



Laboratory Guidebook Notice of Change

Chapter new, **revised**, or archived: MLG Appendix 3.01

Title: FSIS Laboratory Regulatory Sample Pathogen Methods Table and Definitions

Effective Date: 1/15/15

Description and purpose of change(s):

This summary document was issued in association with MLG pathogen methods as performed in the FSIS laboratories. Current changes include additions of an alternative *E. coli* O157:H7 Shiga toxin gene PCR test to MLG 5.09, the average turnaround time for *Salmonella* isolates occasionally submitted to USDA, APHIS for serotyping, and an error correction of the automated biochemical test card used to confirm *L. monocytogenes*.

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.

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

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Title: FSIS Laboratory Regulatory Sample Pathogen Methods Table and Definitions	
Revision: 01	Replaces: 00
Effective: 1/15/15	

Analyte	Microbiology Laboratory Guidebook Chapter #	Screen Test	Confirmatory Tests (following culturing on tube and plating media; and for <i>E. coli</i> O157:H7 IMS bead capture)	Days to Reporting: Screen Negative	Days to Reporting: Potential + Result	Days to Reporting: Presumptive + Result	Days to Reporting: Final + Result
<i>E. coli</i> O157:H7	MLG 5 MLG 5A	BAX [®] Real-Time PCR Assay (alternative: lateral flow devices – RapidChek [®])	<u>Serological confirmation:</u> <i>E. coli</i> O157:H7 latex agglutination test kit (RIM [®] <i>E. coli</i> O157:H7 Latex Test Kit, REMEL) <u>Biochemical confirmation:</u> Vitek [®] 2 GN Cards (bioMerieux) <u>Real-Time PCR Shiga toxin genes/toxin confirmation:</u> <i>stx</i> and <i>eae</i> genes; if needed detection of toxin and H7 genes by Multiplex Real-Time PCR assay (alternative: Shiga Toxin test kit Premier [®] EHEC -Meridian Diagnostics, Inc)	Day 2	Day 2 (Limited distribution)	Day 3	Day 4-5 Depends on when Vitek [®] result is avail, e.g., Day 4 PM; Day 5 AM
<i>Non E. coli</i> O157 STEC	MLG 5B MLG 5B Appendix 3	BAX [®] Real-Time PCR Assays (alternative: Multiplex Real-Time PCR assays): <i>eae</i> , <i>stx</i> then <i>wzx</i> genes	<u>Serological agglutination</u> <u>Real-Time PCR typical colonies</u> <i>eae</i> , <i>stx</i> then <i>wzx</i> genes <u>Biochemical confirmation:</u> Vitek [®] 2 GN Cards (bioMerieux)	Day 2	Day 2 (Limited distribution)	Day 3	Day 4-5 Depends on when Vitek [®] result is avail, e.g., Day 4 PM; Day 5 AM
<i>Listeria monocytogenes</i>	MLG 8 MLG 8A	BAX [®] PCR Assay	<u>Tumbling Motility observation (optional test)</u> <u>Biochemical confirmation:</u> MICRO-ID [®] <i>Listeria</i> , <i>ListeriaAPI</i> [®] , Vitek [®] 2 GP Cards (bioMerieux), CAMP/CAMP Factor Test β-lysin CAMP factor discs (Remel) with MICRO-ID [®] ; Genetic Identification Testing if needed for speciation – GenProbe Accuprobe [®] Ribosomal RNA-based <i>L. monocytogenes</i> -specific test system	Day 3	NA	Day 4-5	Day 5-8

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Analyte	Microbiology Laboratory Guidebook Chapter #	Screen Test	Confirmatory Tests (following culturing on tube and plating media; and for <i>E. coli</i> O157:H7 IMS bead capture)	Days to Reporting: Screen Negative	Days to Reporting: Potential + Result	Days to Reporting Presumptive + Result	Days to Reporting Final + Result
<i>Salmonella</i> spp.	MLG 4 MLG 4C	BAX [®] PCR Assay	<u>Serological testing:</u> Somatic(O)Antigen Agglutination Tests (<i>Salmonella</i> polyvalent O antiserum), special projects only Flagellar (H) Antigen Agglutination Tests (Oxoid <i>Salmonella</i> Latex Test) <u>Biochemical confirmation:</u> Vitek [®] 2 GN Cards (bioMerieux)	Day 2	NA	Day 5 NA for HACCP	Day 5-8 Depends on when Vitek [®] result is avail, e.g., Day 7 PM; Day 8 AM
<i>Campylobacter</i> for Direct Plating method	MLG 41	Direct Plating	Typical colonies subject to same day: <u>Microscopic examination</u> <u>Latex agglutination</u>	Day 3	NA	NA	Day 3

*Table doesn't include additional testing, e.g., serotyping, PFGE subtyping, antibiotic susceptibility testing. *Salmonella* strains not serotyped by FSIS are submitted to USDA, APHIS for serotyping. The average turnaround for traditional serotyping of these occasional isolates is an additional 13 days. 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 in Outbreak Section Eastern Laboratory).

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 when tested with O157 antiserum.

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

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

Potential positive non *E. coli* 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 *wzx*) of one or more of six non-O157 serogroups (O26, O45, O103, O111, O121, O145).

Presumptive positive non *E. coli* O157 STEC – One or more agglutination positive colonies on modified Rainbow agar.

Confirmed positive non *E. coli* O157 STEC – Agglutination positive on Sheep Blood Agar, one or more isolates from the sample is confirmed positive on real-time PCR for the *eae*, *stx*, and *wzx* genes 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 Agar.

Confirmed positive *L. monocytogenes* – A beta hemolytic isolate is Camp test positive, shows tumbling motility (optional) and is characterized biochemically as *L. monocytogenes*. Ribosomal RNA testing is occasionally required to resolve atypical strains.

Presumptive positive *Salmonella* spp. – A sample yields one or more isolates which show typical appearance on TSI and 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., molecular serotyping and PFGE.

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