Laboratory Guidebook
Notice of Change

Chapter new, revised, or archived: MLG 8 Appendix 1.03

Titles: Flow Chart Specific for FSIS Laboratory Isolation and Identification of *Listeria monocytogenes*.

Isolation and Identification of *Listeria monocytogenes* (Culture Method only)

Effective Date: 01/02/2017

Description and purpose of change(s):

This flow chart describes FSIS Laboratory testing included in MLG 8 and MLG 8A.

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 Listeria monocytogenes.

Day 1
- 25 g of product + 225 ml UVM¹ incubate @ 30°C for 20-26 hrs
- 125 g of product + 1125 ml UVM¹ incubate @ 30°C for 23-26 hrs
- Sponge sample + 225 ml UVM¹ incubate @ 30°C for 20-26 hrs

Day 2
- Direct Plate 0.1 ml onto MOX² plate
  Incubate @ 35°C for 24-28 hrs
- Transfer 0.1 ml to MOPS-BLEB¹
  Incubate @ 35°C 18-24 hrs

Day 3
- Perform BAX PCR⁴ Screen
- BAX and MOX must be (-) to report sample as negative
- Transfer colonies to HL³ plates
  Incubate @ 35°C for 18-26 hrs
- Streak onto MOX plates
  Incubate @ 35°C for 24-28 hrs

Day 4
- Examine HL plates for β-hemolytic colonies
  May perform GenProbe
  (-)*
  (+)*
- Examine MOX for typical colonies
  (+)
  (-)
- Transfer colonies to HL³ plates
  Incubate @ 35°C for 18-26 hrs
- Reincubate MOX @ 35°C for total time of 48-56 hrs

Day 5
- Examine morphology/motility
  (+)
- Inoculate VITEK® 2 biochemical test
  (-)*
  (+)*
- Inoculate HL, BHI⁴ broth, and SBA⁵

Day 6
- Re-examine for morphology/motility
  (+)
  (-)*
  (-)*
  Follow Day 4 protocol for morphology (+) Confirmed (+) possible on Day 8 or 9
- Examine biochemicals
  CAMP (+)
  L. innocua (+) (amine negative)
  CAMP (+)
  Motility (+)
  L. mono (+)
  (-)*
  Follow Day 5 protocol for morphology (-) Confirmed (+) possible on Day 8
- Examine morphology/motility
  (+)
  (-)*
  Follow Day 5 protocol for morphology (-) Confirmed (+) possible on Day 8
- Inoculate VITEK® 2 biochemical test

Day 7
- Biochemicals and CAMP Test
- Confirmed Positive⁶
- GenProbe⁷
  (+)*
  (-)*

* = results are reported
** = HL purity and BHI motility test are optional.
*** = if using Micro ID® for biochemical test.
FSIS Labs use GenProbe® to differentiate species when needed.

This chart represents the best case scenario. Analyses may take longer due to normal analytical circumstances.

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Isolation and Identification of *Listeria monocytogenes* (Culture Method only)

**Primary enrichment plating 0.1 ml onto MOX® plate Incubate @ 35°C for 24-28 hrs**

**Day 2**

- Option 1: Transfer 0.1 ml to FB³ Incubate @ 35°C for 24-28 hrs
- Option 2: Transfer 0.1 ml to MOPS-BLEB³ Incubate @ 35°C for 18-24 hrs

**Day 3**

- Transfer colonies to HL³ plates Incubate @ 35°C for 18-26 hrs
- Reincubate MOX@ 35°C for total time of 46-50 hrs
- Reincubate MOX plates. Incubate @ 35°C for 24-28 hrs

**Day 4**

- Examine HL plates for β-hemolytic colonies
- Both MOX and FB³ or MOPS-BLEB must be (+) to report sample as negative.
- Streak onto MOX plates. Incubate @ 35°C for 24-28 hrs
- Transfer colonies to HL³ agar plates. Incubate @ 35°C for 18-26 hrs
- Reincubate MOX @ 35°C for total time of 48-56 hrs

**Day 5**

- Examine morphology/motility
- Examine HL plates for β-hemolytic colonies
- Inoculate biochemical test (VITEK® 2, Micro ID®, or API®) and CAMP plate if needed

**Day 6**

- Inoculate MOX, BHI broth, and SBA**
- Inoculate biochemical test (and CAMP plate if needed)**
- Atypical isolates streaked on HL or BHI broth and incubated overnight/ CAMP test reevaluated in 48 hrs**
- Pick isolates to cryobeads for storage.
- Confirm Positive⁶

**Day 7**

- Examine MOX for typical colonies
- Examine MOX for presumptive (+)
- Examine HL plates for β-hemolytic colonies
- Examine biochemicals and CAMP test
- L. innocua (+) (mannose negative)
- L. mono (+)
- CAMP (+)
- Motility (+)
- Follow Day 5 protocol for morph/mot (+). Confirmed (+) possible on Day 8.

- Atypical isolates analyzed by GenProbe® on Day 8

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**Notes:**

* = results are reported
** = HL purity and BHI motility tests are optional. CAMP test required if using Micro ID for biochemical test
*** = (+) reincubated MOX follows the same steps one day behind

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**Abbreviations:**

- MOX = Modified Oxford Agar
- BHI = Brain Heart Infusion Broth
- FB = Fraser Broth
- UVM = Modified University of Vermont Broth
- MOPS-BLEB = (3-N-Morpholino) Propanesulfonic Acid-Buffered Listeria Enrichment Broth
- L. mono = *Listeria monocytogenes*
- L. innocua = *Listeria innocua*
- API = API®
- VITEK® 2 = VITEK® 2 System
- Micro ID = Micro ID®
- GenProbe®
- CAMP = CAMP Test
- Beta = β-hemolytic colonies

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**Table:**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Streak HL for purity if needed</td>
</tr>
<tr>
<td>2</td>
<td>Streak SBA or TSA-YE for biochemical tests and incubate @ 35°C, 22-26 hrs.</td>
</tr>
<tr>
<td>3</td>
<td>Inoculate BHI broth for morphology and motility if needed</td>
</tr>
<tr>
<td>4</td>
<td>Follow Day 4 protocol for MOX. Possibility of Presumptive (+) Day 6; Confirmed (+) Day 8/9</td>
</tr>
<tr>
<td>5</td>
<td>Re-examine for morphology/motility</td>
</tr>
<tr>
<td>6</td>
<td>Atypical isolates streaked on HL or BHI broth and incubated overnight/ CAMP test reevaluated in 48 hrs.</td>
</tr>
<tr>
<td>7</td>
<td>Confirm Positive by GenProbe® on Day 8</td>
</tr>
</tbody>
</table>

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**Legend:**

- MOX Plate
- (-) = result cannot be determined
- (+) = result is positive
- (-)* = result is negative
- Presumptive (+) = result is possible
- Confirmed Positive⁶ = result is confirmed positive
- Biochemicals and CAMP Test
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