



## Laboratory Guidebook Notice of Change

Chapter new, **revised**, or archived: MLG 5A.04

Title: FSIS Procedure for the Use of *Escherichia coli* O157:H7 Screening Tests for Meat Products and Carcass and Environmental Sponges

Effective Date: 06/29/14

Description and purpose of change(s):

The method has been extended for use on raw beef mixed with raw pork and/or raw poultry products.

A centrifuge step was added for analyzing raw beef mixes containing poultry on the post-enrichment PCR rapid screen.

**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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**Procedure Outline**

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- 5A.6 The BAX<sup>®</sup> System and BAX<sup>®</sup> System Real-Time PCR Assay for *E. coli* O157:H7
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**5A.1 Introduction**

This method describes the use of a commercial PCR-based screening procedure to screen test enrichment cultures for *Escherichia coli* O157:H7 (*E. coli* O157:H7). In the event that the BAX<sup>®</sup> system equipment or BAX<sup>®</sup> *E. coli* O157:H7 test kits are unavailable, this method describes the use of lateral flow devices that FSIS may use to screen for *E. coli* O157:H7.

Samples identified as potentially positive for the presence of *E. coli* O157:H7 by any of these tests are subject to cultural confirmation as described in MLG 5.

Unless otherwise stated all measurements cited in this method have a tolerance of  $\pm 2\%$ .

**5A.2 Safety Precautions**

*E. coli* O157:H7 is a human pathogen with a low infectious dose. (Ingestion of 100 cells can cause disease.) The use of gloves and eye protection is mandatory for all post enrichment viable culture work. Work surfaces must be disinfected prior to and immediately after use. Laboratory personnel must abide by CDC guidelines for manipulating Biosafety Class II pathogens. A Class II laminar flow biosafety cabinet is recommended for activities with potential for producing aerosols of pathogens. All available Safety Data Sheets (SDS) should be obtained from the manufacturer for the media, chemicals, reagents and microorganisms used in the analysis. The personnel who will handle the materials should read all SDS.

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### 5A.3 Quality Control Procedures

- a. All media must be pre-warmed to 18-35°C prior to use.
- b. *E. coli* O157:H7 strain 465-97, or equivalent, shall be used as the positive control. See MLG 5, Section 5.3.c for the positive culture control procedures.
- c. *E. coli* ATCC strain 25922, or equivalent, is used as the optional negative control for the latex agglutination assay.
- d. Prepare at least one “blank” (incubated but un-inoculated pre-enrichment/ enrichment broth) to provide a sterility control for the process.

### 5A.4 Equipment, Reagents and Media

In addition to equipment, reagents and media used in analysis of samples as described in MLG 5, the following materials will be needed.

- a. PCR tube holder (Qualicon or equivalent)
- b. Cell lysis tube cooling block (Qualicon or equivalent) held at  $5 \pm 3^\circ\text{C}$
- c. PCR cooling block (Qualicon or equivalent) held at  $< -10^\circ\text{C}$
- d. Heating block set at  $37 \pm 2^\circ\text{C}$
- e. Heating block set at  $95 \pm 3^\circ\text{C}$
- f. Repeating pipettor to deliver  $200 \pm 20 \mu\text{L}$ , and sterile tips
- g. Pipettor to deliver  $20 \pm 1 \mu\text{L}$ , and sterile disposable filtered tips
- h. Pipettor to deliver  $150 \pm 15 \mu\text{L}$ , and sterile disposable filtered tips
- i. Eight-channel pipettor to deliver  $30 \pm 3 \mu\text{L}$ , and sterile disposable tips
- j. 12 X 75 mm Falcon 352063, or equivalent, tubes
- k. Cell lysis tubes and caps, cell lysis tube rack and box (Genemate<sup>®</sup> 8 strip tubes, ISC Bioexpress, T-3120-5 or equivalent)
- l. Pipettor or pipettes to deliver 5 mL
- m. BAX<sup>®</sup> System Real-Time PCR assay for *E. coli* O157:H7 (Qualicon # D14203648) held at  $5 \pm 3^\circ\text{C}$
- n. Optional for Lateral Flow Device Testing:
  - ii. RapidChek<sup>®</sup> Pathogen Screening Test Kit (Romer Labs # 7000160)  
Polypropylene tubes and sterile pipets
  - iii. Heating block set at 97-100°C, boiling water bath, or autoclave (isotherm cycle)

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

Perform sample preparation and pre-enrichment as described in MLG 5, Section 5.5.

### **5A.6 The BAX<sup>®</sup> System Real-Time PCR Assay for *E. coli* O157:H7**

#### **5A.6.1 Procedure**

Following incubation, perform the rapid screen using 20 µl of enriched samples for all matrices except raw beef mixes containing poultry. Follow the current BAX<sup>®</sup> 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<sup>®</sup> screening to use the rapid screen procedure:

- 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.

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### 5A.6.2 Interpretation of Results

- a. Samples that test BAX<sup>®</sup>-negative shall be reported as negative. Cultural analysis shall continue per MLG 5, Sections 5.6 and 5.7 for sample pre-enrichments that test BAX<sup>®</sup>-positive, indeterminate, or have an invalid result. Or, the laboratory may review the cause and perform a correction. Based on the findings, the laboratory may:
  - repeat the BAX<sup>®</sup> analysis from the rack loading step
  - prepare new BAX<sup>®</sup> tubes and repeating the analysis or
  - screen test with a lateral flow device (LFD) described in Section 5A.7.
  
- 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:
  - repeat the BAX<sup>®</sup> analysis from the rack loading step
  - prepare new BAX<sup>®</sup> tubes and repeating the analysis
  - screen test with a lateral flow device (LFD) described in Section 5A.7
  - analyze all of the samples culturally.

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

### 5A.7 Use of Lateral Flow Devices for Screening *Escherichia coli* O157:H7

In the event that the BAX<sup>®</sup> system equipment fails or BAX<sup>®</sup> System Real-Time PCR Assay for *E. coli* O157:H7 test kits are unavailable, the following lateral flow devices may be used until the BAX<sup>®</sup> system is available to screen for *Escherichia coli* O157:H7. Follow the manufacturer's guidelines or other validated laboratory procedures. **RapidChek<sup>®</sup>** lateral flow device was validated for use in FSIS laboratories.

#### 5A.7.1 Test Procedure

- a. Remove the appropriate number of test devices from storage.
- b. Transfer 1-3 ml of the enrichment broth (from each Stomacher bag) into a polypropylene tube with a sterile pipette. For use with a heating

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- block, use ~ 1 ml. Heat the capped tubes at 97-100°C for the time specified by the manufacturer.
- c. Follow the current manufacturers' instructions for performing the test.

**5A.7.2 Interpretation of Results**

Follow the current manufacturer's instructions for reading the test device results.

**5A.8 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. U.S. Government Printing Office, Washington, D.C. also found at the CDC internet site.

BAX<sup>®</sup> System Real-time PCR Assay for Screening *E. coli* O157:H7 User Guide Supplement and Automated Detection for Bacterial Screening User Guide, Dupont Qualicon.

RapidChek<sup>®</sup> *E. coli* O157: (including H7) Test User Guide, Strategic Diagnostics Inc.