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Laboratory Procedure **MFLP-101**
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Health Products and Food

Branch Ottawa

Detection of *Listeria* spp. in Environmental Surface Samples Using the 3M™ Molecular Detection System Test Kit Version 2

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1. Application

This method is applicable to the rapid detection of *Listeria* spp. 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 on a variety of environmental surfaces.

2. Description

The 3M™ Molecular Detection Assays use isothermal amplification of nucleic acid sequences with high specificity, efficiency, rapidity, and 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 the result of novel developments in molecular biology and is a highly robust, efficient, sensitive, specific, and simple 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* spp. 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, resulting in a process that is both highly specific and rapid, generating more than 10^9 copies of target in as little as 15 min.

4. Definition of Terms

See Appendix A of Volume1.

5. Collection of Samples

See Appendix B of Volume 1. Follow MFLP-41 for collection and transportation of environmental surface samples. The environmental collection device should be moistened with Dey-Engley (D/E) Neutralizing broth.

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 17025:2005 (or 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 *Compendium of Analytical Methods* for consideration of modification of this method.

6.1 3M™ Molecular Detection Assay *Listeria* species test kit (MDA2LIS96)

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

6.2 MDS100 3M™ Molecular Detection Instrument and Accessory Kit (MDS100)

- 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

6.3 Media and other equipment

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

- Demi-Fraser Broth (with Ferric Ammonium Citrate)

- 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) capable of maintaining 37 ± 1°C
- Dry Block Heating unit that can fit Insert (15 cm × 9.5 cm or 5.875”×3.75”) and maintain 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, keep sample units refrigerated prior to analysis.

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 medium 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 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

7.3.1 Prepare a 1 in 10 (1:9) dilution of the environmental sponge by aseptically adding 100 mL of the enrichment media or composite up to 10 sponges with 100 mL of supplemented Demi-Fraser **per sponge**.

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

7.3.3 Incubate all samples for 24-28h at 37°C.

7.3.4 Following the appropriate enrichment, proceed to section 7.4.

7.4 DNA Extraction – Lysis Step

Note: Using one 3M™ Matrix Control (MC) per matrix type is suggested to determine if the matrix has the ability to impact the assay results.

7.4.1 Allow the lysis solution (LS) tubes to warm up to room temperature by setting the rack on the laboratory bench for 2 h. One LS tube is required for each sample, and a negative control 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 To avoid cross-contamination, decap one LS tubes 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 sample into a LS tube.

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.

7.4.5.5 When all samples have been transferred, transfer 20 µL of sterile Demi- Fraser broth into a LS tube to act as the negative control (NC).

7.4.5.6 Cover the rack of LS tubes with the rack lid.

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 to yellow.

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

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 Select 1 Reagent Control (RC) tube and place in rack.

7.5.4 The procedure for transferring each sample lysate into reagent tubes is described in steps 7.5.5 to 7.5.8. Transfer each sample lysate into individual Reagent tubes **first,** followed by the NC. Hydrate the RC tube **last**. 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.

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

7.5.6 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.

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

7.5.8 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.

7.5.9 Repeat steps 7.5.5 to 7.5.8 as needed, for the number of samples to be tested.

7.5.10 When all sample lysates have been transferred, repeat transfer 20 µL of NC lysate into a Reagent tube to serve as a negative control. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.

7.5.11 Transfer **20 µL of NC lysate into a RC tube** to serve as a positive control. Dispense at an angle to avoid disturbing the pellets. Mix by gently pipetting up and down 5 times.

7.5.12 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.

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

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

7.5.15 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* spp. 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”. 3M™ recommends the user to repeat the assay for any “Inspect” samples. If the result continues to be “Inspect”, proceed with cultural confirmation.

7.7 Confirmation of Presumptive Positive Results

Commence with direct plating of the presumptive positive primary 24 - 28h Demi-Fraser enrichment to the selective agars as described in MFHPB-30, concurrently transferring an aliquot of the primary enrichment to a secondary enrichment in modified Fraser broth (MFB) as per MFHPB-30. If the direct plating results confirm the presumptive positive result, the selective secondary enrichment can be discontinued. If the direct plating results are negative, continue with incubation of enrichments, plating, isolation and confirmation as described in MFHPB-30.

8. References

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