

3M Food Safety Technical Bulletin

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Technology Platform: Pathogens
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3M™ Molecular Detection Assay *Listeria monocytogenes* Performance Summary

Testing for *Listeria monocytogenes* is a critical component of food safety programs. Food borne disease caused by *Listeria monocytogenes* can result in significant adverse health conditions with a high mortality rate. Foods associated with outbreaks of *Listeria monocytogenes* include ready-to-eat (RTE) meat and poultry, raw and smoked fish and other seafood, raw milk and soft cheeses made from raw milk, and raw vegetables.

In order to reduce public health concerns related to *Listeria monocytogenes*, a comprehensive farm-to-table approach to food safety is necessary. Farmers, industry, food inspectors, retailers, food service workers and consumers are all critical links in the food safety chain. Faster and more accurate testing methods are needed as traditional microbiology methods are cumbersome and may take more than four days to get results.

The 3M™ Molecular Detection Assay *Listeria monocytogenes* was developed for the rapid and specific detection of *Listeria monocytogenes* in food matrices and environmental samples after enrichment in Demi Fraser Broth Base and Fraser Broth Base. Performance of the 3M method was evaluated for selectivity with a wide range of food matrices, non-food samples, and compared to a reference method. The limit of detection of the method was also determined.

Inclusivity and Exclusivity Testing

a. Inclusivity – false negative rate

Inclusivity is the ability of a method to detect the target analyte from a wide range of strains. One hundred (100) *Listeria monocytogenes* isolates (See Appendix 1) were tested. The *Listeria monocytogenes* strains were cultured in 3M™ Demi Fraser Broth Base and also in 3M™ Fraser Broth Base. Cultures were diluted to a level of approximately 10⁶ CFU/mL (colony forming unit/mL) prior to testing. A false-negative rate of 0% (100% inclusivity) was determined.

b. Exclusivity – false positive rate

Exclusivity is the ability of a method to yield correct results in the presence of non-target organisms, including close relatives. One hundred two (102) non-*Listeria monocytogenes* strains (See Appendix 2) were tested. The non-*Listeria monocytogenes* strains were cultured in Tryptic Soy Broth (TSB) and tested at levels of > 10⁷ CFU/mL prior to testing. A false-positive rate of 0% (100% exclusivity) was determined.



3M™ Molecular Detection Assay *Listeria monocytogenes* Performance Summary (cont.)

Method Comparison Study

Studies were conducted to assess the performance of the method (compared to ISO 11290-1)REF in the presence of possible interferences from intrinsic and extrinsic factors including other organisms. Food samples commonly reported in outbreaks and/or reported as being challenging due to their composition, i.e. high fat, high native microflora, etc. were included. Environmental samples were obtained from meat, fish and dairy processing facilities as well as dairy farms.

647 non-contaminated, naturally-contaminated or artificially-contaminated samples (1 to 10 CFU *Listeria monocytogenes*, conditioned with each matrix, see Appendix 2) were evaluated. Enrichments were prepared and tested according to the 3M Molecular Detection Assay *Listeria monocytogenes* Product Instructions, shown below. This study utilized non-paired samples, which were analyzed using a Mantel-Haenszel Chi Square (X²) statistic. Significant differences are indicated by a Chi Square > 3.84 when 3M method results are compared to ISO 11290-1 cultural reference method results.

| Sample Matrix | Sample Size | Primary Enrichment Demi-Fraser Broth Base | | | Secondary Enrichment Fraser Broth Base | | |
|-----------------------|-------------|--|-------------------------------|----------------------|---|-------------------------------|----------------------|
| | | Enrichment Broth Volume (mL) | Enrichment Temperature (±1°C) | Enrichment Time (hr) | Enrichment Medium (mL) | Enrichment Temperature (±1°C) | Enrichment Time (hr) |
| Environmental* | 1 swab | 10 | 37 | 26-30 | Not required | | |
| | 1 sponge | 100 | | | | | |
| | 1 sponge | 225 | | | | | |
| Heat-processed dairy* | 25 g | 225 | 37 | 24-28 | Not required | | |
| Other foods** | 25 g | 225 | 37 | 24-26 | Fraser Broth Base: 0.1 mL into 10 mL | 37 | 24-26 |

* A 24-26 hr primary enrichment in Demi-Fraser Broth Base followed by a 24-26 hr secondary enrichment in Fraser Broth Base is also acceptable for these matrices.

** Other Foods includes meat, poultry, fish, seafood, produce, vegetable and vegetable-based products, and raw dairy.

Results

| Sample Category | N | X ² | Comparison to ISO reference method |
|----------------------|-----|----------------|------------------------------------|
| Environmental | 89 | 0.12 | Not statistically different |
| Heat-Processed Dairy | 92 | 0.78 | Not statistically different |
| Other Foods | 466 | 0.92 | Not statistically different |

3M™ Molecular Detection Assay *Listeria monocytogenes* Performance Summary (cont.)

Limit of Detection (LOD)

The limit of detection of a method is defined as the lowest concentration point where reliable analytical results can be obtained. This may vary with different strains and methods. For the 3M Molecular Detection Assay *Listeria monocytogenes* method this has been demonstrated to be 1-5 CFU/ sample size.

Appendix 1 – Inclusive and Exclusive Test Strains

| | |
|--------------------------|---|
| Inclusives, n=100 | Organism |
| | <i>Listeria monocytogenes</i> serotypes 1, 1/2a, 1/2b, 2, 2a, 2b, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e, 7 |

| | Organism | Organism |
|--|--------------------------|---|
| | Exclusives, n=102 | <i>Listeria coloradensis</i> |
| <i>Listeria grayi</i> (10) | | <i>Listeria innocua</i> (10) |
| <i>Listeria innocua</i> (10) | | <i>Pseudomonas fluorescens</i> , <i>P. fragi</i> , <i>P. putida</i> , <i>P. stutzeri</i> , <i>P. aeruginosa</i> |
| <i>Listeria rocourtiae</i> (3) | | <i>Rhodococcus equi</i> |
| <i>Listeria seelgeri</i> (9) | | <i>Staphylococcus aureus</i> , <i>S. capitis</i> , <i>S. epidermidis</i> , <i>S. saprophyticus</i> |
| <i>Listeria welshimeri</i> (9) | | <i>Streptococcus agalactiae</i> , <i>S. uberis</i> |
| <i>Alicyclobacillus acidoterrestris</i> | | <i>Enterobacter amnigenus</i> , <i>E. cloacae</i> |
| <i>Bacillus cereus</i> , <i>B. coagulans</i> , <i>B. spizizeni</i> , <i>B. thuringiensis</i> | | <i>Edwardsiella tarda</i> |
| <i>Brochothrix thermosphacta</i> | | <i>Cronobacter sakazakii</i> |
| <i>Citrobacter brakii</i> (2) | | <i>Hafnia alvei</i> |
| <i>Enterococcus faecalis</i> , <i>faecium</i> , <i>mundtii</i> | | <i>Klebsiella oxytoca</i> |
| <i>Lactobacillus plantarum</i> | | <i>Escherichia coli</i> , <i>E. coli</i> O157:H7 (4) |
| <i>Lactococcus cremoris</i> | | <i>Salmonella enterica</i> , ser Enterica, S. Panama, S. Muenchen, S. Reading, S. Brookfield, S. Agona, S. Sendai, S. Thompson, S. Wein, S. Typhimirium |
| <i>Microbacterium estereaeromaticum</i> | | <i>Paenibacillus mascerans</i> |
| <i>Micrococcus luteus</i> | | <i>monocytogenes</i> |

Strain Sources:

3M Food Safety collection, American Type Culture Collection (ATCC), National Culture Type Collection (NCTC), National Collection of Industrial, Food and Marine Bacteria (NCIMB), ILSI-NA at Cornell University, Western Europe Reference Laboratory, *E. coli* Reference Center at the Pennsylvania State University, *Salmonella* Genetic Stock Centre at the University of Calgary

Reference:

ISO 11290-1:1996. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes*

ISO 11290-1:1996/Amd 1:2004. Modification of the Isolation Media and the Haemolysis Test, and Inclusion of Precision Data

3M™ Molecular Detection Assay *Listeria monocytogenes* Performance Summary (cont.)

Appendix 2 – Foods

| Fish, Shellfish and Related | Dairy and Dairy Related | Produce and Produce-Related |
|--|--|--|
| Fish filets / steaks, raw including swordfish, tuna, tilapia, perch, walleye, trout, halibut, cod, buffalo fish, salmon | Cheeses pasteurized, including Brie, Monterey Jack, Muenster, Limburger, Havarti, mascarpone, processed slices, processed block, processed spread, fresh mexican cheese, triple cream, Emmental, mozzarella; raw milk cheeses, cottage cheeses (3 fat levels) ricotta con latte & ricotta salata | Frozen vegetables including artichoke, asparagus, broccoli rabe, Brussels sprouts, carrots, cauliflower, collards, corn, edamame, green beans, lima beans, peas, okra, spinach, squash, zucchini, (some with sauces) |
| Escargot in shell with garlic | Butter; buttermilk, crème fraiche, kefir and yogurts and sour cream; heavy whipping cream and half & half | Fresh leafy vegetables including arugula, bok choy, butter lettuce, chard, escarole, kale, radicchio, red leaf lettuce, romaine and spinach, (some bagged), |
| Salmon, cooked cakes and salmon ready-to-heat meal, Smoked fish (trout and whitefish); Salmon puff, fish with bread-crumbs, fish & chips, Salmon, smoked and raw salmon burger | Cream cheese (salmon & vegetable flavors), dairy dip | Other fresh vegetables including snow peas, sugar snap peas, pea shoots, okra, asparagus, green beans, corn, squash |
| Tuna salad and tuna salad sandwiches | Chocolate milk (fat free and 2%); Milk, pasteurized and raw milk, Ice cream and frozen desserts | Whole cantaloupe, honeydew and kiwano melons, Avocado whole |
| Sushi (cooked fish with rice and veg); Sushi (raw tuna or salmon); Surimi (imitation crab) and surimi | | Potatoes including raw, refrigerated, frozen, fried frozen; fresh root vegetables including carrots, jicama, sweet potato, taro, (whole, cut, shredded) |
| Octopus (iidako salad and raw); Calamari salad and raw calamari steak | | Fresh herbs, (cilantro, parsley) |
| Meats/Poultry and Meat-Related | Ready-to-Eat Meats and Related | Multi-Ingredient |
| Raw ground meats (including beef, pork, wild boar, lamb, bison, rabbit, alligator) and breaded beef steak, raw; beef offal, raw (tripe, feet) | Meat salads (ham, turkey, chicken) and Deli meat slices (chicken, corned beef, pastrami, ham, turkey) | Deli salads including potato, cole slaw, pasta, quinoa, seaweed, barley, freekeh and bean salads |
| Poultry (chicken, turkey, ostrich) of various percent fat | Pate, mousse de foie de canard au porto, pork liver pate | Meat substitutes including vegan/vegetarian hot dogs, burritos, sausage, tofu scramble |
| Sausages, raw including andouille, chorizo, merguez, chitterling, bratwurst, seasoned pork and turkey sausage | Sausages, cooked including andouille, chorizo | |



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