

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

SOP No: MDP-MTH-03A		Page 1 of 8
Title: Isolation and Identification of <i>Salmonella</i> from Fresh Produce using Cultural Methods		
Revision: Revision 01	Replaces: 02/01/05	Effective: 01/01/06

**1. Purpose**

To provide a standard procedure for the isolation and identification of *Salmonella* species from fresh produce for all laboratories participating in the 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**

*Salmonella* is isolated on selective media and identified by biochemical tests including automated systems such as VITEK<sup>®</sup> developed by bioMérieux. The reliability and accuracy are a result of the use of a panel of biochemical tests that are used to characterize the test organism. The resulting profile is compared to known profiles of numerous microorganisms and a subsequent identification is made.

**4. Outline of Procedures**

Equipment and Materials	6.1
Media and Reagents	6.2
List of Controls	6.3
Isolation of <i>Salmonella</i>	6.4
Identification	6.5
Reporting and Shipping	6.6

**5. References**

- 5.1. VITEK<sup>®</sup> Users Manual
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- 5.2. Wallace Andrews and T. S. Hammack. Bacteriological Analytical Manual Chapter 5. *Salmonella*, updated September 2005, <http://www.cfsan.fda.gov/~ebam/bam-5.html> (last accessed 11/10/05)
- 5.3. SOP MDP-MTH-04, Detection of *Salmonella* in Fresh Produce by BAX<sup>®</sup> PCR
- 5.4. SOP MDP-DATA-01, Record Keeping and Results Reporting
- 5.5. SOP MDP-SHIP-03, Procedures for Packaging, Shipping, and Archiving Microbiological Cultures
- 5.6. SOP MDP-QA-03, Quality Assurance (QA) Controls

## **6. Specific Procedures**

### 6.1. Equipment and Materials

- 6.1.1. VITEK<sup>®</sup> System
- 6.1.2. VITEK<sup>®</sup> assay cards: GNI+ Card (Gram-Negative Plus) V1316
- 6.1.3. Water bath, capable of  $42 \pm 0.2^{\circ}\text{C}$
- 6.1.4. Additional equipment as referenced in a participating laboratory's internal procedures
- 6.1.5. Incubator,  $35 \pm 2^{\circ}\text{C}$

### 6.2. Media and Reagents

- 6.2.1. Rappaport-Vassiliadis (RV) medium - 16 x 150 mm sterile test tubes containing 10 mL aliquots.  
*Note:* If using commercial media, Oxoid brand of RV broth is preferred.
  - 6.2.2. Tetrathionate (TT) broth - 16 x 150 mm sterile test tubes containing 10 mL aliquots. On the day of use, add 20 mL iodine solution and 10 mL of sterile 0.1% aqueous Brilliant Green solution per 1 liter basal broth. To prepare the iodine solution, dissolve 5 g potassium iodide in 5 mL sterile distilled water, add 6 g resublimed iodine and stir to dissolve. Dilute to 20 mL.
  - 6.2.3. Xylose-lysine deoxycholate agar (XLD) or Xylose-lysine tergitol 4 (XLT4) agar
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- 6.2.4. Hektoen enteric agar (HE)
  - 6.2.5. Triple Sugar Iron agar (TSI)
  - 6.2.6. Lysine Iron Agar (LIA)
  - 6.2.7. Blood Agar
  - 6.2.8. Chromogenic media selective for *Salmonella* [e.g. CHROMagar<sup>®</sup> *Salmonella* (DRG), *Salmonella* SM ID2 (bioMerieux), or any equivalent media for differentiating *Salmonella* from *Citrobacter*, *Enterobacter* etc. and capturing *Salmonella typhi* or *S. paratyphi* A, if present]
  - 6.2.9 Polysomatic O and flagellar H antisera for *Salmonella* (Difco or demonstrated equivalent)
  - 6.3. List of Controls (Specific strains are listed in SOP MDP-QA-03)
    - 6.3.1. Carry all cultural controls from all screening methods previously completed through this entire procedure. Refer to SOP MDP-LABOP-02 for control setup.
    - 6.3.2. If any of the controls fail to yield a satisfactory result refer to SOP MDP-QA-03.
  - 6.4. Isolation of *Salmonella*
    - 6.4.1. Streak and/or plate (0.1 mL or can be diluted) the UPB preenriched BAX<sup>®</sup> *Salmonella*-positive culture identified using SOP MDP-MTH-04 on selective agar plates (XLD, HE, and chromogenic agar plates) for isolation. Incubate at 35 ± 2°C for 18-24 hours.
    - 6.4.2. Selective Enrichment and Plating
      - 6.4.2.1. Transfer 1 mL of the UPB preenriched BAX<sup>®</sup> *Salmonella*-positive culture identified using SOP MDP-MTH-04 into 10 mL TT broth and incubate at 42 ± 0.2°C for 18-24 hours.
      - 6.4.2.2. Transfer 0.1 mL of the UPB preenriched overnight culture identified using SOP MDP-MTH-04 that tested BAX<sup>®</sup> *Salmonella*-positive into 10 mL of RV broth and incubate for 18-24 hours at 42 ± 0.2°C.
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6.4.2.3. Transfer positive and negative control cultures to TT and RV broths as above.

6.4.2.4. Streak and/or plate (0.1 mL or can be diluted) the TT and RV enriched cultures on selective agar plates (XLD, HE, and chromogenic agar plates) for isolation. Incubate at  $35 \pm 2^{\circ}\text{C}$  for 18-24 hours.

6.4.3. Examine the agar plates for presence of typical colonies.

Typical colony characteristics of <i>Salmonella</i>		Other Organisms
Medium/Test	Colony Characteristics	
HE	blue/green w/ or w/o black center	
XLD agar	pink w/ or w/o black center	
CHROMagar <sup>®</sup> Salmonella	mauve	<i>Citrobacter</i> may appear blue
SM ID2 (SM2)	mauve	<i>Citrobacter</i> may appear white

## 6.5. Identification

6.5.1.1. Select 5-10 typical colonies, if available, from the selective agar plates; inoculate TSI and LIA and incubate  $35 \pm 2^{\circ}\text{C}$  for 18-24 h.

6.5.1.2. From TSI or LIA slants that show reactions typical of *Salmonella*, streak onto BA plates.

6.5.1.3. Select 3-5 typical colonies from BA and identify each using VITEK<sup>®</sup> or another official standard method of identification. If biochemical assay results are consistent with *Salmonella* profile, proceed with serology.

6.5.1.4. Perform serology per manufacturer's directions and the internal laboratory procedures for *Salmonella*-specific polyvalent somatic O and flagellar H antigens. As an option, an agglutination-based test with antisera specific for major serogroups such serogroups B, C etc. or combinations of antisera can be used for preliminary serotype identification. For agglutination tests, use small amount of growth from single colonies.

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6.5.1.5. For archiving and shipping, select one typical isolate that has been identified as *Salmonella* by both biochemical means and serotyping.

6.6. Reporting and Shipping

- 6.6.1. Immediately following completion of biochemical and serological tests, submit final results on SOP MDP-DATA-01 Attachment 01, "Preliminary/Final Results Notification Form".
- 6.6.2. Report results according to SOP MDP-DATA-01.
- 6.6.3. Refer to SOP MTH-SHIP-03 for preparation of cultures for shipment.

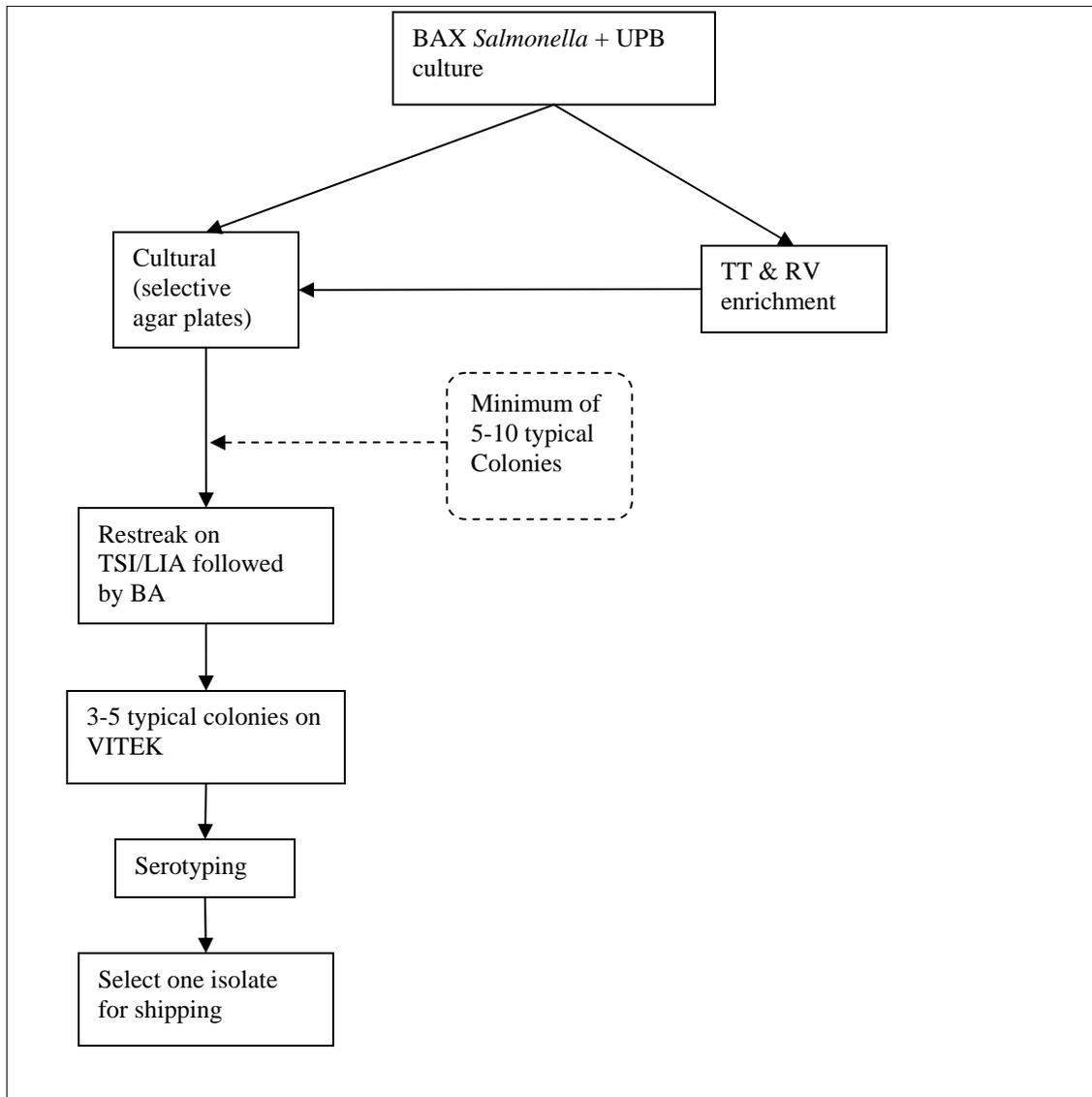
*Disclaimer: Reference to brand names (kits, equipment, media, reagents, etc.) does not constitute endorsement by this agency.*



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**Salmonella Isolation**



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*12/9/05*

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MDP-MTH-03A Revision 01                      January 2006                      Monitoring Programs Office

- Expanded isolation and identification sections
- Included serotyping

MDP-MTH-03A Original                      January 2005                      Monitoring Programs Office

- Change from MDP-MTH-03, Revision 02 to MDP-MTH-03A
- Removed specific details concerning FDA cultural confirmation methodology
- Added control requirements
- Removed references to specific strains
- Defined VITEK<sup>®</sup> positive results

MDP-MTH-03 Revision 02                      December 2003                      Monitoring Programs Office

- Added procedures to isolate *Salmonella* culturally from the sample

MDP-MTH-03 Revision 01                      May 2003                      Monitoring Programs Office

- Updated references
  - Removed instructions on operating instrument
  - Changed from selenite cystine (SC) broth to Rappaport-Vassiliadis (RV) medium; added recommendation for use of Oxoid RV Broth
  - Removed reference to SOP MDP-QA-01
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