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Laboratory Procedure **MFLP-111**
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Health Products and Food Branch

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Detection of *Listeria monocytogenes* in Foods Using the 3M™ Molecular Detection System Test Kit Version 2

Microbiological Methods Committee
Microbiology Evaluation Division
Bureau of Microbial Hazards, Food Directorate,
Health Products and Food Branch, Health Canada
Postal Locator: 2204E
Ottawa, Ontario K1A 0K9

Contact the Microbiological Methods Committee: hc.mmc-cmm.sc@canada.ca

1. Application

This method is applicable to the rapid detection of *Listeria monocytogenes* to determine compliance with the requirements of Sections 4 and 7 of the *Food and Drugs Act* and/or other relevant federal regulations. This method has been validated for use in the food categories raw meat products, fruit and vegetable-based products, for the food type "other" of the food category ready-to-eat (RTE) meat and poultry, the food type "raw" and the food type "frozen" of the food category dairy products and the food type "raw fish and crustaceans" and the food type "frozen" of the food category fish and seafood products.

Note: While this method is only approved for certain food products, as listed above, it is assumed that this method could be used with other foods. To ensure the method is fit for purpose for commodities outside the application, it is imperative that other commodities be properly validated following the criteria in the *Compendium of Analytical Methods*. It is requested that these validation data be forwarded to the Microbiological Methods Committee (MMC) so that the Application Section can be expanded to include these new foods if the data comply with MMC requirements (refer to Development of Methods in Volume 1 of the *Compendium of Analytical Methods*).

2. Description

The 3M™ Molecular Detection Assays use isothermal amplification of nucleic acid sequences combined with bioluminescence to detect the amplification of the target organism's genetic material after 24 h enrichment. Presumptive positive results are reported in real-time while negative results are displayed after the assay is completed.

3. Principle

The chemistry in the 3M™ Molecular Detection Assays is based on loop-mediated isothermal amplification (LAMP) (8.1). LAMP is a nucleic acid amplification technique (8.2, 8.3, 8.4, 8.5). This technology allows the 3M™ Molecular Detection System to offer a simple sample preparation process with only two transfer steps. LAMP utilizes multiple primers that recognize distinct regions of the target *Listeria monocytogenes* gene and uses *Bst* polymerase, a unique enzyme with DNA strand-displacement activity, to enable the continuous, rapid isothermal amplification (8.6).

The 3M™ Molecular Detection System uses bioluminescence technology to report the DNA amplification of the target organism in real-time. This involves a two-step enzymatic process in which pyrophosphate molecules, produced as a byproduct of the DNA amplification, are used to generate light. This light emission is then read by the 3M™ Molecular Detection Instrument and signals the detection of the target organism (8.7).

The unique method of bioluminescence detection, combined with the single-temperature amplification of LAMP used by the 3M™ Molecular Detection System, allows the target DNA to be amplified continuously, generating more than 10^9 copies of target in as little as 15 min.

4. Definition of Terms

See Appendix A of Volume 1.

5. Collection of Samples

See Appendix B of Volume 1.

6. Materials and Special Equipment

Note: The laboratory supervisor must ensure that completion of the analysis described in this method is done in accordance with the International Standards reference ISO/IEC 17025 (latest version): "General Requirements for the Competence of Testing and Calibration Laboratories".

Note: It is the responsibility of the laboratory to ensure equivalency if any variations of the media formulations listed here are used (either product that is commercially available or made from scratch). Please forward equivalency data to the Editor of the *Compendium of Analytical Methods* for consideration of modification of this method.

1) 3M™ Molecular Detection Assay *Listeria monocytogenes* test kit (MDA2LMO96) includes:

- Lysis Solution (LS) tubes (96 – 12 strips of 8 tubes)
- *Listeria monocytogenes* Reagent tubes (96 – 12 strips of 8 tubes)
- Extra caps (96 – 12 strips of 8 caps)
- Reagent Control (RC) (16 tubes)
- Quick Start Guide

2) MDS100 3M™ Molecular Detection Instrument and Accessory Kit (MDS100) includes:

- 3M™ Molecular Detection Speed Loader Tray
- 3M™ Molecular Detection Chill Block Insert
- 3M™ Molecular Detection Chill Block Tray
- 3M™ Molecular Detection Cap/Decap Tool—Lysis
- 3M™ Molecular Detection Cap/Decap Tool—Reagent
- Empty lysis and reagent tray

3) Enrichment Media

The media listed below are commercially available and are to be prepared and sterilized according to the manufacturer's instructions. See also Section 9 of this method and Appendix G of Volume 1 for the formulae of individual media.

Demi-Fraser Broth containing Ferric Ammonium Citrate (See Section 9)
Fraser broth containing Ferric Ammonium Citrate (See Appendix G of Volume 1)

4) Additional Materials

- 3M™ Molecular Detection Heat Block Insert (MDSHBIN)
- Powder-free gloves
- Sterile / barrier filter tips - adaptable to 20 µL micropipettes
- Sterile stomacher bag
- Single Channel 20 µL Micropipette
- 8- Channel Multi-Channel 20 µL Micropipette
- Computer workstation
- Incubator(s) that can be set at 37 ± 1°C
- Dry Block Heating unit that can fit Insert (15 cm × 9.5 cm or 5.875” × 3.75”) and reach temperatures of 100°C
- Stomacher, blender or equivalent

Note: It is the responsibility of each laboratory to ensure that the temperatures of the incubators or water baths are maintained at the recommended temperatures. The following applies to steps of the method which apply to growth only. Where a temperature of ≤ 37°C is recommended in the text of the method, the temperature may be ± 1.0°C, *e.g.,* 35 ± 1.0°C. However, where higher temperatures are recommended, it is imperative that the incubators or water baths be maintained within 0.5°C due to potential lethality of the higher temperatures on the microorganism(s) being isolated.

7. Procedure

The test shall be carried out in accordance with the following instructions:

7.1 Handling of Sample Units

7.1.1 In the laboratory prior to analysis except for shelf-stable foods, keep sample units refrigerated or frozen, depending on the nature of the product. Thaw frozen

samples in a refrigerator, or under time and temperature conditions which prevent microbial growth or death.

7.1.2 Analyze sample units as soon as possible after their receipt in the laboratory.

7.2 Preparation for Analysis

7.2.1 Have sterile Demi-Fraser broth base with Ferric Ammonium Citrate (FAC) prepared, and acclimatized to room temperature before use.

7.2.2 Clean the surface of the working area with a suitable disinfectant.

7.2.3 Turn on block heater with the Heat Block Insert in, such that it can reach 100°C prior to analysis.

7.2.4 Log in to the 3M™ Molecular Detection Software and turn on the 3M™ Molecular Detection Instrument.

7.3 Preparation of Sample

To ensure a representative analytical unit, agitate liquids or free flowing materials until the contents are homogeneous. If the sample unit is a solid, obtain the analytical unit by taking a portion from several locations within the sample unit.

7.3.1 Prepare a dilution by adding aseptically 25 g or mL of the food to 225 mL or 475 mL of the appropriate enrichment media per Table 1 below. For composite samples, analytical units may be combined up to 125 g or ml (*e.g.,* 125 g or mL of food to 1125 mL or 2375 mL of enrichment broth per Table 1 below). If alternate analytical units are required, maintain a ratio of 1 part sample material to 9 parts enrichment media (or 19 parts for food using a 1 in 20 dilution per Table 1 below).

7.3.2 Blend, stomach or vortex as required for thorough mixing.

7.3.3 Enrich all samples according to Table 1. Incubate all samples at 37 ± 1 °C.

7.3.4 Following the appropriate enrichment, proceed to section 7.4.

Table 1 Enrichment Conditions

Food Category	Primary Enrichment			Secondary Enrichment		Sample Analysis Volume [(a)]
	Media	Enrichment Volume (mL)	Time (h)	Media	Time (h)	
Fruit and Vegetable-based products, the food type “Frozen” of the food category Dairy Products and the food type “Other” of the food category RTE Meat and Poultry	Demi-Fraser with FAC	225 (1 in 10)	24 - 26	Not required	N/A	20 µL

Raw Meat Products and the food type "Frozen" and the food type "Raw Fish and Crustaceans" of the food category Fish and Seafood Products	Demi-Fraser with FAC	475 (1 in 20)	28 - 32	Not required	N/A	20 µL
Raw Dairy Products	Demi-Fraser with FAC	225 (1 in 10)	20 – 24	After primary enrichment, transfer 0.1 mL into 10 mL of Fraser broth with FAC	20 - 24	10 µL

(a) Volume of sample transferred to Lysis Solution tubes. Refer to Step 7.4.5.2 of DNA Extraction – Lysis Step section.

7.4 DNA Extraction – Lysis Step

7.4.1 Allow the lysis solution (LS) tubes to warm up to room temperature. One LS tube is required for each sample and a negative control (NC) sample.

7.4.2 LS tube strips can be cut to desired LS tube number. Select the number of individual LS tubes or 8-tube strips needed. Place the LS tubes in an empty rack.

7.4.3 Remove the enrichment broth from the incubator and gently agitate the contents.

7.4.4 Invert the LS capped tubes to mix. To avoid cross-contamination, decap one LS tube strip at a time and use a new pipette tip for each transfer step.

7.4.5 Transfer the enriched sample to LS tubes as described below:

7.4.5.1 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. Caps can be retained in order to store lysis tubes if needed.

7.4.5.2 Transfer 20 µL of enriched sample into a LS tube. In the case of Raw Dairy Products, transfer 10 µL of enriched sample (from the secondary enrichment) instead.

7.4.5.3 Repeat step 7.4.5.2 until each individual sample has been added to a corresponding LS tube in the strip.

7.4.5.4 Repeat steps 7.4.5.1 to 7.4.5.3 as needed, for the number of samples to be tested including all controls.

7.4.6 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. The colour of the lysis solution should change from pink (cool) to yellow (hot).

7.4.7 Remove the uncovered rack of LS tubes from the heating block and allow to cool in the 3M™ Molecular Detection Chill Block Insert for 5 ± 1 min. The lysis solution should change from yellow to pink, when cooled.

7.4.8 Remove the rack of LS tubes from the 3M™ Molecular Detection Chill Block Insert.

7.5 Amplification and Detection

7.5.1 Reagent tube strips can be cut to the desired number of tubes. Select the number of individual Reagent tubes or 8-tube strips needed. One Reagent tube is required for each sample and the NC.

7.5.2 Place Reagent tubes in an empty rack.

7.5.3 The procedure for transferring each sample lysate into Reagent tubes is described in steps 7.5.4 to 7.5.7. Transfer each sample lysate into individual Reagent tubes including all applicable controls. To avoid cross-contamination, decap one Reagent tube strip at a time and use a new pipette tip for each transfer step**.**

Note: Care must be taken when pipetting LS, as carry-over of the protein residue at the bottom of the lysis tubes may interfere with amplification.

7.5.4 Use the 3M™ Molecular Detection Cap/Decap Tool-Reagent to decap the Reagent tubes – one Reagent tube strip at a time. Discard cap.

7.5.5 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 5 times. Using a multi-channel pipette can expedite this process.

7.5.6 Repeat step 7.5.5 until all individual Sample lysates have been added to a corresponding Reagent tube in the strip.

7.5.7 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.

7.5.8 Repeat steps 7.5.4 to 7.5.7 as needed, for the number of samples to be tested including all controls.

7.5.9 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.

7.5.10 Review and confirm the configured run in the 3M™ Molecular Detection Software.

7.5.11 Click the Start button in the software and select instrument for use. The selected instrument's lid automatically opens.

7.5.12 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 60 - 75 min, although positives may be detected sooner.

7.6 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. Negative samples will not give a zero reading as the system and 3M™ Molecular Assay *Listeria monocytogenes* amplification reagents have a background relative light unit (RLU). Presumptive positive results are reported in real-time while “Negative” and “Inspect” results will be displayed after the run is completed.

In the rare event of any unusual light output, the algorithm labels this as “Inspect”. Repeat the assay for any “Inspect” samples beginning at step 7.4. If the result continues to be “Inspect”, proceed with cultural confirmation as described in step 7.7.

7.7 Confirmation of Presumptive Positive Results

For foods requiring only primary enrichment prior to screening (as per Table 1):

Proceed with direct plating of the presumptive positive primary 24 h to 32 h Demi-Fraser with FAC broth to the selective agars as described in MFHPB-30 and continue with isolation and confirmation as described in MFHPB-30.

For foods requiring the secondary enrichment prior to screening (raw dairy products):

Proceed with direct plating of the presumptive positive Fraser with FAC broth to the selective agars as described in MFHPB-30 and continue with isolation and confirmation as described in MFHPB-30.

8. References

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9. Media Formulae

Demi-Fraser Broth with Ferric ammonium citrate supplement	
Basal Medium	
Pancreatic digest of casein	5.0 g
Peptic digest of animal tissue	5.0 g
Beef extract	5.0 g
Yeast extract	5.0 g
Sodium chloride (NaCl)	20.0 g
Mono-potassium phosphate (KH_2PO_4)	1.35 g
Sodium phosphate dibasic, anhydrous (Na_2HPO_4)	9.6 g
Esculin	1.0 g
Lithium chloride	3.0 g
Nalidixic acid	0.01 g
Acriflavine HCl	0.0125 g
Distilled water	1.0 L
pH 7.2 ± 0.2	
Supplement	
Ferric ammonium citrate (FAC)	0.5 g
Distilled water	10.0 mL

FAC solution: suspend ferric ammonium citrate in 10.0 mL of distilled water and filter sterilize.

Complete Medium:
Suspend the basal medium ingredients in 1.0 L of distilled water. Mix thoroughly. Heat to boiling to dissolve completely and autoclave at 121°C for 15 min. Cool to room temperature. Aseptically add 10.0 mL of the FAC solution to the cooled media, mix well and dispense. Store under refrigeration in the dark.

Note:
Acriflavine can photo-oxidize to form compounds inhibitory to *Listeria* spp.

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