P2-124: 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 3M™ Molecular Detection System utilizes isothermal DNA amplification and bioluminescence to detect targeted pathogens, after an enrichment step. The project aim was to evaluate the 3M Molecular Detection System and 3M™ Molecular Detection Assay *Listeria* using environmental samples obtained from retail delis and meat, seafood, and dairy processing plants. 391 environmental samples were collected using 3M™ Sponge-Sticks with D/E Neutralizing Buffer and tested for *Listeria* with the 3M Molecular Detection System after 22- and 48-hour enrichment in 3M™ Modified *Listeria* Recovery Broth; 3M Modified *Listeria* Recovery Broth enrichments were also used for cultural detection of *Listeria* spp.

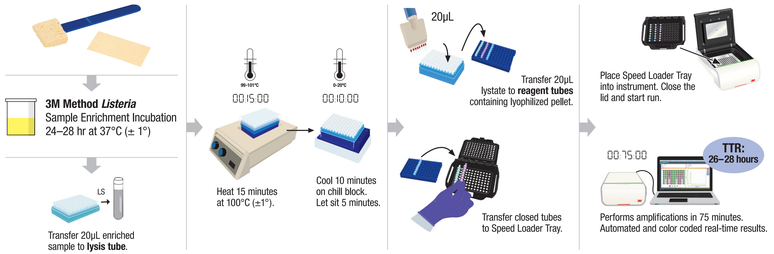
Overall, 3M Molecular Detection System and culture based detection after enrichment in 3M Modified *Listeria* Recovery Broth did not differ significantly ($P<0.05$) with regard to the number of positive samples, when separate chi-square analyses were performed for (i) number of positive samples after 22 hours, (ii) number of positive samples after 48 hours and (iii) number of positive samples after 22 hours and/or 48 hours of enrichment in 3M Modified *Listeria* Recovery Broth. Among 288 sampling sites that were tested with duplicated sponges, 67 tested positive with the 3M Molecular Detection System and 67 also tested positive with the traditional FDA BAM method, further supporting that the 3M Molecular Detection System performs equivalent to traditional methods when used for testing of environmental sponge samples.

INTRODUCTION

PCR-based methods have revolutionized the detection of foodborne pathogens from food or environmental samples by allowing for both more rapid as well as often more specific detection than traditional cultural methods. While other amplification based methods for detection of nucleic acids, including a variety of isothermal amplification methods, have been reported in the peer-reviewed literature[1-4] and have been offered commercially for detection of pathogens in clinical settings, systems using isothermal amplification methods for detection of foodborne pathogens have only recently started to become commercially available. In particular, the 3M Molecular Detection System, 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[5] and to be less susceptible to inhibition by culture media and certain biological substances as compared to PCR[6].

The goal of this project was to evaluate a pre-production version of the 3M Molecular Detection System for its ability to detect *Listeria* in environmental sponge samples, using the 3M Molecular Detection Assay *Listeria.* Detection of *Listeria* spp. in environmental samples collected in retail deli establishments may provide one avenue to help control transmission of *L. monocytogenes* in the deli 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 RTE meat and poultry products is linked to contamination that occurs after products leave the processing environment[7].

3M MOLECULAR DETECTION ASSAY *Listeria* PROTOCOL



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 D/E Neutralizing Buffer. Samples were collected in conjunction with ongoing environmental sampling projects.

3M Molecular Detection System analysis and cultural *Listeria* confirmation. Sample sponges to be tested with the 3M Molecular Detection Assay *Listeria* were enriched in 225mL 3M Modified *Listeria* Recovery Broth directly in the 3M sample collection bag. Sample enrichments were incubated at 30°C and tested with the 3M Molecular Detection System at 22 hours and 48 hours, following the manufacturer's protocol. The same enrichments were used for isolation of *Listeria* spp. as a confirmation of 3M Molecular Detection System results; procedures used for isolation were similar to the plating procedures detailed in FDA BAM.

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.

Detection of *Listeria* in a second sponge using standard microbiological methods. In addition to the sponge sample used for enrichment with the 3M Modified *Listeria* Recovery Broth, followed by analysis with the 3M Molecular Detection System and cultural detection, duplicate sponges from 288 sampling sites were also tested according to the FDA BAM standard method with minor modifications.

REFERENCES

1. Morris PT, Giglio S. (2006). Nucleic acid amplification-based techniques for pathogen detection and identification. Infect Genet Evol. 6(1):2–12.
2. Gill P, Ghaemi A. (2008). Nucleic acid isothermal amplification technologies: a review. Nucleos. Nucleot. Nuc. Acids, 27:224–43.
3. Parida M, Sannarangaiah S, Dash PK, Rao PV, Morita K. 2008. Loop mediated isothermal amplification (LAMP): a new generation of innovative gene amplification technique; perspectives in clinical diagnosis of infectious diseases. Rev Med Virol. 18:407–421.
4. Fu S, Qu G, Guo S, Ma L, Zhang N, Zhang S, Gao S, Shen Z. 2011. Applications of loop-mediated isothermal DNA amplification. Appl Biochem Biotechnol. 163:845–50
5. Ohtsuka K, Yanagawa K, Takatori K, Y. Hara-Kudo. 2005. Detection of *Salmonella enterica* in naturally contaminated liquid eggs by loop-mediated isothermal amplification, and characterization of *Salmonella* isolates. Appl. Environ. Microbiol. 71:6730–6735.
6. Kaneko H, Kawana T, Fukushima E, Suzutani T. 2007. Tolerance of loop-mediated isothermal amplification to a culture medium and biological substances. J Biochem Biophys Methods. 70:499–501.
7. Pradhan, A. K., R. Ivanek, Y. T. Gröhn, R. Bukowski, I. Geornaras, J. Sofos, M. Wiedmann. 2010. Quantitative Risk Assessment of Listeriosis-associated Deaths due to *Listeria monocytogenes* contamination of deli meats originating from manufacture and retail. J. Food Prot. 73:620–630.
8. Karanis P, Thekisoe O, Kiouptsi K, Ongerth J, Igarashi I, Inoue N. 2007. Development and preliminary evaluation of a loop-mediated isothermal amplification procedure for sensitive detection of cryptosporidium oocysts in fecal and water samples. Appl Environ Microbiol. 73:5660–5662.
9. Francois P, Tangomo M, Hibbs J, Bonetti EJ, Boehme CC, Notomi T, Perkins MD, Schrenzel J. 2011. Robustness of a loop-mediated isothermal amplification reaction for diagnostic applications. FEMS Immunol. Med. Microbiol. 62:41–48.

RESULTS AND DISCUSSION

A total of 391 environmental samples from retail, seafood processing, meat processing and dairy processing were tested for *Listeria* with the 3M Molecular Detection Assay *Listeria* after 22- and 48-hour enrichment in 3M Modified *Listeria* Recovery Broth. In addition, the 3M Modified *Listeria* Recovery Broth enrichment for each sample was also tested for *Listeria* spp. using standard plating procedures similar to the FDA BAM method.

Enrichments of a variety of environmental sponge samples from different sources do not cause interference or inhibition of the 3M Molecular Detection System. Among all 391 samples tested, none were shown to inhibit the 3M Molecular Detection Assay *Listeria* results, based on the results for the Matrix Control (data not shown).

Detection with the 3M Molecular Detection System after 22- and 48-hour enrichment does not yield statistically different numbers of samples positive for *Listeria* spp. The number of samples positive at 22 and 48 hours (Table 1) was not significantly different for either the 3M Molecular Detection System or cultural detection from 3M Modified *Listeria* Recovery Broth (chi-square $P<0.05$).

3M Molecular Detection System results after 22 and 48 hours of sample enrichment are not significantly different from culture-based detection after 3M Modified *Listeria* Recovery Broth enrichment. Overall, 3M Molecular Detection System and culture based detection after enrichment in 3M Modified *Listeria* Recovery Broth did not differ significantly ($P<0.05$) with regard to the number of positive samples (Table 1).

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 using an FDA BAM protocol (Table 2).

While there were a number of samples that were positive by the 3M Molecular Detection System after enrichment in 3M Modified *Listeria* Recovery Broth and negative by FDA BAM and vice versa, these data clearly indicate that the overall methods employing enrichment in 3M Modified *Listeria* Recovery Broth and in BLEB yielded comparable results on environmental sponges.

3M Molecular Detection System positive samples represented diversity of *Listeria* species. *sigB* sequencing and allelic typing of a single isolate for each of the 74 3M Molecular Detection System positive samples yielded a diversity of Listeria species and allelic types (Fig. 1).

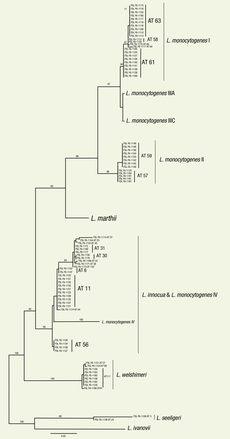
Table 1. Correlation between the 3M Molecular Detection System and traditional culture results after enrichment in 3M Modified *Listeria* Recovery Broth.

No. of Samples	3M MDS Results after Enrichment		Culture Results after 3M mLRB Enrichment	
	22 hours	48 hours	22 hours	48 hours
Concordant Results				
310	–	–	–	–
65	+	+	+	+
3	–	+	–	+
1	+	–	+	–
Discrepant Results				
2	–	+	+	+
1	–	–	+	+
3	+	+	–	+
2	+	–	–	–

Table 2. Correlation between the 3M Molecular Detection System after enrichment in 3M Modified *Listeria* Recovery Broth and FDA-BAM methodology.

No. of Samples	3M Molecular Detection System	FDA-BAM
Positive	67	67
Negative	221	221
Total	**288**	**288**

Figure 1. *sigB* phylogeny for 75 *Listeria* isolates representing one isolate from each of the 74 3M Molecular Detection System positive samples that were also positive by cultural methods (after enrichment in 3M Modified *Listeria* Recovery Broth) as well as one isolate from a sample that was positive by cultural methods (after enrichment in 3M Modified *Listeria* Recovery Broth), but not by the 3M Molecular Detection System.



CONCLUSIONS

Overall, our data show that the 3M Molecular Detection Assay *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 early as 25 minutes, following enrichment and a simple lysis protocol. 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[6,8,9] that suggest that LAMP, the isothermal amplification technology used in the 3M Molecular Detection System, is highly robust and less sensitive to inhibition as compared to many PCR-based amplification methods.

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