Laboratory Guidebook
Notice of Change

Chapter new, revised, or archived: MLG 4 Appendix 2.03

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

Effective Date: 01/02/2017

Description and purpose of change(s):

This flow chart describes FSIS laboratory testing included in MLG 4 and MLG 4C.

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

**Primary Enrichment**

- Various Samples + Enrichment
- Incubate BPW at 35°C for 18-24 hrs (RTE), 20-24 hrs (raw poultry) or 22-26 hrs (fish). Incubate mTSB at 42°C for 15-24 hrs (raw meat).

**Secondary (Selective) Enrichment**

- Transfer 0.5 ml of enrichment broth to 10ml TT tubes.
- Transfer 0.1 ml of enrichment broth to 10ml RV tubes.

**Differential & Selective Isolation Media**

- Streak tubes of RV and TT to BGS and DMLIA plates. Incubate at 35°C for 18-24 hrs.

**Sugar Fermentation & H₂S/Gas Production**

- Re-incubate all BGS and DMLIA plates at 35°C for 18-24 hrs.
- Pick typical colonies (T), stab and streak TSI / LIA slants. Incubate at 35°C for 22-26 hrs.
  - Optional: also streak SBA plate now.

**Examination for Typical Reactions**

- Read TSI/LIA slants
  - *add one day for follow-up

**Purity/Growth**

- Transfer growth from TSI or LIA slants to SBA plate, streak for purity. Incubate at 35°C for 16-24 hrs.

**Confirmed Positive**

- Examine SBA growth for purity.
- Prepare saline suspension.

- Test on VITEK²:
  - (+)
  - (-)**

- CONFIRMED POSITIVE
  - Reported
  - CONFIRMED ISOLATE

**Biochemical Identification**

- FSIS Labs: Pick isolates to nutrient agar deep for further characterization testing at EL-MCB.
- Pick isolates to cryobeads for storage.

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**Notes:**

- This chart represents the best-case scenario but analyses may take longer due to normal analytical circumstances such as restreaking isolates for purity or one day shorter if Day 4 option is followed.

**Legend:**

- (+)
- (-)**
- (-)**
- NT or (-)

**Key:**

- BPW = Buffered Peptone Water
- PCR Screen Test = BAX³
- TT = TT broth (Hajna)
- RV = Rappaport-Vassiliadis R10 Broth
- BGS = Brilliant Green Sulfa Agar
- TSI = Triple Sugar Iron Agar
- LIA = Lysine Iron Agar
- SBA = Sheep Blood Agar
- VITEK² = VITEK² Compact System Automated Biochemical Identification System
- EL-MCB = Eastern Laboratory Microbiology Characterization Branch (EL-MCB), USDA, FSIS
- Modified Tryptone Soya Broth