

## 3M™ Molecular Detection Assay 2 – Listeria

The 3M™ Molecular Detection Assay 2 - Listeria was developed for the rapid and specific detection of Listeria in food matrices and environmental samples after enrichment in Demi-Fraser (DF) Broth. The assay uses loop-mediated isothermal amplification to rapidly amplify unique DNA nucleic acid sequences with high specificity and sensitivity, combined with bioluminescence to detect the amplification.

Performance of the 3M method was evaluated for inclusivity, exclusivity and performance compared to ISO, USDA, FDA or AOAC reference methods with a wide range of food matrices and environmental samples. The limit of detection of the method and of the assay were also determined.

### Inclusivity and Exclusivity Testing

**Inclusivity:** The ability of a method to detect the target analyte from a wide range of strains<sup>1,2</sup>

One hundred fifty-three (153) different Listeria isolates were tested. The strains were cultured in DF broth overnight at 37 ± 1°C and tested according to ISO 16140<sup>1,2</sup> or AOAC<sup>3</sup> guidelines.

**Exclusivity:** The lack of interference from a relevant range of non-target strains<sup>1,2</sup>

One hundred (100) different non-Listeria strains including closely related organisms were tested. The strains were cultured in Tryptic Soy Broth and then diluted to levels of ~ 10<sup>7</sup> CFU/mL prior to testing with the 3M method.

### Results

Table 1. Inclusivity and Exclusivity results

Results	Analysis
153/153 Listeria strains were detected (results were “positive”)	100% Inclusivity
100/100 non-Listeria strains were not detected (results were “negative”)	100% Exclusivity

See Appendix, Tables 4a and 4b, for list of cultures tested

### Internal Methods Comparison Study

Studies were conducted to assess the performance of the method compared to ISO 11290-1 reference method for the detection of Listeria. Foods commonly reported in outbreaks and/or reported as being challenging due to their composition, e.g., high bioburden, added preservatives, etc. were included. Three hundred eighty-three (383) artificially and naturally contaminated food and environmental samples (Table 5, Appendix) were evaluated as non-paired samples, with one portion analyzed by the 3M method and one analyzed by the ISO method. Results were analyzed using a discordant analysis<sup>1,2</sup>. There was no significant difference between the 3M method and the reference ISO 11290-1<sup>4</sup> method for this study (Table 2). In addition, there was no observed interference as confirmed by valid results for all 383 samples tested in parallel using the 3M™ Molecular Detection Matrix Control.

Table 2. Results and statistical analysis

Statistical analysis according to	Acceptance criterion	Analysis	3M versus ISO 11290-1
ISO 16140: 2003 <sup>1</sup>	No significant difference is established between methods when Chi Square ( $\chi^2$ ) is less than 3.84	$\chi^2 = 0.33$	No statistical differences
ISO 16140-2: 2015 <sup>2</sup>	No significant difference is established between methods when ND-PD* is less than or equal to 6, given 6 categories	ND - PD = +3	No statistical differences

For detailed protocols, see 3M Molecular Detection Assay 2 - *Listeria* Product Instructions

\*ND= Negative deviation: PD= Positive deviation

### AOAC Methods Comparison Study

According to AOAC® *Performance Tested Methods*<sup>SM</sup> (PTM) Probability of Detection (POD)<sup>5</sup> protocols, the 3M method was compared to the US FDA BAM<sup>6</sup>, USDA FSIS MLG<sup>7</sup> or AOAC OMA<sup>8</sup> reference methods. There were no significant differences between the 3M method and the corresponding reference method for any of the matrices evaluated (Table 3).

### Results

Table 3. Enrichment protocols and AOAC® *Performance Tested*<sup>SM</sup> Certificate No. 111501 results for the 3M method

Matrix	Reference method	Size	Enrichment volume, mL	Enrichment time, hours	3M versus Reference
Beef hot dogs	USDA MLG	25 g	225	24-30	No statistical differences
Queso fresco, Vanilla ice cream & Cottage cheese	AOAC OMA	25 g	225	24-30	No statistical differences
Bagged raw spinach & Cold-smoked salmon	FDA BAM	25 g	225	24-30	No statistical differences
Deli turkey	USDA MLG	125 g	1225	24-30	No statistical differences
Raw chicken	USDA MLG	25 g	475	28-32	No statistical differences
Cantaloupe	FDA BAM	Whole melon	Sufficient for melon to float	26-30	No statistical differences
Stainless steel, 3M™ Sponge-Stick with DE	USDA MLG	Sponge	225	24-30	No statistical differences
Sealed ceramic, 3M Sponge-Stick with DE	USDA MLG	Sponge	100	24-30	No statistical differences
Plastic, 3M™ Enviro Swab	USDA MLG	Swab	10	24-30	No statistical differences

For detailed protocols, see 3M Molecular Detection Assay 2 - *Listeria* Product Instructions

### Limit of Detection (LOD)

The method LOD is defined as the lowest concentration where reliable analytical results can be obtained. This may vary with different species, strains, samples and methods. For the 3M method this has been demonstrated to be 1-5 CFU/sample.

The assay LOD can be determined following enrichment assuming that at least one CFU in the original sample has sufficiently multiplied and reached a level that can be detected. LOD may vary when considering sample matrix composition and strain variations. 3M internal validation studies using a variety of *Listeria* have shown that the LOD of the 3M assay ranges from 10<sup>3</sup> to 10<sup>4</sup> CFU/mL in DF enrichment.

## Appendix

Table 4a. Inclusive cultures

Inclusives N=153	<i>Listeria monocytogenes</i> , n=100	Serotypes 1, 1/2a, 1/2b, 2, 2a, 2b, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e, 7	
	<i>Listeria</i> , non- <i>monocytogenes</i> , n=53	<i>L. innocua</i> , n=10 <i>L. welshimeri</i> , n=9 <i>L. seeligeri</i> , n=9 <i>L. ivanovii</i> , n=10 <i>L. marthii</i>	<i>L. grayi</i> , n=10 <i>L. fleischmanii</i> subsp. <i>coloradensis</i> <i>L. cornellensis</i> <i>L. grandensis</i> <i>L. riparia</i>

Table 4b. Exclusive cultures

	Family / group	Genus / species / serotype	
Exclusives N=100	<i>Enterococcaceae</i>	<i>Enterococcus faecalis</i> , n=9 <i>Enterococcus faecium</i> , n=5 <i>Enterococcus mundtii</i> , n=2 <i>Enterococcus gallinarum</i> , n=3 <i>Enterococcus hirae</i> , n=4	<i>Enterococcus raffinosus</i> <i>Enterococcus avium</i> <i>Enterococcus casseliflavus</i> <i>Enterococcus saccharolyticus</i> <i>Enterococcus durans</i>
	<i>Bacillaceae</i>	<i>Bacillus cereus</i> <i>Bacillus coagulans</i> <i>Bacillus circulans</i> <i>Bacillus licheniformis</i> <i>Bacillus weihenstephanensis</i>	<i>Bacillus spizizeni</i> <i>Bacillus thuringiensis</i> <i>Bacillus mycoides</i> <i>Bacillus pseudomycooides</i> <i>Bacillus pumilus</i>
	<i>Staphylococcaceae</i>	<i>Staphylococcus aureus</i> <i>Staphylococcus capitis</i>	<i>Staphylococcus epidermidis</i> <i>Staphylococcus hemolyticus</i>
	<i>Streptococcaceae</i>	<i>Streptococcus uberis</i> <i>Streptococcus agalactiae</i> <i>Streptococcus bovis</i>	<i>Streptococcus pyogenes</i> <i>S. salivarius</i> subsp. <i>thermophilus</i> <i>Lactococcus lactis</i> subsp. <i>cremoris</i>
	Miscellaneous microorganisms	<i>Alicyclobacillus acidoterrestris</i> <i>Brochothrix thermosphacta</i> <i>Brochothrix campestris</i> <i>Pseudoclavibacter helvolus</i> <i>Rhodococcus equi</i> <i>Lactobacillus plantarum</i> <i>Lactobacillus brevis</i> <i>Lactobacillus curvatus</i>	<i>Lactobacillus fermentum</i> <i>Lactobacillus sakei</i> <i>Micrococcus luteus</i> <i>Leuconostoc carnosum</i> <i>Leuconostoc citreum</i> <i>Pediococcus pentosaceus</i> <i>Carnobacterium divergens</i> <i>Carnobacterium piscicola</i>
	<i>Pseudomonadaceae</i>	<i>Pseudomonas aeruginosa</i> <i>Pseudomonas fluorescens</i> <i>Pseudomonas fragi</i>	<i>Pseudomonas putida</i> <i>Pseudomonas stutzeri</i>
	<i>Enterobacteriaceae</i>	<i>Hafnia alvei</i> <i>Edwardsiella tarda</i> <i>Citrobacter brakii</i> <i>Klebsiella oxytoca</i> <i>Cronobacter sakazakii</i> <i>Enterobacter amnigenus</i> <i>Enterobacter cloacae</i> <i>Escherichia coli</i> , n=5 <i>Escherichia coli</i> O157:H7, n=4	<i>Salmonella enterica</i> serotypes: Typhimurium, Enterica, Infantis, Newport, Heidelberg, Panama, Derby, Muenchen, Thompson, Wein, Brookfield, Agona, Virchow, Saintpaul

N = total number of tested strains; n = number of strains tested if > 1

Table 5. Matrices used in Internal Methods Comparison

Category	Specific Matrices
Dairy and related	<ul style="list-style-type: none"> <li>• Cheeses pasteurized: Brie, Monterey jack, mascarpone, processed slices, Queso Fresco, triple cream cheese, mozzarella, cottage cheeses (various fat levels) and ricotta con latte.</li> <li>• Raw milk cheeses: French cheeses made from cow, sheep and goat milk, including Reblochon, Pont l’Eveque, Tomme d’ estaing, Crottin, Vacherin-Mont d’Or and L’Encalat</li> <li>• Butter: Butter with olive oil, Danish butter, buttermilk, crème fraiche</li> <li>• Yogurt, Greek yogurt and Kefir</li> <li>• Heavy whipping cream and half &amp; half</li> <li>• Cream cheese (salmon &amp; vegetable flavors) and spinach dip</li> <li>• Fluid milk: Fat free and 2% pasteurized milk and pasteurized chocolate milk</li> <li>• Ice cream and frozen dairy products</li> <li>• Raw Milk</li> </ul>
Fish, shellfish and related	<ul style="list-style-type: none"> <li>• Raw fish/filets / Fish steaks: salmon swordfish, tuna, tilapia, mahi mahi, trout, walleye, cod and catfish</li> <li>• Cooked fish: smoked salmon, trout and whitefish herring filets, various sushi</li> <li>• Octopus (raw and deli salad), scallops, crab meat, shrimp (raw and cooked), surimi (imitation crab, lobster)</li> </ul>
Ready-To-Eat meats and related	<ul style="list-style-type: none"> <li>• Meat salads (ham, turkey, chicken)</li> <li>• Deli meats and ready to eat meats: chicken, corned beef, pastrami, ham, turkey, salami, pepperoni, summer sausage, smoked sausage, bratwurst, cured and uncured hot dogs, fully cooked bacon, liverwurst, duck liver pâté</li> <li>• Imported French charcuterie: duck rillettes, dry-cured Saucisson Sec, pâté de Champagne, country-style pâté, duck terrine Mousquetaire, jambon de Bayonne prosciutto and Ventreche pancetta</li> </ul>
Meat / Poultry	<ul style="list-style-type: none"> <li>• Raw ground meats (including beef, pork) and raw meats including beef, veal, pork, lamb chunks, steaks and chops</li> <li>• Raw poultry: ground chicken, turkey of various percent fat, chicken wings and thighs</li> <li>• Raw sausages: seasoned pork and turkey sausage, bratwurst</li> </ul>
Produce	<ul style="list-style-type: none"> <li>• Bok choy, cauliflower, sugar snap peas, leafy green romaine, butternut squash cubes, jicama sticks, celery sticks, cantaloupe chunks, coleslaw kit, avocados, sprouts, matchstick carrots, baby spinach, mini cucumbers, edamame, sweet corn, broccoli cuts, whole green beans, classic mixed vegetables, asparagus spears, baby sweet peas, shredded hashbrowns, Potatoes O’ Brien, cut organic green beans, baby lima beans, butter beans, chopped spinach, honey glazed carrots, sweet potatoes.</li> </ul>
Multi-ingredient and Composite	<ul style="list-style-type: none"> <li>• Deli salads including potato, coleslaw, pasta</li> <li>• Sandwiches and prepared meals: tuna salad, turkey wrap, chicken salad on croissant, smoked ham with cheese on rye bread, sliced turkey and gravy, meatloaf with tomato sauce, sushi (various)</li> </ul>
Environmental	<ul style="list-style-type: none"> <li>• Sponge samples from dairy and fish processing plants</li> </ul>

References:

1. ISO 16140:2003 Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods.
2. ISO 16140-2:2015 Microbiology of the food chain – Method validation – Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.
3. AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, 2012.
4. ISO 11290-1:1996 & Amd 1:2004 Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: detection of *Listeria monocytogenes* in foods.
5. Wehling, et al. 2011. Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods. Journal of AOAC International, Vol. 94, No. 1.
6. US FDA Bacteriological Analytical Manual Chapter 10: Detection and Enumeration of *Listeria monocytogenes* in Foods. February, 2013.
7. USDA FSIS Microbiological Laboratory Guidelines 8.09: Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry and Egg Products, and Environmental Samples. May 1st, 2013.
8. AOAC Official Method 993.12 *Listeria monocytogenes* in Milk and Dairy Products: Selective Enrichment and Isolation Method, 1996.



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