CHAPTER 8

Isolation and Identification of *Listeria monocytogenes*

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Introduction

Listeria is an important food-borne pathogen which affects the elderly, pregnant women, neonates and immuno-compromised populations. Listeria monocytogenes remains the main pathogenic species of the genus listeria. The genus Listeria comprises up to 21 species. L. monocytogenes, was originally described in 1926, but assigned the name in 1940. *Listeria monocytogenes* can be found in moist environments, soil, water, decaying vegetation and animals including fish. This can survive and even grow under refrigeration. When people eat food contaminated with *L. monocytogenes*, they may develop a disease called listeriosis. *L. monocytogenes* is generally transmitted when food is harvested, processed, prepared, packed, transported or stored in environments contaminated.

Listeria is a Gram-positive, facultative intracellular bacterial pathogen with the ability to adapt to a wide range of conditions of temperatures (2–4 °C), acidity and high-salt concentration. *Listeria* cells are slow growers and may be rapidly outgrown by competitors.

Isolation and characterization (BAM)

Qualitative detection from foods and environmental samples:

a. Individual subsample analysis: For solids, semi-solids, or liquids add 25 g representative portion to 225 ml BLEB containing pyruvate without selective additives (basal BLEB). Thoroughly homogenise the samples.
b. Aseptically add the three filter sterilized selective agents to achieve final concentrations of 10 mg/L acriflavin, 50 mg/L cycloheximide and 40 mg/L sodium nalidixic acid in the BLEB pre-enrichments.
c. Incubate at 30°C for 4 h.
d. Mix the enrichment with additives and continue incubation at 30°C for the remainder of the 24 to 48 h enrichment period.
e. A 50 g portion of the sample should be reserved for possible pathogen enumeration. Store it at 5°C if it is not frozen or, if frozen, in a non-defrosting freezer.

f. At 24 h and 48 h, streak BLEB enrichments onto one esculin-based and one chromogenic selective agar from each of the categories listed in Sections G.1.A and G.1.B. Incubate plates for up to 48 h. Check plates at both 24 h and 48 h.

g. Oxford agar (OXA) (18) (M118): After 24 h incubation at 35°C typical Listeria species colonies are approximately 1 mm diameter, gray to black colonies surrounded by a black halo. Following 48 h incubation typical Listeria species colonies are approximately 2-3 mm diameter, black with a black halo and sunken center. b. PALCAM (50) (M138a): Incubation conditions and appearance of Listeria species colonies are the same as for Oxford agar except that the background plate color is red. c. Modified Oxford Agar (MOX) (46) (M103a): Incubation conditions and appearance of Listeria species colonies are the same as for Oxford agar. CHROMagar™ Listeria (M40a): Incubation conditions and appearance of Listeria colonies are the same as for Agar Listeria according to Ottaviani and Agosti except that the background plate color is light blue (agars is indicative of phosphatidylinositol-specific phospholipase C (PI-PLC) activity. On these agars Listeria species with PI-PLC activity, L. monocytogenes and L. ivanovii, will appear blue-green and all other Listeria species will not develop the blue-green color and remain white in appearance. In the case of Agar Listeria according to Ottaviani and Agosti and CHROMagar™ the presence of a Listeria species is based on a specific β-glucosidase enzyme activity detected by the chromogen, therefore, all Listeria species will appear blue-green on these agars. The phospholipase activity specific for L. monocytogenes and L. ivanovii is determined by the additional opaque white halo surrounding the colony).

h. 2. Select up to 5 typical colonies from each esculin based agar and streak for purity to TSAye (M153) and incubate plates at 30°C for 24 to 48 h. Select up to 2 typical colonies for streaking if using L. monocytogenes-L. ivanovii differential chromogenic agars. The plates may be incubated at 35°C if colonies will not be used for wet-mount motility observations.

i. If isolated colonies are available use remaining colony growth to stab a 5% sheep blood agar (M135) plate. Incubate at 35°C for 24 to 48 h.

Table 1. Differentiation of Listeria species

Species	Mannitol	Rhamnose	Xylose	Virulence[a]	β-Hemolysis[b]
L. monocytogenes	-	+	-	+	+
L. ivanovii	-	-	+	+	+
L. innocua	-	V	-	-	-
L. welshimeri	-	V	+	-	-
L. seeligeri	-	-	+	-	+
L. grayi	+	V	-	-	-

a Mouse test

b Sheep blood agar stab

CAMP (Christie-Atkins-Munch-Peterson) test:

6. Streak weakly β-hemolytic S. aureus (FDA strain ATCC 49444 (CIP 5710; NCTC 7428) or ATCC 25923) and R. equi (ATCC 6939; NCTC 1621) vertically on sheep blood agar.
7. Separately streak test strains horizontally between the S. aureus and R. equi streaks without quite touching them. Incubate plate 24 to 48 h at 35°C.
8. Examine plates for hemolysis in the zone of influence of the vertical streaks. Hemolysis of L. monocytogenes and L. seeligeri is enhanced near the S. aureus streak; L. ivanovii hemolysis is enhanced near the R. equi streak. Other species are non-hemolytic and do not react in this test

Buffered Listeria Enrichment Broth (BLEB):

Media Base

Trypticase soy broth	30 g
Yeast extract	6 g
Monopotassium phosphate (anhydrous)	1.35 g/liter
Disodium phosphate (anhydrous)	9.6 g/liter
Sodium Pyruvate (Sodium salt)	1.11 g/liter
Distilled water	1 liter

Autoclave 15 min at 121°C. Final pH, 7.3 ± 0.1.

Selective Supplements

Acriflavin HCl	10 mg/liter
Nalidixic acid (sodium salt)	40 mg/liter
Cycloheximide	50 mg/liter

Oxford Medium:

Columbia blood agar base	39.0 g
Esculin	1.0 g
Ferric ammonium citrate	0.5 g
Lithium chloride	15.0 g
Cycloheximide	0.4 g
Colistin sulfate	0.02 g
Acriflavin	0.005 g
Cefotetan	0.002 g
Fosfomycin	0.010 g
Distilled water	1 liter

Sterilize by autoclaving at 121°C for 15 min

PALCAM Listeria Selective Agar:

Basal medium

Peptone	23 g
Starch	1 g
NaCl	5 g
Columbia agar	13 g
Mannitol	10 g
Ferric ammonium citrate	0.5 g
Esculin (aesculin)	0.8 g
Dextrose (glucose)	0.5 g

Lithium chloride	15.0 g
Phenol red	0.08 g
Distilled water	1000 ml

Sterilize by autoclaving at 121°C for 15 min.

Selective agents

Polymyxin B sulfate	10 mg
Acriflavin	5 mg
Ceftazidine	20 mg
Distilled water	2 ml

Modified Oxford Listeria Selective Agar:

Columbia Blood Agar Base (brand dependent)	39.0-44.0 g
Agar	2.0 g
Esculin	1.0 g
Ferric ammonium citrate	0.5 g
Lithium chloride (Sigma L0505 quality or equivalent)	15.0 g
Buffered colistin methane sulfonate (1 % w/v) solution	1.0 ml
Distilled water	1.0 L

Adjust pH to 7.2±0.1 if need be. Autoclave at 121° C for 10 min.

References:

Refer: https://www.microbiologyresearch.org/docserver/ fulltext/acmi/2/9/acmi000153.pdf?expires=1682128195&id=id&accname=guest&checksum=9AE0E616F28A348E80FD6DB4B469609D

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