FOOD BIOLOGICAL CONTAMINANTS

Evaluation of Modification of the 3M™ Molecular Detection Assay (MDA) *Salmonella* Method (2013.09) for the Detection of *Salmonella* in Selected Foods: Collaborative Study

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The 3M™ Molecular Detection Assay (MDA) *Salmonella* utilizes isothermal amplification of nucleic acid sequences with high specificity, efficiency, rapidity and bioluminescence to detect amplification of *Salmonella* spp. in food, food-related, and environmental samples after enrichment. A method modification and matrix extension study of the previously approved AOAC *Official Method*℠ 2013.09 was conducted, and approval of the modification was received on March 20, 2014. Using an unpaired study design in a multilaboratory collaborative study, the 3M MDA *Salmonella* method was compared to the U.S. Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) *Microbiology Laboratory Guidebook* (MLG) 4.05 (2011), *Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Catfish Products* for raw ground beef and the U.S. Food and Drug Administration (FDA)/*Bacteriological Analytical Manual* (BAM) Chapter 5, *Salmonella* reference method for wet dog food following the current AOAC guidelines. A total of 20 laboratories participated. For the 3M MDA *Salmonella* method, raw ground beef was analyzed using 25 g test portions, and wet dog food was analyzed using 375 g test portions. For the reference methods, 25 g test portions of each matrix were analyzed. Each matrix was 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). In this study, 1512 unpaired replicate samples were analyzed. Statistical analysis was conducted according to the probability of detection (POD). For the low-level raw ground beef test portions, the following dLPOD (difference between the LPODs of the reference and candidate method) values with 95% confidence intervals were obtained: –0.01 (–0.14, +0.12). For the low-level wet dog food test portions, the following dLPOD with 95% confidence intervals were obtained: –0.04 (–0.16, +0.09). No significant differences were observed in the number of positive samples detected by the 3M MDA *Salmonella* method versus either the USDA/FSIS-MLG or FDA/BAM methods.

For over 100 years, *Salmonella*, one of the most frequently reported causes of foodborne outbreaks, has been known to cause foodborne illness in humans (1). The bacterium has been implicated in outbreaks from a variety of foods including raw animal products, such as meat, poultry, eggs, dairy products, seafood and some fruits and vegetables (2). In order to reduce outbreaks of Salmonellosis, a comprehensive farm-to-fork approach is needed. The detection of *Salmonella* can often be very time-consuming and expensive, as the presence of the microorganism in food usually does not affect the taste, smell, or appearance (3). The 3M™ Molecular Detection Assay (MDA) *Salmonella* method, in conjunction with 3M Buffered Peptone Water ISO (BPW ISO) (4), uses isothermal amplification of nucleic acid sequences to detect *Salmonella* in enriched food, feed, and environmental samples.

The 3M MDA *Salmonella* method allows for next-day detection of *Salmonella* species. After 10–24 h of enrichment using prewarmed (37 ± 1°C or 41.5°C) 3M BPW ISO medium, *Salmonella* detection is performed by the 3M MDA *Salmonella* method. For one matrix (raw head-on shrimp) a short secondary enrichment in Rappaport-Vassiliadis 10 broth (RV10; 4–24 h) is

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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.
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required. Presumptive positive results are reported in real time; negative results are displayed after completion of the assay.

Prior to the collaborative study, the 3M MDA *Salmonella* method was certified as a *Performance Tested Method*SM (PTM) following the AOAC guidelines for harmonized PTM studies (5). The aim of the PTM study was to demonstrate that the 3M MDA *Salmonella* method could detect *Salmonella* in selected foods as claimed by the manufacturer. For the 3M MDA *Salmonella* evaluation, six matrixes were analyzed: raw ground beef (25 g), cooked breaded chicken (325 g), pasteurized liquid whole egg (100 g), raw shrimp (head-off, 25 g), fresh spinach (bagged, 25 g), and wet dog food (375 g) with the primary enrichment incubated at 37 ± 1°C for 18–24 h.

A matrix extension/method modification was performed after the collaborative study with the following: (*1*) a matrix extension to the existing claim, including pasteurized American cheese (25 g), dry dog food (25 and 375 g), creamy peanut butter (25 g), sprout irrigation water (375 mL) enriched at 37 ± 1°C; (*2*) a matrix extension to the existing claim, including chicken carcass rinsate (30 mL), chicken carcass sponges, raw ground chicken (25 g, 10–18 h, and 325 g, 14–18 h), concrete, sealed/glazed ceramic tile, and stainless steel enriched at 41.5 ± 1°C, and raw head-on shrimp enriched at 37 ± 1°C for 18–24 h with a secondary enrichment at 41.5 ± 1°C for 4–24 h; (*3*) a method modification involving a shorter enrichment time and higher enrichment temperature to 41.5 ± 1°C for raw ground beef (25, 325, and 375 g; 10–18 h).

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. 031208 on March 30, 2012. The matrix extension/modification study was approved on March 20, 2014.

The aim of this collaborative study was to compare the 3M MDA *Salmonella* method to the U.S. Department of Agriculture (USDA)/Food Safety and Inspection Service (FSIS) *Microbiology Laboratory Guidebook* (MLG) 4.05 (6) for raw ground beef, and the U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual* (BAM) Chapter 5 (7) method for wet dog food.

Collaborative Study

Study Design

For this collaborative study, two matrixes, raw ground beef (80% lean) and wet dog food (canned beef chunks), were analyzed. The matrixes were obtained from local retailers and screened for the absence of *Salmonella* by preparing one bulk sample and analyzing five sample replicates (25 g) by the appropriate reference method. The screening indicated an absence of the target organism. The raw ground beef was artificially contaminated with *Salmonella* Ohio Sequence Types (STS) 81 (University of Pennsylvania, culture collection; origin: unknown food poisoning outbreak, Illinois) and the wet dog food with *Salmonella* Poona National Collection of Type Cultures (NCTC) 4840 (origin: infant enteritis). There were two inoculation levels for each matrix: a high inoculation level of approximately 2–5 CFU/test portion and a low inoculation level of approximately 0.2–2 CFU/test portion. A set of uninoculated control test portions was also included for each matrix at 0 CFU/test portion.

Twelve replicate samples from each of the three contamination levels of product were analyzed. Two sets of samples (72 total) were sent to each laboratory for analysis by the 3M MDA *Salmonella* method and either the USDA/FSIS-MLG (raw ground beef) or FDA/BAM (wet pet food) reference method due to different sample enrichments for the candidate method and the reference methods. For both matrixes, collaborators were sent an additional 30 g test portion and instructed to conduct a total aerobic plate count (APC) following the FDA/BAM Chapter 3 (8) on the day samples were received to determine the total aerobic 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 broth from a Q Laboratories frozen stock culture held at –70°C. The broth was incubated for 18–24 h at 35 ± 1°C. Appropriate dilutions 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 both test portion sizes, 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. The matrixes were inoculated on the day of shipment so that all test portions would be held for 96 h before testing was initiated. For the analysis of the raw ground beef, the bulk lot of test material was divided into 30 g portions for shipment to the collaborators. For the analysis of the wet dog food, 25 g of inoculated test product was mixed with 350 g of uninoculated test product for shipment to the collaborators for analysis by the 3M MDA *Salmonella* method. For analysis by the reference method, collaborators received 30 g portions.

To determine the level of *Salmonella* spp. in the matrixes, a five-tube most probable number (MPN) was conducted by the coordinating laboratory on the day of initiation of analysis. From both the high and low inoculated levels, five 100 g test portions, the reference method test portions, and five 10 g test portions were analyzed using the appropriate reference method enrichment broth. The MPN and 95% confidence intervals were calculated from the high, low, and uninoculated levels using the MPN Calculator (www.lcftld.com/customer/LCFMPNCalculator.exe; 9). Confirmation of the samples was conducted according to either the USDA/FSIS-MLG 4.05 or FDA/BAM Chapter 5 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. All 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. 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.

Additional shipments of raw ground beef test portions were made by the sponsoring laboratory when aberrant results were observed. Further investigation of the results indicated that each participating collaborator detected the presence of the target analyte in the uninoculated control samples sent in the first shipment. In each case, the same species was reported for the control samples, which may have been due to cross contamination. As a result, new test portions of raw ground beef were shipped and analyzed by each of the collaborating laboratories, as previously described.

Test Portion Analysis

Collaborators followed the appropriate preparation and analysis protocol according to the method for each matrix. For both matrixes, 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 MDA *Salmonella* method, a 25 g portion was enriched with 225 mL of prewarmed (37 ± 1°C) 3M BPW ISO, homogenized for 2 min, and incubated for 18–24 h at 37 ± 1°C. For the wet dog food test portions analyzed by the 3M MDA *Salmonella* method, a 375 g portion was enriched with 3375 mL prewarmed (37 ± 1°C) 3M BPW ISO, homogenized for 2 min, and incubated for 18–24 h at 37 ± 1°C.

Following enrichment, samples were assayed by the 3M MDA *Salmonella* method and confirmed following the standard reference method. Both test portion sizes analyzed by the 3M MDA *Salmonella* method were compared to samples (25 g) analyzed using either the USDA/FSIS-MLG or FDA/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**. Serological testing was also performed.

Statistical Analysis

Each collaborating laboratory recorded results for the reference method and the 3M MDA *Salmonella* method on the data sheets provided. 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 MDA *Salmonella* results were compared to the reference method for statistical analysis. Data for each test portion size were analyzed using the probability of detection (POD; 10). If the confidence interval of a dLPOD did not contain zero, then that would indicate a statistically significant difference between the candidate method and the reference method at the 5% confidence level (11).

AOAC Official Method 2013.09
Salmonella in Selected Foods
3M™ Molecular Detection Assay (MDA)
Salmonella Method
First Action 2013
Revised First Action 2014

[Applicable to detection of *Salmonella* in raw ground beef (25, 325, and 375 g), raw ground chicken (25 and 325 g), cooked breaded chicken (325 g), pasteurized liquid whole egg (100 g), raw shrimp (head-off, 25 g), fresh spinach (bagged, 25 g), wet dog food (375 g), pasteurized American cheese (25 g), peanut butter (25 g), dry dog food (25 and 375 g), sprout irrigation water (375 g), raw head-on shrimp (25 g), chicken carcass rinsate (30 mL), chicken carcass sponge, sealed/glazed ceramic tile, concrete, and stainless steel.]

See Tables **2013.09A** and **B** for a summary of results of the interlaboratory study.

See Tables 1 and 2 of the Appendix for detailed results of the interlaboratory study.

A. Principle

The 3M MDA *Salmonella* method is intended for use with the 3M Molecular Detection System for the rapid and specific detection of *Salmonella* spp. in food, food-related, and environmental samples after enrichment. The 3M MDA *Salmonella* test uses isothermal amplification of unique DNA target sequences with high specificity, efficiency, and rapidity, and bioluminescence to detect the amplified sequences. Presumptive positive results are reported in real-time; negative results are displayed after the assay is completed.

The LOD 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 MDA *Salmonella* method this has been demonstrated to be 1–5 CFU/25 g of sample or 1–5 CFU/swab.

As with all test methods, the source of enrichment medium can influence the results. The 3M MDA *Salmonella* method has only been evaluated for use with the enrichment medium, 3M Buffered Peptone Water ISO (BPW ISO). Matrixes are incubated in 3M BPW for 10–24 h to enrich for *Salmonella* prior to initiating the assay, with the exception of raw head-on shrimp, which requires an additional 4–24 h secondary enrichment in Rappaport-Vassiliadis 10 broth (RV10).

B. Apparatus and Reagents

Items (**b**)–(**g**) are available as the 3M MDA *Salmonella* kit from 3M Food Safety (St. Paul, MN).

(**a**) *3M Molecular Detection System*.—Available from 3M Food Safety.

(**b**) *3M MDA Salmonella reagent tubes*.—Twelve strips of eight tubes.

(**c**) *Lysis solution (LS) tubes*.—Twelve strips of eight tubes.

(**d**) *Extra caps*.—Twelve strips of eight caps.

(**e**) *Negative control (NC)*.—One vial (2 mL).

(**f**) *Reagent control*.—Eight reagent tubes.

(**g**) *Quick start guide.*

(**h**) *3M Molecular Detection Speed Loader Tray*.—Available from 3M Food Safety.

Table 2013.09A. POD summary of raw ground beef (25 g) results for the 3M MDA *Salmonella*[a]

	Inoculation level		
	Uninoculated	Low	High
Candidate presumptive positive/total No. of samples analyzed	1/120	69/120	120/120
Candidate presumptive POD (CP)	0.01 (0.00, +0.05)	0.58 (+0.48, +0.67)	1.00 (+0.97, +1.00)
s_r[b]	0.09 (+0.08, +0.17)	0.51 (+0.45, +0.52)	0.00 (0.00, +0.18)
s_L[c]	0.00 (0.00, +0.04)	0.00 (0.00, +0.14)	0.00 (0.00, +0.18)
s_R[d]	0.09 (+0.08, +0.10)	0.51 (+0.45, +0.52)	0.00 (0.00, +0.24)
Candidate confirmed positive/total No. of samples analyzed	0/120	67/120	120/120
Candidate confirmed POD (CC)	0.00 (0.00, +0.03)	0.56 (+0.47, +0.65)	1.00 (+0.97, +1.00)
s_r	0.00 (0.00, +0.17)	0.51 (+0.45, +0.52)	0.00 (0.00, +0.18)
s_L	0.00 (0.00, +0.17)	0.00 (0.00, +0.11)	0.00 (0.00, +0.18)
s_R	0.00 (0.00, +0.24)	0.51 (+0.46, +0.52)	0.00 (0.00, +0.24)
Positive reference samples/total No. of samples analyzed	0/120	68/120	119/120
Reference POD	0.00 (0.00, +0.03)	0.57 (+0.48, +0.66)	0.99 (+0.95, +1.00)
s_r	0.00 (0.00, +0.17)	0.50 (+0.45, +0.52)	0.09 (+0.08, +0.17)
s_L	0.00 (0.00, +0.17)	0.00 (0.00, +0.18)	0.00 (0.00, +0.04)
s_R	0.00 (0.00, +0.24)	0.51 (+0.45, +0.52)	0.09 (+0.08, –0.11)
dLPOD (C versus R)	0.00 (–0.03, +0.03)	–0.01 (–0.14, +0.12)	0.01 (–0.02, +0.05)
dLPOD (CP versus CC)	0.01 (–0.02, +0.05)	0.02 (–0.11, +0.15)	0.00 (–0.03, +0.03)

[a] Results include 95% confidence intervals.

[b] Repeatability standard deviation.

[c] Among-laboratory standard deviation.

[d] Reproducibility standard deviation.

(**i**) *3M Molecular Detection Chill Block Tray and Chill Block Insert*.—Available from 3M Food Safety.

(**j**) *3M Molecular Detection Heat Block Insert*.—Available from 3M Food Safety.

(**k**) *3M Molecular Detection Cap/Decap Tool for reagent tubes*.—Available from 3M Food Safety.

(**l**) *3M Molecular Detection Cap/Decap Tool for lysis tubes*.—Available from 3M Food Safety.

(**m**) *Empty lysis tube rack*.—Available from 3M Food Safety.

(**n**) *Empty reagent tube rack*.—Available from 3M Food Safety.

(**o**) *3M BPW ISO*.—Available from 3M Food Safety. Formulation equivalent to ISO 6579:2002 Annex B (4).

(**p**) *Rappaport-Vassiliadis 10 broth (RV10)*.—Available from 3M Food Safety.

(**q**) *Disposable pipet*.—Capable of 20 µL.

(**r**) *Multichannel (eight-channel) pipet*.—Capable of 20 µL.

(**s**) *Sterile filter tip pipet tips*.—Capable of 20 µL.

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

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

(**v**) *Thermometer*.—Calibrated range to include 100 ± 1°C.

(**w**) *Dry double block heater unit or water bath*.—Capable of maintaining 100 ± 1°C.

(**x**) *Incubators*.—Capable of maintaining 37 ± 1°C and 41.5 ± 1°C.

(**y**) *Freezer*.—Capable of maintaining –10 to –20°C, for storing the 3M Molecular Detection Chill Block Tray.

(**z**) *Refrigerator*.—Capable of maintaining 2–8°C, for storing the 3M MDA.

(**aa**) *Computer*.—Compatible with the 3M Molecular Detection Instrument.

C. Safety Precautions

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

D. Method Preparation and Precautions

The 3M MDA *Salmonella* is intended for use in a laboratory environment by professionals trained in laboratory techniques. The user should read, understand, and follow all safety information in the instructions for the 3M Molecular Detection System and the 3M MDA *Salmonella* method and retain the safety instructions for future reference. Follow all instructions carefully. Failure to do so may lead to inaccurate results.

Table 2013.09B. POD summary of wet pet food (375 g) results for the 3M MDA *Salmonella*[a]

	Inoculation level		
	Uninoculated	Low	High
Candidate Presumptive positive/total No. of samples analyzed	1/132	65/132	131/132
Candidate presumptive POD (CP)	0.01 (0.00, +0.04)	0.49 (+0.40, +0.58)	0.99 (+0.96, +1.00)
s_r[b]	0.09 (+0.08, +0.16)	0.51 (+0.46, +0.52)	0.09 (+0.08, +0.16)
s_L[c]	0.00 (0.00, +0.04)	0.00 (0.00, +0.14)	0.00 (0.00, +0.04)
s_R[d]	0.09 (+0.08, +0.10)	0.51 (+0.46, +0.52)	0.09 (+0.08, +0.10)
Candidate confirmed positive/total No. of samples analyzed	0/132	65/132	131/132
Candidate confirmed POD (CC)	0.00 (0.00, +0.03)	0.49 (+0.40, +0.58)	0.99 (+0.96, +1.00)
s_r	0.00 (0.00, +0.17)	0.51 (+0.46, +0.52)	0.09 (+0.08, +0.16)
s_L	0.00 (0.00, +0.17)	0.00 (0.00, +0.14)	0.00 (0.00, +0.04)
s_R	0.00 (0.00, +0.23)	0.51 (+0.46, +0.52)	0.09 (+0.08, +0.10)
Positive reference samples/total No. of samples analyzed	0/132	70/132	132/132
Reference POD	0.00 (0.00, +0.03)	0.53 (+0.44, +0.62)	1.00 (+0.97, +1.00)
s_r	0.00 (0.00, +0.17)	0.52 (+0.46, +0.52)	0.00 (0.00, +0.17)
s_L	0.00 (0.00, +0.17)	0.00 (0.00, +0.09)	0.00 (0.00, +0.17)
s_R	0.00 (0.00, +0.23)	0.52 (+0.47, +0.52)	0.00 (0.00, +0.23)
dLPOD (C versus R)	0.00 (-0.03, +0.03)	-0.04 (-0.16, +0.09)	-0.01 (-0.04, +0.02)
dLPOD (CP versus CC)	0.01 (-0.02, +0.05)	0.00 (-0.13, +0.13)	0.00 (-0.03, +0.03)

[a] Results include 95% confidence intervals.

[b] Repeatability standard deviation.

[c] Among-laboratory standard deviation.

[d] Reproducibility standard deviation.

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

Use proper aseptic technique. Use proper precautions for Biosafety Level 2 microorganisms. Periodically, laboratory benches and equipment (pipets, cap/decap tools, etc.) should be decontaminated with a 1–5% (v/v in water) household bleach solution or DNA removal solution.

E. Sample Preparation

Table **2013.09C** presents guidance for the enrichment of food and feed samples at a 1:10 dilution. It is the user's responsibility to validate alternate sampling protocols or dilution ratios to ensure this test method meets the user's criteria.

Prewarm 3M BPW ISO enrichment medium to 37 ± 1°C or 41.5 ± 1°C. Aseptically combine the enrichment medium and sample. Homogenize thoroughly for 2 min. Incubate at 37 ± 1°C or 41.5 ± 1°C. For all meat and highly particulate samples, the use of filter bags is recommended.

In an AOAC PTM study, the 3M MDA Salmonella (Certificate No. 031208) was found to be an effective method for the detection of Salmonella in the matrixes shown in Table **2013.09C**.

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

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

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

Before using the 3M Molecular Detection Chill Block Insert, ensure that it has been stored on the 3M Molecular Detection Chill Block Tray in the freezer (–10 to –20°C) for a minimum of 2 h before use. When removing the 3M Molecular Detection Chill Block Insert from the freezer for use, remove it and the 3M Molecular Detection Chill Block Tray together. Use the 3M Molecular Detection Chill Block Insert/3M Molecular Detection Chill Block Tray within 20 min.

H. Preparation of the 3M™ Molecular Detection Heat Block Insert

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

Note: Depending on the heater unit, allow approximately

Table 2013.09C. Sample enrichment protocols

Sample matrix	Sample size	Enrichment broth vol., mL	Enrichment temp., ±1°C	Enrichment time, h
Raw shrimp (head off)	25 g	225	37	18–24
Fresh spinach (bagged)	25 g	225	37	18–24
Peanut butter	25 g	225	37	18–24
Pasteurized American cheese	25 g	225	37	18–24
Pasteurized liquid whole egg	100 g	900	37	18–24
Cooked breaded chicken	325 g	2925	37	18–24
Dry pet food (dog)	25 g	225	37	18–24
	375 g	1500	37	18–24
Wet pet food (dog)	375 g	3375	37	18–24
Sprout irrigation water	375 mL	3375	37	18–24
Raw ground beef (27% fat)	25 g	225	37	18–24
Raw ground beef (20% fat)	25 g	225	41.5	10–18
	325 g	975	41.5	10–18
	375 g	1500	41.5	10–18
Raw ground poultry	25	225	41.5	10–18
	325	975	41.5	14–18
Chicken carcass rinse	30 mL	30	41.5	18–24
Chicken carcass sponge	1 Sponge	50	41.5	18–24
Stainless steel	1 Swab	50	41.5	18–24
Sealed/glazed ceramic tile	1 Sponge	50	41.5	18–24
Concrete	1 Sponge	225 mL	41.5	18–24

Sample matrix	Sample size	Enrichment broth vol., mL	Enrichment temp., °C	Enrichment time, h	Secondary enrichment medium, mL	Secondary enrichment temp., °C	Secondary enrichment time, h
Raw shrimp (head on)	25 g	225	37	18–14	RV R10: 0.1 mL into 10 mL	41.5	4–24

30–50 min for the 3M Molecular Detection Heat Block Insert to reach temperature. Using a calibrated thermometer, verify that the 3M Molecular Detection Heat Block Insert is at 100 ± 1°C.

I. Preparation of the 3M Molecular Detection Instrument

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

Note: The 3M Molecular Detection Instrument must reach and maintain temperature of 60°C before inserting the 3M Molecular Detection Speed Loader Tray with reaction tubes. This heating step takes approximately 20 min and is indicated by an orange light on the instrument's status bar. When the instrument is ready to start a run, the status bar will turn green.

J. Lysis

Allow the LS tubes to warm up to room temperature by setting the rack on the laboratory bench for 2 h. Remove the enrichment broth from the incubator and gently agitate the contents. One LS tube is required for each sample and the NC sample. LS tube strips can be cut to desired LS tube number. Select the number of individual LS tubes or eight-tube strips needed. Place the LS tubes in an empty rack. To avoid cross-contamination, decap one LS tubes strip at a time and use a new pipet tip for each transfer step. Transfer enriched sample to LS tubes as described below:

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

Use the 3M™ Molecular Detection Cap/Decap Tool-Lysis to decap one LS tube strip—one strip at a time. Set the tool with cap attached aside on a clean surface. Transfer 20 µL of sample into an LS tube. Repeat until each individual sample has been added to a corresponding LS tube in the strip. Use the 3M Molecular Detection Cap/Decap Tool-Lysis to re-cap the LS tube strip. Use the rounded side of the tool to apply pressure in a back and forth motion ensuring that the cap is tightly applied (Figure **2013.09A**).

Repeat as needed, for the number of samples to be tested. When all samples have been transferred, transfer 20 µL of NC into an LS tube. Use the 3M Molecular Detection Cap/Decap Tool-Lysis tool to re-cap the LS tube. Cover the rack of LS tubes with the rack lid and firmly invert 3–5 times to mix. Suspension has to flow freely inside the tube.

Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100 ± 1°C. Place the rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for

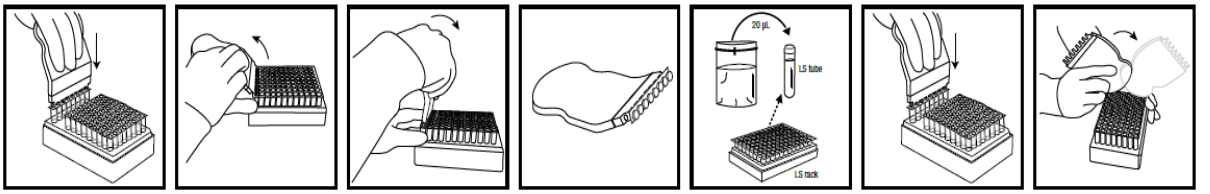

Figure 2013.09A. Transfer of enriched sample to lysis solution tube.

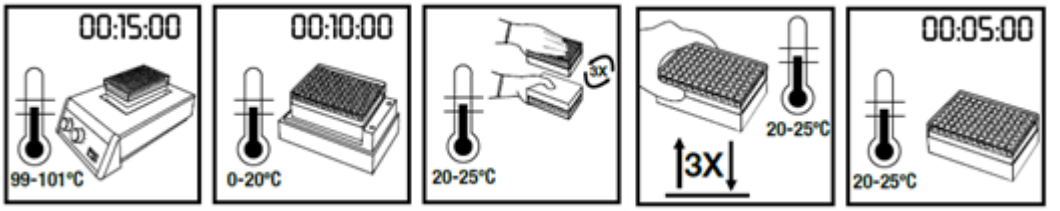

Figure 2013.09B. Sample lysis.

15 ± 1 min. Samples that have not been properly heat-treated during the assay lysis step may be considered a potential biohazard and should not be inserted into the 3M Molecular Detection Instrument.

Remove the rack of LS tubes from the heating block and allow to cool in the 3M Molecular Detection Chill Block Insert for 10 ± 1 min. Remove the rack lid during incubation on the 3M Molecular Detection Chill Block Insert.

Remove the rack of LS tubes from the 3M Molecular Detection Chill Block Insert/3M Molecular Detection Chill Block Tray system. Replace the lid on the rack of LS tubes and firmly invert 3–5 times to mix. Suspension has to flow freely inside the tube. Firmly tap the lysis tubes rack on the laboratory bench 3–5 times. Place the rack on the laboratory bench. Let it sit undisturbed for at least 5 min to allow the resin to settle. Do not mix or disturb the resin at the bottom of the tube (Figure **2013.09B**).

(**a**) Alternatives to equilibrate the LS tubes to room temperature are to incubate the LS tubes in a 37 ± 1°C incubator for 1 h or at room temperature overnight (16–18 h).

(**b**) An alternative to using dry heat for the lysis step is to use a water bath at 100 ± 1°C. Ensure that sufficient water is used to cover up to the liquid level in the LS tubes. Place the rack of LS tubes in the water bath at 100 ± 1°C and heat for 15 ± 1 min.

(**c**) The LS solution may freeze when processing fewer than 48 LS tubes. Freezing of the LS solution will not affect your test. If freezing is observed, allow the LS tubes to thaw for 5 min before mixing.

K. Amplification

Note: It is generally accepted that the matrix may have an impact on any test method. The 3M Molecular Detection Matrix Control (MDMC96) is a verification tool that is separate from the specific pathogen 3M MDAs. The Matrix Control (MC) test is to check for inhibition by the matrix sample. 3M recommends using the 3M Molecular Detection Matrix Control kit during any validation period when adopting the 3M method or in the event of testing new or unknown matrixes or for matrixes that have undergone raw material or process changes.

A matrix can be defined as a sample drawn from a population which is meant to represent the whole. Differences between matrixes may be as simple as the effects caused by differences in their processing, for example, intact muscle vs ground; raw vs pasteurized; fresh vs dried, etc.

If using the MC, see the 3M Molecular Detection Matrix Control product instructions for details. If not, proceed as follows:

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

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

Warning: Care must be taken when pipetting LS, as carry-over of the resin may interfere with amplification.

Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent tubes, one Reagent tubes strip at a time. Discard cap. Transfer 20 µL of sample lysate from the upper portion of the fluid in the LS tube into corresponding reagent tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times. Repeat until individual sample lysate has been added to a corresponding reagent tube in the strip. Cover the reagent tubes with the provided extra cap and use the rounded side of the 3M Molecular Detection Cap/Decap Tool-Reagent to apply pressure in a back and forth motion ensuring that the cap is tightly applied. Repeat as needed for the number of samples to be tested. When all sample lysates have been transferred, transfer 20 µL of NC lysate into a reagent tube. Then transfer 20 µL of NC lysate into an RC tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times. Load capped tubes into a clean and decontaminated 3M Molecular Detection Speed Loader Tray. Close and latch the 3M Molecular Detection Speed Loader Tray lid (Figure **2013.09C**).

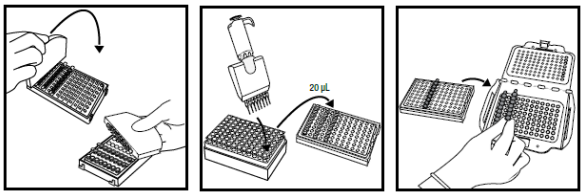

Figure 2013.09C. Transfer of lysate to reagent tube.

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

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

L. Results and Interpretation

An algorithm interprets the light output curve resulting from the detection of the nucleic acid amplification. Results are analyzed automatically by the software and are color-coded based on the result. A positive or negative result is determined by analysis of a number of unique curve parameters. Presumptive positive results are reported in real-time while negative and inspect results will be displayed after the run is completed. Presumptive positive results should be confirmed using your preferred method or as specified by local regulations.

Note: Even a negative sample will not give a zero reading as the system and 3M Molecular Assay *Salmonella* amplification reagents have a "background" relative light unit.

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

Results of Collaborative Study

In this collaborative study, the 3M MDA *Salmonella* method was compared to the to the USDA/FSIS-MLG 4.05 reference method for raw ground beef and to the FDA/BAM Ch. 5 reference method for wet dog food. A total of 20 laboratories throughout the United States participated in this study, with 14 laboratories submitting data for the raw ground beef and 16 laboratories submitting data for the wet dog food as presented in Table 1. 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.

Table 1. Participation of each collaborating laboratory[a]

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

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

[b] Data obtained from additional shipment of raw ground beef. Initial shipment of raw ground beef was not used for evaluation purposes and therefore the data are not presented.

[c] Results were not used in statistical analysis due to laboratory error, or uninoculated control test portions were confirmed as *Salmonella*.

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 2 and 3. Tables **2013.09A** and **B** summarize the interlaboratory results for all foods tested, including POD statistical analysis (10). The results of the collaborating laboratories APC analysis for each matrix are presented in Table 3 in the Appendix.

Raw Ground Beef (25 g Test Portions)

Raw ground beef test portions were inoculated at a low and high level and were analyzed (Table 2) for the detection of *Salmonella* spp. Uninoculated controls were included in each analysis. The results presented for the raw ground beef were from a second shipment of test portions to the collaborating laboratories. The initial shipment of raw ground beef test portions sent to collaborators was discovered to contain contamination of the target analyte in the uninoculated control samples for each laboratory, and therefore no data have been presented. Fourteen laboratories participated in the retest analysis of this matrix, and the results of 10 laboratories were included in the statistical analysis. For the retest of the raw ground beef, laboratories 12, 16, 18, and 19 detected the presence of *Salmonella* spp. in either

the candidate or reference method control replicates. Because of the potential for error, results from these laboratories were excluded from the statistical analysis. The MPN levels obtained for this test portion, with 95% confidence intervals, were 0.81 CFU/test portion (+0.62, +1.04) for the low level and 4.68 CFU/test portion (+3.22, +6.80) for the high level.

For the high level, 120 out of 120 test portions were reported as presumptive positive by the 3M MDA *Salmonella* method with all test portions confirming positive. For the low level, 67 out of 120 test portions were reported as presumptive positive by the 3M MDA *Salmonella* method with 65 test portions confirming positive. For the uninoculated controls, 1 out of 120 samples produced a presumptive positive result by the 3M MDA *Salmonella* method with all test portions confirming negative. For test portions analyzed by the USDA/FSIS-MLG method, 119 out of 120 high inoculum and 68 out of 120 low inoculum test portions confirmed positive. For the uninoculated controls, 0 out of 120 test portions confirmed positive.

For the low-level inoculum, a $dLPOD_C$ value of –0.01 with 95% confidence intervals of (–0.14, +0.13) was obtained between the 3M MDA *Salmonella* method and the USDA/FSIS-MLG method. The confidence intervals obtained for $dLPOD_C$ indicated no significant difference between the two methods. A $dLPOD_{CP}$ value of 0.02 with 95% confidence intervals of (–11, +0.15) was obtained between presumptive and confirmed 3M MDA *Salmonella* results. The confidence intervals obtained for $dLPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results using either confirmation process.

For the high-level inoculum, a $dLPOD_C$ value of 0.01 with 95% confidence intervals of (–0.02, +0.05) was obtained between the 3M MDA *Salmonella* method and the USDA/FSIS-MLG method. The confidence intervals obtained for $dLPOD_C$ indicated no significant difference between the two methods. A $dLPOD_{CP}$ value of 0.00 with 95% confidence intervals of (–0.03, +0.03) was obtained between presumptive and confirmed 3M MDA *Salmonella* results. The confidence intervals obtained for $dLPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results. Detailed results of the POD statistical analysis are presented in Table 1 and Figures 1 and 2 in the Appendix.

Wet Dog Food (375 g Test Portions)

Wet dog food test portions were inoculated at a low and high level and were analyzed (Table 3) 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 11 laboratories were included in the statistical analysis. Laboratories 4, 5, 10, and 16 detected the presence of *Salmonella* spp. in either the candidate or reference method control replicates. Because of the potential for error, results from these laboratories were excluded from the statistical analysis. Laboratory 12 did not submit results due to cross-contamination of sample enrichments as reported by the analyst. The MPN levels obtained for this test portion, with 95% confidence intervals, were 0.72 CFU/test portion (+0.57, +0.90) for the low level and 5.34 CFU/test portion (+3.46, +8.24) for the high level.

For the high level, 131 out of 132 test portions were reported as presumptive positive by the 3M MDA *Salmonella* method with all test portions confirming positive. For the low level, 65 out of 132 test portions were reported as presumptive positive by the 3M MDA *Salmonella* method with all test portions confirming positive. For the uninoculated controls, 1 out of 132 samples produced a presumptive positive result by the 3M MDA *Salmonella* method with all test portions confirming negative. For test portions analyzed by the FDA/BAM method, 132 out of 132 high inoculum and 70 out of 132 low inoculum test portions confirmed positive. For the uninoculated controls, 0 out of 132 test portions confirmed positive.

For the low-level inoculum, a $dLPOD_C$ value of –0.04 with 95% confidence intervals of (–0.16, +0.09) was obtained between the 3M MDA *Salmonella* method and the FDA/BAM method. The confidence intervals obtained for $dLPOD_C$ indicated no significant difference between the two methods. A $dLPOD_{CP}$ value of 0.00 with 95% confidence intervals of (–0.13, +0.13) was obtained between presumptive and confirmed 3M MDA *Salmonella* results. The confidence intervals obtained for $dLPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results using either confirmation process.

For the high-level inoculum, a $dLPOD_C$ value of –0.01 with 95% confidence intervals of (–0.04, +0.02) was obtained between the 3M MDA *Salmonella* method and the FDA/BAM method. The confidence intervals obtained for $dLPOD_C$ indicated no significant difference between the two methods. A $dLPOD_{CP}$ value of 0.00 with 95% confidence intervals of (–0.03, +0.03) was obtained between presumptive and confirmed 3M MDA *Salmonella* results. The confidence intervals obtained for $dLPOD_{CP}$ indicated no significant difference between the presumptive and confirmed results. Detailed results of the POD statistical analysis are presented in Table 2 and Figures 3 and 4 in the Appendix.

Discussion

For this collaborative study, samples were analyzed at both 25 and 375 g test portions as required by the current AOAC guidelines (5), which require methods with more than one sample preparation or enrichment scheme to analyze one matrix per procedure. No negative feedback was provided by the collaborating laboratories in regard to the performance of the candidate method. Several collaborating laboratories expressed questions in regard to the AOAC study design of the collaborative study while other laboratories expressed concern with analyzing 375 g test portions. The concern with handling the larger test portions may have contributed to errors observed during testing that resulted in data not used in the statistical analysis.

During testing, four different laboratories detected the presence of *Salmonella* spp. In seven raw ground beef uninoculated control test portions. Additionally, four different laboratories detected the presence of *Salmonella* spp. in 15 wet pet food uninoculated control test portions. Due to detecting positive samples in the control test portions, the data provided by these laboratories were not included during the statistical analysis. A root cause investigation to determine the source of contamination yielded the following possibilities: Due to the high number of samples analyzed, including test portions inoculated at a high inoculum level, contamination may have occurred during the transfer of enriched samples into

Table 2. 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 MDA *Salmonella*[b]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	+	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
2	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	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	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	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	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
9	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–[c]	+	+	+	+	–	–	–[c]	–	–	–	–	–	–	–	–	–	–	–	–
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
12[d]	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–[c]	–[c]	–[c]	+	+	+	–[c]	–	–	–	–[c]	–[c]	–[c]	–[c]	–	–[c]	–	–[c]	–	–[c]
13	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
14	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–[c]	–	–	–	–	–
16[d]	+	+	+	+	+	+	+	+	+	+	+	+	–[c]	–	+	+	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–[c]	–[c]	–[c]	–[c]	–	–
17	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
18[d]	+	+	+	+	+	+	+	+	+	+	+	+	–[c]	+	+	+	–[c]	+	+	–[c]	+	+	–[c]	+	–[c]	+	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	–[c]	+
19[d]	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	+	+	–	+	–[c]	–	–	–	–[c]	–[c]	–[c]	–[c]	–	–[c]	–[c]	–[c]	–[c]	–[c]
20	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	–	+	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
USDA/FSIS-MLG[d]																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	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	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	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	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
9	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–

Table 2. (*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
10	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	+	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
12[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	+	–	–	–
13	+	+	+	+	+	+	–	+	+	+	+	+	+	–	–	+	–	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
14	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
15	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	–	+	+	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
16[b]	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	+	–	+	–	–	–	–	–	+	–	–	–	–	–	–
17	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
18[b]	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
19[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	–	+	–	+	+	–	+	+	–	–	–	–	–	–	—	–	–
20	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] + = *Salmonella* spp. were detected in samples; – = *Salmonella* spp. were not detected in sample; n/a = laboratory did not participate in this matrix, or results were not received.

[b] Sample results were obtained from the second shipment of raw ground beef test portions.

[c] Sample was presumptive positive on 3M MDA *Salmonella* but confirmed negative indicating a false-positive result.

[d] Results were not used in statistical an/alysis due to laboratory error.

Table 3. Individual collaborator results for wet 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 MDA *Salmonella*																																				
1	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
3	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
4[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	+	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–
5[b]	+	+	+	+	+	+	–	–	+	+	+	+	+	–	+	+	+	–	+	+	+	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–
6	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
7	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	+	–	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
8	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
10[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–[c]	+	+	–[c]	–	+	+	+	–[c]	+	–	–	–[c]	–[c]	–	–	–	–[c]	–	–
11	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
13	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	–	+	+	–	–	+	+	–	–	–	–	–	–[c]	–	–	–	–	–	–	–
14	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
15	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
16[b]	+	+	+	+	–	–	+	+	+	+	+	+	+	+	–[c]	+	–	–	–	+	+	–	+	–	–	–[c]	–	+		–[c]	+		+	–[c]	+	–
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
18	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
19	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
20	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	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	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	+	–	+	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
3	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
4[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	+	+	+	–	+	–	–	–	–	–	–	–	–	–	+	–	–
5[b]	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
6	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	+	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
8	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	+	+	+	+	+	–	+	–	–	–	–	–	–	-	–	–	–	–	–
9	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	–	–	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] Results are presented as + (positive) or – (negative) for each test portion.

[b] Collaborator reported difficulty with the assay.

[c] Result was reported as indeterminate.

Table 3. (*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
10[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	–	+	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
12	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
13	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	–	+	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
14	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
15	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	+	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
16[b]	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	+	+	–	–	+	+	+	–	–	+	–	+	–	–	–	–	–	–	–
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
18	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
19	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
20	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	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; n/a = laboratory did not participate in this matrix, or results were not received.

[b] Results were not used in statistical an/alysis due to laboratory error.

[c] Sample was presumptive positive on 3M MDA *Salmonella* but confirmed negative indicating a false-positive result.

the secondary selective enrichments, or during the streaking of the reference agar plates. For the wet pet food, based on feedback from the collaborators, issues with storage during the incubation of the larger test portion sizes may have led to cross-contamination of the primary enrichments. Based on the fact that uninoculated control test portions were packaged one day prior to the inoculated test portions, contamination during test portion preparation at the coordinating laboratory is not believed to be the cause of the positive control samples.

During the analysis of both the raw ground beef and wet pet food, some laboratories produced false-positive results with the candidate method. The 3M MDA is intended for use in a laboratory environment by professionals trained in laboratory technique. Cross-contamination of samples resulting in false-positive results may occur if careful molecular techniques are not followed. To reduce the risk of cross-contamination, 3M recommends the use of sterile, aerosol barrier (filtered) molecular biology grade pipet tips. A new pipet tip should be used for each sample transfer, and the user may choose to add an intermediate transfer step in order to avoid pipet contamination, i.e., each enriched sample can be transferred into a sterile tube before proceeding into the lysis step. Discrepant results may be obtained if deviations from the method occur. Use of calibrated pipettors and thermometers is critical to ensure that correct volumes of samples, especially when hydrating the reagent tubes, and appropriate temperatures are utilized. It is recommended that users read and become familiar with the 3M MDA *Salmonella* product instructions and follow them carefully.

For either matrix, the collaborative study failed to show a statistically significant difference between the candidate method and the reference method using the POD model when the aforementioned four laboratories were removed from consideration.

Recommendations

It is recommended that the 3M MDA *Salmonella* method be adopted as Official First Action status for the detection of *Salmonella* in selected foods including raw ground beef (25, 325, and 375 g), raw ground chicken (25 and 325 g), cooked breaded chicken (325 g), pasteurized liquid whole egg (100 g), raw shrimp (head-off, 25 g), fresh spinach (bagged, 25 g), wet dog food (375 g), pasteurized American cheese (25 g), peanut butter (25 g), dry dog food (25 and 375 g), sprout irrigation water (375 g), raw head-on shrimp (25 g), chicken carcass rinsate (30 mL), chicken carcass sponge, sealed/glazed ceramic tile, concrete, and stainless steel.

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References

(1) Centers for Disease Control and Prevention (October 26, 2011) http://www.cdc.gov/nczved/divisions/dfbmd/ (accessed November 6, 2012)

(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) U.S. Department of Agriculture, Food Safety and Inspection Service (May 2007) http://askfsis.custhelp.com/app/answers/detail/a_id/334/related/1 (accessed November 6, 2012)

(4) International Organization for Standardization (2002) *ISO 6579: Microbiology of Food and Animal Feeding Stuffs-Horizontal Method for the Detection of Salmonella spp*., 4th Ed., Geneva, Switzerland

(5) Brunelle, S., LaBudde, R., Nelson, M., & Wehling P. (2012) *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces*, AOAC INTERNATIONAL, Gaithersburg, MD, Appendix X

(6) *Microbiology Laboratory Guidebook*, *Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg and Catfish Products* (2011) Revision 4.05, U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC. http://www.fsis.usda.gov/PDF/MLG_4_05.pdf (accessed July 2012)

(7) Andrews, W.H., & Hammack, T. (2011) *Bacteriological Analytical Manual*, Chapter 5, *Salmonella.* http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm (accessed July 2012)

(8) Maturin, L., & Peeler, J.T. (2001) *Bacteriological Analytical Manual*, Chapter 3, *Aerobic Plate Count*. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.htm

(9) Least Cost Formulations, Ltd (2011) *MPN Calculator-Version 1.6*. www.lcfltd.com/customer/LCFMPNCalculator.exe (accessed November 2012)

(10) Least Cost Formulations, Ltd (2011) *AOAC Binary Data Interlaboratory Study Workbook*. http://lcfltd.com/aoac/aoac-binary-v2-2.xls (accessed November 2012)

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