Laboratory Guidebook
Notice of Change

Chapter new, revised, or archived: MLG 5C Appendix 1.02

Title: Flow Chart Specific for FSIS Laboratory Isolation and Identification of Shiga Toxin-Producing Escherichia coli (STEC)

Effective Date: 08/16/21

Description and purpose of change(s):

Replaced VITEK® 2 Compact system with Bruker® MALDI Biotyper
Flow Chart Specific for FSIS Laboratory Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STEC)

**Day 1**
Per 325 g sample, add 975 ml of mTSB¹, stomach for 2 min.
For sponges with 10 ml of buffer, add 50 ml of mTSB¹ to bring the volume to 60 ml, mix well.
Incubate @ 42 ± 1°C for 15-24 hours.
NOTE: Sample receipt temperature of ≤15°C is required.

**Day 2**

- **iQ-Check™ STEC VirX Kit²**
  - *stx (+) & eae (+)*
  - Report as **NEGATIVE**

- **iQ-Check™ STEC SerO II Kit²**
  - **O group (-)**
  - Report as **NEGATIVE**

- **O group (+)**

**Day 3**

- No growth, agglutination (-) colonies, or agglutination (+)/rapid screen (-)
  - Report as **NEGATIVE**

- Streak treated IMS bead capture to mRBA³ (2 dilutions) then incubate @ 35 ± 2°C for 20-24 hrs.
- Perform acid treatment step and streak treated IMS bead capture to mRBA³ (2 dilutions) then incubate @35 ± 2°C for 20-24 hrs.

**Day 4**

- Select a well-isolated colony from each identified phenotype on mRBA³ and perform agglutination with antisera and presumptive rapid screen test specific for the serogroup of interest.

**Acronym Table**
1. mTSB = Modified Tryptone Soy Broth
2. iQ-Check™ = Rapid Screen Assay
3. mRBA = Modified Rainbow Agar
4. SBA = Sheep Blood Agar
5. Bruker® MALDI Biotyper = Proteomic Identification System
6. WGS = Whole Genome Sequencing

*This chart represents the best-case scenario but analyses may take longer due to normal analytical circumstances such as restreaking isolates for purity.*

**CONFIRMED POSITIVE ISOLATES**
Pick isolates to cryobeads for storage.

**A STEC confirmation**
- Either stx (-) or eae (-)
  - Report as **STEC NEGATIVE**

- stx (+) & eae (+)

- **O group (-)**
  - Report as **STEC NEGATIVE**

- **O group (+)**
  - Bruker® MALDI Biotyper⁵ (+)
  - O group (+)
  - Report as **STEC CONFORMED POSITIVE**

- **stx (+) & eae (+)**

- **stx (+) & eae (+)**
  - O group (-)
  - Report as **STEC NEGATIVE**

- **stx (+) & eae (+)**
  - O group (+)
  - Bruker® MALDI Biotyper⁵ (+)
  - Further testing required by WGS⁶

**SBA⁴ incubation**, select and test a colony for:
1) Shiga toxin gene confirmation using iQ-Check³ STEC VirX Kit (stx/eae) and O group serological and genetic test using antigen latex agglutination and iQ-Check³ SerO II Kit
2) Isolate identification using Bruker® MALDI Biotyper⁵

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