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Laboratory Procedure **MFLP-115**
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Health Products and Food Branch

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Detection of *Escherichia coli* O157:H7/NM in Select Foods Using the BAX® System Real-Time PCR assay for *E. coli* O157:H7 Exact

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1. Application

This method is applicable to the detection of *E. coli* O157:H7/NM 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 on the BAX® System Q7 instrument.

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 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 BAX® system is a presence/absence screening tool that uses Polymerase Chain Reaction (PCR) technology for rapid amplification and fluorescence detection. The BAX® System Real-Time PCR assay for *E. coli* O157:H7 Exact kit detects *E. coli* O157:H7/NM in raw processed meats and raw unprocessed meats and raw frozen meats, following enrichment in BAX® System MP media.

3. Principle

This assay uses PCR to amplify a specific fragment of bacterial DNA, which is stable and unaffected by growth environment. The fragment is an *E. coli* O157:H7 specific gene sequence, indicating the presence of the organism. This method demonstrates a high degree of selectivity and specificity, detecting all known atypical variants of *E. coli* O157:H7, including non-motile (NM) strains that still carry the H7 genes (*E. coli* O157:H7/NM).

The BAX® System simplifies the PCR process by combining the primers, polymerase, nucleotides and positive control into a single sample tablet that is already packaged inside the PCR tubes. Additionally, the automated fluorescent detection allows for closed-tube testing, eliminating the potential for carry-over contamination with amplified DNA.

The assay employs the use of a Scorpion™ probe, which, when incorporated into a PCR product, causes an increase in emission signal. The BAX® system then measures the magnitude and characteristics of the fluorescent signal change, and BAX® system software algorithm evaluates the data to determine a positive or negative result.

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".

The media and reagents listed below are commercially available and are to be prepared and sterilized according to the manufacturer's instructions.

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 BAX® System Q7 Start-Up Package (Cat. No. ASY2018) – includes:

- Cycler/detector
- Computer workstation with Microsoft Windows® operating system and BAX® system software
- Automated Thermal Block (cat.no. DMCH2023) or dry block heaters with thermometer inserts for lysis tubes
- Capping/decapping tools
- Pipettes for reagent and sample transfers (5 µL, 10 µL, 10-50 µL 8 channel, repeat pipettor)
- Cooling block with inserts for lysis tubes and PCR tubes
- PCR tube holders
- Lysis tubes with caps and rack
- Barrier or filter tips for pipettes
- Powder-free nitrile gloves
- BAX® System User Guide

6.2 BAX® System Real-Time PCR Assay for *E. coli* O157:H7 Exact (Cat. No. KIT2039; sufficient for 96 tests) includes PCR tubes with tablets, flat optical caps, 2 lysis buffer bottles (12 mL/bottle) and protease solution (400 µL/vial) used to prepare working lysis reagent.

6.3 Enrichment Broths

- BAX® System MP Media [MED2003 (2.5 kg) and MED2016 (StatMedia™)]

6.4 Additional Materials

- Incubators or water baths capable of maintaining the temperatures and associated tolerances prescribed within this method.
- Stomacher for homogenizing samples

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 of Samples

7.2.1 Prepare the required volume of BAX® System MP media according to manufacturer's instructions. Pre-warm the enrichment broth to 40-42°C for individual samples and to 43-45°C for composite samples.

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

7.2.3 Prepare samples according to Table 1 or Table 2.

7.2.4 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.

7.2.5 Incubate samples according to Tables 1 and 2. Continue to incubate samples while the BAX® 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 to 65 g

Food Type	Primary Enrichment	Enrichment Temperature	Enrichment Time
Raw Meat (Processed and Unprocessed)	Prepare a 1 in 10 dilution (1:9) (e.g., 25 g sample and 225 mL of BAX® System MP media)	42 ± 0.5°C	8-24 h
Frozen Meat (Raw or raw processed)			

Table 2. Enrichment Protocol for Composite Samples 325 g - 375 g

Food Type	Primary Enrichment	Enrichment Temperature	Enrichment Time
Raw Meat (Processed and Unprocessed)	Prepare a 1 in 5 dilution (1:4) (e.g., 325 g sample in 1300 mL of BAX® System MP media)	42 ± 0.5°C	8-24 h
Frozen Meat (Raw or raw processed)			10-24 h

7.3 Preparation of Equipment

7.3.1 Refrigerate cooling blocks overnight.

7.3.2 Pre-heat heating blocks to 37°C and 95°C.

7.3.3 Initialize cycler/detector as outlined in BAX® System Q7 *User Guide*.

7.3.4 Create a rack file.

7.3.5 Select RUN FULL PROCESS in the menu bar to warm up the cycler/detector prior to placing your samples into the system.

7.4 Sample Lysis

7.4.1 Prepare the lysis tubes.

7.4.2 Arrange the required number of lysis tubes (one for each sample and one for the "blank") in the rack according to the rack file.

7.4.3 Prepare lysis reagent by pipetting 150 µL of protease into one 12 mL bottle of the lysis buffer.

7.4.4 Add 200 µL of lysis reagent to each lysis tube.

7.4.5 Transfer 20 µL of enriched sample to the corresponding lysis tube.

7.4.6 Secure the caps. When using a manual heating block, heat the tubes at 37°C for 20 minutes, then at 95°C for 10 minutes. When using the Automated Thermal Block, use preset parameters which includes cooling of samples (step 7.4.7).

7.4.7 Place the lysis tubes in cooling block for at least 5 minutes.

7.5 Hydration of PCR Tablets with Lysates

7.5.1 Place PCR tube holder into PCR cooling block.

7.5.2 Place one PCR tube per sample in the holder.

7.5.3 Remove and discard lid from one strip of tubes at a time.

7.5.4 Using a multichannel pipettor, transfer 30 µL of each lysed sample into a corresponding PCR tube.

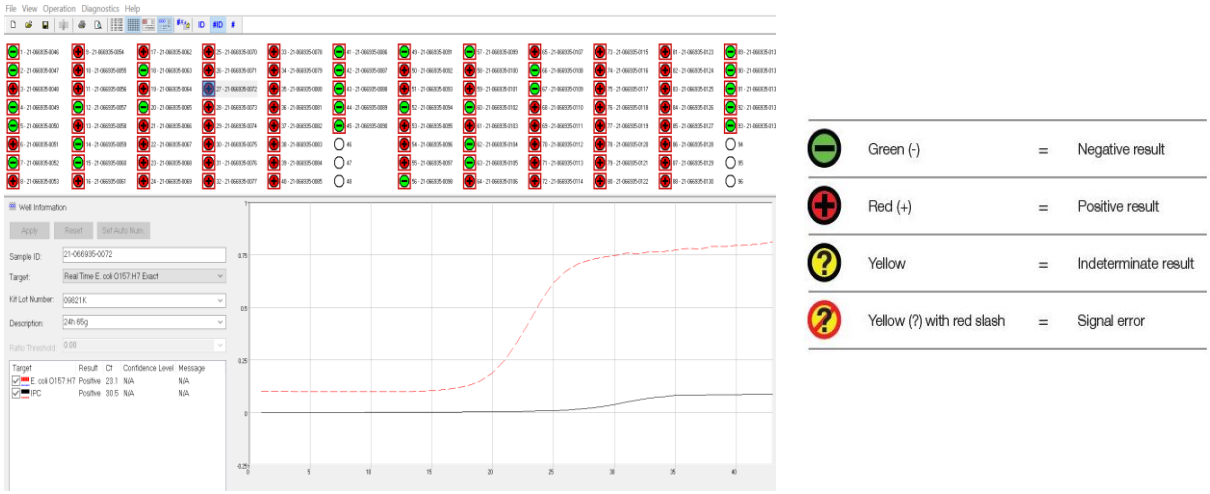
7.5.5 Cover tubes with a new optical cap strip and secure tightly. Repeat for all samples.

7.5.6 After the completion of hydration of all PCR tablets, let PCR tubes sit in the cooling block for 10-30 minutes before loading into the BAX® System instrument. Note: Do not let PCR tubes sit for more than 30 minutes.

7.6 Amplification and Detection

Follow the screen prompts in the PCR Wizard to load your samples, run the program and unload your samples. See BAX® System Q7 *User Guide* for reference.

7.7 Review of Results



For indeterminate results, repeat analysis beginning with step 7.4.5.

7.8 Confirmation of Presumptive Positive Results

Using the enrichment broth, proceed with the immunomagnetic separation, plating and confirmation steps described in the confirmation method MFHPB-10 (8.2).

7.9 Negative and Indeterminate Results

For indeterminate results, repeat analysis beginning with step 7.4.5. Repeated

indeterminate (i.e., more than one) or error results must be treated as presumptive positive. Proceed with confirmation as described in 7.8.

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

8. References

8.1 BAX® System Q7 User Guide (current version).

8.2 Microbiological Methods Committee. 2017. Isolation of *Escherichia coli* O157:H7/NM from foods and environmental surface samples (MFHPB-10). Volume 2. *The Compendium of Analytical Methods*.

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