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

SOP No.: MDP-MTH-12		Page 1 of 6
Title: BAX® System Real-time PCR Assay for *Escherichia coli* O157:H7 in Fresh Produce		
Revision: 1	Replaces: 7/1/2010	Effective:09/01/2011

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).

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® Realtime 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 unique to the target organism. The specific amplification of target genes is monitored in realtime via the generation of fluorescent signals.

4. Safety

E. coli O157:H7 is a human pathogen with a low infectious dose. Laboratory personnel should utilize Biosafety Level II (BSL-2) practices for microbiological manipulations of known and potential pathogens. A BSL-2 laminar flow biosafety cabinet is recommended for activities with potential for producing aerosols of pathogens. Material Safety Data Sheets (MSDS) should be obtained from manufacturers for media, chemicals and reagents used in the analysis and personnel who will handle the materials should know the location of and have ready access to the MSDS sheets for reference.

5. Outline of Procedures

6. References

- BAX® System Real-Time PCR Assay – *E. coli* O157:H7, Part D14203648 DuPont Qualicon.
- USDA Microbiological Data Program (MDP) 2010 Multi-Laboratory Method Verification Study: Realtime PCR assays for detecting shiga toxin DNA sequences of E. coli (STEC) O157:H7 and/or non-O157 serotypes. May 2010.
- BAM Online, Chapter 4a: Diarrheagenic *Escherichia coli.* Last updated: 08/2009. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM070080 (last accessed 06/2010)
- U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health: Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition. http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm
- SOP MDP-DATA-01 Record Keeping and Results Reporting.
- SOP MDP-LABOP-02 Sample Receipt, Elution, Preenrichment, and DNA Extraction.
- SOP MDP-MTH-06 *Escherichia coli* O157 Immunomagnetic Separation (IMS) Method and Presumptive Confirmation.
- SOP MDP-QA-03 Quality Assurance (QA) Controls.

7. Procedures

7.1 Equipment and Materials

- BAX® Q7 System, DuPont Qualicon
- BAX® System Real-time PCR assay kit for *E. coli* O157:H7, DuPont Qualicon
- BAX® lysis buffer without protease. Do not add protease to lysis buffer.
- Additional materials needed to perform procedure as listed in BAX® System Real-Time PCR Assay for E. coli O157:H7 Kit Insert

7.2 Controls (Specific strains are listed in SOP MDP-QA-03) - 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.

- Uninoculated Media Control (DNA from uninoculated UPB)

- No Template Control: Transfer 27 uL of lysis buffer without protease and 3 uL of PCR grade water.
- Negative Cultural Control: DNA from *E. coli* (MDP-017) negative culture control from SOP MDP-LABOP-02.
- Positive Cultural control: DNA from MDP-004 *E. coli* O157:H7 positive culture control from SOP MDP-LABOP-02.
- Positive Produce Control: DNA from inoculated produce culture control from SOP MDP MTH-11 and refer to SOP MDP LABOP-02.

7.3 Positive Samples - STEC pooled and/or individual positive samples (Rt-PCR positive for any one or combinations of stx1, stx2 and uidA genes) from SOP MDP MTH-11. Refer to SOP MDP-LABOP-02 for commodity-specific DNA sample source. (Keep DNA refrigerated or in cold block until ready to use.)

7.4 BAX® Analysis

7.4.1 Refer to the BAX® System Real-Time PCR Assay for *E.coli* O157:H7 Kit insert for equipment set-up and sample loading procedures. Do not perform Kit Insert steps, 4.1 through 4.7 and at step 5.4, instead do the following:

7.4.1.1 Hydrate each BAX® reagent pellet by adding 27 µL of BAX® lysis buffer (<u>without protease</u>) to the PCR tubes. Do not add DNA sample directly to the BAX® reagent pellet without prior addition of lysis buffer (without protease).

7.4.1.2 Transfer 3µL of undiluted extracted DNA prepared from each UPB enriched samples and controls (from SOP MDP-LABOP-02) to the appropriate BAX® PCR tubes and seal the tubes.

Note: To minimize contamination, keep samples and controls separate. PCR tablets shall be hydrated and re-sealed within 10 minutes after removing the caps from the PCR tubes.

7.4.2 Return to BAX® Real-time O157 Kit insert and complete remaining steps, 6.1 through 6.3, to begin automated processing of samples.

7.4.3 For positive BAX® Real-time O157 assay results proceed to SOP MDP-MTH-06, *Escherichia coli* O157 Immunomagnetic Separation (IMS) and Cultural Methods.

7.4.4 For negative BAX® Real-time O157 assay results, refer to SOP MDP-DATA-01 for reporting requirements.

7.4.5 For Indeterminate BAX® Results

7.4.5.1 Remove the refrigerated original pooled and/or individual extracted DNA samples and centrifuge the DNA samples for approximately 10 seconds. Re-analyze the centrifuged DNA samples according to section 7.4. of this SOP.

7.4.5.2 If any individual sample shows an indeterminate result, proceed with SOP MDP-MTH-06 *Escherichia coli* O157 Immunomagnetic Separation (IMS) Method and Presumptive Confirmation for cultural confirmation of individual BAX® positive samples.

7.5 Data Analysis - Refer to section 7 of the BAX® Realtime PCR Kit insert for results interpretation guidance.

7.6 Reporting

7.6.1 A BAX® Real-time PCR O157 assay positive result without cultural confirmation and identification is considered a preliminary positive.

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

Mary Tijerina *31 August 2011*

Revised by: Mary Tijerina Date
Microbiologist, Monitoring Programs Division
8609 Sudley Rd, Ste 206, Manassas, VA 20110
(703) 330-2300 x111

Cynthia Mangione *1 September 2011*

Reviewed by: Cynthia Mangione Date
Chairperson, MDP Technical Advisory Group
New York Department of Agriculture & Markets
Food Laboratory, Bldg 7, State Office Campus
1220 Washington Ave, Albany, NY 12235
(518) 457-0906

Shanker Reddy *1 September 2011*

Reviewed by: Shanker Reddy Date
Molecular Biologist, Monitoring Programs Division
8609 Sudley Road, Ste 206, Manassas, VA 20110
(703) 330-2300 x135

Diana Haynes *1 September 2011*

Approved by: Diana Haynes Date
Deputy Director, Monitoring Programs Division
8609 Sudley Road, Suite 206, Manassas, VA 20110
(703) 330-2300x134

Revision 1 August 2011 Monitoring Programs Division

- Updated Outline of Procedures, Section 5.
- Replaced the word "must" with the word "shall" in *Note* under section 7.4.1.2
- Deleted Section 7.4.3 and following *Note* due to no longer using mEC+n, "For positive pooled and/or individual BAX® Real-*time* samples, transfer 25ml from individual UPB enriched samples to 225ml mEC+n bro*th an*d incubate at 42 ± 2°C for 18-24 hours. *Note: If this step 7.4.3 has been previously completed per SOP MDP-MTH-11, do not repeat."*
- Renumbered SOP sections.
- Added text to (new) Section 7.4.3 to indicate when to refer to SOP MDP-MTH-06.