

## 17.10.08

### AOAC Official Method 997.03 Detection of *Listeria monocytogenes* and Related *Listeria* Species in Selected Foods and from Environmental Surfaces

#### Visual Immunoprecipitate Assay (VIP)

First Action 1997

Revised 2001

Final Action 2001

(Applicable to detection of *Listeria monocytogenes* and related *Listeria* species in dairy foods, red meats, pork, poultry and poultry products, seafood, fruits, vegetables, nutmeats, pasta, chocolate, eggs, bone meal, and from environmental surfaces.)

**Caution:** *L. monocytogenes* infections can cause fetal death. It is recommended that pregnant women avoid handling this organism. Attention should be given to sterilization of contaminated equipment and media before disposal or reuse.

See Table 997.03 for the results of the interlaboratory study supporting acceptance of the method.

#### A. Principle

In the VIP assay, proprietary antibodies, with high specificity to antigens of *L. monocytogenes* and related *Listeria* species, are bound to a chromogenic carrier and separately to a solid support matrix. These reagents are configured in a single use device that will produce a visually determined reaction in the presence of *Listeria*. During the initial hydration of the device, *Listeria* will react with an antibody-chromogen complex contained in the device. Antigen-antibody-chromogen complex is formed, which flows across lateral flow membrane and is subsequently bound by antibody immobilized on membrane. If *Listeria* is present in the test portion, a detection line will form which is positioned across the solid support in a viewing window of the device. The formation of a visually detectable line indicates a positive reaction. Additionally, a procedural control window exists wherein a second line is formed indicating proper test completion. Absence of a procedural control line indicates an invalid test.

#### B. Media and Reagents

(a) *Visual Immunoprecipitate assay (VIP) unit*.—One for each test portion (available as VIP for *Listeria* from BioControl Systems, Inc., 12822 SE 32nd St, Bellevue, WA 98005, USA).

(b) *Modified Fraser broth with lithium chloride (mFB + LiCl)*.—Suspend 55 g of commercial Fraser broth base into 1 L water. Stir until completely dissolved. If necessary, warm to dissolve powder. Do not overheat. Only after powder has completely dissolved, add 4 g LiCl and stir until completely dissolved. Sterilize by autoclaving at 121°C for 15 min. Do not overheat. Do not add ferric ammonium citrate additive to broth. Alternatively, prepare a 45% (w/v) LiCl solution by dissolving 45 g LiCl in enough water for final volume of 100 mL. Filter sterilize the solution through a 0.2 µm filter. Add 2 mL sterile LiCl stock solution to 225 mL sterilized mFB. If using a commercially prepared sterile 8M LiCl stock solution (Sigma), add 2.65 mL per 225 mL sterilized mFB.

(c) *Buffered Listeria enrichment broth (BLEB)*.—Suspend 36.1 g commercial *Listeria* enrichment broth in 1 L water. Add 8.5 g 3-(N-morpholino)propanesulfonic acid (MOPS) free acid and 13.7 g MOPS sodium. Mix well and heat to dissolve if necessary.

Dispense in 9 mL aliquots. Sterilize by autoclaving at 121°C for 15 min.

#### C. Apparatus

(a) *Incubators*.—Maintaining 30–32°C and 35–37°C.

(b) *Micropipets*.—Accurately dispensing 0.1 and 1.0 mL.

(c) *Vortex mixer*.—For mixing test portion tubes.

(d) *Waterbath*.—Maintaining 100°C. Alternatively, flowing steam autoclave set at 100°C or dry heat block may be used.

(e) *Top loading balance*.—For weighing test portions, measuring up to 1000 g, sensitivity of ±0.1 g.

(f) *Blender/stomacher*.—For homogenizing test portions.

#### D. General Instructions

Store VIP units inside foil pouch with desiccant at ambient temperature (15–25°C). After use, discard units into a decontamination container and sterilize before disposal. Do not reuse, and do not use VIP units after expiration date.

Run positive and negative control cultures to become familiar with interpretations of results.

#### E. Preparation of Test Portions

(a) *Primary enrichment—Food products*.—Aseptically weigh 25 g test portion or pipet a 25 mL aliquot into 225 mL mFB + LiCl, **B(b)**. Blend or stomach solid food to homogenize test portion.

*Environmental monitoring*.—For environmental sponges, ensure that the sponge is in a horizontal position in the bag and add 60 mL mFB + LiCl, **B(b)**, to sponge bag. If using a swab, add swab test portion to 10 mL mFB + LiCl, **B(b)**.

Mix well via stomacher or vortex mixer. Incubate 28 ± 2 h at 30°C. If test material produces a viscous liquid (i.e., powdered cheese and meats), add ≤2.25 mL steamed Triton X-100 per 225 mL mFB + LiCl, **B(b)**, preparation prior to incubation.

(b) *Secondary enrichment*.—Transfer 1 mL incubated mFB + LiCl to 9 mL BLEB, **B(c)**. Vortex mix tubes and incubate at 30°C for 24 ± 2 h.

(c) *Inactivation*.—Vortex mix incubated BLEB tubes and transfer 1.0 mL to a clean tube. Inactivate microorganisms at 100°C for 5 min. Cool tube 25–37°C before testing. Inactivated tubes can be stored at 2–8°C up to 4 days prior to testing. Store remaining BLEB enrichment tubes at 2–8°C for confirmation of presumptive positive tubes.

#### F. VIP Assay Procedure

(1) Open sealed pouch containing VIP units, **B(a)**, and remove required number of tests. One device is necessary for each test portion. Do not reuse VIP units. Reseal unused VIP units in pouch containing desiccant. Store at ambient temperature (15–25°C) in a cool dark location.

(2) Gently mix inactivated enrichment broth. If the broth has been previously stored in the cold, bring to room temperature before testing.

(3) Transfer 0.1 mL inactivated broth to sample addition well.

(4) Incubate at ambient temperature for 10 min.

#### G. Reading and Interpreting Results

**Note:** Examine device immediately after 10 min incubation. Otherwise, faint lines may develop because of nonspecific color development that should be disregarded.

Examine VIP unit for the presence of distinct detection lines in both test sample and test verification windows. Lines should be dark

**Table 997.03 Interlaboratory study results for the detection of *Listeria monocytogenes* and related *Listeria* spp. from environmental surfaces (VIP)**

| Surface/<br>collection device | No. of<br>labs | Total No. test<br>samples | Samples positive   |                    | USDA/<br>FSIS | $\chi^2$ <sup>b</sup> | Sensitivity rate <sup>c</sup> |               | Incidence of false<br>negatives among total<br>positive test samples, % <sup>d</sup> |           | Specificity<br>rate <sup>e</sup> | Incidence of false<br>positives among total<br>negative test samples, % <sup>f</sup> |               | Agreement<br>between VIP<br>and<br>USDA/FSIS<br>methods, % <sup>g</sup> |
|-------------------------------|----------------|---------------------------|--------------------|--------------------|---------------|-----------------------|-------------------------------|---------------|--|-----------|----------------------------------|--|---------------|---|
|                               |                |                           | VIP                |                    |               |                       | VIP                           | USDA/<br>FSIS | VIP  | USDA/FSIS | VIP                              | VIP  |               |   |
|                               |                |                           | Pres. <sup>a</sup> | Conf. <sup>a</sup> | USDA/<br>FSIS | VIP                   |                               |               |  |           |                                  |  | USDA/<br>FSIS | VIP   |
| Steel/swab                    | 24             | 120                       | 36                 | 34                 | 36            | 0.2                   | 90                            | 95            | 10.0   | 5.0       | 98                               | 2  | 95            |   |
| Rubber/sponge                 | 24             | 120                       | 89                 | 88                 | 88            | 0.2                   | 97                            | 97            | 3.2  | 3.2       | 98                               | 3  | 95            |   |
| Uninoculated                  | 24             | 120                       | 1                  | 0                  | 0             | —                     | —                             | —             | —  | —         | —                                | —  | —             |   |
| Concrete/sponge               | 17             | 85                        | 55                 | 56                 | 57            | 0.5                   | 96                            | 100           | 3.5  | 0.0       | 100                              | 0  | 98            |   |
| Concrete/swab                 | 17             | 85                        | 51                 | 51                 | 51            | NA <sup>h</sup>       | 100                           | 100           | 0.0  | 0.0       | 100                              | 0  | 100           |   |
| Uninoculated                  | 17             | 85                        | 0                  | 0                  | 0             | —                     | —                             | —             | —  | —         | —                                | —  | —             |   |

<sup>a</sup> Pres. = presumptive positive data; Conf. = culturally confirmed data.

<sup>b</sup>  $\chi^2$  as defined by McNemar is  $(|a - b| - 1)^2 / (a + b)$  where a = test samples positive by VIP and negative by USDA/FSIS, and b = test samples negative by VIP and positive by USDA/FSIS. A  $\chi^2$  value >3.84 indicates significance at  $p < 0.05$ .

<sup>c</sup> Sensitivity rate is defined as total number of analyzed positive test portions among "known" positive test portions/laboratory divided by total number of "known" positive test portions/laboratory, where "known" positive is defined as test samples confirmed positive by the reference method.

<sup>d</sup> Incidence of false negatives is 100 – sensitivity rate.

<sup>e</sup> Specificity rate is defined as total number of analyzed negative test portions among "known" negative test portions/laboratory divided by total number of "known" negative test portions/laboratory, where "known" negative is defined as test samples confirmed negative by the reference method and negative controls.

<sup>f</sup> Incidence of false positives is 100 – specificity rate.

<sup>g</sup> Rate reflects number of confirmed determinations that were equivalent between VIP and USDA/FSIS.

<sup>h</sup> Statistical analysis not applicable. Methods gave equivalent results.

when contrasted with white background and should extend across window. Intensity of test sample and test verification lines may differ. Tests is valid if line is present in test verification window.

Test sample is considered positive when lines are present in test sample window and in test verification window. Test sample is considered negative when control is valid and no line is seen in test sample window. If no line is present in test verification window, test is invalid.

Autoclave used VIP units 15 min at 121°C prior to discarding.

#### **H. Confirmation of Positive VIP Test Portions**

Presumptive positive tests must be confirmed using culture methods as described in the current edition of *Bacteriological Analytical Manual*, AOAC INTERNATIONAL, Gaithersburg, MD 20877, USA, or *Microbiology Laboratory Guidebook*, U.S. Department of Agriculture/Food Safety Inspection Service, Athens, GA 30604, USA. Isolate from previously enriched BLEB tubes.

References: *J. AOAC Int.* **80**, 791(1997); (future issue).