



Laboratory Guidebook Notice of Change

Chapter new, **revised**, or archived: MLG 4C.04

Title: FSIS Procedure for the Use of a Polymerase Chain Reaction (PCR) Assay for Screening *Salmonella* in Meat, Poultry, Pasteurized Egg and Catfish Products

Effective Date: 05/01/2013

Description and purpose of change(s):

Section 4C.5 Table 1 was removed as it is already available in Microbiology Laboratory Guidebook Chapter, MLG 4.

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. FSIS provides guidance at:

[http://www.fsis.usda.gov/PDF/Validation Studies Pathogen Detection Methods.pdf](http://www.fsis.usda.gov/PDF/Validation_Studies_Pathogen_Detection_Methods.pdf)

**United States Department of Agriculture
Food Safety And Inspection Service, Office of Public Health Science**

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Revision: 04	Replaces: 03	Effective: 05/01/2013

Procedure Outline

- 4C.1 Introduction
- 4C.2 Safety Precautions
- 4C.3 Quality Control Procedures
- 4C.4 Equipment, Reagents, and Media
- 4C.5 Sample Preparation and Primary Enrichment
- 4C.6 The BAX[®] System for Screening *Salmonella* Test Procedure
- 4C.7 Interpretation of Results
- 4C.8 Completion of Testing if BAX[®] Unavailable
- 4C.9 Selected References

4C.1 Introduction

This method describes the use of a commercial PCR-based screening procedure as described in MLG 4, Section 4.5 to screen test Ready-to-Eat meat and poultry products, pasteurized egg products, raw meat, carcass sponge samples, whole bird rinses, and raw catfish products 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. Unless otherwise stated all measurements cited in this method have a tolerance range of $\pm 2\%$.

4C.2 Safety Precautions

CDC guidelines for the handling of BioSafety Level 2 organisms should be followed whenever live cultures of *Salmonella* are used. The Material Safety Data Sheet (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 the MSDS prior to startup.

4C.3 Quality Control Procedures

Use the method controls described in MLG 4 Section 4.3.1.

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.

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- a. PCR tube holder (Qualicon)
- b. Cell lysis tube cooling block (Qualicon) held at $5 \pm 3^{\circ}\text{C}$
- c. Techne DB-2A, or equivalent, heating block set at $37 \pm 2^{\circ}\text{C}$
- d. Techne DB-2A, or equivalent, heating block set at $95 \pm 3^{\circ}\text{C}$
- e. Repeating pipettor to deliver $200 \pm 20 \mu\text{l}$, and sterile tips
- f. Pipettor to deliver $5 \pm 1 \mu\text{l}$, and sterile disposable filtered tips
- g. Pipettor to deliver $150 \pm 15 \mu\text{l}$, and sterile disposable filtered tips
- h. Eight channel pipettor to deliver $50 \pm 5 \mu\text{l}$, 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) held at $5 \pm 3^{\circ}\text{C}$

4C.5 Sample Preparation and Primary Enrichment

Sample preparation and enrichment incubation times may vary by matrix and/or program. Refer to MLG 4, Section 4.5 for additional sample preparation and Buffered Peptone Water (BPW) enrichment details. After incubation in BPW, proceed to Section 4C.6.

4C.6 The BAX[®] System for Screening *Salmonella* Test Procedure

Perform the screening on BPW pre-enriched samples following the current BAX[®] User's Guide. Also, 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 4.6, for a BPW pre-enrichment sample that tests BAX[®]-positive, BAX[®]-indeterminate, or has a BAX[®] signal-error result. Alternatively, for samples with BAX[®]-indeterminate or BAX[®] signal-error result the laboratory may review the cause and perform a correction. Based on the findings, the laboratory may analyze the indeterminate or signal-error result samples by:

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- 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 BAX[®] -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[®] analysis from the rack loading step
 - preparing new BAX[®] tubes and repeating the analysis or
 - analyzing all of the samples culturally.

If reanalysis is unsuccessful then discard the samples or prepare and analyze samples from the reserves.

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 4.6.

4C.9 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/biosafety/>

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