*Government of Canada  Gouvernement du Canada*

**HPB Method**

**MFHPB-30**
**February 2011**

**Health Products and Food Branch**

**Ottawa**

# Isolation of *Listeria monocytogenes* and other *Listeria* spp. from foods and environmental samples

**Franco Pagotto, Karine Hébert and Jeff Farber**

**Bureau of Microbial Hazards,**
**Food Directorate,**
**Health Canada,**
**Postal locator: 2204E**
**Ottawa, ON, KIA 0K9**

**Email: micro_methods_committee@hc-sc.gc.ca**

## 1. Application

This method is applicable to the isolation and identification of *Listeria monocytogenes* and other *Listeria* species to determine compliance with the requirements of Sections 4 and 7 of the Food and Drugs Act. This method is Health Canada's standard reference cultural method and can be used for all foods and environmental surfaces. This revised method replaces MFHPB-30, dated September 2010, the Supplement to MFHPB-30, dated March 2002, and Appendix L (Supplement to All *Listeria* Methods), dated August 2005.

## 2. Principle

This method determines the presence of viable *Listeria* species in food and environmental samples. A portion of the product (or environmental sample such as a sponge or swab) is enriched first in a primary broth, then in a secondary enrichment broth, plated onto a specified agar medium and one additional plating medium, and then incubated under specified conditions of time and temperature. It is assumed that viable *Listeria* cells will multiply under these conditions and give rise to visible colonies which can be identified. This method is based on those of Lovett (8.4, 8.5), Hitchins (8.3) and McClain and Lee (8.6), that have been modified on the basis of data collected in comparative studies by Warburton *et al*. (8.13, 8.14, 8.15). It was revised by the addition of modified Fraser broth (8.7), lithium chloride-phenylethanol-moxalactam medium (8.6), and Oxford (8.13), modified Oxford (8.7), and/or PALCAM (8.15) agars. Further revisions to include other chromogenic media have also been made. A mandatory step has been added that requires 24 h enriched LEB (UVM1 formulation) broth to be directly streaked to selective agars, in addition to a transfer step to modified Fraser broth.

Published on the Food Directorate's (Health Canada's) website at:
http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php

## 3. Definition of terms

See Appendix A of Volume 2.

## 4. Collection of samples

**4.1** See Appendix B of Volume 2.
**4.2** For environmental samples, refer to the sampling procedures given in MFLP-41.

## 5. Materials and special equipment

> **Note:** The Laboratory Supervisor must ensure that the analysis described in this method is carried out in accordance with the International Standard referred to as "ISO/IEC 17025:2005 (or latest version): General Requirements for the Competence of Testing and Calibration Laboratories".

The media and reagents listed below that are commercially available and are to be used, prepared and/or sterilized according to the manufacturer's instructions. See Appendix G of Volume 2 for the media formula.

> **Note:** If the analyst uses any variations of the media listed here (either product that is commercially available or made from scratch), it is the responsibility of the analyst or Laboratory Supervisor to ensure equivalency. Please forward equivalency data to the Editor of *Compendium of Analytical Methods* for consideration of modification of this method.

***Listeria* broths and agars** (base media and supplements are commercially available)

1) *Listeria* enrichment broth (LEB) - UVM1 formulation

2) Modified Fraser broth (MFB)

3) Oxford agar (OXA) - mandatory plating media

4) Plating media for second selective agar (one of the following is mandatory)
   - Agar Listeria according to Ottaviana and Agosti (ALOA)
   - A.L. Agar (Bio-Rad)
   - BBL CHROMagar Listeria (BD)
   - Chromogeneic Listeria Agar Plate (OCLA; Oxoid)
   - Lithium chloride-phenylethanol-moxalactam medium (LPM)
   - Modified Oxford agar (MOX)
   - PALCAM agar (PAL)
   - RAPID'L.Mono (Bio-Rad)

5) Control cultures (use ATCC strains or equivalent)
   Positive controls: *Listeria monocytogenes, Listeria ivanovii*, *Listeria innocua*, *Listeria grayi* (*Staphylococcus aureus* and *Rhodococcus equi* - optional)

6) Stomacher, blender or equivalent, vortex mixer

7) Microscope

8) Incubators capable of maintaining 30°C and 35°C

**Note:** It is the responsibility of each laboratory to ensure that the temperature of the incubators or water baths is maintained at the recommended temperatures. Where 35°C is recommended in the text of the method, the incubator may be at 35 +/-1.0° C. Similarly, lower temperatures of 30 or 25°C may be +/- 1.0°C. However, where higher temperatures are recommended, such as 43 or 45.5°C, it is imperative that the incubators or water baths be maintained within 0.5°C due to potential lethality of higher temperatures on the microorganism being isolated.

**Confirmation Media and Reagents**

9) Tryptose broth and agar (TA)

10) Trypticase soy broth and agar, with 0.6% yeast extract (TSB-YE and TSA-YE)

11) Horse or sheep blood agar (recommended for hemolysis test)

12) Motility test medium

13) Carbohydrate fermentation agars or broths (mannitol, rhamnose and xylose).
**Note**: these biochemicals may be done via rapid identification kits (6.8.2)

**Optional**

14) Rapid identification kits, such as the Vitek or API *Listeria* (Bio Mérieux Vitek, Inc.), Micro-ID *Listeria* (Organon Teknika Corp.), the *Listeria* Accuprobe™ Test (Gen-Probe; MFLP-88) or Oxoid Biochemical Identification System (O.B.I.S.) Mono kit (Oxoid) or equivalent

15) Other Chromogenic or novel agar - novel chromogenic and other isolation agars may be used, but only in conjunction with the plating media that are mandatory in the method.

16) Sheep blood agar - for CAMP test

17) Latex Agglutination kit (e.g., Oxoid *Listeria* Test Kit)

18) *Listeria monocytogenes* antisera (e.g., Denka Seiken)

19) Gram stain solutions

20) 3% hydrogen peroxide (for the catalase test)

21) Biochemicals - dextrose, esculin, maltose, α-methyl-D-mannoside

22) Beta-lysine discs (e.g., Remel)

## 6. Procedure

Each sample unit may be analysed individually or the analytical units may be composited according to the sampling scheme described in the *Listeria* Policy. Maintain a ratio of 1 part sample material to 9 parts

sterile enrichment broth. Information regarding *Listeria* distribution can be obtained by analysing each analytical unit separately. Carry out the test in accordance with the following instructions:

**6.1 Handling of Sample Units**

6.1.1 In the laboratory prior to analysis, except for shelf-stable foods, keep sample units refrigerated or frozen, depending on the nature of the product. Thaw frozen samples in a refrigerator, or under time and temperature conditions which prevent microbial growth or death.

6.1.2 Analyze sample units as soon as possible after their receipt in the laboratory.

**6.2 Preparation for Analysis**

6.2.1 Have sterile *Listeria* enrichment broth (LEB) ready, pre-warmed to 30 ± 1 °C before use.

6.2.2 Clean the surface of the working area with disinfectant.

**6.3 Preparation of Sample**

To ensure a representative analytical unit, agitate liquids or free flowing materials until the contents are homogeneous. If the sample unit is a solid, obtain the analytical unit by taking a portion from several locations within the sample unit.

**6.4 Enrichment Procedure (see Figure 1)**

> **Note**: To allow flexibility for incubation times stated, the following guidelines can be used. Incubation times of 24 h are ± 2h; incubation times of 48 h are ± 4 h.

Environmental Samples: Add the environmental sponge or large swabs to 100 mL of LEB or composite up to 10 sponges with 100 mL LEB per sponge (see MFLP-41). Place smaller environmental swabs (e.g., cotton tip) in 10 mL portions of LEB or composite up to 10 swabs with 10 mL LEB per swab.

Food Samples: Add 25 g or mL of the food (the analytical unit) to 225 mL of LEB in a blender jar or stomacher bag. For composite samples, analytical units may be combined up to 125 g or ml (e.g., 125 g or mL of food to 1125 mL of LEB). If alternate analytical units are required, maintain a ratio of 1 part sample material to 9 parts LEB.

For both environmental samples and food blend, stomach or vortex as required for thorough mixing. LEB culture may be incubated in the stomacher bag or other sterile container. Incubate LEB culture for 48 h at 30°C.

**6.4.1 Refrigeration of incubated enrichment broth (LEB) - OPTIONAL**

6.4.1.1 This approach allows for the refrigeration of incubated pre-enrichment broth (LEB) for up to 4 days and provides greater flexibility.

6.4.1.2 Mix thoroughly after refrigeration before proceeding with 6.5.

**6.5 Selective Enrichment**

6.5.1 At 24 and 48 h, mix the LEB culture by swirling or vortexing, and inoculate 10 mL of modified Fraser Broth (MFB) with 0.1 mL of the LEB culture. Incubate 24-26 h at 35°C. In addition, at 24 h (at the same time that the transfer is made from LEB to MFB), proceed with Step 6.6 by directly streaking to the selective agars as described in 6.6.1. It is optional to streak 48 h LEB enrichment to selective plates.

**Helpful hint:** Vortex the MFB at 20 to 24 h, then reincubate for an additional 2 to 6 h before reading reaction. Reading the MFB at 26 h can substantially reduce the plating done at 48 h.

6.5.2 Streak MFB onto plates if positive. A positive broth has darkened and may be black, dark brown or dark green. A negative MFB has the straw colour of a newly made broth. If negative, reincubate another 24 h and streak all positive broth. Proceed with Step 6.6.

### 6.6 Isolation Procedure

6.6.1 After vortexing, streak positive MFB onto two different plating media. Use Oxford agar and one of the following agars as listed in Section 5: ALOA formulation agar, A.L. Agar, BBL CHROMagar Listeria, Chromogeneic Listeria Agar Plate, lithium chloride-phenylethanol-moxalactam medium, modified Oxford agar, PALCAM agar, or RAPID'L.Mono. Incubate LPM plates at 30°C and all other selective agars at 35°C for 48 h, unless the time and temperature are otherwise directed by the manufacturer. Examine plates at 24 h for typical colonies, as well as at 48 h as applicable to the medium used.

Note that there are up to 2 plates produced for each selective agar (one from the streaking of 24 h LEB enriched broth which will always be present and potentially one from positive MFB tube).

6.6.2 **OXA agars** - *Listeria* species form 1 mm diameter black colonies surrounded by black haloes after 24 h. At 48 h colonies are 2-3 mm in diameter, black with a black halo and sunken centre. The colonies can also appear brown-black or green-black. When examined before 24 h, growth of *Listeria* spp. is sometimes apparent but without the characteristic blackening. Some strains of this genus, other than *L. monocytogenes,* are inhibited on this medium when incubated at 35°C.

| **Note**: One of the following media is used in conjunction with Oxford agar, which is mandatory in the method. |
|---|

6.6.3 **Agar Listeria according to Ottaviani & Agosti** - *Listeria* colonies appear blue-green, with *L. monocytogenes* and *L. ivanovii* colonies having opaque halos surrounding the colonies after 24 h. All other *Listeria* species are blue-green but do NOT have the halo. Consult manufacturer insert for a more detailed description

6.6.4 **A.L. agar** - all *Listeria* spp. form blue to blue-green colonies with *L. monocytogenes* and *L. ivanovii* colonies having opaque halos around the colonies after 24 and 48 h, respectively.

6.6.5 **BBL CHROMagar** - *L. monocytogenes* and *L. ivanovii* are blue-green colonies surrounded by a opaque white halo. Other *Listeria* spp. are blue-green colonies without a halo.

6.6.6 **LPM** - Examine LPM plates for suspect colonies using beamed white light powerful enough to illuminate the plate well, striking the plate bottom at a 45° angle. Under optimum transillumination the more isolated and larger (48 h old) *Listeria* colonies appear as whitish piles of crushed glass often showing mosaic-like internal structures occasionally having blue-grey iridescence that tends to sparkle. Alternatively, the colonies can look smooth with a blue tinge around the perimeter. When growth becomes near confluent, an even blue-grey iridescent sheen can be observed.

6.6.7 **MOX agars** - *Listeria* species form 1 mm diameter black colonies surrounded by black haloes after 24 h. At 48 h colonies are 2-3 mm in diameter, black with a black halo and sunken centre. The colonies can also appear brown-black or green-black. When examined before 24 h, growth of *Listeria* spp. is sometimes apparent but without the characteristic blackening. Some strains of this genus, other than *L. monocytogenes,* are inhibited on this medium when incubated at 35°C.

6.6.8 **Brilliance Listeria Agar (formerly OCLA agar)** - *L. monocytogenes* and *L. ivanovii* appear as blue colonies surrounded by an opaque halo, whilst other *Listeria* species produce blue colonies without a halo after 24 h.

6.6.9 **PAL agar** - *Listeria* species form 2 mm grey-green colonies with a black sunken centre and a black halo on a cherry-red background. Some *Enterococcus* and *Staphylococcus* strains form grey colonies with a brown-green halo or yellow colonies with a yellow halo.

6.6.10 **RAPID'L.Mono** - *L. monocytogenes* forms blue colonies without yellow halo while *L. ivanovii* are greenish-blue colonies with yellow halo; other *Listeria* species are yellow to white in colour.

## 6.7 Identification Procedure - Confirmation

6.7.1 If the colonies are well isolated on the selective agars: Pick a minimum of 5 typical colonies from each selective plate to blood agar (6.7.2) at each step of the method that selective plating is done (e.g., after 24 h LEB, after darkening of MFB broth etc.).

If the colonies are NOT well isolated on the selective agars: Pick a minimum of 5 typical colonies from each selective plate to Tryptose agar or Trypticase soy agar with 0.6% yeast extract, streaking for separation. As above, pick a minimum of 5 typical colonies from each step of the method that required purification from selective agars. Incubate plates at 30°C for 24-48 h or until growth is satisfactory. Examine the plates for typical colonies using the light arrangement already described in 6.6.6.

Confirmation of *Listeria* spp. can be accomplished by using motility, hemolysis and 3 carbohydrate agars (mannitol, rhamnose and xylose) or other valid confirmation steps that are published in the Compendium of Analytical Methods as equivalent. Other biochemical tests are optional. Rapid identification kits may be helpful to reinforce confirmation of these results and differentiate the different *Listeria* species (see 6.8.1).

6.7.2 **Hemolysis:**

On blood agar plates (sheep or horse), draw a grid of 20-25 spaces on the plate bottom. Pick typical colonies from the selective agars (if colonies are well isolated) or from the TA or TSA-YE plates (if streaked for purity) and inoculate the blood agars by stabbing one colony per grid.

Stab positive and negative controls (*L. monocytogenes, L. ivanovii* and *L. innocua* or *L. grayi*) on each plate. Incubate for 24 h at 35°C.

**NOTE:** It is recommended that you stab blood agar plates and carbohydrate plates (6.7.4) concurrently from the same colony (motility agar may also be stabbed at this time). Ensure that each colony is placed in the same position on all grid plates.

Examine blood agar plates containing culture stabs by transillumination using a bright light (holding the plate so that the light shines through from the back of the plate). *L. monocytogenes* produces a slight cleared zone around the stab; *L. innocua* shows no zone of hemolysis, whereas *L. ivanovii* produces a well-defined zone of clearing around the stab.

6.7.3 **Motility:**

Agar: Stab motility test medium from selective agars, TA or TSA-YE. Incubate motility test media for a minimum of 48 h at room temperature. Observe daily. If the results are ambiguous after 48 h, incubation can proceed for up to 7 days. Only *Listeria* spp. gives the typical umbrella growth pattern.

and/or

Wet mount: Pick at least one typical colony from selective agar, TA or TSA-YE that is incubated at 30°C or less, and do a wet mount examination using 0.85% saline for the suspending medium and the oil immersion objective of a phase-contrast microscope.

Alternately: Inoculate TSB-YE or TB broths and incubate overnight at 30°C. Transfer a loopful of the overnight cultures to a fresh TSB-YE or TB and incubate at 25°C for 6 hours. Put a drop of each 6 hour culture onto a glass slide and examine for typical *Listeria* motility using the oil immersion objective of a phase contrast microscope. *Listeria* appears as slim, short rods with tumbling motility. Always compare to a known *Listeria* culture. Cocci, large rods, or rods with rapid swimming motility are not *Listeria* species.

6.7.4 **Carbohydrate Utilisation:**

Plates: On carbohydrate (mannitol, rhamnose and xylose) agar plates, draw a grid of 20-25 spaces on the plate bottom. Pick typical colonies from the selective agars, TA or TSA-YE plates and inoculate agars by stabbing one culture per grid. Ensure that each colony is placed in the same position on all grid plates. Always stab positive and negative controls (*L. ivanovii*, *L. monocytogenes* and *L. grayi*). Incubate for 24 h at 35°C.

and/or

Broths: From TSB-YE culture, inoculate the following carbohydrates set up as 0.5% solutions in purple carbohydrate broth: dextrose, esculin, maltose, mannitol, rhamnose, α-methyl-D-mannoside and xylose. Incubate 7 days at 35°C. Examine daily. *Listeria* spp. produces acid with no gas, or no reaction.

Consult Table 1 for the carbohydrate reactions of the *Listeria* spp.

| **Note:** | Carbohydrate plates and broths may also be replaced by rapid identification kits (see 6.8.2) |
|---|---|

## 6.8 Identification Procedure - Optional Tests

6.8.1 **PCR:**
From a single colony from selective agar, follow a validated PCR confirmation method for detection of *Listeria* spp. (see Compendium). It is suggested that the colonies that have been identified by PCR be streaked onto TSA or TSA-YE from the blood agar (6.7.2) to obtain isolates of positives. Biochemical assays may be required if mentioned in the PCR method; check the specific PCR method for guidance. Also see the PCR method for interpretation of PCR results.

6.8.2 **Rapid Identification Kits:**
Rapid identification kits, such as the Vitek or API *Listeria*, Micro-ID *Listeria* or the *Listeria* Accuprobe™ Test, or equivalent. Follow manufacturer's instructions for use.

6.8.3 **Catalase:**
Test a typical colony for catalase. Transfer a colony onto a clean glass slide and add one drop of 3% hydrogen peroxide. Development of bubbles is indicative of a positive reaction. *Listeria* cells are catalase-positive. Avoid picking test colonies from agars containing blood as they can produce a false positive result.

6.8.4 **Gram stain:**
*Listeria* is a small Gram-positive rod.

6.8.5 **CAMP test:**
For the CAMP test, streak fresh isolates of beta-hemolytic *Staphylococcus aureus* and *Rhodococcus equi* vertically on a sheep blood agar plate. Separate the vertical streaks so that test strains may be streaked horizontally between them without touching the vertical streaks. After 24-48 h incubation at 35°C, examine the plates for hemolysis in the zone of the vertical streaks.

6.8.5.1 The hemolysis of *L. monocytogenes* and *L. seeligeri* is enhanced in the vicinity of the *Staphylococcus* streak; while *L. ivanovii* hemolysis is enhanced near the *Rhodococcus* streak. The other *Listeria* species are CAMP test negative. The test can differentiate *L. ivanovii* from *L. seeligeri*, and a weakly-hemolytic *L. seeligeri* from *L. welshimeri*.

6.8.5.2 An alternative and convenient CAMP test may be performed using the *S. aureus* factor in commercially prepared sterile beta-lysine discs. In this test, a beta-lysine disc is placed in the center of the sheep blood plate and 4-5 *Listeria* cultures are streaked as radiating lines from the disc, being careful not to touch the disc with the inoculum. After 18-24 h incubation at 35°C, a very sharp CAMP reaction between *L. monocytogenes* or *L. seeligeri* cultures and the disc can be observed. *L. ivanovii* are strongly hemolytic and form a clear beta hemolytic line along the entire streak.

6.8.6 **Serology:**
Follow manufacture's instructions provided with the antisera.

### 6.9 Interpretation of Results for Speciation

*Listeria* spp. are small, Gram-positive motile rods that are catalase-positive, urea-negative, and produce an acid slant and butt in TSI without production of $H_2S$. They utilize dextrose, esculin, and maltose, with some species also using mannitol, rhamnose, and xylose with production of acid. All species give +/+ reactions in MR-VP broth. *L. grayi* and *L. murrayi* are the only two species which utilize mannitol. *L. murrayi* is the only species which can reduce $NO_3^-$ to $NO_2^-$. It should be noted that *L. grayi* and *L. murrayi* are proposed to be considered members of a single species (8.10).

*L. monocytogenes*, *L. ivanovii*, and *L. seeligeri* (weak) produce hemolysis in horse or sheep blood agar and are also positive in the CAMP test. Of the three, only *L. monocytogenes* cannot utilize xylose, but is rhamnose-positive. *L. ivanovii* can be differentiated from *L. seeligeri* by the CAMP test, where *L. seeligeri* shows enhanced hemolysis only at the *Staphylococcus* streak and *L. ivanovii* shows enhanced hemolysis in the area of the *R. equi* streak.

*L. innocua* can be differentiated from *L. monocytogenes* by its lack of hemolysis on blood agar plates and negative reaction in the CAMP test. *L. welshimeri* that is rhamnose-negative may be confused with a weakly-hemolytic *L. seeligeri* unless the CAMP test is run.

All biochemical, serological, and pathogenicity data are summarized in the attached tables.

## 7. Reporting of results

### 7.1 Reporting of results

A final report contains information on the presence or absence of *Listeria monocytogenes* in the test portion analysed. If other *Listeria* species are isolated, these may be noted in the test report, if agreed between the parties concerned.

The final report shall also include all information necessary for the complete identification of the sample, the method that was used, including any deviations in method, information specifying the mass in grams, or the volume in millilitres. Any information that may have influenced the outcome of the test should be included in the final report.

## 8. References

8.1 Bille, J. 2007. *Listeria* and *Erysipelothrix*. *In* P.R. Murray (ed.), Manual of Clinical Microbiology, 9th Edition. ASM Press, pp. 474-484.

8.2 Health Canada, Health Protection and Food Branch, Food Directorate. 2010. Policy on Listeria monocytogenes in Ready-to-Eat Foods.

8.3 Hitchins, A.D. 1995. *Listeria monocytogenes*. Chapter 10. Revised 1998. Bacteriological Analytical Manual (BAM). AOAC International. Gaithersburg, MD.

8.4 Lovett, J. 1987. *Listeria* isolation. Chapter 29. Bacteriological Analytical Manual (BAM). AOAC. Arlington, Virginia.

8.5 Lovett, J. and A.D. Hitchins. 1988. *Listeria* isolation. Chapter 29. Revised October 13, 1988. Bacteriological Analytical Manual (BAM). AOAC. Arlington, Virginia.

8.6 McClain, D. and W.H. Lee. 1988. Development of USDA-FSIS Method for Isolation of *Listeria Monocytogenes* from Raw Meat and Poultry. JAOAC. **71**(3):660-664.

8.7 McClain, D. and W.H. Lee. 1989. FSIS Method for the Isolation and Identification of *Listeria Monocytogenes* From Processed Meat and Poultry Products. USDA-FSIS Laboratory Communication No. 57. Revised May 24, 1989.

8.8 McLauchlin J. 2005. *Listeria*. *In* S.P. Borriello, P.R. Murray and G. Funke (ed.), Topley and Wilson's Microbiology and Microbial Infections, 10th Edition. ASM Press, pp. 956-969.

8.9 Rocourt, J., and C. Buchrieser. 2007. The genus *Listeria* and *Listeria monocytogenes*: Phylogenetic position, taxonomy and identification. *In* E.T. Ryser and E.H. Marth (ed.), *Listeria*, Listeriosis and Food Safety, 3rd edition. CRC Press, pp. 1-20.

8.10 Rocourt, J., P. Boerlin, F. Grimont, Ch. 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: 69-73

8.11 Seeliger, H.P.R. and D. Jones.1986. The Genus *Listeria*. Bergey's Manual of Systematic Bacteriology. Volume 2. Williams and Wilkins, Baltimore. pp. 1235-1245.

8.12 Wagner, M. and J. McLauchlin. 2008. Biology. *In* D. Liu (ed.), Handbook of *Listeria monocytogenes*. CRC Press, pp. 3-25.

8.13 Warburton, D.W., J.M. Farber, A. Armstrong, R. Caldeira, T. Hunt, S. Messier, R. Plante, N.P. Tiwari and J. Vinet. 1991. A Comparative Study of the "FDA" and "USDA" Methods for the Detection of *Listeria monocytogenes* in Foods. Int. J. Food Microbiol. **13(**2):105-118.

8.14 Warburton, D.W., J.M. Farber, A. Armstrong, R. Caldeira, N.P. Tiwari, T. Babiuk, P. LaCasse and S. Read. 1991. A Canadian Comparative Study of Modified Versions of the "FDA" and "USDA" Methods for the Detection of *Listeria monocytogenes*. J. Food Prot. **54**(9):669-676.

8.15 Warburton, D.W., J.M. Farber, C. Powell, N.P. Tiwari, S. Read, R. Plante, T. Babiuk, P. Laffey, T. Kauri, P. Mayers, M.-J. Champagne, T. Hunt, P. LaCasse, K. Viet, R. Smando and F. Coates. 1992. Comparison of Methods for Optimum Detection of Stressed and Low Levels of *Listeria monocytogenes*. J. Food Microbiol. **9**:127-145.

**Table 1**
**Characteristics differentiating the species of the genus *Listeria*[a]**

| Characteristic | *L. monocytogenes* | *L. innocua* | *L. ivanovii* subsp. *ivanovii* | *L. ivanovii* subsp. *londoniensis* | *L. welshimeri* | *L. seeligeri* | *L. grayi* |
|---|---|---|---|---|---|---|---|
| Gram stain | + | + | + | + | + | + | + |
| Beta-hemolysis | + | - | ++[b] | ++ | - | + | - |
| Acid production from: | | | | | | | |
| Mannitol | - | - | - | - | - | - | + |
| L-Rhamnose | + | V | - | - | V | - | V |
| D-Xylose | - | - | + | + | + | + | - |
| CAMP reaction | | | | | | | |
| *S. aureus* | + | - | - | - | - | + | - |
| *R. equi* | V | - | + | + | - | - | - |
| | | | | | | | |
| Acid production from: | | | | | | | |
| α -Methyl-D-mannoside | + | + | - | - | + | - | + |
| Soluble starch | - | - | - | - | ND | ND | + |
| Ribose | - | - | + | - | - | - | V |
| N-Acetyl-β-D-mannosamine | ND | ND | V | + | ND | ND | ND |
| Hippurate hydrolysis | + | + | + | + | ND | ND | - |
| Lipase production | + | - | + | + | - | + | - |
| Arylesterase activity | - | + | + | + | + | + | + |
| Reduction of nitrate | - | - | - | - | ND | ND | V |

[a]+, ≥90% of strains are positive; -, ≥90% of strains are negative; ND, not determined; V, variable. Adapted from 8.1, 8.8, 8.9, 8.11 and 8.12

[b]++, usually a wider zone of hemolysis observed

**Table 2**
**Serology, hemolytic activity and mouse virulence for *Listeria* species**

| Species | Serotype | Hemolysis of horse blood (7%) stab | Mouse virulence |
|---|---|---|---|
| *L. monocytogenes* | 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4b(x), 4c, 4d, 4e, 7 | + | + |
| *L. ivanovii* | 5 | + | + |
| *L. innocua* | 4ab, 6a, 6b, un* | – | – |
| *L. welshimeri* | 6a, 6b | – | – |
| *L. seeligeri* | 1/2b, 4c, 4d, 6b, un* | + | – |

* un = undefined.

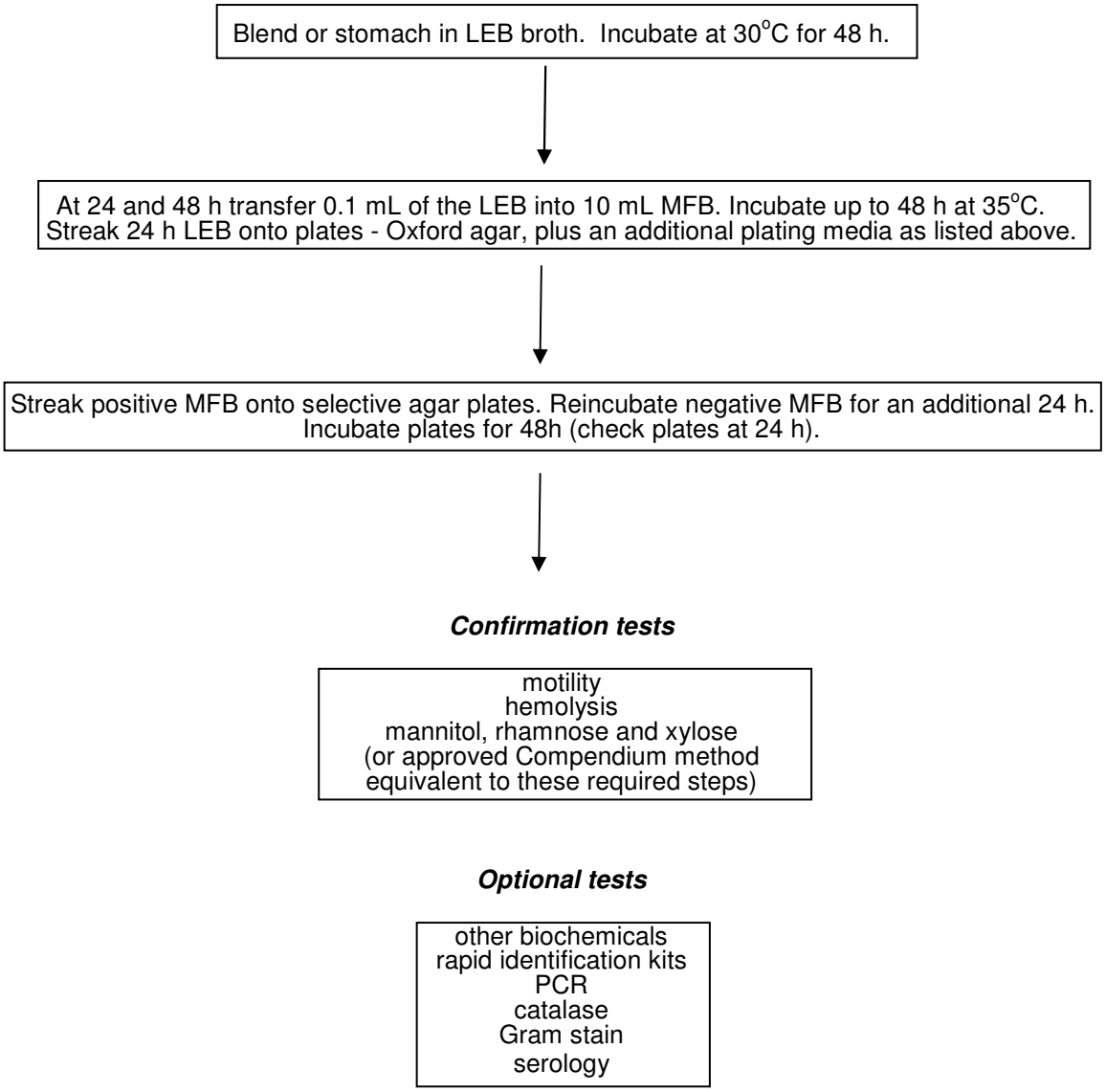


**Figure 1. A Flow Diagram Showing the Isolation Procedure**

# END OF DOCUMENT