Listeria monocytogenes in Raw Milk

OTSC staff seminar: Testing for Listeria monocytogenes in raw cow milk

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Listeria species

Six *Listeria* species – *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeeri*, *L. ivanovii*, *L. grayi*. The last two species each contain 2 subspecies. *L. monocytogenes* and *L. ivanovii* are pathogenic to mice and other animals. However, only *Listeria monocytogenes* (*L. mono.*) is commonly associated with human Listeriosis. Listeriosis associated infection by *L. ivanovii* and even *L. seeligeri* is extremely rare in humans.
What is listeriosis?
- Listeriosis, a serious infection usually caused by eating food contaminated with the bacterium *Listeria monocytogenes*, is an important public health problem in the United States. The disease primarily affects older adults, pregnant women, newborns, and adults with weakened immune systems. However, rarely, persons without these risk factors can also be affected.

What are the symptoms of listeriosis?
- A person with listeriosis usually has fever and muscle aches, often preceded by diarrhea or other gastrointestinal symptoms. Almost everyone who is diagnosed with listeriosis has "invasive" infection, in which the bacteria spread beyond the gastrointestinal tract. The symptoms vary with the infected person:
  - Pregnant women: Pregnant women typically experience only a mild, flu-like illness. However, infections during pregnancy can lead to miscarriage, stillbirth, premature delivery, or life-threatening infection of the newborn.
  - Persons other than pregnant women: Symptoms, in addition to fever and muscle aches, can include headache, stiff neck, confusion, loss of balance, and convulsions.

How does *Listeria* get into food?
- *Listeria monocytogenes* is found in soil and water. Animals can carry the bacterium without appearing ill and can contaminate foods of animal origin, such as meats and dairy products. When *Listeria* bacteria get into a food processing factory, they can live there for years, sometimes contaminating food products. The bacterium has been found in a variety of raw foods, such as uncooked meats and vegetables, as well as in foods that become contaminated after cooking or processing, such as soft cheeses, processed meats such as hot dogs and deli meat (both products in factory-sealed packages and products sold at deli counters), and smoked seafood. Unpasteurized (raw) milk and cheeses and other foods made from unpasteurized milk are particularly likely to contain the bacterium.
- *Listeria* is killed by pasteurization and cooking; however, in some ready-to-eat foods, such as hot dogs and deli meats, contamination may occur after factory cooking but before packaging. Unlike most bacteria, *Listeria* bacteria can grow and multiply in some foods in the refrigerator.

http://www.cdc.gov/nczved/divisions/dfbmd/diseases/listeriosis/#what
Method Overview

- 25 ml of sample enriched for 48 hours in Buffered Listeria Enrichment broth w/ pyruvate (BLEB). At the 4 hour enrichment mark, add antibiotic supplements. Incubation @ 30°C.
- Sample DNA Extract Prep with Wash, Spin, Boil technique
- Real-time PCR screen with MicroSEQ Pathogen Detection Kit on the ABI 7500 Fast
- Confirm positives referencing FDA Bacteriological Analytical Manual (BAM). Chapter 10
- Streak enrichment media to selective/differentiative agars to obtain typical isolates. 24-48 hr. incubation @ 36°C

- Agars: Modified Oxford agar (MOX) and R&F Chromogenic agar
  - MOX (Listeria species) – isolates black (grey) with black halo. 1mm (24hr), 2–3 mm in dia. with sunken center (48hr)
  - R&F – (L. mono & L. ivanovii) – isolates blue/green with small blue/green halo, 1-3 mm dia. convex
Confirm at minimum 2 typical isolates per positive sample: 
Hemolysis, Motility and Biochemical profile

Plate typical isolates to Tryptic Soy Agar/Yeast Extract (TSA/YE) plate to observe purity of isolate and use the same isolate to start Hemolysis.

- **Hemolysis** – Stab into 5% Sheep Blood Agar (SBA) plate – *L. mono.* and *L. seeligeri* - produce a slightly cleared zone around the stab. Other Listeria species show no hemolysis zone or a large well defined hemolysis zone.

- **Motility** – Motility Test Medium (MTM) or Tumbling motility with wet mount compound microscope
  MTM stab - incubated at room temp., observed daily up to 7 days for an umbrella-like growth pattern (mushroom).
  Wet mount microscope – short rods with slight rotating tumbling motility

- **Biochemical profile** – API *Listeria* – specific 4 digit numerical profile for *L. mono.* determined by the colors from chemical reactions.
ABI 7500 Fast
Instrument Results
ABI 7500 Fast and MicroSEQ kit
MicroSEQ Reaction Tubes and Strips
R&F Chromogenic Plate - Listeria monocytogenes (24hr)
R&F Chromogenic Plate - Listeria innocua (24hr)
Modified Oxford agar plate - Listeria monocytogenes (24hr)
Hemolysis Results (24hrs)
Motility Medium
API Listeria

Negative Test results

monocytogenes

Positive Test results