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

SOP No: MLG Appendix 3.08		Page 1 of 4
Title: FSIS Laboratory Sample Pathogen Methods Table and Definitions		
Revision: .08	Replaces: .07	Effective: 12/16/24

Laboratory Guidebook
Notice of Change

Chapter new, **revised**, or archived: MLG Appendix 3.08

Title: FSIS Laboratory Sample Pathogen Methods Table and Definitions

Effective Date:

Description and purpose of change(s):

- Horse Blood Overlay (HBO) agar, a differential media, and Modified Oxford (MOX) agar, a selective media, have been replaced with Harlequin® *Listeria* Chromogenic Agar (HLCA), a media that is both selective and differential for *L. monocytogenes.*
- Updated Figure 1 to show the decreased time for reporting a screened negative result for *L. monocytogenes* samples from 3 days to 2 days and the decreased time for reporting all confirmed positive samples to Days 3-6.
- Title Change to remove the word Regulatory.

APP 3.1 Introduction

This appendix summarizes key steps of the Microbiology Laboratory Guidebook (MLG) 4 *Salmonella*, MLG 8 *Listeria*, MLG 5C Shiga toxin-producing *Escherichia coli* (STEC), and MLG 41 *Campylobacter* methods. The terminology used for describing method results are defined in the glossary.

Table 1 lists the screening technology and result reporting timeline for each method. FSIS Field Inspectors collect samples on Day 0, and Day 1 is when samples arrive in the laboratory. Days listed for each analyte does not include delays (e.g., restreak for purity, waiting for completion of all analyses, rare strains requiring additional testing). Turnaround for these delays could include up to an additional 13 days. All confirmed positive isolates are forwarded to the Microbiology Characterization Branch (MCB) for further testing and upon completion are maintained in long-term storage. Table 1 does not include further characterization testing, e.g., Whole Genome Sequencing (WGS) and/or antimicrobial resistance (AMR) analyses.

AMR analyses are performed on *Salmonella*, STEC, and *Campylobacter* isolates as part of the National Antimicrobial Resistance Monitoring System (NARMS). This testing provides continuous monitoring of AMR profiles and allows detection of novel and emerging phenotypes and/or genotypes.

FSIS Laboratories utilize WGS data to infer the *Salmonella* serotype.

WGS is performed on all *Salmonella*, *L. monocytogenes*, STEC, and *Campylobacter jejuni/coli/lari* isolates and has replaced PFGE as the primary tool for determining source attribution and relatedness to other clinical, food, and environmental isolates. Sequence data is uploaded to the National Center for Biotechnology Information's (NCBI) website and is available to the public. Tools on the site compare the sequences to all uploads and ascertain relatedness and identify genes of interest. The data is also uploaded to CDC's PulseNet for further sharing and analysis with Federal and State public health partners.

Table 1. Summary of Laboratory Sample Pathogen Methods Reporting Times for STEC, *Listeria*, *Salmonella*, and *Campylobacter.* The reporting times are the number of days from when the sample was collected.

Analyte	**Microbiology Laboratory Guidebook Chapter #**	**Screen Test**	**Confirmatory Tests** (following culturing on tube and plating media; and following Immuno-Magnetic Separation [IMS] bead capture for STEC)	Days to Reporting**: Screen Negative**	Days to Reporting**: Potential + Result**	Days to Reporting: **Presumptive + Result**	Days to Reporting: **Final + Result**
STEC	MLG 5C	bioMérieux GENE-UP® (*stx*, *eae*, PEC+, and O group)	O group serological and genetic confirmation: Latex agglutination test kit and Real-Time PCR Isolate confirmation: Bruker® MALDI Biotyper Real-Time PCR Shiga toxin genes confirmation: *stx* and *eae* genes; if needed for inconclusive results, WGS	**Day 2**	**Day 2** (Limited distribution)	**Day 3**	**Day 4**
Listeria spp.	MLG 8	Neogen® Molecular Detection Assay	Isolate confirmation: Bruker® MALDI Biotyper; *Listeria monocytogenes* Genetic Identification Testing, if needed, for speciation – WGS	**Day 2**	**NA**	**Day 3-4**	**Day 4-6**
Salmonella spp.	MLG 4	Neogen® Molecular Detection Assay	Isolate confirmation: Bruker® MALDI Biotyper	**Day 2**	**NA**	**Day 5** NA for non-regulatory samples	**Day 5-6**
Campylobacter spp.	MLG 41	bioMérieux GENE-UP®	Typical colonies subject to same day confirmation: Bruker® MALDI Biotyper	**Day 2**	**NA**	**NA**	**Day 4**

APP 3.2 Definitions:

Potential positive STEC – Enrichment medium from a sample which yields a positive when screened on real-time PCR for each of the targeted genes (*eae*, *stx*1/2, PEC, and O group) of one or more of seven serogroups (O26, O45, O103, O111, O121, O145, O157).

Presumptive positive STEC – One or more typical colonies on modified Rainbow agar (mRBA) which agglutinate with latex agglutination reagents and are positive on real-time PCR for the *eae*, *stx*, and O group of one or more of the seven serogroups.

Confirmed positive STEC – One or more isolates from Sheep Blood Agar (SBA) positive on real-time PCR for the *eae*, *stx*, and O group of one or more of seven serogroups, and identified as *Escherichia coli*. All STEC isolates are forwarded for further characterization.

Presumptive positive *L. monocytogenes* – A sample yields one or more colonies which show typical appearance on Harlequin® *Listeria* Chromogenic Agar (HLCA).

Confirmed positive *L. monocytogenes –L. monocytogenes* typical colonies are identified as *L. monocytogenes.* All *L. monocytogenes* isolates are forwarded for further characterization.

Confirmed positive *Listeria* spp. – *Listeria* typical colonies are identified as *Listeria* spp. Further characterization will not be performed for *Listeria* spp.

Presumptive positive *Salmonella* spp. – A sample yields one or more colonies which show typical appearance on Xylose Lysine Deoxycholate Agar (XLD) and/or Double Modified Lysine Iron Agar (DMLIA).

Confirmed positive *Salmonella* spp. – *Salmonella* typical colonies are identified as *Salmonella* spp. All *Salmonella* isolates are forwarded for further characterization.

Confirmed positive *Campylobacter* spp. – *Campylobacter* typical colonies are identified as *Campylobacter* spp. All *Campylobacter* isolates are forwarded for further characterization.