

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

SOP No: MDP-MTH-02		Page 1 of 7
Title: Detection of <i>Salmonella</i> in Fresh Produce Using VIDAS [®] Method		
Revision: 03	Replaces: 01/01/04	Effective: 05/01/05

1. Purpose

To provide standard procedures for screening Microbiological Data Program (MDP) fruit and vegetable samples for *Salmonella* using the Vitek Immuno Diagnostic Assay System (VIDAS[®]).

2. Scope

This 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 is implemented in that laboratory.

3. Principle

The VIDAS[®] is an automated system developed by bioMérieux for detecting microorganisms isolated from food, environmental, and clinical samples. The reliability and accuracy of detecting the presence of a target organism are a result of the specific antigen-antibody reactions coupled to an enzyme linked fluorescent assay (ELFA) and monitored by a calorimeter.

4. Outline

VIDAS [®]	6.1
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5. References

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- 5.1. Wallace Andrews and T. S. Hammack. Bacteriological Analytical Manual, Chapter 5. *Salmonella*, <http://www.cfsan.fda.gov/~ebam/bam-5.html> (last accessed 04/12/05).
- 5.2. VIDAS[®]. bioMérieux. User Guide and Package Insert.
- 5.3. Curiale, M.S., Gangar, V. and Gravens, C. 1997. VIDAS[®] enzyme-linked immunofluorescent assay for detection of *Salmonella* in food: collaborative study. Journal of AOAC International, Volume 80, No.3, pp. 491-504.
- 5.4. Maijala R, Johansson T, and Hirn J. 1992. Growth of *Salmonella* and competing flora in five commercial RV media. International Journal of Food Microbiology. Volume17, pp.1-8.
- 5.5. SOP MDP-DATA-01, Record Keeping and Results Reporting.
- 5.6. SOP MDP-LABOP-02, Sample Receipt and Elution Procedure.
- 5.7. SOP MDP-MTH-03A, Isolation and Identification of *Salmonella* from Fresh Produce.
- 5.8. SOP MDP-QA-03, Quality Assurance Controls.

6. Specific Procedures

- 6.1. VIDAS[®]: Follow the manufacturer's instructions in the use of instrument
 - 6.2. Equipment and Materials
 - 6.2.1. VIDAS[®] Instrument
 - 6.2.2. VIDAS[®] *Salmonella* (SLM) assay kit (available from bioMérieux, Inc., 595 Anglum Road, Hazelwood, MO 63042-2320)
 - 6.2.3. Water bath set to 42 ± 0.5°C
 - 6.2.4. Additional materials needed to perform procedure as listed in VIDAS[®] SLM Kit User's Guide
 - 6.3. Media and Reagents
 - 6.3.1. Lactose broth
 - 6.3.2. Rappaport-Vassiliadis (RV) broth: 16 x 150 mm sterile test tubes containing 10 mL aliquots. (NOTE: RV broth by Oxoid has been shown to provide
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better enrichment of *Salmonella* compared to RV produced by other manufacturers. Therefore, Oxoid RV broth is recommended).

- 6.3.3. Tetrathionate (TT) broth (with iodine and brilliant green): 16 x 150 mm sterile test tubes containing 10 mL aliquots (On the day the medium is used, add 20 mL iodine solution per 1 L basal broth and 10 mL brilliant green solution per 1 L basal broth.)
 - 6.3.4. M-broth: 16 x 150 mm test tubes containing 10 mL aliquots
 - 6.3.5. Buffered peptone water plus 0.1% Tween 80 (MDP-LABOP-02)
 - 6.4. List of Controls (Specific strains are listed in SOP MDP-QA-03)
 - 6.4.1. Negative culture control: 1 mL of 0.5 MacFarland Standard or an equivalent amount of negative control culture in 225 mL sterile lactose broth
 - 6.4.2. Positive culture control: 1 mL of 0.5 MacFarland Standard or an equivalent amount of positive control culture in 225 mL sterile lactose broth
 - 6.4.3. Positive produce culture control: From each produce type, randomly choose 25 mL of wash eluate and add 1 mL of 0.5 MacFarland or equivalent of the positive control culture. Gently mix the wash; do not use a shaker. Combine 25 mL of this mixture with 225 mL of sterile lactose broth.
 - 6.4.4. Manufacturer's supplied VIDAS *Salmonella* (SLM) standard and controls.
 - 6.4.5. Media Control
 - 6.4.5.1. Combine 25 mL BPW plus 0.1% Tween 80 with 225 mL sterile lactose broth and incubate at $35 \pm 1.0^{\circ}\text{C}$ for 18-24 h. Add 1 mL of this lactose broth into 10 mL TT broth and incubate at $42 \pm 0.5^{\circ}\text{C}$ for 18-24 h. Add 0.1 mL of sterile lactose broth into 10 mL of RV broth and incubate at $42 \pm 0.5^{\circ}\text{C}$ for 18-24 h.
 - 6.4.6. 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.
 - 6.5. VIDAS® Analysis
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- 6.5.1. Pre-enrichment: Aseptically transfer 25 mL of the sample eluate (See SOP MDP-LABOP-02) to 225 ± 5 mL of sterile lactose broth in a suitable sterile container. Incubate lactose broth at 35 ± 1°C for 18-24 h.
- 6.5.2. Selective Enrichment
- 6.5.2.1. After incubation, swirl lactose broth to mix, and transfer 0.1 mL of the culture suspension into 10 mL RV broth. Incubate in a 42 ± 0.5°C water bath for 18-24 h.
- 6.5.2.2. Transfer 1.0 mL of the culture suspension into 10 mL TT broth and incubate tubes in a 42 ± 0.5°C water bath for 18-24 h.
- 6.5.3. Post-enrichment
- 6.5.3.1. Mix selective enrichment broths and transfer 1 mL from the RV broth into 10 mL M-broth. Transfer 1 mL TT broth into another 10 mL M-broth.
- 6.5.3.2. Incubate both M-broth samples at 42 ± 0.5°C for 6-8 h. After incubation, M-broths can be stored at 2-8 °C up to 48 h.
- 6.5.4. Sample preparation for enzyme immunoassay analysis
- 6.5.4.1. Transfer 1 mL each of the two M-broth cultures into a separate sterile test tube. Repeat this for each sample and control.
- 6.5.4.2. Heat the samples and quality control tubes containing 2 mL M-broth by submerging below the broth level in a boiling waterbath for 15 min.
- 6.5.4.3. Cool heated extracts to room temperature (20-25°C) prior to running enzyme immunoassay analysis.
- 6.5.4.4. Mix the kit standards, kit controls and all heated samples. Pipette 500 µL of the standard, control, or sample into the center of a sample well of the appropriately labeled SLM Reagent Strip.
- 6.5.4.5. Perform the VIDAS® analysis.
- 6.6. VIDAS® Detections
- 6.6.1. A positive VIDAS® reading indicates *Salmonella* may be present.
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6.6.2. Proceed to SOP MDP-MTH-03A.

6.7. Reporting

6.7.1. A preliminary positive is defined as a positive VIDAS® reading.

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

6.8. Transfer and Storage of Cultures

6.8.1. Refer to SOP MDP-SHIP-03 for archival procedures and shipping of isolates.



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Revision History

Revision 03	February 2005	MPO
<ul style="list-style-type: none"> • Removed references to specific control strains • Removed instructions for reagent preparation • Allowed sample eluates to be combined for blanks 		
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Revision 02	January 2004	MPO
<ul style="list-style-type: none"> • Adjusted purpose to indicate VIDAS® as backup method in case of failure of BAX instrument methods • Changed from Selenite Cystine Broth to Rappaport-Vassiliadis broth and recommended use of Oxoid RV Broth. • Changed from Butterfield's Phosphate Buffer + 1% Tween to Buffered Peptone Water + 0.1% Tween • Changed from 50 mL sample eluate + 450mL lactose broth to 25 mL sample eluate + 225 mL lactose broth (25g is a serving size) • Removed the step for testing pH after 1 hr incubation of inoculated lactose broth • Increased incubation of TT & RV broth to 18-24 hours at 42±0.5°C • Decreased incubation of M-broth to 6-8 hours • Changed amount of broth used for controls to 225 mL lactose broth • Removed sections on operation of VIDAS instrument; laboratories are advised to refer to the manufacturer's instructions. Work instructions may be written by each laboratory for the operation of the VIDAS instrument. 		
