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Research Note

Validation of the 3M Molecular Detection System for the Detection of *Listeria* in Meat, Seafood, Dairy, and Retail Environments

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ABSTRACT

There is a continued need to develop improved rapid methods for detection of foodborne pathogens. The aim of this project was to evaluate the 3M Molecular Detection System (3M MDS), which uses isothermal DNA amplification, and the 3M Molecular Detection Assay *Listeria* using environmental samples obtained from retail delicatessens and meat, seafood, and dairy processing plants. Environmental sponge samples were tested for *Listeria* with the 3M MDS after 22 and 48 h of enrichment in 3M Modified *Listeria* Recovery Broth (3M mLRB); enrichments were also used for cultural detection of *Listeria* spp. Among 391 samples tested for *Listeria,* 74 were positive by both the 3M MDS and the cultural method, 310 were negative by both methods, 2 were positive by the 3M MDS and negative by the cultural method, and one sample was negative by the 3M MDS and positive by the cultural method. Four samples were removed from the sample set, prior to statistical analyses, due to potential cross-contamination during testing. *Listeria* isolates from positive samples represented *L. monocytogenes, L. innocua, L. welshimeri,* and *L. seeligeri*. Overall, the 3M MDS and culture-based detection after enrichment in 3M mLRB did not differ significantly ($P > 0.05$) with regard to the number of positive samples, when chi-square analyses were performed for (i) number of positive samples after 22 h, (ii) number of positive samples after 48 h, and (iii) number of positive samples after 22 and/or 48 h of enrichment in 3M mLRB. Among 288 sampling sites that were tested with duplicate sponges, 67 each tested positive with the 3M MDS and the traditional U.S. Food and Drug Administration *Bacteriological Analytical Manual* method, further supporting that the 3M MDS performs equivalently to traditional methods when used with environmental sponge samples.

Technologies using nucleic acid amplification have revolutionized the detection of foodborne pathogens from food or environmental samples by allowing for more rapid, as well as often more specific, detection than traditional cultural methods. While many amplification-based methods use PCR, a variety of isothermal amplification methods have been reported in the peer-reviewed literature *(6, 7, 11, 14)* and have been offered commercially for detection of pathogens in clinical settings. Only recently have systems using isothermal amplification methods for detection of foodborne pathogens started to become commercially available. In particular, the 3M Molecular Detection System (3M MDS), which has recently been released, uses loop-mediated isothermal amplification (LAMP), an isothermal amplification method, for detection of foodborne pathogens. LAMP has previously been shown to allow for sensitive detection of *Salmonella enterica* in liquid eggs *(12)* and to be less susceptible to inhibition by culture media and certain biological substances (e.g., plasma, urine) as compared with PCR *(9)*.

The goal of this project was to evaluate a preproduction version of the 3M MDS for its ability to detect *Listeria* spp. in environmental sponge samples, using the 3M Molecular Detection Assay (3M MDA) *Listeria*. Rapid and reliable detection of *Listeria* spp. from environmental samples collected in ready-to-eat food processing facilities is often used to facilitate control of the foodborne pathogen *Listeria monocytogenes,* and this assay was specifically designed to allow for detection of *Listeria* spp. In addition, detection of *Listeria* spp. in environmental samples collected in retail delicatessen establishments may provide one avenue to help control transmission of *L. monocytogenes* in the delicatessen meat retail environment, which is increasingly recognized as a concern, particularly since a recent risk assessment suggested that a considerable proportion of human listeriosis cases linked to consumption of ready-to-eat meat and poultry products is linked to contamination that occurs after products leave the processing environment *(15)*.

MATERIALS AND METHODS

Environmental samples. A total of 391 samples were collected from retail ($n = 120$), seafood processing ($n = 72$), meat processing ($n = 100$), and dairy processing ($n = 99$) environments using 3M sponge-sticks with Dey/Engley neutralizing buffer. Samples were collected in conjunction with ongoing environmental sampling projects. For 288 of these sites, two sponge samples were taken, one for enrichment with the 3M Modified *Listeria* Recovery Broth (3M mLRB) and one for enrichment with standard U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA BAM) procedures, as

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detailed below. Samples (areas ranged from 10 by 10 cm to 60 by 60 cm) were shipped overnight on ice and processed upon arrival.

Enrichment with 3M mLRB and cultural *Listeria* confirmation. Sample sponges to be tested with the 3M MDA *Listeria* were enriched in 225 ml of 3M mLRB with 3M mLRB supplement, directly in the 3M sample collection bag. Sample enrichments were homogenized thoroughly and incubated at 37°C for up to 48 h. At 22 and 48 h, aliquots were removed and tested with the 3M MDS as detailed below.

In addition, at these same two time points (i.e., after 22 and 48 h of enrichment), the same enrichments were used for isolation of *Listeria* spp. as a confirmation of 3M MDS results; procedures used for isolation were similar to the plating procedures detailed in FDA BAM. Briefly, 50 μl of 3M mLRB enrichment broth was streaked onto modified Oxford agar (MOX; Difco, BD, Franklin Lakes, NJ). MOX agar plates were incubated at 30°C for 48 h. Colonies that displayed typical *Listeria* morphology and appearance on MOX agar plates (gray-green colonies with black halo) were substreaked to *Listeria monocytogenes* plating medium (LMPM; R & F Products, Inc., Downers Grove, IL). LMPM plates were incubated at 35°C for 48 h. Colonies on LMPM that were either blue (presumptive *L. monocytogenes* or *Listeria ivanovii*) or white (presumptive *Listeria* species, excluding *L. monocytogenes* and *L. ivanovii*) were plated onto brain heart infusion agar plates (BHI; Difco, BD). BHI agar plates were incubated for 37°C for 24 h. Presumptive *Listeria* colonies were confirmed by PCR and partial *sigB* gene sequencing, as detailed below. All *Listeria* isolates were preserved at −80°C in BHI with 15% glycerol.

For each batch of samples tested, positive and negative controls were used. *L. monocytogenes* 10403S (FSL X1-001) was used as the positive control. This control strain was freshly streaked from frozen stock (stored at −80°C) for each sample batch, and one colony was inoculated into a sample bag with sterile enrichment media. Uninoculated enrichment media processed in parallel with sample bags were used as negative controls.

3M MDA *Listeria*. After 22 and 48 h, 3M mLRB enrichments were gently agitated and 20 μl of each enrichment was aliquoted into a separate 3M lysis tube. Lysis tubes were incubated at 100 ± 1°C for 15 ± 1 min. Immediately following heating, the lysis tubes were allowed to cool in a prechilled 3M Molecular Detection Chill Block for 10 ± 1 min. After completion of these incubation steps, the lysis tubes were mixed by inversion and then left undisturbed for 5 min to allow resin to settle. Without disturbing the resin, 20 μl of sample lysate was removed and aliquoted into a reagent tube and its corresponding 3M Molecular Detection Matrix Control tube and was mixed by pipetting. When all samples had been transferred and mixed, capped tubes were loaded into the 3M Molecular Detection Speed Loader Tray and placed into the 3M Molecular Detection Instrument. For this study, a corresponding matrix control test was performed for each sample. In addition, for each set of samples processed at the same time, one *Listeria* positive process control (inoculated broth), one negative process control (uninoculated broth), one assay kit negative control, and one assay kit reagent control were run. Presumptive-positive results were reported in real time, while negative results were displayed at the end of the 75-min run.

Confirmation of *Listeria* isolates by PCR and *Listeria* species identification. One putative *Listeria* colony per sample was confirmed as *Listeria* spp. using a PCR assay that amplifies a fragment of the *sigB* gene; sequencing of this PCR product allows classification of isolates into *Listeria* spp. as previously shown *(1, 3, 16)*. Briefly, PCR products were purified using ExoSAP-IT for PCR Product Clean-up (Affymetrix, Cleveland, OH) and Sanger sequencing of PCR products was performed at the Cornell University Life Sciences Core Laboratories Center (Ithaca, NY). Sequences were assembled and edited with Sequencher version 4.8 (Gene Codes Corporation, Ann Arbor, MI). Sequence analyses included (i) comparison of *sigB* sequence data for each isolate to a laboratory internal reference database of *Listeria sigB* allelic types (available upon request from M. Wiedmann), using a 660-bp fragment of *sigB* (as described by Sauders et al. 2012 *(16)*) and (ii) construction of a *sigB*-based phylogeny, using RAxML version 7.3.0 *(17),* to further evaluate the *sigB* allelic type diversity among the isolates obtained.

Detection of *Listeria* in a second sponge using standard microbiological methods. In addition to the sponge sample used for enrichment with the 3M mLRB, followed by analysis with the 3M MDS and cultural detection, duplicate sponges from 288 sampling sites were also tested according to the FDA BAM standard method *(8)* with minor modifications. Briefly, a total of 90 ml of buffered *Listeria* enrichment broth (Difco, BD) was added to each sponge, followed by homogenization for 60 s at 2 Hz in a Seward Stomacher 400 circulator (Seward, Bohemia, NY). After initial incubation at 30°C for 4 h, *Listeria* selective enrichment supplement (Oxoid, Basingstoke, Hampshire, UK) was added (4 μl per 1 ml of buffered *Listeria* enrichment broth). After overall incubation, at 30°C, for 24 and 48 h, 100 μl of enrichment media was streaked onto each LMPM and MOX plate. LMPM plates were incubated at 35°C, and MOX plates were incubated at 30°C (to facilitate *Listeria* spp. detection; see Curtis et al. *(2)*) for 48 h and examined for the presence of *Listeria*.

Statistical analysis. A chi-square test (see AOAC, *Official Methods of Analysis Program Manual,* section 5.3.1, test for significant difference *(4)*) was used to determine whether the proportion of positive samples was different between the 3M MDS and the traditional microbiological detection of *Listeria* spp. from the 3M mLRB (as detailed above).

RESULTS AND DISCUSSION

Enrichments of a variety of environmental sponge samples from different sources do not cause interference or inhibition of the 3M MDS. Among all 391 samples tested, none were shown to inhibit the 3M MDA *Listeria,* based on the results for the matrix control. For this study, the matrix control was run in parallel with the 3M MDA *Listeria,* as a method to establish the absence of interference from the sample matrix. As samples from a variety of different food-associated environments were tested, these data suggest that this assay is unlikely to experience inhibition. This is consistent with previous reports *(5, 9, 10)* that suggest that LAMP, the isothermal amplification technology used in the 3M MDS, is highly robust and less sensitive to inhibition as compared with many PCR-based amplification methods.

Of the 391 samples tested, four showed positive results on the 3M MDS but were negative by cultural testing of the 3M mLRB enrichment after both 22 and 48 h. For each of the four samples, retesting of the enrichment media with the 3M MDS yielded a negative result. For three of these four samples, a positive sample was located in an adjacent well, supporting cross-contamination. All four of these samples were considered cross-contamination events and were

TABLE 1. *Correlation between the 3M MDS and traditional culture results after enrichment in 3M mLRB*[a]

No. of samples	3M MDS result after enrichment for:		Culture results after enrichment in 3M mLRB for:	
	22 h	48 h	22 h	48 h
Samples with full agreement of MDS and culture results				
310	−	−	−	−
65	+	+	+	+
3	−	+	−	+
1	+	−	+	−
Samples that did not show full agreement of MDS and culture results				
2	−	+	+	+
1	−	−	+	+
3	+	+	−	+
2	+	−	−	−

[a] 3M MDS, 3M Molecular Detection System; 3M mLRB, 3M Modified *Listeria* Recovery Broth; +, positive result; −, negative result.

removed from further analysis. All subsequent analyses reported here represent the remaining 387 samples. The system tested in this study represented a preproduction version of the 3M MDS. A number of modifications were made to the process and materials to prevent the potential for cross-contamination.

Detection with the 3M MDS after 22 and 48 h of enrichment does not yield statistically different numbers of samples positive for *Listeria* spp. Traditional *Listeria* detection methods typically involve detection of *Listeria* spp. or *L. monocytogenes,* on appropriate selective and differential media, after both 24 and 48 h of enrichment. An initial comparison of the number of samples that were positive by the 3M MDS after 22 or 48 h of enrichment showed that a total of 68 samples were positive by the 3M MDS after both 22 and 48 h of enrichment, whereas (i) three samples were positive after 22 but not 48 h and (ii) five samples were positive after 48 but not 22 h (Table 1). By comparison, among the samples plated onto MOX after 3M mLRB enrichment, 68 samples were positive after both 22 and 48 h of enrichment, whereas (i) one sample was positive after 22 but not 48 h and (ii) six samples were positive after 48 but not 22 h (Table 1). Overall, these results are consistent with the notion that some samples naturally contaminated with *Listeria* spp. or *L. monocytogenes* may be positive after 22 h of enrichment, but not after 48 h of enrichment, possibly due to overgrowth or competitive inhibition of *Listeria* by other organisms after enrichment for 48 h. In addition, environmental samples with low levels of injured *Listeria* spp. may be negative if tested, by either culture-based or molecular methods, after enrichment of 22 to 24 h, and may require 48 h of enrichment to show positive results *(19)*. Some early studies of traditional culture-based detection methods *(18, 19)* have even shown that extension of enrichment beyond 2 days may further increase sensitivity of these detection methods.

TABLE 2. *Correlation between detection with the 3M MDS after enrichment in 3M mLRB and FDA BAM*

	3M MDS	FDA BAM procedure
No. of samples positive	67	67
No. of samples negative	221	221
Total no. of samples	288	288

3M MDS results after 22 and 48 h of sample enrichment are not significantly different from culture-based detection after 3M mLRB enrichment. Among the 387 samples included in the final analysis, a total of 71 and 73 samples were positive when tested by the 3M MDS after 22 and 48 h, respectively, for a total of 76 positive samples (i.e., samples positive after 22 and/or 48 h). By comparison, a total of 69 and 74 samples were positive when tested by culture-based detection methods after 22 and 48 h of enrichment in 3M mLRB, respectively, for a total of 75 positive samples (i.e., samples positive after 22 and/or 48 h). Specifically, (i) 74 samples were positive, after 22 and/or 48 h, by both the 3M MDS and the cultural method, (ii) 310 samples were negative by both methods, (iii) 2 samples were positive by the 3M MDS (at 22 h) and negative by the cultural method, and 1 sample was negative by the 3M MDS and positive by the cultural method (after both 24 and 48 h). For the two samples that were positive by the 3M MDS (at 22 h only) and negative by the cultural method, enrichments were retested using the 3M MDS and were replated on MOX for confirmation. For one of these samples (sample ID ''A-LM16''; Suppl. Table 1 available at http://foodscience.cornell.edu/cals/foodsci/research/labs/wiedmann/links/fortes-et-al-2013.cfm), a frozen aliquot of the 22-h enrichment was available and was retested with the 3M MDS (after preparation of a new lysate); this sample tested positive again upon retesting. For the second of these samples (sample ID ''2-27''; Suppl. Table 1), no frozen sample of the 22-h enrichment was available; retesting with the 3M MDS of the 48-h enrichment for this sample was again negative. These two samples likely represent situations in which *Listeria* spp. grew to levels detectable with the 3M MDS within 22 h, with *Listeria* levels subsequently declining, possibly due to overgrowth or competitive inhibition of *Listeria* by other organisms. Overall, the 3M MDS and culture-based detection after enrichment in 3M mLRB did not differ significantly ($P > 0.05$) with regard to the number of positive samples, when separate chi-square analyses were performed for (i) number of positive samples after 22 h, (ii) number of positive samples after 48 h, and (iii) number of positive samples after 22 and/or 48 h of enrichment in 3M mLRB.

3M MDS results are not different from culture-based detection using the FDA BAM protocol. For a total of 288 of the 387 samples included in the final data analyses, duplicate sponges taken from the same sites had also been tested, as part of other ongoing projects, using an FDA BAM protocol. Among these 288 samples, 67 and 221 samples were positive and negative, respectively, by 3M MDS after 22 and/or 48 h of enrichment in 3M mLRB.

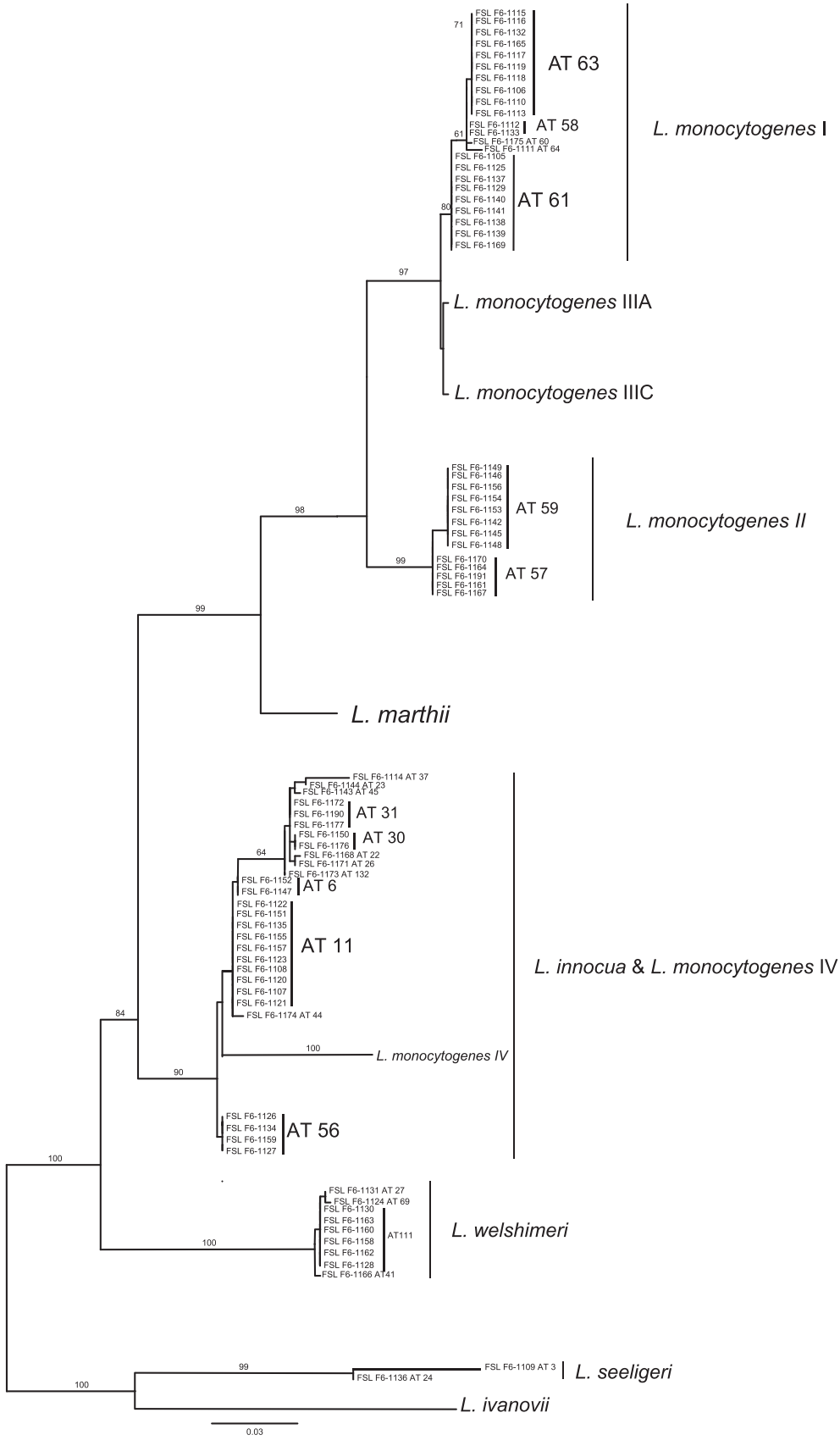

FIGURE 1. sigB *phylogeny for 75* Listeria *isolates representing one isolate from each of the 74 3M Molecular Detection System (3M MDS)–positive samples that were also positive by cultural methods (after enrichment in 3M Modified* Listeria *Recovery Broth [3M mLRB]), as well as one isolate from a sample that was positive by cultural methods (after enrichment in 3M mLRB) but not by the 3M MDS. The tree was inferred using maximum likelihood methods; values on the branches represent bootstrap values >60, based on 500 bootstrap replicates. Construction of the phylogeny used 152 previously identified* sigB *allelic types, as well as the sequence data for the 75 isolates characterized here. To reduce the size of the tree, most allelic types that were not found among the 75 isolates are not shown.*

Among the same 288 samples, 67 and 221 samples were positive and negative, respectively, by the traditional BAM method (Table 2). While there were a number of samples that were positive by the 3M MDS after enrichment in 3M mLRB and negative by FDA BAM, and vice versa, these data clearly indicate that the overall methods employing enrichment in 3M mLRB and in buffered *Listeria* enrichment broth (the enrichment specified by FDA BAM) yielded comparable results on environmental sponges.

3M MDS positive samples represented diversity of *Listeria* species. *sigB* sequencing and allelic typing of a single isolate for each of the 74 3M MDS positive samples, and for the 1 sample that was negative by 3M MDS but positive by cultural isolation after enrichment in 3M mLRB, yielded a diversity of *Listeria* species and allelic types. There were 28 *L. innocua* represented by 12 allelic types, 9 *L. welshimeri* represented by 4 allelic types, 2 *L. seeligeri*, each a different allelic type, and 36 *L. monocytogenes* isolates represented by 7 allelic types. The 36 *L. monocytogenes* isolates were further classified into lineage *(13)* based on *sigB* sequence data: 23 *L. monocytogenes* isolates, representing five allelic types, grouped into lineage I and 13 *L. monocytogenes* isolates, representing two allelic types, grouped into lineage II (Fig. 1). The *L. monocytogenes* isolate from the sample that was negative by 3M MDS but positive by cultural isolation from the 3M mLRB enrichment was identified as *L. monocytogenes* lineage II allelic type 57; another four samples that were positive by both 3M MDS and cultural confirmation after enrichment in 3M mLRB also yielded this allelic type. Testing of the *L. monocytogenes* lineage II allelic type 57 isolate from the sample that was negative by the 3M MDS, but positive by cultural isolation from the 3M mLRB enrichment, showed that this isolate yielded a positive result in the 3M MDA *Listeria*. Combined, these data indicate that the result with this sample likely represents the presence of low numbers of *L. monocytogenes* after enrichment (i.e., below the detection limit for the 3M MDA *Listeria*) and not a failure of the 3M MDA *Listeria* to detect this specific *Listeria* strain.

Overall, our data show that the 3M MDA *Listeria* performs equally as well as the gold standard method when used with sponge samples collected from naturally contaminated environmental sites. The system was able to detect a diversity of *Listeria* species and reported real-time positive results in as few as 25 min, following enrichment and a simple lysis protocol.

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