United States Department of Agriculture
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Title: FSIS Laboratory Regulatory Sample Pathogen Methods Table and Definitions		
Revision: .06	Replaces: .05	Effective: 03/07/22

Laboratory Guidebook
Notice of Change

Chapter new, **revised**, or archived: MLG Appendix 3.06

Title: FSIS Laboratory Regulatory Sample Pathogen Methods Table and Definitions

Effective Date: 03/07/22

Description and purpose of change(s):

This Appendix was revised to reflect an update of the reporting time for *Campylobacter jejuni*/*coli*/*lari* negative and positive results. The reporting time was reduced from 3 days to 2 days for a screened negative result, and from 5 days to 4 days for a confirmed positive result.

APP 3.1 Introduction

This appendix summarizes key steps of the Microbiology Laboratory Guidebook (MLG) 4 *Salmonella*, MLG 8 *Listeria monocytogenes*, MLG 5C Shiga toxin-producing *Escherichia coli* (STEC), and MLG 41 *Campylobacter jejuni*/*coli*/*lari* 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. On Day 1, regulatory 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 also performed on *Salmonella*, STEC, and *Campylobacter* 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 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 Regulatory Sample Pathogen Methods for STEC, *L. monocytogenes*, *Salmonella*, and *Campylobacter*.

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 Based on specifications of MT sampling project	MLG 5C	iQ-Check VirX and iQ-Check SerO II Detection Assay (*stx*, *eae*, 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 monocytogenes	MLG 8	3M Molecular Detection Assay	Observation of β-hemolytic colonies Isolate confirmation: Bruker® MALDI Biotyper; Genetic Identification Testing, if needed, for speciation – WGS	**Day 3**	**NA**	**Day 4-5**	**Day 5-6**
Salmonella spp.	MLG 4	3M Molecular Detection Assay	Isolate confirmation: Bruker® MALDI Biotyper	**Day 2**	**NA**	**Day 5** NA for HACCP	**Day 5-6**
Campylobacter jejuni/coli/lari for Enrichment method	MLG 41	3M Molecular Detection Assay	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, 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*.

Presumptive positive *L. monocytogenes* – A sample from which one or more typical colonies produces beta-hemolysis on Horse Blood Overlay Agar (HBO).

Confirmed positive *L. monocytogenes* – A beta-hemolytic isolate is identified as *L. monocytogenes.*

Presumptive positive *Salmonella* spp. – A sample yields one or more isolates which show typical appearance on Triple Sugar Iron (TSI) and Lysine Iron Agar (LIA) slants.

Confirmed positive *Salmonella* spp. – *Salmonella* typical colonies is identified as *Salmonella* spp. All *Salmonella* isolates are forwarded for further characterization, e.g., serotype determination, WGS and AMR analyses.

Confirmed positive *Campylobacter* spp. – *Campylobacter* typical colonies is identified as *C. jejuni, C. coli, and/or C. lari.* All *Campylobacter* isolates are forwarded for further characterization, e.g., WGS and AMR analyses.