

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

SOP No: MDP-MTH-09		Page 1 of 8
Title: Detection of <i>Salmonella</i> Using VIDAS [®] Method		
Revision: 1	Replaces: 02/09/09	Effective: 09/25/09

1. Purpose

To provide standard procedures for screening USDA/AMS Microbiological Data Program (MDP) 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 colorimeter.

4. References

- 4.1 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).
 - 4.2 VIDAS[®] Instrument and PC User Guide, bioMerieux
 - 4.3 VIDAS *Salmonella* (SLM) package insert, 12/2007, bioMerieux
 - 4.4 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.
 - 4.5 Maijala R, Johansson T, and Hirn J. 1992. Growth of *Salmonella* and competing flora in five commercial RV media. International Journal of Food Microbiology. Volume 17, pp.1-8.
 - 4.6 SOP MDP-DATA-01, Record Keeping and Results Reporting.
 - 4.7 SOP MDP-LABOP-02, Sample Receipt, Elution, Preenrichment, and DNA Extraction
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4.8 SOP MDP-MTH-04, Detection of *Salmonella* in Fresh Produce by Bax® PCR.

4.9 SOP MDP-MTH-10, Isolation and Identification of *Salmonella* using Cultural Methods

4.10 SOP MDP-QA-03, Quality Assurance Controls.

5. Procedures

5.1 **VIDAS®** - Follow the manufacturer's instructions for proper use of this instrument and follow the VIDAS® *Salmonella* (SLM) kit insert for kit use, kit controls use and kit storage. Allow all kit components to reach room temperature before use.

NOTE: All Salmonella spp. are pathogenic to humans and pose a serious health risk. Always handle samples, pure cultures and controls with extreme care and follow standard safety practices and procedures.

5.2 Equipment and Materials

- VIDAS® Instrument, bioMérieux
- VIDAS® *Salmonella* (SLM) assay kit reference number 30 702, bioMérieux
- Water bath set to 42 ± 0.5°C
- VIDAS® Heat and Go, or water bath set at 95-100°C
- Pipettor (100-1000µl)
- Serological pipets, sterile, 1 ml

5.3 Media and Reagents

- Rappaport-Vassiliadis (RV) broth: 16 x 150 mm sterile test tubes containing 10mL aliquots. (RV broth by Oxoid has been shown to provide better enrichment of *Salmonella* compared to RV produced by other manufacturers. Therefore, Oxoid RV broth is recommended).
 - Tetrathionate (TT) broth (with iodine and brilliant green): 16 x 150 mm sterile test tubes containing 10mL aliquots (On the day the medium is used, add 20mL iodine solution per 1L basal broth and 10mL brilliant green solution per 1L basal broth.)
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- M broth: 16 x 150 mm test tubes containing 10 mL aliquots
- Preenriched samples and controls from SOP MDP-LABOP-02

5.4 **Controls** (Specific strains are listed in SOP MDP-QA-03) - 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.

- Negative culture control: *E. coli* (MDP 017) from SOP MDP-LABOP-02
- Positive culture control: *Salmonella typhimurium* (MDP 014) from SOP MDP-LABOP-02
- Uninoculated media control from SOP MDP-LABOP-02
- Positive Produce Control from SOP MDP-LABOP-02.
- Manufacturer's supplied VIDAS® *Salmonella* (SLM) standards (S1) and controls (C1 and C2)

5.5 **Pre-enrichment** - Refer to SOP MDP-LABOP-02 for pre-enrichment of all commodities. Use these test cultures for this SOP.

5.6 Post Enrichment

5.6.1 Gently mix pre-enriched samples and controls from 6.5 above or from BAX® preliminary positive enrichments.

5.6.2 Transfer 0.1mL of the culture suspension into 10 mL RV broth. Incubate in a 42 ± 0.5°C water bath for 18-24 h.

5.6.3 In parallel, 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.

5.6.4 Following incubation, mix selective enrichment broths and transfer 1 mL from the RV broth into 10mL M broth. In addition, transfer 1 mL from the TT broth into another 10 mL M-broth.



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5.6.5 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 hrs if the VIDAS® analysis cannot be performed immediately.

5.7 VIDAS® Analysis

5.7.1 Following incubation, remove the M broth cultures and mix well.

5.7.2 For VIDAS® Heat and Go use, transfer 250 µl from each of the two M broth cultures into the sample well of one appropriately labeled SLM VIDAS® strip. Repeat this for each sample and controls. **Do not** heat the S1 or C1/C2 kit controls. Heat the sample for 15 minutes. Refer to VIDAS® Heat and Go instruction manual for proper use and strip placement. Cool the strips to room temperature before VIDAS® analysis.

5.7.3 For water bath use, transfer 1mL each of the two M-broth cultures into a separate sterile test tube. Repeat this for each sample and controls. **Do not** heat the S1 or C1/C2 kit controls. Heat the samples and control tubes containing 2mL M broth by submerging below the broth level in a boiling waterbath for 15 min.

5.7.4 Cool sampled and controls to room temperature (20-25°C). Mix the contents of the tube and pipet 500µl from the heat-killed sample/control tube into the sample well of the appropriately labeled SLM VIDAS® strip.

5.7.5 Mix the S1 kit standards (if necessary) and the C1 and C2 kit controls. Pipet 500µL of the each standard (in duplicate strips) and C1, C2 control into the sample well of the appropriately labeled SLM VIDAS® Strip.

5.7.6 Perform the VIDAS® analysis according to manufacturer's instructions.

5.8 Interpretation of Results

5.8.1 Once the assay is completed, results are analyzed automatically by the instrument. Two fluorescence measurements in the Reagent Strip's optical cuvette are taken for each specimen tested. The first reading is a background reading of each substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The relative fluorescence value (RFV) is

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calculated by subtracting the background reading from the final result. The calculation appears on the result sheet.

5.8.2 A report is printed which records the type of test performed, test sample identification, analyst initials, date and time, lot number and expiration date of reagent kit being used and each test's RFV, Test Value, and interpreted result.

5.8.3 Test results are reported as invalid when the background reading is above predetermined cutoff value indicating low-level substrate contamination. In such a case, the VIDAS® portion of the analysis shall be repeated.

5.8.4 The RFV obtained for each sample is interpreted by the VIDAS® software as follows:

Thresholds and Interpretations

Test Value	Threshold	Interpretation
<0.23		Negative
≥0.23		Positive

5.8.5 Samples with test values greater than or equal to the threshold value are reported as positive for *Salmonella* and shall be confirmed culturally. Refer to SOP MDP-MTH-04 and SOP MDP-MTH-10.

5.8.6 Samples with test values lower than the threshold value indicate samples with undetectable *Salmonella* antigen.

5.9 Reporting

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

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



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5.10 Transfer and Storage of Cultures - Refer to SOP MDP-SHIP-03 for archival procedures and shipping of isolates.

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

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Mary Tijerina

25 Sep 09

Revised by: Mary Tijerina
Microbiologist, Monitoring Programs Office
8609 Sudley Road, Suite 206
Manassas, VA 20110
(703) 330-2300 x111

Date

Maya Achen

25 Sep 09

Approved By: Maya Achen
Chairperson, MDP Technical Advisory Group
Ohio Department of Agriculture
Consumer Analytical Laboratory, Bldg 3
8995 E Main St
Reynoldsburg, OH 43068
(614) 728-2790

Date

Diana Haynes

25 Sep 09

Approved By: Diana Haynes
Deputy Director, Monitoring Programs Office
8609 Sudley Road, Suite 206
Manassas, VA 20110
(703) 330-2300 x134

Date

