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Laboratory Procedure **MFLP-51**
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Health Products and Food Branch

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Detection of *Listeria* species in Stainless steel Surfaces by the ADIAFOOD Rapid Pathogen Detection System

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1. Application

This method is applicable to the rapid detection of *Listeria* species to determine compliance with the requirements of Sections 4 and 7 of the *Food and Drugs Act*. Positive results must be confirmed with a cultural method. This method has been validated for use on stainless steel surfaces.

Note: While this method is only approved for stainless steel surfaces, as listed above, it is assumed that this method could be used with other environmental surfaces. To ensure the method is fit for purpose for surfaces outside the application, it is imperative that other surfaces 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 so the Application Section can be expanded to include these new surfaces if the data fulfil MMC requirements (refer to Development of Methods in Volume 1 of the *Compendium of Analytical Methods*).

2. Principle

Sterile sponges or swabs pre-moistened with D/E Neutralising buffer are used to sample surfaces and then placed in Listerboost enrichment media (AES Chemunex). Following a 42 to 48 hour enrichment, the broth is subjected to a real time PCR procedure in which the target pathogen DNA is amplified and detected using specific primers and molecular beacons. Molecular beacons consist of unique sequence probes that allow for the identification of the pathogen with a high level of specificity. Once bound to their target, the molecular beacons emit a fluorescent signal that is proportional to the amount of amplified pathogenic DNA. In the absence of target bacteria on the sponges or swabs, no fluorescent signal is detected since the oligonucleotide primers used within this system are highly specific for *Listeria* species, and do not amplify DNA present in other organisms under the same reaction conditions.

The entire procedure, after the enrichment step in proprietary enrichment media, identifies presumptive positive surface swabs within 3 hours.

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http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php

3. Definition of Terms

See Appendix A of Volume 3.

4. Collection of Samples

See Appendix B of Volume 3.

5. Specialized Molecular Markers, Reagents, Buffers, Materials and 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 17025:1999 (or latest version): General Requirements for the Competence of Testing and Calibration Laboratories”.

Note: If the analyst uses any variations of the media listed here (either product that is commercially available or made from scratch), it is the responsibility of the analyst or Laboratory Supervisor to ensure equivalency.

5.1. Materials and Equipment

5.1.1 Listerboost enrichment broth (AEB 910623/4, manufactured by AES Chemunex Canada, and distributed by Innovation Diagnostics, 1-888-965-1871)

5.1.2 ADIAFOOD Kit components (DFS 6102A, manufactured by AES Chemunex Canada, and distributed by Innovation Diagnostics)
Extraction buffer (EX-1)
Extraction reagent, lyophilized (EX-2)
Detection buffer (DT-1)
Detection reagent, lyophilized (DT-2)
Detection microplates or strip tubes containing pre-dispensed PCR reagents
Control strip tubes (only with Detection strip kits)
PCR optical-grade caps

5.1.3 Additional equipment and consumables required

Sponges pre-moistened with 10 ml of D/E Neutralizing buffer and stomacher bags
Swabs pre-moistened with D/E Neutralizing buffer and culture tubes
AES-validated Real-Time PCR thermocycler with the following specifications:

- 96-well (or 48-well) microplate (low and high profile) or 96 (48) × 0.2 mL tubes (low and high profile) sample capacity
- the ability to excite fluorophores with a peak excitation range of 485 to 520 nm
- the ability to detect fluorophores with a peak emission range of 500 to 600 nm
- Sentinel software

Conventional thermocycler for DNA extraction (optional)
Centrifuge with rotor and microplate carriers
Vortex equipped for microplates
Stomacher or equivalent
Multi-channel pipettors
Single-channel pipettor
PCR enclosure (optional)
PCR capping tool
Strip holders

ADIAFOOD Extraction kit (EXT9997A, containing extraction plates, domed caps and seals)
Aerosol barrier tips for pipettors
Disposable multi-channel pipettor basins
Powder-free gloves
Alcohol (denatured) and bleach
Incubator capable of maintaining 37 ± 1°C

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. Where 35°C is recommended in text of the method the incubator may be at 35 ± 1.0°C. Similarly, lower temperatures of 30 or 25 may be ± 1.0°C. However, where higher temperatures are recommended, such as 43 or 45.5°C, 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.

5.2 Molecular Markers, Temperature Cycling Program, Buffers and Reagents

5.2.1 Molecular markers

The oligonucleotide primers and molecular beacon probes are supplied in the kit and specific for *Listeria* spp.

5.2.2 Temperature cycling programs

The temperature cycling programs for the PCR include an automated extraction cycle, resulting in the lysis of the bacterial cells, followed by an automated PCR detection process.

5.2.3 Buffers and reagents

The ADIAFOOD detection kits must be stored refrigerated in their original packaging until use. Reconstituted extraction reagent EX-2 and detection reagent DT-2 have a 14 day shelf life when stored refrigerated in their original containers, sealed with Parafilm.

Note:	All water, pipettes, pipette tips and other materials should be sterile and DNase free.

6. Procedure

6.1 Handling of Sample Units

Follow MFLP-41 using sponges pre-moistened with 10 ml of D/E Neutralizing buffer or swabs pre-moistened with D/E Neutralizing buffer.

6.2 Preparation for Analysis

Follow MFLP-41.

6.3 Preparation of Samples

6.3.1 Transfer each sponge to a sterile stomacher bag with 90 ml of Listerboost enrichment broth and mix thoroughly by the use of a stomacher unit. Transfer each swab to a sterile culture tube with 10 ml of Listerboost enrichment broth and vortex.

6.3.2 For composited environmental samples, aseptically add 10 mL of Listerboost enrichment broth for each swab or 90 mL of Listerboost enrichment broth for each sponge. Stomach for thorough mixing.

6.4 Enrichment Procedure

Enrich environmental samples in Listerboost broth for 42 to 48 hours at 37 ± 1°C.

6.5 DNA Extraction

6.5.1 Dispense the contents from EX-1 (squeeze bottle) into the EX-2 vial to obtain reconstituted EX-2.

6.5.2 Recap the EX-2 vial and mix by inversion to ensure complete dissolution of desiccated reagent.

6.5.3 Pour reconstituted EX-2 into a multi-channel pipettor basin.

6.5.4 Aliquot 90 µL of reconstituted EX-2 into each well of the Extraction microplate.

6.5.5 Transfer 10 µL of the cell suspension into the Extraction microplate according to the sample assignment layout.

6.5.6 Seal the wells of the Extraction microplate with the domed cap strips provided.

6.5.7 Place the Extraction microplate into the thermocycler and begin the DNA extraction program on the thermocycler.

6.5.8 When the Extraction program has been completed, take out the Extraction microplate. All organisms are now lysed and inactivated.

6.5.9 Centrifuge the Extraction microplate for 5 minutes at 1,800 × g. The supernatant can now be used in the PCR amplification method.

6.6 PCR Amplification Method

6.6.1 Dispense the contents from DT-1 (squeeze bottle) into the DT-2 vial to obtain reconstituted DT-2.

6.6.2 Recap the DT-2 vial and mix by inversion to ensure complete dissolution of desiccated reagent.

6.6.3 Pour reconstituted DT-2 into a multi-channel pipettor basin.

6.6.4 Take out a Detection microplate or strips from its pouch (place them on the strip holder).

6.6.5 Aliquot 15 µL of reconstituted DT-2 into the wells of the Detection microplate or strips.

6.6.6 Transfer 10 µL of extracted DNA from the Extraction microplate into the wells of the Detection microplate or strips.

6.6.7 Seal the Detection microplate or strips with the optical-grade caps provided.

6.6.8 Seal the Extraction microplate using the provided microplate seals and store at 4°C until the analysis is completed.

6.6.9 Vigorously invert the Detection microplate or strips 4 times to ensure that the contents are thoroughly mixed in the wells.

6.6.10 Gently tap the Detection microplate or strips to remove any air bubbles from the bottom of wells.

6.6.11 Vortex for 1 minute at maximum speed.

6.6.12 Centrifuge the Detection microplate or strips for 1 minute at 1,800 × g to ensure that all the PCR mixture, including the sample's DNA extract, is in the bottom of the wells.

6.6.13 Secure the Detection microplate or strips tightly in the PCR instrument. Make sure well A1 is in the upper left corner. Click on "Start Detection" to initialize the PCR protocol.

6.6.14 When the Detection program has been completed, remove and discard the Detection microplate or strips.

6.7 Interpretation of Results

A positive PCR test will result in a fluorescence response higher than the baseline produced by the negative controls within 40 cycles of the PCR amplification. The baseline cut-off fluorescence value is determined and set by the system. A negative PCR test will normally not produce visible fluorescence. If fluorescence occurs above the baseline cut-off value in the negative controls, the results of the test are invalidated and the analysis must be repeated with precautions taken to eliminate possible sources of error.

6.8 Confirmation

Using the Listerboost enrichment broth, presumptive positive results may be confirmed with the plating and confirmation steps of a cultural method (*i.e.* MFHPB-07 or MFHPB-30), as appropriate.

7. References

7.1 AdiaFood user manual published by AES Chemunex, including the product insert for the enrichment and detection of *Listeria* spp.

7.2 Pagotto, F., Hébert, K., and Farber J. 2011. MFHPB-30. Isolation of *Listeria monocytogenes* and other *Listeria* spp. from foods and environmental samples. In: Volume 2, *Compendium of Analytical Methods*.

7.3 Warburton, D., Boville, A., Pagotto, F., Daley, E., and Chow C. 2011. MFHPB-07. The isolation of *Listeria monocytogenes* and other *Listeria* spp. from foods and environmental samples using Palcam broth. In: Volume 2, *Compendium of Analytical Methods*.

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