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**Laboratory Procedure** **MFLP-100**
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# Detection of *Salmonella* spp. in Foods Using the 3M™ Molecular Detection System Test Kit Version 2

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## 1. Application

This method is applicable to the detection of *Salmonella* spp. to determine compliance with the requirements of Sections 4 and 7 of the *Food and Drugs Act* and/or other relevant federal regulations. Positive results must be confirmed with a cultural method. This method has been validated for use in all foods except chocolate based products, spices, powdered dairy products and whole nuts.

## 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 18-26 hours 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 *Salmonella* spp. gene and uses *Bst* polymerase, a unique enzyme with DNA strand-displacement activity, to enable 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 *Compendium of Analytical Methods* for consideration of modification of this method.

1) 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 trays

2) 3M™ Molecular Detection Assay 2 *Salmonella* spp. test kit (MDA2SAL96) includes:

- 3M™ Lysis Solution (LS) tubes (96 – 12 strips of 8 tubes)
- 3M™ *Salmonella* spp. Reagent tubes (96 – 12 strips of 8 tubes)
- Extra caps (96 – 12 strips of 8 caps)
- 3M™ Reagent Control (RC) (16 tubes)
- Quick Start Guide

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 Appendix G of Volume 1 for the formulae of individual media.

- Buffered Peptone Water (BPW)

4) Additional Materials

- 3M™ Molecular Detection Heat Block Insert (MDSHBIN)
- 3M™ Molecular Detection Matrix Control (MDMC96) (optional)
- 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 and 41.5 ± 0.5°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 BPW 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 Add 9 volumes (i.e., 225 mL) of room temperature Buffered Peptone Water to each food sample (usually 25 g) in a stomacher bag.

7.3.2 To reduce the workload, up to 15 analytical units may be composited into a single test sample (e.g., 375 g or mL) while maintaining the 1:10 dilution ratio.

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

7.3.4 Incubate all samples for 18 - 26 h at 37 ± 1°C. Incubate kefir 18 - 26 h at 41.5 ± 0.5°C.

7.3.5 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. Invert the capped tubes to mix. 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 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 caps 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 BPW into a LS tube to act as the negative control (NC) (unless running own internal controls).

7.4.6 Verify that the temperature of the 3M™ Molecular Detection Heat Block Insert is at 100 ± 1°C. Place the uncovered 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 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 from 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 to ensure 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 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 *Salmonella* 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". 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

Commence with direct plating of the presumptive positive primary BPW enrichment to the selective agars as described in MFHPB-20 (8.8), concurrently transferring an aliquot of the primary enrichment to the selective enrichments as described in MFHPB-20. If the direct plating results confirm the presumptive positive result, the selective enrichment can be discontinued. If the direct plating results are negative, continue with incubation of enrichments, plating, isolation and confirmation as described in MFHPB-20.

## 8. References

8.1 Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res*. 28(12):E63. 3.

8.2 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(1):41-48.

8.3 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(3):499-501.

8.4 Kiddle G, HardingeP, Buttigieg N, Gandelman O, Pereira C, McElgunn CJ, Rizzoli M, Jackson R, Appleton N, Moore C, Tisi LC, Murray JAH. 2012. GMO detection using a bioluminescent real time reporter (BART) of loop mediated isothermal amplification (LAMP) suitable for field use. *BMC Biotechnology*. 12:15.

8.5 Plutzer J, Karanis P. 2009. Rapid identification of *Giardia duodenalis* by loop-mediated isothermal amplification (LAMP) from faecal and environmental samples and comparative findings by PCR and real-time PCR methods. *Parasitol Res*. 104 (6):1527-1533.

8.6 Nkouawa A, Sako Y, Li T, Xingwang C, Wandra T, Swastika K, Nakao M, Yanagida T, Nakaya K, Qiu D, Ito A. 2010. Evaluation of a loop-mediated isothermal amplification method using fecal specimens for differential detection of *Taenia* species from humans. *J. Clin. Microbiol*. 48(9):3350-3352.

8.7 Gandelman OA, Church VL, Moore CA, Kiddle G, Carne CA, Parmar S, Jalal H, Tisi LC, Murray JAH. 2010. Novel bioluminescent quantitative detection of nucleic acid amplification in real-time. *PloS ONE*. 5(11): e14155.

8.8 Reid A. 2009. Isolation of *Salmonella* from food and environmental samples (MFHPB-20) in Volume 2. *The Compendium of Analytical Methods*.

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