



## Laboratory Guidebook Notice of Change

Chapter new, **revised**, or archived: MLG 8A.06

Title: FSIS Procedure for the Use of a *Listeria monocytogenes* Polymerase Chain Reaction (PCR) Screening Test

Effective Date: 01/02/2017

Description and purpose of change(s):

FSIS Laboratories validated ready-to-eat Siluriformes (fish) as a matrix for the *Listeria monocytogenes* screening test.

**The methods described in this guidebook are for use by the FSIS laboratories. FSIS does not specifically endorse any of the mentioned test products and acknowledges that equivalent products may be available for laboratory use. Method validation is necessary to demonstrate the equivalence of alternative tests as detailed in the document titled “FSIS Guidance for Evaluating Test Kit Performance” available on the FSIS website.**

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| Revision: .06   | Replaces: .05 | Effective: 1/2/17 |

## **Procedure Outline**

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### **8A.1 Introduction**

This method describes the use of a commercial PCR based screening procedure to screen-test processed meat, poultry, Ready to Eat (RTE) Siluriformes (fish), and egg products and environmental sponge samples for the presence of *Listeria monocytogenes*. All samples identified as presumptively positive for *Listeria monocytogenes* are subject to cultural confirmation.

### **8A.2 Safety Precautions**

CDC guidelines for the handling of BioSafety Level 2 organisms should be followed whenever live cultures of Salmonella are used. The Safety Data Sheet (SDS) may be obtained from the manufacturer for the media, chemicals, reagents, and microorganisms used in the analysis. The personnel who will handle the material should read the SDS prior to startup.

Laboratories should develop a policy and inform pregnant women and potentially immunocompromised individuals of the risk from working in laboratory rooms or areas where *Listeria monocytogenes* isolation or identification procedures are in progress.

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### 8A.3 Quality Control Procedures

#### 8A.3.1 Culture Controls

- a. At least one *Listeria monocytogenes* positive control strain is required. Appropriate cultures include ATCC<sup>®</sup> 19111, NCTC 7973 or other *Listeria monocytogenes* cultures validated to perform in an equivalent manner.
- b. At least one *Listeria innocua* negative control culture is required. Appropriate cultures include *Listeria innocua* strain ATCC<sup>®</sup> 33090 or other *Listeria. innocua* strains validated to perform in an equivalent manner.

#### 8A.3.2 Sterility Control

Prepare one “blank” (incubated but un-inoculated pre-enrichment/ enrichment broth) to provide a sterility control for the process.

### 8A.4 Equipment, Reagents, and Media

In addition to equipment, reagents, and media used in analysis of samples as described in MLG 8, the following materials will be needed.

- a. PCR tube holder (Qualicon)
- b. Cell lysis tube cooling block (Qualicon) held at  $5 \pm 3^{\circ}\text{C}$
- c. Techne<sup>®</sup> DB-2A heating block set at  $55 \pm 2^{\circ}\text{C}$
- d. Techne<sup>®</sup> DB-2A heating block set at  $95 \pm 3^{\circ}\text{C}$
- e. Repeater pipettor set at 200:1  $\mu\text{l}$ , and tips
- f. Pipettor set at  $5 \pm 1 \mu\text{l}$ , and tips
- g. Pipettor set at  $150 \pm 1 \mu\text{l}$ , and tips
- h. 12 X 75 mm Round-Bottom polypropylene tubes
- i. Cell Lysis Tubes and Caps, Cell Lysis Tube Rack and box (Genemate 8 strip tubes, ISC Bioexpress, T-3120-5)
- j. Pipettor and 5 ml pipettes
- k. BAX<sup>®</sup> Assay for Screening *L. monocytogenes* (Qualicon # 17710609) held at  $5 \pm 3^{\circ}\text{C}$
- l. Morpholinepropanesulfonic acid-buffered *Listeria* enrichment broth (MOPS-BLEB) medium:  
BBL *Listeria* enrichment broth (BBL #12333, or equivalent)  
MOPS free acid (Sigma #1254, or equivalent)  
MOPS sodium salt (Sigma #M9381, or equivalent)

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**8A.5 Sample Preparation and Primary Enrichment**

Perform sample preparation and pre-enrichment in as described in MLG 8, Section 8.5.1 and 8.5.2.

**8A.6 Secondary Enrichment and Primary Enrichment Plating**

- a. Transfer  $0.1 \pm 0.02$  ml of the UVM enrichment to  $10 \pm 0.5$  ml of MOPS-BLEB. Incubate inoculated MOPS-BLEB tubes at  $35 \pm 2^\circ\text{C}$  for 18-24 h.
- b. Streak a MOX plate. Streak a loopful or a drop approximating 0.1 ml of the UVM over the surface of the plate. Alternatively, dip a sterile cotton-tipped applicator or equivalent into the UVM and swab 25-50% of the surface of a MOX plate. Use a loop to streak for isolation from the swabbed area onto the remainder of the plate. Incubate the MOX at  $35 \pm 2^\circ\text{C}$  for  $26 \pm 2$  h.

**8A.7 The BAX<sup>®</sup> System for Screening *Listeria monocytogenes* Test Procedure**

Follow the current BAX<sup>®</sup> User's Guide for preparing reagents, performing the test, and reading the results. The equipment must be set up, and operated, and all records must be documented, according to laboratory work instructions.

**8A.8 Cultural Confirmation**

- a. Streak a MOX plate using a loopful of the MOPS-BLEB, or streak a drop approximating 0.1 ml or aseptically dip a sterile cotton-tipped applicator into the MOPS-BLEB and swab 25-50% of the surface of a MOX plate. Use a loop to streak for isolation from the swabbed area onto the remainder of the plate. Incubate the MOX at  $35 \pm 2^\circ\text{C}$  for  $26 \pm 2$  h.
- b. Proceed with all isolation and purification procedures as per MLG 8, Sections 8.5.4.a.i, 8.5.6, and 8.6.

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### **8A.9 Interpretation of Results**

- a. Samples that test BAX<sup>®</sup>-negative will be reported as negative if the concurrent 24h Primary Enrichment Plating is negative. Cultural analysis will continue on samples that are BAX<sup>®</sup>-negative but have typical colonies on the 24 h Primary Enrichment Plating MOX plates, or have a BAX<sup>®</sup>-positive, BAX<sup>®</sup>-indeterminate or have an invalid result. Alternatively, for samples with a BAX<sup>®</sup>-indeterminate or an invalid result, the laboratory may review the cause and perform a correction. Based on the findings, the laboratory may analyze the indeterminate or invalid result samples by:
- repeating the BAX<sup>®</sup> analysis from the rack loading step or
  - preparing new BAX<sup>®</sup> tubes and repeating the analysis.
- b. In analytical runs where the positive control tests BAX<sup>®</sup>-negative, indeterminate, or has a signal-error result, the entire batch of samples is affected and a review of the cause and a correction shall be performed. Based on the findings the laboratory may analyze the samples by:
- repeating the BAX<sup>®</sup> analysis from the rack loading step
  - preparing new BAX<sup>®</sup> tubes and repeating the analysis or
  - analyzing all of the samples culturally.

If reanalysis is unsuccessful then sample discards or preparing fresh samples from the reserves are required.

### **8A.10 Completion of Testing if BAX<sup>®</sup> Unavailable**

If circumstances (e.g. a power outage or equipment failure) do not allow testing using the BAX<sup>®</sup> system, the laboratory shall complete the cultural method by streaking all samples and controls from MOPS-BLEB medium onto MOX plates. Proceed with all isolation and purification procedures as per MLG 8, Sections 8.5.6 and 8.6.

### **8A.11 Selected References**

Centers for Disease Control and Prevention and National Institutes of Health (CDC/NIH). 2007. BioSafety in Microbiological and Biomedical Laboratories, 5<sup>th</sup> ed., or current.  
<http://www.cdc.gov/biosafety/publications/bmb15/index.htm>

BAX<sup>®</sup> System PCR Automated Detection for Bacterial Screening User Guide, Dupont Qualicon.