United States Department of Agriculture
Food Safety and Inspection Service
Office of Public Health Science
Laboratory QA Staff
950 College Station Road
Athens, GA 30605

Laboratory Guidebook
Notice of Change

Chapter **new**, revised**,** or archived: MLG 41A.00

Title: FSIS Procedure for the Use of a Polymerase Chain Reaction (PCR) Assay for Screening *Campylobacter jejuni/coli/lari* in Poultry Rinse, Sponge and Raw Product Samples

Effective Date: 05/01/2016

Description and purpose of change(s):

This chapter is issued in association with MLG 41. It describes a *Campylobacter jejuni/coli/lari* screening method validated by the FSIS laboratory system.

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.

Procedure Outline

41A.1 Introduction

This method describes the use of a commercial PCR-based screening procedure to screen-test poultry rinse, sponge and raw product for the presence of *Campylobacter jejuni/coli/lari*. All samples identified as potentially positive for *Campylobacter jejuni/coli/lari* are subject to cultural confirmation as described in MLG 41. Unless otherwise stated, all measurements cited in this method have a tolerance range of ± 2%.

41A.2 Safety Precautions

CDC guidelines for the handling of Biosafety Level 2 organisms should be followed whenever live cultures of *Campylobacter* are used. The Safety Data Sheet (SDS) 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 SDS prior to startup.

41A.3 Quality Control Procedures

Use the method controls and control culture preparation as described in MLG 41 Section 41.3.2 and 41.3.3a, c-d respectively.

41A.4 Equipment, Reagents and Media

In addition to equipment, reagents and media used in analysis of samples as described in MLG 41, 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 Real-Time PCR Assay for Screening *Campylobacter jejuni/coli/lari* kit (Qualicon) held at 5 ± 3°C

41A.5 Sample Preparation and Primary Enrichment

Perform sample preparation and primary enrichment as described in MLG 41.6. After initial incubation in enrichment broth, proceed to Section 41A.6. The enrichment culture may be analyzed immediately upon removal from the incubator without waiting for tempering to room temperature.

While the BAX® System for Screening *Campylobacter jejuni/coli/lari* test is running, the samples and controls can be maintained at room temperature. To limit the exposure of the aerobic environment, keep the sample bag/sample aliquot closed/covered until ready to continue the analysis. At the completion of the PCR test, move quickly to plate and place the positive samples and controls in the proper microaerobic environment following MLG 41, Section 41.6.5.

41A.6 The BAX® System for Screening *Campylobacter jejuni/coli/lari* Test Procedure

Following the initial incubation, perform the rapid screen using 5 µL of sample enrichment. Follow the current BAX® System User's Guide for preparing reagents, performing the remainder of the PCR test, and reading the results.

41A.7 Interpretation of Results

a. Samples that test BAX®-negative will be reported as negative for the enrichment method. For a sample enrichment that tests BAX®-positive, BAX®-indeterminate,

or has a BAX® signal-error result, continue cultural analysis of all samples and controls by proceeding with plating, isolation and confirmation steps starting at MLG 41, Section 41.6.5. 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 re-analysis is unsuccessful, then prepare fresh analytical portions from the sample reserve or discard the sample.

41A.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 and controls by proceeding with plating, isolation and confirmation steps starting at MLG 41, Section 41.6.5.

41A.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.