



Laboratory Guidebook Notice of Change

Chapter new, **revised**, or archived: MLG Appendix 3.03

Title: FSIS Laboratory Regulatory Sample Pathogen Methods Table and Definitions

Effective Date: 02/24/20

Description and purpose of change(s):

Revised this Appendix to reflect change to routinely determining *Salmonella* serotype using whole genome sequencing data.

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

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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* 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 sample arrives in the laboratory. Days listed do 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 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* 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 *Salmonella* serotype. MCB coordinates shipping of isolates that can't be identified using this method to the National Veterinary Services Laboratories (NVSL) for traditional antisera agglutination serotyping.

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.

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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 (<i>E. coli</i> O157:H7 and non-O157) Based on specifications of MT sampling project	MLG 5C	iQ-Check VirX and iQ-Check SerO iQ-Check VirX and iQ-Check SerO Detection Assay (<i>stx</i> , <i>eae</i> , O group)	<u>O group serological and genetic confirmation:</u> Latex agglutination test kit and Real-Time PCR <u>Biochemical confirmation:</u> Vitek® 2 GN Cards (bioMerieux) <u>Real-Time PCR Shiga toxin genes confirmation:</u> <i>stx</i> and <i>eae</i> genes; if needed, detection of toxin and H7 genes for O157 confirmation by Multiplex Real-Time PCR assay or WGS	Day 2	Day 2 (Limited distribution)	Day 3	Day 4-5 Depends on when Vitek® 2 result is avail, e.g., Day 4 PM; Day 5 AM
<i>Listeria monocytogenes</i>	MLG 8	3M Molecular Detection Assay	<u>Observation of β-hemolytic colonies</u> <u>Biochemical confirmation:</u> Vitek® 2 GP Cards (bioMerieux); Genetic Identification Testing, if needed, for speciation – GenProbe Accuprobe® Ribosomal RNA-based <i>L. monocytogenes</i> -specific test system or WGS	Day 3	NA	Day 4-5	Day 5-8
<i>Salmonella</i> spp.	MLG 4	3M Molecular Detection Assay	<u>Biochemical confirmation:</u> Vitek® 2 GN Cards (bioMerieux)	Day 2	NA	Day 5 NA for HACCP	Day 5-8 Depends on when Vitek® 2 result is avail, e.g., Day 7 PM; Day 8 AM
<i>Campylobacter</i> for Enrichment method	MLG 41	BAX PCR Assay	Typical colonies subject to same day: <u>Microscopic examination and Latex agglutination</u> (Scimedx Corp. or Microgen Bioproducts)	Day 3	NA	NA	Day 5

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APP 3.2 Definitions:

Potential positive *E. coli* O157:H7 – Enrichment medium from one or more subsamples yields a positive when screen tested.

Presumptive positive *E. coli* O157:H7 – One or more typical colonies on plating agar agglutinate with *E. coli* O157:H7 latex agglutination reagents and positive on the presumptive PCR assay.

Confirmed positive *E. coli* O157:H7 – One or more isolates from the sample is biochemically identified as *Escherichia coli*, serologically and genetically determined to be “O157” and meets at least one of the following criteria:

- 1) Positive for Shiga toxin (ST) production
- 2) Positive for Shiga toxin gene(s) (*stx*)
- 3) Genetically determined to be “H7”

Potential positive non-O157 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 six non-O157 serogroups (O26, O45, O103, O111, O121, O145).

Presumptive positive non-O157 STEC – One or more colonies on modified Rainbow agar (mRBA) agglutinate with latex agglutination reagents and are positive on the presumptive PCR screen.

Confirmed positive non-O157 STEC – Latex agglutination positive colony on Sheep Blood Agar (SBA), one or more isolates from the sample is positive on real-time PCR for the *eae*, *stx*, and O group of one or more of six non-O157 serogroups, and biochemically 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 characterized biochemically as *L. monocytogenes*. Ribosomal RNA testing is occasionally required to resolve atypical strains.

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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 are characterized biochemically as *Salmonella* spp. All *Salmonella* isolates are forwarded for further characterization, e.g., serotype determination, WGS and AMR analyses.

Confirmed positive *Campylobacter* spp. – Rapid screen positive, typical colony morphology, microscopic ID, latex agglutination positive for *C. jejuni*, *C. coli*, and/or *C. lari*.