive samples confirmed as positive by the USDA/FSIS MLG 4.05 reference method. There were 11 ground chicken samples that were determined to be positive by the reference method. When comparing the number of samples determined to be positive by the 3M Petrifilm SALX System to that of the reference method, there was no statistical difference in the results obtained ($\chi^2<3.8$, $p<0.05$). The number of samples that were presumptive positive from the 3M Petrifilm SALX System was compared to the number confirmed as positive using probability of detection (POD) and difference in probability (dPODcp). The 95% confidence interval for the dPOD for the 3M Petrifilm SALX System presumptive positives compared to the confirmed positive contains zero. Therefore, there was no significant difference in the results at the 5% level. The number of samples that were confirmed positive from the 3M Petrifilm SALX System was compared to the number confirmed as positive by the reference method using probability of detection (POD) and difference in probability (dPODc). The 95% confidence interval for the dPOD for the 3M Petrifilm SALX System confirmed positives compared to the reference method confirmed positive contains zero. Therefore, there was no significant difference in the results at the 5% level.

Pasteurized Liquid Whole Eggs

All uninoculated control samples of pasteurized liquid eggs were confirmed negative for *Salmonella*. The samples were inoculated with an estimated 0.008 cells/g of *S. enterica* subsp. Enteritidis (ATCC 13076, heat stressed) as determined by MPN. The 3M Salmonella Petrifilm System detected 7 suspect colonies of which 7 samples were presumptive when the confirmation disk was added. All 7 of the presumptive samples confirmed as positive by the USDA/FSIS MLG 4.05 reference method. There were 8 pasteurized liquid eggs samples that were determined to be positive by the reference method. When comparing the number of samples determined to be positive by the 3M Petrifilm SALX System to that of the reference method, there was no statistical difference in the results obtained ($\chi^2<3.8$, $p<0.05$). The number of samples that were presumptive positive from the 3M Petrifilm SALX System was compared to the number confirmed as positive using probability of detection (POD) and difference in probability (dPODcp). The 95% confidence interval for the dPOD for the 3M Petrifilm SALX System

presumptive positives compared to the confirmed positive contains zero. Therefore, there was no significant difference in the results at the 5% level. The number of samples that were confirmed positive from the 3M Petrifilm SALX System was compared to the number confirmed as positive by the reference method using probability of detection (POD) and difference in probability (dPODc). The 95% confidence interval for the dPOD for the 3M Petriflm SALX System confirmed positives compared to the reference method confirmed positive contains zero. Therefore, there was no significant difference in the results at the 5% level.

Stainless Steel

All uninoculated control samples of swabs of stainless steel were confirmed negative for *Salmonella*. The samples were inoculated with an estimated 841 cells/coupon of *Salmonella enterica* spp. Kahla (ATCC 17980)based on the plating of the inoculum. The 3M Petrifilm SALX System detected 6 presumptive colonies, all of which confirmed positive after incubation with the 3M Petrifilm SALX Confirmation Disk. All 6 of the 3M Petrifilm SALX System confirmed samples confirmed positive with the MLG 4.05 method. There were 7 sponge samples from stainless steel that were determined to be positive by the FDA BAM Chapter 10 method. When comparing the number of samples determined to be positive by the 3M method to that of the reference method, there was no statistical difference in the results obtained (χ^2<3.8, p<0.05). The number of samples that were presumptive positive from the 3M Petrifilm SALX System was compared to the number confirmed as positive using probability of detection (POD) and difference in probability (dPODcp). The 95% confidence interval for the dPOD for the 3M Petrifilm SALX System presumptive positives compared to the confirmed positive contains zero. Therefore, there was no significant difference in the results at the 5% level. The number of samples that were confirmed positive from the 3M Petrifilm SALX System was compared to the number confirmed as positive by the reference method using probability of detection (POD) and difference in probability (dPODc). The 95% confidence interval for the dPOD for the candidate method confirmed positives compared to the reference method confirmed positive contains zero. Therefore, there was no significant difference in the results at the 5% level.

Conclusions

The 3M Petrifilm Salmonella Express System was evaluated following the AOAC *Performance Tested Methods* requirements: inclusivity/exclusivity, ruggedness, stability/lot to lot consistency and method comparison. Nine matrices were tested by both the 3M Petrifilm SALX System and either the USDA/FSIS-MLG or the FDA-BAM for the detection of *Salmonella* species in food. Overall the assay was equivalent to the reference methods based on the Chi square test for significant difference and the Probability of Detection (POD). There were no unexpected results in all of the other PTM requirements.

The 3M Petrifilm Salmonella Express System is sufficiently rugged to withstand small variations in the experimental parameters of enrichment incubation time and temperature, plate incubation time and temperature, disk incubation time and temperature and various plate hydration options. Each parameter was testing using both the high and low microbial load methods as described in the Instructions for Use.

The method was considered easy to perform and the package insert was found to be straight forward and easy to follow. The 3M Petrifilm SALX System offers a significant savings in time when compared to the traditional reference methods as confirmed positive results can be obtained in as little as 44 hours for low microbial load matrices and 52 hours for high microbial load matrices. For foods with low microbial load, the 3M Petrifilm SALX method eliminated the need for transfer of enriched test portions to secondary enrichments. Overall, the 3MPetriflm Salmonella Express System was found to

be comparable to the reference methods and was found have the benefits of rapid results, high sensitivity and high specificity for the detection of *Salmonella* in a broad range of foods.

References

1. Centers For Disease Control and Prevention – Food borne Estimates. www.cdc.gov/features/foodborneestimates
2. Bad Bug Book on-line Jan 2012 – Salmonella http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/ucm071284.htm
3. US FDA Bacterial Analytical Manual (BAM) on-line Feb 2011, Chapter 5: Salmonella. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm
4. USDA Microbiological Laboratory Guide (MLG) on-line Oct 1, 2010, Chapter 4.05: Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg and Catfish Products. http://www.fsis.usda.gov/PDF/MLG_4_05.pdf

Appendix

Table of Contents

Document #2

Table 2. Inclusivity organisms and test results.

Genus, species, subspecies	Serotype	Group	Sub-group	Serology	Origin	ID	Low Microbial Load Result	High Microbial Load Result
Salmonella enterica enterica	Abaetetuba	I	O:11 (F)	1 1 k 1,5	IMVS	TICC 2736	+	+
Salmonella enterica enterica	Aberdeen	I	O:11 (F)	1 1 i 1,2	unknown	TICC 2500	+	+
Salmonella enterica enterica	Abony	I	O:4 (B)	1, 4, 5, 12:b:e, n, x	unknown	NCTC 6017	+	+
Salmonella enterica enterica	Abortusequi	I		4,12L-:e,n,x	unkown	ATCC 9842	+	+
Salmonella enterica enterica	Adelaide	I	O:35 (O)	35 f,g – [z27]	unknown	TICC 2501	+	+
Salmonella enterica enterica	Agona	I	O:4 (B)	1,4,12:f,g, s:- -	unknown	SGSC 2458, electrophoretic type Ag1; SARB1	+	+
Salmonella enterica enterica	Amsterdam	I	O:3,10 (E1)	3 , { 10}{15}{15 ,34} g , m , s –		TICC 2660	+	+
Salmonella enterica enterica	Anatum	I	O:3,10 (E1)	3, {10} {15} {15,34}	unknown	TICC 2668	+	+
Salmonella enterica enterica	Augustenborg	I	O:7 (C1)	6 , 7 ,14 i 1,2		TICC 2670	+	+
Salmonella enterica enterica	Bareilly	I		6,7:y:1,5	unkown	ATCC 9115	+	+
Salmonella enterica enterica	Berta	I		9,12:f,g,t:-	unkown	ATCC 8392	+	+
Salmonella enterica enterica	Birkenhead	I	O:7 (C1)	6 ,7 c 1,6	unknown	TICC 1273	+	+

Genus, species, subspecies	Serotype	Group	Sub-group	Serology	Origin	ID	Low Microbial Load Result	High Microbial Load Result
Salmonella enterica enterica	Blockley	I	O:8 (C2-C3)	6,8:k:1,5 6,8:k:1,5	Beth Israel Hosp., New York	ATCC 51961	+	+
Salmonella enterica enterica	Bovis-morbificans	I	O:8 (C2-C3)	6, 8, 20	unknown	TICC 1293	+	+
Salmonella enterica enterica	Braenderup	I	O:7 (C1)	6 , 7,14 e,h e,n,z15		TICC 2508	+	+
Salmonella enterica enterica	Brandenburg	I	O:4 (B)	4 ,[5],12 l,v e,n,z15		TICC 2674	+	+
Salmonella enterica enterica	Brazil	I	O:16 (I)	16:a:1, 5	unknown	NCTC 8446	+	+
Salmonella enterica enterica	Bredeney	I	O:4 (B)	1,4,12,27	unknown	TICC 2510	+	+
Salmonella enterica enterica	Cerro	I	O:18 (K)	6,14,18 z 4,z23 [1,5] [z45],[z82]		TICC 2176	+	+
Salmonella enterica enterica	Cholerasuis	I	O:7 (C1)	6,7:c:1,5	swine, California	ATCC 10708	+	+
Salmonella enterica enterica	Derby	I	O:4 (B)	1,4,[5],12	unknown	TICC 2519	+	+
Salmonella enterica enterica	Dublin	I	O:9 (D1)	1,9,12[Vi]	unknown	TICC 2757	+	+
Salmonella enterica enterica	Emek	I	O:8 (C2-C3)	8,20 g,m,s –	unknown	TICC 1447	+	+
Salmonella enterica enterica	Enteritidis	I	O:9 (D1)	1,9,12 g,m –	unknown	ATCC 13076	+	+

Genus, species, subspecies	Serotype	Group	Sub-group	Serology	Origin	ID	Low Microbial Load Result	High Microbial Load Result
Salmonella enterica enterica	Gallinarum	I	O:9 (D1)	1 ,9,12 – –		TICC 1320	+	+
Salmonella enterica enterica	Gaminara	I		16:d:1,7	unkown	ATCC 8324	+	+
Salmonella enterica enterica	Give	I	O:3,10 (E1)	3 ,{10}{15}{ 15,34} l,v 1,7 [d],[z77]		TICC 1282	+	+
Salmonella enterica enterica	Hadar	I	O:8 (C2-C3)	6,8 z10 e,n,x		TICC 2528	+	+
Salmonella enterica enterica	Havana	I	O:13 (G)	1,13,23 f , g,[s] – [z79]		TICC 443	+	+
Salmonella enterica enterica	Heidelberg	I	O:4 (B)	1 ,4,[5],12 r 1,2		TICC 561	+	+
Salmonella enterica enterica	Hessarek	I	O:4 (B)	4,12,[27] a 1,5		TICC 2655	+	+
Salmonella enterica enterica	Indiana	I		1,4,12:z:1, 7	unknown	ATCC 51959	+	+
Salmonella enterica enterica	Infantis	I	O:7 (C1)	6,7,14 r 1,5 [R1…],[z37],[z45],[z4 9]		TICC 2616	+	+
Salmonella enterica enterica	Johannesburg	I	O:40 (R)	1 , 4 0 b e,n,x		TICC 2543	+	+
Salmonella enterica enterica	Kahla	I		4,42:z35:1 ,6		ATCC 17980	+	+

Genus, species, subspecies	Serotype	Group	Sub-group	Serology	Origin	ID	Low Microbial Load Result	High Microbial Load Result
Salmonella enterica enterica	Kedougou	I	O:13 (G)	1 , 13,23 i l,w		TICC 4102	+	+
Salmonella enterica enterica	Kentucky	I	O:8 (C2-C3)	8 ,20 i z6	IMVS	TICC 2620	+	+
Salmonella enterica enterica	Kiambu	I	O:4 (B)	1,4,12 z 1,5	IMVS	TICC 2692	+	+
Salmonella enterica enterica	Kimberley	I	O:38 (P)	3 8 l,v 1,5	unknown	TICC 778	+	+
Salmonella enterica enterica	Kirkee	I		17:b:1,2			+	+
Salmonella enterica enterica	Kokomlemle	I	O:39 (Q)	3 9 l,v e,n,x	IMVS	TICC 2544	+	+
Salmonella enterica enterica	Kottbus	I	O:8 (C2-C3)	6,8 e,h 1,5	IMVS	TICC 2621	+	+
Salmonella enterica enterica	Lansing	I	O:38 (P)	38 i 1,5	IMVS	TICC 2622	+	+
Salmonella enterica enterica	Lexington	I	O:3,10 (E1)	3, {10} {15} {15,34}	IMVS	TICC 2624	+	+
Salmonella enterica enterica	Lille	I	O:7 (C1)	6,7,14 z38 – [z82]	IMVS	TICC 2684	+	+
Salmonella enterica enterica	Litchfield	I	O:8 (C2-C3)	6,8 l,v 1,2	IMVS	TICC 2547	+	+
Salmonella enterica enterica	Livingstone	I	O:7 (C1)	6 ,7,14 d l,w	IMVS	TICC 2549	+	+
Salmonella enterica enterica	Llandoff	I	O:1,3,19 (E4)	1,3,19 z29 [z6] [z37]		TICC 1291	+	+

Genus, species, subspecies	Serotype	Group	Sub-group	Serology	Origin	ID	Low Microbial Load Result	High Microbial Load Result
Salmonella enterica enterica	London	I	O:3,10 (E1)	3, {10} {15}	IMVS	TICC 2550	+	+
Salmonella enterica enterica	Manhattan	I	O:8 (C2-C3)	6,8 d 1,5 [z58]	unknown	TICC 2551	+	+
Salmonella enterica enterica	Mbandaka	I	O:7 (C1)	6,7,14 z10 e,n,z15 [z37],[z45]	unknown	TICC 1289	+	+
Salmonella enterica enterica	Miami	I	O:9 (D1)	1,9,12:a:1, 5	unknown	SGSC 2485	+	+
Salmonella enterica enterica	Montevideo	I	O:7 (C1)	{ 6 ,7,14}{54} g,m,[p],s [1 ,2,7]	unknown	TICC 763	+	+
Salmonella enterica enterica	Muenchen	I	O:8 (C2-C3)	6,8:d:1,2	France	SGSC 2490, electrophoretic type Mu2; SARB33	+	+
Salmonella enterica enterica	Naestved	I	O:9 (D1)	1,9,12 g,p,s –	human; Ontario, Canada	SGSC 3612, 88/8	+	+
Salmonella enterica enterica	Newport	I	O:8 (C2-C3)	6,8:e,h:1, 2 6,8:e,h:1, 2	food poisoning, England	ATCC 6962	+	+
Salmonella enterica enterica	Nottingham	I	O:16 (I)	16:d:e, n, z15	unknown	NCTC 7832	+	+
Salmonella enterica enterica	Ohio	I	O:7 (C1)	6,7,14 b l,w [z59]		TICC 444	+	+
Salmonella enterica enterica	Oranienburg	I	O:7 (C1)	6 , 7,14 m,t [z57]		TICC 2560	+	+

Genus, species, subspecies	Serotype	Group	Sub-group	Serology	Origin	ID	Low Microbial Load Result	High Microbial Load Result
Salmonella enterica enterica	Orion	I	O:3,10 (E1)	3 ,{10}{15}{ 15,34} y 1,5		TICC 720	+	+
Salmonella enterica enterica	Panama	I	O:9 (D1)	1,9,12:l,v: 1,5	Italy	SGSC 2496, electrophoretic type Pn1; SARB39	+	+
Salmonella enterica enterica	Paratyphi A	I	O:2 (A)	1,2,12:a:[1,5]	unknown	SGSC 2499, electrophoretic type Pa1; SARB42	+	+
Salmonella enterica enterica	Paratyphi B	I	O:4 (B)	1 ,4,[5],12	unknown	TICC 2240	+	+
Salmonella enterica enterica	Paratyphi C	I	O:7 (C1)	6,7,[Vi]	human clinical isolate, Switzerland	SGSC 3592, 4034-88	+	+
Salmonella enterica enterica	Poona	I	O:13 (G)	1,13,22	unknown	TICC 2472	+	+
Salmonella enterica enterica	Reading	I	O:4 (B)	1,4,[5],12	unknown	SGSC 2510	+	+
Salmonella enterica enterica	Rissen	I	O:7 (C1)	6,7,14 f,g –		TICC 2644	+	+
Salmonella enterica enterica	Rubislaw	I	O:11 (F)	1 1 r e,n,x	unknown	TICC 2645	+	+
Salmonella enterica enterica	Saint Paul	I	O:4 (B)	1,4,[5],12	unknown	TICC 559	+	+
Salmonella enterica enterica	Schwarzengrund	I	O:4 (B)	1 , 4 , 1 2,27	IMVS	TICC 4513	+	+

Genus, species, subspecies	Serotype	Group	Sub-group	Serology	Origin	ID	Low Microbial Load Result	High Microbial Load Result
Salmonella enterica enterica	Sendai	I	O:9 (D1)	1,9,12:a:1,5	California	SGSC 2515, electrophoretic type Se1; SARB58	+	+
Salmonella enterica enterica	Senftenberg	I	O:1,3,19 (E4)	1 ,3,19 g,[s],t – [z27],[z34],[z37],[z43],[z45],[z46],[z82]	unknown	TICC 2178,	+	+
Salmonella enterica enterica	Singapore	I	O:7 (C1)	6 ,7 k e,n,x		TICC 2652,	+	+
Salmonella enterica enterica	Stanley	I	O:4 (B)	1,4,[5],12,[27]	Scotland	SGSC 2517, electrophoretic type St1; Biotype 26bei.; SARB60	+	+
Salmonella enterica enterica	Tennessee	I	O:7 (C1)	6 ,7,14 z29 [1,2,7]	IMVS	TICC 2577	+	+
Salmonella enterica enterica	Thompson	I	O:7 (C1)	6 , 7,14 k 1,5 [R1…]	human, Florida	SGSC 2519, electrophoretic type Th1; SARB62	+	+
Salmonella enterica enterica	Typhi	I	O:9 (D1)	9,12[Vi] d – [z66]		ATCC 9993	+	+
Salmonella enterica enterica	Typhimurium	I	O:4 (B)	1 , 4 ,[5],12	bovine, septicaemic liver	ATCC 14028	+	+
Salmonella enterica enterica	Typhimurium (nm)*	I					+	+
Salmonella enterica enterica	Typhimurium (nm)*	I					+	+

Genus, species, subspecies	Serotype	Group	Sub-group	Serology	Origin	ID	Low Microbial Load Result	High Microbial Load Result
Salmonella enterica enterica	Typhimurium (nm)*						+	+
Salmonella enterica enterica	UNNAMED	I	O:7 (C1)	6,7:c:1,5		SGSC 3038	+	+
Salmonella enterica enterica	Virchow	I	O:7 (C1)	6,7,14 r 1,2	unknown	TICC 552	+	+
Salmonella enterica enterica	Virginia	I	O:8 (C2-C3)	8 d 1,2		TICC 2659	+	+
Salmonella enterica enterica	Wandsworth	I	O:39 (Q)	3 9 b 1,2	IMVS	TICC 2661	+	+
Salmonella enterica enterica	Waycross	I	O:41 (S)	41 z4,z23 [e,n,z15]	unknown	TICC 566	+	+
Salmonella enterica enterica	Welikade	I	O:16 (I)	1 6 l,v 1,7	IMVS	TICC 2586	+	+
Salmonella enterica enterica	Weltevreden	I	O:3,10 (E1)	3 , {10}{15}	IMVS	TICC 2663	+	+
Salmonella enterica enterica	Westhampton	I	O:3,10 (E1)	3,{10}{15} {15,34} g,s,t – [z37]		TICC 3053	+	+
Salmonella enterica enterica	Wien	I	O:4 (B)	1,4,12,[27]	France	SGSC 2528, electrophoretic type Wi1; SARB71	+	+
Salmonella enterica enterica	Worthington	I	O:13 (G)	1 ,13,23 z l,w [z43]	IMVS	TICC 2587	+	+
Salmonella enterica enterica	Zanzibar	I	O:3,10 (E1)	3,{10}{15} k 1,5	IMVS	TICC 2589	+	+

Genus, species, subspecies	Serotype	Group	Sub-group	Serology	Origin	ID	Low Microbial Load Result	High Microbial Load Result
Salmonella enterica enterica	Zehlendorf	I	O:30 (N)	3 0 a 1,5	IMVS	TICC 2395	+	+
Salmonella enterica houtenae	(IV Argentina)	IV	6, 7	6,7:z36:-	unknown	SGSC 2555	+	+
Salmonella enterica houtenae	(IV Wassenaar)	IV	O ag grp Z	IV 50:g,z51:-	Massachusettes	SGSC 2576	+	+
Salmonella enterica houtenae		IV		43:z4,z23:- -	unknown	SGSC 3084	+	+
Salmonella enterica houtenae		IV	40 R	43:z4, z23:-	unknown	NCTC 10401	+	+
Salmonella enterica indica	(VI Flint)	VI	6, 14 (H)	50:z4,z23:-	unknown	SGSC 2554	+	+
Salmonella enterica indica	(VI Vrindaban)	VI	O ag grp W	45:a:e,n,x	England	SGSC 2582	+	+
Salmonella enterica indica		VI		45:a:e,n,x	unknown	SGSC 3116	+	+
Salmonella enterica indica	(India)	VI			unknown	BAA-1578	+	+
Salmonella enterica salamae		II		1,9,12:l,w:e,n	Africa	ATCC 6959	+	+
Salmonella enterica salamae	(Betioky)	II		59:k:(z)	Madagascar	ATCC 700152	+	+
Salmonella enterica salamae	(Tranoroa)	II		55:k:z39	Unknown	ATCC 700148	+	+
Salmonella enterica salamae	(II Setubal)	II		60:g,m,t:z6	Mississipi	SGSC 2567	+	+

ATCC - American Type Culture Collection; BAA-from American Type Culture Collection; FSD- 3M Food Safety Culture Collection; NCTC - National Collection of Type Cultures; SGSC- Salmonella Genetic Stock Centre TICC - Tecra International Culture

Table. 3 Exclusivity organisms and test results.

Organism	ID	Test Result
Cedecea neteri	ATCC 33855	-
Citrobacter brakii	ATCC 29063	-
Citrobacter diversus	TICC 1604	-
Citrobacter freundii	ATCC 8090	-
Citrobacter koseri	TICC 4469	-
Citrobacter sedlakii	TICC 4088	-
Citrobacter youngae	TICC 4255	-
Cronobacter sakazakii	ATCC 29544	-
Edwardsiella tarda	NCTC 10396	-
Enterobacter aerogenes	ATCC 13048	-
Enterobacter amnigenus	ATCC 51816	-
Enterococcus faecalis	ATCC 14506	-
Escherichia coli	ATCC 25922	-
Escherichia coli O157:H7	PSU 93.0134	-
Escherichia hermanii	TICC 2489	-
Escherichia vulneris	TICC 447	-
Hafnia alvei	ATCC 51815	-
Klebsiella oxytoca	ATCC 51817	-
Klebsiella pneumoniae	ATCC 23357	-
Morganella morganii	ATCC 25830	-
Pantoea spp	TICC 4488	-
Proteus mirabilis	ATCC 43071	-
Proteus vulgaris	ATCC 13315	-
Providencia rettgeri	TICC 4472	-
Serratia ficaria	TICC 4475	-
Serratia liquefaciens	ATCC 51814	-
Serratia marcesens	ATCC 14041	-
Shigella sonnei	ATCC 25931	-
Staphylococcus aureus	ATCC 25923	-
Yersinia enterocolitica	ATCC 23715	-
Yersinia kristensenii	ATCC 33639	-
Yersinia ruckerii	ATCC 29473	-

ATCC - American Type Culture Collection; NCTC - National Collection of Type Cultures; PSU-Penn State University Culture Collection; TICC - Tecra International Culture Collection

Table 4. Ruggedness Results for the Evaluation of Varied Primary Enrichment Times and Temperatures.

Isolate	# of Samples	Incubation Temperature (°C) Number Positive			Incubation Time, Hours Number Positive			
		40	41.5	43	16	18	24	26
Salmonella Typhimurium ATCC 14028	10	10	10	10	10	10	10	10
Salmonella Poona NCTC 4840	10	10	10	10	10	10	10	10
Citrobacter braakii NCTC 4840	0	0	0	0	0	0	0	0

Table 5. Ruggedness Results for the Evaluation of Varied Times and Temperatures for the 3M Petrifilm Salmonella Express Plate.

Isolate	# of Samples	Incubation Temperature (°C) Number Positive			Incubation Time, Hours Number Positive				
		40	41.5	43	20	22	24	26	28
Salmonella Typhimurium ATCC 14028	10	10	10	10	10	10	10	10	10
Salmonella Poona NCTC 4840	10	10	10	10	10	10	10	10	10
Citrobacter braakii NCTC 4840	0	0	0	0	0	0	0	0	0

Table 6. Ruggedness Results for the Evaluation of Varied Times and Temperatures 3M Petrifilm Salmonella Express Confirmation Disk.

Isolate	# of Samples	Incubation Temperature (°C) Number Positive			Incubation Time, Hours Number Positive				
		40	41.5	43	2	3	4	5	6
Salmonella Typhimurium ATCC 14028	10	10	10	10	10	10	10	10	10
Salmonella Poona NCTC 4840	10	10	10	10	10	10	10	10	10
Citrobacter braakii NCTC 4840	10	0	0	0	0	0	0	0	0

Table 7. Ruggedness Results for the Evaluation of Various Hydration Fluids Used With the 3M Petrifilm Salmonella Express Plate.

Isolate	# of Samples	Positive		
		Sterile Distilled	Sterile Reverse Osmosis	Butterfield's Phosphate Buffer
Salmonella Typhimurium ATCC 14028	30	30	30	30
Salmonella Enteritidis ATCC 13076	30	30	30	30
Citrobacter braakii ATCC 43162	30	0	0	0

Table 8. Stability Data for 3M Petrifilm Salmonella Express Plate and Disk.

		3M Petrifilm SALX Plate Point in Shelf Life - # positive			3M Petrifilm SALX Confirmation Disk Point in shelf life - # positive		
Strain	**Number of replicates**	Newly Manufactured Time 0	Middle of Shelf Life 9 Months (To be submitted when available)	End of shelf life 18 months (To be submitted when available)	Newly Manufactured Time 0	Middle of Shelf Life 9 Months (To be submitted when available)	End of shelf life 18 months (To be submitted when available)
Salmonella Typhimurium ATCC 14028	5	5	-	-	5	-	-
Salmonella Poona NCTC 4840	5	5	-	-	5	-	-
Citrobacter braakii ATCC 43162	5	0	-	-	0	-	-

Figure 1. 3M Petrifilm *Salmonella* vs. USDA – raw ground chicken, raw ground beef, raw ground pork, chicken nuggets, and pasteurized liquid whole eggs.

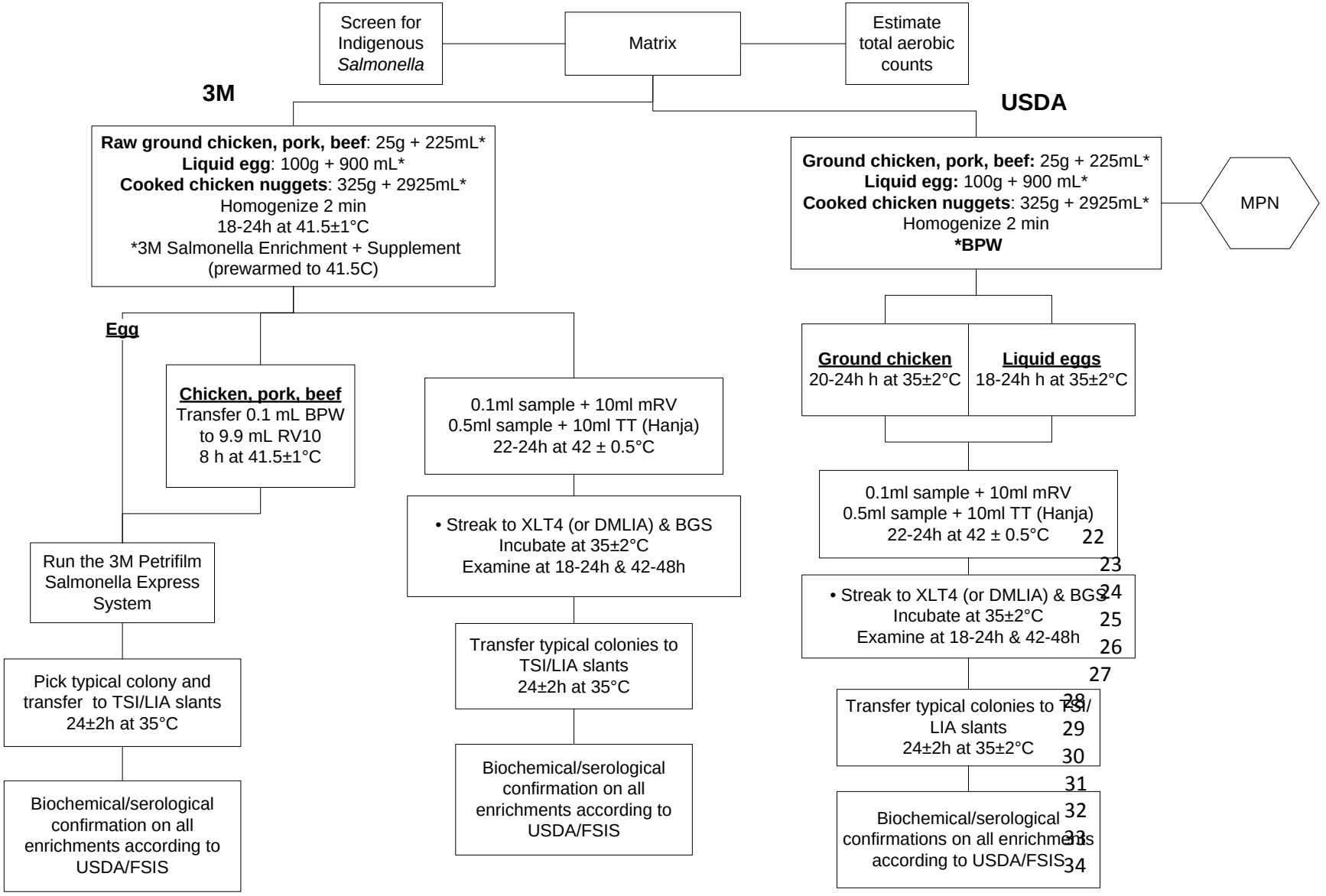


Figure 2. 3M Petrifilm *Salmonella* vs. FDA-BAM – raw shrimp, bunched spinach, dry dog food (375g)

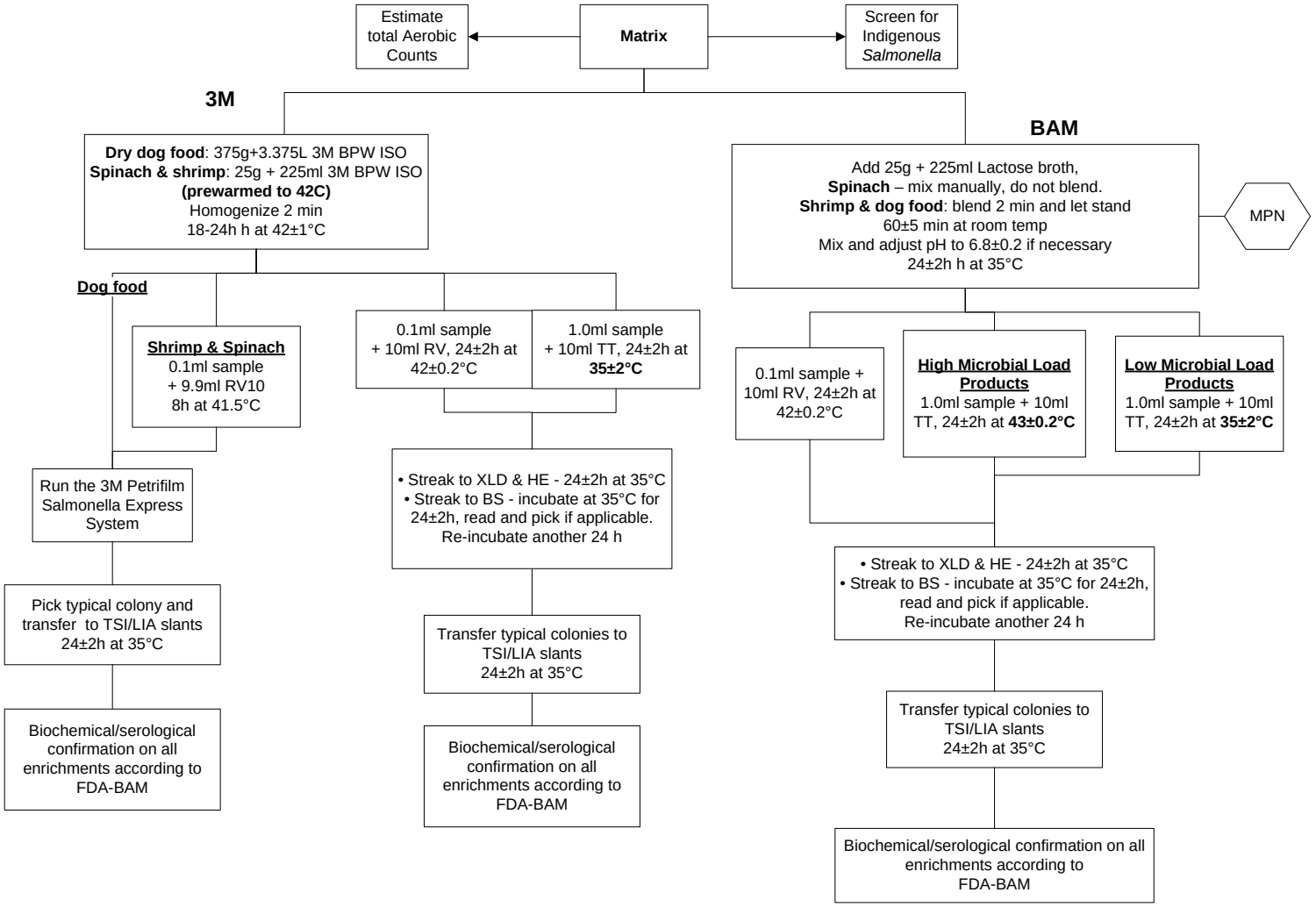
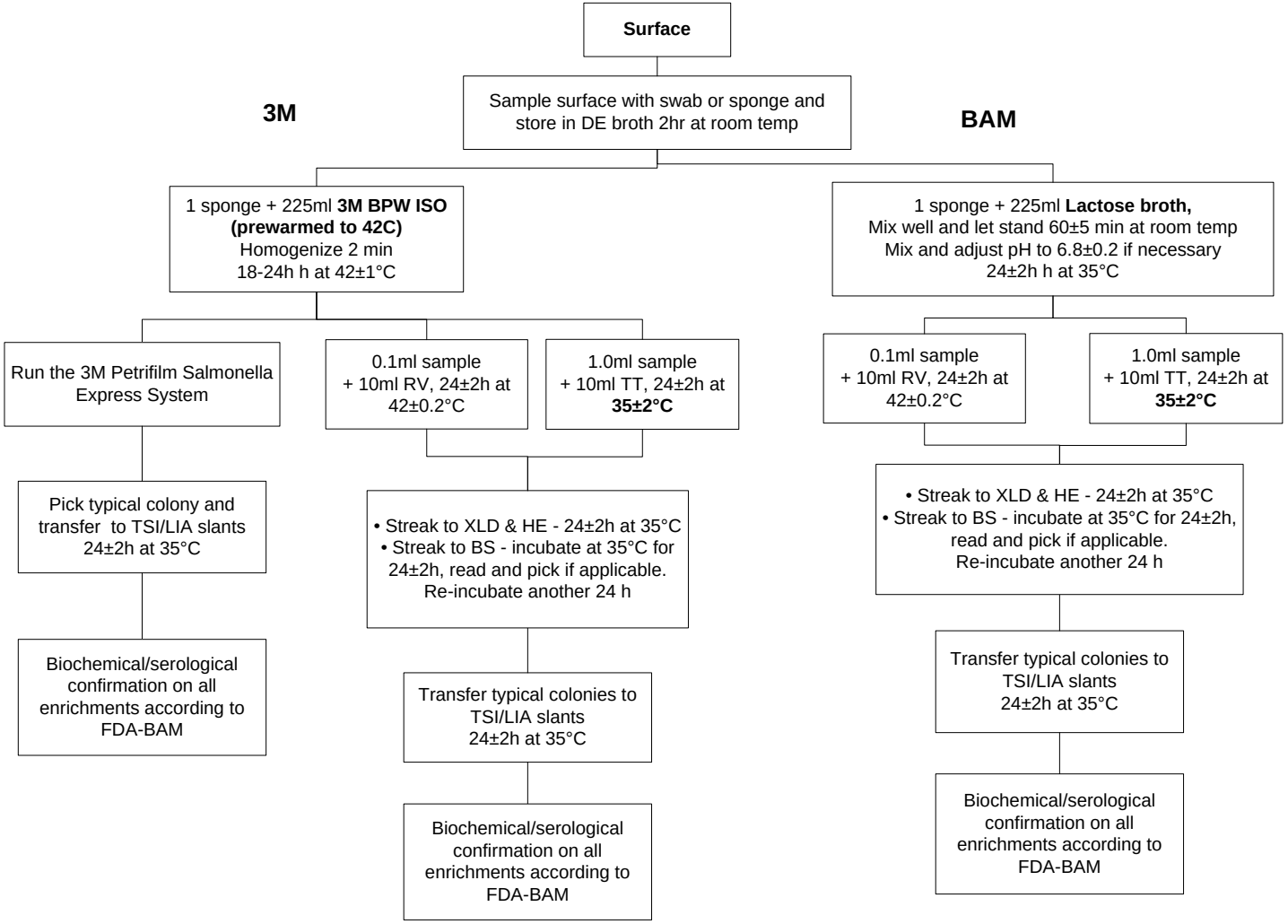


Figure 3. 3M™ Petrifilm™ Salmonella Express vs. FDA-BAM – stainless steel



Continued - Evaluation of the 3M™ Petrifilm™ Salmonella Express System Method for the Detection of Salmonella in Select Foods, AOAC Research Institute Performance Tested Method: Tables 9-30

Table 9 . Summary of Method Comparison for High Microbial Load Matrices (≥10^4CFU/g)

Method	USDA/FSIS- MLG	3M Petrifilm SALX								
Raw Ground Chicken	14	Number of presumptive positive test portions on 3M Petrifilm SALX Plates				Confirmed positive by 3M Petrifilm SALX Confirmation Disk				USDA/FSIS MLG Confirmation
		Enrichment		Enrichment		Enrichment		Enrichment		
		8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	
		15	15	15	15	15	15	15	15	14
Method	**FDA/BAM**	**3M Petrifilm SALX**								
Frozen Uncooked Shrimp	10	Number of presumptive positive test portions on 3M Petrifilm SALX Plates				Confirmed positive by 3M Petrifilm SALX Confirmation Disk				FDA/BAM Confirmation
		Enrichment		Enrichment		Enrichment		Enrichment		
		8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	
		12	12	12	12	12	12	12	12	12
Method	**FDA/BAM**	**3M Petrifilm SALX**								
Fresh Spinach	10	Number of presumptive positive test portions on 3M Petrifilm SALX Plates				Confirmed positive by 3M Petrifilm SALX Confirmation Disk				FDA/BAM Confirmation
		Enrichment		Enrichment		Enrichment		Enrichment		
		8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	
		7	11	7	11	7	11	7	11	11
Method	**USDA/FSIS- MLG**	**3M Petrifilm SALX**								
Raw Ground Beef	13	Number of presumptive positive test portions on 3M Petrifilm SALX Plates				Confirmed positive by 3M Petrifilm SALX Confirmation Disk				USDA/FSIS MLG Confirmation
		Enrichment		Enrichment		Enrichment		Enrichment		
		8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	
		13	13	13	13	13	13	13	13	13
Method	**USDA/FSIS- MLG**	**3M Petrifilm SALX**								
Raw Ground Pork	9	Number of presumptive positive test portions on 3M Petrifilm SALX Plates				Confirmed positive by 3M Petrifilm SALX Confirmation Disk				USDA/FSIS MLG Confirmation
		Enrichment		Enrichment		Enrichment		Enrichment		
		8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	
		9	9	9	9	9	9	9	9	9

Table 10 . Summary of Method Comparison for Low Microbial Load Matrices(≤10^4CFU/g)

Method	USDA/FSIS- MLG	3M Petrifilm SALX Method				
Pastuerized Liquid Whole Eggs	4	Presumptive positive test portions on 3M Petrifilm SALX Plates		Confirmed positive by 3M SALX Confirmation Disk		
		18 Hr Primary Enrichment	24 Hr Primary Enrichment	18 Hr Primary Enrichment	24 Hr Primary Enrichment	USDA/MLG Confirmation
		6	6	6	6	6
Method	**FDA/BAM**	**3M Petrifilm SALX Method**				
Dry Dog Food	14	Presumptive positive test portions on 3M Petrifilm SALX Plates		Confirmed positive by 3M SALX Confirmation Disk		
		18 Hr Primary Enrichment	24 Hr Primary Enrichment	18 Hr Primary Enrichment	24 Hr Primary Enrichment	FDA/BAM Confirmation
		16	16	16	16	16
Method	**FDA/BAM**	**3M Petrifilm SALX Method**				
Stainless Steel	7	Presumptive positive test portions on 3M Petrifilm SALX Plates		Confirmed positive by 3M SALX Confirmation Disk		
		18 Hr Primary Enrichment	24 Hr Primary Enrichment	18 Hr Primary Enrichment	24 Hr Primary Enrichment	FDA/BAM Confirmation
		6	6	6	6	6
Method	**USDA/FSIS- MLG**	**3M Petrifilm SALX Method**				
Cooked Chicken Nuggets	6	Presumptive positive test portions on 3M Petrifilm SALX Plates		Confirmed positive by 3M SALX Confirmation Disk		
		18 Hr Primary Enrichment	24 Hr Primary Enrichment	18 Hr Primary Enrichment	24 Hr Primary Enrichment	USDA/MLG Confirmation
		6	6	6	6	6

Table 11. 3M Petrifilm SALX Method Comparison Chi-Square Results for Raw Ground Chicken vs USDA/FSIS MLG (Internal Data)

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/25g*	Total Samples	3M Petrifilm SALX Method			USDA/FSIS MLG	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive positive test portions on 3M Petrifilm SLAX Plates	Confirmed				
						SALX Confirmation Disk Positive	Reference			
18 Hr Primary Enrichment – 8 Hr RV-R10 2° Enrichment										
Control	N/A	7.1 x 10^3	< 0.075	5	0	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Heidelberg NCTC 5717**		1.15 (0.70-2.07)	20	15	15	14	14	0.12	0
18 Hr Primary Enrichment – 24 Hr RV-R10 2° Enrichment										
Control	N/A	7.1 x 10^3	< 0.075	5	0	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Heidelberg NCTC 5717**		1.15 (0.70-2.07)	20	15	15	14	14	0.12	0
24 Hr Primary Enrichment – 8 Hr RV-R10 2° Enrichment										
Control	N/A	7.1 x 10^3	< 0.075	5	0	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Heidelberg NCTC 5717**		1.15 (0.70-2.07)	20	15	15	14	14	0.12	0
24 Hr Primary Enrichment – 8 Hr RV-R10 2° Enrichment										
Control	N/A	7.1 x 10^3	< 0.075	5	0	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Heidelberg NCTC 5717**		1.15 (0.70-2.07)	20	15	15	14	14	0.12	0

***Includes 95% confidence interval**

Table 12. 3M Petrifilm SALX Method Comparison Chi-Square Results for Raw Ground Beef vs USDA (Internal Data)

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/25g*	Total Samples	3M Petrifilm SALX Method		USDA/FSIS-MLG	X^2 Alternative Confirmation	X^2 Traditional Conformation
					Presumptive positive test portions on 3M Petrifilm SLAX Plates	SALX Confirmation Disk Positive			
18 Hr Primary Enrichment – 8 Hr 2^0 RV-R10 Enrichment									
Control	N/A	3.4×10^6	< 0.075	5	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Ohio STS 81**		1.07 (0.65-1.79)	20	13	13	13	0	0
18 Hr Primary Enrichment – 24 Hr 2^0 RV-R10 Enrichment									
Control	N/A	3.4×10^6	< 0.075	5	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Ohio STS 81**		1.07 (0.65-1.79)	20	13	13	13	0	0
24 Hr Primary Enrichment – 8 Hr 2^0 RV-R10 Enrichment									
Control	N/A	3.4×10^6	< 0.075	5	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Ohio STS 81**		1.07 (0.65-1.79)	20	13	13	13	0	0
24 Hr Primary Enrichment – 24 Hr 2^0 RV-R10 Enrichment									
Control	N/A	3.4×10^6	< 0.075	5	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Ohio STS 81**		1.07 (0.65-1.79)	20	13	13	13	0	0

***Includes 95% confidence interval**

Table 13. 3M Petrifilm SALX Method Comparison Chi-Square Results for Raw Ground Pork vs USDA (Internal Data)

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/25g*	Total Samples	3M Petrifilm SALX Method		USDA/FSIS-MLG	X^2 Alternative Confirmation	X^2 Traditional Conformation
					Presumptive	Confirmed			
18 Hr Primary Enrichment – 8 Hr RV-R10 2^0 Enrichment									
Control	N/A	2.4×10^7	< 0.075	5	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Montevideo ATCC 8387**		0.66 (0.38-1.16)	20	9	9	9	0	0
18 Hr Primary Enrichment – 24 Hr RV-R10 2^0 Enrichment									
Control	N/A	2.4×10^7	< 0.075	5	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Montevideo ATCC 8387**		0.66 (0.38-1.16)	20	9	9	9	0	0
24 Hr Primary Enrichment – 8 Hr RV-R10 2^0 Enrichment									
Control	N/A	2.4×10^7	< 0.075	5	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Montevideo ATCC 8387**		0.66 (0.38-1.16)	20	9	9	9	0	0
24 Hr Primary Enrichment – 24 Hr RV-R10 2^0 Enrichment									
Control	N/A	2.4×10^7	< 0.075	5	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Montevideo ATCC 8387**		0.66 (0.38-1.16)	20	9	9	9	0	0

***Includes 95% confidence intervals**

Table 14. 3M Petrifilm SALX Method Comparison Chi-Square Results for Uncooked Frozen Shrimp vs BAM (Internal Data)

Inoculation Level	Inoculating Organism	APC cfu/g	MPN/25g*	Total Samples	3M Petrifilm SALX Method			FDA/BAM	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive positive test portions on 3M SALX Plates	Confirmed positive by 3M Confirmation Disk	Confirmed Reference Method			
18 Hr Primary Enrichment – 8 Hr RV-R10 2^0 Enrichment										
Control	N/A	4.0×10^4	< 0.075	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Virchow ATCC 51955**		0.77 (0.41-1.36)	20	12	12	12	10	0.39	
18 Hr Primary Enrichment – 24 Hr RV-R10 2^0 Enrichment										
Control	N/A	4.0×10^4	< 0.075	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Virchow ATCC 51955**		0.77 (0.41-1.36)	20	12	12	12	10	0.39	
24 Hr Primary Enrichment – 8 Hr RV-R10 2^0 Enrichment										
Control	N/A	4.0×10^4	< 0.075	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Virchow ATCC 51955**		0.77 (0.41-1.36)	20	12	12	12	10	0.39	
24 Hr Primary Enrichment – 24 Hr RV-R10 2^0 Enrichment										
Control	N/A	4.0×10^4	< 0.075	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Virchow ATCC 51955**		0.77 (0.41-1.36)	20	12	12	12	10	0.39	

***Includes 95% confidence intervals**

Table 15. 3M Petrifilm SALX Method Comparison Chi-Square Results for Spinach vs BAM (Internal Data)

Inoculation Level	Inoculating Organism	APC CFU/25g	MPN/25g*	Total Samples	3M Petrifilm SALX Method			FDA/BAM	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive positive test portions on 3M SALX Plates	Confirmed				
						Confirmed positive by 3M Confirmation Disk	Reference Method			
18 Hr Primary Enrichment – 8 Hr RV-R10 2^0 Enrichment										
Control	N/A	1.0×10^7	< 0.075	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Saintpaul ATCC 9712**		0.77 (0.41-1.36)	20	7	7	11	10	0.9	
18 Hr Primary Enrichment – 24 Hr RV-R10 2^0 Enrichment										
Control	N/A	1.0×10^7	< 0.075	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Saintpaul ATCC 9712**		0.77 (0.41-1.36)	20	11	11	11	10	0.1	
24 Hr Primary Enrichment – 8 Hr RV-R10 2^0 Enrichment										
Control	N/A	1.0×10^7	< 0.075	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Saintpaul ATCC 9712**		0.77 (0.41-1.36)	20	7	7	11	10	0.9	
24Hr Primary Enrichment – 24 Hr RV-R10 2^0 Enrichment										
Control	N/A	1.0×10^7	< 0.075	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Saintpaul ATCC 9712**		0.77 (0.41-1.36)	20	11	11	11	10	0.1	

***Includes 95% confidence intervals**

Table 16. 3M Petrifilm SALX Method Comparison Chi-Square Results for Pasteurized Liquid Whole Eggs vs BAM (Internal Data)

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/25g*	Total Samples	3M Petrifilm SALX Method			USDA/FSIS MLG	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive positive test portions on 3M SALX Plates	Confirmed				
						Confirmed positive by 3M Confirmation Disk	Reference Method			
18 Hr Primary Enrichment										
Control	N/A	6.0×10^1	< 0.075	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Enteritidis ATCC 13076**		1.15 (0.70-2.07)	20	6	6	6	4	0.98	0.98
24 Hr Primary Enrichment										
Control	N/A	6.0×10^1	< 0.075	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Enteritidis ATCC 13076**		1.15 (0.70-2.07)	20	6	6	6	4	0.98	0.98

***Includes 95% confidence intervals**

Table 17. 3M Petrifilm SALX Method Comparison Chi-Square Results for Dry Dog Food vs BAM (Internal Data)

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/325g*	Total Samples	3M Petrifilm SALX Method			FDA/BAM	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive positive test portions on 3M SALX Plates	Confirmed positive by 3M Confirmation Disk	Reference Method			
18 Hr Primary Enrichment										
Control	N/A	1.7×10^3	< 0.075	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Poona ATCC 4840**		0.03 (0.01-0.68)	20	16	16	16	14	0.52	0.52
24 Hr Primary Enrichment										
Control	N/A	1.7×10^3	< 0.075	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Poona ATCC 4840**		0.03 (0.01-0.68)	20	16	16	16	14	0.52	0.52

***Includes 95% confidence intervals**

Table 18. 3M Petrifilm SALX Method Comparison Chi-Square Results for Cooked Chicken Nuggets vs USDA (Internal Data)

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/25g*	Total Samples	3M Petrifilm SALX Method		USDA/FSIS-MLG	X^2 Alternative Confirmation	X^2 Traditional Conformation
					Presumptive positive test portions on 3M SALX Plates	Confirmed positive by 3M Confirmation Disk			
18 Hr Primary Enrichment									
Control	N/A	2.4×10^2	< 0.075	5	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Typhimurium ATCC 14028**		0.03 (0.01-0.08)	20	6	6	6	0	0
24 Hr Primary Enrichment									
Control	N/A	2.4×10^2	< 0.075	5	0	0	0	-	-
0.2-2 CFU/ Test Portion	***S. enterica* ser. Typhimurium ATCC 14028**		0.03 (0.01-0.08)	20	6	6	6	0	0

***Includes 95% confidence intervals**

Table 19. 3M Petrifilm SALX Method Comparison Chi-Square Results for Stainless Steel vs BAM (Internal Data)

Inoculation Level	Inoculating Organism	APC CFU/g	CFU/ 100cm^2*	Total Samples	3M Petrifilm SALX Method			FDA/BAM	X^2 Alternative Confirmation	X^2 Traditional Conformation
					Presumptive positive test portions on 3M SALX Plates	Confirmed				
						Confirmed positive by 3M Confirmation Disk	Reference Method			
18 Hr Primary Enrichment										
Control	N/A	NA	0	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Kahla ATCC 17980**		51	20	6	6	6	7	0.11	0.11
24 Hr Primary Enrichment										
Control	N/A	NA	0	5	0	0	0	0	-	
0.2-2 CFU/ Test Portion	***S. enterica* ser. Kahla ATCC 17980**		51	20	6	6	6	7	0.11	0.11

Includes 95% confidence intervals

Table 20. 3M Petrifilm SALX Method Comparison POD Results for Raw Ground Chicken (Internal Data)

Matrix	Time Point	Strain	MPN[a]/25g	N[b]	3M Petrifilm SALX Method			Reference Method			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
Raw Ground Chicken Vs. USDA/FSIS-MLG	18 Hr Primary Enrichment – 8 Hr 2^0 RV-R10 Enrichment	*Salmonella* Heidelberg NCTC 5717	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			1.15 (0.70-2.07)	20	14	0.7	0.48 - 0.85	14	0.7	0.48 - 0.85	0	-0.27 – 0.27
	18 Hr Primary Enrichment – 24 Hr 2^0 RV-R10 Enrichment		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			1.15 (0.70-2.07)	20	14	0.7	0.48 - 0.85	14	0.7	0.48 - 0.85	0	-0.27 – 0.27
	24 Hr Primary Enrichment – 8 Hr 2^0 RV-R10 Enrichment		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			1.15 (0.70-2.07)	20	14	0.7	0.48 - 0.85	14	0.7	0.48 - 0.85	0	-0.27 – 0.27
	24 Hr Primary Enrichment – 24 Hr 2^0 RV-R10 Enrichment		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			1.15 (0.70-2.07)	20	14	0.7	0.48 - 0.85	14	0.7	0.48 - 0.85	0	-0.27 – 0.27

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator with 95% confidence interval

[b]N = Number of test potions

[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 candidate method confirmed results 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. 3M Petrifilm SALX Method Comparison POD Results for Raw Frozen Shrimp (Internal Data)

Matrix	Time Point	Strain	MPN[a]/25g	N[b]	3M Petrifilm SALX Method			Reference Method			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_{CP}$[d]	95% CI	x	POD$_{CC}$[e]	95% CI		
Frozen Uncooked Shrimp - Confirmed by the 3M Confirmation Disk	18 Hr Primary Enrichment –	*Salmonella* Virchow ATCC 51955	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
	8 Hr 2^0 RV-R10 Enrichment		0.77 (0.41-1.36)	20	12	0.6	0.39 – 0.78	12	0.6	0.39 – 0.78	0	-0.28 – 0.28
	18 Hr Primary Enrichment –		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
	24 Hr 2^0 RV-R10 Enrichment		0.77 (0.41-1.36)	20	12	0.6	0.39 – 0.78	12	0.6	0.39 – 0.78	0	-0.28 – 0.28
	24 Hr Primary Enrichment –		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
	8 Hr 2^{0}RV-R10 Enrichment		0.77 (0.41-1.36)	20	12	0.6	0.39 – 0.78	12	0.6	0.39 – 0.78	0	-0.28 – 0.28
	24 Hr Primary Enrichment –		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
	24 Hr 2^0 RV-R10 Enrichment		0.77 (0.41-1.36)	20	12	0.6	0.39 – 0.78	12	0.6	0.39 – 0.78	0	-0.28 – 0.28
Frozen Uncooked Shrimp - Confirmed by the Reference Method	18 Hr Primary Enrichment –	*Salmonella* Virchow ATCC 51955	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
	8 Hr 2^0 RV-R10 Enrichment		0.77 (0.41-1.36)	20	12	0.6	0.39 – 0.78	12	0.6	0.39 – 0.78	0	-0.28 – 0.28
	18 Hr Primary Enrichment –		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
	24 Hr 2^0 RV-R10 Enrichment		0.77 (0.41-1.36)	20	12	0.6	0.39 – 0.78	12	0.6	0.39 – 0.78	0	-0.28 – 0.28
	24 Hr Primary Enrichment –		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
	8 Hr 2^0 RV-R10 Enrichment		0.77 (0.41-1.36)	20	12	0.6	0.39 – 0.78	12	0.6	0.39 – 0.78	0	-0.28 – 0.28
	24 Hr Primary Enrichment –		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
	24 Hr 2^0 RV-R10 Enrichment		0.77 (0.41-1.36)	20	12	0.6	0.39 – 0.78	12	0.6	0.39 – 0.78	0	-0.28 – 0.28

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator with 95% confidence interval

[b]N = Number of test potions

[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 candidate method confirmed results 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 22. 3M Petrifilm SALX Method Comparison POD Results for Raw Spinach (Internal Data)

Matrix	Time Point	Strain	MPN[a]/25g	N[b]	3M Petrifilm SALX Method			Reference Method			dPOD$_C$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
Fresh Spinach Vs. FDA/BAM	18 Hr Primary Enrichment – 8 Hr 2^0 RV-R10 Enrichment	*Salmonella* Saint paul	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.77 (0.41-1.36)	20	7	0.35	0.18 - 0.57	10	0.5	0.30 – 0.70	0.15	-0.41 - 0.15
	18 Hr Primary Enrichment – 24 Hr 2^0 RV-R10 Enrichment	ATCC 9712	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.77 (0.41-1.36)	20	7	0.35	0.18 - 0.57	10	0.5	0.30 – 0.70	0.15	-0.41 - 0.15
	24 Hr Primary Enrichment – 8 Hr 2^0 RV-R10 Enrichment		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.77 (0.41-1.36)	20	7	0.35	0.18 - 0.57	10	0.5	0.30 – 0.70	0.15	-0.41 - 0.15
	24 Hr Primary Enrichment – 24 Hr 2^0 RV-R10 Enrichment		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.77 (0.41-1.36)	20	7	0.35	0.18 - 0.57	10	0.5	0.30 – 0.70	0.15	-0.41 - 0.15

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator

[b]N = Number of test potions

[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 candidate method confirmed results 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 23. 3M Petrifilm SALX Method Comparison POD results for Raw Ground Beef (Internal Data)

Matrix	Time Point	Strain	MPN[a]/25g	N[b]	3M Petrifilm SALX Method			Reference Method			dPOD$_C$[f]	95% CI[g]
					X[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
Raw Ground Beef Vs. USDA/FSIS-MLG	18 Hr Primary Enrichment –	*Salmonella*	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
	8 Hr 2^0 RV-R10 Enrichment	Ohio	1.07 (0.65-1.79)	20	13	0.65	0.43 – 0.82	13	0.65	0.43 – 0.82	0	-0.28 – 0.28
	18 Hr Primary Enrichment –	STS 81	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
	24 Hr 2^0 RV-R10 Enrichment		1.07 (0.65-1.79)	20	13	0.65	0.43 – 0.82	13	0.65	0.43 – 0.82	0	-0.28 – 0.28
	24 Hr Primary Enrichment –		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
	8 Hr 2^0 RV-R10 Enrichment		1.07 (0.65-1.79)	20	13	0.65	0.43 – 0.82	13	0.65	0.43 – 0.82	0	-0.28 – 0.28
	24 Hr Primary Enrichment –		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
	24 Hr 2^0 RV-R10 Enrichment		1.07 (0.65-1.79)	20	13	0.65	0.43 – 0.82	13	0.65	0.43 – 0.82	0	-0.28 – 0.28

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator with 95% confidence interval

[b]N = Number of test potions

[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 candidate method confirmed results 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 24. 3M Petrifilm SALX Method Comparison POD Results for Raw Ground Pork (Internal Data)

Matrix	Time Point	Strain	MPN[a]/25g	N[b]	3M Petrifilm SALX Method			Reference Method			dPOD$_C$[f]	95% CI[g]
					X[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
Raw Ground Pork Vs. USDA/FSIS-MLG	18 Hr Primary Enrichment – 8 Hr 2^0 RV-R10 Enrichmnet	*Salmonella* Montevideo ATCC 8387	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.66 (0.38-1.16)	20	9	0.45	0.26 – 0.66	9	0.45	0.26 – 0.66	0	-0.28 – 0.28
	18 Hr Primary Enrichment – 24 Hr 2^0 RV-R10 Enrichmnet		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.66 (0.38-1.16)	20	9	0.45	0.26 – 0.66	9	0.45	0.26 – 0.66	0	-0.28 – 0.28
	24 Hr Primary Enrichment – 8 Hr 2^0 RV-R10 Enrichmnet		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.66 (0.38-1.16)	20	9	0.45	0.26 – 0.66	9	0.45	0.26 – 0.66	0	-0.28 – 0.28
	24 Hr Primary Enrichment – 24 Hr 2^0 RV-R10 Enrichmnet		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.66 (0.38-1.16)	20	9	0.45	0.26 – 0.66	9	0.45	0.26 – 0.66	0	-0.28 – 0.28

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator with 95% confidence interval

[b]N = Number of test potions

[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 candidate method confirmed results 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 25. 3M Petrifilm SALX Method Comparison POD Results for Liquid Whole Egg (Internal Data)

Matrix	Time Point	Strain	MPN[a]/100g	N[b]	3M Petrifilm SALX Method			Reference Method			dPOD$_{CP}$[f]	95% CI[g]
					x[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
Pasteurized Liquid Whole Egg Vs. USDA/FSIS-	18 Hr Primary Enrichment –	*Salmonella* Enteritidis ATCC 13076	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.32 (0.14-0.56)	20	6	0.3	0.15-0.52	4	0.2	0.08 – 0.42	0.1	-0.17 – 0.35
	24 Hr Primary Enrichment –		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.32 (0.14-0.56)	20	6	0.3	0.15-0.52	4	0.2	0.08 – 0.42	0.1	-0.17 – 0.35

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator with 95%

[b]N = Number of test potions

[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 candidate method confirmed results 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 26. 3M Petrifilm SALX Method Comparison POD Results for Dry Dog Food and Stainless Steel (Internal Data)

Matrix	Time Point	Strain	MPN[a]/ 325g or CFU 100cm^2	N[b]	3M Petrifilm SALX Method			Reference Method			dPOD$_C$[f]	95% CI[g]
					X[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
Dry Dog Food Vs. FDA/BAM	18 Hr Primary Enrichment	*Salmonella* Poona	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.03 (0.01-0.68)	20	16	0.8	0.58 - 0.92	14	0.7	0.48 - 0.85	0.1	-0.17 – 0.35
	24 Hr Primary Enrichment	ATCC 4840	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.03 (0.01-0.68)	20	16	0.8	0.58 - 0.92	14	0.7	0.48 - 0.85	0.1	-0.17 – 0.35
Stainless Steel Vs. FDA/BAM	18 Hr Primary Enrichment	*Salmonella* Kahla	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			51	20	6	0.3	0.15 – 0.52	7	0.35	0.18 – 0.57	-0.05	-0.32 – 0.23
	24 Hr Primary Enrichment	ATCC 17980	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			51	20	6	0.3	0.15 – 0.52	7	0.35	0.18 – 0.57	-0.05	-0.32 – 0.23

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator with 95% confidence interval

[b]N = Number of test potions

[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 candidate method confirmed results 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 27. 3M Petrifilm SALX Method Comparison POD Results for Cooked Chicken Nuggets (Internal Data)

Matrix	Time Point	Strain	MPN[a]/325g	N[b]	3M Petrifilm SALX Method			Reference Method			dPOD$_C$[f]	95% CI[g]
					X[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
Cooked Chicken Nuggets Vs. USDA/FSIS-MLG	18 Hr Primary Enrichment	*Salmonella* Typhimurium ATCC 14028	0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.03 (0.01-0.08)	20	6	0.3	0.15 – 0.52	6	0.3	0.15 – 0.52	0	-0.27 – 0.27
	24 Hr Primary Enrichment		0	5	0	0	0.00 - 0.43	0	0	0.00 - 0.43	0	-0.43 – 0.43
			0.03 (0.01-0.08)	20	6	0.3	0.15 – 0.52	6	0.3	0.15 – 0.52	0	-0.27 – 0.27

[a]MPN = Most Probable Number is based on the POD of reference method test portions across labs using the AOAC MPN calculator with 95% confidence interval

[b]N = Number of test potions

[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 candidate method confirmed results 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 28. Statistical Analysis (Chi Square) of 3M Salmonella Petrifilm Method Compared to the USDA/FSIS Method for Detection of Salmonella in Raw Ground Chicken and Pasteurized Liquid Eggs (Independent Laboratory Data)

				3M Salmonella Petrifilm Method					
Matrix	Level of Target Organism	MPN/25g[a]	Total Samples[b]	Presumptive Positive	Confirmed Positive[c]	Reference Positive[d]	X^{2e}	Relative Sensitivity Rate[f]	False Positive Rate[g]
Raw Ground Chicken	Control	NA	5	0	0	0	-	-	
	Low	0.8	20	12	12	11	0.1	100	0.0
Pasteurized Liquid Eggs	Control	NA	5	0	0	0	-	-	
	Low	0.2	20	7	7	8	0.1	100	0.0

[a] Most probable number of colony forming units (cfu)/g food

[b] Number of test assay positive samples not considering subsequent confirmations

[c] Number of test assay positive samples subsequently confirmed

[d] Number of samples positive by reference method

eMantel-Hansezel chi square

results, expressed as a percentage.

positive reference results

[g] Test false positive rate is 100 – test specificity rate. Not calculated where the number of samples negative by both methods is 0.

Table 29. Statistical Analysis (Chi Square) of 3M Salmonella Petrifilm Method Compared to the BAM Method for Detection of Salmonella on Stainless Steel (Independent laboratory Data)

			3M Petrifilm SALX Method					
Matrix	Level of Target	Total Samples[b]	Presumptive Positive	Confirmed Positive[c]	Reference Positive[d]	X^{2}[e]	Relative Sensitivity	False Positive
Stainless Steel	Control	5	0	0	0	-	-	
	Low	20	6	6	7	0.11	100	0.0

[b] Number of test assay positive samples not considering subsequent confirmations

[c] Number of test assay positive samples subsequently confirmed

[d] Number of samples positive by reference method

[e] Mantel-Hansezel chi square

[f] Test sensitivity rate is defined as the number of confirmed positive test results divided by the number of test screened positive results, expressed as a percentage.

[g] Test false positive rate is 100 – test specificity rate. Not calculated where the number of samples negative by both methods is 0.

Table 30. Statistical Analysis (POD) of 3M Salmonella Petrifilm Method Confirmed vs. Reference Method (Independent Laboratory Data)

Matrix	MPN[a]/25g (or 100g for egg)	N[b]	3M Petrifilm SALX Method Confirmed			Reference Method			$dPOD_C$[f]	95% CI[g]
			X[c]	POD_c[d]	95% CI	X[c]	POD_R[e]	95% CI		
Ground Chicken	N/A[h]	5	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	-0.435-0.435
	0.79	20	12	0.600	0.387-0.781	11	0.550	0.342-0.742	0.050	-0.237-0.326
Pasteurized Liquid Egg	N/A[h]	5	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	-0.435-0.435
	0.80	20	7	0.350	0.181-0.567	8	0.400	0.219-0.313	-0.050	-0.322-0.233
Stainless Steel	N/A[h]	5	0	0.000	0.000-0.435	0	0.000	0.000-0.435	0.000	-0.435-0.435
	N/A[h]	20	6	0.300	0.145-0.519	7	0.350	0.181-0.567	-0.050	-0.317-0.226

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the AOAC MPN calculator, with 95% confidence interval

[b]N = Number of test portions

[c]x = Number of positive test portions

[d]PODC = Confirmed candidate method positive outcomes divided by the total number of trials

[e]PODR = Confirmed reference method positive outcomes divided by the total number of trials

[f]dPODC = Difference between the candidate method and reference method POD values

[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

[h]N/A = Not applicable

3M
Petrifilm™

Product Instructions
6536/6537/6538/6539

- EN *Salmonella* Express System
- FR Système *Salmonella* Express
- DE *Salmonella* Express System
- IT Sistema *Salmonella* Express
- ES Sistema *Salmonella* Express
- NL *Salmonella* Express Systeem
- SV *Salmonella* Express System
- DA *Salmonella* Express System
- NO *Salmonella* Express system
- FI *Salmonella* Express Järjestelmä
- PT Sistema *Salmonella* Express
- EL *Σαλμονέλλα* Εξπρές Σύστημα
- PL System do szybkiego wykrywania obecności bakterii *Salmonella*
- HU *Salmonella* Express rendszer
- CS *Salmonella* Systém rychlé kultivace
- RO Sistem Expres pentru detecţie *Salmonella*
- RU Система *Сальмонелла* Экспресс
- TR *Salmonella* Tanımlama Sistemi
- JA サルモネラエクスプレスシステム
- ZH 沙门氏菌快速系统
- TH ชุดทดสอบ *Salmonella* Express
- KO 살모넬라 익스프레스 시스템

Issue Date: 2012-10

Petrifilm™ Product Instructions

Salmonella Express System

PRODUCT DESCRIPTION AND INTENDED USE

The 3M™ Petrifilm™ *Salmonella* Express (SALX) System is used for the rapid qualitative detection and biochemical confirmation of *Salmonella* in enriched food and food process environmental samples. The 3M Petrifilm SALX System consists of the 3M™ *Salmonella* Enrichment Base, the 3M™ *Salmonella* Enrichment Supplement, the 3M™ Petrifilm™ *Salmonella* Express (SALX) Plate, and the 3M™ Petrifilm™ *Salmonella* Express (SALX) Confirmation Disk, which are all packaged separately.

The 3M Petrifilm SALX Plate is a sample ready-to-use chromogenic culture medium system that contains a cold-water-soluble gelling agent and is selective and differential for *Salmonella*, providing a presumptive result. The 3M Petrifilm SALX Confirmation Disk contains a biochemical substrate that facilitates the biochemical confirmation of Salmonella organisms.

The 3M Petrifilm SALX Plate is used with or without the 3M Petrifilm SALX Confirmation Disk. The 3M Petrifilm SALX Confirmation Disk may be used only in conjunction with the 3M Petrifilm SALX Plate.

The 3M Petrifilm SALX System 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. For example, 3M has not documented this product for testing water, pharmaceutical, cosmetic, clinical or veterinary samples. The 3M Petrifilm SALX System has not been evaluated with all possible food products, food processes and food processing environments, testing protocols or with all possible strains of bacteria and may not detect all *Salmonella* strains. 3M has not validated the 3M Petrifilm SALX System using composite samples.

As with all test methods, the enrichment medium formulation can influence the results. The 3M Petrifilm SALX Plate has only been evaluated for use with the 3M *Salmonella* Enrichment Base, the 3M *Salmonella* Enrichment Supplement, and Rappaport-Vassiliadis R10 (R-V R10) Broth (typical formulation of R-V R10 broth follows below):

Typical Formula

Magnesium chloride (anhydrous)	13.4 grams
Sodium chloride	7.2 grams
Casein peptone	4.54 grams
Monopotassium phosphate	1.45 grams
Malachite green	0.036 grams
Demineralized water	1000.0 mL

pH 5.1 ± 0.2 @ 25°C

Adjust pH as required to meet performance standards.

3M Petrifilm SALX Plate and 3M Petrifilm SALX Confirmation Disk components are decontaminated though not sterilized. 3M Food Safety is certified to International Organization for Standardization (ISO) 9001 for design and manufacturing.

SAFETY

The user should read, understand, and follow all safety information in the instructions for the 3M Petrifilm SALX System. 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.

⚠ WARNING

Do not use the 3M Petrifilm SALX System in the diagnosis of conditions in humans or animals.

3M Petrifilm SALX System will not specifically differentiate some lactose positive *Salmonella* sp. (primarily *S. arizonae* and *S. diarizonae*) from other lactose positive organisms. Lactose positive *Salmonella* strains will appear as non-*Salmonella* (blue colonies, green colonies, blue to green colonies, and/or black colonies with or without a yellow zone and/or associated gas bubble). It has been stated that these strains account for less than 1% of the total *Salmonella* serotypes[1].

The user must train its personnel in current proper testing techniques: for example, Good Laboratory Practices or ISO 17025 [2,3].

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

- Upon receipt always verify hydrated 3M Petrifilm *Salmonella* Express Plate gel color using provided instructions.

1. Hydrate one plate with 2 mL (+/- 0.1 mL) of sterile distilled water, reverse osmosis water or Butterfield's Phosphate Diluent.
2. Wait 5-10 minutes at 20-25°C.

3. Compare color of the gel on the hydrated 3M Petrifilm SALX Plate to the red color sample on the 3M Petrifilm SALX Hydrated Plate Color Card. If the center gel area is similar to color card, proceed with method.
4. If the color of the gel on the hydrated 3M Petrifilm SALX Plate appears orange to brown, DO NOT USE. This indicates the package has been exposed to extreme high temperatures. Contact your local 3M representative.

To reduce the risks associated with a false negative result leading to the release of contaminated product and/or the possibility of false positive results requiring a retest:

- Always use the 3M Petrifilm SALX System before the expiration date
- Use the 3M Petrifilm SALX System with food samples and food process environmental samples that have been validated either by the user or by a third party
- Use the 3M Petrifilm SALX System only with surfaces, neutralizing buffers, and protocols that have been validated either by the user or by a third party
- Store the 3M Petrifilm SALX System as indicated on the package and in the product instructions
- Follow the procedures and perform the tests exactly as stated in the product instructions
- **Always use a permanent, ultra fine tip marker** to circle the characteristic presumptive *Salmonella* colonies on the top film before placing the 3M Petrifilm SALX Confirmation Disk onto the gel

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

- Perform pathogen testing in a properly equipped laboratory under the control of trained personnel
- Always follow standard good laboratory safety practices (GLP)[2], including proper containment procedures, wearing appropriate protective apparel and eye protection while handling testing materials and test samples
- Avoid direct contact with the contents of the enrichment medium and inoculated plates
- Dispose of enrichment media and inoculated plates according to all applicable government, regulatory regulations and applicable laboratory procedures
- Wear appropriate protective apparel while handling the 3M Petrifilm SALX Plate as some of the components may be considered allergenic and irritants to some individuals

To reduce the risks associated with environmental contamination:

- Follow current industry standards and local regulations for disposal of contaminated waste.

⚠ CAUTION

3M Petrifilm SALX System does not differentiate any one *Salmonella* strain from another.

Consult the Material Safety Data Sheet for additional information.

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.

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.

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 (1-800-328-1617 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.

STORAGE AND DISPOSAL

Plate storage

Upon receipt, store **unopened** 3M Petrifilm SALX Plate pouches at 2 to 8°C. They are sensitive to both moisture and light. Just prior to use, allow unopened pouches to come to room temperature (20-25°C / <60% RH) before opening. Return unused 3M Petrifilm SALX Plates to pouch. **To prevent exposure to moisture, store** opened 3M Petrifilm SALX Plate pouches in a sealed bag, protected from light, at -20 to -10°C for no longer than 4 weeks.

Confirmation Disk storage
3M Petrifilm SALX Confirmation Disks are individually packaged within a foil pouch. They are sensitive to both moisture and light. Store unopened pouches of 3M Petrifilm SALX Confirmation Disks at 2 to 8°C. Remove only those individually packaged 3M Petrifilm SALX Confirmation Disks that will be used immediately and store the remaining 3M Petrifilm SALX Confirmation Disks in the foil pouch by folding the end of the pouch over and applying adhesive tape. **To prevent exposure to moisture, do not refrigerate opened** 3M Petrifilm SALX Confirmation Disk **pouches.** Store resealed pouches in a cool (20-25°C) dry place (less than 60% RH) for no longer than 4 weeks.

Do not use 3M Petrifilm SALX Plates or 3M Petrifilm SALX Confirmation Disks that show discoloration, either before or after hydration. Expiration date and lot number are noted on each package of 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disks. The lot number is also noted on individual plates and on individual confirmation disk packages.

⚠ Disposal
After use, 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disks may contain microorganisms that may be a potential biohazard. Follow current industry standards and local regulations for disposal of contaminated waste. Consult the Material Safety Data Sheet for additional information.

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

Wear appropriate protective apparel and follow standard good laboratory safety practices (GLP)[2].

Sample Enrichment

Foods
Table 1 presents guidance for test matrix samples. It is the user's responsibility to validate alternate sampling protocols (e.g., compositing) or dilution ratios to ensure this test method meets the user's criteria.

For Low Microbial Load Foods:
Low microbial load foods have a total aerobic colony count of ≤ 10^4 colony forming units/gram. Examples include: pasteurized, cooked, or processed foods.

1. Pre-warm 3M *Salmonella* Enrichment Base with the added 3M *Salmonella* Enrichment Supplement to 41.5 ± 1.0°C (see Table 1).
2. Aseptically combine the enrichment medium and sample. For all meat and highly particulate samples, the use of stomacher filter bags is recommended. Homogenize thoroughly for 2 minutes. Incubate at 41.5 ± 1.0°C for 18-24 hours (see Table 1).

For High Microbial Load Foods:
High microbial load foods have a total aerobic colony count of > 10^4 colony forming units/gram and require the use of Rappaport-Vassiliadis R10 (R-V R10) Broth. Examples include: raw, unprocessed foods.

1. Pre-warm 3M *Salmonella* Enrichment Base with the added 3M *Salmonella* Enrichment Supplement to 41.5 ± 1.0°C (see Table 1).
2. Aseptically combine the enrichment medium and sample. For all meat and highly particulate samples, the use of stomacher filter bags is recommended. Homogenize thoroughly for 2 minutes. Incubate at 41.5 ± 1.0°C for 18-24 hours (see Table 1).
3. After primary enrichment incubation, transfer 0.1 mL of the primary enrichment into 10.0 mL Rappaport-Vassiliadis R10 (R-V R10) Broth. Incubate at 41.5 ± 1.0°C for 8-24 hours.

Environmental samples
For sample collection, use a biocide-free cellulose sponge hydrated with Dey-Engley (D/E) Neutralizing Broth. Following user established procedures, remove any remaining D/E Neutralizing Broth residue from the sampled surface.

The recommended size of the sampling area to verify the presence or absence of the pathogen on the surface is 100 cm^2 (10 cm x 10 cm or 4 in. x 4 in.)[4]. When sampling with a sponge, cover the entire area going in two directions (left to right then up and down).

1. Pre-warm 3M *Salmonella* Enrichment Base with the added 3M *Salmonella* Enrichment Supplement to 41.5 ± 1.0°C (see Table 1).
2. Aseptically combine the enrichment medium and sample. Mix thoroughly. Incubate at 41.5 ± 1.0°C for 18-24 hours.
3. If the environmental sample has a high microbial load (total aerobic colony count of > 10^4 colony forming units/sample), then after primary enrichment incubation, transfer 0.1 mL of the primary enrichment into 10.0 mL Rappaport-Vassiliadis R10 (R-V R10) Broth. Incubate at 41.5 ± 1.0°C for 8-24 hours.

If the environmental sample has a low microbial load (total aerobic colony count of ≤ 10^4 colony forming units/sample), then step 3 can be skipped.

Table 1: Sample Enrichment Protocols

Test Matrix	HIGH MICROBIAL LOAD	SAMPLE SIZE	PRIMARY ENRICHMENT			SECONDARY ENRICHMENT		
			ENRICHMENT VOLUME (mL)	Temperature (±1°C)	Enrichment Time (hr)	ENRICHMENT MEDIUM	Temperature (±1.0°C)	Enrichment Time (hr)
Foods: Raw Meat, Poultry	✓	25 g	225	41.5	18-24	R-V R10 Broth : 0.1 mL into 10.0 mL	41.5	8-24
Foods: Seafood and Fish	✓	25 g	225			R-V R10 Broth : 0.1 mL into 10.0 mL	41.5	8-24
Foods: Pasteurized Eggs		100 g	900			Not required for low microbial load foods.		
Animal Feed		375 g	3375			Not required for low microbial load foods.		
Foods: Produce	✓	25 g	225			R-V R10 Broth : 0.1 mL into 10.0 mL	41.5	8-24
Environmental	*	1 Sponge	225			Not required for low microbial load foods.		
Other Foods	*	Follow appropriate reference method for sample size and enrichment volume						

* SOME ENVIRONMENTAL AND OTHER FOOD SAMPLES MAY HAVE A HIGH MICROBIAL LOAD AND REQUIRE THE USE OF R-V R10 SECONDARY ENRICHMENT.

PLATE HYDRATION

1. Use prescribed sterile diluents to hydrate the 3M Petrifilm SALX Plates: Butterfield's Phosphate Diluent, distilled water, or reverse osmosis water.
2. Place the 3M Petrifilm SALX Plate on a flat, level surface (Figure A).
3. Lift the top film and with the pipette perpendicular dispense 2.0 mL ± 0.1 mL of sterile diluent onto the center of bottom film (Figure B). Do not close the top film before dispensing the entire 2.0 mL volume.
4. Gently roll down the top film onto the diluent to prevent trapping air bubbles (Figure C).
5. Place the 3M™ Petrifilm™ Flat Spreader (Catalog #6425) on the center of the plate. Press gently on the center of the spreader to distribute the diluent evenly. Spread the diluent over the entire 3M Petrifilm SALX Plate growth area before the gel is formed. Do not slide the spreader across the film (Figure D).
6. Remove the spreader and leave the 3M Petrifilm SALX Plate undisturbed for at least 1 minute.
7. Place 3M Petrifilm SALX Plate on a flat surface for at least 1 hour at room temperature (20-25°C / <60% RH), protected from light, to allow the gel to form. Hydrated 3M Petrifilm SALX Plates can be stored at room temperature (20-25°C / <60% RH) protected from light, for up to 8 hours before use. If hydrated 3M Petrifilm SALX Plates will not be used within 8 hours, store them in a sealed plastic bag. Protect 3M Petrifilm SALX Plates from light and store at -20 to -10°C for up to 5 days.
8. After 3M Petrifilm SALX Plates are removed from storage, allow them to warm to room temperature before use.

PLATE INOCULATION

1. Remove the enrichment medium from the incubator and agitate contents by hand.
2. Use a sterile 10 µL loop (3 mm diameter) to withdraw each sample. Use a smooth loop (one that does not have jagged edges and is not distorted) to prevent the gel surface from breaking.
3. Open the 3M Petrifilm SALX Plate and streak onto the gel (Figure E). Perform a single streak to obtain isolated colonies (Figure 1).

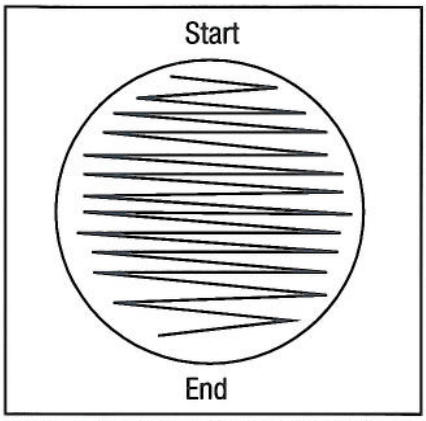


Figure 1: Streaking pattern on the 3M Petrifilm SALX Plate

4. Roll down the top film to close the 3M Petrifilm SALX Plate.
5. Using a gloved hand (while practicing good laboratory practices to avoid cross contamination and/or direct contact with the plate), gently apply a sweeping motion with even pressure onto the top film to remove any air bubbles in the inoculation area (Figure F).

PLATE INCUBATION

Incubate plates at 41.5 ± 1.0°C for 24 hours ± 2 hours in a horizontal position with the colored side up in stacks of no more than 20 plates.

INTERPRETATION

1. Remove the 3M Petrifilm SALX Plates from the incubator and proceed with visually reading the results.
2. **Using indirect back lighting may enhance reading of colony color, discrete yellow zones, and gas bubbles associated with a colony.**
3. For interpretation, visually examine the isolated colonies. See Table 2. Do not count artifact bubbles that may be present.
4. Presumptive positive *Salmonella* species are red to brown colonies with a yellow zone or associated gas bubble, or both (Figure G). An associated gas bubble is defined as being located within one colony diameter distance from the colony (see Table 2 below).

Table 2: Interpretation for Presumptive Positive *Salmonella* species

Colony Color			Colony Metabolism		Result
Red	Dark Red	Brown	Yellow zone	Gas bubble	
✓			✓		Presumptive +
✓				✓	Presumptive +
✓			✓	✓	Presumptive +
	✓		✓		Presumptive +
	✓			✓	Presumptive +
	✓		✓	✓	Presumptive +
		✓	✓		Presumptive +
		✓		✓	Presumptive +
		✓	✓	✓	Presumptive +

Non-*Salmonella* species (Figure H):

- Blue colonies, green colonies, blue to green colonies, and/or black colonies with or without a yellow zone and/or associated gas bubble are non-*Salmonella* organisms.
- Red, dark red, and brown colonies with no yellow zone and no associated gas are non-*Salmonella* organisms.
- Red, dark red, and brown colonies with a magenta zone are non-*Salmonella* organisms.

If presumptive positive *Salmonella* colonies are not present, then *Salmonella* organisms were not detected in the matrix.

Presumptive *Salmonella* species:

If presumptive positive *Salmonella* colonies are present, then perform the following steps and continue to the Biochemical Confirmation step:

a. On the 3M Petrifilm SALX Plate top film, **circle a minimum of five isolated presumptive positive *Salmonella* colonies (if present) using a permanent, ultra fine tip marker** (Figure I).

b. Biochemically confirm all *Salmonella* presumptive positive results using the 3M Petrifilm SALX Confirmation Disk. See the Biochemical Confirmation section.

c. If the 3M Petrifilm SALX Plates cannot be analyzed within 1 hour of removal from the incubator, **first circle the presumptive *Salmonella* colonies on the top film by using a permanent, ultra fine tip marker** and then place the plates in a sealed plastic bag for later analysis. Protect 3M Petrifilm SALX Plates from light and store at -20 to -10°C for no longer than 72 hours. Allow plates to warm to room temperature (20-25°C / <60% RH) before adding the disk to the plate.

⚠ **WARNING:** To reduce the risks associated with a false-negative result leading to the release of contaminated product and the possibility of false positive results requiring a retest, always use a permanent, ultra fine tip marker to circle the characteristic presumptive *Salmonella* colonies on the top film of the 3M Petrifilm SALX Plate before appropriate storage of the plate and/or before placing the 3M Petrifilm SALX Confirmation Disk onto the gel.

BIOCHEMICAL CONFIRMATION

1. Perform good laboratory practices to avoid cross contamination and/or direct contact with the 3M Petrifilm SALX Plate and/or 3M Petrifilm SALX Confirmation Disk.
2. Remove an individually packaged 3M Petrifilm SALX Confirmation Disk from its pouch and allow it to come to room temperature (20-25°C / <60% RH). Then remove the 3M Petrifilm SALX Confirmation Disk from its individual package by peeling the package to expose the 3M Petrifilm SALX Confirmation Disk's tab, grasping the tab, and removing the 3M Petrifilm SALX Confirmation Disk.
3. Lift the top film (with the already circled presumptive *Salmonella* colonies) of the 3M Petrifilm SALX Plate and insert the 3M Petrifilm SALX Confirmation Disk by rolling it onto the gel to avoid entrapping air bubbles (Figure J). Close the 3M Petrifilm SALX Plate.
4. Using a gloved hand, gently apply a sweeping motion with even pressure onto the top film to remove any air bubbles in the inoculation area and assure good contact between the gel and the 3M Petrifilm SALX Confirmation Disk (Figure K).
5. Incubate the 3M Petrifilm SALX System (plate and disk) at 41.5 ± 1.0°C for 4 - 5 hours in a horizontal position, right side up, no higher than 20 plates.
6. Remove the 3M Petrifilm SALX System from the incubator and proceed with reading the results.
   Only read the circled presumptive *Salmonella* colonies. (See Table 3):

Table 3: Interpretation of 3M Petrifilm SALX System:

Colony Color			Biochemical Confirmation Result
Green to Blue	Blue to Dark Blue	Black	
✓			Biochemically Confirmed +
	✓		Biochemically Confirmed +
		✓	Biochemically Confirmed +

Biochemically confirmed positive results:

- Colonies that are green to blue, blue to dark blue, or black or have a blue precipitate around them are biochemically confirmed positive for *Salmonella* species.

Biochemically confirmed negative results:

- Colonies that remain the same red, dark red or brown color without a blue precipitate are negative for *Salmonella* species.

7. Colonies may be subcultured for further identification. When subculturing, wear appropriate protective apparel and follow standard good laboratory safety practices (GLP)[2].
   a. Lift the top film and aseptically remove the colony either from the gel or the 3M Petrifilm SALX Confirmation Disk. If a 3M Petrifilm SALX Confirmation Disk is covering the gel, aseptically peel the disk away and then aseptically remove the colony from the gel.
   b. Streak the colony onto/into media per appropriate reference method [5,6,7].
8. If the colonies cannot be subcultured within 1 hour of plate removal from the incubator, then store the 3M Petrifilm SALX Plates for later analysis by placing in a sealed plastic bag at -20 to -10°C for no longer than 72 hours in the dark. Allow 3M Petrifilm SALX Plates to warm to room temperature (20-25°C / <60% RH) before continuing subculturing for identification.
9. After the test is complete, dispose of the 3M Petrifilm SALX Plates and 3M Petrifilm SALX Confirmation Disks in accordance with current industry standards and/or local regulations.

For further information refer to the 3M™ Petrifilm™ SALX System "Interpretation Guide." If you have questions about specific applications or procedures, please visit our website at www.3M.com/foodsafety or contact your local 3M representative or distributor.

REFERENCES

1. McDonough P.L, et al. (2000). Diagnostic and Public Health Dilemma of Lactose-Fermenting *Salmonella* enterica Serotype *Typhimurium* in Cattle in the Northeastern United States. J. Clin. Microbiol. 38:1221-1226.
2. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.
3. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
4. ISO 18593:2004. Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs.
5. US Food and Drug Administration Bacteriological Analysis Manual. Chapter 5 *Salmonella*, Section C-24.
6. US Department of Agriculture (USDA) Microbiology Laboratory Guidebook 4.05. Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg and Catfish Products.
7. ISO 6579:2002. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp.

Refer to the current versions of the standard methods listed above.

EXPLANATION OF SYMBOLS

Consult product instructions for use

LOT

The lot in a box and the hourglass symbols are symbols that represent lot number and expiration date. The hourglass is followed by a year and month which represents the expiration date (year and month: YYYY- MM). The entire line after the hourglass represents the lot number (YYYY- MM AZ).

Storage conditions


Figure A.

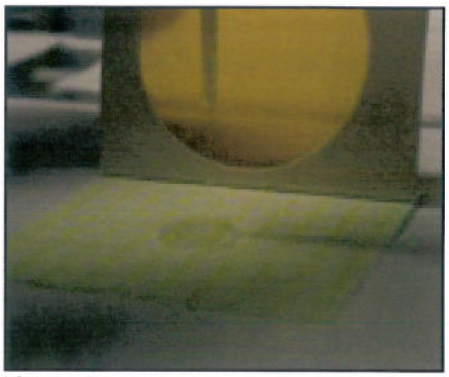


Figure B.

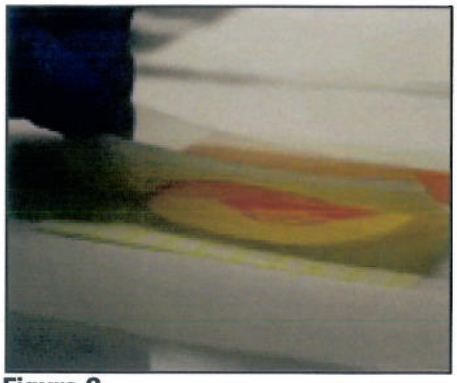


Figure C.

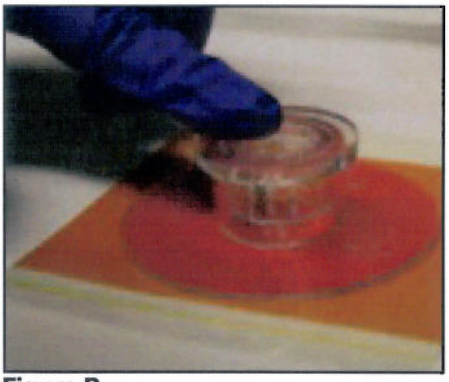


Figure D.

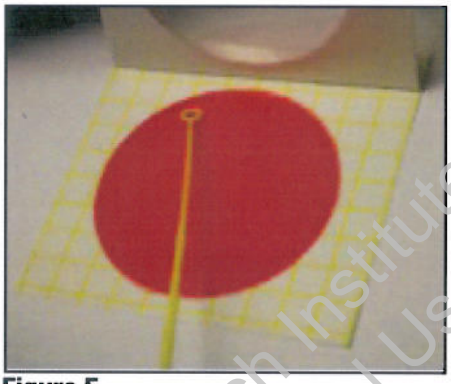


Figure E.

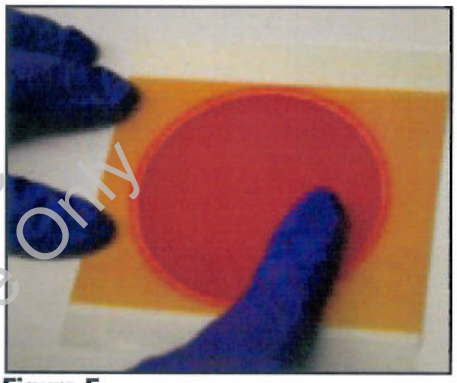


Figure F.

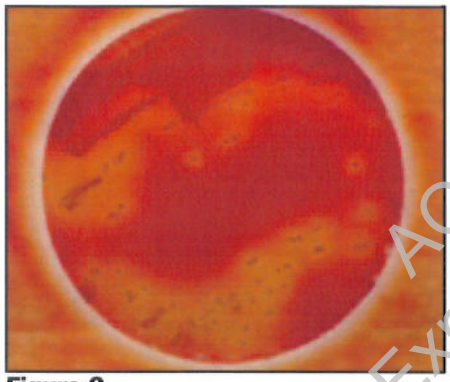


Figure G.


Figure H.

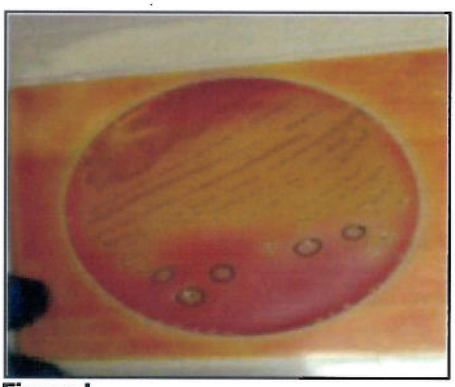


Figure I.

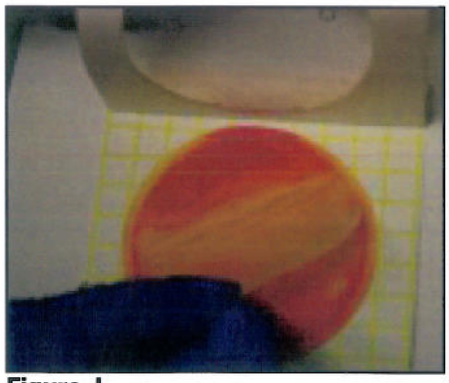


Figure J.

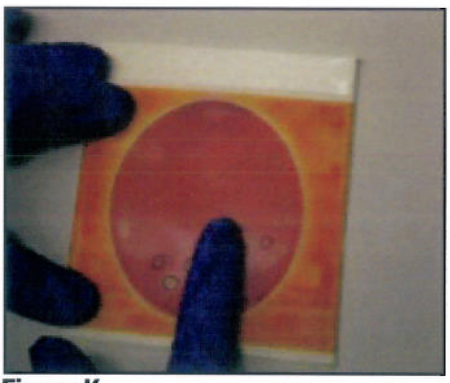


Figure K.

3M United States
3M Center
Bldg. 275-5W-05
St. Paul, MN 55144-1000
USA
1-800-328-6553

3M Canada
Post Office Box 5757
London, Ontario N6A 4T1
Canada
1-800-563-2921

3M Europe and MEA
3M Deutschland GmbH
Carl-Schurz - Strasse 1
D41453 Neuss/Germany
+49-2131-14-3000

3M Latin America
3M Center
Bldg. 275-5W-05
St. Paul, MN 55144-1000
USA
1-954-340-8263

3M Asia Pacific
No 1, Yishun Avenue 7
Singapore, 768923
65-64508869

3M Japan
3M Health Care Limited
33-1, Tamagawadai 2-chrome
Setagaya-ku, Tokyo
158-8583, Japan
81-570-011-321

3M Australia
Bldg A, 1 Rivett Road
North Ryde, NSW 2113
Australia
61 1300 363 878

3M Health Care
2510 Conway Ave
St. Paul, MN 55144 USA
www.3M.com/foodsafety

34-8709-1465-1

AOAC Official Method 2014.05
Enumeration of Yeast and Mold in Food
3M™ Petrifilm™ Rapid Yeast and Mold Count Plate
First Action 2014

[Applicable to the enumeration of yeast and mold in the following high-water activity matrices: yogurt, frozen bread dough, fermented salami, sour cream, ready-made pie, raw frozen ground beef patties (77% lean), ready-to-eat deli sandwiches, sliced apples, and the following low-water activity matrixes: raw almonds and dehydrated soup.]

Caution: After use, the diluents and 3M Petrifilm RYM Count Plates may contain microorganisms that may be a potential biohazard as several foodborne molds have the ability to produce toxic metabolites known as mycotoxins. If further identification of a mold species is required, appropriate personal protective equipment (PPE) should be used when top film is retracted and exposure to spores or mycotoxins may occur. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Material Safety Data Sheet for additional information and local regulations for disposal. For information on potential biohazards, reference *Biosafety in Microbiological and Biomedical Laboratories*, 5th Ed., Section VIII-B: Fungal Agents.

The 3M Petrifilm RYM Count plates contain chloramphenicol and chlortetracycline, potent broad spectrum antibiotic drugs commonly used in yeast and mold enumeration. The drug, when used in humans, is associated with many toxic effects. Care should be taken to avoid coming into direct contact with the gel on the plates.

See Tables **2014.05A** and **2014.05B** for a summary of results of the collaborative study. The result for each collaborating laboratory's aerobic plate count analysis for each matrix is shown in Table **2014.05C**.

See Tables 2–9 for detailed results of the collaborative study [*J. AOAC Int*. **98**, 767(2015)].

A. Principle

The 3M Petrifilm Rapid Yeast and Mold Count (RYM) Plate is a sample-ready culture medium system, which contains nutrients

Table 2014.05A. Interlaboratory study results of 3M Petrifilm RYM versus FDA-BAM and ISO 21527 methods for frozen raw ground beef patties

		3M Petrifilm RYM method				FDA-BAM/ISO 21527 methods[a]						
Matrix	Lot	*N*[b]	Mean[c]	s_r	s_R	*N*	Mean	s_r	s_R	*P*-value[d]	Difference of means	Reverse transformed mean difference[e]
Frozen raw ground beef patties												
25°C, 48 h	Control	11(0)	<1.00	—	—	11(0)	<1.00	—	—	—	—	—
	Low	11(0)	2.12	0.41	0.41	11(1)	2.07	0.36	0.38	0.5323	0.05	14.34
	Medium	11(0)	3.52	0.10	0.10	11(0)	3.47	0.09	0.11	0.1637	0.05	360.10
	High	11(0)	4.65	0.13	0.14	11(0)	4.59	0.10	0.14	0.2266	0.06	5763.84
25°C, 60 h	Control	11(0)	<1.00	—	—	11(0)	<1.00	—	—	—	—	—
	Low	11(0)	2.14	0.36[f]	0.37	11(1)	2.07	0.36	0.38	0.3773	0.07	20.55
	Medium	11(0)	3.52	0.10	0.10	11(0)	3.47	0.09	0.11	0.1573	0.05	360.10
	High	11(0)	4.65	0.14	0.15	11(0)	4.59	0.10	0.14	0.1750	0.06	5763.84
28°C, 48 h	Control	11(0)	<1.00	—	—	11(0)	<1.00	—	—	—	—	—
	Low	11(0)	2.17	0.29[f]	0.30	11(1)	2.07	0.36	0.38	0.1391	0.10	30.42
	Medium	11(0)	3.53	0.10	0.10	11(0)	3.47	0.09	0.11	0.0824	0.06	437.23
	High	11(0)	4.67	0.08[f]	0.11	11(0)	4.59	0.10	0.14	0.0966	0.08	7869.00
28°C, 60 h	Control	11(0)	<1.00	—	—	11(0)	<1.00	—	—	—	—	—
	Low	11(0)	2.16	0.29[f]	0.29	11(1)	2.07	0.36	0.38	0.1843	0.09	27.05
	Medium	11(0)	3.53	0.09	0.10	11(0)	3.47	0.09	0.11	0.1095	0.06	437.23
	High	11(0)	4.67	0.08[f]	0.11	11(0)	4.59	0.10	0.14	0.1088	0.08	7869.00

[a] Samples were analyzed by harmonized FDA-BAM Chapter 18 and ISO 21527 methods using 0.1% peptone as the sample diluent.

[b] *N* = Number of laboratories that reported complete results. Outliers are in parentheses.

[c] Log_{10} yeast and mold CFU/g.

[d] Significant difference (P <0.05).

[e] Results presented as CFU/g.

[f] Results indicate that the candidate method is more repeatable than the reference methods. s_r = Repeatability standard deviation; s_R = reproducibility standard deviation.

Table 2014.05B. Interlaboratory study results of 3M Petrifilm RYM versus FDA-BAM and ISO 21527 methods for raw almonds

		3M Petrifilm RYM method				FDA-BAM/ISO 21527 methods[a]						
Matrix	Lot	*N*[b]	Mean[c]	s_r	s_R	*N*	Mean	s_r	s_R	*P*-value[d]	Difference of means	Reverse transformed mean difference[e]
						Raw almonds						
25°C, 48 h	Control	12(0)	<1.00	—	—	12(0)	<1.00	—	—	—	—	—
	Low	14(0)	1.45	0.17[f]	0.26	14(0)	1.55	0.19	0.34	0.4165	0.10	–7.30
	Medium	14(1)	2.12	0.26	0.39	14(0)	2.21	0.20	0.24	0.3322	0.09	–30.36
	High	14(2)	3.00	0.18	0.49	14(1)	3.08	0.12	0.31	0.2833	0.08	–202.26
25°C, 60 h	Control	12(0)	<1.00	—	—	12(0)	<1.00	—	—	—	—	—
	Low	14(0)	1.53	0.23	0.28	14(0)	1.55	0.19	0.34	0.8391	0.02	–1.60
	Medium	14(0)	2.20	0.21	0.27	14(0)	2.21	0.20	0.24	0.7789	0.01	–3.69
	High	14(2)	3.04	0.18	0.41	14(1)	3.08	0.12	0.31	0.5418	0.04	–105.79
28°C, 48 h	Control	12(0)	<1.00	—	—	12(0)	<1.00	—	—	—	—	—
	Low	14(0)	1.58	0.16[f]	0.21	14(0)	1.55	0.19	0.34	0.7381	0.03	2.54
	Medium	14(0)	2.17	0.17[f]	0.29	14(0)	2.21	0.20	0.24	0.6139	0.04	–11.73
	High	14(2)	3.01	0.17	0.45	14(1)	3.08	0.12	0.31	0.3904	0.07	–178.97
28°C, 60 h	Control	12(0)	<1.00	—	—	12(0)	<1.00	—	—	—	—	—
	Low	14(0)	1.60	0.17[f]	0.20	14(0)	1.55	0.19	0.34	0.5474	0.05	4.33
	Medium	14(0)	2.21	0.17[f]	0.23	14(0)	2.21	0.20	0.24	0.9483	0.00	0.00
	High	14(2)	3.03	0.18	0.42	14(1)	3.08	0.12	0.31	0.4687	0.05	–130.75

[a] Samples were analyzed by harmonized FDA-BAM Chapter 18 and ISO 21527 methods using 0.1% peptone as the sample diluent.

[b] *N* = Number of laboratories that reported complete results. Outliers are in parentheses.

[c] Log_{10} yeast and mold CFU/g.

[d] Significant difference (P <0.05).

[e] Results presented as CFU/g.

[f] Results indicate that the candidate method is more repeatable than the reference methods. s_r = Repeatability standard deviation; s_R = reproducibility standard deviation.

supplemented with antibiotics, a cold-water-soluble gelling agent, and an indicator system that facilitates yeast and mold enumeration. 3M Petrifilm RYM Count Plates are used for the enumeration of yeast and mold in as little as 48 h in the food and beverage industries. 3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

B. Apparatus and Reagents

(**a**) *3M Petrifilm RYM Count Plate.*—25 plates/pouch; two pouches/box (3M Food Safety, St. Paul, MN, USA).

(**b**) *Sterile diluents.*—0.1% peptone water.

(**c**) *Pipets.*—Capable of 1000 µL or a serological pipet.

(**d**) *Sterile pipet tips.*—Capable of 1000 µL.

(**e**) *Stomacher.*—Seward or equivalent.

(**f**) *Filter stomacher bags.*—Seward or equivalent.

(**g**) *3M Petrifilm Flat Spreader.*

(**h**) *Incubators.*—Capable of maintaining 25 ± 1°C and 28 ± 1°C and having a solid front to maintain a dark interior.

(**i**) *Refrigerator.*—Capable of maintaining 2–8°C, for storing the 3M Petrifilm RYM Plates.

(**j**) *L-shaped spreaders.*

(**k**) *Standard colony counter or illuminated magnifier.*

C. General Instructions

(**a**) Store unopened 3M Petrifilm RYM Plate pouches refrigerated or frozen (–20 to 8°C/–4 to 46°F). Just prior to use, allow unopened pouches to come to room temperature before opening (20–25°C/<60% RH). Return unused 3M Petrifilm RYM Plates to the pouch. Seal by folding the end of the pouch over and applying adhesive tape. To prevent exposure to moisture, do not refrigerate opened pouches. Store resealed pouches in a cool dry place (20–25°C/<60% RH) for no longer than 4 weeks. It is recommended that resealed pouches of 3M Petrifilm RYM Plates be stored in a freezer if the laboratory temperature exceeds 25ºC (77ºF) and/or the laboratory is located in a region where the relative humidity exceeds 60% (with the exception of air-conditioned premises).

To store opened pouches in a freezer, place 3M Petrifilm RYM Plates in a sealable container.

Post-incubation 3M Petrifilm RYM Plates can be stored at –10 to –20°C for up to 7 days.

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

Table 2014.05C. Results of aerobic plate count for collaborating laboratories

Lab	Frozen raw ground beef, CFU/g	Raw almonds, CFU/g
1	3.8×10^2	6.0×10^1
2	1.1×10^3	6.0×10^2
3	<10	3.0×10^1
4	Not reported	Not reported
5	2.8×10^3	2.8×10^1
6	8.0×10^1	2.2×10^1
7	9.1×10^2	1.6×10^2
8	Not reported	Not reported
9	9.0×10^2	2.0×10^2
10	1.3 x 10^3	4.0×10^2
11	>2500	1.0×10^1
12	Not reported	7.0×10^1
13	9.5×10^1	1.0×10^1
14	7.3×10^2	2.3×10^2
15	3.7×10^2	8.0×10^1

Table 2014.05D. Appearance of yeast and mold on 3M Petrifilm RYM Plates

Yeast	Mold
Small colonies	Large colonies
Colonies have defined edges	Colonies have diffused edges
Pink/tan to blue/green in color	Blue/green to variable upon prolonged incubation
Colonies appear raised (3-dimensional)	Colonies appear flat
Colonies have a uniform color	Colonies have a dark center with diffused edges

D. Sample Preparation

(*1*) Aseptically prepare a 1:10 dilution of each test portion.

Dairy products.—Pipet 11 mL or weigh 11 g of sample into 99 mL sterile 0.1% peptone water. Shake 25 times to homogenize.

All other foods.—Weigh out 25 g of sample from test portion into a sterile stomacher bag and dilute with 225 mL of 0.1% peptone water; stomach at high speed to homogenize.

(*2*) Prepare 10-fold serial dilutions in 0.1% peptone water.

(*3*) Place a 3M Petrifilm RYM Count Plate on a flat, level surface for each dilution to be tested.

(*4*) Lift the top of the film. Dispense 1 mL of each dilution onto the center of the bottom film of each plate.

(*5*) Roll the film down onto the sample.

(*6*) Place the 3M Petrifilm Flat Spreader (Cat. No. 6425) on the center of the plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm RYM Count Plate growth area before the gel is formed. *Do not slide the spreader across the film.*

(*7*) Remove the spreader and leave the plate undisturbed for at least 1 min to permit the gel to form.

(*8*) Incubate the 3M Petrifilm RYM Count Plates at 25 or 28°C in a horizontal position with the clear side up in stacks of no more than 40. Enumerate plates after 48 h of incubation. If colonies appear faint, allow up to an additional 12 h of incubation time for enhanced interpretation. 3M Petrifilm RYM Count Plates can be counted using a standard colony counter with the use of a back light or an illuminated magnifier to assist with the estimated enumeration.

(*9*) Yeast colonies appear raised and small with defined edges. Colonies may appear pink/tan to blue/green in color.

(*10*) Mold colonies appear flat with a dark center and diffused edges. Colonies may appear blue/green to variable upon prolonged incubation. *See* Table **2014.05D** for yeast and mold appearance.

(*11*) The circular growth area is approximately 30 cm^2. Plates containing greater than 150 colonies can be either estimated or recorded as TNTC (too numerous to count). Estimation can only be done by counting the number of colonies in one or more representative squares and determining the average number per square. The average number can be multiplied by 30 to determine the estimated count per plate. If a more accurate count is required, the sample will need to be retested at higher dilutions. When the sample contains substantial amounts of mold, depending on the type of mold, the upper countable limit may be at user discretion.

(*12*) Food samples may occasionally show interference on the 3M Petrifilm RYM Count Plates, for example:

(*a*) Uniform blue background color (often seen from the organisms used in cultured products). These should not be counted as TNTC.

(*b*) Intense pinpoint blue specks (often seen with spices or granulated products).

(*c*) Report final results as colony-forming units/gram (CFU/g).

(*13*) If required, colonies may be isolated for further identification by direct microscopy or biochemical analysis. Lift the top film and pick the colony from the gel.

Reference: *J. AOAC Int*. **98**, 767(2015)
DOI: 10.5740/jaoacint.15-006

Posted: June 20, 2015

6. Based on your experience with the method, are there any recommended changes to the AOAC First Action method as written?	No
Would you like to provide comment(s) or feedback on additional methods? This form contains feedback for three (3) methods. You may now provide feedback on two (2) additional methods.	Additional feedback
AOAC Official Methods Number (XXXX.XX)	2014.05
Method Name or Manuscript Title	3M Petrifilm Rapid Yeast & Mold Count Plate for Enumeration
Please include the manuscript publication reference, if available. (OMA methods have manuscripts unless otherwise noted in the OMA)	JAOAC Int. 98 (3) 767-783 (2015)
1. In your experience using the method, does the method perform according to the method''s applicability as written?	Yes
2. In your experience with the method, are there any safety concerns identified while using or regarding use of the method?	No
3. In your experience with the method, are there available reference materials? Were there any concerns identified while using or regarding use of the method?	No
4. In your experience with the method, do you have data demonstrating linearity, accuracy, repeatability, LOD/LOQ?	No
If No, please explain.	ERP member w/o laboratory

5. Do you have any information that supports the reproducibility of this method as written? If so, please specify and submit information.	Yes
Please specify the information to be provided to support the reproducibility of this method as written.	Beuchat and Mann. J Food Protect 178 (1) 95-111 (2016)
6. Based on your experience with the method, are there any recommended changes to the AOAC First Action method as written?	No
Would you like to provide comment(s) or feedback on additional methods? This form contains feedback for three (3) methods. You may now provide feedback on one (1) additional method.	Additional feedback
AOAC Official Methods Number (XXXX.XX)	2014.06
Method Name or Manuscript Title	3M MDA Listeria
Please include the manuscript publication reference, if available. (OMA methods have manuscripts unless otherwise noted in the OMA)	J AOAC Int. 98 (4) 993-1002 (2015)
1. In your experience using the method, does the method perform according to the method''s applicability as written?	Yes
2. In your experience with the method, are there any safety concerns identified while using or regarding use of the method?	No
3. In your experience with the method, are there available reference materials? Were there any concerns identified while using or regarding use of the method?	No

AOAC Official Methods of Analysis (OMA) Method Use & Performance Feedback (for NON-SMPR approved O...

Submission Date	2016-07-14 13:36:53
Please indicate your perspective	Method Developer
AOAC Official Methods Number (XXXX.XX)	2014.01
Method Name or Manuscript Title	Evaluation of the 3M™ Petrifilm™ Salmonella Express System for the Detection of Salmonella Species in Selected Foods: Collaborative Study
Please include the manuscript publication reference, if available. (OMA methods have manuscripts unless otherwise noted in the OMA)	Journal of AOAC International Vol. 97, No. 6, 2014
Upload documentation to support the Safety Concerns	MethodSafetyandRiskAssessmentv2.doc
Please attach any documentation to support your feedback, if available. (The maximum file size is 20MB. Multiple files may be attached.)	3M™ Petrifilm™ Salmonella Express Final Action 9-2016.pdf
Would you like to provide comment(s) or feedback on additional methods? This form contains feedback for three (3) methods. You may now provide feedback on two (2) additional methods.	Additional feedback
AOAC Official Methods Number (XXXX.XX)	2014.05
Method Name or Manuscript Title	Evaluation of the 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate for the Enumeration of Yeast and Mold in Food: Collaborative Study, First Action 2014.05
Please include the manuscript publication reference, if available. (OMA methods have manuscripts unless otherwise noted in the OMA)	Journal of AOAC International Vol. 98, No. 3, 2015
6. Based on your experience with the method, are there any recommended changes to the AOAC First Action method as written?	No

Please attach any documentation to support your feedback, if available. (The maximum file size is 20MB. Multiple files may be attached.)	3M™ Petrifilm™ Rapid Yeast and Mold Final Action 9-2016.pdf
Would you like to provide comment(s) or feedback on additional methods? This form contains feedback for three (3) methods. You may now provide feedback on one (1) additional method.	No, thank you!
AOAC Official Methods Number (XXXX.XX)	q
Method Name or Manuscript Title	q
1. In your experience using the method, does the method perform according to the method"s applicability as written?	Yes
2. In your experience with the method, are there any safety concerns identified while using or regarding use of the method?	No
3. In your experience with the method, are there available reference materials? Were there any concerns identified while using or regarding use of the method?	No
4. In your experience with the method, do you have data demonstrating linearity, accuracy, repeatability, LOD/LOQ?	Yes
5. Do you have any information that supports the reproducibility of this method as written? If so, please specify and submit information.	No
Name	Bob Jechorek
Organization	3M Food Safety
E-mail Address	rpjechorek@mmm.com

FOOD BIOLOGICAL CONTAMINANTS

Evaluation of the 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate for the Enumeration of Yeast and Mold in Food: Collaborative Study, First Action 2014.05

PATRICK BIRD, JONATHAN FLANNERY, ERIN CROWLEY, JAMES AGIN, and DAVID GOINS
Q Laboratories, Inc., 1400 Harrison Ave, Cincinnati, OH 45214
ROBERT JECHOREK[1]
3M Food Safety Department, 3M Center – Bldg. 260-6B-01, St. Paul, MN 55144

Collaborators: K. Beers, J. Blumfield, R. Brooks, B. Brown, A. Calle, R. Dermer, B. Dieckleman, C. Fagundes, P. Fatemi, A. Gaydos, K. Gonzales, A. Hankins, T. Hirsch, W. Lee, A. Mastalerz , J. Medellin, D. Metzger, A. Morris, J. Pickett, K. Powers, J. Reed, J. Reynolds, N. Shipley, S. Spencer, J. Williams

The 3M™ Petrifilm™ Rapid Yeast and Mold (RYM) Count Plate is a simple, ready-to-use chromogenic culture method for the rapid detection and enumeration of yeast and mold in food products. The 3M Petrifilm RYM Count Plate method was compared to the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA BAM) Chapter 18, *Yeasts, Molds and Mycotoxins* and the ISO 21527:2008 *Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration for Yeast and Molds* – Part 1: *Colony Count Technique in Products with Water Activity Greater Than 0.95* and Part 2: *Colony Count Technique in Products with Water Activity Less Than or Equal to 0.95* reference methods for raw almonds and raw frozen ground beef patties (77% lean). The 3M Petrifilm RYM Count Plate method was evaluated using a paired study design in a multi-laboratory collaborative study following the current AOAC Validation Guidelines. Three target contamination levels (low, 10–100 CFU/g; medium, 100–1000 CFU/g; high 1000–10 000 CFU/g) as well as an uninoculated control level (0 CFU/g) were evaluated for each matrix. Samples evaluated by the 3M Petrifilm RYM Count Plate method were prepared in duplicate and incubated at both 25°C and 28°C. Plates at both temperatures were enumerated after 48 and 60 h of incubation. No significant difference was observed between the 3M Petrifilm RYM Count Plate method and the FDA BAM or ISO 21527 reference methods for each contamination level. No statistical differences were observed between samples analyzed by the 3M Petrifilm RYM Count Plate method (at either 25°C or 28°C) and the reference methods. No statistical significant differences were observed between enumeration of colonies at 48 and 60 h on the 3M Petrifilm RYM Count Plate method and the reference methods.

Received January 21, 2015.
The method was approved by the Expert Review Panel for Microbiology for Food and Environmental Surfaces as First Action.
The Expert Review Panel for Microbiology for Food and Environmental Surfaces invites method users to provide feedback on the First Action methods. Feedback from method users will help verify that the methods are fit for purpose and are critical to gaining global recognition and acceptance of the methods. Comments can be sent directly to the corresponding author or methodfeedback@aoac.org.
[1] Corresponding author's e-mail: rpjechorek@mmm.com
DOI: 10.5740/jaoacint.15-006

Foodborne yeasts and mold constitute a large and diverse group of microorganisms. Due to their heterotrophic nature, these microorganisms are able to adapt and survive in a wide range of environmental conditions including acidic and alkaline conditions, temperatures ranging from 5°C to 35°C and in low moisture ($<0.85_{Aw}$) products (1, 2). Many of these species cause varying degrees of food decomposition leading to substantial economic loses (2). Several foodborne molds, and possibly some yeasts, may also be hazardous to human health due to their ability to produce toxic metabolites (1). The presence of these microorganisms in food commodities can indicate contamination of the product, inadequately cleaned food processing equipment, or inadequate food storage facilities (2). Cultural enumeration of yeasts and molds can take 5 to 7 days and the need for rapid enumeration is critical to the food industry. The 3M™ Petrifilm™ Rapid Yeast and Mold Count (RYM) Plate uses a cold-water-soluble gelling agent and an indicator system to facilitate the enumeration of yeast and molds after 48 h of incubation.

The 3M Petrifilm RYM Count Plate allows for the simple and rapid enumeration of yeasts and molds in the food and beverage industries. Samples are diluted in 0.1% peptone water and a sample aliquot is plated onto the 3M Petrifilm RYM Count Plate. The sample aliquot is dispersed throughout the growth area and the plates can be incubated at 25–28°C. Enumeration of colonies occurs in as little as 48 h of incubation. If colonies appear faint, the plates can be incubated up to an additional 12 h for enhanced interpretation. The 3M Petrifilm RYM Count Plate provides gridlines to assist with the enumeration.

Prior to the collaborative study, the 3M Petrifilm RYM Count Plate method was validated according to AOAC Validation Guidelines (3) in a harmonized AOAC *Performance Tested Method*℠ (PTM) study. The objective of the PTM study was to demonstrate that the 3M Petrifilm RYM Count Plate accurately detected and enumerated yeasts and molds in selected foods as claimed by the manufacturer and that no difference in

repeatability was observed between the 3M Petrifilm RYM Count Plate method and the reference methods. For the 3M Petrifilm RYM Count Plate method PTM evaluation, 10 matrixes were evaluated: yogurt (1.5% fat), sour cream, raw almonds, sliced apples, frozen bread dough, ready-made cherry pie, ready-to-eat deli sandwiches, dehydrated chicken noodle soup, fermented salami, and raw frozen ground beef patties (77% lean).

All other PTM parameters (inclusivity, exclusivity, ruggedness, stability, and lot-to-lot variability) tested in the PTM studies satisfied the performance requirements for PTM approval. The method was awarded PTM certification No. 121301 on December 18, 2013.

The purpose of this collaborative study was to compare the 3M Petrifilm RYM Count Plate method to a harmonized version of the U.S. Food and Drug Administration (FDA) *Bacterial Analytical Manual* (BAM) Chapter 18, *Yeasts, Molds and Mycotoxins* (1) and the ISO 21527:2008 *Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Enumeration for Yeast and Molds* – Part 1: *Colony Count Technique in Products with Water Activity Greater Than 0.95* (4) and Part 2: *Colony Count Technique in Products with Water Activity Less Than or Equal to 0.95* (5) methods using 0.1% peptone water as the diluent for raw almonds and raw frozen ground beef patties (77% lean).

Collaborative Study

Study Design

In this collaborative study, two matrixes, raw frozen ground beef patties (77% lean) and raw almonds, were evaluated. The matrixes were obtained from local retailers and screened for the presence of yeast and molds by the BAM and ISO reference methods. The raw frozen ground beef patties were artificially contaminated with a yeast, *Trichosporon mucoides* American Type Culture Collection (ATCC, Manassas, VA) 201382, and the almonds with a mold, *Aspergillus aculeatus* ATCC 56925. Four separate levels of contamination, including an uninoculated control level and three levels of artificial contamination, were evaluated for each matrix. The target for the three levels of artificial contamination was as follows: low, 10–100 CFU/g; medium, 100–1000 CFU/g; high 1000–10 000 CFU/g. Two replicate samples from each of the four levels were analyzed by both the candidate and reference methods. One set of paired samples (eight total) per matrix was sent to each laboratory for analysis by the 3M Petrifilm RYM Count Plate method and the BAM and ISO reference methods. For both matrixes, collaborators were sent an additional 60 g test portion and instructed to conduct a total aerobic plate count (APC) using 3M™ Petrifilm™ Aerobic Count Plate (AOAC *Official Method* **990.12**; 6) on the day samples were received as a quality measure to verify appropriate shipping conditions.

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

Preparation of Inocula and Test Portions

The *Trichosporon mucoides* used in this evaluation was propagated in 10 mL of Brain Heart Infusion (BHI) broth from a frozen stock culture stored at –70°C at Q Laboratories, Inc. (Cincinnati, OH) The broth was incubated for 48 ± 2 h at 30 ± 1°C. Appropriate dilutions of the cultures were prepared in Butterfields Phosphate Buffer (PBW) based on previously established growth curves to obtain low, medium and high contamination levels. For the raw frozen ground beef patties, a bulk lot of the matrix was thawed, inoculated with the diluted liquid inoculum and mixed thoroughly by hand kneading to ensure an even distribution of microorganisms. The inoculated raw frozen ground beef patties samples were separated into 30 g test portions and held at –20 ± 2°C for 2 weeks when testing was initiated.

The *Aspergillus aculeatus* used in this evaluation was propagated on Potato Dextrose Agar (PDA) in a culture tissue flask from a frozen stock spore suspension stored at –70°C at Q Laboratories, Inc. The culture tissue flask was incubated for 7 days at 25 ± 1°C. The mold spore suspension was prepared by rinsing the culture tissue flask twice with 20 mL of 0.9% saline containing 0.05% Tween 20. Each suspension was centrifuged at 4000 × *g* for 10 ± 0.5 min to pellet the spores and the supernatant was decanted. The two separate spore pellets were resuspended with 2.5 mL of PBW and combined. The spore suspension was combined with a cryoprotectant, reconstituted non-fat dry milk (NFDM), homogenized by vortex to mix and placed onto a freeze dry system for 72 ± 4 h to lyophilize the culture. After the lyophilization process, appropriate dilutions of the culture were prepared in sterile NFDM powder to produce the low, medium and high contamination levels. A bulk lot of whole raw almonds were inoculated with the lyophilized inoculum and mixed thoroughly by hand mixing to ensure an even distribution of microorganisms. The inoculated almonds were separated into 30 g test portions and held at ambient temperature (24 ± 2°C) so that the organism had equilibrated for 2 weeks when testing was initiated.

The shipment conditions and hold times of the inoculated test materials were verified as a quality control measure prior to study initiation.

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 International Air Transport Association. Frozen raw ground beef patties samples were packed with cold packs to ensure the samples remained frozen (–20 ± 2°C) during shipment. Upon receipt, raw frozen ground beef patties samples were held at –20 ± 2°C until the following Monday when analysis was initiated. Raw almond samples were packed and shipped at ambient temperature. Upon receipt, samples were held at room temperature (24 ± 2°C) until the following Monday when analysis was initiated. In addition to each of the test portions and the total plate count replicate, collaborators also received

a test portion for each matrix labeled as "temperature control". Participants were instructed to record the temperature of this portion upon receipt of the shipment, document results on the Sample Receipt Confirmation form provided and fax to the study director.

Test Portion Analysis

Collaborators followed the appropriate preparation and analysis protocol according to the method specified for each matrix. For both matrixes, each collaborator received eight test portions (two high, two medium, two low, and two uninoculated replicates). For the analysis of the each matrix by the 3M Petrifilm RYM Count Plate method, a 25 g test portion was diluted with 225 mL of 0.1% peptone water and homogenized for 2 min. Ten-fold serial dilutions of each sample were prepared and a 1.0 mL aliquot of each dilution was plated onto four separate 3M Petrifilm RYM Count Plates. For each dilution, two of the plates were incubated at 25 ± 1°C and two of the plates were incubated at 28 ± 1°C. Plates were removed from incubation after 48 ± 2 h and typical yeast and mold colonies in the countable range (plates that contain up to 150 colonies) were enumerated using a standard colony counter. Plates containing greater than 150 colonies were either estimated or recorded as too numerous to count (TNTC). All plates were re-incubated at the appropriate temperatures for an additional 10–14 h (to reach a total incubation time of 60 h) and colonies were enumerated a second time in the same manner as the 48 h time point.

Both matrixes analyzed by the 3M Petrifilm RYM Count Plate method were also analyzed using the BAM and ISO reference methods in a paired study design. Serial dilutions for each sample were spread plated in triplicate onto Dichloran-rose Bengal Chloramphenicol (DRBC) agar (raw frozen ground beef patties) or Dichloran 18% Glycerol (DG18) agar (raw almonds). Agar plates were incubated for 5 days at 25 ± 1°C and typical colonies in the countable range (10–150) were enumerated using a standard colony counter. Plates containing no colonies were re-incubated for 2 days and observed for typical growth.

Statistical Analysis

Each collaborating laboratory recorded the CFU/g results for the reference methods and the 3M Petrifilm RYM Count Plate method on the electronic spreadsheet provided in the collaborator study outline. The data sheets were submitted to the study director at the end of each week of testing for analysis. The data from each duplicate plates (3M Petrifilm RYM Count Plate) or triplicate plates (BAM and ISO) were averaged. The averaged counts were converted into logarithms for data analysis. Outliers were identified using the Cochran and Grubbs' tests. A paired *t*-test was conducted to determine if the mean of replicate samples at each contamination level for each matrix was different between the 3M Petrifilm RYM Count Plate method and the reference methods. (3). A *P*-value greater than the standard alpha value of 0.05 indicated no statistical difference between the two methods. The repeatability (s_r), reproducibility (s_R), RSD_r, and RSD_R of the 3M Petrifilm RYM Count Plate method and reference methods were determined by the mean of the logarithm transformations of the counts for each contamination level of each matrix (7). A lower standard deviation value indicated a greater propensity for repeatability of a method. In addition, the difference of means and reverse transformed difference of means for each contamination level was determined (3).

AOAC Official Method 2014.05
Enumeration of Yeast and Mold in Food
3M™ Petrifilm™ Rapid Yeast and Mold Count Plate
First Action 2014

[Applicable to the enumeration of yeast and mold in the following high-water activity matrices: yogurt, frozen bread dough, fermented salami, sour cream, ready-made pie, raw frozen ground beef patties (77% lean), ready-to-eat deli sandwiches, sliced apples, and the following low-water activity matrixes: raw almonds and dehydrated soup.]

See Tables **2014.05A** and **2014.05B** for a summary of results of the collaborative study. The result for each collaborating laboratory's aerobic plate count analysis for each matrix is shown in Table **2014.05C**.

See Tables 2–9 for detailed results of the collaborative study.

A. Principle

The 3M Petrifilm Rapid Yeast and Mold Count (RYM) Plate is a sample-ready culture medium system, which contains nutrients supplemented with antibiotics, a cold-water-soluble gelling agent, and an indicator system that facilitates yeast and mold enumeration. 3M Petrifilm RYM Count Plates are used for the enumeration of yeast and mold in as little as 48 h in the food and beverage industries. 3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

B. Apparatus and Reagents

(**a**) *3M Petrifilm RYM Count Plate.*—25 plates/pouch; two pouches/box (3M Food Safety, St. Paul, MN).

(**b**) *Sterile diluents.*—0.1% peptone water.

(**c**) *Pipets.*—Capable of 1000 µL or a serological pipet.

(**d**) *Sterile pipet tips.*—Capable of 1000 µL.

(**e**) *Stomacher.*—Seward or equivalent.

(**f**) *Filter stomacher bags.*—Seward or equivalent.

(**g**) *3M Petrifilm Flat Spreader.*

(**h**) *Incubators.*—Capable of maintaining 25 ± 1°C and 28 ± 1°C and having a solid front to maintain a dark interior.

(**i**) *Refrigerator.*—Capable of maintaining 2–8°C, for storing the 3M Petrifilm RYM Plates.

(**j**) *L-shaped spreaders.*

(**k**) *Standard colony counter or illuminated magnifier.*

C. General Instructions

(**a**) Store unopened 3M Petrifilm RYM Plate pouches refrigerated or frozen (–20 to 8°C/–4 to 46°F). Just prior to use, allow unopened pouches to come to room temperature before opening (20–25°C/<60% RH). Return unused 3M Petrifilm RYM Plates to the pouch. Seal by folding the end of the pouch over and applying adhesive tape. To prevent exposure to moisture, do not refrigerate opened pouches. Store resealed pouches in a cool dry place (20–25°C/<60% RH) for no longer than 4 weeks. It is recommended that resealed pouches of 3M

Table 2014.05A. Interlaboratory study results of 3M Petrifilm RYM versus FDA-BAM and ISO 21527 methods for frozen raw ground beef patties

		3M Petrifilm RYM method				FDA-BAM/ISO 21527 methods[a]						
Matrix	Lot	N[b]	Mean[c]	s_r	s_R	N	Mean	s_r	s_R	*P*-value[d]	Difference of means	Reverse transformed mean difference[e]
Frozen raw ground beef patties												
25°C, 48 h	Control	11(0)	<1.00	—	—	11(0)	<1.00	—	—	—	—	—
	Low	11(0)	2.12	0.41	0.41	11(1)	2.07	0.36	0.38	0.5323	0.05	14.34
	Medium	11(0)	3.52	0.10	0.10	11(0)	3.47	0.09	0.11	0.1637	0.05	360.10
	High	11(0)	4.65	0.13	0.14	11(0)	4.59	0.10	0.14	0.2266	0.06	5763.84
25°C, 60 h)	Control	11(0)	<1.00	—	—	11(0)	<1.00	—	—	—	—	—
	Low	11(0)	2.14	0.36[f]	0.37	11(1)	2.07	0.36	0.38	0.3773	0.07	20.55
	Medium	11(0)	3.52	0.10	0.10	11(0)	3.47	0.09	0.11	0.1573	0.05	360.10
	High	11(0)	4.65	0.14	0.15	11(0)	4.59	0.10	0.14	0.1750	0.06	5763.84
28°C, 48 h	Control	11(0)	<1.00	—	—	11(0)	<1.00	—	—	—	—	—
	Low	11(0)	2.17	0.29[f]	0.30	11(1)	2.07	0.36	0.38	0.1391	0.10	30.42
	Medium	11(0)	3.53	0.10	0.10	11(0)	3.47	0.09	0.11	0.0824	0.06	437.23
	High	11(0)	4.67	0.08[f]	0.11	11(0)	4.59	0.10	0.14	0.0966	0.08	7869.00
28°C, 60 h	Control	11(0)	<1.00	—	—	11(0)	<1.00	—	—	—	—	—
	Low	11(0)	2.16	0.29[f]	0.29	11(1)	2.07	0.36	0.38	0.1843	0.09	27.05
	Medium	11(0)	3.53	0.09	0.10	11(0)	3.47	0.09	0.11	0.1095	0.06	437.23
	High	11(0)	4.67	0.08[f]	0.11	11(0)	4.59	0.10	0.14	0.1088	0.08	7869.00

[a] Samples were analyzed by harmonized FDA-BAM Chapter 18 and ISO 21527 methods using 0.1% peptone as the sample diluent.

[b] N = Number of laboratories that reported complete results. Outliers are in parentheses.

[c] Log_{10} yeast and mold CFU/g.

[d] Significant difference (P <0.05).

[e] Results presented as CFU/g.

[f] Results indicate that the candidate method is more repeatable than the reference methods. s_r = Repeatability standard deviation; s_R = reproducibility standard deviation.

Petrifilm RYM Plates be stored in a freezer if the laboratory temperature exceeds 25ºC (77ºF) and/or the laboratory is located in a region where the relative humidity exceeds 60% (with the exception of air-conditioned premises).

To store opened pouches in a freezer, place 3M Petrifilm RYM Plates in a sealable container.

Post-incubation 3M Petrifilm RYM Plates can be stored at –10 to –20°C for up to 7 days.

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

D. Safety Precautions

After use, the diluents and 3M Petrifilm RYM Count Plates may contain microorganisms that may be a potential biohazard as several foodborne molds have the ability to produce toxic metabolites known as mycotoxins. If further identification of a mold species is required, appropriate personal protective equipment (PPE) should be used when top film is retracted and exposure to spores or mycotoxins may occur. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Material Safety Data Sheet for additional information and local regulations for disposal. For information on potential biohazards, reference *Biosafety in Microbiological and Biomedical Laboratories*, 5th Ed., Section VIII-B: Fungal Agents.

The 3M Petrifilm RYM Count plates contain chloramphenicol and chlortetracycline, potent broad spectrum antibiotic drugs commonly used in yeast and mold enumeration. The drug, when used in humans, is associated with many toxic effects. Care should be taken to avoid coming into direct contact with the gel on the plates.

E. Sample Preparation

(*1*) Aseptically prepare a 1:10 dilution of each test portion.

Dairy products.—Pipet 11 mL or weigh 11 g of sample into 99 mL sterile 0.1% peptone water. Shake 25 times to homogenize.

All other foods.—Weigh out 25 g of sample from test portion into a sterile stomacher bag and dilute with 225 mL of 0.1% peptone water; stomach at high speed to homogenize.

(*2*) Prepare 10-fold serial dilutions in 0.1% peptone water.

(*3*) Place a 3M Petrifilm RYM Count Plate on a flat, level surface for each dilution to be tested.

Table 2014.05B. Interlaboratory study results of 3M Petrifilm RYM versus FDA-BAM and ISO 21527 methods for raw almonds

		3M Petrifilm RYM method				FDA-BAM/ISO 21527 methods[a]						
Matrix	Lot	N[b]	Mean[c]	s_r	s_R	N	Mean	s_r	s_R	*P*-value[d]	Difference of means	Reverse transformed mean difference[e]
Raw almonds												
25°C, 48 h	Control	12(0)	<1.00	—	—	12(0)	<1.00	—	—	—	—	—
	Low	14(0)	1.45	0.17[f]	0.26	14(0)	1.55	0.19	0.34	0.4165	0.10	–7.30
	Medium	14(1)	2.12	0.26	0.39	14(0)	2.21	0.20	0.24	0.3322	0.09	–30.36
	High	14(2)	3.00	0.18	0.49	14(1)	3.08	0.12	0.31	0.2833	0.08	–202.26
25°C, 60 h	Control	12(0)	<1.00	—	—	12(0)	<1.00	—	—	—	—	—
	Low	14(0)	1.53	0.23	0.28	14(0)	1.55	0.19	0.34	0.8391	0.02	–1.60
	Medium	14(0)	2.20	0.21	0.27	14(0)	2.21	0.20	0.24	0.7789	0.01	–3.69
	High	14(2)	3.04	0.18	0.41	14(1)	3.08	0.12	0.31	0.5418	0.04	–105.79
28°C, 48 h	Control	12(0)	<1.00	—	—	12(0)	<1.00	—	—	—	—	—
	Low	14(0)	1.58	0.16[f]	0.21	14(0)	1.55	0.19	0.34	0.7381	0.03	2.54
	Medium	14(0)	2.17	0.17[f]	0.29	14(0)	2.21	0.20	0.24	0.6139	0.04	–11.73
	High	14(2)	3.01	0.17	0.45	14(1)	3.08	0.12	0.31	0.3904	0.07	–178.97
28°C, 60 h	Control	12(0)	<1.00	—	—	12(0)	<1.00	—	—	—	—	—
	Low	14(0)	1.60	0.17[f]	0.20	14(0)	1.55	0.19	0.34	0.5474	0.05	4.33
	Medium	14(0)	2.21	0.17[f]	0.23	14(0)	2.21	0.20	0.24	0.9483	0.00	0.00
	High	14(2)	3.03	0.18	0.42	14(1)	3.08	0.12	0.31	0.4687	0.05	–130.75

[a] Samples were analyzed by harmonized FDA-BAM Chapter 18 and ISO 21527 methods using 0.1% peptone as the sample diluent.

[b] N = Number of laboratories that reported complete results. Outliers are in parentheses.

[c] Log_{10} yeast and mold CFU/g.

[d] Significant difference (P <0.05).

[e] Results presented as CFU/g.

[f] Results indicate that the candidate method is more repeatable than the reference methods. s_r = Repeatability standard deviation; s_R = reproducibility standard deviation.

(*4*) Lift the top of the film. Dispense 1 mL of each dilution onto the center of the bottom film of each plate.

(*5*) Roll the film down onto the sample.

(*6*) Place the 3M Petrifilm Flat Spreader (Cat. No. 6425) on the center of the plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm RYM Count Plate growth area before the gel is formed. *Do not slide the spreader across the film.*

(*7*) Remove the spreader and leave the plate undisturbed for at least 1 min to permit the gel to form.

(*8*) Incubate the 3M Petrifilm RYM Count Plates at 25 or 28°C in a horizontal position with the clear side up in stacks of no more than 40. Enumerate plates after 48 h of incubation. If colonies appear faint, allow up to an additional 12 h of incubation time for enhanced interpretation. 3M Petrifilm RYM Count Plates can be counted using a standard colony counter with the use of a back light or an illuminated magnifier to assist with the estimated enumeration.

(*9*) Yeast colonies appear raised and small with defined edges. Colonies may appear pink/tan to blue/green in color.

(*10*) Mold colonies appear flat with a dark center and diffused edges. Colonies may appear blue/green to variable

Table 2014.05C. Results of aerobic plate count for collaborating laboratories

Lab	Frozen raw ground beef, CFU/g	Raw almonds, CFU/g
1	3.8×10^2	6.0×10^1
2	1.1×10^3	6.0×10^2
3	<10	3.0×10^1
4	Not reported	Not reported
5	2.8×10^3	2.8×10^1
6	8.0×10^1	2.2×10^1
7	9.1×10^2	1.6×10^2
8	Not reported	Not reported
9	9.0×10^2	2.0×10^2
10	1.3×10^3	4.0×10^2
11	>2500	1.0×10^1
12	Not reported	7.0×10^1
13	9.5×10^1	1.0×10^1
14	7.3×10^2	2.3×10^2
15	3.7×10^2	8.0×10^1

Table 2014.05D. Appearance of yeast and mold on 3M Petrifilm RYM Plates

Yeast	Mold
Small colonies	Large colonies
Colonies have defined edges	Colonies have diffused edges
Pink/tan to blue/green in color	Blue/green to variable upon prolonged incubation
Colonies appear raised (3-dimensional)	Colonies appear flat
Colonies have a uniform color	Colonies have a dark center with diffused edges

upon prolonged incubation. *See* Table **2014.05D** for yeast and mold appearance.

(*11*) The circular growth area is approximately 30 cm^2. Plates containing greater than 150 colonies can be either estimated or recorded as TNTC (too numerous to count). Estimation can only be done by counting the number of colonies in one or more representative squares and determining the average number per square. The average number can be multiplied by 30 to determine the estimated count per plate. If a more accurate count is required, the sample will need to be retested at higher dilutions. When the sample contains substantial amounts of mold, depending on the type of mold, the upper countable limit may be at user discretion.

(*12*) Food samples may occasionally show interference on the 3M Petrifilm RYM Count Plates, for example:

(*a*) Uniform blue background color (often seen from the organisms used in cultured products). These should not be counted as TNTC.

(*b*) Intense pinpoint blue specks (often seen with spices or granulated products).

(*c*) Report final results as colony-forming units/gram (CFU/g).

(*13*) If required, colonies may be isolated for further identification by direct microscopy or biochemical analysis. Lift the top film and pick the colony from the gel.

Results of the Collaborative Study

In this collaborative study, the 3M Petrifilm RYM Count Plate method was compared to two reference methods for enumerating total yeast and mold: FDA BAM Chapter 18 and ISO 21527 Part 1 or Part 2. A total of 15 laboratories throughout the United States participated, with 11 laboratories submitting valid data for the frozen raw ground beef patties and 14 laboratories submitting valid data for the raw almonds as presented in Table 1. For the frozen raw ground beef patties, laboratories 4 (failed to enumerate 3M Petrifilm RYM Count Plates at 60 h), 6 and 8 (plated the reference method samples in duplicate and not the required triplicate plating) and 12 [plated the 3M Petrifilm RYM Count Plates and reference method plates on two different days (enrichments were held at 2–8°C overnight and the 3M Petrifilm RYM Plates were plated 24 h after the reference method plates)] reported deviations from the protocol and were therefore excluded from statistical analysis. For the raw almonds, laboratory 4 reported a deviation from the protocol (failure to enumerate 3M Petrifilm RYM Count Plates at 60 h) and was therefore excluded from the statistical analysis.

The 3M Petrifilm RYM Count Plate results along with FDA BAM and ISO results reported by each laboratory were converted to logarithmic values for statistical analysis and were plotted using a Youden's plot (*see* Figures 1–4). The $\log_{10}$ individual lab results are presented in Tables 2–9. Using the Youden's plots, an initial review of the data to determine outliers was conducted by observing the mean replicate results for each laboratory at each contamination level for each matrix. The transformed data was than statistically analyzed for outliers by the Cochran and Grubb's tests. No evidence of physical cause or suspicion of cause was noted, so all outliers identified were included in the statistical analysis. A paired *t*-test, the difference of means, and the reverse transformed mean difference were calculated on each contamination level for each matrix to determine if a statistically significant difference existed between the methods. Repeatability (s_r), s_R, RSD_r, and RSD_R were determined for each contamination level for both the 3M Petrifilm RYM Count Plate and FDA BAM/ISO 21527 methods. The results of the interlaboratory data analyses are presented in Tables **2014.05A** and **2014.05B**. The result for each collaborating laboratory's aerobic plate count analysis for each matrix is presented in Table **2014.05C**.

Frozen Raw Ground Beef Patties (77% Lean)

Frozen raw ground beef patties test portions were inoculated at a low, medium and high contamination level and were analyzed (Tables 2–5) for the enumeration of yeast and mold. Uninoculated controls were included in each analysis. Fifteen laboratories participated in the analysis of this matrix.

3M RYM Count Plate incubated at 25°C and enumerated at 48 h.—The mean $\log_{10}$ counts of the 3M Petrifilm RYM Count Plate and FDA BAM/ISO 21527 results were compared

Table 1. Participation of each collaborating laboratory[a]

Laboratory	Raw ground beef	Almonds
1	Y	Y
2	Y	Y
3	Y	Y
4	Y[b]	Y[b]
5	Y	Y
6	Y[b]	Y
7	Y	Y
8	Y[b]	Y
9	Y	Y
10	Y	Y
11	Y	Y
12	Y[b]	Y
13	Y	Y
14	Y	Y
15	Y	Y

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

[b] Results were not used in statistical analysis due to deviation of testing protocol or laboratory error.

statistically. One laboratory in the FDA BAM/ISO 21527 low contamination level was identified as an outlier as defined by the Cochran's tests for outliers. However, no evidence of physical cause or suspicion of cause was noted and it was determined that they would be included in the statistical analysis.

There were no statistically significant differences at the 5% level in log transformed means between the 3M Petrifilm RYM Count Plate method and FDA BAM/ISO 21257 methods, at any of the three contamination levels.

3M RYM Count Plate incubated at 25°C and enumerated at 60 h.—The mean $\log_{10}$ counts of the 3M Petrifilm RYM Count Plate and FDA BAM/ISO 21527 results were compared statistically. One laboratory in the FDA BAM/ISO 21527 low contamination level was identified as an outlier as defined by the Cochran's tests for outliers. However, no evidence of physical cause or suspicion of cause was noted and it was determined that they would be included in the statistical analysis.

There were no statistically significant differences at the 5% level in log transformed means between the 3M Petrifilm RYM Count Plate method and FDA BAM/ISO 21257 methods, at any of the three contamination levels.

3M RYM Count Plate incubated at 28°C and enumerated at 48 h.—The mean $\log_{10}$ counts of the 3M Petrifilm RYM Count Plate and FDA BAM/ISO 21527 results were compared statistically. One laboratory in the FDA BAM/ISO 21527 low contamination level was identified as an outlier as defined by the Cochran's tests for outliers. However, no evidence of physical cause or suspicion of cause was noted and it was determined that they would be included in the statistical analysis.

There were no statistically significant differences at the 5% level in log transformed means between the 3M Petrifilm RYM Count Plate method and FDA BAM/ISO 21257 methods, at any of the three contamination levels.

3M RYM Count Plate incubated at 28°C and enumerated at 60 h.—The mean $\log_{10}$ counts of the 3M Petrifilm RYM Count Plate and FDA BAM/ISO 21527 results were compared statistically. One laboratory in the FDA BAM/ISO 21527 low contamination level was identified as an outlier as defined by the Cochran's tests for outliers. However, no evidence of physical cause or suspicion of cause was noted and it was determined that they would be included in the statistical analysis.

There were no statistically significant differences determined between the 3M Petrifilm RYM Count Plate and FDA BAM/ISO 21527 methods at the 95% level or between the differences of means at all three contamination levels.

Raw Almonds

Raw almond test portions were inoculated at a low, medium and high contamination level and were analyzed (Tables 6–9) for the enumeration of yeast and mold. Uninoculated controls were included in each analysis. Fifteen laboratories participated in the analysis of this matrix.

3M RYM Count Plate incubated at 25°C and enumerated at 48 h.—The mean $\log_{10}$ counts of the 3M Petrifilm RYM Count Plate and FDA BAM/ISO 21527 results were compared statistically. One laboratory was identified in the 3M Petrifilm RYM Count Plate medium contamination results as an outlier by the Cochran's test for outliers, one laboratory was identified in the FDA BAM/ISO 21527 high contamination level as an outlier by the Single Grubbs' test, and two laboratories in the 3M Petrifilm RYM Count Plate high contamination level were identified as an outlier by the double Grubbs' test for outliers. However, no evidence of physical cause or suspicion of cause was noted and it was determined that they would be included in the statistical analysis.

There were no statistically significant differences at the 5% level in log transformed means between the 3M Petrifilm RYM Count Plate method and FDA BAM/ISO 21257 methods, at any of the three contamination levels.

3M RYM Count Plate incubated at 25°C and enumerated at 60 h.—The mean $\log_{10}$ counts of the 3M Petrifilm RYM Count Plate and FDA BAM/ISO 21527 results were compared statistically. One laboratory was identified in the FDA BAM/ISO 21527 high contamination level as an outlier by the Single Grubbs' test and two laboratories in the 3M Petrifilm RYM Count Plate high contamination level were identified as an outlier by the double Grubbs' test for outliers. However, no evidence of physical cause or suspicion of cause was noted and it was determined that they would be included in the statistical analysis.

There were no statistically significant differences determined between the 3M Petrifilm RYM Count Plate method and FDA BAM/ISO 21527 methods at the 95% level or between the differences of means at all three contamination levels.

3M RYM Count Plate incubated at 28°C and enumerated at 48 h.—The mean $\log_{10}$ counts of the 3M Petrifilm RYM Count Plate and FDA BAM/ISO 21527 results were compared statistically. One laboratory was identified in the FDA BAM/ISO 21527 high contamination level as an outlier by the Single Grubbs' test and two laboratories in the 3M Petrifilm RYM Count Plate high contamination level were identified as an outlier by the double Grubbs' test for outliers. However, no evidence of physical cause or suspicion of cause was noted and it was determined that they would be included in the statistical analysis.

There were no statistically significant differences at the 5% level in log transformed means between the 3M Petrifilm RYM Count Plate and FDA BAM/ISO 21257 methods, at any of the three contamination levels.

3M RYM Count Plate incubated at 28°C and enumerated at 60 h.—The mean $\log_{10}$ counts of the 3M Petrifilm RYM Count Plate and FDA BAM/ISO 21527 results were compared statistically. One laboratory was identified in the FDA BAM/ISO 21527 high contamination level as an outlier by the Single Grubbs' test and two laboratories in the 3M Petrifilm RYM Count Plate high contamination level were identified as an outlier by the double Grubbs' test for outliers. However, no evidence of physical cause or suspicion of cause was noted and it was determined that they would be included in the statistical analysis.

There were no statistically significant differences at the 5% level in log transformed means between the 3M Petrifilm RYM Count Plate method and FDA BAM/ISO 21257 methods, at any of the three contamination levels.

Discussion

No negative feedback was reported to the study directors from the collaborating laboratories in regards to the performance of the 3M Petrifilm RYM Count Plate. A few laboratories indicated difficulty in spreading the test portion liquid aliquot over the entire surface of the plate before the sample began to

Table 2. Total yeast and mold log_{10} counts for frozen raw ground beef patties by 3M Petrifilm RYM Count Plate after 48 h incubation at 25°C versus FDA BAM (ref. 1) and ISO 21527 (ref. 4) reference methods[a]

	Uninoculated control				Low				Medium				High			
	Petrifilm RYM		FDA BAM/ISO 21527[b]		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527	
Lab	A[c]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	<1.00	<1.00	<1.00	<1.00	2.18	1.78	2.23	1.60	3.45	3.38	3.49	3.32	4.64	4.62	4.32	4.54
2	<1.00	<1.00	<1.00	<1.00	2.43	2.48	2.43	2.83	3.62	3.71	3.49	3.45	4.62	4.73	4.53	4.52
3	<1.00	<1.00	<1.00	<1.00	2.34	2.30	2.41	2.32	3.52	3.30	3.63	3.53	4.64	4.40	4.82	4.81
4[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	<1.00	<1.00	<1.00	<1.00	1.95	1.78	1.70	2.00	3.41	3.53	3.30	3.41	4.43	4.51	4.46	4.64
6[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7	<1.00	<1.00	<1.00	<1.00	2.23	2.36	1.80	1.82	3.51	3.56	3.36	3.61	4.79	4.32	4.43	4.49
8[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	<1.00	<1.00	<1.00	<1.00	2.45	2.23	2.28	2.23	3.56	3.60	3.51	3.45	4.72	4.73	4.57	4.79
10	<1.00	<1.00	<1.00	<1.00	2.20	2.20	2.11	2.00	3.60	3.45	3.45	3.43	4.76	4.61	4.66	4.40
11	<1.00	<1.00	<1.00	<1.00	2.36	1.00	2.40	1.90	3.57	3.66	3.49	3.54	4.68	4.56	4.49	4.59
12[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	<1.00	<1.00	<1.00	<1.00	2.45	2.08	2.00	1.67	3.49	3.51	3.52	3.48	4.75	4.89	4.67	4.77
14	<1.00	<1.00	<1.00	<1.00	2.20	1.00	2.34[e]	1.00[e]	3.41	3.67	3.57	3.70	4.70	4.60	4.65	4.62
15	<1.00	<1.00	<1.00	<1.00	2.30	2.28	2.15	2.23	3.38	3.53	3.38	3.23	4.72	4.85	4.61	4.66

[a] Log_{10} yeast and mold CFU/g.

[b] FDA BAM and ISO 21257 reference method replicates were enumerated at 5 and 7 days (uninoculated test portions) of incubation at 25°C

[c] A and B = Indicated duplicate test portions.

[d] Laboratory data not included in statistical analysis for all contamination levels.

[e] Cochran's outlier.

Table 3. Total yeast and mold log_{10} counts for frozen raw ground beef patties by 3M Petrifilm RYM Count Plate after 60 h incubation at 25°C versus FDA BAM (ref. 1) and ISO 21527 (ref. 4) reference methods[a]

	Uninoculated control				Low				Medium				High			
	Petrifilm RYM		FDA BAM/ISO 21527[b]		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527	
Lab	A[c]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	<1.00	<1.00	<1.00	<1.00	2.18	1.78	2.23	1.60	3.45	3.40	3.49	3.32	4.62	4.61	4.32	4.54
2	<1.00	<1.00	<1.00	<1.00	2.43	2.48	2.43	2.83	3.62	3.71	3.49	3.45	4.62	4.73	4.53	4.52
3	<1.00	<1.00	<1.00	<1.00	2.30	2.26	2.41	2.32	3.56	3.30	3.63	3.53	4.68	4.40	4.82	4.81
4[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	<1.00	<1.00	<1.00	<1.00	1.95	1.78	1.70	2.00	3.41	3.53	3.30	3.41	4.43	4.51	4.46	4.64
6[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7	<1.00	<1.00	<1.00	<1.00	2.23	2.36	1.80	1.82	3.51	3.56	3.36	3.61	4.79	4.32	4.43	4.49
8[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	<1.00	<1.00	<1.00	<1.00	2.45	2.23	2.28	2.23	3.56	3.58	3.51	3.45	4.72	4.75	4.57	4.79
10	<1.00	<1.00	<1.00	<1.00	2.20	2.20	2.11	2.00	3.60	3.45	3.45	3.43	4.76	4.61	4.66	4.40
11	<1.00	<1.00	<1.00	<1.00	2.36	1.30	2.40	1.90	3.56	3.57	3.49	3.54	4.68	4.56	4.49	4.59
12[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	<1.00	<1.00	<1.00	<1.00	2.45	2.32	2.00	1.67	3.51	3.51	3.52	3.48	4.76	4.92	4.67	4.77
14	<1.00	<1.00	<1.00	<1.00	2.20	1.00	2.34[e]	1.00[e]	3.41	3.68	3.57	3.70	4.70	4.62	4.65	4.62
15	<1.00	<1.00	<1.00	<1.00	2.30	2.28	2.15	2.23	3.38	3.53	3.38	3.23	4.72	4.85	4.61	4.66

[a] Log_{10} yeast and mold CFU/g.

[b] FDA BAM and ISO 21257 reference method replicates were enumerated at 5 and 7 days (uninoculated test portions) of incubation at 25°C.

[c] A and B = Indicated duplicate test portions.

[d] Laboratory data not included in statistical analysis for all contamination levels.

[e] Cochran's outlier.

Table 4. Total yeast and mold log_{10} counts for frozen raw ground beef patties by 3M Petrifilm RYM Count Plate after 48 h incubation at 28°C versus FDA BAM (ref. 1) and ISO 21527 (ref. 4) reference methods[a]

	Uninoculated control				Low				Medium				High			
	Petrifilm RYM		FDA BAM/ISO 21527[b]		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527	
Lab	A[c]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	<1.00	<1.00	<1.00	<1.00	2.18	1.48	2.23	1.60	3.49	3.38	3.49	3.32	4.64	4.66	4.32	4.54
2	<1.00	<1.00	<1.00	<1.00	2.38	2.57	2.43	2.83	3.68	3.62	3.49	3.45	4.64	4.68	4.53	4.52
3	<1.00	<1.00	<1.00	<1.00	2.30	2.45	2.41	2.32	3.66	3.30	3.63	3.53	4.68	4.40	4.82	4.81
4[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	<1.00	<1.00	<1.00	<1.00	1.70	2.04	1.70	2.00	3.56	3.51	3.30	3.41	4.53	4.53	4.46	4.64
6[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7	<1.00	<1.00	<1.00	<1.00	2.18	2.26	1.80	1.82	3.46	3.61	3.36	3.61	4.65	4.54	4.43	4.49
8[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	<1.00	<1.00	<1.00	<1.00	2.30	2.26	2.28	2.23	3.59	3.54	3.51	3.45	4.66	4.75	4.57	4.79
10	<1.00	<1.00	<1.00	<1.00	2.20	2.15	2.11	2.00	3.60	3.45	3.45	3.43	4.79	4.60	4.66	4.40
11	<1.00	<1.00	<1.00	<1.00	2.48	1.70	2.40	1.90	3.56	3.64	3.49	3.54	4.73	4.66	4.49	4.59
12[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	<1.00	<1.00	<1.00	<1.00	2.52	2.18	2.00	1.67	3.41	3.41	3.52	3.48	4.80	4.92	4.67	4.77
14	<1.00	<1.00	<1.00	<1.00	2.23	1.60	2.34[e]	1.00[e]	3.60	3.59	3.57	3.70	4.71	4.67	4.65	4.62
15	<1.00	<1.00	<1.00	<1.00	2.34	2.20	2.15	2.23	3.49	3.41	3.38	3.23	4.72	4.73	4.61	4.66

[a] Log_{10} yeast and mold CFU/g.

[b] FDA BAM and ISO 21257 reference method replicates were enumerated at 5 and 7 days (uninoculated test portions) of incubation at 25°C.

[c] A and B = Indicated duplicate test portions.

[d] Laboratory data not included in statistical analysis for all contamination levels.

[e] Cochran's outlier.

Table 5. Total yeast and mold log_{10} counts for frozen raw ground beef patties by 3M Petrifilm RYM Count Plate after 60 h incubation at 28°C versus FDA BAM (ref. 1) and ISO 21527 (ref. 4) reference methods[a]

	Uninoculated control				Low				Medium				High			
	Petrifilm RYM		FDA BAM/ISO 21527[b]		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527	
Lab	A[c]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	<1.00	<1.00	<1.00	<1.00	2.18	1.48	2.23	1.60	3.52	3.38	3.49	3.32	4.64	4.62	4.32	4.54
2	<1.00	<1.00	<1.00	<1.00	2.38	2.57	2.43	2.83	3.69	3.62	3.49	3.45	4.65	4.68	4.53	4.52
3	<1.00	<1.00	<1.00	<1.00	2.30	2.26	2.41	2.32	3.59	3.30	3.63	3.53	4.65	4.40	4.82	4.81
4[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	<1.00	<1.00	<1.00	<1.00	1.70	2.04	1.70	2.00	3.56	3.51	3.30	3.41	4.53	4.53	4.46	4.64
6[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7	<1.00	<1.00	<1.00	<1.00	2.18	2.26	1.80	1.82	3.46	3.61	3.36	3.61	4.65	4.54	4.43	4.49
8[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	<1.00	<1.00	<1.00	<1.00	2.30	2.26	2.28	2.23	3.59	3.54	3.51	3.45	4.66	4.75	4.57	4.79
10	<1.00	<1.00	<1.00	<1.00	2.20	2.15	2.11	2.00	3.60	3.45	3.45	3.43	4.79	4.60	4.66	4.40
11	<1.00	<1.00	<1.00	<1.00	2.48	1.70	2.40	1.90	3.56	3.64	3.49	3.54	4.73	4.66	4.49	4.59
12[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	<1.00	<1.00	<1.00	<1.00	2.54	2.18	2.00	1.67	3.41	3.41	3.52	3.48	4.81	4.92	4.67	4.77
14	<1.00	<1.00	<1.00	<1.00	2.23	1.60	2.34[e]	1.00[e]	3.60	3.59	3.57	3.70	4.71	4.68	4.65	4.62
15	<1.00	<1.00	<1.00	<1.00	2.34	2.20	2.15	2.23	3.49	3.41	3.38	3.23	4.72	4.73	4.61	4.66

[a] Log_{10} yeast and mold CFU/g.

[b] FDA BAM and ISO 21257 reference method replicates were enumerated at 5 and 7 days (uninoculated test portions) of incubation at 25°C.

[c] A and B = Indicated duplicate test portions;

[d] Laboratory data not included in statistical analysis for all contamination levels.

[e] Cochran's outlier.

Table 6. Total yeast and mold log_{10} counts for raw almonds by 3M Petrifilm RYM Count Plate after 48 h incubation at 25°C versus FDA BAM (ref. 1) and ISO 21527 (ref. 4) reference methods[a]

	Uninoculated control				Low				Medium				High			
	Petrifilm RYM		FDA BAM/ISO 21527[b]		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527	
Lab	A[c]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	<1.00	<1.00	1.00[d]	<1.00	1.30	1.30	1.78	1.85	1.60	1.30	2.28	1.95	1.78[e]	1.90[e]	2.04[f]	2.40[f]
2	1.30[d]	1.00[d]	1.30[d]	1.85[d]	1.78	1.60	2.04	1.70	1.30	1.90	2.11	2.34	1.95[e]	2.15[e]	2.64	2.84
3	<1.00	<1.00	<1.00	<1.00	1.30	1.30	1.60	1.60	2.34	2.28	2.38	2.18	3.56	3.11	3.30	3.11
4[g]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	<1.00	<1.00	<1.00	<1.00	1.30	1.00	1.78	1.95	2.30	2.15	2.30	2.45	3.41	3.04	3.18	3.18
6	<1.00	<1.00	<1.00	<1.00	1.48	1.30	2.20	1.78	1.00[h]	2.00[h]	2.34	2.20	3.08	3.15	3.36	3.28
7	<1.00	<1.00	<1.00	<1.00	1.78	1.30	1.70	1.30	2.45	2.20	2.43	2.11	3.20	2.90	3.20	3.11
8	<1.00	<1.00	<1.00	<1.00	1.85	1.95	1.00	1.00	2.49	2.45	2.00	2.08	3.26	3.51	3.00	3.08
9	<1.00	<1.00	<1.00	<1.00	1.30	1.30	1.48	1.00	2.53	2.46	2.56	2.53	3.08	3.00	3.04	3.00
10	<1.00	<1.00	<1.00	<1.00	1.30	1.00	1.30	1.00	2.08	2.43	2.00	2.56	3.26	3.11	3.30	3.04
11	<1.00	<1.00	<1.00	<1.00	1.00	1.48	1.48	1.60	2.00	2.08	1.95	1.90	3.08	3.04	3.15	3.08
12	<1.00	<1.00	<1.00	<1.00	1.60	1.78	1.30	1.00	2.08	2.30	2.00	2.00	3.60	3.36	3.40	3.08
13	<1.00	<1.00	<1.00	<1.00	1.48	1.30	1.48	1.30	2.00	2.20	1.78	1.95	2.90	2.90	2.90	2.95
14	<1.00	<1.00	<1.00	<1.00	1.48	1.60	1.70	1.78	2.45	2.23	2.76	2.08	3.43	3.00	3.40	3.23
15	<1.00	<1.00	<1.00	<1.00	1.78	1.70	1.78	1.85	2.45	2.26	2.45	2.30	3.23	2.90	3.41	3.41

[a] Log_{10} yeast and mold CFU/g.

[b] FDA BAM and ISO 21257 reference method replicates were enumerated at 5 and 7 days (uninoculated test portions) of incubation at 25°C.

[c] A and B = Indicated duplicate test portions.

[d] Data not included in statistical analysis for this contamination level.

[e] Double Grubbs' outlier.

[f] Single Grubbs' outlier.

[g] Laboratory data not included in statistical analysis for all contamination levels.

[h] Cochran's outlier.

Table 7. Total yeast and mold log_{10} counts for raw almonds by 3M Petrifilm RYM Count Plate after 60 h incubation at 25°C versus FDA BAM (ref. 1) and ISO 21527 (ref. 4) reference methods[a]

	Uninoculated control				Low				Medium				High			
	Petrifilm RYM		FDA BAM/ISO 21527[b]		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527	
Lab	A[c]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	<1.00	<1.00	1.00[d]	<1.00	1.48	1.78	1.78	1.85	1.90	1.60	2.28	1.95	2.00[e]	2.20[e]	2.04[f]	2.40[f]
2	1.30[e]	1.00[e]	1.30[d]	1.85[d]	1.90	1.60	2.04	1.70	1.48	2.15	2.11	2.34	2.28[e]	2.38[e]	2.64	2.84
3	<1.00	<1.00	<1.00	<1.00	1.30	1.30	1.60	1.60	2.38	2.28	2.38	2.18	3.56	3.11	3.30	3.11
4[g]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	<1.00	<1.00	<1.00	<1.00	1.60	1.70	1.78	1.95	2.30	2.15	2.30	2.45	3.41	3.04	3.18	3.18
6	<1.00	<1.00	<1.00	<1.00	1.78	1.00	2.20	1.78	2.30	1.78	2.34	2.20	3.08	3.08	3.36	3.28
7	<1.00	<1.00	<1.00	<1.00	1.78	1.30	1.70	1.30	2.45	2.20	2.43	2.11	3.23	3.08	3.20	3.11
8	<1.00	<1.00	<1.00	<1.00	1.85	1.95	1.00	1.00	2.45	2.49	2.00	2.08	3.26	3.51	3.00	3.08
9	<1.00	<1.00	<1.00	<1.00	1.30	1.30	1.48	1.00	2.56	2.48	2.56	2.53	3.00	3.04	3.04	3.00
10	<1.00	<1.00	<1.00	<1.00	1.30	1.00	1.30	1.00	2.08	2.43	2.00	2.56	3.26	3.11	3.30	3.04
11	<1.00	<1.00	<1.00	<1.00	1.00	1.48	1.48	1.60	2.00	2.08	1.95	1.90	3.08	3.04	3.15	3.08
12	<1.00	<1.00	<1.00	<1.00	1.78	1.90	1.30	1.00	2.08	2.30	2.00	2.00	3.65	3.36	3.40	3.08
13	<1.00	<1.00	<1.00	<1.00	1.48	1.30	1.48	1.30	2.20	2.00	1.78	1.95	2.90	2.90	2.90	2.95
14	<1.00	<1.00	<1.00	<1.00	1.48	1.60	1.70	1.78	2.45	2.23	2.76	2.08	3.45	3.00	3.40	3.23
15	<1.00	<1.00	<1.00	<1.00	1.78	1.70	1.78	1.85	2.26	2.45	2.45	2.30	3.23	2.90	3.41	3.41

[a] Log_{10} yeast and mold CFU/g.

[b] FDA BAM and ISO 21257 reference method replicates were enumerated at 5 and 7 days (uninoculated test portions) of incubation at 25°C.

[c] A and B = Indicated duplicate test portions.

[d] Data not included in statistical analysis for this contamination level.

[e] Double Grubbs' outlier.

[f] Single Grubbs' outlier.

[g] Laboratory data not included in statistical analysis for all contamination levels

Table 8. Total yeast and mold log_{10} counts for raw almonds by 3M Petrifilm RYM Count Plate after 48 h incubation at 28°C versus FDA BAM (ref. 1) and ISO 21527 (ref. 2) reference methods[a]

	Uninoculated control				Low				Medium				High			
	Petrifilm RYM		FDA BAM/ISO 21527[b]		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527[g]	
Lab	A[c]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	1.00[d]	1.00[d]	1.00[d]	<1.00	1.30	1.48	1.78	1.85	1.78	1.90	2.28	1.95	1.90[e]	2.20[e]	2.04[f]	2.40[f]
2	1.30[d]	1.30[d]	1.30[d]	1.85[d]	1.78	1.60	2.04	1.70	1.78	1.78	2.11	2.34	2.11[e]	2.11[e]	2.64	2.84
3	<1.00	<1.00	<1.00	<1.00	1.78	1.30	1.60	1.60	2.34	2.30	2.38	2.18	3.57	3.15	3.30	3.11
4[g]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	<1.00	<1.00	<1.00	<1.00	1.60	1.78	1.78	1.95	2.30	2.00	2.30	2.45	3.34	3.15	3.18	3.18
6	<1.00	<1.00	<1.00	<1.00	1.78	1.30	2.20	1.78	1.78	1.30	2.34	2.20	3.04	3.11	3.36	3.28
7	<1.00	<1.00	<1.00	<1.00	1.60	1.48	1.70	1.30	2.36	2.20	2.43	2.11	3.18	3.04	3.20	3.11
8	<1.00	<1.00	<1.00	<1.00	1.85	1.90	1.00	1.00	2.43	2.45	2.00	2.08	3.20	3.40	3.00	3.08
9	<1.00	<1.00	<1.00	<1.00	1.30	1.30	1.48	1.00	2.53	2.45	2.56	2.53	3.08	3.00	3.04	3.00
10	<1.00	<1.00	<1.00	<1.00	1.30	1.30	1.30	1.00	2.11	2.38	2.00	2.56	3.26	3.18	3.30	3.04
11	<1.00	<1.00	<1.00	<1.00	1.60	1.30	1.48	1.60	2.15	2.30	1.95	1.90	3.15	3.15	3.15	3.08
12	<1.00	<1.00	<1.00	<1.00	1.70	1.85	1.30	1.00	2.28	2.26	2.00	2.00	3.70	3.34	3.40	3.08
13	<1.00	<1.00	<1.00	<1.00	1.60	1.60	1.48	1.30	2.15	2.28	1.78	1.95	2.78	2.90	2.90	2.95
14	<1.00	<1.00	<1.00	<1.00	1.60	1.70	1.70	1.78	2.53	2.08	2.76	2.08	3.49	3.08	3.40	3.23
15	<1.00	<1.00	<1.00	<1.00	1.78	1.78	1.78	1.85	2.11	2.45	2.45	2.30	3.08	2.70	3.41	3.41

[a] Log_{10} yeast and mold CFU/g.

[b] FDA BAM and ISO 21257 reference method replicates were enumerated at 5 and 7 days (uninoculated test portions) of incubation at 25°C.

[c] A and B = Indicated duplicate test portions.

[d] Data not included in statistical analysis for this contamination level.

[e] Double Grubbs' outlier.

[f] Single Grubbs' outlier.

[g] Laboratory data not included in statistical analysis for all contamination levels.

Table 9. Total yeast and mold log_{10} counts for raw almonds by 3M Petrifilm RYM Count Plate after 60 h incubation at 28°C versus FDA BAM (ref. 1) and ISO 21527 (ref. 2) reference methods[a]

	Uninoculated control				Low				Medium				High			
	Petrifilm RYM		FDA BAM/ISO 21527[b]		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527		Petrifilm RYM		FDA BAM/ISO 21527	
Lab	A[c]	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	1.00[d]	1.00[d]	1.00[d]	<1.00	1.60	1.48	1.78	1.85	1.90	1.95	2.28	1.95	1.90[e]	2.26[e]	2.04[f]	2.40[f]
2	1.30[d]	1.30[d]	1.30[d]	1.85[d]	1.85	1.60	2.04	1.70	1.78	2.11	2.11	2.34	2.28[e]	2.34[e]	2.64	2.84
3	<1.00	<1.00	<1.00	<1.00	1.78	1.30	1.60	1.60	2.38	2.30	2.38	2.18	3.57	3.15	3.30	3.11
4[g]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	<1.00	<1.00	<1.00	<1.00	1.60	1.78	1.78	1.95	2.30	2.00	2.30	2.45	3.34	3.15	3.18	3.18
6	<1.00	<1.00	<1.00	<1.00	1.78	1.30	2.20	1.78	1.95	1.60	2.34	2.20	3.04	3.15	3.36	3.28
7	<1.00	<1.00	<1.00	<1.00	1.70	1.48	1.70	1.30	2.36	2.20	2.43	2.11	3.18	3.04	3.20	3.11
8	<1.00	<1.00	<1.00	<1.00	1.85	1.90	1.00	1.00	2.43	2.45	2.00	2.08	3.20	3.40	3.00	3.08
9	<1.00	<1.00	<1.00	<1.00	1.48	1.30	1.48	1.00	2.54	2.51	2.56	2.53	3.00	3.04	3.04	3.00
10	<1.00	<1.00	<1.00	<1.00	1.30	1.30	1.30	1.00	2.11	2.38	2.00	2.56	3.26	3.18	3.30	3.04
11	<1.00	<1.00	<1.00	<1.00	1.60	1.30	1.48	1.60	2.15	2.30	1.95	1.90	3.15	3.15	3.15	3.08
12	<1.00	<1.00	<1.00	<1.00	1.70	1.90	1.30	1.00	2.28	2.26	2.00	2.00	3.70	3.34	3.40	3.08
13	<1.00	<1.00	<1.00	<1.00	1.60	1.60	1.48	1.30	2.15	2.28	1.78	1.95	2.78	2.90	2.90	2.95
14	<1.00	<1.00	<1.00	<1.00	1.60	1.70	1.70	1.78	2.53	2.08	2.76	2.08	3.51	3.08	3.40	3.23
15	<1.00	<1.00	<1.00	<1.00	1.78	1.78	1.78	1.85	2.11	2.45	2.45	2.30	3.08	2.70	3.41	3.41

[a] Log_{10} yeast and mold CFU/g.

[b] FDA BAM and ISO 21257 reference method replicates were enumerated at 5 and 7 days (uninoculated test portions) of incubation at 25°C.

[c] A and B = Indicated duplicate test portions.

[d] Data not included in statistical analysis for this contamination level.

[e] Double Grubbs' outlier.

[f] Single Grubbs' outlier.

[g] Laboratory data not included in statistical analysis for all contamination levels.

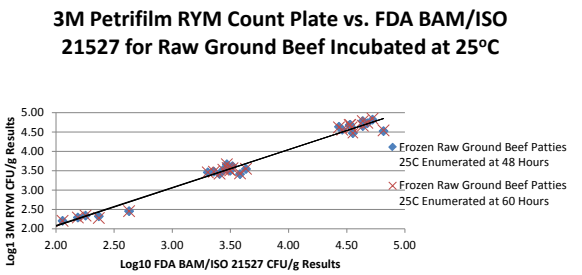


Figure 1. 3M Petrifilm RYM Count Plate versus FDA BAM/ISO 21527 results for frozen raw ground beef patties incubated at 25°C and enumerated at 48 and 60 h.

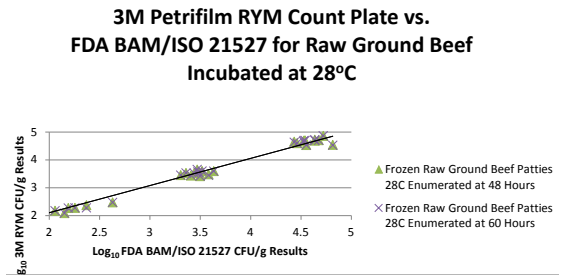


Figure 2. 3M Petrifilm RYM Count Plate versus FDA BAM/ISO 21527 Results for frozen raw ground beef patties Incubated at 28°C and Enumerated at 48 and 60 h.

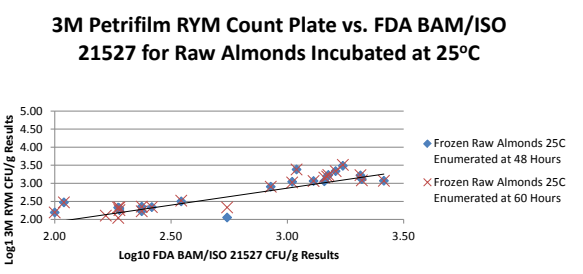


Figure 3. 3M Petrifilm RYM Count Plate versus FDA BAM/ISO 21527 results for raw almonds incubated at 25°C and enumerated at 48 and 60 h.

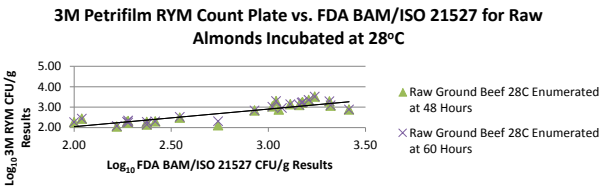


Figure 4. 3M Petrifilm RYM Count Plate versus FDA BAM/ISO 21527 results for raw almonds incubated at 28°C and enumerated at 48 and 60 h.

solidify in the 3M Petrifilm RYM Count Plate medium. Several laboratories also indicated that the colonies were very easy to interpret due to their size and color. One laboratory indicated that the "Space saving benefits of Petrifilm over the traditional FDA BAM method are readily apparent."

During the analysis of samples, several laboratories indicated deviating from the approved protocol and therefore, their data was subsequently removed from statistical analysis. No laboratories were removed from statistical analysis as a result of their data. For the raw ground beef, four laboratories were removed from statistical analysis. Laboratory 12 was unable to enumerate samples by both the 3M Petrifilm RYM Count Plate method and reference methods on the same day. All samples were plated onto DRBC, then stored at 2–8°C overnight and plated onto the Petrifilm RYM plates the following day. Laboratories 6 and 8 prepared the reference method samples using duplicate plating and not the required triplicate plating. Laboratory 4 (for both raw ground beef and raw almonds) failed to enumerate the 3M Petrifilm RYM Count Plates at the 60 h time point. Due to these deviations, these laboratories were removed from statistical analysis.

There were no statistically significant differences at the 5% level in log transformed means between the 3M Petrifilm RYM Count Plate method and FDA BAM/ISO 21257 methods for either matrix evaluated in this study, at any of the three contamination levels. No statistically significant differences were observed between the 3M Petrifilm RYM Count Plate results when enumerated at 48 h versus 60 h and compared to the FDA BAM/ISO 21527 methods. No statistically significant differences were also observed between 3M Petrifilm RYM Count Plates that were incubated at 25°C and 28°C when compared to the FDA BAM/ISO 21527 methods. Based on the data presented, the s_r values obtained at all incubation conditions and for all three contamination levels were generally similar between the two methods.

For raw frozen ground beef patties and raw almonds, the 3M Petrifilm RYM Count Plate method was more repeatable than the FDA BAM/ISO 21527 reference methods for one of the six contamination levels analyzed at 25°C and four of the six contamination levels analyzed at 28°C.

Recommendations

It is recommended that the 3M Petrifilm Rapid Yeast and Mold Count (RYM) Plate method be adopted as Official First Action status for the enumeration of yeast and molds in the following high water activity matrixes: yogurt (1.5% fat), frozen bread dough, fermented salami, sour cream, ready-made pie, raw frozen ground beef patties (77%), ready-to-eat deli sandwiches, sliced apples, and the following low water activity matrixes: raw almonds and dehydrated soup.

Acknowledgments

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

Dave Metzger, Wendy Lee, and Thomas Hirsch, Accelerated Analytical, Milwaukee, WI

Peyman Fatemi and Sharon Spencer, Aemtek, Inc., Fremont, CA

Joel Blumfield, EDL Labs, Inc., Purvis, MS

Bryan Dieckleman, Eurofins Microbiology Laboratories, Inc., Des Moines, IA

Karilyn Gonzales and Chelsea Fagundes, Food Microbiological Laboratories, Inc., Cypress, CA

Jerry Reed and Jeraldine Medellin, Metrix 360 Laboratories, San Antonio, TX

Adam Hankins, McCoy and McCoy Laboratories, Inc., Madisonville, KY

Rob Dermer, Natalie Shipley, and James Williams, Microbac Laboratories, Inc., Fayetteville, NC

Ashley Morris, Robert Brooks, and Karen Powers, Microbac Laboratories, Inc., Maryville, TN

Brenda Brown, Murray Brown Laboratories, LLC, Denver, CO

Karen Beers, Safe Foods Corp., Rogers, AR

Alexandra Calle, Texas Tech University, Food Safety and Microbiology, Lubbock, TX

Jerry Lynn Picket and Joseph Reynolds, Tyson WBA Analytical, Springdale, AR

Andy Gaydos, Waters Agricultural, Camilla, GA

Allison Mastalerz, Q Laboratories Inc., Cincinnati, OH

We would like to extend a special thanks to the following team members at Q Laboratories, Inc. for their efforts during the collaborative study: M. Joseph Benzinger Jr., Ben Bastin, Kiel Fisher, Kateland Koch, Will Judd and Nicole Klass.

References

(1) Tournas, V., Stack, M.E., Mislivec, P.B., Koch, H.A., & Bandler, R. (2001) FDA/*Bacteriological Analytical Manual*, Chapter 18, *Yeasts, Molds and Mycotoxins*. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071435.htm (accessed July 2014)

(2) Downes, F.P., Beuchat, L.R., & Cousin, M. A. (2001) American Public Health Association *Compendium of Methods for the Microbial Examination of Foods*, Chapter 20, *Yeasts and Molds,* 4th Ed., pg. 209–213

(3) *Official Methods of Analysis* (2012) 19th Ed., Appendix J: AOAC INTERNATIONAL, Gaithersburg, MD, http://www.eoma.aoac.org/app_j.pdf (accessed July 2014)

(4) International Organization for Standardization (2012) ISO 21527-1:2008, *Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Enumeration of Yeasts and Molds – Part 1: Colony Count Technique in Products with Water Activity Greater than 0.95*, Geneva, Switzerland

(5) International Organization for Standardization (2012) ISO 21527-2:2008, *Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Enumeration of Yeasts and Molds – Part 2: Colony Count Technique in Products with Water Activity Less than or Equal to 0.95,* Geneva, Switzerland

(6) *Official Methods of Analysis* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Method **990.12**. http://www.eoma.aoac.org/ (accessed July 2014)

(7) Least Cost Formulations, Ltd (2012) AOAC *Interlaboratory Study Workbook Blind (Unpaired) Replicates,* Version 2.1. http://www.lcfltd.com/customer/LCFMPNCalculator.exe (accessed August 2014)

Expert Review Panel
Microbiology for Food and Environmental Surfaces
OFFICIAL CHAIR'S EXPERT REVIEW PANEL REPORT

ACKNOWLEDGMENT

The undersigned chair hereby confirms that the following document has been reviewed and constitutes the final version of the Official Chair's Report for the Expert Review Panel for Microbiology for Food and Environmental Surfaces was held on Wednesday, December 10, 2014 at AOAC INTERNATIONAL Headquarters located at 2275 Research Blvd, Rockville, Maryland 20850.

WENDY MCMAHON, SILLIKER LABORATORIES
Expert Review Panel Co-Chair

MICHAEL BRODSKY, BRODSKY CONSULTING
Expert Review Panel Co-Chair

Wendy McMahon
Date Jan 23, 2015

AOAC RESEARCH INSTITUTE
2275 Research Blvd, Suite 300
Rockville, Maryland 20850
UNITED STATES

Contact:
La'Kia Phillips, Conformity Assessment Coordinator at lphillips@aoac.org
Deborah McKenzie, Sr. Director, DMcKenzie@aoac.org

EXPERT REVIEW PANEL MEETING ATTENDEES

Expert Review Panel Chair (s)
Michael Brodsky, Brodsky Consulting
Wendy McMahon, Silliker Laboratories

Expert Review Panel Members
Patrice Arbault, BioAdvantage
Yi Chen, FDA/ Tom Hammack, FDA
Maria Christina Fernandez, University of Buenos Aires
Tony Hitchins, FDA/CFSAN (Retired)
Sam Mohajer, Canadian Food Inspection Agency
Paul Wehling, General Mills

Method Authors
DeAnn Benesh, 3M Food Safety
Robert Jechorek, 3M Food Safety
Lisa Montereso, 3M Food Safety
Erin Crowley, Q Labs
Patrick Bird, Q Labs

AOAC Staff
Jim Bradford, Executive Director
Deborah McKenzie
Tien Milor
La'Kia Phillips

Observers
N/A

EXPERT REVIEW PANEL, METHOD BACKGROUND, AND CONCLUSIONS

Criteria for Vetting Methods to be considered:
AOAC convened the *Official Methods of Analysis*℠ (OMA) Expert Review Panel for Microbiology for Foods and Environmental Surfaces on Wednesday, December 10, 2014 from 8:00am to 12:00pm at AOAC INTERNATIONAL Headquarters located at 2275 Research Blvd, Rockville, Maryland 20850.

The purpose of the meeting was to review and evaluate OMAMAN-16: 3M™ Petrifilm™ Rapid Yeast and Mold Collaborative Study submitted by Bob Jechorek of 3M Food Safety located at 3M Center, Building 260-06-B-01, St. Paul, MN 55144 and Erin Crowley, Q Laboratories, 1400 Harrison Avenue, Cincinnati, OH 45214. The candidate method was reviewed against the approved collaborative study protocol. Supplemental information was also provided to the reviewers which included the collaborative study manuscript, method safety checklist, AOAC *Performance Tested Methods*℠ validation report, *Performance Tested Methods*℠ protocol and package insert.

OMAMAN-17: Evaluation Of The 3M™ Molecular Detection Assay (MDA) Listeria for the Detection of Listeria Species submitted by Lisa Monteroso of 3M Food Safety Department located at 3M Center, Building 0260-06-B-01, Saint Paul, Minnesota 55144-1000 U.S.A. The candidate method was reviewed against the approved collaborative study protocol. Supplemental information was also provided to the reviewers which included the collaborative study manuscript, method safety checklist, AOAC *Performance Tested Methods*℠ validation report, two (2) *Performance Tested Methods*℠ modification reports and package insert.

OMAMAN-18: Evaluation Of The 3M™ Molecular Detection Assay (MDA) Listeria Monocytogenes for the Detection of Listeria Monocytogenes submitted by Lisa Monteroso of 3M Food Safety Department located at 3M Center, Building 0260-06-B-01, Saint Paul, Minnesota 55144-1000 U.S.A. The candidate method was reviewed against the approved collaborative study protocol. Supplemental information was also provided to the reviewers which included the collaborative study manuscript, method safety checklist, AOAC *Performance Tested Methods*℠ validation report, and package insert.

Criteria for Vetting Experts and Selection Process:
The following seven (7) candidates and one (1) alternate member were submitted for consideration by the Official Methods Board to evaluate candidate methods for Pesticide Residues methods as per the Expert Review Panel (ERP) Policies and Procedures. The candidates were highly recommended by the Chemical Contaminants and Residues in Foods Community, have participated in various AOAC activities, including but limited to, Method Centric Committees that were formed under the legacy OMA pathway, and were vetted by the Official Methods Board. The experts are Amy Brown, Jo Marie Cook (Alternate), Julie Kowalski, John Reuther, Marina Torres, Jian Wang, and Xiaoyan Wang.

ERP Orientation:
The ERP members have completed the mandatory AOAC Expert Review Panel Orientation Webinar on Wednesday, November 5, 2014.

Expert Review Panel Meeting Quorum
The meeting of the Expert Review Panel was held in person. A quorum is the presence of seven (7) members or 2/3 of the total vetted ERP, whichever is greater. Eight (8) out of the eleven (11) voting members were present and therefore met a quorum to conduct the meeting. It was also noted that Jim Agin who was not present, will not participate on this Expert Review Panel.

Standard Method Performance Requirements (SMPRs): N/A

Conclusion:
The Expert Review Panel reviewed OMAMAN-16: 3M™ Petrifilm™ Rapid Yeast and Mold, OMAMAN-17: Evaluation Of The 3M™ Molecular Detection Assay (MDA) Listeria For The Detection Of Listeria Species, and OMAMAN-18: Evaluation Of The 3M™ Molecular Detection Assay (MDA) Listeria Monocytogenes For The Detection Of Listeria Monocytogenes and have adopted these methods for AOAC First Action Official Method status by unanimous decision as noted in the meeting minutes.

Subsequent ERP Activities:
ERP members will continue to evaluate the method for 2 years.

MEETING MINUTES

I. Welcome and Introductions

The Expert Review Panel Co-chairs, Michael Brodsky and Wendy McMahon welcomed Expert Review Panel members, initiated introductions, and discussed with the panel the goal of the meeting.

II. Review of AOAC Volunteer Policies

A brief overview of AOAC Volunteer Policies, Volunteer Acceptance Agreement and and Expert Review Panel Policies and Procedures which included Volunteer Conflicts of Interest, Policy on the Use of the Association, Name, Initials, Identifying Insignia, Letterhead, and Business Cards, Antitrust Policy Statement and Guidelines, and the Volunteer Acceptance Form (VAF). All members of the ERP were required to submit and sign the Volunteer Acceptance Form.

III. Expert Review Panel Process Overview and Guidelines

Deborah McKenzie presented a quick overview of the Expert Review panel process including meeting logistics, consensus, First Action to Final Action requirements, and documentation.

IV. Review of Methods

All ERP members presented a review and discussed the proposed collaborative study manuscript for The purpose of the meeting was to review and evaluate OMAMAN-16: 3M™ Petrifilm™ Rapid Yeast And Mold Collaborative Study. The method authors Robert Jechorek of 3M Food Safety and Erin Crowley of Q Laboratories were both present and able to address questions and concerns of the ERP members. A summary of comments was provided to the ERP members.[1]

MOTION:
Motion by Brodsky; Second by McMahon, for the method to move forward for First Action Official Method Status.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions (Unanimous). *Motion Passed.*

All ERP members presented a review and discussed the proposed collaborative study manuscript for The purpose of the meeting was to review and evaluate OMAMAN-17: Evaluation Of The 3M™ Molecular Detection Assay (MDA) Listeria For The Detection Of Listeria Species. The method authors Lisa Montereso of 3M Food Safety and Erin Crowley of Q Laboratories were both present and able to address questions and concerns of the ERP members. A summary of comments was provided to the ERP members.[2]

MOTION:
Motion by Brodsky; Second by Arbault, for the method to move forward for First Action Official Method Status.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions (Unanimous). *Motion Passed.*

All ERP members presented a review and discussed the proposed collaborative study manuscript for The purpose of the meeting was to review and evaluate OMAMAN-18: Evaluation Of The 3M™ Molecular Detection Assay (MDA) Listeria Monocytogenes for The Detection Of Listeria Monocytogenes. The method authors Lisa Montereso of 3M Food Safety and Erin Crowley of Q Laboratories were both present and able to

[1] Attachment 1: Summary of Expert Reviewer Comments for OMAMAN-16
[2] Attachment 2: Summary of Expert Reviewer Comments for OMAMAN-17

address questions and concerns of the ERP members. A summary of comments was provided to the ERP members.[3]

MOTION:
Motion by Hitchins, Second by Mohajer, for the method to move forward for First Action Official Method Status.
Consensus demonstrated by: 8 in favor, 0 opposed, and 0 abstentions (Unanimous). ***Motion Passed.***

V. Discuss Final Action Requirements for First Action Official Methods (if applicable)
No further action was discussed at this time.

VI. Adjournment

[3] Attachment 3: Summary of Expert Reviewer Comments for OMAMAN-18

OMAMAN-16: 3M Petrifilm Rapid Yeast And Mold Collaborative Study*

Study Director: Erin Crowley, Q Laboratories, 1400 Harrison Avenue, Cincinnati, OH 45214
Co-Study Director: Bob Jechorek, 3M Food Safety, 3M Center, Building 260-06-B-01, St. Paul, MN 55144

TECHNICAL EVALUATION CRITERIA Is the test kit method scientifically and technically sound?	
ER 1	yes
ER 2	yes
ER 3	yes
ER 4	yes
ER 5	yes
ER 6	yes
ER 7	yes
ER 8	yes
Have sufficient controls been used, including those required to calculate the rate of false-positive and false-negative results where appropriate?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Is sufficient information included for system suitability determination and product performance or acceptance testing?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	
ER 6	Yes
ER 7	Yes
ER 8	Yes
Are the conclusions statements valid based upon data presented?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	No
ER 8	No

OMAMAN-16: 3M Petrifilm Rapid Yeast And Mold Collaborative Study*

Study Director: Erin Crowley, Q Laboratories, 1400 Harrison Avenue, Cincinnati, OH 45214
Co-Study Director: Bob Jechorek, 3M Food Safety, 3M Center, Building 260-06-B-01, St. Paul, MN 55144

Do you agree that the evidence or data from this and previous studies support the proposed applicability statement?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Are there sufficient data points per product evaluated in accordance with AOAC requirements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
General Comments about the Method Scope/Applicability:	
ER 1	The method scope and applicability are clearly described, and the Petrifilm protocols have been clearly presented. Both incubation temperature options were also clearly stipulated. Description of the look like colonies were sufficiently explained.
ER 2	A statement about growth curve for the yeast selected was made on page 3, row 14-15. This culture incubated for 48h while there are strains requiring 5-7 days incubation. The method includes up to 60 h incubation. Reviewer recommends applicability statement to this point. The background section could include this point and then the discussion could address it.
ER 3	Almonds and Ground Beef have been used for the collaborative study which are both appropriate matrices and were used in the pre-collaborative study.
ER 4	No additional comments
ER 5	The method is useful for rapid quantification of spoilage microorganisms
ER 6	Accurately reflects breadth and depth of study
ER 7	None
ER 8	1. Page 5, lines 27-29, Sterile Diluent. The method lists several diluents that can be used with this method, including 0.1% peptone water. All of the diluents, other than 0.1% peptone water, should be removed from the method, since the method was validated with 0.1 peptone water and no other diluent. The implied conclusion is that the method, as given by the draft official method, is that it can be run with any diluent even though it was only validated with 0.1 % peptone water. 2. Page 5, lines, 7 - 9, applicability (here and elsewhere in the report). The method refers to yogurt, ready-made-pie, frozen ground beef patties, sandwiches, and dehydrated soup. These are not specific enough. Yogurt and beef should have fat percentages. The types of pie, sandwiches and dehydrated soup should be specified. For example, apple pie, chicken soup, tuna sandwich... The applicability statement is too broad as written.

OMAMAN-16: 3M Petrifilm Rapid Yeast And Mold Collaborative Study*

Study Director: Erin Crowley, Q Laboratories, 1400 Harrison Avenue, Cincinnati, OH 45214
Co-Study Director: Bob Jechorek, 3M Food Safety, 3M Center, Building 260-06-B-01, St. Paul, MN 55144

General comments about the method:	
ER 1	The method is very well described in the various documents.
ER 2	NA
ER 3	The method is much simpler compared to traditional method and the space savings in the use of Petrifilm are significant as compared to traditional agar plates.
ER 4	No additional comments
ER 5	The method is really useful due to it significantly reduces test times
ER 6	Well written and easy to follow
ER 7	none
ER 8	1. Page 7, line 17. There should be references directing the analyst to methods for the further identification of yeast/fungal isolates. They should be ISO, BAM, MLG and others.
Pros/Strengths of the Manuscript:	
ER 1	Good description of the sample preparation protocols. Efficient description of the collab study workflow and organization. Tables are very useful for summarizing the results.
ER 2	Very well written.
ER 3	The Manuscript is well written and the information flow is in an understandable order.
ER 4	Generally well written.
ER 5	It is a simple method for working
ER 6	Very thoroughly written and detailed
ER 7	Well written in general.
ER 8	It is well written.
Cons/Weaknesses of the Manuscript:	
ER 1	Very minor edits: page 7, line 25, reports lab 5 as one of the 4 labs with deviations, but in table 1 page 13, lab 6 is marked as the lab showing deviations for ground beef??? Reading through the report and the pack insert, it remains unclear if the minimum incubation is 48 hours or 46 hours since it is stipulated that incubation shall be 48+/-2 hours but reading is required at 48 hours: is minimal time of 48 hours of incubation is required?
ER 2	NA
ER 3	Table 1 describes which data sets were not used in the statistical analysis; however, there are not indications as such in tables 2 – 9 where raw data is presented. It may help to identify the labs who's data sets were excluded in each table using a superscript letter.
ER 4	Need to elaborate on the issues and possible causes of those issues of laboratories whose data were not used in the study.
ER 5	no
ER 6	None
ER 7	Page 1, line 24 states "unpaired study design" but page 4 line, line 22 states "paired study design". Please clarify in the manuscript. Page 3, line 29: Clarify that after lyophilization dilutions were done with sterile NFDM powder or reconstituted NFDM. Page 4, line 38: Increase font size. Page 4, line47: Justify or omit reverse transformed mean difference here and in Tables 2014.1 and 2014.2.[Continued] Page 7, line 24: Insert "valid" before both "data" words. Page 7, line 33: Omit Figs 1-4 which are somewhat redundant. Add statement about acceptability of Youden plots. Page 7, line 37: Remove "reverse transformed difference" here and in Tables 2014.1 &.2. Page 8, lines 31-33: State the repeatability SD values supporting this assertion. Page 8, lines 44-46: State the repeatability SD values supporting this assertion.

OMAMAN-16: 3M Petrifilm Rapid Yeast And Mold Collaborative Study*

Study Director: Erin Crowley, Q Laboratories, 1400 Harrison Avenue, Cincinnati, OH 45214
Co-Study Director: Bob Jechorek, 3M Food Safety, 3M Center, Building 260-06-B-01, St. Paul, MN 55144

	Page 9, lines 8-10: State the repeatability SD values supporting this assertion. Page 9, lines 30-32: State the repeatability SD values supporting this assertion. Page 9, lines 42-44: State the repeatability SD values supporting this assertion. Page 10, lines 7-9: State the repeatability SD values supporting this assertion. Page 10, lines 21-23: State the repeatability SD values supporting this assertion. Page 10 lines 42-44: State the repeatability SD values supporting this assertion. Add the requested repeatability SD values as footnotes to relevant Tables.
ER 8	Applicability statement is too general. Tables are not properly footnoted.
Supporting Data and Information: Does data from collaborative study support the method as written?	
ER 1	Yes.
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	yes
ER 6	Yes
ER 7	Yes, in general.
ER 8	Yes.
Supporting Data and information: Does data collected support the criteria given in the collaborative study protocol?	
ER 1	The data supports the criteria.
ER 2	Yes
ER 3	No
ER 4	yes
ER 5	yes
ER 6	Yes
ER 7	Yes.
ER 8	Yes.
Are there any concerns regarding the safety of the method?	
ER 1	To be covered once Safety Advisor review is presented.
ER 2	No
ER 3	No
ER 4	No
ER 5	No
ER 6	No
ER 7	No.
ER 8	No.
Are there any concerns regarding the data manipulation, data tables, or statistical analysis?	
ER 1	No concerns.
ER 2	No
ER 3	The manuscript would be much stronger if a more detail rational is given as to why some of the collaborative labs were excluded. It is understandable to have such occurrences in a large collaborative study.
ER 4	No
ER 5	No
ER 6	No

ER 7	In Table 2014.1 for the RYM method it is not clear what data from Tables 2, 3, 4 & 5 is used to get the SD values for repeatability and reproducibility. In Table 2014.2 for the RYM method it is not clear what data from Tables 6 7, 8 & 9 is used to get the SD values presented for repeatability and reproducibility. Typically the SD for reproducibility is noticeably larger than that for repeatability. This is true for the 2014.2 (almond) results by the RYM and the FDA/ISO methods. However, it is not so for the 2014.1 (beef) results by the RYM or reference method. So perhaps the calculations and data inputs should be checked. [Continued] In many cases, especially in Table 2014.1 the mean RYM and reference methods' mean counts are not significantly different and yet the differences, although not very different, are often significantly different. In Table 2014.1 perhaps this is related to the fact that only 11 collaborators provided valid results.
ER 8	None.
General Comments (2)	
ER 1	
ER 2	NA
ER 3	NA
ER 4	No additional comments
ER 5	
ER 6	
ER 7	None
ER 8	Tables 2014.1 & 2014.2. It should be footnoted that the BAM and ISO methods are identical when using 0.1 % peptone water. as was done in this collaborative study.
EDITORIAL EVALUATION CRITERIA Is the Validation Study Manuscript in a format acceptable to AOAC?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Is the method described in sufficient detail so that it is relatively easy to understand, including equations and procedures for calculation of results (are all terms explained)?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	No
ER 8	Yes
Are the figures and tables sufficiently explanatory without the need to refer to the text?	
ER 1	Yes

OMAMAN-16: 3M Petrifilm Rapid Yeast And Mold Collaborative Study*

Study Director: Erin Crowley, Q Laboratories, 1400 Harrison Avenue, Cincinnati, OH 45214
Co-Study Director: Bob Jechorek, 3M Food Safety, 3M Center, Building 260-06-B-01, St. Paul, MN 55144

ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	No
Are all the figures and tables pertinent?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	No
ER 8	Yes
Could some be omitted and covered by a simple statement?	
ER 1	No
ER 2	No
ER 3	No
ER 4	No
ER 5	No
ER 6	Yes
ER 7	Yes
ER 8	No
Are the references complete and correctly annotated?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
Does the method contain adequate safety precaution reference and/or statements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes

OMAMAN-16: 3M Petrifilm Rapid Yeast And Mold Collaborative Study*

Study Director: Erin Crowley, Q Laboratories, 1400 Harrison Avenue, Cincinnati, OH 45214
Co-Study Director: Bob Jechorek, 3M Food Safety, 3M Center, Building 260-06-B-01, St. Paul, MN 55144

ER 8	Yes
RECOMMENDATION: Do you recommend that the ERP adopt this method as an AOAC *Official Methods of Analysis (First Action status)*?	
ER 1	Yes.
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	yes
ER 6	Yes
ER 7	This method is potentially recommendable for adoption after manuscript clarifications are made.
ER 8	Yes, if my above comments regarding applicability and diluent are addressed. The package insert also needs to be revised in light of these comments. If multiple diluents are recommended, even though only 1 was validated, then I would recommend against giving the method First Action Status.
AFTER FIRST ACTION STATUS: Is there any additional information that the ERP should consider in order to recommend the method for Final Action status?	
ER 1	No.
ER 2	NA
ER 3	NA
ER 4	No additional comments
ER 5	No
ER 6	User comments
ER 7	No
ER 8	Two years worth of use in the field, without substantial problems, should be the criteria for making this a final action method.
General Comments (3)	
ER 1	
ER 2	NA
ER 3	NA
ER 4	No additional comments
ER 5	
ER 6	
ER 7	None
ER 8	1. Package insert, page 4 of 8, Sterile Diluent. The method lists several diluents that can be used with this method, including 0.1% peptone water. All of the diluents, other than 0.1% peptone water, should be removed from the method, since the method was validated with 0.1 peptone water and no other diluent. 2. Package insert, page 6 of 8, applicability. The method refers to yogurt, ready-made-pie, frozen ground beef patties, sandwiches, and dehydrated soup. These are not specific enough. Yogurt and beef should have fat percentages. The types of pie, sandwiches and dehydrated soup should be specified. For example, apple pie, chicken soup, tuna sandwich... The applicability is too broad as written.

AOAC INTERNATIONAL

***Official Methods of Analysis*SM Program**

Collaborative Study Protocol

for

3M™ Petrifilm™ Rapid Yeast and Mold Count Plate

OMA 2014-June-XXX

Prepared for:
Bob Jechorek
Study Director
3M Food Safety

Table of Contents

1. INTRODUCTION

The goal of this collaborative study is to estimate the following performance parameters of the 3M™ Petrifilm™ Rapid Yeast and Mold (RYM) Count Plate Method: repeatability (s_r) and reproducibility (s_R) standard deviations, relative standard deviations of repeatability (RSD_r) and reproducibility (RSD_R) and repeatability and reproducibility values (r and R, respectively). Test portions from 4 different contamination levels will be sent to collaborators where they will perform the 3M Petrifilm RYM Count Plate and parts 1 and 2 of the ISO 21527-2008 reference method and the FDA BAM Chapter 18 **reference methods. The following foods will be tested: raw frozen ground beef (80% lean, 20% fat) as a high moisture product and raw almonds as a low moisture product.**

1.1. Description of the 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate

The 3M™ Petrifilm™ Rapid Yeast and Mold Count (RYM) Plate is a sample-ready culture medium system which contains nutrients supplemented with antibiotics, a cold-water-soluble gelling agent, and an indicator system that facilitates yeast and mold enumeration. 3M Petrifilm RYM Plates are used for the enumeration of yeast and mold in the food and beverage industries. 3M Petrifilm RYM Plate components are decontaminated though not sterilized. 3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

1.2. Summary of 3M Petrifilm RYM Count Plate PTM/Precollaborative Study

The results of the PTM study demonstrated the 3M Petrifilm RYM Plate method to be equivalent to or better than the average log counts of the ISO 21527:2008 parts 1 and 2 and the FDA BAM Chapter 18 reference methods at 48 and 60 hours for the ten matrices evaluated: yogurt, frozen bread dough, fermented salami, sour cream, ready-made pie, frozen ground beef patties, raw almonds, sandwiches, sliced apples, dehydrated soup. In addition, the repeatability of the 3M Petrifilm RYM method was equivalent to or better than the reference methods at both 48 and 60 hours. In the independent laboratory validation study, there was no significant difference in detection, enumeration, or repeatability between the 3M Petrifilm RYM method and ISO 21527:2008 parts 1 and 2 and the FDA BAM Chapter 18 reference methods for yogurt at 48 and 60 hours. For frozen ground beef patties there was a significant difference between the 3M Petrifilm RYM Plate method and the reference methods at 48 and 60 hours with the 3M Petrifilm RYM method enumerating more organisms that the reference methods($p<0.05$). In strain studies, of the 42 yeast and mold tested, all produced typical colonies on 3M Petrifilm RYM Plate method. Of the 4 bacterial strains that should not have been detected by the 3M Petrifilm RYM Plate method, all 4 strains did not grow on the plate. Lot-to-lot study of 3M Petrifilm RYM Plates was evaluated and showed no differences at time zero. In assay ruggedness studies, there was no

difference in performance for three test parameters: incubation time, incubation temperature and type of diluents used to rehydrate the plate medium.

The method was awarded PTM certification number 121301 on December 18, 2013.

1.3 Study Directors for Collaborative Study

Robert P. Jechorek
Study Co-Director
3M Food Safety Department
3M Center, Building 260-6B-01
St. Paul, MN 55144
Phone: 651-733-9764
Fax: 651-733-5819
Email: rpjechorek@mmm.com

Patrick Bird & Erin Crowley
Study Co-Directors
Q Laboratories, Inc.
1400 Harrison Avenue
Cincinnati, Ohio 45214
Phone : 513-471-1300
Fax : 513-471-5600
Email: pbird@qlaboratories.com
ecrowley@qlaboratories.com

2. Collaborators

A total of 12-13 collaborators will be solicited to participate in this study. A minimum of 8 laboratories, with acceptable data, will be needed for the successful completion of this study. Qualified laboratories currently using the 3M Petrifilm Plates or who will be thoroughly trained by a 3M representative, will act as collaborators for this study.

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. Collaborative Study Design

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

Table 1. Overview of Study Design

MATRIX	Inoculating Organism	Target Levels	# Replicates		SAMPLE CODES
			3M RYM*	ISO/ BAM *	
Raw frozen ground	*Trichosporon mucoides*	0 cfu/g	4	3	Random number
		~50 cfu/g	4	3	

beef (80% lean, 20% fat)	ATCC 201382	~500 cfu/g	4	3	between 100-199
		~5000 cfu/g	4	3	
Raw almonds	*Aspergillus aculeatus* ATCC 56925	0 cfu/g	4	3	Random number between 200-299
		~50 cfu/g	4	3	
		~500 cfu/g	4	3	
		~5000 cfu/g	4	3	

*Two plates incubated at 25°C and two plates incubated at 28°C

1.2. Study Schedule

1.2.1. One matrix will be tested each week of the study. All data should be sent to the co-Study Director by the 3rd week of study.

1.2.2. One co-Study Director 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.

4. Test Portion Preparation

4.1. Matrix spiking

4.1.1. Obtain 4 lots (~2100g/lot) of each of food product. Determine the background levels of aerobic bacteria by Bacteriological Analytical Manual Chapter 3 Aerobic Plate Count (January 2001) and any yeast/mold counts by Bacteriological Analytical Manual Chapter 18: Yeasts, Molds and Mycotoxins (April 2001).

4.1.2. **If the lots are naturally contaminated**, each lot should be manipulated (temperature abused to increase levels or mixed to decrease levels) so as to produce 4 different contamination levels. The goal is to have each batch approximately 10-fold higher than the previous.

4.1.3. **If there is no natural contamination** in any of the 4 lots then all 4 lots should be combined to form one large batch. It should then be divided into 4 sub-batches, 3 of which will need to be artificially contaminated with a yeast or mold (Table 1) at the following levels targeted: low, 10-100 cfu/g; medium: 100-1000 cfu/g; high: 1000-10,000 cfu/g. The fourth batch will serve as the control.

4.1.3.1. **Raw frozen ground beef** (80% lean/20% fat) will be thawed, inoculated with a liquid inoculum (*Trichosporon mucoides*

ATCC 201382), manually mixed and held at -20 to -30°C for a minimum of 2 weeks.

4.1.3.2. **Raw almonds** will be inoculated with a lyophilized culture (*Aspergillus aculeatus* ATCC 56925) and stabilized for a minimum of 2 weeks at room temperature (21-23°C).

4.2. Shipment of Test portions

4.2.1. A total of 16 test portions (2 test portions x 4 levels x 2 methods) of each matrix will be sent to each laboratory.

4.2.2. Test portions will be shipped in leak-proof, insulated containers with ice packs to maintain the appropriate temperature range.

4.2.3. Packages will be sent via overnight courier.

4.3. Test Portion Analysis

4.3.1. The laboratories will follow the appropriate test portion preparation protocol according to the method for each food.

5. 3M Petrifilm RYM Count Plate

5.1. *Applicability*

For the enumeration of yeast and mold from yogurt, sour cream, raw almonds, sliced apples, frozen bread dough, ready-made pie, sandwiches, dehydrated soup, fermented salami and frozen ground beef patties (80% lean/20% fat).

***Caution*:**

3M Petrifilm RYM Plates do not differentiate any one yeast or mold strain from another. 3M has not documented 3M Petrifilm RYM Plates for use in industries other than food and beverage. For example, 3M has not documented 3M Petrifilm RYM Plates for testing water, pharmaceuticals or cosmetics. 3M Petrifilm RYM Plates have not been tested with all possible food products, food processes, testing protocols or with all possible strains of yeast and mold. Do not use 3M Petrifilm RYM Plates in the diagnosis of conditions in humans or animals. The user must train its personnel in proper testing techniques. After use, 3M Petrifilm RYM Plates may contain microorganisms that may be a potential biohazard. Follow current industry standards for disposal. For information on potential biohazards, reference Biosafety in Microbiological and Biomedial Laboratories, 5th edition, Section VIII-B: Fungal Agents.

5.2. Test Kit - 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate

5.2.1. Cat. #6475 3M Petrifilm Rapid Yeast & Mold Plates 25/pouch, 2 pouches/box
5.2.2. Cat. # 6477 3M Petrifilm Rapid Yeast & Mold Plates 25/ pouch, 20 pouches /case

5.3. Additional Reagents - 3M Petrifilm RYM Count Plate
5.3.1. Sterile diluent – 0.1% peptone water

5.4. Additional media
5.4.1. Dichloran-rose bengal chloramphenicol (DRBC) agar
5.4.2. Dichloran 18% glycerol (DG18) agar

5.5. Apparatus
5.5.1. *Pipettes*. – capable of pipetting 1,000µL or sterile serological pipette
5.5.2. *Sterile pipette tips.* – capable of 1,000µL
5.5.3. *Stomacher®*. – Seward or equivalent
5.5.4. *Filter Stomacher® bags*. - Seward or equivalent
5.5.5. 3M™ Petrifilm™ Flat Spreader (6425)
5.5.6. *Incubators*. – Incubator capable of maintaining 25 ± 1°C and 28 ± 1°C, and having a solid front so as to maintain a dark interior.
5.5.7. *Refrigerator*. – capable of maintaining 2-8°C, for storing the 3M Petrifilm RYM Count Plates
5.5.8. L shaped spreaders (hockey sticks)

5.6. Determination
5.7. Sample Preparation
5.7.1. Prepare a 1:10 dilution of each test portion – 25g plus 225 mL of 0.1% Peptone Water (10^{-1})
5.7.2. Stomach for 2 minutes in the stomacher bag.
5.7.3. Prepare an additional 3 dilutions ($10^{-2}$to 10^{-4}) using 0.1% Peptone Water.

5.8. **3M Petrifilm Rapid Yeast and Mold Count Plate Method** (please refer to the IFU for additional details)
5.8.1. Place two 3M Petrifilm RYM Plates on a flat, level surface for each dilution to be tested.
5.8.2. Lift the top film and with the pipette perpendicular dispense 1 mL of each dilution onto the center of bottom film of each plate.
5.8.3. Drop the top film down onto the sample.
5.8.4. Place the 3M Petrifilm Flat Spreader (6425) on the center of the plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm RYM Plate growth area before the gel is formed. Do not slide the spreader across the film.
5.8.5. Remove the spreader and leave the plate undisturbed for at least one minute to permit the gel to form.
5.8.6. Incubate 3M Petrifilm RYM Plates at 25 and 28°C in a horizontal position with the clear side up in stacks of no more than 40. Enumerate plates at **48**

± 2 hours and 60 hours 3M Petrifilm RYM plates can be counted using a standard colony counter or other illuminated magnifier. Gridlines are visible with the use of a backlight to assist with estimated enumeration.

5.8.7. To differentiate yeast and mold colonies on the 3M Petrifilm RYM Plates, look for one or more of the following characteristics:

YEAST	**MOLD**
Small colonies	Large colonies
Colonies have defined edges	Colonies have diffuse edges
Pink-tan to blue-green in color	Blue/green to variable upon prolonged incubation
Colonies appear raised (3 dimensional)	Colonies appear flat
Colonies have a uniform color	Colonies have a dark center with diffused edge

5.8.8. The circular growth area is approximately 30 cm^2. Plates containing greater than 150 colonies can either be estimated or recorded as TNTC. Estimation can be done by counting the number of colonies in one or more representative squares and determining the average number per square. The average number can be multiplied by 30 to determine the estimated count per plate. If a more accurate count is required, the sample will need to be retested at higher dilutions. When the sample contains substantial amounts of mold, depending on the type of mold, the upper countable limit may be lowered at user discretion.

5.8.9. Food samples may occasionally show interference on the 3M Petrifilm RYM Plates, for example:

5.8.9.1. uniform blue background color (often seen from the organisms used in cultured products) these should not be counted as TNTC.

5.8.9.2. intense, pinpoint blue specs (often seen with spices or granulated products).

6. Reporting Raw Data

6.1. Report data using the data report form in Appendix 8.2.

6.2. Upon completion of each food type, the laboratory will fax or email the completed data form to the co-Study Director.

6.3. Copies of all data sheets should be retained by the collaborating labs for a minimum of one year.

7. Analyzing Raw Data

7.1. Average duplicate plates for the 3M Petrifilm RYM method and triplicate plates for the reference method.

7.2. Convert yeast/mold counts into logarithms for data analysis.
7.3. Perform a one way analysis of variance (ANOVA) or a paired t-test to determine if the mean of replicate samples at each contamination level for each matrix is not different between the 3M Petrifilm RYM method and the reference methods.
7.4. Calculate the repeatability (s_r) of the 3M Petrifilm RYM method and the reference methods by determining the mean of the logarithm (base 10) of the counts and the standard deviation, s_r, for each contamination level of each matrix.

8. Appendices

8.1. Instructions to Collaborators
8.2. Data Report Forms
8.3. Study Materials
8.4. Study flow diagram
8.5. Collaborator Information Sheet
8.6. Collaborator Comment Form
8.7. 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate Instructions for Use

Appendix 8.1

Instructions to Collaborators

General Instructions about Collaborative Studies

Introduction

The purpose of this document is to provide detailed instructions for performing the collaborative study for the 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate Method.

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

Robert P. Jechorek
Study Co-Director
3M Food Safety Department
3M Center, Building 260-6B-01
St. Paul, MN 55144
Phone: 651-733-9764
Fax: 651-733-5819
Email: rpjechorek@mmm.com

Patrick Bird & Erin Crowley
Study Co-Directors
Q Laboratories, Inc.
1400 Harrison Avenue
Cincinnati, Ohio 45214
Phone : 513-471-1300
Fax : 513-471-5600
Email: pbird@qlaboratories.com
ecrowley@qlaboratories.com

Important Information

1. Read the methods carefully. If you have any questions, contact one of the 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 Petrifilm RYM supplies are on hand.

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

4. THIS IS A STUDY OF THE METHOD, NOT OF THE LABORATORY. THE METHOD MUST BE FOLLOWED AS CLOSELY AS PRACTICABLE, AND ANY

DEVIATIONS FROM THE METHOD AS DESCRIBED, NO MATTER HOW TRIVIAL THEY MAY SEEM, MUST BE NOTED ON THE REPORT FORM.

5. Report all of your results as soon as analyses are completed. Do not do more or less than indicated in the instructions. For example, do not do duplicate analysis and report the best or average result. More or fewer results complicate the statistical analyses and may invalidate your results. Data sheets are provided with these instructions and indicate which results are to be reported. Please include any criticisms, suggested improvements, or general comments about the 3M Petrifilm RYM Count Plate method 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 Study Director. All test portions will be shipped refrigerated. **Analysis of test portions must be initiated on the day scheduled by the Study Director.** 3M Petrifilm RYM Plates will be sent directly from 3M. Other supplies (Appendix 8.3) will be sent by the coordinating laboratory.

Proposed analysis schedule

Food	Date Shipped	Date Tested
Raw frozen ground beef (80% lean/20% fat)	TBD*	TBD
Raw almonds	TBD	TBD

*To be completed by coordinating laboratory

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 frozen ground beef samples should arrive frozen and should be stored at -20°C (frozen) until the day of analysis. Raw almonds should be stored at room temperature (21-23°C).

Test Portion Analysis

Collaborators will receive eight 25g test portions to test each matrix using the 3M Petrifilm RYM and reference methods. 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 sheets provided.

Food Type	Reference Methods	Diluent	3M Petrifilm RYM plates
Raw frozen ground beef (80% lean/20% fat)	ISO 21527-2008 & FDA-BAM Ch. 18	0.1% Peptone	2 at 25°C 2 at 28°C
Raw almonds	ISO 21527-2008 & FDA-BAM Ch. 18	0.1% Peptone	2 at 25°C 2 at 28°C

1.1. Sample Preparation

1.1.1. Prepare a 1:10 dilution of each test portion – 25g plus 225 mL of 0.1% Peptone Water (10^{-1})

1.1.2. Stomach for 2 minutes in the stomacher bag.

1.1.3. Prepare an additional 4 dilutions (10^{-2} to 10^{-4}) using 0.1% Peptone Water.

1.2. **3M Petrifilm Rapid Yeast and Mold Count Plate Method** (please refer to the IFU for additional details and Appendix 8.4 for flow diagram)

1.2.1 Place **four** 3M Petrifilm RYM Plates on a flat, level surface for each dilution to be tested.

1.2.2 Lift the top film and with the pipette perpendicular dispense 1 mL of each dilution onto the center of bottom film of each plate.

1.2.3 Drop the top film down onto the sample.

1.2.4 Place the 3M Petrifilm Flat Spreader (6425) on the center of the plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm RYM Plate growth area before the gel is formed. Do not slide the spreader across the film.

1.2.5 Remove the spreader and leave the plate undisturbed for at least one minute to permit the gel to form.

1.2.6 Incubate **two** 3M Petrifilm RYM Plates of each dilution at 25°C and **two** 3M Petrifilm RYM Plates at 28°C in a horizontal position with the clear side up in stacks of no more than 40. Enumerate plates at **48 ± 2 hours and 60 hours, keeping the time the plates are out of the incubator to a minimum**.

1.2.7 3M Petrifilm RYM plates can be counted using a standard colony counter or other illuminated magnifier. Gridlines are visible with the use of a backlight to assist with estimated enumeration.

1.2.8 To differentiate yeast and mold colonies on the Petrifilm RYM, look for one or more of the following characteristics:

YEAST	**MOLD**
Small colonies	Large colonies
Colonies have defined edges	Colonies have diffuse edges
Pink-tan to blue-green in color	Blue/green to variable upon prolonged incubation
Colonies appear raised (3 dimensional)	Colonies appear flat
Colonies have a uniform color	Colonies have a dark center with diffused edge

1.2.9 The circular growth area is approximately 30 cm^2. Plates containing greater than 150 colonies can either be estimated or recorded as TNTC. Estimation can be done by counting the number of colonies in one or more representative squares and determining the average number per square. The average number can be multiplied by 30 to determine the estimated count per plate. If a more accurate count is required, the sample will need to be retested at higher dilutions. When the sample contains substantial amounts of mold, depending on the type of mold, the upper countable limit may be lowered at user discretion.

1.2.10 Food samples may occasionally show interference on the 3M Petrifilm RYM Plates, for example:

1.2.10.1 uniform blue background color (often seen from the organisms used in cultured products) these should not be counted as TNTC.

1.2.10.2 intense, pinpoint blue specs (often seen with spices or granulated products).

1.3. **ISO 21527:2008. Parts 1 and 2 and FDA-BAM Ch. 18 (April 2001)**[1]

1.3.1Using the diluted sample (prepared in section 5) distribute 0.1 ml inoculum of each dilution onto specific agar plate listed below in triplicate and spread.

1.3.1.1 Low moisture foods (raw almonds): Dichloran 18% glycerol (DG18) agar

1.3.1.2 High moisture foods (raw frozen ground beef (80% lean/20% fat): Dichloran-rose bengal chloramphenicol agar (DRBC).

1.3.2 Incubate at 25°C for 5-7 days. Do not incubate plates in stacks higher than three and do not invert.

1.3.3 After 5 days count and record typical colonies. If there is no growth, re-incubate for another 48 hours.

1.3.4 Record results from the three plates with 10-150 colonies and report average results in colony forming units (CFU) per gram.

Final Results

[1] http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071435.htm

All results are to be recorded on the data sheets provided. Immediately upon completion of each food type, the data sheet should be faxed or emailed to co-Study Director Patrick Bird at: (513)-471-5600 or pbird@qlaboratories.com.

Appendix 8.1a – Raw frozen ground beef Collaborative Study Data Report Form

Petrifilm RYM Lot:

0.1% Peptone Lot:

DRBC Lot:

	Dilution	48 h	Dilution	60 h	Dilution	48 h	Dilution	60 h	Test Portion Number
Test portion 1a									1a
Test portion 1b									1b
Test portion 2a									1c
Test portion 2b									2a
Test portion 3a									2b
Test portion 3b									2c
Test portion 4a									3a
Test portion 4b									3b
Test portion 5a									3c
Test portion 5b									4a
Test portion 6a									4b
Test portion 6b									4c
Test portion 7a									5a
Test portion 7b									5b
Test portion 8a									5c
Test portion 8b									6a
									6b
									6c
									7a
									7b
									7c
									8a
									8b
									8c

Appendix 8.2b – Raw almonds Collaborative Study Data Report Form

Petrifilm RYM Lot:

0.1% Peptone Lot:

DG18 Lot:

	Dilution	48 h	Dilution	60 h	Dilution	48 h	Dilution	60 h	Test Portion Number
Test portion 1a									1a
Test portion 1b									1b
Test portion 2a									1c
Test portion 2b									2a
Test portion 3a									2b
Test portion 3b									2c
Test portion 4a									3a
Test portion 4b									3b
Test portion 5a									3c
Test portion 5b									4a
Test portion 6a									4b
Test portion 6b									4c
Test portion 7a									5a
Test portion 7b									5b
Test portion 8a									5c
Test portion 8b									6a
									6b
									6c
									7a
									7b
									7c
									8a
									8b
									8c

Appendix 8.3

Study Materials

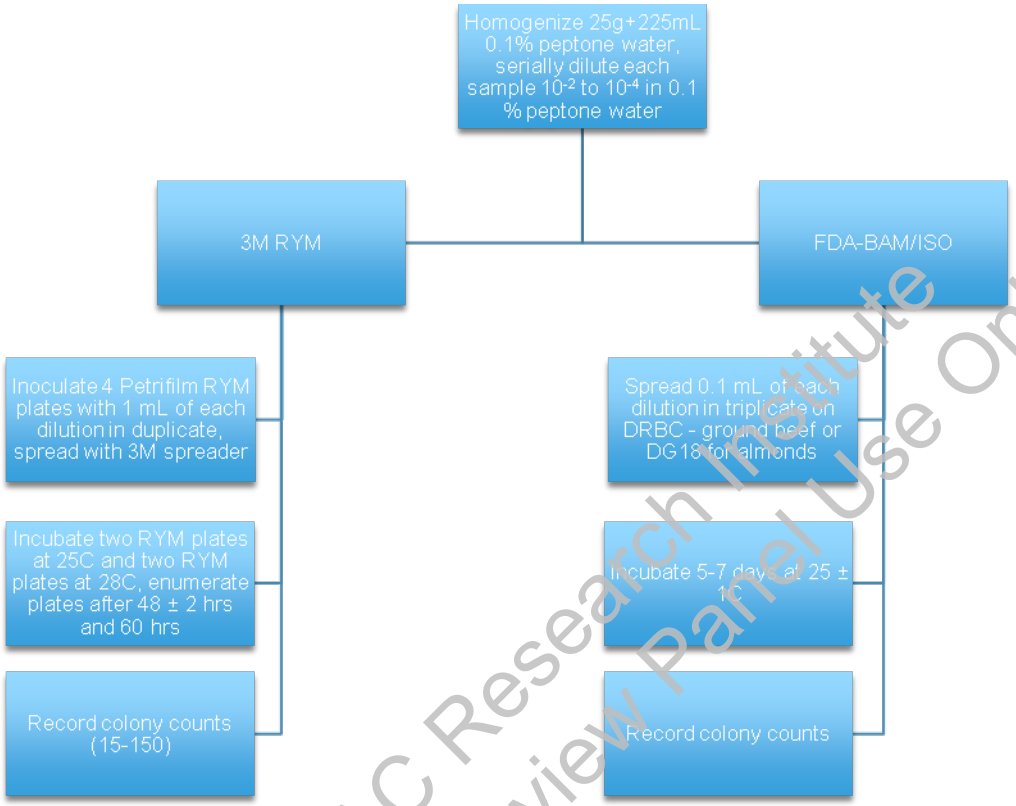
Materials and reagents provided by the study sponsor

1. 3M™ Petrifilm™ Flat Spreader (6425)
2. 3M™ Petrifilm™ Rapid Yeast and Mold Count Plates
3. Sterile diluent – 0.1% peptone water
4. Dichloran-rose bengal chloramphenicol agar (DRBC)
5. Dichloran 18% glycerol (DG18) agar
6. L shaped spreaders (hockey sticks)

Materials required but not provided

1. *Pipettes*. – capable of pipetting 1,000µL or sterile serological pipette
2. *Sterile pipette tips.* – capable of 1,000µL
3. *Stomacher®*. – Seward or equivalent
4. *Filter Stomacher® bags*. - Seward or equivalent
5. *Incubators*. – Incubator capable of maintaining 25 ± 1°C and another capable of maintaining 28 ± 1°C, and having a solid front so as to maintain a dark interior.
6. *Refrigerator*. – capable of maintaining 2-8°C, for storing the 3M Petrifilm RYM Count Plates

Appendix 8.4 – Study flow diagram



Appendix 8.5

3M™ Petrifilm™ Rapid Yeast and Mold Count Plate

Collaborative Study
Collaborator Information Sheet

Please complete the information below and return to TBD.

Contact name:

Laboratory name:

Fax number:

Phone number:

Email address:

Mailing address:

Shipping address:

Appendix 8.6

3M™ Petrifilm™ Rapid Yeast and Mold Count Plate

Collaborative Study
Collaborator Comments Form

Collaborating Laboratory:

Date:

General Comments about Method:

3M™ Petrifilm™ Rapid Yeast Mold Count Plate

AOAC Performance Tested Method

Abstract

The 3M™ Petrifilm™ Rapid Yeast Mold Count Plate (Petrifilm RYM) detects and enumerates yeast and mold from a variety of foods. Ten matrices – yogurt, sour cream, almonds, sliced apples, frozen bread dough, ready-made pie, sandwiches, dehydrated soup, fermented salami and frozen ground beef patties – were compared to the ISO 21527:2008 parts 1 and 2 and the FDA BAM Chapter 18 reference methods at 48 and 60 hours. Naturally contaminated samples that covered three levels of contamination (low level 10 -100 CFU/g, medium level 100-1,000 CFU/g, and a high level 1,000-10,000 CFU/g) were analyzed. Matrices that were not naturally contaminated were artificially contaminated with a yeast or mold, and five replicates were analyzed at four levels (uninoculated, low, medium, and high). The results demonstrated that the 3M Petrifilm RYM Plate method as shown to be equivalent to or better than the average log counts of the ISO 21527:2008 parts 1 and 2 and the FDA BAM Chapter 18 reference methods at 48 and 60 hours. In addition, the repeatability of the Petrifilm RYM method was equivalent to or better than the reference methods at both 48 and 60 hours. In the independent laboratory validation study, there was no significant difference in detection, enumeration, or repeatability between the Petrifilm RYM method and ISO 21527:2008 parts 1 and 2 and the FDA BAM Chapter 18 reference methods for yogurt at 48 and 60 hours. For frozen ground beef patties there was a significant difference between the Petrifilm RYM Plate method and the reference methods at 48 and 60 hours with the Petrifilm RYM method enumerating more organisms that the reference methods($p<0.05$). In strain studies, of the 42 yeast and mold tested, all produced typical colonies on Petrifilm RYM Plate method. Of the 4 bacterial strains that should not have been detected by the Petrifilm RYM Plate method, all 4 strains did not grow on the plate. Lot-to-lot study of Petrifilm RYM Plates was evaluated and showed no differences at time zero. In assay ruggedness studies, there was no difference in performance for three test parameters: incubation time, incubation temperature and type of diluents used to rehydrate the plate medium.

Method Author

Bob Jechorek
3M Food Safety Department
3M Center - Bldg 260-6B-01
St. Paul, MN 55144-1000

Submitting Company

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

Independent Laboratory

Aegis laboratories
224 N. Derby Lane
North Sioux City, South Dakota 57049

Reviewers of Final Submission

Yi Chen, US-FDA (AOAC General Referee)
Michael Brodsky, Brodsky Consultants
Wayne Ziemer

General Information

The large and diverse group of microscopic foodborne yeasts and molds (fungi) includes several hundred species. The ability of these organisms to grow in many foods is due in large part to their relatively versatile environmental requirements.

Both yeasts and molds cause various degrees of deterioration and decomposition of foods. They are ubiquitous organisms that can contaminate virtually any type of food through the entire food chain. Their ability to be detected in or on foods depends on food type, organisms involved, and degree of

contamination; the contaminated food may be slightly blemished, severely blemished, or completely decomposed, with the actual growth manifested by rot spots of various sizes and colors, unsightly scabs, slime, white cottony mycelium, or highly colored spore releasing mold. Abnormal flavors and odors may also be produced. Contamination of foods by yeasts and molds can result in substantial economic losses to producer, processor, and consumer[1].

Several food borne molds and some yeasts, may also be hazardous to human or animal health because of their ability to produce toxic metabolites known as mycotoxins.

The 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate method was developed to provide rapid quantitative yeast and mold results from foods. The method is reproducible, and is faster than the agar methods, providing results in 48 hours compared to the typical five to seven days.

Principle

The 3M™ Petrifilm™ Rapid Yeast and Mold Count (RYM) Plate is a sample-ready culture medium system which contains nutrients supplemented with antibiotics, a cold-water soluble gelling agent, and an indicator system that facilitates yeast and mold enumeration, but does not speciate yeast and mold from one another. 3M Petrifilm RYM is used for the enumeration of yeast and mold in the food and beverage industries and has been validated for yogurt, sour cream, almonds, sliced apples, frozen bread dough, ready-made pie, sandwiches, dehydrated soup, fermented salami and frozen ground beef patties.

Definitions

Sensitivity .— *Number of True* Positives / (Number *of True P*ositives + Number of False Negatives)

Specificity .— Number of True N*egatives /* (Number of False Positives + Number of True Negatives)

Materials and Methods

Test Kit Information

Kit Name: 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate

Catalog Number: Box 6475, Case 6477

Test Kit Reagents

3M™ Petrifilm™ Rapid Yeast and Mold Count Plate

Additional Supplies and Reagents

1. Pipettor capable of pipetting 1,000µl or sterile serological pipette
2. Sterile pipette tips
3. Sterile diluent
4. 3M™ Petrifilm™ Flat Spreader (6425)

Apparatus

Incubator capable of maintaining 25-28 ± 1°C.

Safety Precautions

3M Petrifilm RYM Plates do not differentiate any one yeast or mold strain from another.

3M has not documented 3M Petrifilm RYM Plates for use in industries other than food and beverage. For example, 3M has not documented 3M Petrifilm RYM Plates for testing water, pharmaceuticals or cosmetics. 3M Petrifilm RYM Plates have not been tested with all possible food products, food processes, testing protocols or with all possible strains of yeast and mold.

Do not use 3M Petrifilm RYM Plates in the diagnosis of conditions in humans or animals.

The user must train its personnel in proper testing techniques. For example, Good Laboratory Practices[2], ISO 7218[3], or ISO 17025[4]. After use, 3M Petrifilm™ RYM Plates may contain microorganisms that may be a potential biohazard. Follow current industry standards for disposal.

General Preparation

Use proper aseptic technique. Use proper precautions for biosafety level 2 microorganisms.

Store unopened packages at ≤8°C (≤46°F). Use before expiration date on package. In areas of high humidity where condensate may be an issue, it is best to allow packages to reach room temperature before opening.

Sample Preparation

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.

Analysis

To use 3M Petrifilm RYM Plates, place the plates on a flat, level surface. Lift the top film and with the pipette perpendicular dispense 1 mL of sample suspension onto the center of bottom film, then roll the top film down onto the sample. Place the 3M™ Petrifilm™ Flat Spreader (6425) or other flat spreader on the center of the 3M Petrifilm RYM Plate and press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm RYM Plate growth area before the gel is formed. Do not slide the spreader across the film. Remove the spreader and leave the 3M Petrifilm RYM Plate undisturbed for at least one minute to permit the gel to form.

Incubate 3M Petrifilm RYM Plates at 25-28°C for 48 +/- 2 hours* in a horizontal position with the clear side up in stacks of no more than 40.

*If colonies appear faint, allow an additional 12 hours of incubation time for enhanced interpretation.

Interpretation

3M Petrifilm RYM Plates can be counted using a standard colony counter or other illuminated magnifier. Gridlines are visible with the use of a backlight to assist with estimated enumeration.

Do not count colonies on the foam dam since they are removed from the nutrient medium. To differentiate yeast and mold colonies on the 3M Petrifilm RYM Plate, look for one or more of the following characteristics listed in Table 1.

Table 1. Colonial Characteristics of Yeast and Mold on Petrifilm RYM Plates.

YEAST	MOLD
Small colonies	Large colonies
Colonies have defined edges	Colonies have diffuse edges
Pink-tan to blue-green in color	Blue/green to variable upon prolonged incubation
Colonies appear raised (3 dimensional)	Colonies appear flat
Colonies have a uniform color	Colonies have a dark center with diffused edge

Examine the plates for yeast and mold results at 48 hours. Certain slower growing yeasts and molds may appear faint at 48 hours. To enhance interpretation of these molds allow for an additional 12 hours of incubation time. The circular growth area is approximately 30 cm^2. 3M Petrifilm RYM Plates containing greater than 150 colonies can either be estimated or recorded as TNTC. Estimation can be done by counting the number of colonies in one or more representative squares and determining the average number per square. There are 30 squares in the counting area. The average number can be multiplied by 30 to determine the estimated count per plate. If a more accurate count is required, the

sample will need to be retested at higher dilutions. When the sample contains substantial amounts of mold, depending on the type of mold, the upper countable limit may be lowered at user discretion. Food samples may occasionally show interference on the 3M Petrifilm RYM Plates, for example: a) a uniform blue background color (often seen from the organisms used in cultured products) these should not be counted as TNTC. b) intense, pinpoint blue specs (often seen with spices or granulated products).

When necessary, colonies may be isolated for further identification. Lift the top film and pick the colony from the gel.

Internal Validation Studies

Ruggedness Testing

Minor perturbations were introduced within parameters that might be expected to occur when Petrifilm RYM Plates are used. The results are listed in Tables 2 and 3.

Methodology

The effects of perturbations in three (3) method parameters were investigated:

1. Incubation time (44 and 62 hours)
2. Incubation temperature (24 ± 1°C and 29 ± 1°C)
3. Dilution fluid (0.1% peptone Water, 0.1% peptone water (45°C), Butterfields Buffer, Letheen Broth, saline, peptone salt diluent, water, buffered peptone water).

The Petrifilm RYM Plate method was evaluated for ruggedness by examining the performance of the plates when small changes in the operating conditions were made. All ruggedness parameters were evaluated with one yeast (*Candida tropicalis*) and one mold (*Geotrichum candidum*) at high and low level inoculums, and one non-target strain (*Pseudomonas aeruginosa* ATCC # 27853) at a high level inoculum. For the target organisms, five replicates were inoculated to achieve the target levels of 50-75 CFU/mL for high level yeast and 25-50 CFU/mL for high level mold, and 1-20 CFU/ml for the low level yeasts and 1-10 CFU/ml for the low level mold. For the non-target strain, five replicates were inoculated to achieve target levels of 80-100 CFU/mL for the high level.

Table 2. Summary of Ruggedness Test Results for *Candida tropicalis* – Inoculation Level, Incubation Time, Incubation Temperature, and Dilution Fluid.

24°C 44 and 62 hours	44 hours							62 hours						
***C. tropicalis* - LOW Inoculation**	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev
0.1% Peptone Water	16	16	23	16	15	17.200	3.271	17	16	24	17	16	18.000	3.391
0.1% Peptone water (45°C)	17	17	13	21	18	17.200	2.864	18	18	13	21	19	17.800	2.950
Butterfields Buffer	28	21	18	18	20	21.000	4.123	28	21	19	18	20	21.200	3.962
Letheen Broth	20	25	31	16	17	21.800	6.221	21	26	31	17	18	22.600	5.857
Saline	7	12	6	18	11	10.800	4.764	8	12	6	18	12	11.200	4.604
Peptone Salt Diluent	19	8	15	17	12	14.200	4.324	19	9	15	17	14	14.800	3.768
Water	11	15	7	18	18	13.800	4.764	11	15	8	19	19	14.400	4.879
BPW	12	15	15	17	13	14.400	1.949	13	15	15	18	13	14.800	2.049
29°C 44 and 62 hours	44 hours							62 hours						
***C. tropicalis* - LOW Inoculation**	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev
0.1% Peptone Water	18	26	29	16	19	21.600	5.595	18	26	29	17	19	21.800	5.357
0.1% Peptone water (45°C)	23	18	22	14	18	19.000	3.606	23	18	22	14	19	19.200	3.564
Butterfields Buffer	16	16	11	13	21	15.400	3.782	16	16	11	13	21	15.400	3.782
Letheen Broth	18	17	18	20	20	18.600	1.342	20	17	18	20	22	19.400	1.949
Saline	7	12	14	11	16	12.000	3.391	7	12	14	10	15	11.600	3.209
Peptone Salt Diluent	24	21	16	14	11	17.200	5.263	24	21	16	14	11	17.200	5.263
Water	11	13	16	27	14	16.200	6.301	11	13	16	27		16.750	7.136
BPW	9	17	22	15	21	16.800	5.215	9	16	2	15	21	12.600	7.301
24°C 44 and 62 hours	44 hours							62 hours						
***C. tropicalis* - HIGH Inoculation**	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev
0.1% Peptone Water	59	65	65	62	65	63.200	2.683	60	66	65	63	66	64.000	2.550
0.1% Peptone water (45°C)	49	56	59	57	72	58.600	8.385	50	59	62	60	74	61.000	8.602
Butterfields Buffer	52	45	58	49	63	53.400	7.162	54	45	62	50	63	54.800	7.727
Letheen Broth	47	64	54	48	50	52.600	6.914	47	64	56	50	50	53.400	6.768
Saline	44	39	41	50	38	42.400	4.827	46	39	42	50	38	43.000	5.000
Peptone Salt Diluent	56	58	41	36	35	45.200	11.032	56	61	44	36	35	46.400	11.718
Water	29	38	36	51	30	36.800	8.815	32	42	46	52	31	40.600	9.044
BPW	40	36	39	42	44	40.200	3.033	41	37	39	42	46	41.000	3.391
29°C 44 and 62 hours	44 hours							62 hours						
***C. tropicalis* - HIGH Inoculation**	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev
0.1% Peptone Water	59	60	80	64	69	66.400	8.562	60	60	82	65	70	67.400	9.154
0.1% Peptone water (45°C)	58	72	65	63	54	62.400	6.877	57	72	66	64	57	63.200	6.380
Butterfields Buffer	50	53	59	44	63	53.800	7.463	50	52	59	44	63	53.600	7.503
Letheen Broth	60	49	58	55	65	56.200	6.380	61	50	51	55	65	56.400	6.465
Saline	36	40	52	42	37	41.400	6.387	35	41	53	42	36	41.400	7.162
Peptone Salt Diluent	43	50	44	38	45	44.000	4.301	43	49	45	39	45	44.200	3.633
Water	45	37	35	39	32	37.600	4.879	45	37	35	39	32	37.600	4.879
BPW	53	52	42	51	60	51.600	6.427	53	52	42	50	59	51.200	6.140

Table 3. Summary of Ruggedness Test Results for *Geotrichum candidum* – Inoculation Level, Incubation Time, Incubation Temperature, and Dilution Fluid.

24°C 44 and 62 hours	44 hours							62 hours						
***G. candidum* - LOW**	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev
0.1% Peptone Water	9	7	2	9	9	7.200	3.033	9	9	2	11	9	8.000	3.464
0.1% Peptone water (45°C)	9	8	12	8	6	8.600	2.191	10	9	12	10	9	10.000	1.225
Butterfields Buffer	4	2	10	8	2	5.200	3.633	4	2	10	8	4	5.600	3.286
Letheen Broth	7	5	7	11	7	7.400	2.191	11	7	7	14	10	9.800	2.950
Saline	2	3	3	1	3	2.400	0.894	3	5	2	1	3	2.800	1.483
Peptone Salt Diluent	2	1	3	1	3	2.000	1.000	2	2	3	2	3	2.400	0.548
Water	3	3	6	3	3	3.600	1.342	3	3	6	4	3	3.800	1.304
BPW	6	2	4	8	4	4.800	2.280	7	3	6	9	6	6.200	2.168
29°C 44 and 62 hours	44 hours							62 hours						
***G. candidum* - LOW**	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev
0.1% Peptone Water	5	9	9	10	4	7.400	2.702	5	10	10	10	5	8.000	2.739
0.1% Peptone water (45°C)	8	7	4	6	6	6.200	1.483	8	6	4	6	6	6.000	1.414
Butterfields Buffer	3	2	8	8	8	5.800	3.033	3	2	8	8	8	5.800	3.033
Letheen Broth	8	12	10	13	9	10.400	2.074	9	12	10	13	9	10.600	1.817
Saline	2	5	2	2	1	2.400	1.517	2	5	3	2	1	2.600	1.517
Peptone Salt Diluent	4	4	1	4	3	3.200	1.304	4	5	1	4	3	3.400	1.517
Water	2	3	1	5	4	3.000	1.581	2	4	1	5	4	3.200	1.643
BPW	7	9	9	3	8	7.200	2.490	7	9	9	7	8	8.000	1.000
24°C 44 and 62 hours	44 hours							62 hours						
***G. candidum* - HIGH**	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev
0.1% Peptone Water	29	29	30	16	24	25.600	5.857	36	34	28	19	26	30.600	7.925
0.1% Peptone water (45°C)	26	22	26	34	31	27.800	4.712	31	28	32	37	34	32.400	3.362
Butterfields Buffer	20	28	33	28	24	26.600	4.879	21	29	37	19	27	26.600	7.127
Letheen Broth	28	30	25	29	20	26.400	4.037	34	33	26	26	21	28.000	5.431
Saline	21	14	11	15	14	15.000	3.674	20	15	14	18	16	16.600	2.408
Peptone Salt Diluent	17	22	16	17	16	17.600	2.510	17	24	17	17	17	18.400	3.130
Water	17	16	20	19	18	18.000	1.581	19	17	26	20	21	20.600	3.362
BPW	16	20	16	16	15	16.600	1.949	20	21	21	21	23	21.200	1.095
29°C 44 and 62 hours	44 hours							62 hours						
***G. candidum* - HIGH**	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	Sdev
0.1% Peptone Water	31	28	23	29	33	28.800	3.768	31	28	24	30	33	29.200	3.421
0.1% Peptone water (45°C)	21	24	23	26	25	23.800	1.924	21	24	24	28	25	24.400	2.510
Butterfields Buffer	23	32	21	24	26	25.200	4.207	23	31	21	24	26	25.000	3.808
Letheen Broth	31	30	33	32	29	31.000	1.581	31	29	33	32	30	31.000	1.581
Saline	18	12	18	18	22	17.600	3.578	18	12	18	18	22	17.600	3.578
Peptone Salt Diluent	18	6	13	13	17	13.400	4.722	17	7	13	13	17	13.400	4.099
Water	17	22	20	27	19	21.000	3.808	17	23	21	28	19	21.600	4.219
BPW	26	24	20	21	20	22.200	2.683	26	23	21	21	19	22.000	2.646

A General Linear Model statistic was used to compare the ruggedness parameters. The p values indicated that there were no significant differences in the results obtained by the Petrifilm RYM Plate method for the detection of the low or high yeast or mold levels when minor modifications to incubation temperature (p=0.975), incubation time (p=0.721), dilution buffers (p=0.289) were made.

Shelf Life and Lot-to-Lot Consistency

3M Petrifilm RYM Plates were made from multiple lots of raw materials and were manufactured at different times. Sixty different samples representing three different lots of manufacture and shelf life were created. One yeast, *Candida troplicalis* (Microbiologics 45062-1 2015-03), and one mold, *Geotrichum candidum* (Microbiologics 519-1-7 2014-07), were tested at high and low inoculum levels.

One non-target strain, *E. coli* ATCC 25922,was tested at a high level inoculum level (target 150 cfu/mL). For the yeast *Candida troplicalis*, five replicates were inoculated to Petrifilm RYM Plates achieve the target level of approximately 50-100 CFU/mL for high level and 10-20 CFU/ml for the low level. For *E. coli*, five replicates were inoculated to achieve target levels of 100-150 CFU/mL for the high level. The plates were incubated at 25 ± 1°C for 48 hours and then the colonies were enumerated.

Table 4. Summary of Combined Shelf Life and Lot-to-Lot Consistency for *Geotrichum candidum* at the Low Inoculation Level.

DRBC Raw Count	Log_{10}	Petrifilm 2014-01 KA Raw Count	Log_{10}	Petrifilm 2014-01 KB Raw Count	Log_{10}	Petrifilm 2014-01 KC Raw Count	Log_{10}
14	1.146	17	1.230	21	1.322	13	1.114
24	1.380	14	1.146	11	1.041	21	1.322
15	1.176	17	1.230	21	1.322	20	1.301
18	1.255	15	1.176	13	1.114	16	1.204
18	1.255	15	1.176	14	1.146	15	1.176
	1.243		**1.192**		**1.189**		**1.223**
		t-test	**0.422**		**0.603**		**0.631**

Table 5. Summary of Combined Shelf Life and Lot-to-Lot Consistency for *Geotrichum candidum* at the High Inoculation Level.

DRBC Raw Count	Log_{10}	Petrifilm 2014-01 KA Raw Count	Log_{10}	Petrifilm 2014-01 KB Raw Count	Log_{10}	Petrifilm 2014-01 KC Raw Count	Log_{10}
43	1.633	42	1.623	47	1.672	58	1.763
45	1.653	55	1.740	48	1.681	54	1.732
54	1.732	46	1.663	51	1.708	45	1.653
33	1.519	48	1.681	50	1.699	50	1.699
40	1.602	56	1.748	57	1.756	46	1.663
	1.628		**1.691**		**1.703**		**1.702**
		t-test	**0.232**		**0.128**		**0.164**

Table 6. Summary of Combined Shelf Life and Lot-to-Lot Consistency for *Candida tropicalis* at the Low Inoculation Level.

DRBC Raw Count	Log_{10}	Petrifilm 2014-01 KA Raw Count	Log_{10}	Petrifilm 2014-01 KB Raw Count	Log_{10}	Petrifilm 2014-01 KC Raw Count	Log_{10}
18	1.255	17	1.230	18	1.255	21	1.322
17	1.230	9	0.954	21	1.322	20	1.301
22	1.342	20	1.301	16	1.204	19	1.279
21	1.322	15	1.176	13	1.114	24	1.380
12	1.079	21	1.322	17	1.230	14	1.146
	1.246		**1.197**		**1.225**		**1.286**
		t-test	**0.597**		**0.775**		**0.200**

Table 7. Summary of Combined Shelf Life and Lot-to-Lot Consistency for *Candida tropicalis* at the High Inoculation Level.

DRBC Raw Count	Log_{10}	Petrifilm 2014-01 KA Raw Count	Log_{10}	Petrifilm 2014-01 KB Raw Count	Log_{10}	Petrifilm 2014-01 KC Raw Count	Log_{10}
88	1.944	114	2.057	92	1.964	90	1.954
105	2.021	102	2.009	91	1.959	104	2.017
87	1.940	91	1.959	99	1.996	101	2.004
95	1.978	95	1.978	92	1.964	115	2.061
75	1.875	92	1.964	94	1.973	97	1.987
	1.952		**1.993**		**1.971**		**2.005**
		t-test	**0.170**		**0.520**		**0.073**

Table 8. Summary of Combined Shelf Life and Lot-to-Lot Consistency for *E. coli* at the High Inoculation Level.

Organism	Replicate	Petrifilm AC	DRBC Agar	2014-01 KA CFU/plate	2014-01 KB CFU/plate	2014-01 KC CFU/plate
E. coli 25922 High Inoculation Level	1	142	0	0	0	0
	2	127	0	0	0	0
	3	139	0	0	0	0
	4	120	0	0	0	0
	5	142	0	0	0	0

Summary of Combined Lot-to-Lot and Shelf Life Stability

A paired t-test for equal or unequal variances was conducted to analyze statistical differences between Lot 2014-01 KA (time 0 shelf life), Lot 2014-01 KB (time 0 shelf life), Lot 2014-01 KC (time 0 month shelf life). The p values obtained indicated that there were no significant differences in the results obtained by the Petrifilm RYM Plate method for the detection of yeast or mold at the low or high level at any lot/shelf life combination. The non-target organism, *E. coli*, was not detected one any of the plates at

any lot/shelf life combination. The lot-to-lot /shelf life study is continuing and results will be reported as they become available.

Inclusivity Testing

The objective of this experiment was to determine the ability of the 3M Petrifilm RYM Plate method to detect a variety of yeast and molds. A total of 19 yeast and 23 molds were tested (Table 9). Of the 42 organisms tested, 32 of the organisms were obtained from the Microbiologics, Inc. (St. Cloud, MN). The 10 strains not Microbiologics derived were from 3M culture collections. While an inclusivity list is not required for a yeast/mold Performance Tested Method submission, one is included here to demonstrate the broad range of the yeasts and molds detected by the Petrifilm RYM Count Plate method.

Inclusivity Testing Methodology

For the organisms obtained from Microbiologics, Inc. as LYFO DISK® preparations, one pellet was added to 10ml of pre-warmed (37°C) 0.1% peptone water and then vortexed until pellet was completely dissolved. The pellet suspension was serially diluted as needed (the pellet counts provided by manufacturer served as guidance in establishing dilution scheme). The RYM plates were inoculated in triplicate with 1ml from each dilution to obtain counts within countable range. The RYM plates were then incubated at 25°C and enumerated at 48 and 60 hours.

For the pour plates, sterile DRBC (dichloran-rose bengal chloramphenicol agar), tempered to 50-55°C, was added to the dishes and the agar was allowed to solidify at room temperature. Then, 100µl of each dilution was added to triplicate DRBC plates and spread with a sterile L shaped spreader. The plates were then incubated at 25°C, and enumerated after five days of incubation.

All 42 organisms tested on the Petrifilm RYM Count Plate were detected as typical colonies, yielding a sensitivity of 100%.

Table 9. Inclusivity Organisms.

Organism-Yeasts- 48 hours	Source
Y41: *Saccharomyces cerevisiae*	3M culture collection
Y28: *Hansenuela anomala*	3M culture collection
Candida albicans	3M culture collection
Rh. Mucaliginosa	Microbiologics
Trichosporon mucoides	Microbiologics
Candida guillermondii	Microbiologics
Saccharomyces lactis	Microbiologics
Candida sphaerica	Microbiologics
Candida tropicalis	Microbiologics
Kluyveromyces lactis ATCC 8563	Microbiologics
Kluyveromyces lactis ATCC 10689	Microbiologics
Debaryomyces hansenii	Microbiologics
Candida catenulata ATCC 10565	Microbiologics
Candida glabralta	Microbiologics
Candida kefyr ATCC 204093	Microbiologics
Candida krusei ATCC 14243	Microbiologics
Candida lusitaniae ATCC 34449	Microbiologics
Ustilago spp.	Microbiologics
Yarrowia lypolitica	3M culture collection

Organism-Molds- 48 hours	Source
*Aspergillus niger (M6) (re-*classified as *brasiliensis*)	Microbiologics
Aspergillus oryzae	Microbiologics
Aspergillus brasiliensis	Microbiologics
Paecilomyces sp (M10)	Microbiologics
Penicillium chrysogenum	Microbiologics
Geotrichum candidum	Microbiologics
Cladosporium spp	Microbiologics
Scorulopsiosis acremonium	Microbiologics
Mucor racemosus	Microbiologics/NCPF
Aspergillus ustus	Microbiologics
Aspergillus flavus	3M culture collection
Aspergillus japonicus	3M culture collection
Aspergillus aculeatus	3M culture collection
Aspergillus carbonarius	3M culture collection
Cladosporium herbarum	3M culture collection
Trichoderma virens	3M culture collection
Chaemotium globosum	Microbiologics
Geotrichum capitatum	Microbiologics
Yeasts and Molds- Slow growing- 60 hrs	**Source**
Zygosaccharomyces rouxii [a]	Microbiologics
Zygosaccharomyces bailii [a]	Microbiologics
Penicillium aurantiogriseum [b]	Microbiologics
Aspergillus fumigatus [c]	Microbiologics
Botrytis sp (M97) [c]	Microbiologics

[a] *Z bailii* and *Z rouxii* are slow growing yeasts - require 60 hours at 25 or 28C

[b] *P aurantiogriseum* is a slow growing mold - requires 60 hours at 25 or 28C

[c]: *A fumigatus* and M97 prefer a higher temperature for growth. These molds are visible on the Rapid Yeast and Mold plates within 48hrs at 28C but require 60 hours to grow at 25C

Exclusivity Testing

The objective of this experiment was to illustrate the specificity of the 3M Petrifilm RYM Plate method. A total of 4 species of bacteria were tested for exclusivity using the Petrifilm RYM Plate method (Table 10). All 4 species of bacteria were chosen from the 3M culture collection. While an exclusivity list is not

required for a yeast/mold Performance Tested Method submission, one is included here. The list is abbreviated for the following reasons:

- The antibiotic cocktail in the Petrifilm RYM Count Plate is the same as that for the current 3M™ Petrifilm™ Yeast and Mold Count Plate.
- There have been no complaints filed for breakthrough of bacteria on the current plate.
- The organisms selected represent both Gram positive and Gram negative organisms that are likely to be detected if there was breakthrough.

Exclusivity Testing Methodology

The exclusivity portion consisted of quantifying 4 non-target (bacterial) organisms using the Petrifilm RYM Plate method. Non-target (exclusivity) organisms were cultured in Tryptic Soy Broth (TSB) at 35 ± 1 °C for 18 to 22 hours. Ten-fold serial dilutions for each culture were made 0.1%peptone water to achieve a target inoculum of 100-150 CFU/mL. This was performed to verify that the target inoculum of 100-150 CFU/ml was achieved. The dilutions were then plated in triplicate onto Petrifilm RYM Plates and into sterile Petri dishes, to which ~20 mL of sterile, tempered Tryptic Soy Agar (TSA) was added. The TSA plates were swirled to mix and allowed to harden at room temperature.

The RYM plates were incubated at 25°C and enumerated at 48 and 60 hours. The TSA plates were incubated at 32°C and the colonies enumerated at 48 hours. None of the four non-target organisms were detected on the Petrifilm RYM Plates.

Table 10. Exclusivity Organisms

Organism	Source
Escherichia coli ATCC *25922*	3M Culture Collection
Bacillus subtilis ATCC *6633*	3M Culture Collection
Aeromonas hydrophila ATCC 7965	3M Culture Collection
Enterococcus faecalis ATCC 14506	3M Culture Collection

Inclusivity/Exclusivity Results

The Petrifilm RYM Plate method was used to evaluate 42 target yeasts and molds and 4 non-target organisms. All 42 target organisms produced typical (see colonial morphology descriptions listed in

Table 1) colonies by the Petrifilm RYM Plate method. There was no growth for any of the 4 non-target (exclusivity) strains on the Petrifilm RYM Plate method

Method Comparison

A study was conducted to determine the ability of the Petrifilm RYM Plate method to recover and enumerate yeasts and molds compared to the ISO 21527:2008 parts 1 and 2 and the FDA BAM Chapter 18.

Apparatus and Reagents

(a.) 3M Petrifilm Rapid Yeast and Mold Count Plate – Plates available from 3M Food Safety (St. Paul, MN), contain chromogenic detection medium and a cold-water-soluble gelling agent.
(b.) Pipets – 1 mL serological pipette or calibrated 1.0 mL positive displacement pipettor, or 3M Electronic Pipettor and tips, or equivalent, may be used to deliver a 1.0 mL inoculum onto plates.
(c.) Plastic spreader – Flat spreader available from 3M Food Safety, catalog number 6425.
(d.) Incubator – Capable of maintaining 25° - 28° ± 1°C for 48 ± 1 hours.
(e.) Colony Counter – Standard apparatus, Quebec Model, available from many suppliers, or one providing equivalent magnification and visibility.
(f.) DRBC Agar
(g.) Stomacher or equivalent
(h.) Appropriate diluent as described in the 3M Petrifilm Yeast and Mold Count Plate Instructions for Use

For the method comparison analysis, ten matrices were tested from a single production lot: Yogurt, sour cream, almonds, sliced apples, frozen bread dough, ready-made pie, sandwiches, dehydrated soup, fermented salami and frozen ground beef patties. For each matrix a minimum 3 lots were analyzed for the presence of the target analytes. Matrices containing naturally occurring yeasts and molds were temperature abused in order to get 3 distinctive lots. Naturally contaminated foods (frozen bread dough, sliced apples, sandwiches) had a target contamination level of low (10-100 CFU/g), medium (100-1,000 CFU/g), and high (1,000-10,000 CFU/g level). For the sliced apples naturally contaminated lots were held at 2-5°C for one week and diluted with uncontaminated product to reduce contamination levels. Frozen bread dough was diluted with uncontaminated product to reduce contamination levels. The sandwiches were held at 2-5°C for 48 hours, for 1 to 2 weeks to achieve three separate lots of contamination. A total of four lots each of artificially contaminated matrixes (yogurt, sour cream, almonds, ready-made pie, dehydrated soup, fermented salami and frozen ground beef patties), were analyzed for the target analyte at the following target levels: uninoculated (0 CFU/g), low (10-100 CFU/g), medium (100-1,000 CFU/g) and high (1,000-10,000 CFU/g). Frozen beef patties, yogurt, and ready-made pie were inoculated with a broth culture. The broth culture was prepared by transferring a single colony to yeast mold (YM) broth for 48 hours at 30°C. The inoculated test portions were mixed

thoroughly by hand kneading then held to stabilize according to their food type. Perishable foods (yogurt, sour cream, ready-made pie, and fermented salami) were refrigerated at 2-5°C for 48-72 hrs to allow time for the organism to equilibrate within the matrix. Frozen beef patties were thawed, inoculated and held at -20 to -30°C for a minimum of 2 weeks. For shelf stable foods, (almonds, dehydrated soup) a bulk lot of each matrix was inoculated with a lyophilized culture and stabilized for a minimum of 2 weeks at room temperature. The lyophilized mold cultures were purchased in pelleted form (Microbiologics, Inc) crushed and mixed into the test matrix.

Table 11. Matrix Preparation

Matrices	Agar Type	Inoculating Organism	Stabilization	Target Yeast and Mold Levels	Replicates
Yogurt	DRBC[a]	*Kluyveromyces lactis* ATCC 8563	2-4 days 2-6°C	10-100 cfu/ g 100-1000 cfu/ g 1000-10,000 cfu/g 0 cfu/g	5 5 5 5
Sour Cream	DRBC	*Geotrichum candidum* ATCC 34614	2-4 days 2-6°C	10-100 cfu/ g 100-1000 cfu/ g 1000-10,000 cfu/g 0 cfu/g	5 5 5 5
Almonds	DG18[b]	*Aspergillus aculeatus* ATCC 56925	2 weeks 25°C	10-100 cfu/ g 100-1000 cfu/ g 1000-10,000 cfu/g 0 cfu/g	5 5 5 5
Sliced Apples	DRBC	Natural	n/a	10-100 cfu/ g 100-1000 cfu/ g 1000-10,000 cfu/g	5 5 5
Frozen Bread Dough	DRBC	Natural	n/a	10-100 cfu/ g 100-1000 cfu/ g 1000-10,000 cfu/g	5 5 5
Ready-made Pie	DRBC	*Hansenuela anomala* (3M-Y28)	2-4 days 2-6°C	10-100 cfu/ g 100-1000 cfu/ g 1000-10,000 cfu/g 0 cfu/g	5 5 5 5
Sandwiches	DRBC	Natural	n/a	10-100 cfu/ g 100-1000 cfu/ g 1000-10,000 cfu/g	5 5 5
Dehydrated Soup	DG18	*Paecilomyces* spp (3M-M10)	2 weeks 25°C	10-100 cfu/ g 100-1000 cfu/ g 1000-10,000 cfu/g 0 cfu/g	5 5 5 5
Frozen Ground Beef Patty	DRBC	*Trichosporon mucoides* ATCC 201382	2 weeks -20 to -30°C	10-100 cfu/ g 100-1000 cfu/ g 1000-10,000 cfu/g 0 cfu/g	5 5 5 5
Fermented Salami	DRBC	*Candida tropicalis* ATCC 13803	2-4 days 2-6°C	10-100 cfu/ g 100-1000 cfu/ g 1000-10,000 cfu/g 0 cfu/g	5 5 5 5

[a] DRBC - dichloran-rose bengal chloramphenicol agar

[b] DG18 - dichloran 18% glycerol agar

For the two reference methods, ISO 21527 and FDA BAM, 25 gram test portions were diluted with 225 mL of 0.1% peptone water in a sterile bag, and stomached for 2 minutes. Serial dilutions were prepared in 0.1% peptone water and aliquots of each dilution were plated in triplicate on dichloran-rose bengal chloramphenicol agar (DRBC) or dichloran 18% glycerol (DG18) agar for low moisture foods. The agar plates were incubated for 5-7 days at 25°C. A Quebec Colony counter was used to assist in counting

colonies and results were reported by averaging counts between triplicate plates. Final results were presented as colony forming units per gram (CFU/g).

3M Petrifilm Rapid Yeast Mold Plate Method

Paired test portions for each matrix were prepared as described in the ISO and FDA BAM reference methods. A 1 mL aliquot of each sample dilution was placed on the RYM Petrifilm. The top film was gently lowered and the aliquot was spread with the 3M Petrifilm Flat Spreader. The plates were left undisturbed for one minute, and incubated for 48 ±2 hours at 25±1°C. Typical colonies were enumerated and reported as CFU/g. The RYM plates were reincubated for an additional 12 hours (60 total hours) and then enumerated using a Quebec colony counter. Typical yeast colonies were small pink tan or blue green in color with defined edges. Molds s typically formed large blue-green colonies with diffuse edges.

RESULTS

For each matrix, the colony forming units/g were converted to $\log_{10}$ and analyzed. A t-test was performed using the appropriate assumption for equal or unequal variance to evaluate the mean of the $\log_{10}$ values for statistical difference between two methods at the 95% confidence level.

Repeatability of the Petrifilm RYM method and the reference methods was calculated as the standard deviation (S_r) for each contamination level for each matrix.

Yogurt 48 hours

No statistically significant difference was observed between all 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low, medium, and high levels were 0.6343, 0.4633, and 0.6776, respectively. The t-test indicated no significant differences between the 3M RYM method and the ISO and FDA BAM methods. The 3M RYM method had a higher repeatability value than the ISO and FDA BAM methods for the medium and high levels, with S_r values, respectively, of 0.0279 and 0.0850 for the medium level and 0.0787 and 0.0895 for the high level. For the low level the ISO and FDA BAM had higher repeatability value compared to the RYM method, with S_r values, respectively, of 0.1026 and 0.0445. Detailed results are presented in Table 12.

Table 12. Method Comparison Results for Yogurt at 48 Hours.

Level	Sample	3M RYM Petrifilm				ISO/ FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	
Uninoculated	1	<10	<0			<10	<0			
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0	-	-	<10	<0	-	-	NA
Low	1	2.8×10^2	2.4472			2.6×10^2	2.415			
	2	2.0×10^2	2.301			3.2×10^2	2.5051			
	3	3.0×10^2	2.4771			3.0×10^2	2.4771			
	4	3.2×10^2	2.5051			3.2×10^2	2.5051			
	5	3.8×10^2	2.5798	2.462	0.1026	3.4×10^2	2.5315	2.4868	0.0445	0.6343
Medium	1	2.8×10^3	3.4472			2.4×10^3	3.3802			
	2	2.4×10^3	3.3802			3.6×10^3	3.5563			
	3	2.8×10^3	3.4472			2.6×10^3	3.415			
	4	2.6×10^3	3.415			3.4×10^3	3.5315			
	5	2.6×10^3	3.415	3.4209	0.0279	2.4×10^3	3.3802	3.4526	0.085	0.4633
High	1	2.0×10^4	4.301			2.2×10^4	4.3424			
	2	1.6×10^4	4.2041			1.6×10^4	4.2041			
	3	2.0×10^4	4.301			2.2×10^4	4.3424			
	4	1.6×10^4	4.2041			1.4×10^4	4.1461			
	5	1.3×10^4	4.1139	4.2248	0.0787	1.6×10^4	4.2041	4.2478	0.0895	0.6776

Yogurt 60 hours

No statistically significant difference was observed between all 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low, medium, and high levels were 0.3973, 0.4633, and 0.6776, respectively. The t-test indicated no significant differences between the 3M RYM method and the ISO and FDA BAM methods. The 3M RYM method had a higher repeatability value than the ISO and FDA BAM methods for the medium and high levels, with S_r values, respectively, of 0.0279 and 0.0850 for the medium level and 0.0787 and 0.0895 for the high level. For the low level the ISO and FDA BAM had higher repeatability value compared to the RYM method, with S_r values, respectively, of 0.0924 and 0.0445. Detailed results are presented in Table 13.

Table 13. Method Comparison Results for Yogurt at 60 Hours.

Level	Sample	3M RYM Petrifilm				ISO / FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	
Uninoculated	1	<10	<0			<10	<0			
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0	-	-	<10	<0	-	-	NA
Low	1	2.8×10^2	2.4472			2.6×10^2	2.415			
	2	2.0×10^2	2.301			3.2×10^2	2.5051			
	3	2.8×10^2	2.4472			$3.0 \times 1 0^2$	2.4771			
	4	3.0×10^2	2.4771			3.2×10^2	2.5051			
	5	3.6×10^2	2.5563	2.4458	0.0942	3.4×10^2	2.5315	2.4868	0.0445	0.3973
Medium	1	2.8×10^3	3.4472			2.4×10^3	3.3802			
	2	2.4×10^3	3.3802			3.6×10^3	3.5563			
	3	2.8×10^3	3.4472			2.6×10^3	3.415			
	4	2.6×10^3	3.415			3.4×10^3	3.5315			
	5	2.6×10^3	3.415	3.4209	0.0679	2.4×10^3	3.3802	3.4526	0.085	0.4633
High	1	2.0×10^4	4.301			2.2×10^4	4.3424			
	2	1.6×10^4	4.2041			1.6×10^4	4.2041			
	3	2.0×10^4	4.301			2.2×10^4	4.3424			
	4	1.6×10^4	4.2041			1.4×10^4	4.1461			
	5	1.3×10^4	4.1139	4.2248	0.0787	1.6×10^4	4.2041	4.2478	0.0895	0.6776

Sour Cream 48 and 60 hours

No statistically significant difference was observed between 2 of the 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low and medium levels were 0.8528 and 0.3007. The 3M RYM method had a higher repeatability value than the ISO and FDA BAM methods for the low and medium levels, with S_r values, respectively, of 0.0681 and 0.0733 for the low level and 0.3970 and 0.4310 for the medium level. The t-test indicated a significant difference between the 3M RYM method and the ISO and FDA BAM methods on the high level with a p-value of 0.0457. The means were 2.9943 for the 3M RYM method and 3.1259 for the ISO and FDA BAM methods. For the high level the ISO and FDA BAM had higher repeatability values compared to the RYM method, with S_r values, respectively, of 0.0367 and 0.1050. Detailed results are presented in Table 14.

Table 14. Method Comparison Results for Sour Cream at 48 and 60 Hours.

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	
Uninoculated	1	<10	<0			<10	<0			
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0	-	-	<10	<0	-	-	NA
Low	1	8.2x101	1.9138			9.2×10^1	1.9638			
	2	1.0×10^1	1.0000			1.0×10^1	1.0000			
	3	1.0×10^1	1.0000			1.0×10^1	1.0000			
	4	1.0×10^1	1.0000			1.0×10^1	1.0000			
	5	2.0×10^1	1.301	1.243	0.397	1.0×10^1	1.0000	1.1928	0.431	0.8528
Medium	1	1.0×10^2	2.0000			5.4×10^3	1.8451			
	2	7.0×10^1	1.8451			2.7×10^3	2.0000			
	3	8.0×10^1	1.9031			3.2×10^3	1.9542			
	4	9.0×10^1	1.9542			5.6×10^3	2.0414			
	5	7.0×10^1	1.8451	1.9095	0.0681	4.0×10^3	1.9542	1.959	0.0733	0.3007
High	1	1.2×10^3	3.0792			3.9×10^4	3.1761			
	2	7.5×10^2	2.8751			2.6×10^4	3.0792			
	3	1.0×10^3	3.0000			4.2×10^4	3.1139			
	4	8.0×10^2	2.9031			3.8×10^4	3.1461			
	5	1.3×10^3	3.1139	2.9943	0.105	3.4×10^4	3.1139	3.1259	0.0367	0.0457

Almonds 48 and 60 hours

No statistically significant difference was observed between 2 of the 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low and medium levels were 0.1471 and 0.0661. The t-test indicated a significant difference between the 3M RYM method and the ISO and FDA BAM methods on the high level with a p-value of 0.0045. The 3M RYM method had a higher repeatability value than the ISO and FDA BAM methods for the low and high levels, with S_r values, respectively, of 0.0788 and 0.2220 for the low level and 0.0331 and 0.0603 for the high level. For the medium level the ISO and FDA BAM had higher repeatability values compared to the RYM method, with S_r values, respectively, of 0.0885 and 0.0590. Detailed results are presented in Table 15.

Table 15. Method Comparison Results for Almonds at 48 and 60 Hours.

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	
Uninoculated	1	<10	<0			<10	<0			
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0	-	-	<10	<0	-	-	NA
Low	1	$2.0x10^1$	1.301			$2.0x10^1$	1.301			
	2	$2.0x10^1$	1.301			$1.0x10^1$	1.0000			
	3	$3.0x10^1$	1.4771			$3.0x10^1$	1.4771			
	4	$2.0x10^1$	1.301			$1.0x10^1$	1.0000			
	5	$2.0x10^1$	1.301	1.3362	0.0788	$2.0x10^1$	1.0000	1.1556	0.222	0.1471
Medium	1	$1.4x10^2$	2.1461			1.0×10^3	2.301			
	2	1.4×10^2	2.1461			1.4×10^3	2.301			
	3	2.3×10^2	2.3617			1.3×10^3	2.415			
	4	1.6×10^2	2.2041			1.0×10^3	2.3222			
	5	$1.7x10^2$	2.2304	2.2177	0.0385	2.3×10^3	2.2553	2.3189	0.059	0.0661
High	1	6.2×10^2	2.7924			5.0×10^4	2.699			
	2	6.8×10^2	2.8325			6.2×10^4	2.6435			
	3	6.0×10^2	2.7782			5.8×10^4	2.6335			
	4	6.2×10^2	2.7924			4.9×10^4	2.6902			
	5	7.2×10^2	2.8573	2.8106	0.0331	5.3×10^4	2.7853	2.6903	0.0603	0.0045

Sliced Apples 48 hours

No statistically significant difference was observed between all 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low, medium, and high levels were 0.8546, 0.5476, and 0.1104, respectively. The t-test indicated no significant differences between the 3M RYM method and the ISO and FDA BAM methods. The 3M RYM method had a higher repeatability value than the ISO and FDA BAM methods for the all levels, with S_r values, respectively, of 0.2519 and 0.3476 for the low level, 0.2367 and 0.3799 for the medium level, and 0.1187 and 0.1555for the high level. Detailed results are presented in Table 16.

Table 16. Method Comparison Results for Sliced Apples at 48 Hours.

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	
Low	1	4.0×10^1	1.6021			4.0×10^1	1.6021			
	2	1.0×10^1	1.0000			1.0×10^1	1.0000			
	3	1.0×10^1	1.0000			1.0×10^1	1.0000			
	4	2.0×10^1	1.301			<10	<0			
	5	2.0×10^1	1.301	1.2408	0.2519	<10	<0	1.2007	0.3476	0.8546
Medium	1	4.4×10^2	2.6435			4.6×10^2	2.6628			
	2	1.2×10^2	2.0792			1.1×10^2	2.0414			
	3	1.4×10^2	2.1461			1.1×10^2	2.0414			
	4	1.2×10^2	2.0792			4.0×10^1	1.6021			
	5	1.6×10^2	2.2041	2.2304	0.2367	1.5×10^2	2.1761	2.4414	0.3799	0.5476
High	1	1.8×10^4	4.2553			3.0×10^4	4.4771			
	2	1.6×10^4	4.2041			1.9×10^4	4.2788			
	3	2.5×10^4	4.3979			3.6×10^4	4.5563			
	4	2.6×10^4	4.415			4.1×10^4	4.6128			
	5	1.4×10^4	4.1461	4.2837	0.1187	1.9×10^4	4.2788	4.4407	0.1555	0.1104

Sliced Apples 60 hours

No statistically significant difference was observed between all 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low, medium, and high levels were 0.8546, 0.3675, and 0.1104, respectively. The t-test indicated no significant differences between the 3M RYM method and the ISO and FDA BAM methods. The 3M RYM method had a higher repeatability value than the ISO and FDA BAM methods for the all levels, with S_r values, respectively, of 0.2519 and 0.3476 for the low level, 0.2133 and 0.3799 for the medium level, and 0.1187 and 0.1555 for the high level. Detailed results are presented in Table 17.

Table 17. Method Comparison Results for Sliced Apples at 60 Hours.

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	
Low	1	4.0×10^1	1.6021			4.0×10^1	1.6021			
	2	1.0×10^1	1.0000			1.0×10^1	1.0000			
	3	1.0×10^1	1.0000			1.0×10^1	1.0000			
	4	2.0×10^1	1.301			<10	<0			
	5	2.0×10^1	1.301	1.2408	0.2519	<10	<0	1.2007	0.3476	0.8546
Medium	1	4.4×10^2	2.6435			4.6×10^2	2.6628			
	2	1.5×10^2	2.1761			1.1×10^2	2.0414			
	3	1.4×10^2	2.1461			1.1×10^2	2.0414			
	4	1.4×10^2	2.1461			4.0×10^1	1.6021			
	5	2.2×10^2	2.3424	2.2908	0.2133	1.5×10^2	2.1761	2.4414	0.3799	0.3675
High	1	1.8×10^4	4.2553			3.0×10^4	4.4771			
	2	1.6×10^4	4.2041			1.9×10^4	4.2788			
	3	2.5×10^4	4.3979			3.6×10^4	4.5563			
	4	2.6×10^4	4.415			4.1×10^4	4.6128			
	5	1.4×10^4	4.1461	4.2837	0.1187	1.9×10^4	4.2788	4.4407	0.1555	0.1104

Frozen Bread Dough 48 hours

No statistically significant difference was observed between 2 of the 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low and medium levels were 0.9389 and 0.2490. The t-test indicated a significant difference between the 3M RYM method and the ISO and FDA BAM methods on the high level with a p-value of 0.0020. The 3M RYM method had a higher repeatability value than the ISO and FDA BAM methods for the medium and high levels, with S_r values, respectively, of 0.0628 and 0.2073 for the medium level and 0.1136 and 0.1690 for the high level. For the low level the ISO and FDA BAM had higher repeatability values compared to the RYM method, with S_r values, respectively, of 0.0873 and 0.1116. Detailed results are presented in Table 18.

Table 18. Method Comparison Results for frozen Bread Dough at 48 Hours.

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	
Low	1	2.2×10^2	2.3424			2.1×10^2	2.3222			
	2	1.8×10^2	2.2553			2.1×10^2	2.3222			
	3	2.1×10^2	2.3222			1.9×10^2	2.2788			
	4	2.0×10^2	2.301			2.3×10^2	2.3617			
	5	3.5×10^2	2.5441	2.353	0.1116	3.2×10^2	2.5051	2.358	0.0873	0.9389
Medium	1	2.8×10^3	3.4472			2.0×10^3	3.301			
	2	2.4×10^3	3.3802			2.3×10^3	3.3617			
	3	2.6×10^3	3.415			2.2×10^3	3.3424			
	4	2.4×10^3	3.3802			9.4×10^2	2.9731			
	5	3.4×10^3	3.5315	3.4308	0.0628	3.5×10^3	3.5441	3.3045	0.2073	0.249
High	1	2.5×10^4	4.3979			1.8×10^4	4.2553			
	2	2.2×10^4	4.3424			8.0×10^3	3.9031			
	3	2.1×10^4	4.3222			8.6×10^3	3.9345			
	4	1.8×10^4	4.2553			6.5×10^3	3.8129			
	5	3.6×10^4	4.5563	4.3748	0.1136	8.3×10^3	3.9191	3.965	0.169	0.002

Frozen Bread dough 60 hours

No statistically significant difference was observed between 2 of the 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low and medium levels were 0.6873 and 0.2274. The t-test indicated a significant difference between the 3M RYM method and the ISO and FDA BAM methods on the high level with a p-value of 0.0015. The 3M RYM method had a higher repeatability value than the ISO and FDA BAM methods for the medium and high levels, with S_r values, respectively, of 0.0608 and 0.2073 for the medium level and 0.1154 and 0.1690 for the high level. For the low level the ISO and FDA BAM had higher repeatability values compared to the RYM method, with S_r values, respectively, of 0.0873 and 0.1054. Detailed results are presented in Table 19.

Table 19. Method Comparison Results for frozen Bread Dough at 60 Hours.

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM			p-value
		Ct/g	LOG	MEAN	SD (S_r)	Ct/g	MEAN	SD (S_r)	
Low	1	2.2×10^2	2.3424			2.1×10^2			
	2	1.9×10^2	2.2788			2.1×10^2			
	3	2.5×10^2	2.3979			1.9×10^2			
	4	2.2×10^2	2.3424			2.3×10^2			
	5	3.6×10^2	2.5563	2.3836	0.1054	3.2×10^2	2.358	0.0873	0.6873
Medium	1	2.9×10^3	3.4624			2.0×10^3			
	2	2.5×10^3	3.3979			2.3×10^3			
	3	2.6×10^3	3.415			2.2×10^3			
	4	2.4×10^3	3.3802			9.4×10^2			
	5	3.4×10^3	3.5315	3.4374	0.0608	3.5×10^3	3.3045	0.2073	0.2274
High	1	2.6×10^4	4.415			1.8×10^4			
	2	2.5×10^4	4.3979			8.0×10^3			
	3	2.1×10^4	4.3222			8.6×10^3			
	4	1.9×10^4	4.2788			6.5×10^3			
	5	3.8×10^4	4.5798	4.3987	0.1154	8.3×10^3	3.965	0.169	0.0015

Ready-made Pie 48 hours

No statistically significant difference was observed between 2 of the 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low and medium levels were 0.5370 and 0.4128. The t-test indicated a significant difference between the 3M RYM method and the ISO and FDA BAM methods on the high level with a p-value of 0.0001. The 3M RYM method had a higher repeatability value than the ISO and FDA BAM methods for the low, medium, and high levels, with S_r values, respectively, of 0.1649 and 0.1783 for the low level and 0.1457 and 0.1482 for the medium level, and 0.0324 and 0.0433 for the high level. Detailed results are presented in Table 20.

Table 20. Method Comparison Results for Ready-made Pie at 48 Hours.

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	
Uninoculated	1	<10	<0			<10	<0			
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0	-	-	<10	<0	-	-	NA
Low	1	$2.0x10^1$	1.301			$1.0x10^1$	1.0000			
	2	$1.0x10^1$	1.0000			$2.0x10^1$	1.301			
	3	$1.0x10^1$	1.0000			$2.0x10^1$	1.301			
	4	$1.0x10^1$	1.0000			<10	<0			
	5	$2.0x10^1$	1.301	1.1204	0.1649	<10	<0	1.2007	0.1738	0.537
Medium	1	$1.2x10^2$	2.0792			1.0×10^3	2.0000			
	2	1.4×10^2	2.1461			1.4×10^3	2.1461			
	3	1.2×10^2	2.0792			1.3×10^3	2.1139			
	4	$2.1x10^2$	2.3222			1.0×10^3	2.0000			
	5	$2.5x10^2$	2.3979	2.2049	0.1467	2.3×10^3	2.3617	2.1244	0.1482	0.4128
High	1	7.2×10^3	3.8573			5.0×10^4	3.699			
	2	7.7×10^2	3.8865			6.2×10^4	3.7924			
	3	8.0×10^3	3.9031			5.8×10^4	3.7634			
	4	7.6×10^2	3.8808			4.9×10^4	3.6902			
	5	8.8×10^3	3.9445	3.8944	0.0324	5.3×10^4	3.7243	3.7339	0.0433	0.0001

Ready-made Pie 60 hours

No statistically significant difference was observed between 2 of the 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low and medium levels were 0.5370 and 0.3651. The t-test indicated a significant difference between the 3M RYM method and the ISO and FDA BAM methods on the high level with a p-value of 0.0001. The 3M RYM method had a higher repeatability value than the ISO and FDA BAM methods for the low, medium, and high levels, with S_r values, respectively, of 0.1399 and 0.1482 for the low level and 0.1649 and 0.1482 for the medium level, and 0.0282 and 0.0433 for the high level. Detailed results are presented in Table 21.

Table 21. Method Comparison Results for Ready-made Pie at 60 Hours.

Sandwiches 48 hours

No statistically significant difference was observed between 2 of the 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low and medium levels were 0.6033 and 0.4879. The t-test indicated a significant difference between the 3M RYM method and the ISO and FDA BAM methods on the high level with a p-value of 0.0290. The 3M RYM method had a higher repeatability value than the ISO and FDA BAM methods for the high level, with S_r values, respectively, of 0.0494 and 0.1363. The ISO and FDA BAM methods had a higher repeatability values than the 3M RYM method for the low and medium levels, with S_r values, respectively, of 0.2270 and 0.2692 for the low level and 0.0522 and 0.1535 for the medium level. Detailed results are presented in Table 22.

Table 22 . Method Comparison Results for Sandwiches at 48 Hours

		3M RYM Petrifilm				ISO FDA BAM				
Level	Sample	Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	p-value
Low	1	2.0×10^1	1.301			4.0×10^1	1.6021			
	2	4.0×10^1	1.6021			3.0×10^1	1.4771			
	3	4.0×10^1	1.6021			2.0×10^1	1.301			
	4	4.0×10^1	1.6021			2.0×10^1	1.301			
	5	1.0×10^1	1.0000	1.4214	0.2692	1.0×10^1	1.0000	1.3362	0.227	0.6033
Medium	1	3.6×10^2	2.5563			2.7×10^2	2.4314			
	2	3.2×10^2	2.5051			2.7×10^2	2.4314			
	3	1.6×10^2	2.2041			2.5×10^2	2.3979			
	4	2.6×10^2	2.415			2.6×10^2	2.415			
	5	1.8×10^2	2.2553	2.3872	0.1535	3.4×10^2	2.5315	2.4414	0.0522	0.4879
High	1	1.1×10^4	4.0414			1.1×10^4	4.0414			
	2	1.1×10^4	4.0414			1.4×10^4	4.1461			
	3	9.1×10^3	3.959			2.6×10^4	4.415			
	4	1.1×10^4	4.0414			1.6×10^4	4.2041			
	5	8.8×10^3	3.9445	4.0055	0.0494	1.6×10^4	4.2041	4.2021	0.1363	0.029

Sandwiches 60 hours

No statistically significant difference was observed between 2 of the 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low and medium levels were 0.6033

and 0.4879. The t-test indicated a significant difference between the 3M RYM method and the ISO and FDA BAM methods on the high level with a p-value of 0.0308. The 3M RYM method had a higher repeatability value than the ISO and FDA BAM methods for the high level, with S_r values, respectively, of 0.0396 and 0.1363 for the high level. The ISO and FDA BAM method had a higher repeatability values than the 3M RYM method for the low and medium levels, with S_r values, respectively, of 0.2270 and 0.2692 for the low level and 0.0522 and 0.1535 for the medium level. Detailed results are presented in Table 23.

Table 23. Method Comparison Results for Sandwiches at 60 Hours.

		3M RYM Petrifilm				ISO/FDA BAM				
Level	Sample	Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	p-value
Low	1	2.0×10^1	1.301			4.0×10^1	1.6021			
	2	4.0×10^1	1.6021			3.0×10^1	1.4771			
	3	4.0×10^1	1.6021			2.0×10^1	1.301			
	4	4.0×10^1	1.6021			2.0×10^1	1.301			
	5	1.0×10^1	1.0000	1.4214	0.2692	1.0×10^1	1.0000	1.3362	0.227	0.6033
Medium	1	3.6×10^2	2.5563			2.7×10^2	2.4314			
	2	3.2×10^2	2.5051			2.7×10^2	2.4314			
	3	1.6×10^2	2.2041			2.5×10^2	2.3979			
	4	2.6×10^2	2.415			2.6×10^2	2.415			
	5	1.8×10^2	2.2553	2.3872	0.1535	3.4×10^2	2.5315	2.4414	0.0522	0.4879
High	1	1.1×10^4	4.0414			1.1×10^4	4.0414			
	2	1.1×10^4	4.0414			1.4×10^4	4.1461			
	3	9.6×10^3	3.9823			2.6×10^4	4.415			
	4	1.1×10^4	4.0414			1.6×10^4	4.2041			
	5	9.1×10^3	3.959	4.0131	0.0396	1.6×10^4	4.2041	4.2021	0.1363	0.0308

Dehydrated Soup 48 and 60 hours

For dehydrated soup, the 3M RYM method recovered 3 separate levels of the target organism, but no recovery of the target analyte was detected by the reference methods. The 3M RYM plates recovered an average of 1.2760 logs for the low level, with a standard deviation of 0.1721. The medium level had an average of 2.4234 logs with a standard deviation of 0.1132. The high level had an average of 2.9145

logs with a standard deviation of 0.1366. Only one typical colony morphology, large blue colonies with dark centers and diffuse edges, was observed on the 3M RYM plates. That colony morphology matched the morphology of the inoculating organisms, *Paecilomyces*. Further macroscopic and microscopic investigation into the culture verified that it was *Paecilomyces.* Detailed results are in Table 24.

Table 24. Method Comparison Results for Dehydrated Soup at 48 and 60 Hours.

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	
Low	1	2.0×10^1	1.301			<10	<0			
	2	3.0×10^1	1.4771			<10	<0			
	3	1.0×10^1	1.0000			<10	<0			
	4	2.0×10^1	1.301			<10	<0			
	5	2.0×10^1	1.301	1.276	0.1721	<10	<0	-	-	NA
Medium	1	3.1×10^2	2.4914			<10	<0			
	2	3.4×10^2	2.5315			<10	<0			
	3	2.3×10^2	2.3617			<10	<0			
	4	3.0×10^2	2.4771			<10	<0			
	5	1.8×10^2	2.2553	2.4234	0.1132	<10	<0	-	-	NA
High	1	1.3×10^4	3.1139			<10	<0			
	2	7.2×10^4	2.8573			<10	<0			
	3	9.9×10^4	2.9956			<10	<0			
	4	6.4×10^4	2.8062			<10	<0			
	5	6.3×10^4	2.7993	2.9145	0.1366	<10	<0	-	-	NA

Fermented Salami 48 hours

No statistical difference was observed between all 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low, medium, and high levels were 0.7354, 0.7194, and 0.0805, respectively. One outlier was identified by the Cochran and Grubs test for outliers. The t-test indicated no significant differences between the 3M RYM method and the ISO/FDA BAM methods.

The ISO/FDA BAM method had a lower repeatability value than the 3M RYM method for all three levels, with S_r values, respectively, of 0.4273 and 0.4671 for the low level, 0.0554 and 0.0686 for the medium level, and 0.0352 and 0.0385 for the high level. Detailed results are presented in Table 25.

Table 25. Method Comparison Results for Fermented Salami at 48 Hours.

Level	Sample	3M RYM Petrifilm				ISO/FDA BAM				p-value
		Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	
Uninoculated	1	<10	<0			<10	<0			
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0	-		<10	<0	-	-	NA
Low	1	<10	<0			<10	<0			
	2	9.5×10^1	1.9777			1.0×10^2	2.0000			
	3	1.0×10^1	1.0000			2.0×10^1	1.3010			
	4	1.0×10^1	1.0000			1.0×10^1	1.0000			
	5	3.0×10^1	1.4771	1.3637	0.4671	4.0×10^1	1.6021	1.4758	0.4273	0.7354
Medium	1	3.2×10^2	2.5051			3.4×10^2	2.5315			
	2	3.6×10^2	2.5563			3.4×10^2	2.5315			
	3	3.9×10^2	2.5911			3.2×10^2	2.5051			
	4	3.0×10^2	2.4771			2.5×10^2	2.3979			
	5	2.6×10^2	2.415	2.5089	0.0686	3.2×10^2	2.5051	2.4942	0.0554	0.7194
High	1	2.5×10^3	3.3979			3.5×10^4	3.3010			
	2	2.6×10^3	3.4150			4.3×10^4	3.3802			
	3	2.6×10^3	3.4150			4.9×10^4	3.3802			
	4	2.2×10^3	3.3424			4.5×10^4	3.3424			
	5	2.8×10^3	3.4472	3.4035	0.0385	5.2×10^4	3.3802	3.3568	0.0352	0.0805

Fermented salami 60 hours

No statistically significant difference was observed between all 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low and medium levels and high levels were 0.1461, 0.7194, and 0.0837, respectively. The t-test indicated no significant differences between the 3M RYM method and the ISO and FDA BAM methods. The ISO/FDA BAM method had a lower repeatability value than the 3M RYM method for all levels, with S_r values, respectively, of 0.4273and 0.5072 for the low level and 0.0554 and 0.0686 for the medium level, and 0.0481 and 0.0352 for the high level. Detailed results are presented in Table 26.

Table 26. Method Comparison Results for Fermented Salami at 60 Hours

		3M RYM Petrifilm				ISO/FDA BAM				
Level	Sample	Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	p-value
Uninoculated	1	<10	<0			<10	<0			
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0	-	-	<10	<0	-	-	NA
Low	1	<10	<0			<10	<0			
	2	1.1×10^2	2.0414			1.0×10^2	2.0000			
	3	1.0×10^1	1.0000			2.0×10^1	1.3010			
	4	1.0×10^1	1.0000			1.0×10^1	1.0000			
	5	4.0×10^1	1.6021	1.4109	0.5072	4.0×10^1	1.6021	1.4758	0.4273	0.1461
Medium	1	3.2×10^2	2.5051			3.4×10^2	2.5315			
	2	3.6×10^2	2.5563			3.4×10^2	2.5315			
	3	3.9×10^2	2.5911			3.2×10^2	2.5051			
	4	3.0×10^2	2.4771			2.5×10^2	2.3979			
	5	2.6×10^2	2.415	2.5089	0.0686	3.2×10^2	2.5051	2.4942	0.0554	0.7194
High	1	2.5×10^3	3.3979			3.5×10^4	3.301			
	2	2.6×10^3	3.415			4.3×10^4	3.3802			
	3	2.6×10^3	3.415			4.9×10^4	3.3802			
	4	2.2×10^3	3.3424			4.5×10^4	3.3424			
	5	3.0×10^3	3.4771	3.4095	0.0481	5.2×10^4	3.3802	3.3568	0.0352	0.0837

Frozen Ground Beef Patties 48 hours

No statistically significant difference was observed between all 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low, medium, and high levels were 0.4824, 0.7657, and 0.0613. The t-test indicated no significant differences between the 3M RYM method and the ISO and FDA BAM methods. The 3M RYM method had a lower repeatability value than the ISO and FDA BAM methods for the high level, with S_r values, respectively, of 0.0276 and 0.0538. The ISO and FDA BAM methods had lower repeatability values than the 3M RYM for the low and medium levels with S_r values respectively, of 0.1390 and 0.2270 for the low level and 0.1104 and 0.1214 for the medium level. Detailed results are presented in Table 27.

Table 27. Method Comparison Results for Frozen Ground Beef Patties at 48 Hours.

		3M RYM Petrifilm				ISO/FDA BAM				
Level	Sample	Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	p-value
Uninoculated	1	<10	<0			<10	<0			
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0	-	-	<10	<0	-	-	NA
Low	1	$4.0x10^1$	1.6021			$5.0 x10^1$	1.699			
	2	$2.0x10^1$	1.301			$5.0x10^1$	1.699			
	3	$4.0x10^1$	1.6021			$5.0 x10^1$	1.699			
	4	$6.0x10^1$	1.7782			$4.0 x10^1$	1.6021			
	5	$8.0x10^1$	1.9031	1.6373	0.227	$8.0x10^1$	1.9031	1.7204	0.1104	0.7482
Medium	1	$1.9x10^2$	2.2788			$2.2x10^2$	2.3424			
	2	$2.2 x10^2$	2.3424			$2.3x10^2$	2.3617			
	3	$2.0 x10^2$	2.301			$1.6 x10^2$	2.2041			
	4	$1.8 x10^2$	2.2553			$3.9 x10^2$	2.5911			
	5	$3.6x10^2$	2.5563	2.3468	0.1215	$2.3x10^2$	2.3617	2.3722	0.139	0.7657
High	1	$5.0 x 10^3$	3.699			$3.5 x 10^4$	3.5441			
	2	$4.4 x 10^3$	3.6435			$4.3 x 10^4$	3.6335			
	3	$5.2 x 10^3$	3.716			$4.9 x 10^4$	3.6902			
	4	$5.0 x 10^3$	3.699			$4.5x 10^4$	3.6532			
	5	$4.8 x 10^3$	3.6812	3.6877	0.0276	$5.2 x 10^4$	3.6232	3.6288	0.0538	0.0613

Frozen Ground Beef Patties 60 hours

No statistically significant difference was observed between all 3 levels analyzed between the two methods. The p-values calculated at a 95% confidence level for the low, medium, and high levels were 0.7542, 0.9330, and 0.0613. The t-test indicated no significant differences between the 3M RYM method and the ISO and FDA BAM methods. The 3M RYM method had a lower repeatability value than the ISO and FDA BAM methods for the high level, with S_r values, respectively, of 0.0276 and 0.0538. The ISO and FDA BAM methods had a lower repeatability value than the 3M RYM for the low and medium levels with S_r values of 0.1390 and 0.1630 for the low level and 0.1104 and 0.1214 for the medium level. Detailed results are presented in Table 28.

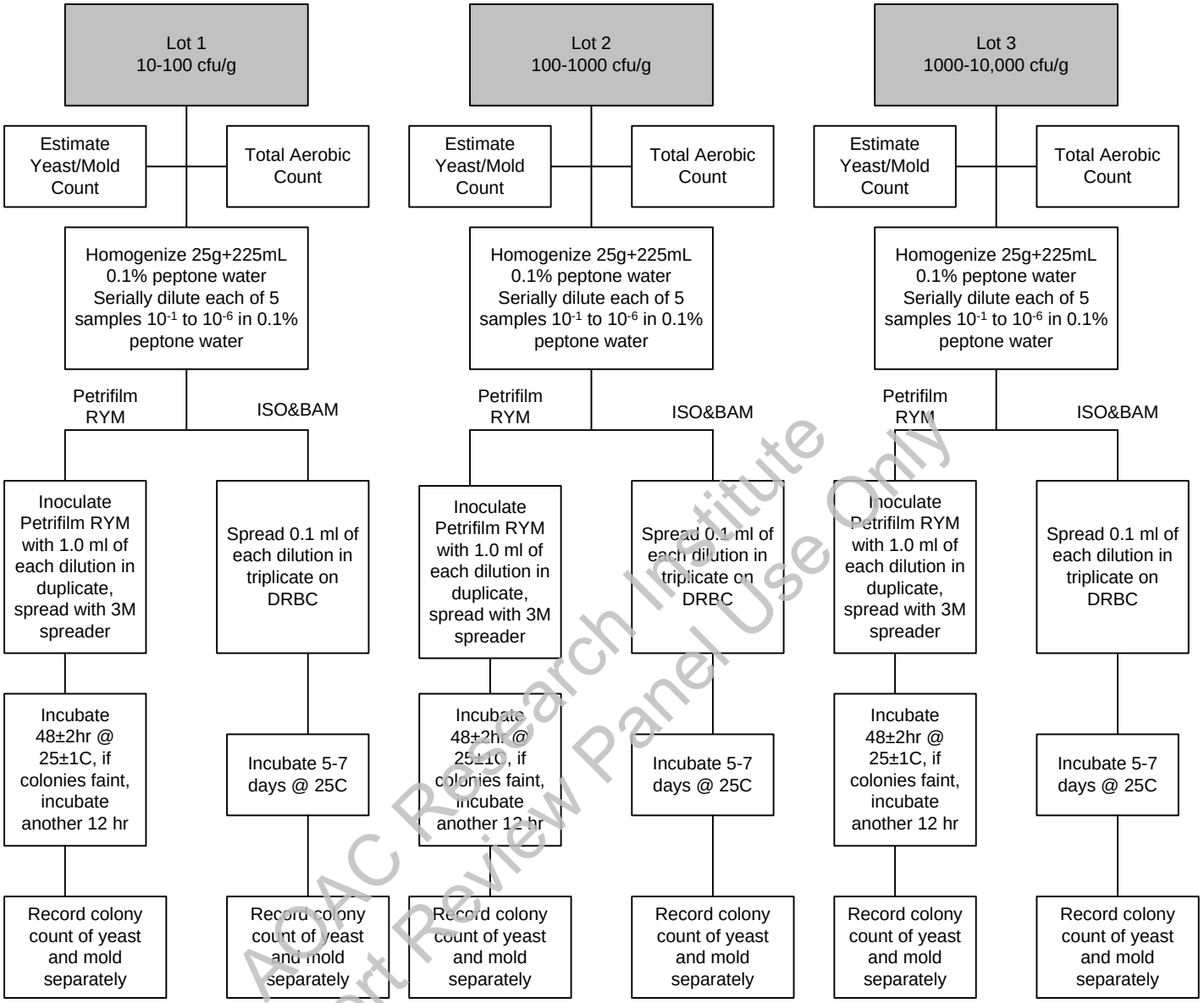
Table 28 . Method Comparison Results for Frozen Ground Beef Patties at 60 Hours.

		3M RYM Petrifilm				ISO/FDA BAM				
Level	Sample	Ct/g	LOG	MEAN	SD (S_r)	Ct/g	LOG	MEAN	SD (S_r)	p-value
Uninoculated	1	<10	<0			<10	<0			
	2	<10	<0			<10	<0			
	3	<10	<0			<10	<0			
	4	<10	<0			<10	<0			
	5	<10	<0	-	-	<10	<0	-	-	NA
Low	1	$4.0x10^1$	1.6021			$5.0 x10^1$	1.699			
	2	$3.0x10^1$	1.4771			$5.0x10^1$	1.699			
	3	$5.0x10^1$	1.699			$5.0 x10^1$	1.699			
	4	$6.0x10^1$	1.7782			$4.0 x10^1$	1.6021			
	5	$8.0x10^1$	1.9031	1.6919	0.163	$8.0x10^1$	1.9031	1.7204	0.1104	0.7542
Medium	1	$2.0 x10^2$	2.301			$2.2x10^2$	2.3424			
	2	$2.2 x10^2$	2.3424			$2.3x10^2$	2.3617			
	3	$2.0 x10^2$	2.301			$1.6 x10^2$	2.2041			
	4	$2.0 x10^2$	2.301			$3.9 x10^2$	2.5911			
	5	$3.8x10^2$	2.5798	2.3451	0.1214	$2.3x10^2$	2.3617	2.3722	0.1309	0.933
High	1	$5.0 x 10^3$	3.699			$3.5 x 10^4$	3.5441			
	2	$4.4 x 10^3$	3.6435			$4.3 x 10^4$	3.6335			
	3	$5.2 x 10^3$	3.716			$4.9 x 10^4$	3.6902			
	4	$5.0 x 10^3$	3.699			$4.5x 10^4$	3.6532			
	5	$4.8 x 10^3$	3.6812	3.6877	0.0276	$5.2 x 10^4$	3.6232	3.6288	0.0538	0.0613

Independent Laboratory Study

The independent laboratory study was conducted at Aegis laboratories, North Sioux City, South Dakota. The study was conducted according to the approved protocol outlined in Figures 1 and 2 below.

Figure 1. 3M Petrifilm RYM Plate vs. ISO (ISO 21527:2008 parts 1 and 2)and BAM Reference Methods (FDA BAM Chapter 18) – High Water Activity Foods: Yogurt, frozen ground beef patty.



METHOD COMPARISON TESTING

Yeast strains specified for use in this independent validation study were obtained from Microbiologics, Inc. One technician prepared samples and a second technician performed the assays on "blind" coded samples.

All samples were fully randomized and labeled with a code so that the analyst performing the assays was unaware of the level or type of bacterial culture present.

Test Organisms

Trichosporon mucoides (ATCC® 201382™) and *Kluyveromyces lactis* (ATCC 8563) were grown individually in YM broth. Serial dilutions were prepared in tubes of sterile distilled water diluent to achieve an inoculation level of 10-100, 100-1000, 1000-10000 CFU/g in each of the foods.

Product Inoculation

Frozen beef patties were inoculated in bulk with *T. mucoides* (Table 30) to have a target of 10-100, 100-1000.1000-10000 CFU/g of the culture in the product, thoroughly mixed, and stored at -20°C for 2 weeks. The yogurt was inoculated *K. lactis* (Table 29) for a target of 10000-CFU/g of culture, mixed and diluted with uninoculated yogurt for 2 additional target levels of culture of 10-100 CFU/g and 100-1000 CFU/g.

Aerobic Plate Count

One 25g sample of each lot of uninoculated frozen ground beef patties and yogurt was analyzed for aerobic plate count (AOAC 990.12).

Microbial Analysis

A 1:10 dilution of each 25g sample was prepared with 225ml of 0.1% peptone. The samples were homogenized for 2 min and the sample diluted with 0.1% peptone for 5 additional dilutions. One ml of each dilution was plated onto duplicate plates of RYM. The plates were then be incubated at 25 ± 1°C for 60h. The plates were read after 48 and 60h of incubation. In addition, 100 µl of each dilution was plated onto triplicate plates of DRBC with 1ml of sample plated across 3 plates for the 1/10 dilution and incubated at 25 ± 1°C for 5-7 days.

For each matrix, five samples were analyzed per replicate, 5 at 10-100 CFU/g, 5 at 100-1000 CFU/g, and 5 at 1000-10000 CFU/g, and 5 un-inoculated control samples.

The yogurt and frozen beef patties had <1 Log CFU/g of yeast and mold in the uninoculated product

and the level of mold was less than the detection limit for all inoculated samples.

Table 29. 3M Petrifilm RYM Method Compared to ISO 21527:2008 part 1 and FDA-BAM Chapter 18 for Yogurt at 48 and 60 Hours.

		RYM Method-48h		RYM Method-60h		Reference Method	
Level	Replicate	cfu/g	log cfu/g	cfu/g	log cfu/g	cfu/g	log cfu/g
Uninoculated	1	<10	<1	<10	<1	<10	<1
	2	<10	<1	<10	<1	<10	<1
	3	<10	<1	<10	<1	<10	<1
	4	<10	<1	<10	<1	<10	<1
	5	<10	<1	<10	<1	<10	<1
	Mean		NA		NA		NA
	Sr		NA		NA		NA
	RSDr, %		NA		NA		NA
	p -value		NA		NA		NA
LOW							
	1	180	2.26	180	2.26	180	2.26
	2	210	2.32	210	2.32	230	2.36
	3	240	2.38	250	2.40	280	2.45
	4	230	2.36	240	2.38	170	2.23
	5	240	2.38	240	2.38	200	2.3
	Mean	220	2.34	224	2.35	212	2.32
	Sr		0.05		0.06		0.09
	RSDr, %		2.14		2.55		3.88
	p -value		0.60		0.60		
Medium							
	1	3100	3.49	3100	3.49	3100	3.49
	2	3000	3.48	2900	3.46	3600	3.56
	3	4400	3.64	4300	3.63	3800	3.58
	4	3600	3.56	3600	3.56	3000	3.48
	5	2900	3.46	2900	3.46	2900	3.46
	Mean	3400	3.53	3360	3.52	3280	3.51
	Sr		0.07		0.07		0.05
	RSDr, %		1.98		1.99		1.42
	p -value		0.67		0.67		
High							
	1	40000	4.60	40000	4.60	38000	4.58
	2	35000	4.54	35000	4.54	44000	4.64
	3	35000	4.54	35000	4.54	46000	4.66
	4	31000	4.49	31000	4.49	29000	4.46
	5	33000	4.52	33000	4.52	33000	4.52
	Mean	34800	4.54	34800	4.54	38000	4.57
	Sr		0.04		0.04		0.08
	RSDr, %		0.88		0.88		1.75
	p -value		0.35		0.34		

Table 30. 3M Petrifilm RYM Method Compared to ISO 21527:2008 part 1 and FDA-BAM Chapter 18 for frozen Ground Beef Patties at 48 and 60

		RYM Method-48h		RYM Method-60h		Reference Method	
Level	Replicate	cfu/g	log cfu/g	cfu/g	log cfu/g	cfu/g	log cfu/g
Uninoculated	1	<10	<1	<10	<1	<10	<1
	2	<10	<1	<10	<1	<10	<1
	3	<10	<1	<10	<1	<10	<1
	4	<10	<1	<10	<1	<10	<1
	5	<10	<1	<10	<1	<10	<1
	Mean		NA		NA		NA
	Sr		NA		NA		NA
	RSDr, %		NA		NA		NA
	p -value		NA		NA		NA
LOW							
	1	80	1.9	90	1.95	90	1.95
	2	100	2.00	100	2.00	60	1.78
	3	70	1.85	70	1.85	60	1.78
	4	100	2.00	120	2.08	100	2.00
	5	70	1.85	100	2.00	80	1.90
	Mean	84	1.92	96	1.98	78	1.88
	Sr		0.08		0.09		0.10
	RSDr, %		4.16		4.55		5.32
	p -value		0.52		0.60		
Medium							
	1	960	2.98	1020	3.01	570	2.76
	2	810	2.91	870	2.94	510	2.71
	3	780	2.89	820	2.91	680	2.83
	4	780	2.89	840	2.92	640	2.81
	5	780	2.89	870	2.94	600	2.78
	Mean	822	2.91	884	2.95	600	2.78
	Sr		0.04		0.04		0.05
	RSDr, %		1.37		1.36		1.80
	p -value		0.01		0.01		
High							
	1	7100	3.85	7000	3.85	5400	3.73
	2	6300	3.80	6700	3.83	4400	3.64
	3	6600	3.82	6900	3.84	4100	3.61
	4	5700	3.76	6000	3.78	3700	3.57
	5	5900	3.77	6100	3.79	4100	3.61
	Mean	6320	3.8	6540	3.81	4340	3.63
	Sr		0.04		0.03		0.06
	RSDr, %		1.05		0.79		1.65
	p -value		0.00		0.00		

Hours.

Table 31. Comparison of repeatability between the 3M Petrifilm RYM method ISO 21527:2008 part 1 and FDA-BAM Chapter 18.

Product	Level	48 Hr RYM vs Reference Method Repeatability	48 Hr RYM vs Reference Value Repeatability
Yogurt	Low	3.24	2.25
	Medium	1.96	1.96
	High	4.00	4.00
Frozen Beef Patty	Low	1.56	1.23
	Medium	1.56	1.56
	High	2.25	4.00

Summary of Independent Laboratory Results

The low inoculated yogurt had a mean level of yeast of 2.3 Log CFU/g (Table 29). There was no significant difference between the 48h RYM counts and the reference method or the 60h RYM and the reference method ($p>0.05$). The medium inoculated yogurt had a mean level of yeast of 3.5 Log CFU/g. There was no significant difference between the 48h RYM counts and the reference method or the 60h RYM and the reference method ($p>0.05$). The high inoculated yogurt had a mean level of yeast of 4.5 Log CFU/g. There was no significant difference between the 48h RYM counts and the reference method or the 60h RYM and the reference method ($p>0.05$). The repeatability of all methods was less than 0.1 and when compared between the RYM and the reference methods was less than the critical value (<6.39, Table 31).

The low inoculated frozen ground beef patties had a mean level of yeast of 1.9Log CFU/g (Table 30). There was no significant difference between the 48h RYM counts and the reference method or the 60h RYM and the reference method ($p>0.05$). The medium inoculated frozen ground beef patties had a mean level of yeast of 2.78-2.95 Log CFU/g. There was a significant difference between the counts on the 48h RYM and the 60h RYM and those on the reference method ($p<0.05$) with the RYM plates with a higher number of organisms. The high inoculated yogurt had a mean level of yeast of 3.63-3.81 Log CFU/g. There was a significant difference between the counts on the 48h RYM and the 60h RYM and those of the reference method ($p<0.05$) with the RYM plates with a higher number of organisms. The repeatability of all methods was less than or equal to 0.1 and when compared between the RYM and the reference methods was less than the critical value (<6.39, Table 31).

The independent laboratory stated that the 3M Petrifilm RYM Plate method:

- Was very straightforward and easy to follow.
- The plates were easy to use and were preferred over the spread plate method
- The plates were less susceptible to contamination by environmental yeast and mold
- The plates were easy to read at both 48h and 60h although the 60h required less determination if potential colony was a colony or a food particle.

Discussion

The 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate method was compared to the ISO 21527:2008 parts 1 and 2 and the FDA BAM Chapter 18 reference methods at 48 and 60 hours for the detection and enumeration of yeast and mold from a variety of foods. Ten food matrices – yogurt, sour cream, almonds, sliced apples, frozen bread dough, ready-made pie, sandwiches, dehydrated soup, fermented salami and frozen ground beef patties – were inoculated with coliforms at low, medium and high levels. The log counts from the 3M Petrifilm RYM Plate method were compared with log counts from reference methods.

Paired t-tests indicated there were no significant differences in detection or enumeration between the 3M Petrifilm RYM Plate method and the ISO and BAM reference methods at any level at both 48 and 60 hours for yogurt, sliced apples, fermented salami, and frozen ground beef patties. For these matrices, the Petrifilm RYM method had better repeatability with the exceptions of yogurt at the low level inoculum at both 48 and 60 hours, and the frozen ground beef patties at the high level inoculation level at both 48 and 60 hours, for which the reference methods had better repeatability.

For almonds, frozen bread dough, and ready-made pies, detection and enumeration was the same for both the Petrifilm RYM method and the reference methods at low and medium inoculation levels at 48 and 60 hours. At the high inoculation level for these matrices, the Petrifilm RYM method had statistically better detection and enumeration at 48 and 60 hours (p=0.004, 0.002/0.0015, 0.0001 respectively). The repeatability for almonds the low and high inoculation levels at both 48 and 60 hours favored the Petrifilm RYM method, but the reference methods had better repeatability at the medium inoculation level at both time points.

There were no differences in recovery at the low and medium inoculation levels for sour cream and sandwiches at 48 and 60 hours. For both matrices there was a statistically significant difference at the high inoculation level (p=0.0457 and p=0.029 respectively) which favored the reference methods. For

sour cream at the low and medium inoculation levels at both time points, the Petrifilm RYM method had better repeatability. The reference methods had better repeatability at the high inoculation level. For sandwiches, the Petrifilm RYM method had better repeatability at the high inoculation level at both 48 and 60 hours, whereas the reference methods had better repeatability at the low and medium inoculation levels at both time points.

There was no recovery of the inoculated organism (*Paecilomyces* spp) on the reference medium (DG-18) from dehydrated soup. Five lots of dehydrated soup, with increasing inoculation levels, were tested in an attempt to gain recovery of the target organism recovery on DG-18. In addition, prepoured DG-18 agar medium was utilized instead of the dehydrated, laboratory made medium as another attempt to recover the target organism. The General Referee was contacted for advice. The decision was made that since the *Paecilomyces* spp was not recovered with attempts to increase the inoculums titer that the data would be accepted with no recovery from the DG-18 reference medium. There is no explanation for why the DG-18 reference medium does not recover the organism from dehydrated soup. The organism does grow on the medium when grown in pure culture.

In strain studies, of the 19 yeast and 23 mold strains that should have been detected by the 3M Petrifilm RYM Plate method, all were detected and had appropriate colonial morphology, giving a sensitivity of 100%. Of the 4 non-target organisms that should not been detected the 3M Petrifilm RYM Plate method, none of the 4 strains were detected, yielding a specificity of 100%. The 3M Petrifilm RYM Plate has a shelf life of 18 months when stored at 2-8°C. A combined lot-to-lot and stability study has begun on three unique lots of Petrifilm RYM Plates. At time 0, there was no difference in the performance of the product with regard to lot or age of the product. The study is continuing and the results will be reported as they become available.

Method ruggedness was demonstrated for three test parameters including incubation temperature, incubation time, and water for rehydration.

Summary of Validated Performance Claims

The 3M Petrifilm RYM Plate method is used for the enumeration of yeast and mold in the food and beverage industries and has been validated for yogurt, sour cream, almonds, sliced apples, frozen bread dough, ready-made pie, sandwiches, dehydrated soup, fermented salami and frozen ground beef patties. A t-test was performed using the appropriate assumption for equal or unequal variance to

evaluate the mean of the $\log_{10}$ values for statistical difference between two methods at the 95% confidence level. Repeatability of the Petrifilm RYM method and the reference methods was calculated as the standard deviation (S_r) for each contamination level for each matrix.

The performance of the Petrifilm RYM method in terms of enumeration and repeatability was equal to or better than the ISO 21527:2008 parts 1 and 2 and the FDA BAM Chapter 18 reference methods at 48 and 60 hours, with the exception of high inoculation level of sour cream at 48 and 60 hours and the medium and high levels of sandwiches at 48 and 60 hours.

Conclusion

These studies have demonstrated that the 3M Petrifilm Rapid Yeast and Mold Count Plate method is a, specific, sensitive, and rugged method that detects and enumerates yeast and mold at 48 and 60 hours from a variety of matrices, yielding equivalent values to the ISO 21527:2008 parts 1 and 2 and the FDA BAM Chapter 18 reference methods.

References

1. Bacteriological Analytical Manual, Chapter 18. Yeasts, Molds and Mycotoxins. April, 2001 revision.

2. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.

3. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.

Appendix 8.7 - 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate Instructions for Use

EN (English)

Issue Date: 2013-07

3M

Petrifilm™ **Product Instructions**

Rapid Yeast and Mold Count Plate

PRODUCT DESCRIPTION AND INTENDED USE

The 3M™ Petrifilm™ Rapid Yeast and Mold Count (RYM) Plate is a sample-ready culture medium system which contains nutrients supplemented with antibiotics, a cold-water-soluble gelling agent, and an indicator system that facilitates yeast and mold enumeration. 3M Petrifilm RYM Plates are used for the enumeration of yeast and mold in the food and beverage industries. 3M Petrifilm RYM Plate components are decontaminated though not sterilized. 3M Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

SAFETY

The user should read, understand, and follow all safety information in the Product Instructions for the 3M Petrifilm RYM Plate. 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.

⚠ WARNING

To reduce the risks associated with the release of contaminated product:

- Use 3M Petrifilm RYM Plates for food and beverage sample testing that you have validated.
- Follow all product storage instructions contained in these Product Instructions.
- Do not use beyond expiration date.

To reduce the risks associated with clinical misdiagnosis:

- Do not use 3M Petrifilm RYM Plates in the diagnosis of conditions in humans or animals.

⚠ CAUTION

To reduce the risks associated with exposure to biohazards and environmental contamination:

- Follow current industry standards for disposal of biohazardous waste.

To reduce the risks associated with misinterpretation of results:

- 3M Petrifilm RYM Plates do not differentiate any one yeast or mold strain from another.

3M has not documented 3M Petrifilm RYM Plates for use in industries other than food and beverage. For example, 3M has not documented 3M Petrifilm RYM Plates for testing water, pharmaceuticals or cosmetics.

3M Petrifilm RYM Plates have not been tested with all possible food products, food processes, testing protocols or with all possible strains of yeast and mold.

Do not use 3M Petrifilm RYM Plates in the diagnosis of conditions in humans or animals.

The user must train its personnel in proper testing techniques. For example, Good Laboratory Practices[1], ISO 7218[2], or ISO 17025[3].

For information on documentation of product performance, 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.

1

EN (English)

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.

STORAGE AND DISPOSAL

Store **unopened** 3M Petrifilm RYM Plate pouches refrigerated or frozen (-20 to 8°C / -4 to 46°F). Just prior to use, allow unopened pouches to come to room temperature before opening (20-25°C / <60% RH). Return unused 3M Petrifilm RYM Plates to pouch. Seal by folding the end of the pouch over and applying adhesive tape. **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool dry place (20 -25°C / <60% RH) for no longer than 4 weeks. It is recommended that resealed pouches of 3M Petrifilm RYM Plates be stored in a freezer (see below) if the laboratory temperature exceeds 25°C (77°F) and/or the laboratory is located in a region where the relative humidity exceeds 60% (with the exception of air-conditioned premises).

To store opened pouches in a freezer, place 3M Petrifilm RYM Plates in a sealable container. To remove frozen 3M Petrifilm RYM Plates for use, open the container, remove the plates that are needed and immediately return remaining plates to the freezer in the sealed container. Allow 3M Petrifilm RYM Plates to come to room temperature before plating. 3M Petrifilm RYM Plates should not be used past their expiration date. Do not store open pouches in a freezer with an automatic defrost cycle, as this could damage the 3M Petrifilm RYM Plates due to repeated exposure to moisture.

Do not use 3M Petrifilm RYM Plates that show discoloration. Expiration date and lot number are noted on each package of 3M Petrifilm RYM Plates. The lot number is also noted on individual 3M Petrifilm RYM Plates.

After use, 3M Petrifilm RYM Plates may contain microorganisms that may be a potential biohazard. Follow current industry standards for disposal.

⚠ DISPOSAL

To reduce the risks associated with exposure to biohazards and environmental contamination:

- Follow current industry standards for disposal of biohazardous waste.

INSTRUCTIONS FOR USE

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

Wear appropriate protective apparel and follow standard good laboratory safety practices (GLP).[1]

Sample Preparation

1. Use appropriate sterile diluents:

   Butterfield's phosphate buffer (ISO 6341-1), Buffered Peptone Water (ISO), 0.1% peptone water, peptone salt diluent, saline solution (0.85-0.90%), bisulphite-free letheen broth, or distilled water. **Do not use diluents containing citrate, bisulphite or thiosulfate with 3M Petrifilm RYM Plates;** they can inhibit growth. If citrate buffer is indicated in the standard procedure, substitute with 0.1% peptone water, warmed to 40-45°C.

2. Blend or homogenize sample.

Plating

1. Place the 3M Petrifilm RYM Plate on a flat, level surface.
2. Lift the top film and with the pipette perpendicular dispense 1 mL of sample suspension onto the center of bottom film.
3. Roll the top film down onto the sample.
4. Place the 3M™ Petrifilm™ Flat Spreader (6425) or other flat spreader on the center of the 3M Petrifilm RYM Plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm RYM Plate growth area before the gel is formed. Do not slide the spreader across the film.
5. Remove the spreader and leave the 3M Petrifilm RYM Plate undisturbed for at least one minute to permit the gel to form.

Incubation

Incubate 3M Petrifilm RYM Plates at 25-28°C for 48 +/- 2 hours* in a horizontal position with the clear side up in stacks of no more than 40.

*If colonies appear faint, allow an additional 12 hours of incubation time for enhanced interpretation.

2

EN (English)

Interpretation

1. 3M Petrifilm RYM Plates can be counted using a standard colony counter or other illuminated magnifier. Gridlines are visible with the use of a backlight to assist with estimated enumeration.
2. Do not count colonies on the foam dam since they are removed from the nutrient medium.
3. To differentiate yeast and mold colonies on the 3M Petrifilm RYM Plate, look for one or more of the following characteristics:

YEAST	MOLD
Small colonies	Large colonies
Colonies have defined edges	Colonies have diffuse edges
Pink-tan to blue-green in color	Blue/green to variable upon prolonged incubation
Colonies appear raised (3 dimensional)	Colonies appear flat
Colonies have a uniform color	Colonies have a dark center with diffused edge

4. Read yeast and mold results at 48 hours. Certain slower growing yeasts and molds may appear faint at 48 hours. To enhance interpretation of these molds allow for an additional 12 hours of incubation time.
5. The circular growth area is approximately 30 cm^2. 3M Petrifilm RYM Plates containing greater than 150 colonies can either be estimated or recorded as TNTC. Estimation can be done by counting the number of colonies in one or more representative squares and determining the average number per square. The average number can be multiplied by 30 to determine the estimated count per plate. If a more accurate count is required, the sample will need to be retested at higher dilutions. When the sample contains substantial amounts of mold, depending on the type of mold, the upper countable limit may be lowered at user discretion.
6. Food samples may occasionally show interference on the 3M Petrifilm RYM Plates, for example:

   a) a uniform blue background color (often seen from the organisms used in cultured products) these should not be counted as TNTC.

   b) intense, pinpoint blue specs (often seen with spices or granulated products).
7. When necessary, colonies may be isolated for further identification. Lift the top film and pick the colony from the gel.

REFERENCES

1. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.
2. ISO 7218. Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations.
3. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.

EXPLANATION OF SYMBOLS

Caution or Warning, consult Product Instructions.

Consult Product Instructions.

LOT The lot in a box and the hourglass symbols are symbols that represent lot number and expiration date. The hourglass is followed by a year and month which represents the expiration date (year and month: YYYY-MM). The entire line after the hourglass represents the lot number (YYYY-MM A Z).

Storage between given temperatures.

3

Issue Date: XXXX-XX

Petrifilm™
Product Instructions
Rapid Yeast and Mold Count Plate

PRODUCT DESCRIPTION AND INTENDED USE

The 3M™ Petrifilm™ Rapid Yeast and Mold Count (RYM) Plate is a sample-ready-culture-medium system which contains nutrients supplemented with antibiotics, a cold-water soluble gelling agent, and an indicator system that facilitates yeast and mold enumeration. 3M Petrifilm RYM Plates are used for the enumeration of yeast and mold in the food and beverage industries. 3M Petrifilm RYM Plate components are decontaminated though not sterilized.

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

SAFETY

The user should read, understand, and follow all safety information in the Product Instructions for the 3M Petrifilm RYM Plate. 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.

⚠ WARNING

To reduce the risks associated with the release of contaminated product:

- Use 3M Petrifilm RYM Plates for food and beverage sample testing that you have validated.
- Follow all product storage instructions contained in these Product Instructions.
- Do not use beyond the expiration date.

To reduce the risks associated with clinical misdiagnosis:

- Do not use 3M Petrifilm RYM Plates in the diagnosis of conditions in humans or animals.

⚠ CAUTION

To reduce the risks associated with exposure to biohazards and environmental contamination:

- Follow current industry standards and local regulations for disposal of biohazardous waste.

To reduce the risks associated with misinterpretation of results:

- 3M Petrifilm RYM Plates do not differentiate any one yeast or mold strain from another.
- 3M has not documented 3M Petrifilm RYM Plates for use in industries other than food and beverage. For example, 3M has not documented 3M Petrifilm RYM Plates for testing water, pharmaceuticals or cosmetics.
- 3M Petrifilm RYM Plates have not been tested with all possible food products, food processes, testing protocols or with all possible strains of yeast and mold.
- Do not use 3M Petrifilm RYM Plates in the diagnosis of conditions in humans or animals.
- The user must train its personnel in proper testing techniques. For example, Good Laboratory Practices[1], ISO 7218[2], or ISO 17025[3].

Consult the Safety Data Sheet for additional information.

For information on documentation of product performance, visit our website at www.3M.com/foodsafety or contact your local 3M representative or distributor.

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.

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.

STORAGE
Store unopened 3M Petrifilm RYM Plate pouches refrigerated or frozen (-20 to 8°C / -4 to 46°F). Just prior to use, allow unopened pouches to come to room temperature before opening (20-25°C / <60% RH). Return unused 3M Petrifilm RYM Plates to pouch. Seal by folding the end of the pouch over and applying adhesive tape. **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool dry place (20-25°C / <60% RH) for no longer than 4 weeks. It is recommended that resealed pouches of 3M Petrifilm RYM Plates be stored in a freezer (see below) if

the laboratory temperature exceeds 25ºC (77ºF) and/or the laboratory is located in a region where the relative humidity exceeds 60% (with the exception of air-conditioned premises).

To store opened pouches in a freezer, place 3M Petrifilm RYM Plates in a sealable container. To remove frozen 3M Petrifilm RYM Plates for use, open the container, remove the plates that are needed and immediately return remaining plates to the freezer in the sealed container. Allow 3M Petrifilm RYM Plates to come to room temperature before plating. 3M Petrifilm RYM Plates should not be used past their expiration date. Do not store open pouches in a freezer with an automatic defrost cycle, as this could damage the 3M Petrifilm RYM Plates due to repeated exposure to moisture.

Do not use 3M Petrifilm RYM Plates that show discoloration. Expiration date and lot number are noted on each package of 3M Petrifilm RYM Plates. The lot number is also noted on individual 3M Petrifilm RYM Plates.

⚠ DISPOSAL

After use, 3M Petrifilm RYM Plates may contain microorganisms that may be a potential biohazard. Follow current industry standards for disposal.

For information on potential biohazards, reference Biosafety in Microbiological and Biomedical Laboratories, 5th edition, Section VIII-B: Fungal Agents or equivalent.

INSTRUCTIONS FOR USE

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

Wear appropriate protective apparel and follow standard good laboratory safety practices (GLP).[1]

Sample Preparation

1. Prepare appropriate dilution(s) of the sample as needed.

   Use appropriate sterile diluents:

   Butterfield's phosphate buffer (ISO 5541-1), Buffered Peptone Water (ISO), 0.1% peptone water, peptone salt diluent, saline solution (0.85-0.90%), bisulfite-free letheen broth, or distilled water. **Do not use diluents containing citrate, bisulfite or thiosulfate with 3M Petrifilm RYM Plates; they can inhibit growth.** If citrate buffer is indicated in the standard procedure, substitute with 0.1% peptone water, warmed to 40–45°C.

   See "Specific Instructions for Validated Methods" for specific requirements.

2. Blend or homogenize sample.

Plating

1. Place the 3M Petrifilm RYM Plate on a flat, level surface.
2. Lift the top film and with the pipette perpendicular dispense 1 mL of sample suspension onto the center of bottom film.
3. Roll the top film down onto the sample.
4. Place the 3M™ Petrifilm™ Flat Spreader (6425) or other flat spreader on the center of the 3M Petrifilm RYM Plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm RYM Plate growth area before the gel is formed. Do not slide the spreader across the film.
5. Remove the 3M Petrifilm Flat Spreader and leave the 3M Petrifilm RYM Plate undisturbed for at least one minute to permit the gel to form.

Incubation

Incubate 3M Petrifilm RYM Plates at 25°C +/- 1°C or 28°C +/-1°C for 48 +/- 2 hours* in a horizontal position with the clear side up in stacks of no more than 40.

*If colonies appear faint, allow an additional 12 hours of incubation time for enhanced interpretation. If

a 60 hour time-point for interpretation is not convenient, extending the incubation time to 72 hours is an acceptable alternative.

See “Specific Instructions for Validated Methods“ for specific requirements.

Interpretation

1. 3M Petrifilm RYM Plates can be counted using a standard colony counter or other illuminated magnifier. Gridlines are visible with the use of a backlight to assist with estimated enumeration.
2. Do not count colonies on the foam dam since they are removed from the nutrient medium.
3. To differentiate yeast and mold colonies on the 3M Petrifilm RYM Plate, look for one or more of the following characteristics:

YEAST	MOLD
Small colonies	Large colonies
Colonies have defined edges	Colonies have diffuse edges
Pink/tan to blue/green in color	Blue/green to variable upon prolonged incubation
Colonies appear raised (3 dimensional)	Colonies appear flat
Colonies have a uniform color	Colonies have a dark center with diffused edge

4. Read yeast and mold results at 48 hours. Certain slower growing yeasts and molds may appear faint at 48 hours. To enhance interpretation of these molds allow for an additional 12 hours of incubation time. If a 60 hour time-point for interpretation is not convenient, extending the incubation time to 72 hours is an acceptable alternative.
5. The circular growth area is approximately 30 cm^2. 3M Petrifilm RYM Plates containing greater than 150 colonies can either be estimated or recorded as Too Numerous To Count (TNTC). Estimation can be done by counting the number of colonies in one or more representative squares and determining the average number per square. The average number can be multiplied by 30 to determine the estimated count per plate. If a more accurate count is required, the sample will need to be retested at higher dilutions. When the sample contains substantial amounts of mold, depending on the type of mold, the upper countable limit may be lowered at user discretion.
6. Food samples may occasionally show interference on the 3M Petrifilm RYM Plates, for example:

   a) a uniform blue background color (often seen from the organisms used in cultured products) these should not be counted as TNTC.

   b) intense, pinpoint blue specs (often seen with spices or granulated products).
7. When necessary, colonies may be isolated for further identification. Lift the top film and pick the colony from the gel.

Specific Instructions for Validated Methods

AOAC-RI Performance Tested Methods (PTM)

In an AOAC RI PTM study, the 3M Petrifilm RYM Plate method was found to be equivalent to or better than the average log counts of the ISO 21527:2008 parts 1 and 2 and to the FDA BAM Chapter 18 reference methods at 48 and 60 hours.

Scope of Validation:

Yogurt, frozen bread dough, fermented salami, sour cream, ready-made pie, frozen ground beef patties, almonds, sandwiches, sliced apples, and dehydrated soup.

Incubation:

Incubate 3M Petrifilm RYM Plates between 48 and 60 hours at 25°C +/-1 °C or 28°C +/-1°C.

Interpretation:

Plates containing greater than 150 colonies can either be estimated or recorded at too numerous to count (TNTC). Estimation can be done by counting the number of colonies in one or more representative squares and determining the average number per square. The average number can be multiplied by 30 to determine the estimated count per plate. If a more accurate count is required, the sample can be retested at higher dilutions.

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE
LICENSE NUMBER 121301

NF Validation by AFNOR Certification:

NF Validation certified method in compliance with ISO 16140 [4] in comparison to 21527 part 1 and part 2[5]

Use the following details when implementing the above instructions for Use:

Scope of the validation:

All human food products, animal feed and environmental products (primary production samples excepted)

Sample preparation:

Use only ISO listed diluents[6]

Incubation:

Incubate 3M Petrifilm RYM Plates between 60 and 72 hours at 25°C +/-1 °C or 28°C +/-1°C.

The plates can be stored in the incubator up to 5 days.

Interpretation:

Calculate the number of microorganisms present in the test sample according to ISO 7218[2] for one plate per dilution. For calculation, take into account only 3M Petrifilm RYM Plates that contain up to 150 colonies. Estimates are outside of the scope of the NF Validation Certification (cf. interpretation part paragraph 5).

BY AFNOR CERTIFICATION
NF VALIDATION

3M 01/13 – 07/14

ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS

www.afnor-validation.com

For more information about end of validity, please refer to NF VALIDATION certificate available on the website mentioned above

REFERENCES

1. U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.
2. ISO 7218. Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations.
3. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
4. ISO 16140. Microbiology of food and animal feeding stuffs - Protocole for the validation of alternative method
5. ISO 21527. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of yeasts and moulds.

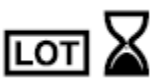
   Part 1: Colony count technique in products with water activity greater than 0.95

   Part 2: Colony count technique in products with water activity less than or equal to 0.95
6. ISO 6887-1. Microbiology of food and animal feeding stuffs- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination.

EXPLANATION OF SYMBOLS

⚠ Caution or Warning, consult Product Instructions.

Consult Product Instructions.



The lot in a box and the hourglass symbols are symbols that represent lot number and expiration date. The hourglass is followed by a year and month which represents the expiration date (year and month: YYYY-MM). The entire line after the hourglass represents the lot number (YYYY-MM AZ).

Store between given temperatures.