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® LMO2 System, Isolation and Confirmation		
Revision: 1	Replaces: LM Special Project 2	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 monocytogenes* II (LMO2) 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 bioMérieux, Inc. 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 Official Method, *Listeria monocytogenes* in Foods, 2004.02
- 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® LMO2 User Guide & Protocol Summary, bioMérieux, Inc.

7. Procedures

7.1 Equipment and Materials

- VIDAS® System, bioMérieux
- VIDAS® LMO2 kit , bioMérieux
- Universal Pre-enrichment Broth (UPB)
- Half/Demi-Fraser Broth (dFB)
- Fraser Broth (FB) without ferric ammonium citrate
- Modified Oxford Agar (MOX)
- 5% Sheep Blood agar (SBA); Optional: Horse Blood Agar (HBA) plates
- Erythromycin - Item # 97061-222 (50g) from VWR or equivalent
- Trypticase Soy Agar (TSA)
- Trypticase Soy Agar with 0.6% Yeast extract (TSAYE)
- Brain Heart Infusion Broth (BHI) (optional)
- Listeria chromogenic agar (For example: R&F *L. monocytogenes* chromogenic agar, BioRad Rapid L. mono agar or Rapid Listeria agar, etc.)
- Incubator capable of maintaining a temperature of 30±2°C
- Incubator capable of maintaining a temperature of 35±2°C
- VITEK® 2 Gram Positive (GP) cards, bioMérieux
- VITEK® System, bioMérieux
- Additional materials needed to perform procedure as listed in VIDAS® LMO2 User Guide & Protocol Summary
- API Listeria

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

- Media control: Uninoculated demi-Fraser broth

- 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 dFB.
- **Sprouts:** Aseptically transfer approximately 25g of the sprouts to ~225mL of dFB. Stomach the sample for 2 minutes and leave it soaking.
- **Bagged Lettuce**: Aseptically transfer approximately 25g of the bagged lettuce sample to ~225mL of dFB, shake or rub and leave it soaking.
- **Bagged Spinach**: Aseptically transfer approximately 25g of the bagged spinach sample to ~225mL of dFB, shake or rub and leave it soaking.

- Incubate dFB samples at 30±1°C for 25±1 hours.

OPTIONAL: Following 24h dFB incubation of samples, if any sample broth has darkened, streak that 24h dFB to MOX and/or Listeria Chromogenic agars. 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 section 7.4. for cultural confirmation.

- After incubation, mix sample well and transfer 1mL of the dFB suspension into a tube containing 10mL FB.
- Incubate FB tubes at 30±1°C for 25±1 hours.

7.3.1 Perform VIDAS® LMO2 assay on 24h individual FB samples (no pooling). Refer to kit insert for details and refer to the VIDAS® User Manual for run set-up and sample loading

procedures.

Note: *If the VIDAS® LMO2 assay cannot be performed immediately after the FB incubation period, it is acceptable to refrigerate enrichment sample bags approximately 48 hours before advancing to next analytical step.*

7.3.2 Refrigerate remaining enrichment broths until all analyses are complete.

7.3.3 Once the LMO2 assay is completed, results are analyzed automatically by the instrument and a report is printed out. Samples with a test value greater than or equal to the threshold value of 0.05 are reported as positive and shall be confirmed culturally. Samples with test values lower than the threshold value indicate samples with undetectable *L. monocytogenes* antigen.

7.3.4 Initiate cultural confirmation from the positive individual FB enrichment 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 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 from 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) to check for β hemolysis. Incubate blood plates at 35±2°C for 18-24 hours.

7.4.3 After 24 hours 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 monocytogenes,* proceed to SOP MDP-DATA-01, for reporting.

7.4.6 If VITEK® result 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 do not confirm as *L. monocytogenes,* stop further analysis.

7.5 Reporting

7.5.1 Report preliminary results following VIDAS LMO2 Positive 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 (if PFGE is not to be done in testing laboratory).

- LM Special Project 2 was upgraded to MDP Standard Operating Procedure MTH-16 due to the program-wide adoption of this method

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