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

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Title: Detection of *Listeria monocytogenes* in Fresh Produce Using the VIDAS® LIS System, Isolation and Confirmation		
Revision: 2	Replaces: LM Special Project 1	Effective: 10 September 2012

1. Purpose

To provide a standard operating procedure for surveillance of *Listeria monocytogenes* in fresh produce using bioMérieux's VIDAS® *Listeria* spp. (LIS) assay for laboratories participating in the USDA, AMS, Microbiological Data Program (MDP).

2. Scope

This standard operating procedure (SOP) shall be followed by participating laboratories participating in testing for the presence of *L. monocytogenes* in at least two of the following four commodities: cantaloupe (CN), sprouts (SR), bagged lettuce (LT) and bagged spinach (SP). This SOP represents minimum MDP requirements and is presented as a general guideline. Each laboratory shall maintain internal written procedures that provide specific details concerning how this screening procedure has been implemented in the laboratory.

3. Principle

The VIDAS® is an automated system developed by bioMerieux for detecting microorganisms 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. Safety

Laboratory personnel should utilize Biosafety Level II (BSL-2) practices for MDP manipulations of known and potential pathogens. A BSL-2 laminar flow biosafety cabinet is recommended for activities with potential for producing aerosols of pathogens. Material Safety Data Sheets (MSDS) should be obtained from manufacturers for media, chemicals and reagents used in the analysis and personnel who will handle the materials should know the location of and have ready access to the MSDS sheets for reference.

5. Outline of Procedures

6. References

- AOAC OMA Official Method, N°2004.06 for all foods (harmonized protocol)
- AOAC-RI Performance Tested Method N°981202 for environmental samples
- BAM Online, April 2011, Chapter 10, Detection and Enumeration of *Listeria monocytogenes* in Foods
- Ma, L. et al (2011) Green Fluorescent Protein Labeling of Listeria, Salmonella, and Escherichia coli O157:H7 for Safety-Related Studies. PLoS One 6(4): e18083.
- Ueda, S. and Kuwabara, Y. (2010) Evaluation of an Enzyme-Linked Fluorescent Assay for the Detection of *Listeria monocytogenes* from Food. Biocontrol Science 15(3): 91-95.
- VIDAS® LIS User Guide & Protocol Summary, bioMérieux, Inc.

7. Procedure

7.1 Equipment and Materials

- VIDAS® System, bioMerieux
- VIDAS® *Listeria* (LIS) kit (30 700), bioMerieux
- Heat and Go block (bioMerieux), water bath, or equivalent
- Buffered Listeria enrichment Broth (bLEB) with sodium pyruvate (Refer to BAM)
- Additives for bLEB (Acriflavine (0.5%), Cyclohexamide (1.0%), and Nalidixic Acid (0.5%) (Refer to BAM) (Oxoid SR141E or equivalent)
- Modified Oxford Agar (MOX)
- 5% Sheep Blood agar (SBA); Optional: Horse Blood Agar (HBA) plates
- Trypticase Soy Agar with 0.6% Yeast extract (TSAYE)
- Brain Heart Infusion Broth (BHI) (optional)
- Listeria chromogenic agar (For example: R&F Listeria Chromogenic agar, BioRad Rapid L.mono agar or Rapid Listeria agar etc)
- Incubator capable of maintaining a temperature range of 35±2°C

- Incubator capable of maintaining a temperature of 30±1°C
- VITEK® 2 Gram Positive (GP) cards, bioMerieux
- VITEK® System, bioMerieux
- Additional materials needed to perform procedure as listed in VIDAS® LIS System User Guide & Protocol Summary
- API Listeria (optional)
- Erythromycin - Item # 97061-222 (50g) from VWR or equivalent

7.2 Controls: Carry all controls through this entire procedure, including any necessary cultural confirmation.

- Media control: Uninoculated Buffered Listeria enrichment Broth (bLEB)
- Negative control: Use *E.coli* MDP-017: E.coli (ATCC 25922 Δ*ybi*K-KanR)
- Negative *Listeria* control : Use *L. innocua* MDP-021 (OSU ATCC 33090 GFP)
- Positive control: Use *L. monocytogenes* MDP-022 (OSU G3982 GFP)
- Positive Produce Control: Use *L. monocytogenes* MDP-022 (OSU G3982 GFP)

Growing *Listeria* controls: *Listeria* cultures can be grown on any rich agar plate/slant media (example: TSA, BHI or TSAYe) supplemented with 10 µg/mL erythromycin. Incubate the plates/slants for 42-48 hours at 35°C. Check for fluorescence following incubation using the UV light. The isolate should be transferred to fresh slants at a monthly interval. Cool the autoclaved media before adding 0.01g (10 mg) of erythromycin per liter. Mix well before dispensing to plates or culture tubes.

7.3 Sample Set up and VIDAS® Analysis

- **Cantaloupe**: Follow MDP-LABOP-02 for set up of Cantaloupes in UPB. Following UPB addition to the sample and after shaking, rubbing, etc., aseptically transfer approximately 25mL of the UPB from the bag containing the cantaloupe sample to ~225mL of bLEB.
- **Sprouts:** Aseptically transfer approximately 25g of the sprouts to ~225mL of bLEB. Stomach the sample for 2 minutes and leave it soaking.
- **Bagged Lettuce**: Aseptically transfer approximately 25g of the bagged lettuce sample to ~225mL of bLEB, shake or rub and leave it soaking.
- **Bagged Spinach**: Aseptically transfer approximately 25g of the bagged spinach sample to ~225mL of bLEB, shake or rub and leave it soaking.

Incubate bLEB samples at 30±1°C for 4 hours. Add additives (refer to BAM) and incubate

samples for a total incubation period of 48-50 hours at 30±1°C.

OPTIONAL: Following 24 h of incubation of samples in bLEB, streak the 24 h bLEB to MOX and/or Listeria Chromogenic agar. Reincubate bLEB enrichments. Incubate MOX plates for approximately 48 hours at 35±2°C. Incubate *Listeria* Chromogenic agar plates according to manufacturer's instructions. Read plates and follow steps under 7.4 for cultural confirmation.

7.3.1 Refer to the VIDAS® User Manual and kit insert for run set-up and sample loading procedures.

7.3.2 Transfer 1-2 mL individual enrichment broth (no pooling) into a tube and boil in a water bath for 15 ± 1 minutes. Cool the tube. Mix the boiled broth and transfer 0.5 mL into the sample well on the VIDAS® strip. Optionally use the Heat and Go VIDAS® blocks following manufacturer's instructions. Cool the strip for 10 minutes.

7.3.3 Repeat this for the controls. Do not heat the S1 or C1/C2 kit controls. Perform the VIDAS® assay. Refer to the kit insert.

Note: *If the VIDAS® assay cannot be performed immediately after the incubation period, it is acceptable to store the enrichment bags at refrigeration temperature up to a period of 48 hours.*

7.3.4 Store the enrichment broths at refrigeration temperature until all analysis is complete.

7.3.5 Once the assay is completed, results are analyzed automatically by the instrument. A report is printed out. Samples with a test value greater than or equal to the threshold value of 0.04 are reported as positive and shall be confirmed culturally. Samples with test values lower than the threshold value indicate samples with undetectable *Listeria* antigen.

Note: *The VIDAS® LIS kit detects Listeria species in the sample. Hence cultural analyses shall be carried out to detect only L.monocytogenes from the positive VIDAS® sample.*

7.3.6 Initiate cultural confirmation from the positive individual bLEB broths.

7.4 *L. monocytogenes* cultural confirmation

Optionally laboratories may follow FDA BAM instructions on isolation and identification of *Listeria monocytogenes* colonies.

7.4.1 Streak the positive bLEB enrichment broth to MOX and Listeria Chromogenic agars. Incubate MOX agar at 35±2°C for a maximum of 44-48 hours. Follow manufacturer's instructions for incubation of the Listeria chromogenic agars.

7.4.2 Following incubation of selective agars, examine plates. Pick 10 suspect colonies from either, or both, selective agars and transfer to TSAYE plates for purity. Incubate TSAYE at 30±2°C for 18-24 hours. In addition stab and/or streak onto blood agar plates (ex. SBA or HBA) for β hemolysis. Incubate blood plates at 35±2°C for 18-24 hours.

7.4.3 After 24 hour incubation period check colonies on blood plates to verify lack of fluorescence.

7.4.4 Examine the isolates on blood plates for β hemolysis and TSAYE streaks for purity.

7.4.5 Conduct biochemical analysis from blood plate or TSAYE, using the VITEK 2GP cards (optional API Listeria). If any one of the isolates confirm as *Listeria monocytogene,* proceed to SOP MDP-DATA-01 for reporting.

7.4.6 If VITEK is inconclusive the following procedures may aid in confirmation; gram stain, motility, CAMP test, catalase test. See BAM online Chapter 10.

7.4.6.1 Before a final negative result is reported, 20 typical colonies (if available) from selective agars shall be screened for β hemolysis, and five β hemolytic colonies (if available) shall be analyzed for biochemical analysis.

7.4.6.2 If all isolates are not confirmed as *L. monocytogenes,* stop further analysis.

7.5 Reporting

7.5.1 Report preliminary results following observation of typical colonies on Chromogenic Agar as soon as possible per SOP MDP-DATA-01.

7.5.2 Report final confirmed result per SOP MDP-DATA-01. Ship out *L.monocytogenes* isolates per SOP-MDP-SHIP-03 for PFGE.

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

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Cynthia Mangione *8/8/12*

Prepared by: Cynthia Mangione Date
Chairperson, MDP Technical Advisory Group
New York Department of Agriculture & Markets Food Laboratory
State Office Campus, 1220 Washington Ave, Bldg 7, 3rd Floor
Albany, NY 13209 (518) 457-0906

Shanker Reddy *8/8/12*

Reviewed by: Shanker Reddy, PhD Date
Molecular Biologist, Microbiological Data Program
8609 Sudley Road, Suite 206, Manassas, VA 20100
(703) 330-2300 x135

Mary Tijerina *8/8/12*

Reviewed by: Mary Tijerina Date
Microbiologist, Microbiological Data Program
8609 Sudley Road, Suite 206, Manassas, VA 20100
(703) 330-2300 x111

Diana Haynes *8/8/12*

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

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Revision History

Revision 1 March 2011 Monitoring Programs Division

- Section **7.1 Equipment and materials** added water bath or equivalent to Heat and Go bullet, added Oxoid SR141E or equivalent to bLEB additives bullet, added optional to the BHI bullet, and added API Listeria and erythromycin bullets.
- Section **7.3 Sample Set up and VIDAS® Analysis** changed incubation period to 48 – 50 hours.
- Section 7.3.2 removed temperature requirement for boiling water bath. Removed specifics on how to use heat and go and replaced with "by following manufacturer's instructions".
- Sentence under section **7.4 *L. monocytogenes* cultural confirmation** added 'Optionally".
- Section 7.4.1 added "a maximum of" to allow plates to be processed at 24 hours if appropriate growth is present.
- Section 7.4.2 modified text to give an option of using SBA or HBA blood plates and allow for concurrent streaking of blood plates and TSAYE plates. In addition removed catalase test.
- Added section 7.4.3 for fluorescence check.
- Section 7.4.4 modified text to allow for blood plate flexibility and removed BHI.
- Removed section 7.4.5 gram stain and motility. Added TSAYE plate for VITEK analysis and corrected VITEK 2GP name.
- Added section 7.4.6.1
- Moved section 7.4.7 to 7.4.6.2.
- Section 7.5.1 modified text to indicate that MPO shall be notified of a preliminary positive once typical colonies are observed on Chromogenic agar plates.

Revision 2 August 2012 Monitoring Programs Division

- LM Special Project 1 was upgraded to MDP Standard Operating Procedure MTH-15 due to program-wide adoption of this method