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

SOP No: MDP-MTH-10		Page 1 of 12
Title: Isolation and Identification of *Salmonella* using Cultural Methods		
Revision: 04	Replaces: 1/1/2010	Effective: 9/1/2011

1. Purpose

To provide a standard procedure for the isolation and identification of *Salmonella* species from commodities analyzed for 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

Salmonella is isolated on selective media and identified by biochemical tests including automated systems such as VITEK® 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

5. References

- BAM Online, 8th edition. 2001. Chapter 5. *Salmonella,* http://www.cfsan.fda.gov/~ebam/bam-5.html (last accessed 11/10/05)
- SOP MDP-DATA-01, Record Keeping and Results Reporting
- SOP MDP-MTH-04, Detection of *Salmonella* in Fresh Produce by BAX® PCR
- SOP MDP-MTH-09, Detection of *Salmonella* using VIDAS® Method
- SOP MDP-SHIP-03, Procedures for Packaging, Shipping, and Archiving Microbiological Cultures
- SOP MDP-QA-03, Quality Assurance (QA) Controls
- VITEK® Users Manual, bioMérieux

6. Procedures

6.1. Equipment and Materials

- VITEK® System or VITEK® 2 Compact System, bioMérieux
- VITEK® GNI+ Card or GN cards, bioMérieux
- Water bath, 42 ± 0.2°C
- Incubator, 35 ± 2°C
- Vortex mixer
- Serological pipettes, sterile, disposable, various sizes
- Dynal® Automated BeadRetriever or manual Immunomagnetic Separation (IMS) equipment, Invitrogen

6.2. **Media and Reagents**

- *Optional*: SDIX RapidCheck© SELECT™ Salmonella media, XLT4, Brilliant Green Sulfa Agar (BGSA), Chromagenic agars
- Rappaport-Vassiliadis (RV) broth: Follow manufacturer's instructions for preparation. Dispense 10-mL aliquots into 16 x 150 mm test tubes before autoclaving.
- Tetrathionate (TT) broth: Follow manufacturer's instructions for preparation. **DO NOT AUTOCLAVE.** Dispense 10-mL aliquots into sterile 16 x 150 mm test tubes. On day of use, add 0.2 mL iodine solution and 0.1 mL Brilliant Green solution per 10 mL tube of TT broth. Mix by vortexing.
- M broth: 16 x 150 mm test tubes containing 10 mL aliquots
- Iodine solution for basal TT broth, commercially available.
- Brilliant Green Dye solution for basal TT broth, commercially available.
- Xylose lysine deoxycholate (XLD) agar, commercially available or follow manufacturer's instructions for preparation.
- Hektoen enteric (HE) agar, commercially available or follow manufacturer's instructions for preparation.
- Bismuth Sulfite (BS) agar: Follow manufacturer's instructions for preparation.
- Triple Sugar Iron (TSI) agar slants, commercially available or follow manufacturer's instructions for preparation.
- Lysine Iron agar (LIA) slants, commercially available or follow manufacturer's instructions for preparation.
- Urea Broth tubes or Urea Agar slants (use *Proteus vulgaris* ATCC 13315 as positive control and *E. coli* MDP 017 as negative control)
- Nutritive broth such as Tryptic Soy or Brain Heart Infusion, follow manufacturer's instructions for preparation.
- Tryptic Soy agar with 5% Sheep's Blood, commercially available.

- Dynabeads® anti-*Salmonella*, Invitrogen or Pathatrix®
- *Salmonella* polyvalent somatic (O) antiserum, Poly A-I and Vi (poly O antiserum): Contains agglutinins for at least the following somatic (O) antigens: 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,19,22,23,24,25,34, and Vi. They are agglutinins for somatic (O) groups: A, B, C_1, C_2, D, E_1, E_2, E_3, E_4, F, G_1, G_2, H, I and Vi, DIFCO, Becton Dickinson.
- *Salmonella* polyvalent flagellar (H), commercially available rapid test (REMEL) or DIFCO *Salmonella* H Antiserum Poly a-z, Becton Dickinson.
- Formalinized physiological saline solution: Prepare by adding 6-mL formaldehyde (HCHO) solution (36-38%) to 1-L of sterile 0.85% w/v saline (NaCl) solution.
- Physiological saline solution, sterile 0.85% w/v saline (NaCl) solution.

6.3. **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.

NOTE: Alternatively, it is acceptable for laboratories to set up appropriate media controls (positive, negative, and uninoculated) from pure cultures without using the cultural controls from the screening methods. Each laboratory shall have written procedures for ensuring the appropriate controls are used for this procedure.

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 one or more each of HE, XLD, and BS plates (chromogenic agar plates may also be used) from the TT broth culture, the RV broth culture and M broth cultures that were initiated as per MDP MTH-09 SOP. It is recommended that the laboratory streak onto several selective agar plates in order to increase the chances of identifying typical colonies. Incubate plates at 35 ± 2°C for 24 ± 2 hours. If no typical colonies are present on the BS plates, re-incubate the BS plates at 35 ± 2°C for an additional 24 ± 2 hours.

6.4.2 Laboratories may perform IMS in order to enhance isolation of *Salmonella*. Use UPB pre-enriched cultures that tested positive for *Salmonella* by PCR. Follow manufacturer's instructions for performing IMS. Plate or streak the eluted sample on HE, XLD and BS agar plates and incubate plates at 35 ± 2°C for 24 ± 2 hours. If colonies are present on the BS plates, pick typical colonies and then re-incubate the BS plates at 35 ± 2°C for an additional 24 ± 2 hours.

6.5 **Selective Enrichment and Plating** (for BAX® positive samples only).

6.5.1 Transfer 1mL of the pooled and individual (the 3 samples that were positive in the pooled culture) UPB preenriched BAX® *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.

6.5.2 In parallel, transfer 0.1 mL of the pooled and individual UPB preenriched BAX® *Salmonella*-positive culture identified using SOP MDP-MTH-04 into 10 mL of RV broth and incubate for 18-24 hours at 42 ± 0.2°C.

6.5.3 Transfer or set-up all appropriate positive, negative and blank controls to TT and RV broths as above.

6.5.4 Streak the TT, RV and M Broth enriched cultures on selective agar plates (XLD, HE, BS and chromogenic agar plates) for isolation. Incubate plates at 35 ± 2°C for 24 ± 2 hours. If no typical colonies are present on the BS agar plates, re-incubate the BS plates at 35 ± 2°C for an additional 24 ± 2 hours.

6.5.5 Examine the agar plates for the presence of typical *Salmonella* colonies.

Typical colony characteristics of *Salmonella*		**Other Organisms**
Medium	**Colony Characteristics**	
HE	blue/green w/ or w/o black center	Yellow, orange
XLD	pink w/ or w/o black center	Yellow, orange
BS	Brown/gray or black w/ or w/o a metallic sheen	
Chromagenic agar	refer to manufacturer's user guide	refer to manufacturer's user guide

6.6 **Identification** (If you obtain a pooled BAX® positive result followed by an individual BAX® negative with an individual negative VIDAS or a pooled negative VIDAS, you can consider this to be a double negative and testing can stop. Otherwise, continue with steps listed below.)

6.6.1 After examining the plates, pick at least 10 suspect *Salmonella* colonies (if available) from any selective agar plates and inoculate triple sugar iron (TSI) agar slants, lysine iron agar (LIA) slants, and Urea agar slants or Urea broth tubes for each colony. (If no typical or suspect *Salmonella* colonies are present, TSI, LIA, Urea slants/tubes *should* still be inoculated. Pick at least 10 atypical colonies for this step.) Inoculate TSI, LIA, and Urea slants/tubes according to instructions given in FDA BAM, chapter 5.

Note: Use professional judgment and experience in deciding the number of plates and number of colonies needed for screening which depends on the extent of contamination, the background microflora level, the commodity type, post harvest handling and type of enrichment used.

6.6.2 Incubate the TSI, LIA, and Urea agar slants for 18-24 hours at 35 ± 2°C.

6.6.3 Examine slants. *Salmonella* typically produce alkaline (red) slant and acid (yellow) butt, with or without production of H_2S (blackening of agar) in TSI agar. In LIA agar, *Salmonella* typically produce alkaline (purple) slant and butt. **Refer to FDA BAM, chapter 5 for further instruction and interpretation of slant/tube results.**

6.6.4 If typical *Salmonella* reactions are observed, proceed to confirmation steps below. If TSI, LIA, and Urea reactions are atypical of *Salmonella,* no further confirmation is needed as the isolate is not *Salmonella*.

6.7 **Biochemical Confirmation**

6.7.1 From the TSI or LIA or Urea slants/tubes that show reactions typical of *Salmonella*, streak onto BA plates to check for fluorescence or manufacturer's suggested media source.

6.7.2 Incubate BA plates 18-24 hours at 35 ± 2°C or follow manufacturer's recommended incubation conditions.

6.7.3 Select 3-5 isolated colonies from BA or manufacturer's suggested media source and identify each using VITEK® and/or another standard method of identification, such as O/H serology.

NOTE: Laboratories shall notify MPO if other pathogenic bacteria such as Enterobacter sakazakii are identified.

6.7.4 Perform serology per manufacturer's instructions 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 as 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.

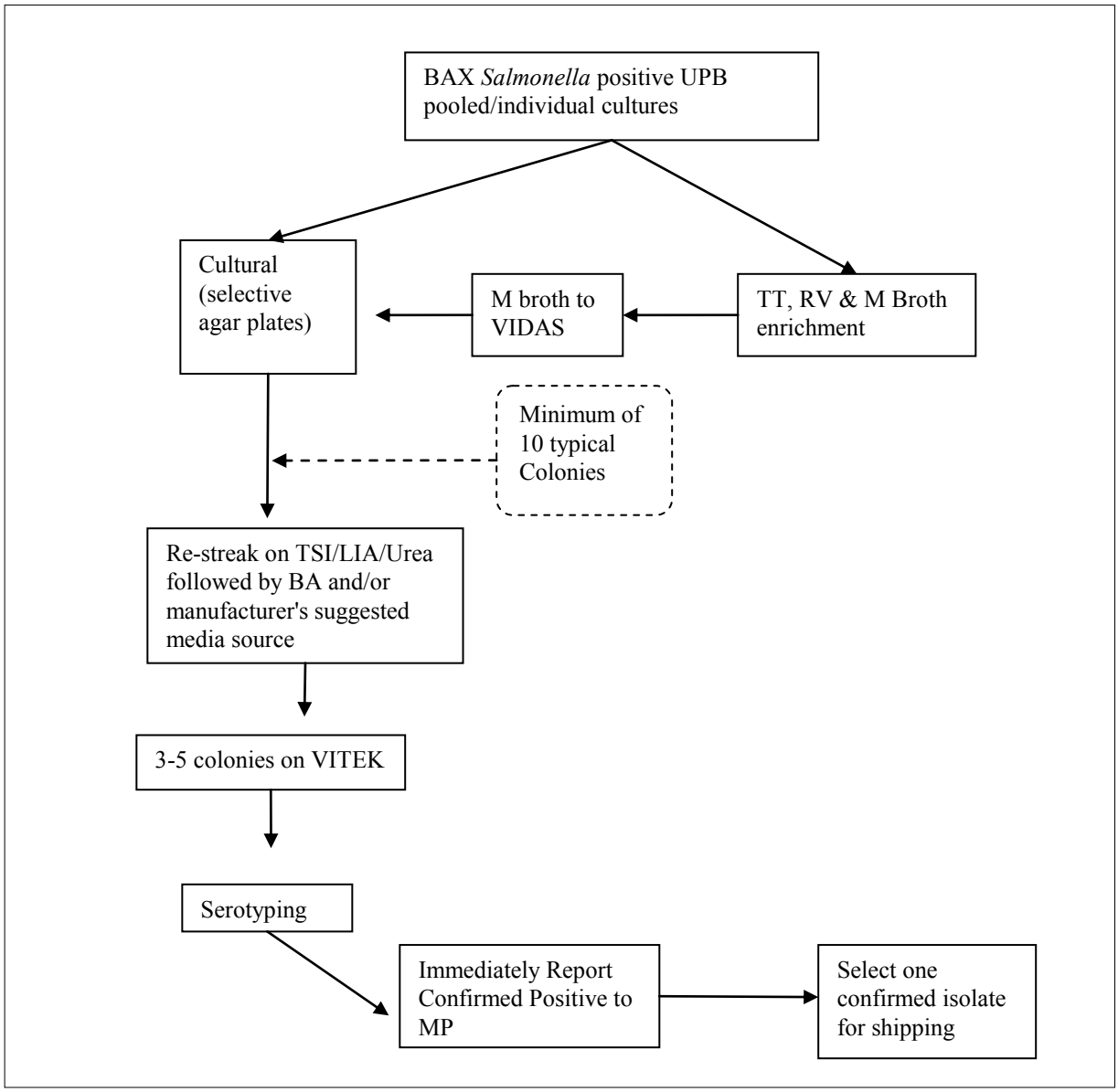
6.8 **Reporting and Shipping**

6.8.1 IMMEDIATELY following completion of biochemical and serological tests, report confirmed results to MP per SOP MDP-DATA-01 on Atch 01, Results Notification Form, and prepare (3) isolate slants for shipment to ODA for antimicrobial susceptibility testing and, if necessary, PFGE and serotyping.

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

6.8.3 Refer to MDP-SHIP-03 SOP for preparation of cultures for shipment.

Optional: *Salmonella* Isolation for BAX Positive Samples Flowchart



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Revision 04 June 2011 Monitoring Programs Division

- Deleted "(optional)" from "Dynal® Automated**…"** sentence, Section 6.1
- Added "*Optional*: SDIX RapidCheck© SELECT™ Salmonella media, XLT4, Brilliant Green Sulfa Agar (BGSA), Chromagenic agars", Section 6.2
- Deleted "Chromogenic *Salmonella* agar such as CHROMagar *Salmonella* (DRG International), *Salmonella* SM ID2 (bioMerieux), or equivalent (optional), Section 6.2
- Added "Urea Broth tubes or Urea Agar slants (use *Proteus vulgaris* ATCC 13315 as positive control and *E. coli* MDP 017 as negative control)", Section 6.2
- Revised sentence to read as "Dynabeads® anti-*Salmonella*, Invitrogen or Pathatrix®" by removing catalog number and the word "(optional), Section 6.2
- Deleted **bold** words and added *italicized* words "**Although not required,** laboratories may **also** perform IMS **using anti-Salmonella Dynabeads** in order to enhance isolation of Salmonella *using UPB pre-enriched cultures that tested positive for Salmonella by PCR.* Follow manufacturer's instructions for performing IMS. *Plate or streak the eluted sample on HE, XLD and BS agar plates and incubate plates at 35 ± 2°C for 24 ± 2 hours. If colonies are present on the BS plates, pick typical colonies and then re-incubate the BS plates at 35 ± 2°C for an additional 24 ± 2 hours."*, Section 6.4.2
- Deleted "CHROMagar Salmonella" and "SM ID2 (SM2)" from table, Section 6.5.5
- Added "Chromagenic agar" to table, Section 6.5.5
- Deleted **bold** words and added *italicized* words "After examining the plates, pick at least **5-**10 suspect Salmonella colonies (if available) from *any* selective agar plates and inoculate triple sugar iron (TSI) agar slants, lysine iron agar (LIA) slants, *and Urea agar slants or Urea broth tubes* for each colony. (If no typical or suspect Salmonella colonies are present, TSI, LIA, *Urea slants/tubes* should still be inoculated. Pick at least *10* atypical colonies for this step.) Inoculate TSI , LIA, *and Urea slants/tubes* according to instructions given in FDA BAM, chapter 5.", Section 6.6.1
- Added Note, Section 6.6.1
- Added words "…and Urea… Section 6.6.2
- Deleted words "…TSI and LIA…" and added **bold** word "…slant/**tube**…", Section 6.6.3
- Added words "…and Urea…", Section 6.6.4
- Added words "…or Urea tubes…", Section 6.7.1
- Deleted **bold** words and added *italicized* words "Select 3-5 isolated colonies from BA or

manufacturer's suggested media source and identify each using VITEK® *and/or* another **official** standard method of identification, **If biochemical assay results are consistent with Salmonella profile, proceed with serology** *such as O/H serology*." , Section 6.7.3

- Updated Flowchart

Revision 03 December 2009 Monitoring Programs Division

- Added Outline of Procedures, Section 4
- Alphabetized and removed numbering in References, Section 5
- Removed numbering in Equipment and Materials, Section 6.1
- Removed numbering in Media and Reagents, Section 6.2
- Added "M broth: 16 x 150 mm test tubes containing 10 mL aliquots", Section 6.2
- Reworded Section 6.4.1 to "Streak one or more each of HE, XLD, and BS plates (Chromogenic agar plates may also be used) from the TT broth culture, the RV broth culture and M broth cultures that were initiated as per MDP MTH-09 SOP. It is recommended that the laboratory streak onto several selective agar plates in order to increase the chances of identifying typical colonies. Incubate plates at 35 ± 2°C for 24 ± 2 hours. If no typical colonies are present on the BS plates, re-incubate the BS plates at 35 ± 2°C for an additional 24 ± 2 hours."
- Removed "Note" statements (Once 35±2°C incubation has been completed, in case of weekend, holiday, etc., plates may be removed from incubator and allowed to sit at room temperature or be refrigerated until next business day. If plates are removed from incubator, please insure that information, comments, observations, etc., are recorded as part of data packets and/or sample data transmitted to MPO.) from Section 6.4.1
- Added "If no typical colonies are present on the BS agar plates, re-incubate the BS plates at 35 ± 2°C for an additional 24 ± 2 hours", Section 6.5.4
- Added "If you obtain a pooled BAX® positive result followed by an individual BAX® negative with an individual negative VIDAS or a pooled negative VIDAS, you can consider this to be a double negative and testing can stop. Otherwise, continue with steps listed below.", Section 6.6
- Added words "to check for fluorescence or manufacturer's suggested media source",

Section 6.7.1

- Added words "or follow manufacturer's recommended incubation conditions", Section 6.7.2
- Added words "or manufacturer's suggested media source" in Section 6.7.3
- Replaced words "Fillable Reporting" with "Results Notification Form" and added words "and prepare (3) isolate slants for shipment to ODA for antimicrobial susceptibility testing and, if necessary, PFGE and serotyping.", Section 6.8.1
- Added "Optional" to Flowchart title
- Added "M Broth" and "M Broth to VIDAS" to Flowchart
- Added "and/or manufacturer's suggested media source" in Flowchart

Revision 02 September 2009 Monitoring Programs Division

- Updated References, Section 4
- Revised Sections 5.4 through 5.8
- Updated Flowchart

Revision 01 March 2009 Monitoring Programs Division

- Added GN cards to 6.1.2
- Added 6.4.1
- Added, "pooled and individual (the 3 samples that were positive in the pooled culture)" to 6.4.5.1.
- Added "pooled and individual" to 6.4.5.2.
- Added the note in 6.6.3.
- Revised the flowchart to include pooled and individual UPB enriched samples.