

**United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program**

SOP No.: MDP-MTH-05		Page 1 of 5
Title: Detection of <i>Escherichia coli</i> O157:H7 in Fresh Produce by BAX [®] PCR		
Revision: 02	Replaces: 01	Effective: 01/01/06

1. Purpose

To provide a standard procedure for detection of *Escherichia coli* (*E. coli*) O157:H7 in fresh produce using the BAX[®] system by all laboratories participating in the USDA/AMS Microbiological Data Program (MDP). The BAX[®] system is used here as a screening tool.

2. Scope

This standard operating procedure (SOP) shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities. This SOP represents minimum MDP requirements and is presented as a general guideline. Each laboratory shall have written procedures that provide specific details concerning how the procedure has been implemented in that laboratory.

3. Principle

The BAX[®] PCR system is a DNA-based method developed by DuPont Qualicon for detecting bacterial pathogens in food and environmental samples. The sensitivity and the accuracy are a result of the use of polymerase chain reaction (PCR) to amplify DNA fragments specific for a given target organism. The amplification of a specific DNA fragment is monitored using a dye that fluoresces upon intercalating with the double-stranded DNA of the amplified PCR product.

4. Outline of Procedures

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5. References

- 5.1 BAX[®] System, User Guide and Protocol Summary, DuPont Qualicon
- 5.2 Peter Feng and S. D. Weagant. Bacteriological Analytical Manual Chapter 4A. Diarrheagenic *Escherichia coli*. <http://www.cfsan.fda.gov/~ebam/bam-4a.html> (last accessed 11/09/04)
- 5.3 SOP MDP-DATA-01 Record Keeping and Results Reporting
- 5.4 SOP MDP-LABOP-02 Sample Receipt, Elution, Preenrichment, and DNA Extraction
- 5.5 SOP MDP-MTH-06 *Escherichia coli* O157 Immunomagnetic Separation (IMS) Method and Presumptive Confirmation
- 5.6 SOP MDP-QA-03 Quality Assurance (QA) Controls
- 5.7 Evaluation of Enrichment Ability of Universal Pre-Enrichment Broth (UPB) for *Salmonella* ser. Typhimurium and *E. coli* O157:H7 from Produce Commodities. Final study report, Division of Consolidated Laboratory Services (DCLS), Department of General Services, Commonwealth of Virginia. October 2005.

6. Procedures

- 6.1. Equipment and Materials
 - 6.1.1. BAX[®] System
 - 6.1.2. BAX[®] PCR assay kit for *E. coli* O157:H7
 - 6.1.3. Additional materials needed to perform procedure as listed in BAX[®] System User Guide & Protocol Summary.

Note: Use BAX[®] lysis buffer without protease.
 - 6.2. List of Controls: (Specific strains are listed in SOP MDP-QA-03)
 - 6.2.1. DNA from Universal Preenrichment Broth (UPB) (Blank)
 - 6.2.2. DNA from negative culture control from SOP MDP-LABOP-02
 - 6.2.3. DNA from positive culture control from SOP MDP-LABOP-02
 - 6.2.4. DNA from positive produce culture control from SOP MDP-LABOP-02
 - 6.2.5. BAX[®] lysis buffer (without protease)
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6.2.6. Carry all controls through this entire procedure, including any necessary cultural confirmation. If any of the controls fail to yield a satisfactory result refer to SOP MDP-QA-03.

6.3. Safety

E. coli O157:H7 is a human pathogen and is shown to cause disease with a low infectious dose. The laboratory personnel must follow CDC guidelines for working with Class II pathogens. Use of lab coats, gloves and eye protection is mandatory. A Class II biosafety laminar flow hood (cabinet) is recommended.

6.4. BAX[®] Analysis

6.4.1. Use DNA extracted from the UPB preenriched cultures. Keep the DNA under refrigeration or in a cooling block until ready to use.

6.4.2. Transfer PCR tubes to the cooling block.

6.4.3. Hydrate BAX[®] reagent pellet by adding 50 µL of BAX[®] lysis buffer (without protease) to the PCR tubes.

6.4.4. Transfer a 5-µL aliquot of extracted DNA prepared from each of the UPB-preenriched samples and controls (from SOP MDP-LABOP-02) to the PCR tubes.

Note: To minimize contamination, keep samples and controls separate.

Note: Do not add DNA sample directly to the BAX[®] reagent pellet without prior addition of lysis buffer (without protease) alone or as a DNA:lysis buffer mix.

6.5. BAX[®]-Positive Samples

6.5.1. When a sample is positive or indeterminate via BAX[®] proceed to attempt to isolate *E. coli* O157:H7 as directed by SOP MDP-MTH-06.

6.6. Reporting

6.6.1. A BAX[®]-positive result is considered a preliminary positive.

6.6.2. Data shall be reported according to SOP MDP-DATA-01.

Disclaimer: Reference to brand names (kits, equipment, media, reagents, etc.) does not constitute endorsement by this agency.

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