United States Department of Agriculture
Food Safety and Inspection Service
Office of Public Health Science
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Laboratory Guidebook
Notice of Change

Chapter new, **revised**, or archived: MLG 8A.03

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

Effective Date: 2/19/08

Description and purpose of change(s):

FSIS has extended the validated use of a commercial PCR based screening procedure to include pasteurized liquid egg products.

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.

Procedure Outline

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8A.1 Introduction

8A.1.1 General

This method describes the use of a commercial PCR based screening procedure as described in MLG 8 Section 8.4.5 to screen-test processed meat, poultry, pasteurized liquid egg products and environmental sponge samples for the presence of *Listeria monocytogenes.* All samples identified as presumptively positive for *Listeria monocytogenes* by these tests are subject to cultural confirmation.

8A.1.2 Limits of Detection

For this method, *L. monocytogenes* detection limits are determined to be better than 1 cfu/g in a 25g meat, poultry, or environmental sample, approximately 4.5 cfu/g in a 25 g pasteurized liquid whole egg blend sample, and $1.0x10^{-2}$cfu/ml in a 500 ml brine sample.

8A.2 Safety Precautions

CDC guidelines for the handling of BioSafety Level 2 organisms should be followed whenever live cultures of *Listeria monocytogenes* are used. All available Material Safety Data Sheets

(MSDS) must 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 all MSDS sheets, and all MSDS requirements should be followed.

Pregnant women and potentially immunocompromised individuals must be prohibited from laboratory rooms or areas where *L. monocytogenes* isolation or identification procedures are in progress. Although a properly sanitized laboratory area should not harbor *L. monocytogenes* or other pathogens, supervisors should use their own discretion in allowing high-risk individuals into these areas when not in use for these activities.

8A.3 Quality Control Procedures

8A.3.1 Culture Controls

a. At least one *L. monocytogenes* positive control strain is required. Appropriate cultures include ATCC 19111, NCTC 7973 or other *L. monocytogenes* cultures validated to perform in an equivalent manner.

b. At least one *L. innocua* negative control culture is required. Appropriate cultures include L. *innocua* strain ATCC 33090 or other *L. innocua* strains validated to perform in an equivalent manner.

8A.3.2 Sterility Control

Additionally, always 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 4 ± 2°C
c. Techne DB-2A Heating block set at 55 ± 2°C
d. Techne DB-2A Heating block set at 95 ± 3°C
e. Eppendorf Repeater Plus Pipettor (or equivalent) set at 200:l µl, and tips

f. Corning Lambda 20 Pipettor (or equivalent) set at 5 ± l µl, and tips

g. Corning Lambda 200 Pipettor (or equivalent) set at 150 ± l µl, and tips

h. 12 X 75 mm (Falcon 352063, or equivalent) 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® Assay for Screening *L. monocytogenes* (Qualicon # 17710609) held at 4 ± 2°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)

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 Direct Plating

a. Transfer 0.1 ± 0.02 ml of the UVM enrichment to 10 ± 0.5 ml of MOPS-BLEB. Incubate inoculated MOPS-BLEB tubes at 35 ± 2°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 ± 2°C for 26 ± 2 h.

8A.7 The BAX® System for Screening L. monocytogenes Test Procedure

Follow the current BAX® 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 by streaking a drop approximating 0.1 ml or aseptically dip a sterile cotton-tipped applicator or

equivalent 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 ± 2°C for a minimum of 24 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.

8A.9 Interpretation of Results

a. Samples that test BAX®-negative will be reported as negative if the concurrent 24h Direct Plating is also negative. Cultural analysis will continue on samples that are BAX®-negative but have typical colonies on the 24 h Direct Plating MOX plates, or have a BAX®-positive, BAX®-indeterminate or have an invalid result. Or based on the findings of a cause analysis, the laboratory may analyze the indeterminate or invalid result samples by:
   - repeating the BAX® analysis from the rack loading step or
   - preparing new BAX® tubes and repeating the analysis.

b. In analytical runs where the positive control tests negative, either the reserve samples will be retested or 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.10 Completion of Testing if BAX® Unavailable

If circumstances (e.g. a power outage or equipment failure) do not allow testing using the BAX® 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, 5th ed. U.S. Government Printing Office, Washington, D.C. (also found on the internet at:
http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm

BAX® System PCR Automated Detection for Bacterial Screening User Guide, Dupont Qualicon.