Bacteriological Analytical Manual (BAM)

Chapter 10: Detection of *Listeria monocytogenes* in Foods and Environmental Samples, and Enumeration of *Listeria monocytogenes* in Foods

Authors: Anthony D. Hitchins (ret.) and Karen Jinneman and Yi Chen

April 2022

https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam

Table of Contents

Revision History

- April 2022: Section E.2 correction to additives amounts, Section J. clarification on enumeration homogenate preparations
- February 2022: Section E.2, correction to additives amounts; Section J, clarification on enumeration homogenate preparations; editing of other sections.
- March 2017: Addition of the sample matrix for environmental samples.
- January 2016: More specific sample preparation and analytical set-up instructions for qualitative detection or quantitative enumeration.
- January 2016: Reorganization and editing of all sections.
- February 2013: update to Table 1; update for BAM Media M52: buffered *Listeria* enrichment broth (BLEB) in the Media section.
- November 2011: Addition of PCR confirmation for *Listeria monocytogenes* and *Listeria* spp. isolates other than *L. grayi*.
- April 2011: Section E. Diagram describing the Henry Optical System for examination of colonies added; references for *Listeria monocytogenes* Risk Assessment and Guidance updated.
- August 2002: Section J. Enumeration: Added instructions for positive result on all MPN tubes.
- April 2001: Section H. CAMP test: Updated address for ATCC and added a link to its web page.

Introduction

The genus *Listeria* contains 6 species: *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, and *L. grayi* (Table 1). *L. grayi* (28, 40) and *L. ivanovii* (13, 27) each contain two subspecies, which do not need to be specified in this analysis. A taxonomic review of the genus by Rocourt (41) in 1999 updated the previous reviews (11, 43). In recent years, many new species were proposed. However, these new species are not widely adopted and the number of type strains for the newly proposed species are very limited. *L. ivanovii* and *L. monocytogenes* are pathogenic for mice and other animals. However, only *L. monocytogenes* is commonly associated with human listeriosis. Listeriosis associated infection by *L. ivanovii*, and even by *L. seeligeri*, is extremely rare in humans. The universal occurrence of *L. monocytogenes* in food (42) and the risk of contracting foodborne listeriosis (47,48) have been thoroughly reviewed recently. This chapter describes the detection and enumeration of *L. monocytogenes* in foods and detection from food processing environment.

This standard methodology and alternative rapid methodologies are intended to be used for detection and isolation of *L. monocytogenes* from foods and environmental samples. Analytical sample size for foods is generally 25 g, and this can be from individual units or as part of a sample composite.

Alternatively, rapid test kits with their respective enrichment media approved as AOAC Official Methods of Analysis (OMA) may be conditionally used to screen for the presence of *Listeria* contaminants. Putative *Listeria* isolates on selective agars from standard or screen positive enrichments are purified on non-selective agars and confirmed by conventional identification tests or by a battery of such tests in kit form. Isolates may be rapidly confirmed as *L. monocytogenes* (or not) by using specific test kits or PCR procedures. Subtyping of *L. monocytogenes* isolates is generally expected, which includes serological typing and whole genome sequencing. Optional pathogenicity testing of *L. monocytogenes* isolates is described in Section H.

Enumeration of *L. monocytogenes* in positive food samples is performed on reserve sample by colony count on *L. monocytogenes* differential selective agars in conjunction with MPN enumeration using selective enrichment in buffered *Listeria* enrichment broth with subsequent plating on *L. monocytogenes* differential selective agars as described below.

A. Equipment and Materials

FDA does not specifically endorse any of commercial products listed. Equivalent products may be available.

1. Sterile swab: The following or equivalent:

   a. 3M™ Swab-sampler in 10 ml D/E neutralizing broth (catalog# RS96010DE, www.mmm.com) (Polyester).

b. Puritan® dry cotton swab (catalog# 25-806 1PC, 25-806 2PC, www.puritanmedproducts.com) (Cotton).
c. World Bioproducts PUR-Blue™ swab sampler with (catalog# BLU-10DE) or without (catalog# BLU-DRY, www.worldbioproducts.com) 10 ml D/E neutralizing broth (Polyurethane).
d. Healthlink® dry swab transporter (catalog# 4159BX, www.hardydiagnostics.com) (Polyester).

2. Sterile sponge: The following or equivalent:

a. World Bioproducts EZ Reach™ sponge sampler with (catalog# EZ-10DE-PUR) or without (catalog# EZ-DRY-PUR, www.worldbioproducts.com) 10 ml D/E neutralizing broth (Polyurethane).
b. Nasco Whirl-Pak® dry sponge probe (catalog# B01475WA, www.enasco.com) (Cellulose).
c. 3M™ Sponge-sticks with (catalog# SSL10DE, www.mmm.com) or without (catalog# SSL100) 10 ml D/E neutralizing broth (Cellulose).

If available, dry samplers can be obtained and D/E neutralizing broth added later, or pre-moistened samplers can be obtained. We do not recommend less than 10 ml D/E neutralizing broth due to the possible presence of sanitizer residues on environmental surfaces.

3. Balance for weighing sample to 0.1 g

4. Incubators, 30, 35, and 37°C

5. Water bath, 80 ± 2°C

6. Phase-contrast microscope with oil immersion phase objective (100×)

7. Blender motor and jars or stomacher and bags

8. Vortex mixer

B. Media and Reagents

FDA does not specifically endorse any of commercial products listed. Equivalent products may be available.

1. Buffered *Listeria* enrichment broth (BLEB) (M52)
2. Acriflavine monohydrochloride
3. Nalidixic acid (sodium salt)
4. Cycloheximide
5. Natamycin (Pimaricin)
6. Dey/Engley (D/E) neutralizing broth (M193)
7. Oxford medium (OXA) (M118)
8. Polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol (PALCAM) (M118a)

9. Modified Oxford MOX agar (M103a)
10. Lithium chloride-phenylethanol-moxalactam (LPM) agar (M81) with added esculin and iron (M82)
11. R&F® *Listeria monocytogenes* Chromogenic Plating Medium (R&F Laboratories, Downers Grove, IL) (M17a)
12. Agar *Listeria* according to Ottaviani and Agosti (M10a)
13. Oxoid™ chromogenic *Listeria* agar (Oxoid Ltd., Basingstoke, England) (M40b)
14. Rapid'*L.mono*™ (Bio-Rad Laboratories Inc.) (M131a)
15. CHROMagar™ *Listeria* (CHROMagar, Paris, France) (M40a)
16. Trypticase soy agar with 0.6% yeast extract (TSAye) (M153)
17. Sheep blood agar (M135)
18. Hydrogen peroxide solution, 3% for catalase test (R12)
19. Gram stain kit
20. Motility test medium (MTM, Difco™) (M103)
21. Trypticase soy broth with 0.6% yeast extract (TSBye) (M157)
22. Purple carbohydrate fermentation broth base (M130), containing 0.5% solutions of dextrose, esculin, maltose, rhamnose, mannitol, and xylose
23. Physiological saline solution, 0.85% (R63)
24. Fluorescent antibody (FA) buffer (Difco™)
25. *Listeria*-typing sera Type 1 (Difco™ catalog# 223031) and Type 4 (Difco™ catalog# 223041)
26. *Listeria* Antisera Set (Denka Seiken™ catalog# 294616)
27. Optional: Nitrate reduction medium (M108) and nitrate detection reagents (R48)

Note: Alternative companies may be used when the products are equivalent.

C. Control Cultures

1. *Listeria monocytogenes* ATCC 19115
2. *Listeria innocua* ATCC 33090
3. *Listeria seeligeri* ATCC 35967
4. *Listeria ivanovii* ATCC 19119
5. *Rhodococcus equi* ATCC 6939
6. *Staphylococcus aureus* ATCC 25923 or ATCC 49444

Green fluorescent protein (GFP) control strains which fluoresce under UVA light, at wavelengths between 360 to 400 nm, have been developed by FDA and have been licensed by FDA to Microbiologics for distribution (https://www.microbiologics.com; 200 Cooper Avenue North St. Cloud, MN 56303, 1-800-599-2847). The following cultures may be purchased from Microbiologics:

Listeria monocytogenes (1/2a) / FDALS808, catalog# 01249UV

D. Sample Preparation

Sample transport and storage practices should maintain the recommended storage conditions for the food commodity. Sample analysis should be initiated as soon as possible upon sample receipt. If sample analysis must be delayed, store frozen samples frozen (-20 ± 5°C); store nonperishable, canned or low-moisture foods at room temperature, and store refrigerated, unfrozen perishable foods at 4 ± 2°C until sample analysis is initiated.

Basic analytical options include: 1. Qualitative detection (limit of detection <1 CFU per analytical unit), 2. Quantitative determination.

1. 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) (M52) (10, 26). Thoroughly mix, blend or stomach, continue enrichment as described in section E or F. Certain foods may require different sample set-up procedures such as soaking and rinsing. 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. Refer to applicable sampling compliance guidance documents for additional instructions.

   b. Composite sample analysis: Composites may be used to analyze multiple subunits from a single sample. Refer to applicable sampling compliance guidance documents for specific composite procedures. Generally, two composites are prepared from a sample consisting of 10 sub-samples (liquid, cream or solid food). To prepare a composite, 50 g or ml representative portions from each of the 5 sub-samples are pooled and 250 ml basal BLEB without selective agents is added and then blended or stomached (M52). A 50 g portion of this composite blend (equivalent to 25 g food plus 25 ml basal BLEB) is combined with 200 ml of basal BLEB. The composite is then incubated as described in Section E or F.

   c. Environmental samples: When sampling dry surfaces, swabs (cotton, polyester or polyurethane) or sponges (cellulose or polyurethane) should be pre-moistened in 10 ml Dey/Engley (D/E) neutralizing broth. Swabs are typically stored in tube containers and sponges are typically stored in bag containers. Before sampling, press swabs against inner tube walls or squeeze the sponges in bags to remove excess broth. Swab environmental surfaces using firm and even pressure vertically (approximately 10 times), then flip the sampler and use the other side to swab horizontally (approximately 10 times) and diagonally (approximately 10 times). When putting the samplers back to the container, make sure they are submerged in the D/E neutralizing broth. When sampling wet surfaces, dry swabs or sponges should be used and put into D/E neutralizing broth immediately after sampling. Swabs that are made from cotton or that have large tips appear to absorb liquid better than others. For samples

that are not suitable to be collected by regular sized swabs/sponges mentioned in section A, refer to applicable sampling compliance guidance documents for additional instructions. After sampling, swab/sponge can be maintained in D/E neutralizing broth at 4°C for up to 48 h before analysis. Swab/sponge submerged in D/E neutralizing broth is then added to 90 ml (or more to fully submerge the swab or sponge) of basal BLEB (M52). Sponge in D/E neutralizing broth can also be added to 225 ml of basal BLEB. Thoroughly massage or stomach to expel the collection broth into enrichment broth. Continue enrichment as described in Section E or F.

d. Interpret result: Confirmation of one or more *L. monocytogenes* isolates from an enrichment indicates that *L. monocytogenes* is present at ≥ 1 CFU per sample size analyzed or present on environmental swab or sponge sample.

2. Quantitative determination from foods:

Enumeration is performed using a combination of MPN and direct plating. Refer to Section J for details. Surveillance samples are generally first analyzed by qualitative detection and reserve portions of the positive samples are then enumerated. For outbreak response situations, all samples may be directly enumerated. Refer to Section J and applicable sampling compliance guidance documents.

E. Enrichment Procedure

1. Incubate food samples or environmental samples homogenized in basal BLEB (M52, 43) at 30°C, for 4 h.

2. Aseptically add the three filter sterilized selective agents (M52) 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.

3. Mix the enrichment with additives and continue incubation at 30°C for the remainder of the 24 to 48 h enrichment period.

F. Alternate Screening Methodologies

The following alternative screening methodologies may be used to screen samples for the presence of *Listeria*. Follow the manufacturers' package insert making certain they have not deviated from the approved versions of the AOAC International Official Methods Manual protocols (Section F.1). The kits are only approved for the specified food and environmental matrices, claimed in the OMA method, which vary from kit to kit. For other matrices that are not validated a matrix extension validation is necessary. Negative results obtained with the products are considered definitive and no further testing is required. Presumptive positive results with these rapid screening methods must be confirmed by streaking to the selective agars and confirming isolates to the species level by the procedures described in Sections G-I.

1. Rapid screening methods for *Listeria* spp.:

   a. AOAC Official Method 993.09. Colorimetric deoxyribonucleic acid hybridization method (GENE-TRAK *Listeria* Assay) (3, 16).
   (Applicable to dairy products, meats, and seafoods)
   Collaborative study: milk (2%), brie cheese, cooked crab meat, frankfurters, roast beef, raw ground pork
   Pre-collaborative: crab meat, raw shrimp, cheddar cheese, cottage cheese, ice cream, chocolate milk, nonfat dried milk, fish fillet, ground raw pork, fermented sausage, raw ground turkey

   b. AOAC Official Method 994.03. Colorimetric Monoclonal Enzyme-Linked Immunosorbent Assay Method (*Listeria*-Tek) (4, 17, 31).
   (Applicable to dairy products, seafoods, meats)
   Collaborative study: frankfurters, roast beef vacuum packed, brie cheese, 2% milk, raw frozen shelled shrimp, cooked frozen crab
   Pre-collaborative: crab meat, ice cream, milk, chocolate milk, non-fat dried milk, raw fish, cooked beef, roast beef, cured ham, raw sausage, raw oyster, raw chicken, raw turkey

   c. AOAC Official Method 995.22. 2000. Colorimetric polyclonal enzyme immunoassay screening method (TECRA™ *Listeria* Visual Immunoassay) (5, 29).
   (Applicable to dairy foods, seafoods, poultry, meats (not raw ground chuck), leafy vegetables)
   Collaborative study: fish fillets, ice cream, lettuce, chicken, ground turkey
   Pre-collaborative: crabmeat, shrimp, soft cheese, chocolate milk, non-fat dried milk, raw beef, roast beef, frankfurters, bologna, oysters, chicken

   d. AOAC Official Method 2002.09. TECRA™ *Listeria* Visual Immunoassay Using TECRA *Listeria* Enrichment Broth (32, 5, 29).
   (Applicable to raw and processed meats, cultured and non-cultured dairy products)
   Note: Method is based upon 995.22 but with altered enrichment, omission of cycloheximide and additional foods.
   Collaborative study: fish fillets, turkey, raw ground beef, ice cream, lettuce

   e. AOAC Official Method 996.14. Assurance® Polyclonal Enzyme Immunoassay Method (EIA) (6, 19).
   (Applicable to dairy foods, red meats, pork, poultry products, fruits, nutmeats, seafood, pasta, vegetables, cheese, animal meal, chocolate, and eggs, bone meal and from environmental surfaces)
   Collaborative study: nonfat dried milk, ice cream, raw poultry, raw shrimp, cooked roast beef, green beans
   Pre-collaborative: crabmeat, soft cheese, dry egg, egg liquid frozen, milk, chocolate milk, raw fish, bone meal, raw beef, raw pork, scallops, chocolate, nuts, pasta, raw chicken, coleslaw

   f. AOAC Official Method 997.03. Visible Immunoprecipitate Assay (VIP) (7, 20).
   (Applicable to dairy foods, red meats, pork, poultry and poultry products, seafood, fruits, vegetables, nutmeats, pasta, chocolate, eggs, and bone meal, and environmental surfaces)

Collaborative study: nonfat dried milk, ice cream, raw poultry, raw shrimp, cooked roast beef, green beans, environmental surfaces

g. AOAC Official Method 999.06. Enzyme Linked Immunofluorescent Assay (ELFA) VIDAS® LIS Assay Screening Method (8, 21).
(Applicable to dairy products, vegetables, seafoods, raw meats and poultry, and processed meats and poultry)
Collaborative study: ice cream, green beans, fish, turkey, cheese, roast beef

h. AOAC Official Method 2004.06. Modified VIDAS® LIS Assay Screening Method (36).
(Applicable to dairy products, vegetables, seafood, raw meats and poultry, and processed meats and poultry)
Collaborative study: brie cheese, ice cream, fish, green beans, roast beef

i. AOAC Official Method 2010.02. VIDAS® LSX Assay Screening Method (35).
(Applicable to dairy products, vegetables, seafood, raw meats and poultry, and processed meats and poultry)
Collaborative study: vanilla ice cream, cheddar cheese, raw ground beef, frozen green beans, deli turkey, cooked shrimp

j. AOAC Official Method 2013.10. VIDAS® UP *Listeria* (LPT) Assay Kit (36).
(Applicable to deli ham (25 and 125 g), pepperoni (25 g), beef hot dogs (25 g), chicken nuggets (25 g), chicken liver pâté (25 g), ground beef (125 g), deli turkey (125 g), cooked shrimp (25 g), smoked salmon (25 g), whole cantaloupe melon, bagged mixed salad (25 g), peanut butter (25 g), black pepper (25 g), vanilla ice cream (25 g), queso fresco (25 and 125 g), stainless steel, plastic, ceramic and concrete environmental surfaces)
Collaborative study: queso fresco

See supplemental data, Tables 2A–D, for detailed results of the collaborative study on J. AOAC Int. website, http://aoac.publisher.ingentaconnect.com/content/aoac/jaoac.

2. Rapid screening methods for *Listeria monocytogenes*: Please note that these methods do not screen for *Listeria* spp. and therefore may not be suitable for situations in which the identification of *Listeria* spp. is desired.

a. AOAC Official Method 2003.12. BAX® Automated System (9).
(Applicable to dairy products, fruits and vegetables [except radishes], seafoods, raw and processed meats, and poultry)
Collaborative study: frankfurters, soft cheese, smoked salmon, ground beef, radishes, peas

b. AOAC Official Method 2004.02. Enzyme Linked Immunofluorescent Assay (ELFA) VIDAS® LMO2 Assay Screening Method (33).
(Applicable to *L. monocytogenes* in dairy products, vegetables, seafood, raw meats and poultry, and processed meats and poultry)
Collaborative study: vanilla ice cream, brie cheese, coked roast beef, frozen green beans, frozen tilapia fish

c. AOAC Official Method 2013.11. VIDAS® *Listeria monocytogenes* (LMX) Assay (37).
(Applicable to deli ham (25 and 125 g), fermented sausage (25 g), liver pâté (25 g),

processed cheese (25 g), vanilla ice cream (25 g), cooked shrimp (25 g), smoked white fish (25 g), frozen spinach (25 g), peanut butter (25 g), deli turkey (25 and 125 g), queso fresco (125 g), and ground beef (125 g))
Collaborative study: queso fresco

G. Isolation Procedure

1. 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.

A. Esculin based *Listeria* selective agars:

a. 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.
d. LPM (30) (M81) fortified with esculin and Fe^{3+}: Incubate at 30°C. Typical *Listeria* species colony appearance is similar to Oxford agar.

B. Chromogenic *L. monocytogenes*-*L. ivanovii* agars:

a. R&F® *Listeria monocytogenes* Chromogenic Plating Medium (R&F® LMCPM) (39, 25) (M17a): Incubate plates at 35°C, *L. monocytogenes* and *L. ivanovii* produce a 1-3 mm diameter, smooth, convex, blue/green colony and small blue/green halo. All other *Listeria* species produce a 1-2 mm, smooth, convex white colony with no halo. Typical *L. monocytogenes* and *L. ivanovii* colonies can be distinguished using a commercial Confirmatory Medium (R&F laboratories) or by the identification methods described in Section H.
b. RAPID'*L.mono*™ (M131a): Incubate plates at 37°C, *L. monocytogenes* and *L. ivanovii* produce a 1-3 mm diameter, smooth, convex, blue/green colony. Typical colonies appear dark blue/green in the red background of RAPID'*L.mono*™ agar and appear blue/green when the background flora change the background color of the agar to yellow. Additionally, a yellow halo will surround *L. ivanovii* colonies. However, yellow halo may be obvious in areas of at least moderate growth. Heavy growth of *L. ivanovii* could turn the entire plate to yellow. In addition, caution has to be taken for *L. monocytogenes*-*L. ivanovii* differentiation because background flora in some food commodities can change the color of certain areas of this agar to yellow, and this could make *L. monocytogenes* appear as *L.*

ivanovii. All other *Listeria* species produce a 1-2 mm, smooth, convex white colony with or without a yellow halo.

c. Agar *Listeria* according to Ottaviani and Agosti (44, 49) (M10a), or Oxoid™ chromogenic *Listeria* agar (OCLA) (M40b): Incubate plates at 37°C, all *Listeria* species appear as 1-3 mm diameter blue/green colonies. Additionally, *L. monocytogenes* and *L. ivanovii* have an opaque white halo surrounding the colony.

d. CHROMagar™ *Listeria* (M40a): Incubation conditions and appearance of *Listeri*a colonies are the same as for Agar *Listeria* according to Ottaviani and Agosti except that the background plate color is light blue.

Note: The chromogen used in both the R&F® LMCPM and RAPID'*L.mono*™ 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.

For the approved rapid methods, use the selective isolation agar recommended by the manufacturer but auxiliary use of chromogenic *L. monocytogenes-L. ivanovii* differential agars is also recommended.

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.

3. 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.

H. Identification Procedure

Identify purified isolates from growth on the TSAye plate by the following classical tests (Section H.1.a-e). Alternatively, rapid biochemical test kits or PCR analyses may be used to confirm isolates to the species level (Section H.2.a-c).

Examine TSAye plates for typical 1-3 mm diameter smooth convex white colonies. Observation with Henry oblique transmitted illumination (Figure 1) (23) can be helpful at this stage but is not mandatory.

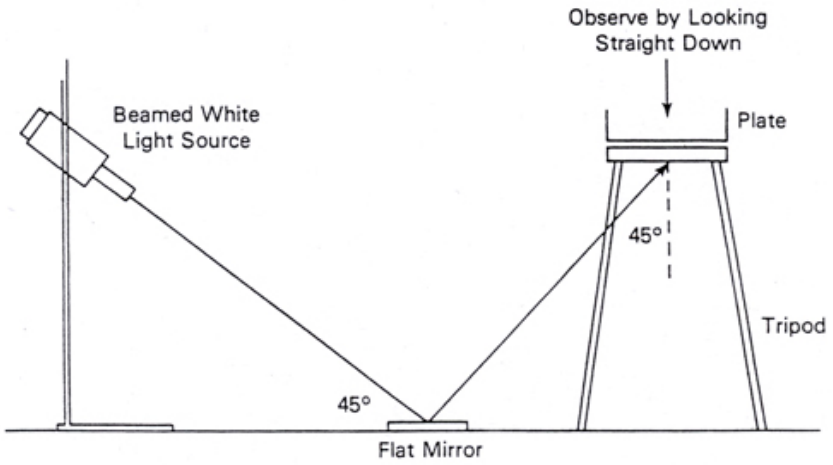

Figure 1. Henry Optical System for examination of colonies

1. Standard classification:

a. Hemolysis:

Inoculate heavily (from TSAye colony) 5% sheep blood agar by stabbing plates that have been poured thick and dried well (check for moisture before using). Draw grid of 20-25 spaces on plate bottom. Stab one culture per grid space. Always stab positive controls (*L. ivanovii* and *L. monocytogenes*) and negative control *(L. innocua*). Incubate for 24 to 48 h at 35°C. Attempt to stab as near to bottom of agar layer as possible, without actually touching bottom of agar layer and possibly fracturing the agar.

Examine blood agar plates containing culture stabs brightly lit from behind the plate. *L. monocytogenes* and *L. seeligeri* produce a slightly cleared zone around the stab. *L. innocua* shows no zone of hemolysis, whereas *L. ivanovii* produces a well-defined clear zone around the stab. If mixed culture was observed on the TSAye plate repeat the hemolysis test with an isolated colony.

CAMP test: Resolve questionable reactions by the Christie-Atkins-Munch-Peterson (CAMP) (15) test. CAMP test strains are available from culture collections, including the American Type Culture Collection (ATCC), Manassas, VA, http://www.atcc.org

i. 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.

ii. 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. Figure 2 shows the arrangement of the culture streaks on a CAMP plate.

iii. 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 (Table 1).

iv. Alternatively, a factor easily prepared from *S. aureus* cultures can be used to enhance hemolysis by *L. monocytogenes* and *L. seeligeri* in sheep blood agar plates. Disks impregnated with the β-lysin of *S. aureus* (Remel, Lenexa, KS) can be used.

Table 1. Differentiation of *Listeria* species

Species	Mannitol	Rhamnose	Xylose	Virulence[a]	β-Hemolysis[b]	Hemolysis enhancement with *Staphylococcus aureus* (S)	Hemolysis enhancement with *Rhodococcus equi* (R)
L. monocytogenes	-	+[c]	-	+	+	+	-[d]
L. ivanovii[e]	-	-	+	+	+	-	+
L. innocua	-	V[f]	-	-	-	-	-
L. welshimeri	-	V	+	-	-	-	-
L. seeligeri	-	-	+	-	+[g]	+	-
L. grayi[h]	+	V	-	-	-		

[a] Mouse test

[b] Sheep blood agar stab

[c] Some lineage III strains of *L. monocytogenes*, which are primarily associated with animal listeriosis, are rhamnose negative.

[d] Rare strains are S+ and R+. The R+ reaction is less pronounced than that of *L. ivanovii*.

[e] Ribose fermenting strains are classified as *L. ivanovii* subsp. *ivanovii* and ribose non-fermenters as *L. ivanovii* subsp. *londiniensis*.

[f] V, variable biotypes, greater than 10% of strains for this trait.

[g] Weakly hemolytic *L. seeligeri* strains may appear non hemolytic.

[h] Includes two subspecies - *L. grayi* subsp. *murrayi* reduces nitrate. *L. grayi* subsp. *grayi* does not reduce nitrate.

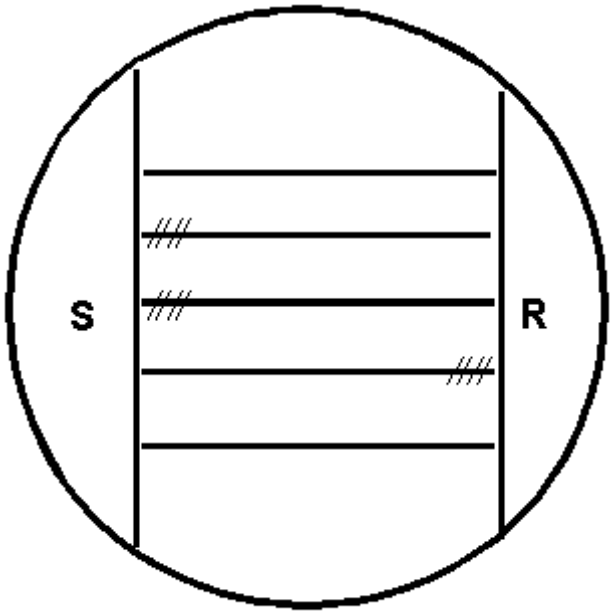

Figure 2. CAMP test for *Listeria monocytogenes*: Inoculation pattern of the sheep blood agar plate. Horizontal lines represent streak inoculations of 5 test strains. Vertical lines represent streak inoculations of *Staphylococcus aureus* (S) and *Rhodococcus equi* (R). Hatched lines indicate (diagrammatically only) the locations of hemolysis enhancement regions.

b. Motility: Pick typical colony from TSAye and examine by wet mount, using 0.85% saline for suspending medium and oil immersion objective of phase-contrast microscope. Choose a colony with enough growth to make a fairly heavy suspension; emulsify thoroughly. *Listeria* spp. are slim, short rods with slight rotating or tumbling motility. Always compare with known culture. *Cocci*, large rods, or rods with rapid, swimming motility are not *Listeria* spp. Alternatively, stab tube of MTM (M103) from TSAye. Incubate for up to 7 days at room temperature (20-25°C). Observe daily until the isolate growth pattern is obvious. *Listeria* is motile, giving a typical umbrella-like growth pattern.

c. Catalase: Test typical colonies for catalase by placing some colony growth in a drop of 3% hydrogen peroxide. *Listeria* species are catalase-positive.

d. Gram stain: Use 16 to 24 h growth from TSAye plates. All *Listeria* spp. are short, Gram-positive rods; however, with older cultures the Gram stain reaction can be variable and also cells may appear spheroidal. The cells have a tendency to palisade in thick-stained smears. This can lead to false rejection as a diphtheroid.

e. Carbohydrate fermentation series: Pick typical colony to a tube of TSB for inoculating carbohydrate fermentation and other test media. Incubate at 35°C for 24 h. This culture may be kept at 4°C several days and used repeatedly as inoculum.

i. From TSBye culture, inoculate the following carbohydrates in 0.5% solutions in purple carbohydrate broth with Durham tubes: dextrose, esculin, maltose, rhamnose, mannitol, and xylose. Incubate 7 days at 35°C.

ii. Positive reactions will be indicated by the production of acid and the media turning a yellow color with no gas production. All *Listeria* spp. should be positive for dextrose, esculin, and maltose. All *Listeria* spp. except *L. grayi* should be mannitol-negative. If pigmentation of the isolate on OXA, PALCAM, MOX or LPM plus esculin/Fe^{3+} is unequivocal, the esculin test may be omitted. Consult Table 1 for interpretations of the results.

f. Optional: Nitrate reduction test: Only *L. grayi* subsp. *murrayi* reduces nitrates. The test distinguishes *L. grayi* subsp. *murrayi* from *L. grayi* subsp. *grayi*.

i. Use a TSAye culture to inoculate nitrate broth (M108). Incubate at 35°C for 5 days.

ii. Add 0.2 ml reagent A, followed by 0.2 ml reagent B (R48). A red-violet color indicates presence of nitrite, i.e., nitrate has been reduced. If no color develops, add powdered zinc and hold for 1 h. A developing red-violet color indicates that nitrate is still present and has not been reduced.

iii. As an alternative procedure, add 0.2 ml reagent A followed by 0.2 ml reagent C. An orange color indicates reduction of nitrate. If no color develops, add powdered zinc as above. Development of an orange color indicates unreduced nitrate.

g. Optional: Since all *Listeria* species test negative for indole, oxidase, urease, and H_2S production from organic sulfur compounds (H_2S is produced from thiosulfate in the MICRO-ID™ test kit) and test positive for methyl red and Voges-Proskauer, these tests are discretionary. *Brochothrix*, which is phylogenetically closely related to *Listeria*, is distinguishable from *Listeria* by its inability to grow at 35°C and by its lack of motility. Distinguishing features of the Gram-positive non-sporeforming rods, *Erysipelothrix* and *Kurthia*, which occur rarely in *Listeria* analysis, can be found elsewhere (11, 43).

h. Optional: Immunocompromised mouse pathogenicity test: The classical tests for *Listeria* pathogenicity are the Anton conjunctivitis test (rabbits), inoculation of mice, and inoculation of embryonated eggs. The immunocompromised mouse test, using intra-peritoneal injection, is recommended because of its greatly improved sensitivity (38). Confirmation of *L. monocytogenes* animal pathogenicity is not needed for clinical isolates and is optional for food isolates. An isolate should be identified as *L. monocytogenes* if it meets all the other criteria outlined in this chapter. See link for detailed protocol.

Biochemical and pathogenicity data are summarized in Table 1. All data collection must be completed before species identities are determined and subsequent subtyping performed. Atypical *Listeria* strains exist that could confuse the identification. For example, there are undocumented references (46) to hemolytic *L. innocua* isolates. Some *L. monocytogenes* and *L.*

welshimeri isolates are rhamnose-negative. Some *L. seeligeri* isolates have very weak hemolytic reaction and can be confused with non-hemolytic species. Sometimes aberrant *Listeria* strains are isolated which are extremely difficult to speciate (26) (See Guideline for BAM Users on Identification of Atypical Hemolytic *Listeria* Isolates). If such an aberrant *Listeria* isolate is obtained, contact Karen Jinneman.

2. Alternate rapid identification:

Purified isolates may be rapidly identified by using commercial kits or real-time PCR. Follow manufacturer instructions for inoculation and interpretation.

a. API® *Listeria* (bioMerieux, Durham, NC) which requires an additional β-hemolysis test for final isolate identification (12). CAMP test is optional.
b. MICRO-ID™ *Listeria* Identification System (Remel, Lenexa, KS) which requires an additional CAMP test and β-hemolysis test. (2, 22).
c. VITEK® 2 Automatic Gram Positive card (bioMerieux, Hazelwood, MO), which requires an additional CAMP test and β-hemolysis test (38).
d. Real-time PCR, which requires an additional β-hemolysis test. CAMP test is optional.
    - Protocol: Simultaneous Confirmation of *Listeria* species and *L. monocytogenes* isolates by real-time PCR.
    - Attachment 1: Single Lab Validation (SLV) - Individual Ct values for each isolate by each enzyme mix. (pdf, 58Kb)
    - Attachment 2: Multi Lab validation (MLV) - Individual Ct values for each isolate by laboratory. (pdf, 60Kb)
    - *Listeria* species identification includes *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. seeligeri* and *L. welshimeri*. Identification of *L. grayi* has not been verified with this assay. Isolates that are identified as *Listeria* species but not *L. monocytogenes* can be fully speciated as described in Section H.1.a-e or H.2.a-c.

Alternative AOAC OMA rapid methods for the detection of *L. monocytogenes* listed in section F.2 can be used to confirm pure culture. Depending on the kit, isolates may be identified in pure culture or from OXA or the other selective isolation agars. Purified isolates identified as *Listeria monocytogenes* by these tests should be retained for regulatory reference.

For environmental samples, refer to applicable sampling compliance guidance documents to determine if identification to the genus level is sufficient and if further differentiation between *L. monocytogenes* and other *Listeria* species is necessary.

I. Subtyping of *L. monocytogenes* Isolates (required)

Confirmed *L. monocytogenes* isolates should be typed serologically and genetically.

1. Serological typing: Serology is useful when addressing epidemiological considerations. Most *L. monocytogenes* isolates obtained from patients, foods, and the environment are type 1 or 4. More than 90% of *L. monocytogenes* isolates can be serotyped with commercially available sera. However, all nonpathogenic *Listeria* species, except *L. welshimeri*, share one or more

somatic antigens with *L. monocytogenes* (43). Therefore, serotyping alone without thorough isolate characterization is not adequate for identification of *L. monocytogenes*.

Use commercial sera (Difco™ Type 1 catalog# 223031 and Type 4 catalog# 223041) to characterize isolates as type 1, type 4 or not type 1 or 4 (types 3, 5, 6, etc.) at a minimum. Use a TSBye culture to inoculate Tryptose broth. Incubate for 24 h at 35°C, at which temperature flagella (H) antigen expression is reduced. Transfer to Tryptose agar slants and incubate for 24 h at 35°C. Wash both slants in a total of 3 ml Difco™ fluorescent antibody (FA) buffer and transfer to a sterile 16 × 125-mm screw-cap tube. Heat in a water bath at 80°C for 1 h. Sediment cells by centrifugation at 1600 g for 30 min. Remove 2.2-2.3 ml of supernatant fluid and resuspend the pellet in the remainder of buffer. Follow manufacturer's recommendations for sera dilution and agglutination.

Complete serological characterization can also be done (Denka Seiken™ catalog# 294616). Pure cultures of *L. monocytogenes* isolates should be cultured for 24 h at 35°C on non-selective agar such as BHI agar. Colony growth is then resuspended, heat inactivated and tested for agglutination as recommended by the antisera manufacturer.

2. Genetic subtyping: Whole genome sequencing of food and environmental isolates should be submitted to GenomeTrakr. Retain all isolates as additional subtyping techniques may also be requested.

J. Enumeration (required)

If a food sample tests positive for *L. monocytogenes*, use a reserve portion of sample for enumeration. Enumeration is performed using a combination of MPN and direct plating.

1. MPN procedure:
   a. Prepare a homogenate of a 50-g amount of reserve food sample in 450 ml pre-warmed basal BLEB with selective agents.
   b. Prepare a 4-dilution, 3-tube MPN using dilutions that will deliver equivalent to 10, 1, 0.1, and 0.01 g sample per aliquot at each respective dilution.
   c. Incubate all twelve aliquots as described in Section E.
   d. At 48 h streak each aliquot as described in Section G followed by isolation and confirmation according to sections G-H. Alternatively each of the enrichments can be rapidly screened by one of the approved methods described in Section F with confirmation of all presumptive positives by the isolation and confirmation steps as described in Sections G-H.
   e. Interpret results based on the number of tubes with confirmed positive *L. monocytogenes* using the tables in BAM Appendix 2 (14).
   f. If all the MPN tubes are *Listeria* positive, refer to direct plating for enumeration results.
      In situations that would require narrower confidence limits, the number of replicate tubes for certain dilutions could be increased. One ml of homogenate of complete

BLEB and sample can be added and diluted by multi-channel pipette or robotically, in 96-well plates.

2. Direct plating procedure:
   a. For solid food, prepare a homogenate of a 25-g amount of reserve food sample in 225 ml basal BLEB without selective agents. Perform another 10-fold dilution if necessary. Certain foods may require different sample set-up and dilution procedures, refer to applicable compliance assignment documents.
   b. Direct plate 1 ml of liquid food or 1 ml homogenate of solid food prepared in step a onto 3 to 5 plates of one of the *L. monocytogenes* differential chromogenic agars as described in section G.
   c. If the colonies on the plates are too numerous to count, use reserve sample, perform additional 10-fold serial dilutions and proceed with direct plating.
   d. Confirm 5 representative colonies per plate.
   e. Alternatively, spiral plating method described in Chapter 3 can be used for direct plating.

Notes: Guideline for BAM Users on Identification of Atypical Hemolytic *Listeria* Isolates

References

1. Anonymous. 2001. Validation certificate for alternative method according to the standard EN ISO 16140:2003. http://www.chromagar.com/fichiers/1450365640CHR_21_01_12_01_en_V2013.pdf
2. AOAC Official Method 992.18. 2000. MICRO-ID *Listeria*. Chapter 17.10.02, pp. 141-144 In: Official Methods of Analysis of AOAC INTERNATIONAL. 17th Edition. W. Horwitz (ed.). Volume 1. Agricultural Chemicals, Contaminants and Drugs. AOAC INTERNATIONAL, Gaithersburg, MD.
3. AOAC Official Method 993.09. 2000. *Listeria* in dairy products, seafoods, and meats. Colorimetric deoxyribonucleic acid hybridization method (GENE-TRAK *Listeria* Assay). Chapter 17.10.04, pp. 147-150 In: Official Methods of Analysis of AOAC INTERNATIONAL. 17th Edition. W. Horwitz (ed.). Volume 1. Agricultural Chemicals, Contaminants and Drugs. AOAC INTERNATIONAL, Gaithersburg, MD.
4. AOAC Official Method 994.03. 2000. *Listeria monocytogenes* in dairy products, seafoods, and meats. Colorimetric monoclonal enzyme-linked immunosorbent assay method (*Listeria*-Tek). Chapter 17.10.05, pp. 150-152 In: Official Methods of Analysis of AOAC INTERNATIONAL. 17th Edition. W. Horwitz (ed.). Volume 1. Agricultural Chemicals, Contaminants and Drugs. AOAC INTERNATIONAL, Gaithersburg, MD.
5. AOAC Official Method 995.22. 2000. *Listeria* in foods. Colorimetric polyclonal enzyme immunoassay screening method (TECRA *Listeria* Visual Immunoassay [TLVIA]). Chapter 17.10.06, pp. 152-155 In: Official Methods of Analysis of AOAC INTERNATIONAL. 17th Edition. W. Horwitz (ed.). Volume 1. Agricultural Chemicals, Contaminants and Drugs. AOAC INTERNATIONAL, Gaithersburg, MD.
6. AOAC Official Method 996.14. 2000. Assurance Polyclonal Enzyme Immunoassay Method. Chapter 17.10.07, pp. 155-158 In: Official Methods of Analysis of AOAC INTERNATIONAL. 17th Edition. W. Horwitz (ed.). Volume 1. Agricultural Chemicals, Contaminants and Drugs. AOAC INTERNATIONAL, Gaithersburg, MD.
7. AOAC Official Method 997.03. 2000. Visual Immunoprecipitate Assay (VIP). Chapter 17.10.08, pp. 158-160 In: Official Methods of Analysis of AOAC INTERNATIONAL. 17th Edition. W. Horwitz (ed.). Volume 1. Agricultural Chemicals, Contaminants and Drugs. AOAC INTERNATIONAL, Gaithersburg, MD.
8. AOAC Official Method 999.06. 2000. Enzyme Linked Immunofluorescent Assay (ELFA) VIDAS LIS Assay Screening Method. Chapter 17.10.09, pp. 160-163. In: Official Methods of Analysis of AOAC INTERNATIONAL. 17th Edition. W. Horwitz (ed.). Volume 1. Agricultural Chemicals, Contaminants and Drugs. AOAC INTERNATIONAL, Gaithersburg, MD.
9. AOAC Official Method 2003.12. 2005. Evaluation of BAX® Automated System for the Detection of *Listeria monocytogenes* in Foods. Chapter 17.10.10, pp. 222-225. In: Official Methods of Analysis of AOAC INTERNATIONAL. 18th Edition. W. Horwitz (ed.). AOAC INTERNATIONAL, Gaithersburg, MD.

10. Asperger, H., H. Heistinger, M. Wagner, A. Lehner and E. Brandl. 1999. A contribution of *Listeria* enrichment methodology - growth of *Listeria monocytogenes* under varying conditions concerning enrichment broth composition, cheese matrices and competing microflora. *Microbiology* **16**:419-431.
11. Bille, J., J. Rocourt, and B. Swaminathan. 1999. *Listeriae*, *Erysipelothrix*, and *Kurthia*, pp. 295-314. In: Manual of Clinical Microbiology. 7th Edition. P. R. Murray (ed.). American Society for Microbiology, Washington, DC.
12. 2008 draft. Bille, J. B. Catimel, E. Bannerman, C. Jacquet, M.N. Yersin, I. Camiaux, D. Monget and J. Rocourt. 1992. API *Listeria*, a new and promising one-day sysem to identify *Listeria* isolates. *Appl. Environ. Microbiol*. **58**(6):1857-1860.
13. Boerlin et al. 1992. *L. ivanovii* subsp. *londoniensis* subsp. *novi*. *Int. J. Syst. Bacteriol*. **42**:69-73.
14. Blodgett, R. 2006. Appendix 2. Most Probable Number from Serial Dilutions. In U.S. Food and Drug Administration Bacteriological Analytical Manual Online.
15. Christie, R., N. E. Atkins, and E. Munch-Petersen. 1944. A note on the lytic phenomenon shown by group B *streptococci*. *Aust. J. Exp. Biol. Med. Sci*. **22**: 197-200.
16. Curiale, M. S., T. Sons, L. Fanning, W. Lepper & D. McIver. 1994. Deoxyribonucleic acid hybridization method for the detection of *Listeria* in dairy products, seafoods, and meats: collaborative study. *J. AOAC INTERNATIONAL* **77**:602-617.
17. Curiale, M. S., W. Lepper & B. Robison. 1994. Enzyme-linked immunoassay for detection of *Listeria monocytogenes* in dairy products, seafoods, and meats: collaborative study. *J. AOAC INTERNATIONAL* **77**:1472-1489.
18. Curtis, G. D. W., R. G. Mitchell, A. F. King, and J. Emma. 1989. A selective differential medium for the isolation of *Listeria monocytogenes*. *Lett. Appl. Microbiol*. **8**:95-98.
19. Feldsine, P. T., A. H. Lienau, R. L. Forgey, and R. D. Calhoon. 1997. Assurance polyclonal enzyme immunoassay (EIA) for detection of *Listeria monocytogenes* and related *Listeria* species in selected foods: collaborative study. *J. AOAC INTERNATIONA*L **80**:775-790.
20. Feldsine, P. T., A. H. Lienau, R. L. Forgey & R. G. Calhoon. 1997. Visual immunoprecipitate assay (VIP) for *Listeria monocytogenes* and related *Listeria* species detection in selected foods: collaborative study. *J. AOAC INTERNATIONAL* **80**:791-805.
21. Gangar, V., M. S. Curiale, A. D'Onorio, A. Schultz, R. L. Johnson, and V. Atrache. 2000. VIDAS® Enzyme-linked immunofluorescent assay for detection of *Listeria* in foods: collaborative study. *J. AOAC INTERNATIONAL* **83**:903-918.
22. Higgins, D. L., and B. J. Robison. 1993. Comparison of MICRO-ID Listeria method with conventional biochemical methods for identification of *Listeria* isolated from food and environmental samples: collaborative study. J. AOAC INTERNATIONAL **76**:831-838.
23. Hitchins, A. D. 1998. *Listeria monocytogenes*. Chapter 10. In: G. J. Jackson (Coordinator) Bacteriological Analytical Manual. 8th Edition. Revision A. AOAC INTERNATIONAL, Gaithersburg, MD.
24. Hitchins, A. D., and R. E. Duvall. 2000. Feasibility of a defined microflora challenge method for evaluating the efficacy of foodborne *Listeria monocytogenes* selective enrichments. *J. Food Protect*. **63**:1064-1070.

25. Jinneman, K., J. M. Hunt, C. A. Eklund, J. S. Wernberg, P. N. Sado, J. M. Johnson, R. S. Richter, S. T. Torres, E. Ayotte, S. J. Eliasberg, P. Istafanos, D. Bass, N. Kexel-Calabresa, W. Lin,, and C. N. Barton. 2003. Evaluation and Interlaboratory Validation of a Selective Agar for Phosphatidylinositol-Specific Phospholipase C Activity Using Chromogenic Substrate to Detect *Listeria monocytogenes* from Foods. *J. Food Protect*. **66**:441-445.
26. Johnson, J.M., K. Jinneman, G. Stelma, B. G. Smith, D. Lye, J. Messer, J. Ulaszek, L. Evsen, S. Gendel, R. W. Bennett, B. Swaminathan, J. Pruckler, A. Steigerwalt, S. Kathariou, S. Yildirim, D. Volokhov, A. Rasooly, V. Chizhikov, M. Wiedmann, E. Fortes, R. E. Duvall, and A. D. Hitchins. 2004. Natural Atypical *Listeria innocua* Strains with *Listeria monocytogenes* Pathogenicity Island 1 Genes. *Appl. Environ. Microbiol*. **70**:4256-4266.
27. Jones, D., and H.P.R. Seeliger. 1986. International committee on systematic bacteriology. Subcommittee the taxonomy of *Listeria*. *Int. J. Syst. Bacteriol*. **36**:117-118.
28. Jones, D. 1992. Current classification of the genus *Listeria*. In: *Listeria* 1992. Abstracts of ISOPOL XI, Copenhagen, Denmark). p. 7-8.
29. Knight, M. T., M. C. Newman, M. Joseph-Benziger Jr., J. R. Agin, M. Ash, P. Sims, and D. Hughes. 1996. TECRA *Listeria* Visual Immunoassay [TLVIA] for detection of *Listeria* in foods: collaborative study. *J. AOAC INTERNATIONAL* **79**:1083-1094.
30. Lee, W. H., and D. McClain. 1986. Improved *L. monocytogenes* selective agar. *Appl. Environ. Microbiol*. **52**:1215-1217.
31. Mattingly, J. A., B. T. Butman, M. C. Plank, and R. J. Durham. 1988. A rapid monoclonal antibody-based ELISA for the detection of *Listeria* in food products. *J. AOAC INTERNATIONAL* **71**:669-673.
32. Official Methods of Analysis of AOAC INTERNATIONAL AOAC INTERNATIONAL, Gaithersburg, MD, USA Official Method 2002.09
33. Official Methods of Analysis of AOAC INTERNATIONAL AOAC INTERNATIONAL, Gaithersburg, MD, USA Official Method 2004.02
34. Official Methods of Analysis of AOAC INTERNATIONAL AOAC INTERNATIONAL, Gaithersburg, MD, USA Official Method 2004.06
35. Official Methods of Analysis of AOAC INTERNATIONAL AOAC INTERNATIONAL, Gaithersburg, MD, USA Official Method 2010.02
36. Official Methods of Analysis of AOAC INTERNATIONAL AOAC INTERNATIONAL, Gaithersburg, MD, USA Official Method 2012.02
37. Official Methods of Analysis of AOAC INTERNATIONAL AOAC INTERNATIONAL, Gaithersburg, MD, USA Official Method 2013.10
38. Official Methods of Analysis of AOAC INTERNATIONAL AOAC INTERNATIONAL, Gaithersburg, MD, USA Official Method 2013.11
39. Restaino, L., E. W. Frampton, R. M. Irbe, G. Schabert, and H. Spitz. 1999. Isolation and detection of *Listeria monocytogenes* using fluorogenic and chromogenic substrates for phosphatidylinositol-specific phospholipase C. *J. Food Protect*. **62**:244-251.

40. Rocourt, J., P. Boerlin, F.Grimont, C. Jacquet, and J-C. Piffaretti. 1992. Assignment of *Listeria grayi* and *Listeria murrayi* to a single species, *Listeria grayi*, with a revised description of *Listeria grayi*. *Int. J. Syst. Bacteriol*. **42**:171-174.
41. Rocourt, J. 1999. The genus *Listeria* and *Listeria monocytogenes*: phylogenetic position, taxonomy, and identification. In: *Listeria*, Listeriosis and Food Safety. E. T. Ryser and E. H. Marth (Eds). 2nd edition, pp. 1-20. Marcel Dekker, Inc., New York, NY.
42. Ryser, E. T., and E. H. Marth. 1999. *Listeria*, Listeriosis and Food Safety. 2nd edition. Marcel Dekker, Inc., New York, NY.
43. Seeliger, H.P.R., and D. Jones. 1986. *Listeria*. pp. 1235-1245. In: Bergey's Manual of Systematic Bacteriology, Vol. 2, 9th ed. P.H.A. Sneath, N.S. Mair, M.E. Sharpe, and J.G. Holt (Eds). Williams & Wilkins Co., Baltimore, MD.
44. Shaw S., Nundy D. and Blais B.: Performance of the ALOA medium in the detection of hemolytic *Listeria* species in food and environmental samples. Laboratory Services Division, Canadian Food Inspection Agency, Ottawa, Ontario, Canada K1A 0C6.
45. Stelma, G.N., Jr., A. L. Reyes, J. T. Peeler, D.W. Francis, J.M. Hunt, P L. Spaulding, C.H. Johnson, and J. Lovett. 1987. Pathogenicity testing for *L. monocytogenes* using immunocompromised mice. *J. Clin. Microbiol*. **25**:2085-2089.
46. USDA/FSIS. 1999. Isolation and identification of *Listeria monocytogene*s from red meat, poultry, egg and environmental samples. Ch. 8. Microbiology Laboratory Guidebook. 3rd Edition, Revision 2.
47. US FDA/CFSAN. 2008. Guidance for Industry: Control of *Listeria monocytogenes* in Refrigerated or Frozen Ready-To-Eat Foods (Draft Guidance). (accessed 04/14/2011).
48. US DHHS/FDA/CFSAN and USDA/FSIS. 2003. *Listeria monocytogenes* Risk Assessment: Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* among Selected Categories of Ready-to-Eat Foods. (accessed 04/14/2011).
49. Vlaemynck G., Lafarge V., Scotter S. (2000): Improvement of the detection of *Listeria monocytogenes* by the application of ALOA, a diagnostic, chromogenic isolation medium. *Journal of Applied Microbiology*, **88** : 430-441.
50. Van Netten et al. 1989. Liquid and solid selective differential media for the detection and enumeration of *Listeria monocytogenes*. *Int. J. Food Microbiol*. **8**:299-316.
51. Wang, S-Y. and A. D. Hitchins. 1994. Differential enrichment kinetics of severely and moderately injured *Listeria monocytogenes* cell fractions of heat injured populations. *J. Food Safety* **14**:259-27.