

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

SOP No.: MDP-MTH-04		Page 1 of 9
Title: BAX <sup>®</sup> System for Detection of <i>Salmonella</i> in Fresh Produce		
Revision: Original	Replaces: NA	Effective: 10/01/03

**1. Purpose:**

To provide standard procedures for use of the BAX system for the analysis of *Salmonella* in fresh produce 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 that may impact the program.

**3. Principle:**

The BAX<sup>®</sup> system is a DNA-based screening method for detecting pathogens in food and environmental samples developed by DuPont Qualicon. The sensitivity and the accuracy in detection are a result of the use of polymerase chain reaction (PCR) to amplify specific DNA fragments.

In the event that a laboratory experiences a BAX instrument failure, the laboratory may screen samples using the Neogen Reveal kit for *Salmonella*. Confirmed results from the Reveal kit may be reported according to standard procedures.

**4. Outline of Procedure:**

4.1	Equipment and Materials	6.1
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4.3	BAX Analysis	6.3
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**5. References:**

- 5.1 BAX *Salmonella* Validation Protocol
- 5.2 SOP MDP-LABOP-02, Sample Receipt and Elution Procedure (Note: use Buffered Peptone Water (BPW) with 0.1% Tween 80 as wash buffer)
- 5.3 BAM Chapter 5 *Salmonella*, October 2001, [www.cfsan.fda.gov](http://www.cfsan.fda.gov)
- 5.4 BAX System and User Guide & Protocol summary, Qualicon, Inc., 2002
- 5.5 BAM Chapter 3 Aerobic Plate Count, June 28, 2002, [www.cfsan.fda.gov](http://www.cfsan.fda.gov)

**6. Specific Procedures:**

- 6.1 Equipment and Materials
    - 6.1.1 Incubator set at 35 ±2°C (used for pre-enrichment)
    - 6.1.2 Incubator set at 37 ±0.5°C (used for BAX 3 hour re-growth step)
    - 6.1.3 Incubator set at 42 ±0.5°C (used for cultural confirmation)
    - 6.1.4 2 heating blocks: one set at 37 ±0.5°C and another set at 95 ±1°C (used for lysing cells to release DNA)
    - 6.1.5 Cooling block
    - 6.1.6 Sterile pipets: 1ml and 10ml and pipet aids
    - 6.1.7 Micropipet with sterile tips for a range of 5-200ul
    - 6.1.8 Dupont Qualicon BAX instrument
    - 6.1.9 BAX System User Guide
    - 6.1.10 BAX system PCR assay kit for *Salmonella*
    - 6.1.11 Lysis tubes, caps, optical caps, capping tools for BAX
    - 6.1.12 Sterile tubes
    - 6.1.13 VITEK System and User Guide
    - 6.1.14 Powder-free gloves
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6.2 Media and Reagents

6.2.1 Lactose Broth (LB)

6.2.2 Brain Heart Infusion Broth (BHI)

6.2.3 Rappaport-Vassiliadis Broth (RV); RV broth can be made by following the BAM-FDA protocol or bought commercially and used as per the manufacturer's directions.

6.2.4 Tetrathionate Broth (TT)

6.2.5 Bismuth Sulfite Agar

6.2.6 Hektoen Enteric Agar (HE)

6.2.7 Xylose Lysine Desoxycholate Agar (XLD)

6.2.8 *Salmonella enterica* serovar Poona strain carrying a pKT-kan plasmid that contains a gene coding for green fluorescent protein (GFP) for positive culture control (CDHS/MDL #00A 3563 (USDA/ARS/PW #RM235

6.2.9 *Enterobacter aerogenes* (negative culture control)

6.3 BAX Analysis

6.3.1 Pre-enrichment and Preparation

6.3.1.1 Remove a 25mL aliquot of each sample wash eluate and place into a suitable sterile container. Add 225 mL lactose broth to each of the containers. Incubate samples 18-24 hours at 35 ±2°C.

6.3.1.2 Ensure that cooling blocks are refrigerated overnight prior to beginning assay.

6.3.1.3 Turn on the BAX and then the associated computer. Launch the BAX application. Perform verification if needed (every two weeks – see BAX User Guide Chapter 3 for instructions).

6.3.1.4 Create a rack file. This may be done manually or by using the rack wizard. Define all wells for analysis, including controls. (For step-

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by- step directions, see BAX User Guide Chapter 2). Cyclor should be ready when samples have been prepared. A delay could result in non-specific binding of the cells to the tablet reagents.

### 6.3.2 Regrowth and Lysis

- 6.3.2.1 Mix lactose broth pre-enrichment culture well. To pool three samples, add a 10ul aliquot of each of the three pre-enriched samples to a 500uL tube of BHI broth. Incubate for 3 hours at 37±0.5C (alternatively, heating blocks may be used). Refrigerate the unused lactose broth culture until the BAX analysis is done. If there is no space to hold the containers, transfer at least 5mL of the broth to a sterile tube and refrigerate until the BAX analysis is done.
  - 6.3.2.2 Set one heating block at 37 ±0.5°C and the other to 95 ±1°C.
  - 6.3.2.3 Prepare lysis tubes. From kit, add 150uL of protease to one 12mL bottle of lysis buffer. If a smaller volume is needed, use 12.5uL protease to 1mL lysis buffer. Prepared lysis reagent may be stored up to two weeks at 2-8°C.
  - 6.3.2.4 Transfer 200uL of lysis reagent to each lysis tube.
  - 6.3.2.5 Transfer 5ul re-grown sample to lysis tubes using the micropipettor. Cap with lysis tube caps.
  - 6.3.2.6 Heat tubes for 20 min. in 37 ±0.5C heating block.
  - 6.3.2.7 Move tubes to 95 ±1C heating block for 10 minutes.
  - 6.3.2.8 Place tubes in lysis tube cooling block for 5 minutes. Complete use of all cooling blocks within 30 minutes of removal from refrigerator.
  - 6.3.2.9 Begin heating cyclor by clicking the “Run full process” icon on the BAX computer.
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- 6.3.2.10 Place a PCR tube holder into the PCR cooling block. Place one PCR tube per sample and one per control into the holder, according to rack file.
- 6.3.2.11 Remove PCR caps using decapping tool and discard. Remove cap from lysis tube using decapping tool.
- 6.3.2.12 Transfer 50uL of lysed sample from lysis tube into corresponding PCR tube. Repeat for all samples and controls. Cap tubes with optical caps using the capping tool. Keep samples in cooling block until cycler is ready for loading.

6.3.4 Use of BAX Instrument

- 6.3.4.1 When prompted by instrument, load rack. Follow prompts to begin cycling process.
- 6.3.4.2 After PCR and detection processes are complete, remove samples and follow prompts to display results.

6.4 The following controls are included and used in the MDP *Salmonella* setup.

- 6.4.1 Negative Media Control: 25mL BPW plus 0.1% Tween 80 (See SOP MDP-LABOP-02) to 225mL sterile lactose broth.
- 6.8.2 Negative Culture Control: 1mL *E. aerogenes* suspension in 225 mL sterile lactose broth.
- 6.8.3 Positive *Salmonella* pure culture control: 1mL *S. Poona* culture suspension in 225ml sterile lactose broth.
- 6.8.4 Positive produce culture control: A single produce sample chosen at random after eluate is inoculated into test cultures has the following additions of the control culture suspensions combined: 1 mL *E. coli* and 1mL *S. Poona*. Gently mix produce by hand – do not use shaker; 1 mL of the produce control eluate is inoculated into 225mL of sterile lactose broth.

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6.8.5 Use the controls for each daily batch of samples and carry forward through the remaining test steps and the BAM culture confirmation steps if a confirmation is needed on a sample. (See SOP MDP-MTH-03). If the positive control fails to yield a satisfactory result, or there is any question about the performance of the testing because of the control results, refer to SOP MDP-QA-01.

6.5 Reporting

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

6.6 Confirmation

6.6.1 When a pooled sample is positive via BAX, repeat the BAX for the three samples individually. Confirmation may be done on the same or following day. From each saved subsample lactose broth, add a 10uL aliquot to a 500ul tube of BHI broth. Incubate for 3 hours at  $37 \pm 0.5^{\circ}\text{C}$ . Refrigerate the unused pre-enriched broth until the BAX analysis is done. Follow this SOP from section 6.3.2.2.

6.6.2 To confirm samples testing positive via BAX:

6.6.2.1 Transfer 1mL of each pre-enriched lactose broth into 10mL tetrathionate broth (TT broth) and incubate at  $42 \pm 0.5^{\circ}\text{C}$  for 18-24 hours. Also, transfer 0.1mL of pre-enriched lactose broth into 10mL of RV (Rappaport-Vassiliadis) broth and incubate for 18-24 hours at  $42.0^{\circ} \pm 0.5^{\circ}\text{C}$ .

6.6.2.2 Streak a loopful of enrichment cultures on BS, HE and XLD plates. Pick at least one suspect colony from each of the differential and selective agar plates.

6.6.2.3 Following the manufacturer's instructions, identify isolate using Vitek. *Note: an alternative confirmatory method (e.g., API 20E or other approved biochemical method) may be used until a laboratory is fully trained/proficient in the use of the VITEK).*

6.6.2.4 Restreak positive *Salmonella* culture on TSA slant for shipping to appropriate reference laboratories (refer to SOP MDP-SHIP-02).

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09/29/03

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