



Health Products and Food Branch

Ottawa

Detection of *Escherichia coli* O157 (including H7) in Select Foods Using the Neogen® Molecular Detection System Test Kit Version 2

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1. Application

This method is applicable to the detection of *E. coli* O157 (including H7) 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 raw meat 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 Neogen® Molecular Detection Assays use isothermal amplification of nucleic acid sequences combined with bioluminescence technology to detect the amplification of the target organism's genetic material after 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 Neogen® 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 Neogen® Molecular Detection System to offer a sample preparation process with only two transfer steps. LAMP utilizes multiple primers that recognize distinct regions of the target *E. coli* O157 gene and uses *Bst* polymerase, a unique enzyme with DNA strand-displacement activity, to enable continuous and rapid isothermal amplification (8.6).

The Neogen® 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 Neogen® 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 Neogen® Molecular Detection System, allows the target DNA to be amplified continuously, generating more than 10⁹ 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.

6.1 Neogen® Molecular Detection Assay 2, *E. coli* O157 (including H7) test kit (MDA2ECO96) includes:

- Lysis Solution (LS) tubes (96 – 12 strips of 8 tubes)
- *E. coli* O157 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 Neogen® Molecular Detection Instrument and Accessory Kit (MDS100) includes:

- Neogen® Molecular Detection Speed Loader Tray
- Neogen® Molecular Detection Chill Block Insert
- Neogen® Molecular Detection Chill Block Tray
- Neogen® Molecular Detection Cap/Decap Tool—Lysis
- Neogen® Molecular Detection Cap/Decap Tool—Reagent

6.3 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 for the formulae of individual media.

- Buffered Peptone Water, ISO formulation (BPW-ISO)

6.4 Additional Materials

- Neogen® Molecular Detection Heat Block Insert (MDSHBIN)
- Neogen® Molecular Detection Matrix Control (MDMC96)
- 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) or water baths capable of maintaining the temperatures and associated tolerances prescribed within this method
- Dry Block Heating unit that can accommodate the Neogen® Molecular Detection Heat Block Insert (15 cm × 9.5 cm or 5.875"×3.75") and is capable of maintaining a temperature of 100 ± 1°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-ISO prepared, pre-warmed to 39.5-41.5°C before use.
- 7.2.3 Turn on block heater with the Heat Block Insert in to achieve 100 ± 1°C prior to analysis.
- 7.2.4 Log in to the Neogen® Molecular Detection Software and turn on the Neogen® Molecular Detection Instrument.

7.3 Preparation of Sample

To ensure a representative analytical unit, obtain the analytical unit by taking a portion from several locations within the sample unit.

- 7.3.1 Prepare samples according to Table 1 or Table 2.

- 7.3.2 Blend or stomach as required for thorough mixing. It is preferable to stomach using a smaller volume of media and then add additional media to the required final volume.
- 7.3.3 Incubate samples according to Tables 1 and 2. Continue to incubate samples while the screening test is proceeding, to ensure that the required 22 – 24 hours of incubation have been completed if confirmation by MFHPB-10 is required.

Table 1. Enrichment Protocol for Individual Samples 25-65 g

Food Type	Preparation	Enrichment Temperature	Enrichment Time
Raw Meat (processed and unprocessed)	Prepare a 1 in 10 dilution (1:9) (e.g., 25 g sample in 225 mL of BPW-ISO)	41.5 ± 0.5°C	10-18 h
Frozen Meat (raw or raw processed)			18-24 h

Table 2. Enrichment Protocol for Composite Samples 325-365 g

Food Type	Preparation	Enrichment Temperature	Enrichment Time
Raw Meat (processed and unprocessed)	Prepare a 1 in 4 dilution (1:3) (e.g., 325 g sample in 975 mL of BPW-ISO)	41.5 ± 0.5°C	10-18 h
Frozen Meat (raw or raw processed)			18-24 h

7.4 DNA Extraction – Lysis Step

Note: Using the Neogen Matrix Control (MC) is suggested to determine if the matrix has the ability to impact the assay results, as the assay does not include an internal amplification control (IAC). The inclusion of a MC for a given sample provides a quality control function similar to an IAC, to provide an indication that the specific matrix did not inhibit the assay resulting in a potential false negative result. A matrix can be defined as a type of product with intrinsic properties such as composition and process. Differences between matrices may be as simple as the effects caused by differences in their processing or presentation for example, raw versus pasteurized; fresh versus dried, etc.

- 7.4.1 Allow the Lysis Solution (LS) tubes to warm up at 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 Neogen® 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 including all controls.
- 7.4.5.5 When all samples have been transferred, transfer 20 µL of sterile BPW-ISO into a LS tube to act as a negative control (NC) (unless running own internal controls).
- 7.4.6 Verify that the temperature of the Neogen® Molecular Detection Heat Block Insert is at 100 ± 1 °C. Place the rack of LS tubes in the Neogen® Molecular Detection Heat Block Insert and heat for 15 ± 1 min. The color 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 Neogen® Molecular Detection Chill Block Insert at ambient temperature 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 Neogen® 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. Select one Reagent Control (RC) tube and place in 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. If a RC is used, 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.

- 7.5.4 Use the Neogen® 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 μ L of Sample lysate from the upper portion of the liquid (avoid precipitate) 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 Neogen[®] 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 7.5.5 to 7.5.7 as needed, for the number of samples to be tested including all controls. Transfer 20 μ L of NC lysate into an RC tube to serve as a positive control unless using own positive controls.
- 7.5.9 Load capped tubes into a clean and decontaminated Neogen[®] Molecular Detection Speed Loader Tray. Close and latch the Neogen[®] Molecular Detection Speed Loader Tray lid.
- 7.5.10 Review and confirm the configured run in the Neogen[®] 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 Neogen[®] Molecular Detection Speed Loader Tray into the Neogen[®] 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 Neogen[®] Molecular Assay *E. coli* O157 (including H7) 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.

If the results of the matrix control are negative, the test is considered invalid. Proceed with confirmation as described in 7.7.

7.7 Confirmation of Presumptive Positive Results

Using the enrichment broth, proceed with the immunomagnetic separation and confirmation steps as described in MFHPB-10 (8.8).

7.8 Negative and Indeterminate Results

Negative results can be reported as *E. coli* O157:H7/NM Not Detected.

In the event of any unusual light output, the algorithm labels this as "Inspect". The presence or absence of the target pathogen was indeterminate. Repeat the assay for any "Inspect" samples beginning at step 7.4. Repeated "Inspect" (i.e., more than one) must be treated as presumptive positive. Proceed with confirmation as described in 7.7.

8. References

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9. Preparation of Media

Buffered Peptone Water (BPW) (ISO)

Basal Medium	
Enzymatic Digest of Casein	10.0 g
Sodium chloride (NaCl)	5.0 g
Disodium Hydrogen Phosphate (anhydrous)	3.6 g
Potassium dihydrogen phosphate	1.5 g
Distilled water	1.0 L
Final pH 7.2 ± 0.2 at ambient temperature	

Complete Medium:

Dissolve the ingredients in 1.0 L of distilled water. Dispense into appropriate glassware and autoclave at 121°C for 15 min.

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