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

Chapter new, **revised**, or archived: MLG 4C.02

Title: FSIS Procedure for the Use of a Polymerase Chain Reaction (PCR) Assay for Screening *Salmonella* in Raw Meat, Carcass Sponge Samples, Whole Bird Rinses, Ready-to-Eat Meat and Poultry Products and Pasteurized Egg Products

Effective Date: 7/13/07

Description and purpose of change(s):

The chapter title was changed. The procedure to follow when a PCR indeterminate or signal-error occurs was clarified in Section 4C.7 Interpretation of Results.

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.

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Title: FSIS Procedure for the Use of a Polymerase Chain Reaction (PCR) Assay for Screening Salmonella in Raw Meat, Carcass Sponge Samples, Whole Bird Rinses, Ready-to-Eat Meat and Poultry Products and Pasteurized Egg Products		
Revision: 02	Replaces: 01	Effective: 7/13/07

Procedure Outline

4C.1 Introduction

4C.1.1 General

This method describes the use of a commercial PCR-based screening procedure as described in MLG 4, Section 4.4.5 to screen test Ready-to-Eat meat and poultry products, pasteurized egg products, raw meat, carcass sponge samples, and whole bird rinses for *Salmonella*. All samples identified as potentially positive for the presence of *Salmonella* by this test are subject to cultural confirmation as described in MLG 4.

4C.1.2 Limits of Detection

For this method, *Salmonella* detection limits are determined to be better than 1 cfu/g in a 25g sample.

4C.2 Safety Precautions

CDC guidelines for the handling of BioSafety Level 2 organisms should be followed whenever live cultures of *Salmonella* 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.

4C.3 Quality Control Procedures

4C.3.1 Culture Controls

See MLG 4, Section 4.3.1 for a description of the culture controls.

4C.3.2 Sterility Control

Additionally, always prepare at least one "blank" (incubated but un-inoculated pre-enrichment/ enrichment broth) to provide a sterility control for the process.

4C.4 Equipment, Reagents, and Media

In addition to equipment, reagents and media used in analysis of samples as described in MLG 4, the following materials will be needed.

a. PCR tube holder (Qualicon)
b. Cell lysis tube cooling block (Qualicon) held at 5 ± 3°C
c. Techne DB-2A, or equivalent, heating block set at 37 ± 2°C
d. Techne DB-2A, or equivalent, heating block set at 95 ± 3°C
e. Repeating pipettor to deliver 200 ± 20 µl, and sterile tips
f. Pipettor to deliver 5 ± l µl, and sterile disposable filtered tips
g. Pipettor to deliver 150 ± 15 µl, and sterile disposable filtered tips
h. Eight-channel pipettor to deliver 50 ± 5 µl, and sterile disposable tips
i. 12 X 75 mm Falcon 352063, or equivalent, tubes
j. Cell lysis tubes and caps, cell lysis tube rack and box (Genemate 8 strip tubes, ISC Bioexpress, T-3120-5)
k. Pipettor and 5 ml pipettes
l. BAX® System PCR Assay for Screening *Salmonella* kit (Qualicon # 17710608) held at 5 ± 3°C

4C.5 Sample Preparation and Primary Enrichment

4C.5.1 Raw Meat, Carcass Sponge Samples, Whole Bird Rinses

Perform sample preparation and pre-enrichment as described in MLG 4, Sections 4.5.5 through 4.5.7.

4C.5.2 Ready-To-Eat Meat and Poultry Products and Pasteurized Egg Products

Perform sample preparation and pre-enrichment as described in MLG 4, Sections 4.5.3, 4.5.4, 4.5.8 and 4.5.9 with the exception of incubation time in BPW. The incubation time in BPW for BAX® System PCR Assay for Screening *Salmonella* analysis of Ready-to-Eat meat and poultry products and pasteurized egg products is 18-24h.

4C.6 The BAX® System for Screening *Salmonella* 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, operated, and all records documented according to laboratory work instructions.

4C.7 Interpretation of Results

a. Samples that test BAX® -negative will be reported as negative. Cultural analysis will continue as per MLG 4, Section 4.5.3.d-i, of a sample BPW pre-enrichment that tests BAX® -positive, BAX® -indeterminate, or has a BAX® signal-error result. Or based on the findings of a cause analysis, the laboratory may analyze the indeterminate or signal-error 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 both positive controls test negative, the reserve samples will be retested beginning with sample preparation and enrichment. In analytical runs where one of the positive controls tests negative, the laboratory shall grow the control culture and continue cultural analysis of all samples by proceeding with isolation and purification steps as per MLG 4, Section 4.5.3.d-i.

4C.8 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, if possible, continue cultural analysis of all samples by proceeding with isolation and purification steps as per MLG 4, Section 4.5.3.d-i.

4C.9 Selected References

Centers for Disease Control and Prevention and National Institutes of Health (CDC/NIH). 1999. BioSafety in Microbiological and Biomedical Laboratories, 4th ed. U.S. Government Printing Office, Washington, D.C. also found on the internet at: http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

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