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

SOP No.: MDP-MTH-04		Page 1 of 9
Title: Detection of *Salmonella* in Fresh Produce by BAX® PCR		
Revision: 05	Replaces: 09/25/09	Effective: 07/01/2010

1. Purpose

To provide a standard procedure for detection of *Salmonella* species 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® PCR system is a DNA-based screening method developed by DuPont Qualicon for detection of 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.

4. Safety

Laboratory personnel should utilize Biosafety Level II (BSL-2) practices for MDP 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 User Guide & Protocol Summary, DuPont Qualicon
- SOP MDP-DATA-01 Record Keeping and Results Reporting
- SOP MDP-LABOP-02, Sample Receipt, Elution, Pre-enrichment, and DNA Extraction
- SOP MDP-MTH-09, Detection of *Salmonella* using VIDAS® Method
- SOP MDP-MTH-10, Isolation and Identification of *Salmonella* using Cultural Methods
- SOP MDP-QA-03, Quality Assurance (QA) Controls

7. Procedures

7.1 Equipment and Materials

- BAX® Q7 System, DuPont Qualicon
- BAX® PCR assay kit for *Salmonella* species, DuPont Qualicon
- Additional materials needed to perform procedure as listed in BAX® System User Guide & Protocol Summary

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

Carry all controls through this entire procedure, including any necessary cultural confirmation. Refer to SOP MDP LABOP-02 for control setup. If any of the controls fail to yield a satisfactory result refer to SOP MDP-QA-03.

- Uninoculated Media Control: DNA from uninoculated Universal Preenrichment Broth (UPB)
- Negative Cultural Control : DNA from *Escherichia coli* (MDP-017) negative culture control from SOP MDP-LABOP-02
- Positive Cultural Control: DNA from *Salmonella typhimurium* (MDP-014) positive culture control from SOP MDP-LABOP-02
- Positive ProduceControl: DNA from inoculated produce culture control from SOP MDP-LABOP-02
- No-template Control: Transfer 45µL of BAX® lysis buffer (without protease) and add 5 uL of PCR grade water.

7.3 BAX® Analysis

7.3.1 Refer to the BAX® User Manual for BAX® run set-up and sample loading procedures.

7.3.2 For all commodities except alfalfa sprouts, use DNA extracted from the pooled UPB pre-enriched samples. Keep the DNA under refrigeration or in a cooling block until ready to use. Refer to SOP MDP-LABOP-02.

7.3.3 For alfalfa sprouts, use DNA extracted from individual UPB pre-enriched samples. Keep the DNA under refrigeration or in a cooling block until ready to use. Refer to SOP MDP-LABOP-02.

7.3.4 Transfer enough BAX® PCR tubes for all pooled/non-pooled (sprouts) samples and controls to the cooling block.

7.3.5 Hydrate each BAX® reagent pellet by adding 45µL of BAX® lysis buffer (without protease) to the PCR tubes

7.3.6 Transfer 5µL of extracted DNA prepared from each of the UPB-pre-enriched samples and controls (from SOP MDP-LABOP-02) to the appropriate BAX® PCR tubes. Do not add

DNA sample directly to the BAX® reagent pellet without prior addition of lysis buffer (without protease).

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

7.3.7 Start BAX® PCR analysis according to the BAX® User Manual.

7.4 BAX® Positive Samples

7.4.1 When a pooled sample is BAX® positive, extract DNA from individual samples that constituted this pool by following SOP MDP-LABOP-02.

7.4.2 Transfer 5µL of extracted DNA from the positive pool and individual samples, along with appropriate controls, to hydrated BAX® PCR tubes (section 7.3.5) and run the analysis.

7.4.3 Proceed to SOP-MTH-09, Detection of *Salmonella* using VIDAS® method, for secondary testing of pooled and/or individual BAX® positive samples. A pooled positive BAX® result followed by pooled and individual negative VIDAS® results and individual negative BAX® results will be recognized as a confirmed negative. If any questions, consult MPO.

7.4.4 Proceed to SOP MDP-MTH-10, Isolation and Identification of *Salmonella* using Cultural Methods for cultural confirmation of individual BAX® positive samples. In order to increase the likelihood of cultural isolation, laboratories should begin cultural methods as soon as possible from the individual positive samples as well as from the BAX® positive pooled culture

7.5 Indeterminate BAX® results

7.5.1 Remove the refrigerated original pooled DNA samples and centrifuge the DNA samples for approximately 10 seconds. Re-analyze the centrifuged DNA samples according to section 7.3 of this SOP.

7.5.2 If results are still indeterminate, extract the DNA according to LABOP-02 from each of the individual refrigerated UPB enriched samples from the indeterminate pooled sample.

7.5.3 Analyze each individual extracted DNA sample according to section 7.3 of this SOP.

7.5.4. If an individual sample shows an indeterminate result, proceed with sections 7.4.3 and 7.4.4 of this SOP.

7.6 Data Analysis Refer to the BAX® User Manual for melting curve interpretation

7.7 Reporting - A BAX® positive result will be reported to MPO as a preliminary positive result as soon as possible per 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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Revision 05 June 2010 Monitoring Programs Office

- Removed attachment
- Added Safety, Section 4
- Added Outline of Procedures, Section 5
- Updated References, Section 6
- Updated Control list
- Updated section 7.5

Revision 04 September 2009 Monitoring Programs Office

- Updated References, Section
- Revised Section 5.3
- Revised Section 5.4
- Revised Section 5.6
- Added Atch 1, Salmonella Flowchart

Revision 03 March 2009 Monitoring Programs Office

- Deleted "The BAX system is used here as a screening tool" from the Purpose.
- Added Data Analysis to the Outline
- Deleted MDP-MTH-03A from the References and added MDP-MTH-10.
- Added "DuPont Qualicon" to 6.1.1 and 6.1.2
- Deleted "List of" from Controls in the Outline and section 6.2.
- Added "Uninoculated Media Control" and "uninoculated" to 6.2.1.
- Added "Negative Cultural Control" and *Escherichia coli* (MDP-017)" to 6.2.2.
- Added "Positive Cultural Control" and "*Salmonella typhimurium* (MDP-014)" to 6.2.3.
- Added "Positive Matrix (produce) Control" and "inoculated" to 6.2.4.
- Deleted the Lysis Buffer control from 6.2.
- Added 6.3.1
- Added "pooled" to 6.3.2. Added SOP reference to 6.3.2.

- Added "enough BAX" and "for all pooled/non-pooled (sprouts) samples and controls" to 6.3.3.
- Added "each" to 6.3.5.
- Deleted "aliquot" from 6.3.6 and added "appropriate BAX" to 6.3.5.
- Added 6.3.7 and deleted "Proceed as directed by BAX User Manual".
- Added 6.4.1 procedure for BAX positive pooled samples.
- Added 6.4.2 for indeterminate BAX results.
- Added 6.5 Data Analysis.

Revision 02 January 2006 Monitoring Programs Office

- Added references to UPB
- Modified BAX® procedure to allow extracted DNA
- Screening procedure now performed on individual samples (rather than 3 pooled samples)
- All cultural procedures now included in SOP MDP-MTH-03A

Revision 01 January 2005 Monitoring Programs Office

- Removed references to outdated control strains
- Removed all duplicative instructions found in BAX® Users Manual
- Added provision to obtain 25 mL wash eluate for positive produce control