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 4C.07

Title: FSIS Procedure for the Use of a Polymerase Chain Reaction (PCR) Assay for Screening *Salmonella* in Meat, Poultry, Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges

Effective Date: 01/02/2017

Description and purpose of change(s):

FSIS Laboratories extended this method to Ready-to-Eat (RTE) Siluriformes (fish).

The FSIS Laboratories validated a 1:4 enrichment broth to sample ratio (1 part sample in 3 parts enrichment broth) for the analysis of RTE products for the presence of *Salmonella* (MLG 4 and 4C).

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: .07	Replaces: .06	Effective: 1/2/17

Procedure Outline

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4C.2 Safety Precautions
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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 (RTE) meat and poultry products, egg products, raw meat, carcass and environmental sponges, whole bird and parts rinses, and raw and RTE Siluriformes (fish) 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 ± 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 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.

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.

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) held at 5 ± 3°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 enrichment details. After incubation in enrichment broth, proceed to Section 4C.6.

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

Following incubation, perform the rapid screen using 5 µl of sample enrichment for all matrices except raw beef/poultry mixes. Follow the current BAX® System User's Guide for preparing reagents, performing the remainder of the PCR test, and reading the results.

Following incubation of raw beef mixes containing poultry, a centrifuge step must be performed prior to BAX® screening:

- Dispense 200 ± 20 µl lysis reagent to each cell lysis tube.

- Heat the filled lysis tubes for 20 ± 1 minute at 37 ± 2°C. Aseptically transfer 1 ml of the poultry mix enrichment sample to a sterile 1.5 ml microcentrifuge tube.
- Centrifuge at a setting of 1,500 x g for 1 minute (at speed) to pellet large debris. Supernatant will still not be clear at this low speed but should no longer have large particles of meat suspended.
- Transfer the supernatant to a new sterile 1.5 ml microcentrifuge tube. It is essential to ensure that none of the pelleted debris is carried over with the supernatant.
- Centrifuge supernatant at 10,000 x g for 5 minutes.
- Discard the supernatant from the centrifuge tube, leaving a little of the supernatant if necessary so the pellet is not disturbed during this step.
- Suspend the pellet in 100 µl of PCR grade water either by vortexing or using the pipet tip.
- Add 5 µl of the suspension directly to the pre-heated lysis buffer that was prepared during the initial steps.
- Heat the inoculated lysis tubes for 10 ± 1 minute at 95 ± 3°C. Perform remainder of the PCR test according to manufacturer's 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 sample enrichment 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:

  • repeat the BAX® analysis from the rack loading step or
  • prepare 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:

  • repeat the BAX® analysis from the rack loading step
  • prepare new BAX® tubes and repeating the analysis or
  • analyze all of the samples culturally.

If reanalysis is unsuccessful then prepare fresh analytical portions from the sample reserve or discard the sample.

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.