FOOD BIOLOGICAL CONTAMINANTS

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

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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

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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.
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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.

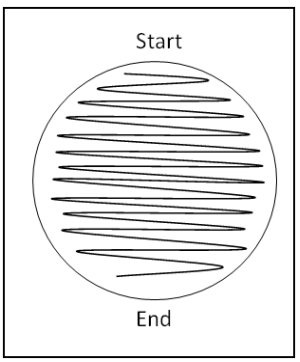

Figure 2014.01. Streaking pattern on the 3M Petrifilm SALX Plate.

(**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.

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