MICROBIOLOGICAL METHODS

Evaluation of 3M™ Molecular Detection Assay (MDA) *Listeria* for the Detection of *Listeria* species in Selected Foods and Environmental Surfaces: Collaborative Study, First Action 2014.06

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The 3M™ Molecular Detection Assay (MDA) *Listeria* is used with the 3M Molecular Detection System for the detection of *Listeria* species in food, food-related, and environmental samples after enrichment. The assay utilizes loop-mediated isothermal amplification to rapidly amplify *Listeria* target DNA with high specificity and sensitivity, combined with bioluminescence to detect the amplification. The 3M MDA *Listeria* method was evaluated using an unpaired study design in a multilaboratory collaborative study and compared to the AOAC *Official Method of Analysis*℠ (OMA) 993.12 *Listeria monocytogenes in Milk and Dairy Products* reference method for the detection of *Listeria* species in full-fat (4% milk fat) cottage cheese (25 g test portions). A total of 15 laboratories located in the continental United States and Canada participated. Each matrix had three inoculation levels: an uninoculated control level (0 CFU/test portion), and two levels artificially contaminated with *Listeria monocytogenes,* a low inoculum level (0.2–2 CFU/test portion) and a high inoculum level (2–5 CFU/test portion) using nonheat-stressed cells. In total, 792 unpaired replicate portions were analyzed. Statistical analysis was conducted according to the probability of detection (POD) model. Results obtained for the low inoculum level test portions produced a difference in cross-laboratory POD value of –0.07 with a 95% confidence interval of (–0.19, 0.06). No statistically significant differences were observed in the number of positive samples detected by the 3M MDA *Listeria* method versus the AOAC OMA method.

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The method was approved by the Expert Review Panel for Microbiology for Food and Environmental Surfaces.
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.
Supplemental Tables and Figures are available on the *J. AOAC Int.* website, http://aoac.publisher.ingentaconnect.com/content/aoac/jaoac
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Listeria is a Gram-positive, rod-shaped bacterium found widespread in the environment, and one species, *Listeria monocytogenes*, is known to be the causative agent of listeriosis in humans (1). Due to its high mortality rate, specifically in susceptible individuals such as older adults, pregnant women, newborns, and adults with weakened immune systems, listeriosis presents itself as an important health problem in the United States, Canada, and throughout the world (2). *Listeria*'s ability to survive in extreme conditions, such as low temperature and a broad pH range (4.4 to 9.4), can cause severe problems for food manufacturers as the organism can survive cleaning conditions and contaminate food commodities (1, 3). While less frequent than other foodborne pathogens, outbreaks from *L. monocytogenes* have been linked to a wide variety of food types, such as raw milks and cheeses, pasteurized dairy products, smoked seafood, ready-to-eat deli meats, hot dogs, and most recently cantaloupes (2). The presence of other *Listeria* species, such as *L. innocua, L. welshimeri,* or *L. ivanovii,* is often used as an indicator for the possible contamination of *L. monocytogenes* (4). The 3M™ Molecular Detection Assay (MDA) *Listeria* method uses loop-mediated isothermal amplification of target nucleic acid sequences to detect *Listeria* in enriched food, feed, and environmental samples. The isothermal amplification is a PCR conducted at a constant temperature, eliminating the need for temperature cycling and decreasing the time to results.

The 3M MDA *Listeria* method allows for the rapid and specific detection of *Listeria* species after as little as 24 h of enrichment using prewarmed (37 ± 1°C) Demi Fraser (DF) broth base [without ferric ammonium citrate (FAC)] or 3M Modified *Listeria* Recovery Broth (mLRB). After enrichment, samples are evaluated using the 3M MDA *Listeria* on the 3M Molecular Detection System. Presumptive positive results are reported in real-time, while negative results are displayed after completion of the assay (75 min).

Prior to the collaborative study, the 3M MDA *Listeria* method was validated according to AOAC guidelines (5) in a harmonized AOAC *Performance Tested Method*SM (PTM) study. The objective of the PTM study was to demonstrate that the 3M MDA *Listeria* method could detect *Listeria* on selected environmental surfaces as claimed by the manufacturer. For the 3M MDA *Listeria* PTM evaluation, three matrixes were evaluated: stainless steel (sponge in 225 mL 3M mLRB), sealed concrete (sponge in 225 mL 3M mLRB), and plastic (swab in 10 mL 3M mLRB). 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. 081203 on March 30, 2012.

A method modification and matrix extension study was performed in 2014 with the following matrixes: beef hot dogs (25 g), deli turkey (25 g), cold smoked salmon (25 g), full-fat cottage cheese (25 g), bagged raw spinach (25 g), whole cantaloupe (whole melon), sealed concrete (sponge in 100 mL and sponge in 225 mL enrichment volume) and stainless steel (sponge in 225 mL enrichment volume) using DF broth base without FAC as the primary enrichment and, where applicable, a secondary enrichment in Fraser broth base without FAC. 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 modification and matrix extension was awarded PTM approval and license No. 081203 on June 30, 2014.

The purpose of this collaborative study was to compare the reproducibility among different laboratories of the 3M MDA *Listeria* method to the AOAC *Official Method of Analysis* (OMA) **993.12** *Listeria monocytogenes in Milk and Dairy Products* (6) reference method for full-fat (4% milk fat) cottage cheese.

Collaborative Study

Study Design

In this collaborative study, one matrix, full-fat cottage cheese, was analyzed using 25 g test portions. The full-fat cottage cheese was obtained from a local retailer and screened for the absence of *Listeria* by the AOAC **993.12** reference method prior to analysis. The matrix was artificially contaminated with nonheat-stressed cells of *L. monocytogenes* American Type Culture Collection (ATCC; Manassas, VA) 19114 at two inoculation levels: 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 were also included at 0 CFU/test portion. Twelve replicate portions from each of the three inoculation levels were analyzed. Two sets of samples (72 total) were sent to each laboratory for analysis by 3M MDA *Listeria* and AOAC **993.12** due to the different sample enrichment procedures for each method. Additionally, collaborators were sent a 30 g test portion and instructed to conduct a total aerobic plate count using 3M™ Petrifilm™ Aerobic Count Plate (AOAC OMA **990.12**; 7) on the day samples were received for the purpose of determining the total aerobic microbial load.

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

Preparation of Inocula and Test Portions

The *L. monocytogenes* culture used in this evaluation was propagated in 10 mL of Brain Heart Infusion broth from a frozen stock culture stored at –70°C at Q Laboratories, Inc. The broth was incubated for 18 ± 0.5 h at 35 ± 1°C. Appropriate dilutions of the culture were prepared based on previously established growth curves for both the low and high inoculation levels. The full-fat cottage cheese was inoculated at a low and high inoculation level with the diluted inoculum and thoroughly hand-mixed to ensure an even distribution of microorganisms. The inoculated test product was divided into separate 30 g portions which were packaged into sterile Whirl-pak bags.

To determine the level of *L. monocytogenes* in the full-fat cottage cheese, a five-tube most probable number (MPN) was conducted on the day of initiation of analysis. From both the high and low inoculated batches, 5 × 50 g test portions, the reference method test portions from the collaborating laboratories, and 5 × 10 g test portions were analyzed. Each test portion was enriched at a 1:10 dilution and evaluated following the AOAC **993.12** reference method. The MPN and 95% confidence intervals were calculated from the high, medium, and low levels using the LCF MPN Calculator, Version 1.6, provided by AOAC Research Institute (8). Confirmation of the samples was conducted according to the AOAC **993.12** reference method.

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 the International Air Transportations Association. Upon receipt, samples were held by the collaborating laboratory at refrigeration temperature (3–5°C) until the following Monday when analysis was initiated a total of 96 h after inoculation. All samples were packed with cold packs to target a temperature of <7°C during shipment. In addition to each of the test portions and the total plate count sample, collaborators also received a test portion for each matrix labeled as “temperature control.” Participants were instructed to obtain the temperature of this portion upon receipt of the package, document results on the Sample Receipt Confirmation form provided, and fax to the study director. The shipment and hold times (through 120 h) of the inoculated test material had been verified as a QC measure prior to study initiation.

Test Portion Analysis

Each collaborator received 72 test portions of full-fat cottage cheese (12 high inoculum, 12 low inoculum, and 12 uninoculated controls for each method). Collaborators followed the appropriate preparation and analysis protocol according to the method specified for the matrix (Table 1).

Table 1. Participation of each collaborating laboratory[a]

Lab	Full-fat cottage cheese[a]
1	Y
2	Y
3	Y
4	Y[b]
5	Y[b]
6	Y[c]
7	Y
8	Y
9	Y
10	Y
11	Y
12	Y
13	Y[c]
14	Y
15	Y

[a] Y = Collaborator analyzed the food type.

[b] Results were not submitted to the coordinating laboratory.

[c] Results were not used in statistical analysis due to laboratory error.

For the analysis of the test portions by the 3M MDA *Listeria* method, a 25 g portion was enriched with 225 mL of prewarmed (37 ± 1°C) DF broth base without FAC, homogenized for 2 ± 0.5 min, and incubated for 26 ± 2 h at 37 ± 1°C. Following enrichment, samples were assayed by the 3M MDA *Listeria* method and confirmed following the standard reference method by streaking an aliquot of the primary enrichment onto Oxford Agar (OXA). Presumptive positive samples were streaked for isolation on Trypticase Soy Agar with yeast extract (TSA/ye), verified morphologically by Gram stain, and biochemically confirmed by hemolysis testing and by VITEK 2 GP Biochemical Identification method (AOAC OMA **2012.02**; 9) or API *Listeria* Identification System biochemical test kits (bioMérieux, Lyon, France). Laboratories utilizing API *Listeria* kits were also required to conduct catalase and oxidase tests.

For samples analyzed using the AOAC **993.12** reference method, 25 g test portions were enriched in prewarmed (45 ± 2°C) selective enrichment broth, homogenized for 2 ± 0.5 min, and incubated at 30 ± 2°C for 48 ± 2 h. Samples were streaked onto OXA, and presumptive positive samples were streaked for isolation onto TSA/ye. Colonies from TSA/ye were verified morphologically by Gram stain and biochemically confirmed by hemolysis test and by VITEK 2 GP Biochemical Identification method or API *Listeria* biochemical test kits. Laboratories utilizing API *Listeria* kits were also required to conduct catalase and oxidase tests.

Statistical Analysis

Each collaborating laboratory recorded results for the reference method and the 3M MDA *Listeria* method on the data sheets provided. The data sheets were submitted to the study director at the end of testing for analysis. The results of each test portion for each sample were compiled by the study director and the 3M MDA *Listeria* 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). The POD was calculated as the number of positive outcomes divided by the total number of trials. The cross-laboratory POD (LPOD) was calculated for the candidate presumptive results, $LPOD_{CP}$, the candidate confirmatory results (including false-negative results), $LPOD_{CC}$, the difference in the candidate presumptive and confirmatory results, $dLPOD_{CP}$, presumptive candidate results that confirmed positive (excluding false-negative results), $LPOD_C$, the reference method, $LPOD_R$, and the difference in the confirmed candidate and reference methods, $dLPOD_C$. A dLPOD confidence interval not containing the point zero would indicate a statistically significant difference between the 3M MDA *Listeria* and the AOAC **993.12** reference methods at the 5% probability level. In addition to POD, the repeatability SD (s_r), the among-laboratory repeatability SD (s_L), the reproducibility standard deviation (s_R), and the P_T value were calculated. The s_r provides the variance of data within one laboratory, the s_L provides the difference in SD between laboratories, and the s_R provides the variance in data between different laboratories. The P_T value provides information on the homogeneity test of laboratory PODs (11).

AOAC Official Method 2014.06
Listeria species in Selected Foods and Environmental Surfaces
3M™ Molecular Detection Assay (MDA)
Listeria Method
First Action 2014

[Applicable to detection of *Listeria* species in selected foods, including beef hot dogs (25 g), deli turkey (25 g), cold smoked salmon (25 g), full-fat cottage cheese (25 g), and two environmental surfaces: sealed concrete (sponge in 100 mL and sponge in 225 mL enrichment volume) and stainless steel (and sponge in 225 mL enrichment volume) enriched in prewarmed DF broth base.]

See Table **2014.06A** for a summary of results of the interlaboratory study supporting acceptance of the method.

See Appendix available on the *J. AOAC Int.* website for supplementary materials for detailed results of the interlaboratory study (http://aoac.publisher.ingentaconnect.com/content/aoac/jaoac).

A. Principle

The 3M MDA *Listeria* is intended for use with the 3M Molecular Detection System for the rapid and specific detection of *Listeria* spp. in selected foods and environmental surfaces. The 3M MDA uses loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification. Presumptive positive results are reported in real-time, while negative results are displayed after the assay is completed. Samples are enriched in prewarmed DF broth base, which does not contain FAC.

Table 2014.06A. Summary of results for the detection of *Listeria* in full-fat cottage cheese (25 g)

Method[a]	3M MDA *Listeria*		
Inoculation level	Uninoculated	Low	High
Candidate presumptive positive/total No. of samples analyzed	1/132	67/132	132/132
Candidate presumptive POD (CP)	0.01 (0.00, 0.04)	0.51 (0.42, 0.60)	1.00 (0.97, 1.00)
s_r[b]	0.09 (0.08, 0.16)	0.51 (0.45, 0.52)	0.00 (0.00, 0.16)
s_L[c]	0.00 (0.00, 0.04)	0.00 (0.00, 0.17)	0.00 (0.00, 0.16)
s_R[d]	0.09 (0.08, 0.10)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P-value[e]	0.4338	0.8931	1.0000
Candidate confirmed positive/total No. of samples analyzed	0/132	64/132	132/132
Candidate confirmed POD (CC)	0.00 (0.00, 0.03)	0.48 (0.40, 0.57)	1.00 (0.97, 1.00)
s_r	0.00 (0.00, 0.16)	0.51 (0.45, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.00 (0.00, 0.15)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.23)	0.51 (0.46, 0.52)	0.00 (0.00, 0.23)
P-value	1.0000	0.8762	1.0000
Positive reference samples/total No. of samples analyzed	0/132	73/132	132/132
Reference POD	0.00 (0.00, 0.03)	0.55 (0.47, 0.64)	1.00 (0.97, 1.00)
s_r	0.00 (0.00, 0.16)	0.50 (0.45, 0.52)	0.00 (0.00, 0.16)
s_L	0.00 (0.00, 0.16)	0.00 (0.00, 0.18)	0.00 (0.00, 0.16)
s_R	0.00 (0.00, 0.23)	0.50 (0.45, 0.52)	0.00 (0.00, 0.23)
P-value	1.0000	0.6678	1.0000
dLPOD (candidate vs reference)[f]	0.00 (–0.03, 0.03)	–0.07 (–0.19, 0.06)	0.00 (–0.03, 0.03)
dLPOD (candidate presumptive vs candidate confirmed)[f]	0.01 (–0.02, 0.04)	0.01 (–0.12, 0.13)	0.00 (–0.03, 0.03)

[a] Results include 95% confidence intervals.

[b] s_r = Repeatability SD.

[c] s_L = Among-laboratory SD.

[d] s_R = Reproducibility SD.

[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.

B. Apparatus and Reagents

Items (**a**) and (**h**)–(**o**) are available from 3M Food Safety (St. Paul, MN). Items (**b**)–(**g**) are available as the 3M MDA *Listeria* kit from 3M Food Safety.

(**a**) *3M Molecular Detection Instrument.*

(**b**) *3M MDA Listeria 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 (RC).*—Two pouches. Each pouch contains eight reagent tubes.

(**g**) *Quick Start Guide.*

(**h**) *3M Molecular Detection Speed Loader Tray.*

(**i**) *3M Molecular Detection Chill Block Tray and Chill Block Insert.*

(**j**) *3M Molecular Detection Heat Block Insert.*

(**k**) *3M Molecular Detection Cap/Decap Tool-Reagent (for reagent tubes).*

(**l**) *3M Molecular Detection Cap/Decap Tool-Lysis (for lysis tubes).*

(**m**) *Empty lysis tube rack.*

(**n**) *Empty reagent tube rack.*

(**o**) *DF broth base.*—Formulation equivalent to ISO 11290-1:1996.

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

(**q**) *Multichannel (8-channel) pipet.*—Capable of 20 µL.

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

(**s**) *Filter Stomacher® bags.*—Seward Ltd (West Sussex, UK) or equivalent.

(**t**) *Stomacher.*—Seward or equivalent.

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

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

(**w**) *Incubators.*—Capable of maintaining 37 ± 1°C.

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

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

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

C. General Instructions

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

(**b**) 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.

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

D. Safety Precautions

Periodically decontaminate laboratory benches and equipment (pipets, cap/decap tools, etc.) with a 1–5% (v/v in water) household bleach solution or DNA removal solution.

L. monocytogenes is of particular concern for pregnant women, the aged, and the infirmed. It is recommended that these groups of concern avoid handling this organism. After use, the enrichment medium and the 3M MDA *Listeria* 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 (MSDS) for additional information and local regulations for disposal.

Ethanol used in the method is flammable and caution should be used. Consult MSDS for additional information.

E. Sample Enrichment

(**a**) Prewarm DF broth base without FAC to 37 ± 1°C.

(**b**) Aseptically combine the enrichment medium and sample following the procedures in Table **2014.06B**. For all meat and highly particulate samples, the use of filter bags is recommended. Homogenize thoroughly (Stomacher, blender) for 2 ± 0.5 min. Incubate at 37 ± 1°C.

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

(**a**) Wet a cloth or paper towel with a 1–5% (v/v in water) household bleach solution and wipe the 3M Molecular Detection Speed Loader Tray.

(**b**) Rinse the 3M Molecular Detection Speed Loader Tray with water.

(**c**) Use a disposable towel to wipe the 3M Molecular Detection Speed Loader Tray dry.

(**d**) Ensure that 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 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.

Table 2014.06B. Enrichment protocols using Demi-Fraser broth base without FAC

		Primary enrichment, Demi-Fraser broth (no FAC)		
Sample matrix	Sample size	Enrichment broth volume, mL	Enrichment temperature (±1°C)	Enrichment time, h
Food				
Full-fat cottage cheese	25 g	225	37	24–28
Beef hot dogs	25 g	225	37	24–28
Deli turkey	25 g	225	37	24–28
Cold smoked salmon	25 g	225	37	24–28
Environmental surfaces				
Stainless steel	1 Swab	10	37	26–30
Sealed concrete	1 Sponge	100	37	26–30
Stainless steel, sealed concrete	1 Sponge	225	37	26–30

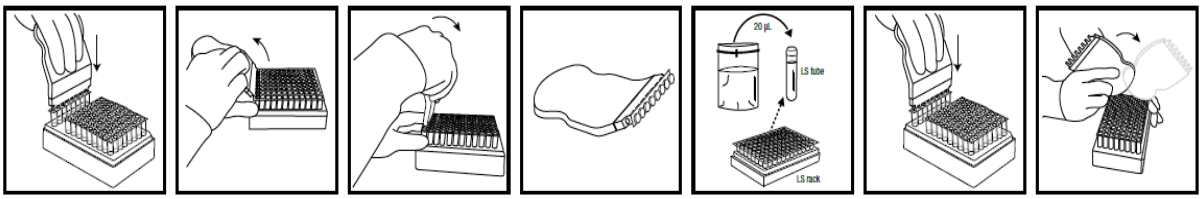

Figure 2014.06A. Transfer of enriched sample to lysis solution tube.

I. Preparation of the 3M Molecular Detection Instrument

(**a**) Launch the 3M Molecular Detection Software and log in.

(**b**) Turn on the 3M Molecular Detection Instrument.

(**c**) 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

(**a**) Allow the LS tubes to warm up to room temperature (20–25°C) by setting the rack on the laboratory bench for 2 h. 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**) Remove the enrichment broth from the incubator and gently agitate the contents.

(**c**) One LS tube is required for each sample and the NC sample.

(*1*) 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.

(*2*) To avoid cross-contamination, decap one LS tube strip at a time and use a new pipet tip for each transfer step.

(**d**) Transfer enriched sample to LS tubes as described below:

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

(*1*) 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.

(*2*) Transfer 20 µL of sample into an LS tube.

(*3*) Repeat step (**d**)(*2*) until each individual sample has been added to a corresponding LS tube in the strip.

(*4*) Use the 3M Molecular Detection Cap/Decap Tool-Lysis to recap 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. *See* Figure **2014.06A**.

(*5*) Repeat steps (**d**)(*1*)–(**d**)(*4*) as needed, for the number of samples to be tested.

(*6*) When all samples have been transferred, then transfer 20 µL NC into an LS tube. Use the 3M Molecular Detection Cap/Decap Tool-Lysis tool to recap the LS tube.

(*7*) Cover the rack of LS tubes with the rack lid and firmly invert three to five times to mix. Suspension has to flow freely inside the tube.

(**e**) Verify that the temperature of the 3M Molecular Detection Heat Block Insert is at 100 ± 1°C. Place the rack of LS tubes in the 3M Molecular Detection Heat Block Insert and heat for 15 ± 1 min. 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. 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.

(**f**) 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. The LS solution may freeze when processing fewer than 48 LS tubes. Freezing of the LS solution will not affect the test. If freezing is observed, allow the LS tubes to thaw for 5 min before mixing. Remove the rack lid during incubation on the 3M Molecular Detection Chill Block Insert.

(**g**) 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 three to five times to mix. Suspension has to flow freely inside the tube.

(**h**) Firmly tap the lysis tubes rack on the laboratory bench three to five times.

(**i**) Place the rack on the laboratory bench and let sit undisturbed for 5–10 min to allow the resin to settle. Do not mix or disturb the resin at the bottom of the tube. *See* Figure **2014.06B**.

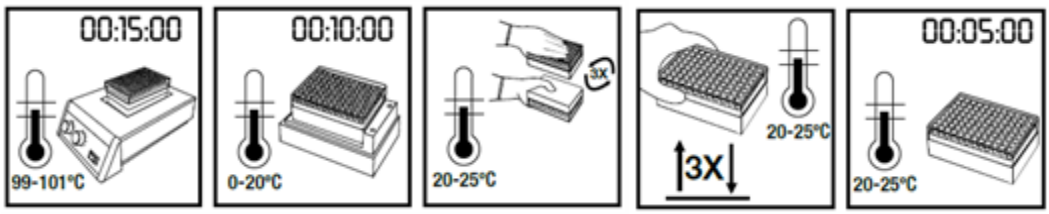

Figure 2014.06B. Sample lysis.

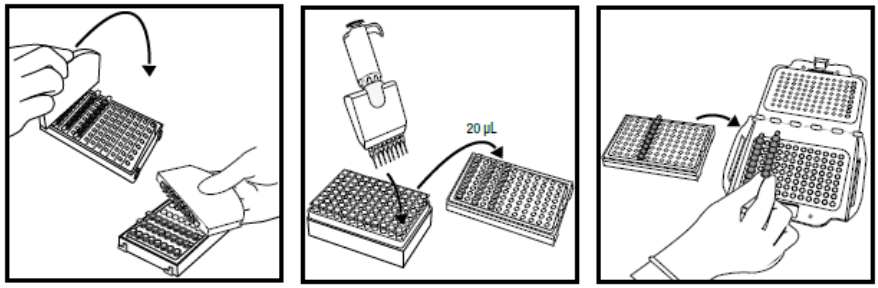

Figure 2014.06C. Transfer of lysate to reagent tube.

K. Amplification

(**a**) One reagent tube is required for each sample and the NC.

(*1*) Reagent tube strips can be cut to desired tube number. Select the number of individual reagent tubes or eight-tube strips needed.

(*2*) Place reagent tubes in an empty rack.

(*3*) Avoid disturbing the reagent pellets from the bottom of the tubes.

(**b**) Select one RC tube and place in rack.

(**c**) To avoid cross-contamination, decap one reagent tube strip at a time and use a new pipet tip for each transfer step.

(**d**) Transfer lysate to reagent tubes and RC tube as described below:

Note: 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 carryover of the resin may interfere with amplification.

(*1*) Use the 3M Molecular Detection Cap/Decap Tool-Reagent to decap the reagent tubes, one reagent tube strip at a time. Discard cap.

(*2*) 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 five times.

(*3*) Repeat step (**d**)(*2*) until individual sample lysate has been added to a corresponding reagent tube in the strip.

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

(*5*) Repeat steps (**d**)(*1*)–(**d**)(*4*) as needed, for the number of samples to be tested.

(*6*) When all sample lysates have been transferred, repeat steps (**d**)(*1*)–(**d**)(*4*) to transfer 20 µL NC lysate into a reagent tube.

(*7*) Transfer 20 µL NC lysate into an RC tube. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down five times.

(**e**) 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 **2014.06C**).

(**f**) Review and confirm the configured run in the 3M Molecular Detection Software.

(**g**) Click the Start button in the software and select instrument for use. The selected instrument's lid automatically opens.

(**h**) 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.

(**i**) 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 Matrix Control 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 one's preferred method or as specified by the U.S. Food and Drug Administration *Bacteriological Analytical Manual*, U.S. Department of Agriculture, Food Safety and Inspection Service *Microbiology Laboratory Guidebook*, AOAC *Official Method* **993.12**, or ISO 11290 methods starting from the 3M primary enrichment, followed by transfer to a secondary enrichment or direct plating onto media through confirmation of isolates using appropriate biochemical and serological methods.

Note: Even a negative sample will not give a zero reading as the system and 3M Molecular Assay *Listeria* 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 the user to repeat the assay for any Inspect samples. If the result continues to be Inspect, proceed to confirmation test using one's preferred method or as specified by local regulations.

Results of Collaborative Study

For this collaborative study, the 3M MDA *Listeria* method was compared to the AOAC **993.12** reference method for full-fat cottage cheese. A total of 15 laboratories throughout the United States and Canada participated in this study, with 13 laboratories submitting data for the full fat cottage cheese.

Table 2. Individual collaborator results for full-fat cottage cheese (4% milk fat)[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 *Listeria*																				
1	+	+	+	+	+	+	+	+	+	+	+	+	–[c]	+	+	–	–	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
2	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
3	+	+	+	+	+	+	+	+	+	+	+	+	–[c]	–	–	–	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
4[b]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5[b]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6[b]	+	+	+	+	+	+	+	+	+	+	+	+	+[d]	+[d]	+[d]	+[d]	+	+	+	+	+	+[d]	+	+	+[d]	+[d]	+[d]	+[d]	+	+[d]	+[d]	+[d]	+[d]	+[d]	+[d]	+[d]
7	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	–	+	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–[c]
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13[b]	+[d]	+[d]	+[d]	+[d]	+[d]	+[d]	+[d]	+	+	+[d]	+[d]	+[d]	+[d]	–	+[d]	+	+	+	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
15	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
																	AOAC OMA **993.12**																			
1	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	+	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
3	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
4[b]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5[b]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6[b]	+	+	+	+	+	–	+	–	–	+	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
8	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
9	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
10	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
11	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–	–	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
12	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
13[b]	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
15	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	–	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–

[a] + = *Listeria* species were detected in samples; – = *Listeria* species were not detected in sample; NA = laboratory did not participate in this matrix or results were not received.

[b] Results were not used in statistical analysis due to laboratory error.

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

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

Each laboratory analyzed 36 test portions for each method: 12 inoculated with a high level of *Listeria*, 12 inoculated with a low level of *Listeria*, and 12 uninoculated controls. The 3M MDA *Listeria* method produced 199 presumptive positive results with 196 confirming positive by traditional confirmation. There were 205 confirmed positives by the reference method.

A background screen of the matrix indicated an absence of indigenous *Listeria* species. For each matrix, the level of *Listeria* 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 Table 2. Table A summarizes the interlaboratory results for all foods tested, including POD statistical analysis (11). As per criteria outlined in Appendix J of the AOAC validation guidelines, fractional positive results were obtained. Detailed results for each laboratory are presented in Table A and Figures 1A and 1B of the Supplementary Materials. The results for each collaborating laboratory's 3M Petrifilm Aerobic Count Plate (AOAC **990.12**) for full-fat cottage cheese are presented in Table B of the Supplementary Materials.

Full-Fat Cottage Cheese (25 g Test Portions)

Full-fat cottage cheese test portions were inoculated at a low and high level and were analyzed for the detection of *Listeria* spp. (Table 2). Uninoculated controls were included in each analysis. Laboratories 4 and 5 did not submit results to the coordinating laboratory. Laboratories 6 and 13 reported deviations in the protocol: Laboratory 6 incorrectly incubated their MDA test portions at 30°C for 48 h instead of the required 37°C for 24 h; Laboratory 13 confirmed all colony growth regardless of supplementary tests (Gram stain, catalase reaction) indicating that the organism would not be classified as *Listeria* (Gram-negative or Gram-positive with spores, catalase negative), and results from these laboratories were excluded from the statistical analysis. The MPN levels obtained for the inoculated samples, with 95% confidence intervals, were 0.80 CFU/test portion (0.63,1.00) for the low level and 4.83 CFU/test portion (3.30, 7.70) for the high level.

For the high level, 132 out of 132 test portions ($LPOD_{CP}$ of 1.00) were reported as presumptive positive by the 3M MDA *Listeria* method with all 132 test portions ($LPOD_{CC}$ of 1.00) confirming positive. Based on the valid data submitted from each of the collaborating laboratories, 0 false-negative results or false-positive results were obtained resulting in 132 confirmed positives ($LPOD_C$ of 1.00). For the low level, 67 out of 132 test portions ($LPOD_{CP}$ of 0.51) were reported as presumptive positive by the 3M MDA *Listeria* method with 64 test portions ($LPOC_{CC}$ of 0.48) confirming positive. Based on the valid data submitted from each of the collaborating laboratories, three false-positive results were obtained resulting in 64 confirmed positives ($LPOD_C$ of 0.48). For the uninoculated controls, one out of 132 samples ($LPOD_{CP}$ of 0.01) produced a presumptive positive result by the 3M MDA *Listeria* method with all 132 test portions ($LPOD_{CC}$ of 0.00) confirming negative. Based on the valid data submitted from each of the collaborating laboratories, 0 false-negative results and 1 false-positive results were obtained resulting in 0 confirmed positives ($LPOD_C$ of 0.00). For test portions analyzed by the AOAC **993.12** Method, 132 out of 132 high inoculum test portions and 73 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 –0.07 with a 95% confidence interval of (–0.19, 0.06) was obtained between the 3M MDA *Listeria* method and the AOAC **993.12** method. The confidence interval obtained for $dLPOD_C$ indicated no significant difference between the two methods. A $dLPOD_{CP}$ value of 0.01 with a 95% confidence interval of (–0.12, 0.13) was obtained between presumptive and confirmed 3M MDA *Listeria* 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 0.00 with a 95% confidence interval of (–0.03, 0.03) was obtained between the 3M MDA *Listeria* method and the AOAC **993.12** method. The confidence interval obtained for $dLPOD_C$ indicated no significant difference between the two methods. A $dLPOD_{CP}$ value of 0.00 with a 95% confidence interval of (–0.03, 0.03) was obtained between presumptive and confirmed 3M MDA *Listeria* results. The confidence interval 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 A and Figures 1A and B of the Appendix.

Discussion

No negative feedback was provided by the collaborating laboratories in regard to the performance of the 3M MDA *Listeria*. Several laboratories reported difficulty in isolating and identifying *Listeria* colonies on OXA from samples enriched in the DF broth base (without FAC) when compared to samples enriched in the AOAC **991.12** selective enrichment broth. This may be related to differences in formulation between the two enrichments. The AOAC **993.12** enrichment broth is designed to reduce the background flora on OXA and is more selective than DF broth base (without FAC). In some instances, this level of selectivity may cause stress on *Listeria* cells, thus requiring a longer enrichment time to reach a detectable level.

Based on the data submitted, two laboratories, Laboratories 6 and 13, were removed from statistical consideration for the full-fat cottage cheese. During analysis, Laboratory 6 did not follow the approved incubation time and temperature for the candidate method (samples were incubated for 48 h at 30°C and the validated enrichment time and temperature are 24–28 h at 37°C), and Laboratory 13 confirmed growth from all plates, regardless of supplementary tests that would have precluded confirmation via API *Listeria* test kits (bioMérieux). Due to this fact, all samples confirmed via API *Listeria* produced a *Listeria* species result even if Gram stain reaction (Gram-negative), motility reaction (negative), catalase reaction (negative), and oxidase reaction (positive) would indicate the organism is not of the genus *Listeria*.

During the analysis of the full-fat cottage cheese, four false positive results were obtained out of 396 test portions analyzed with the candidate method. The 3M MDA *Listeria* correctly identified whether a test portion was positive or negative more than 99% of the time (false-positive rate of 1%). For full-fat cottage cheese, the collaborative study indicated no statistically significant difference between the candidate method and the reference method or the presumptive, and confirmed results of

the candidate method were obtained when the POD statistical model was used.

Recommendations

It is recommended that the 3M Molecular Detection Assay *Listeria* method be adopted as Official First Action status for the detection of *Listeria* species in selected foods, including beef hot dogs (25 g), deli turkey (25 g), cold smoked salmon (25 g), full-fat cottage cheese (25 g), and two environmental surfaces: sealed concrete (sponge in 225 mL enrichment volume) and stainless steel (and sponge in 225 mL enrichment volume) enriched in DF broth base (without FAC).

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