



Laboratory Guidebook Notice of Change

New, **revised**, or archived: MLG 4 Appendix 2.07

Title: Flow Chart Specific for FSIS Laboratory Isolation and Identification of *Salmonella*

Effective Date: 02/01/23

Description and purpose of change(s):

This flowchart is revised to add sample preparation instructions for raw beef product cloth samples. The cloth sampling device was added to the sample preparation section. This method was updated to remove the TSI and LIA additional screening media section.

Flow Chart Specific for FSIS Laboratory Isolation and Identification of *Salmonella*

Day 1

Various Samples + Enrichment
 Incubate BPW¹ @ 35°C for 18 – 24hrs (RTE¹¹), 20 – 24hrs (raw poultry).
 Incubate mTSB¹⁰ @ 42°C for 15 – 24hrs (raw meat).
 NOTE: Sample receipt temperature of ≤15°C is required for raw poultry and raw beef products.

PRIMARY ENRICHMENT

* This chart represents the best-case scenario, but analyses may take longer due to analytical testing circumstances such as re-streaking isolates for purity or one day shorter if Day 4 option is followed.
 **Negatives reported

Day 2

Perform 3M™ Molecular Detection Assay 2 - *Salmonella* ²

(+) (**) (-)**

Transfer 0.5 mL of enrichment broth to 10ml TT³ tubes
 Transfer 0.1 mL of enrichment broth to 10ml RV⁴ tubes
 Incubate all tubes @ 42°C for 22 – 24hrs

SECONDARY (SELECTIVE) ENRICHMENT

- 1 BPW = Buffered Peptone Water
- 2 3M™ Molecular Detection Assay = Rapid Screen Test
- 3 TT = TT broth (Hajna)
- 4 RV = Rappaport-Vassiliadis R10 Broth
- 5 BGS = Brilliant Green Sulfa Agar
- 6 DMLIA = Double Modified Lysine Iron Agar
- 7 SBA = Sheep Blood Agar
- 8 Bruker® MALDI Biotyper = Proteomic Identification System
- 9 Eastern Laboratory – Microbiology Characterization Branch (EL-MCB), USDA, FSIS
- 10 Modified Tryptone Soya Broth
- 11 Ready-to-Eat Processed Products

Day 3

Streak tubes of RV and TT to BGS⁵ and DMLIA⁶ plates. Incubate @ 35°C for 18 – 24 hrs.

DIFFERENTIAL & SELECTIVE ISOLATION MEDIA

Day 4

NT or (-) (T)

Re-incubate all BGS and DMLIA plates @ 35°C for 18 – 24 hrs.

Pick typical (T), streak SBA⁷ plate. Incubate @ 35°C for 16-24 hrs. Re-incubate all BGS and DMLIA plates @ 35°C for 18 – 24 hrs.

EXAMINATION FOR TYPICAL REACTIONS

Day 5

(-) (T) (T) (-)**

*add one day for follow-up

Examine SBA growth for purity. Perform confirmatory tests using a single isolated colony.

PURITY/GROWTH

Test on Bruker® MALDI Biotyper⁸

(-) (T) (-)** (+)

CONFIRMED POSITIVE Reported

CONFIRMED POSITIVE ISOLATE

FSIS Labs: Pick isolates to nutrient agar deep for further characterization testing and storage at EL-MCB⁹. Serotype will be inferred from WGS data.

ISOLATE CONFIRMATION

** Negatives Reported