

Exhibit 1059
Mullins Decl. Appendix O-1
(MLG 1998 from HathiTrust)

Microbiology laboratory guidebook

United States.

Washington, D.C. : U.S. Dept. of Agriculture, Food Safety and Inspection Service, Office of Public Health and Science, Microbiology Division : 1998-

<https://hdl.handle.net/2027/umn.31951d017531255>



Public Domain, Google-digitized

http://www.hathitrust.org/access_use#pd-google

We have determined this work to be in the public domain, meaning that it is not subject to copyright. Users are free to copy, use, and redistribute the work in part or in whole. It is possible that current copyright holders, heirs or the estate of the authors of individual portions of the work, such as illustrations or photographs, assert copyrights over these portions. Depending on the nature of subsequent use that is made, additional rights may need to be obtained independently of anything we can address. The digital images and OCR of this work were produced by Google, Inc. (indicated by a watermark on each page in the PageTurner). Google requests that the images and OCR not be re-hosted, redistributed or used commercially. The images are provided for educational, scholarly, non-commercial purposes.

Options



#1 | ^



United States
Department of
Agriculture

Food Safety
and Inspection
Service

Office of Public
Health and
Science

Microbiology
Division

Microbiology Laboratory Guidebook

3rd Edition, 1998
Volume 1 and 2

UNIVERSITY OF MINNESOTA
GOVERNMENT PUBLICATIONS LIBRARY

JUL 7 - 1998

U.S. DEPOSITORY PROPERTY
U.S.G.P.O.D-295



#

1

/ 556



MICROBIOLOGY LABORATORY GUIDEBOOK

UNITED STATES DEPARTMENT OF AGRICULTURE
FOOD SAFETY AND INSPECTION SERVICE
OFFICE OF PUBLIC HEALTH AND SCIENCE
MICROBIOLOGY DIVISION



B. P. DEY, DVM, MS, MPH, Ph.D., Editor
C. P. LATTUADA, Ph.D., Co-Editor

Editorial Board

A. M. McNAMARA, Sc.D., R. P. MAGEAU., Ph.D. and
S. S. GREEN., Ph.D.

3RD EDITION, 1998
VOLUME 1

FOREWORD

The 1993 *Escherichia coli* O157:H7 outbreak in the Pacific Northwest focused national attention on food safety. Since then, the number of requests for reprints on analytical methods used by the Microbiology Division, Office of Public Health and Science, Food Safety and Inspection Service, United States Department of Agriculture, has increased dramatically. Scientists within the Division have responded to these requests by completely revising and updating our Microbiology Laboratory Guidebook (MLG) for publication.

This MLG is our laboratory guidebook for the microbiological analysis of meat, poultry, and egg products that fall under the jurisdiction of USDA. It contains methods that FSIS prefers to use for the analysis of these foods. Since USDA does not endorse or approve methods for use by the food industry, inclusion of a particular method in the MLG should not be construed in this manner. Similarly, the mention of specific brand or trade names for a product, medium, chemical or reagent associated with methods contained herein does not constitute endorsement or selectivity by the authors or USDA over similar products that might also be suitable.

The use of the MLG comes with several caveats. This guidebook was written for microbiologists, and its interpretation and use should only be undertaken by trained microbiologists. FSIS assumes no responsibility for any economic, personal injury or other damage that may occur to individuals or organizations because of the use of methods contained in this guidebook. Users should note and pay particular attention to the safety caution symbol (†) and written warnings associated with certain hazardous chemicals or dangerous biological materials used in some of the methods. Users must act in a responsible manner at all times to protect themselves and the environment during performance of these methods. This guidebook must be supplemented with quality assurance and quality control programs as well as chemical, biological, and employee safety hazards management programs in order to operate a microbiology laboratory. These programs are beyond the scope of this guidebook and are the sole responsibility of the user to develop and implement.

This guidebook contains protocols for analytical tests that are required by FSIS regulatory activities. Some protocols, such as the Bioassay procedure for antibiotic residue detection and quantitation, may not be of value to commercial laboratories nor do we expect others to try to commercialize them. They are included here primarily as informational material since they are part of our current analytical methods.

The 1998, 3rd edition MLG publication consists of two separate volumes with a newly revised format utilizing a loose-leaf binder. This format should make the updating of chapters easier by allowing the substitution of a single chapter or page versus reprinting of the entire MLG. Because we anticipate the addition of new materials, the chapter numbers between volumes are not continuous in order to accommodate all changes.

Publishing this new 3rd edition MLG replaces all previous MLG versions and supersedes all Laboratory Communications, which should be discarded.

Finally, to produce a work of this magnitude requires a team of dedicated scientists and support staff. I would like to thank the following people for their efforts: Larry H. Dillard, Joseph Y. Chiu and James G. Eye for coordinating the FSIS Technical Support Laboratory reviews of the manual; Microbiology Division staff members Bhabani P. Dey, Stanley S. Green, Charles P. Lattuada, Bonnie E. Rose, Richard P. Mageau, and Gerri M. Ransom for composing, editing and proofreading many chapters; and Julie M. Hall for providing secretarial support in typing most of the chapters under trying conditions and meeting the demands of a diverse group of scientists.

Ann Marie McNamara, Sc.D.
Director
Microbiology Division
Office of Public Health and Science
Editorial Board, MLG

January 1998

GENERAL CONSIDERATIONS

Before any analyst attempts to perform the microbiological methods contained within this Microbiology Laboratory Guidebook (MLG), it might be helpful to call attention to the following general considerations in the use of this guidebook.

In order to maximize the achievement of successful results when using the various methods in this MLG, it should be clearly understood that all methods and procedures should be performed at all times in a manner as close as possible to the prescribed directions. Particular attention should be paid to all details provided in a given analytical procedure. Changes or shortcuts should not be attempted in a method simply to accommodate factors, for example, such as processing a large number of similar samples through the method at the same time.

All chemicals, media, immunoreagents and commercial test kits should be within current shelf expiration dates and be subjected to quality control and quality assurance procedures to insure their proper performance for their intended purpose and use within the methods presented in this MLG. All instrumentation should be subjected to continuous maintenance and appropriate quality control procedures to insure unquestionably correct performance during use in all methods. The use of positive and negative test controls at all times, as specified for a given procedure, should be implemented. Adequate documentation and record keeping should be employed for all analytical results, test controls, quality assurance and quality control procedures, instrument maintenance programs, and any observed laboratory deviations to the above or in methods performance.

Although all of the methods described in this guidebook have exact numerical values given for performance parameters such as weight and volume measures, pH, time and temperature to achieve optimum results, it should be clearly understood that an acceptable range exists within which optimum results can still be expected to be achieved without compromising the integrity of the method. For any given method, unless otherwise clearly stated within the text of this MLG, the following allowable ranges for the given parameters are considered to be acceptable and are applicable:

Weight and volume measures: $\pm 1\%$
pH: ± 0.2 units
Time: hours ± 1 hour; minutes $\pm 1\%$
Temperature: $\pm 1.0^{\circ}\text{C}$

CONTENTS

Volume 1

FOREWORD.....	i-ii
GENERAL CONSIDERATIONS.....	iii
TABLE OF CONTENTS.....	iv-ix
CHAPTER	Page
1. SAMPLE PREPARATION FOR MEAT, POULTRY AND PASTEURIZED EGG PRODUCTS..... Charles P. Lattuada and B. P. Dey	1:1-3
2. PHYSICAL EXAMINATION OF MEAT AND POULTRY PRODUCTS..... Charles P. Lattuada and B. P. Dey	2:1-8
3. EXAMINATION OF FRESH, REFRIGERATED AND FROZEN PREPARED MEAT, POULTRY AND PASTEURIZED EGG PRODUCTS..... Charles P. Lattuada, Larry H. Dillard and Bonnie E. Rose	3:1-13
4. ISOLATION AND IDENTIFICATION OF <i>SALMONELLA</i> FROM MEAT, POULTRY AND EGG PRODUCTS..... Bonnie E. Rose	4:1-14
5. DETECTION, ISOLATION AND IDENTIFICATION OF <i>ESCHERICHIA COLI</i> O157:H7 AND O157:NM (NONMOTILE) FROM MEAT AND POULTRY PRODUCTS..... Amelia K. Sharar and Bonnie E. Rose	5:1-10
6. ISOLATION, IDENTIFICATION, AND ENUMERATION OF <i>CAMPYLOBACTER JEJUNI/COLI</i> FROM MEAT AND POULTRY PRODUCTS..... Gerri M. Ransom and Bonnie E. Rose	6:1-10
7. ISOLATION AND IDENTIFICATION OF <i>AEROMONAS</i> SPECIES FROM MEAT AND POULTRY PRODUCTS..... Bonnie E. Rose and Anita J. G. Okrend	7:1-6
8. ISOLATION AND IDENTIFICATION OF <i>LISTERIA MONOCYTOGENES</i> FROM MEAT, POULTRY AND EGG PRODUCTS..... Jennifer L. Johnson	8:1-18

TABLE OF CONTENTS (Continued)

CHAPTER	Page
9. ISOLATION & IDENTIFICATION OF PATHOGENIC <i>YERSINIA ENTEROCOLITICA</i> FROM MEAT AND POULTRY PRODUCTS..... Jennifer L. Johnson	9:1-22
10. EXAMINATION OF HEAT PROCESSED, HERMETICALLY SEALED (CANNED) MEAT AND POULTRY PRODUCTS..... George W. Krumm, Charles P. Lattuada, Ralph W. Johnston, James G. Eye and John Green	10:1-38
11. TESTS FOR ENZYMES IN MEAT AND MEAT PRODUCTS..... Charles P. Lattuada, James G. Eye, John M. Damare and B. P. Dey	11:1-5
12. EXAMINATION OF MEAT AND POULTRY PRODUCTS FOR <i>BACILLUS CEREUS</i> Charles P. Lattuada and Dennis McClain	12:1-5
13. EXAMINATION OF MEAT AND POULTRY PRODUCTS FOR <i>CLOSTRIDIUM PERFRINGENS</i> Ann Marie McNamara and Charles P. Lattuada	13:1-7
14. METHODS FOR THE DETECTION OF <i>CLOSTRIDIUM BOTULINUM</i> TOXINS IN MEAT AND POULTRY PRODUCTS..... L. Victor Cook, Wei Hwa Lee, Charles P. Lattuada and Gerri M. Ransom	14:1-8
15. IMMUNOASSAYS FOR THE DETECTION AND QUANTITATION OF STAPHYLOCOCCAL ENTEROTOXINS FROM MEAT AND POULTRY PRODUCTS AND/OR BROTH CULTURE FLUIDS..... Richard P. Mageau	15:1-1

PART A

(Presumptive) Staphylococcal Enterotoxin Reverse Passive Latex Agglutination Test.....	15:2-8
---	--------

PART B

(Confirmative) Biotin-Streptavidin Enzyme Linked Immunosorbent Assay for Staphylococcal Enterotoxins.....	15:9-18
---	---------

TABLE OF CONTENTS (Continued)

CHAPTER	Page
16. AGAROSE THIN-LAYER ISOELECTRIC FOCUSING (TLIEF) FOR SPECIES DETERMINATION OF RAW MUSCLE TISSUES.....	16:1-10
Richard P. Mageau	
17. IDENTIFICATION OF ANIMAL SPECIES IN COOKED AND CANNED MEAT AND POULTRY PRODUCTS.....	17:1-11
Ronald G. Berger and Richard P. Mageau	
18. SPECIES IDENTIFICATION FIELD TESTS (SIFT).....	18:1-5
Mark E. Cutrufelli and Richard P. Mageau	
19. COMPETITIVE ENZYME-LINKED IMMUNOASSAY (CELIA) FOR THE DETECTION AND QUANTITATION OF CHLORAMPHENICOL.....	19:1-13
Richard P. Mageau	
20. QUALITY ASSURANCE PROGRAM TO ENSURE CORRECT PERFORMANCE OF THE FLOW (ICN) TITERTEK MULTISKAN MC PLATE READER.....	20:1-2
Richard P. Mageau	
21. ANIMAL SPECIES DETERMINATION, IMMUNOLOGICAL.....	21:1-1
Richard P. Mageau	
PART A	
(Presumptive) Tube Ring Precipitin Test.....	21:1-12
PART B	
(Presumptive) Commercial ELISA Immunostick Screen Test Kit.....	21:13-19
PART C	
(Confirmation) Agar Gel Immunodiffusion Test.....	21:20-31
APPENDIX	
MEDIA AND REAGENTS.....	A:1-35
B. P. Dey	
MOST PROBABLE NUMBER TABLES.....	A:36-41
Richard P. Mageau	

TABLE OF CONTENTS (Continued)

Volume 2

FOREWORD..... i-ii

GENERAL CONSIDERATIONS..... iii

TABLE OF CONTENTS..... iv-ix

CHAPTER Page

32. DETECTION AND IDENTIFICATION OF
EXTRANEIOUS MATERIAL IN MEAT AND
POULTRY PRODUCTS 32:1-5
Mark M. Wheeler and James G. Eye

PART A

Detection of Light Filth in Prepared
Infant Foods Containing Meat and
Poultry..... 32:6-10
Mark M. Wheeler and Barbara Bennett

PART B

Detection and Identification of
Extraneous Materials in Non-Meat Food
Ingredients-Macroscopic Examination..... 32:11-14
James G. Eye and Mark M. Wheeler

PART C

Detection of Glass and Non-Aluminum
Metals in Meat and Poultry Products..... 32:15-18
Mark M. Wheeler

PART D

Method for the Isolation of Glass
From Prepared Meat and Poultry
Baby Foods..... 32:19-24
Mark M. Wheeler

33. DETECTION OF ANTIMICROBIAL RESIDUES IN
MEAT AND POULTRY TISSUE BY SCREEN TESTS..... 33:1-1
B. P. Dey, Clarence A. White,
Richard H. Reamer and Nitin H. Thaker

TABLE OF CONTENTS (Continued)

CHAPTER	Page
33. (Continued)	PART A
Detection of Antimicrobial Residues By Swab Test On Premises (STOP).....	33:1-13
Clarence A. White, B. P. Dey and Richard H. Reamer	
	PART B
Detection of Antimicrobial Residue In Calves By Calf Antibiotic and Sulfonamide Test (CAST).....	33:14-25
Clarence A. White, B. P. Dey and Richard H. Reamer	
	PART C
Tentative Confirmation of CAST Results for Sulfonamide Residues in Meat and Poultry Tissue.....	33:26-35
B. P. Dey, Sandra L. Kamosa and Clarence A. White	
	PART D
Detection of Antimicrobial Residue By Fast Antimicrobial Screen Test (FAST).....	33:36-48
B. P. Dey, Clarence A. White, and Nitin H. Thaker	
	PART E
Evaluation of Antimicrobial Residues in Meat and Poultry Tissue By A Modified Fast Antimicrobial Screen Test (M-FAST).....	33:49-57
B. P. Dey, Richard H. Reamer and Sandra L. Kamosa	
34. BIOASSAY FOR THE DETECTION, IDENTIFICATION AND QUANTITATION OF ANTIMICROBIAL RESIDUES IN MEAT AND POULTRY TISSUE.....	34:1-66
Clarence A. White, B. P. Dey, Richard H. Reamer and Nitin H. Thaker	
35. DETECTION OF ANTIBIOTIC AND SULFONAMIDE RESIDUES IN MEAT TISSUE BY COMMERCIALY AVAILABLE IMMUNOASSAY KITS.....	35:1-6
Anne M. Dulin, Clarence A. White and Nitin H. Thaker	

TABLE OF CONTENTS (Continued)

CHAPTER	Page
36. QUALITY CONTROL.....	36:1-23
Charles P. Lattuada, Joe Y. Chiu, Larry H. Dillard, James G. Eye and George W. Krumm	
APPENDIX	
MEDIA AND REAGENTS.....	A:1-35
B. P. Dey	
MOST PROBABLE NUMBER TABLES.....	A:36-41
Richard P. Mageau	

CHAPTER 8. ISOLATION AND IDENTIFICATION OF *LISTERIA* *MONOCYTOGENES* FROM MEAT, POULTRY AND EGG PRODUCTS

Jennifer L. Johnson

8.1 Introduction

Listeria monocytogenes is a common bacterial contaminant of raw foods, including raw meats and poultry. The organism is also capable of causing serious illness in the immunocompromised, pregnant women, and the elderly.

One of a closely-related group of *Listeria* species, *L. monocytogenes* is the only species found in ready-to-eat meat and poultry products of concern to FSIS. Recovery of the low levels of *L. monocytogenes* found in meat and poultry products involves several enrichment broths and one or more kinds of selective plating media. This protocol incorporates several changes made during 1994 in order to improve the isolation and recovery of *L. monocytogenes* from meat and poultry products which are often contaminated with other *Listeria* spp.

The main differences between this protocol and Laboratory Communication No. 57 are the incubation of Fraser broth for 48 h and streaking of all Fraser broth cultures (regardless of darkening) onto Modified Oxford (MOX) agar in this protocol. ALL Fraser broth-enrichment cultures are streaked onto MOX after 24 h. If no typical colonies are present (after 24 h incubation) on the MOX plate streaked from the 24 h culture, then the 48 h Fraser broth enrichment culture is also streaked onto MOX. In addition, a new method for selecting MOX colonies and optional miniaturized identification systems are described. The new selection method for MOX colonies will increase the number of isolates which are streaked to Horse Blood Overlay (HL) agar. This increases the likelihood of recovering *L. monocytogenes* from samples which may contain a mixture of *Listeria* species.

The vast amount of knowledge on *L. monocytogenes* which has been accumulated since the mid-1980s has raised new questions about exactly what constitutes a "typical" isolate of *L. monocytogenes*. It is known that certain phenotypic traits once considered to be characteristic of *L. monocytogenes* (e.g. β -hemolysis, rhamnose fermentation) may be absent or variably-expressed in organisms which are genotypically *L. monocytogenes*. However studies indicate that phenotypically-atypical strains of *L. monocytogenes* have also been recovered from meat and poultry products.

Phenotypic characterization of any *Listeria* isolate must be done with the knowledge that an isolate may not exactly fit the expected pattern of phenotypic characteristics. Given the current state of

technology and the availability of rapid nucleic acid probe tests for *L. monocytogenes*, it would seem prudent to retain phenotypically-atypical isolates and send them to a laboratory for genetic testing.

NOTE: This chapter supersedes L.C. 57 and all related memoranda. This protocol should not be abbreviated or conducted in any manner other than that detailed here.

8.2 Equipment

- a. Balance capable of weighing to 0.1 g
- b. Sterile supplies: chisels, knives, scissors, spatulas, forceps, pipettes (1 ml), bent glass rods (hockey sticks)
- c. Sterile disposable/reusable dishes, pans or trays for cutting samples
- d. Stomacher™ (model 400 by Tekmar, or comparable model), sterile plastic bags (with twist-ties or self-sealing) **OR** blade-type blender, sterile cutting assemblies and blender jars
- e. Incubator, $30 \pm 1^{\circ}\text{C}$
- f. Incubator, $35 \pm 1^{\circ}\text{C}$
- g. Incubator, 20 or $25 \pm 1^{\circ}\text{C}$
- h. Vortex mixer
- i. Phase-contrast microscope (40X and 100X objectives), glass microscope slides and cover slips, immersion oil
- j. Platinum inoculating needle and loop
- k. Grease pencil or waterproof marker
- l. Fluorescent desk lamp

8.21 Reagents

The list of reagents provided below has been divided into two sections. The first section lists "critical reagents" necessary for confirmation of *L. monocytogenes*. The second section lists "supplemental reagents" which may be needed when an analyst is uncertain as to whether *Listeria* or another genus has been isolated. The supplemental tests may also be used to identify *Listeria* species other than *L. monocytogenes* if the exact identity of an isolate is required.

a. Critical reagents

- i. Hydrogen peroxide (3% solution)
- ii. β -lysin discs (#21-120, Remel, Lenexa, KS or equivalent). These discs are convenient and their use precludes possible difficulties caused by the use of live hemolytic cultures of *Staphylococcus aureus* and *Rhodococcus equi*. If these discs are not available, the conventional CAMP test can be

performed with CAMP-test cultures (*S. aureus* ATCC 25923 or ATCC 49444, and *R. equi* ATCC 6939).

b. Supplemental reagents

- i. Gram stain reagents
- ii. Nitrate reduction reagents (available commercially)
- iii. MR-VP reagents (available commercially in strip or disc format)
- iv. Polyvalent *Listeria* antiserum (Difco), types 1 and 4, [Note: this antisera is not specific for *L. monocytogenes* and will react with several other *Listeria* spp.]

8.22 Media

The list of media provided below has been divided into two sections. The first section lists "critical media" necessary for the isolation and confirmation of *L. monocytogenes*. The second section lists "supplemental media" which may be needed when an analyst is uncertain as to whether *Listeria* or another genus has been isolated, or when exact speciation of a non-*L. monocytogenes* isolate is desired.

a. Critical media

- i. Modified UVM broth (UVM)
- ii. Fraser broth (with Ferric Ammonium Citrate added)
- iii. Modified Oxford (MOX) agar
- iv. Horse blood overlay agar (HL)
- v. Trypticase soy agar with 5% sheep blood (CAMP test agar)
- vi. Phenol red carbohydrate fermentation broth
- vii. Phenol red broth containing 0.5% rhamnose
- viii. Phenol red broth containing 0.5% xylose
- ix. Phenol red broth containing 1% mannitol
- x. Bacto motility test medium (DIFCO)
- xi. Brain heart infusion (BHI) broth
- xii. BHI agar slants
- xiii. Trypticase Soy Agar-Yeast Extract (TSA-YE)

b. Supplemental media

- i. Nitrate broth
- ii. MR-VP broth
- iii. Oxidation/fermentation medium (O/F) with 1% glucose
- iv. Bile esculin agar slants

8.3 Isolation Procedures

- a. This procedure may be used for meat, poultry, liquid, or dried egg products. Frozen meat and poultry products should be cultured while still in the frozen state. Frozen egg samples should be thawed and mixed prior to sampling in order to obtain a more homogeneous sample.
- b. A schematic of the method is presented in Fig. 1.

8.31 Enrichment and Plating

- a. Add 225 ml of UVM broth to a 25 g or a 25 ml sample. Stomach or blend for 2 minutes. If blended, transfer the homogenized material to a suitable sterile container (a stomached sample may be left in the stomacher bag) and incubated at 30°C for 22 ± 2 h.
- b. For secondary enrichment, transfer 0.1 ml of the UVM enrichment culture to a tube (10 ml) of Fraser broth. Incubate all tubes at 35°C for 27 ± 1 h.
- c. **STREAK ALL** 27 ± 1 h Fraser broth enrichment cultures (REGARDLESS of color) onto MOX agar plates. After all of the Fraser broth enrichment cultures have been streaked to MOX, reincubate them at 35°C for an additional 22 ± 2 h.
- d. Incubate MOX plates at 35°C for 24-48 h. After 24 h of incubation, examine the plate for distinctive 1-2 mm round colonies surrounded by zones of esculin hydrolysis.
- e. If there are no colonies having this morphology on the MOX agar plate, then streak a second MOX plate from the 48 h Fraser broth enrichment culture*. Reincubate the MOX plate from the previous day for another 24 h, and incubate the newly-streaked MOX plate at 35°C for 24-48 h.
- f. If colonies display a morphology similar to that described above (8.31 d), proceed to the next step or, if the colonies are very small, reincubate the MOX agar plates at 35°C for an additional 20 ± 4 h.

*NOTE: When testing samples suspected of involvement in foodborne illnesses, streak from ALL Fraser broth cultures (after BOTH 24 and 48 h of incubation) onto MOX agar. Incubate ALL plates for 48 h, reading after both 24 and 48 h.

8.32 Colony Selection and Restreaking

- a. Restreak onto MOX all suspect isolates recovered from MOX agar to ensure purity before any confirmational testing is done.
- b. Select a group of typical colonies on each suspect MOX agar plate. Gently run a platinum inoculating loop across 5-20 of these colonies and streak for isolation on a Horse Blood Overlay agar (HL) plate. HL plates are very thin and easily gouged.
- c. Perform this procedure for each MOX plate containing suspect colonies. Incubate HL plates for 19 ± 3 h at 35°C .

8.33 Examination of HL Plates for Hemolysis

- a. Examine each HL plate by holding it at a right angle under a fluorescent lamp, and viewing the bottom of the plate. Select only translucent colonies, 1-2 mm in diameter, with a narrow zone of β -hemolysis surrounding the colony and complete clearing of the blood underneath the colony.
- b. Confirmation of at least 2 such colonies from an HL plate is recommended.

NOTE: Some *L. monocytogenes* isolates may be only faintly β -hemolytic on HL. If the analyst is in any doubt about the existence of β -hemolysis, the isolate should be carried forward and analyzed by the CAMP test (or, optionally, a *Listeria* Identification System). The colony may be lifted from the agar before determining hemolysis, but the analyst should realize that, apart from *L. monocytogenes*, some strains of *L. innocua* may also give contact hemolysis (Skalka et al., 1983).

8.4 Identification and Confirmation Procedures

- a. The USDA enrichment and plating procedure will recover not only *L. monocytogenes* but other *Listeria* spp. as well. Since current FSIS regulations are only concerned with *L. monocytogenes*, it is imperative that any recovered *Listeria* isolates be accurately speciated. This section of the protocol again lists "critical tests" (Section 8.41, below) for *L. monocytogenes* separately from "supplemental tests" (Section 8.42). The "critical tests" **MUST** be done in order to confirm *L. monocytogenes*. The "supplemental tests" are optional, and provide additional information to analysts unfamiliar with the

characterization and speciation of *Listeria*.

- b. As an alternative to the critical and/or supplemental confirmation tests, the analyst may elect to use one of two miniaturized *Listeria* identification systems which are used in FSIS laboratories; these commercially-available systems are described in Section 8.43. Isolates which are identified by either of these systems must also be tested for catalase production and motility (Section 8.41).

8.41 Critical Tests for Confirming *L. monocytogenes* (see Fig. 2):

a. Motility testing

Well-isolated presumptive *Listeria* colonies which are β -hemolytic on HL agar are picked singly to separate tubes of BHI broth; two to five isolates should be selected. A tube of motility test medium may be inoculated using the same needle as for the BHI broth. Incubate the motility medium and the BHI broth cultures at 20-25°C. After 16-18 h incubation, examine a wet-mount preparation of the BHI culture for tumbling motility using the 40X or 100X objective of a phase-contrast microscope. [If the BHI culture appears to contain more than one type of cell, streak it out on an HL plate, incubate the HL plate for 19 $\pm$ 3 h at 35°C. Select one isolated colony, inoculate it into BHI broth, and proceed with testing.] Examine the motility medium tubes after $\geq$ 48 h incubation. Umbrella-like growth is typical of *L. monocytogenes*.

b. Catalase test

Inoculate a BHI slant, incubate at 35°C for 20 $\pm$ 2 h. Test a loopful of cellular growth for catalase production using 3% H₂O₂. Gas bubble formation indicates a positive test.

c. Further identification

Proceed with further identification only if the culture is catalase positive, has typical tumbling motility and appears to be a pure culture when examined by phase contrast microscopy. Growth from the BHI broth or slant culture may be used to inoculate further biochemical tests.

d. **Rhamnose, xylose, and mannitol tests**

Inoculate one tube each of rhamnose, xylose, and mannitol broth. Incubate at 35°C for 1-7 days, examining after 1,2,3, and 7 days.

e. **Modified CAMP test**

A modified CAMP test using a commercially available β -lysin disc is recommended because of difficulties encountered with CAMP tests using live cultures of *Staphylococcus aureus* and/or *Rhodococcus equi*. The disc is sterile and has no β -hemolytic activity of its own to obscure the CAMP reaction. Use of the β -lysin disc also avoids interpretation difficulties caused by reactions between *R. equi* and some strains of *L. monocytogenes*.

Aseptically remove a disc and place it in the center of a (8 ml) TSA sheep blood plate. Four to eight isolates may be tested on a single plate by streaking as radiating lines which nearly touch the disc. Incubate at 35°C, examining the plate after both 24 and 48 h. *L. monocytogenes* will give a very strong arrowhead-shaped enhanced β -hemolytic zone near the disc while *L. seeligeri* will give a weaker reaction.

f. **Alternate CAMP test**

If the β lysin disc is not available, streak weakly β -hemolytic *S. aureus* ATCC 4944 (or ATCC 25923) and *R. equi* ATCC 6939 vertically in parallel streak lines on a TSA sheep blood plate, leaving sufficient space between the vertical streaks so that test isolates may be streaked horizontally between them without touching them. Incubate at 35°C, examining the plate after both 24 and 48 h.

8.42 Supplemental Tests for Atypical Isolates

a. **Introduction**

These tests are recommended for atypical isolates or for use by analysts inexperienced in *L. monocytogenes* characterization. One or more of the following tests may be performed:

b. **Gram staining**

Smear a loopful of the BHI culture onto a slide and perform a Gram stain.

c. Biochemical tests

- i. Bile esculin agar - Incubate at 35°C for 48 h.
- ii. MR-VP medium - Incubate at 35°C for 48 h.
- iii. Glucose O/F medium - Incubate at 35°C for 48 h.
- iv. Nitrate broth - Incubate at 35°C for 48 h.

d. Serology

- i. Organisms identified as *L. monocytogenes* may be tested using polyvalent somatic antiserum against *L. monocytogenes*.
- ii. Commercially available antisera (Difco, Detroit, MI or equivalent) will react with serotype 1 and 4 *Listeria* spp., including some strains of *L. monocytogenes*, *L. innocua*, and *L. seeligeri*. Use of this antisera is not considered a definitive test for *L. monocytogenes* due to reactivity with *Listeria* spp. other than *L. monocytogenes* and to a limited serological spectrum (e.g. it does not react with serovar O3 - a common serovar of *L. monocytogenes* found on meat and poultry).
- iii. Exact serological characterization of *L. monocytogenes* isolates requires access to a battery of specific typing antisera.

8.43 Alternative Biochemical Tests

a. Introduction

There are at least two miniaturized biochemical test strips available commercially for the identification of *L. monocytogenes*. Any other equivalent test systems may be substituted for either of them. Use of any such test strip is optional for confirmation of catalase-positive isolates with typical *Listeria*-like motility.

- b. api® LISTERIA Identification System (BioMérieux-Vitek).** If the api® *Listeria* system is used, the CAMP test is optional since the strip contains an alternative test for differentiating between *L. monocytogenes* and *L. innocua*.

- i. Following step 8.33, select at least 2 colonies exhibiting β -hemolysis, and streak each isolate onto an HL plate that has been divided in half; (do not divide a plate into more than 2 sections). Incubate the HL plate(s) at 35°C for 24 h.

- ii. Using growth from the fresh HL culture, prepare inoculum for the api® strip as directed in the manufacturer's instructions.
 - iii. On the data sheet, record the type of hemolytic reaction visible on the HL plate used for inoculum preparation.
 - iv. Incubate the inoculated identification strip at 35°C. After 20 ± 2 h incubation, read the reactions on the strip as directed in the manufacturer's instructions.
- c. **Micro-ID *Listeria* identification test strips** (Organon Teknika Corp.). If the Micro-ID strip is used, the CAMP test **MUST** be done since the strip itself cannot differentiate between *L. monocytogenes* and *L. innocua*. The manufacturer's directions must be followed in performing this test starting with 8.43 c, i (below).
- i. Following step 8.33, select at least 2 colonies exhibiting β-hemolysis, and streak each isolate onto a TSA-YE plate that has been divided in half; (do not divide a plate into more than 2 sections). Incubate the TSA-YE plate(s) at 30°C for 24 h or until growth is satisfactory.
 - ii. Using growth from the fresh TSA-YE culture, prepare inoculum for the Micro-ID strip as directed in the manufacturer's instructions. Incubate the inoculated identification strip at 35°C. Perform the CAMP test as described in Section 8.41 e, using the same inoculum as for the Micro-ID strip.
 - iii. After 24 h of incubation, read the reactions on the strip as directed in the manufacturer's instructions. Read the CAMP test reaction and record the results on the Micro-ID data sheet.

8.44 Characteristics of *L. monocytogenes*

L. monocytogenes is a catalase-positive coccobacillus which exhibits tumbling motility when grown at 20-25°C. It is usually β-hemolytic on HL agar, and gives a positive CAMP reaction to the *Staphylococcus aureus* factor on sheep blood agar. *L. monocytogenes* usually ferments rhamnose, and is incapable of fermenting mannitol and xylose or reducing nitrate. Like other members of the genus *Listeria*, it is Gram positive, oxidase negative, MR and VP positive, bile esculin positive, fermentative with respect to glucose, and does not form spores. Table 1 presents information on the characterization of *L. monocytogenes*.

8.5 Optional Procedures for Obtaining Quantitative Estimations of *L. monocytogenes* (in cfu/g of Meat or Poultry)

a. Introduction

Certain circumstances, such as products suspected of containing large numbers of *L. monocytogenes* or baseline surveys may require quantitative information in addition to a standard qualitative determination.

b. Direct Plating

- i. Direct plating usually is done on sample reserves following a confirmed qualitative test.
- ii. The sample is added to UVM broth and homogenized as described in Section 8.31 a, then a 0.1 or a 0.2 ml quantity of the sample homogenate is spread-plated onto a MOX plate.
- iii. The MOX plates are incubated as described in Section 8.31 d; typical *L. monocytogenes*-like colonies are individually picked to HL and confirmed (Section 8.4). Then, for example, if 0.1 ml was plated and all isolates confirmed as *L. monocytogenes*, the cfu of *L. monocytogenes*/g of meat would be equal to the number of confirmed *L. monocytogenes* colonies X 100. If 0.2 ml was plated and all isolates confirmed as *L. monocytogenes*, the cfu of *L. monocytogenes*/g of meat would be equal to the number of confirmed *L. monocytogenes* colonies X 50.

c. Most Probable Numbers (MPN) Determination

- i. Samples containing fewer than 100 target microorganisms per gram are difficult to quantitate by standard plating techniques. It has been shown (Ziegler and Halvorson, 1935) that the MPN technique provided higher counts on such samples than direct plating.
- ii. The general procedure would use three consecutive dilutions, in triplicate, ranging from 0.1 ml of sample extract/wash to 10 ml.
- iii. All sample aliquots would be enriched in UVM and then follow the Isolation Procedures starting with Section 8.31 b. Use standard MPN tables for determining the MPN estimate. Specific protocols may differ, when this occurs, follow the MPN

instructions given in the specific program protocol.

8.51 Optional Procedures for Obtaining Accelerated Results and for Accommodating Weekend Work

a. Introduction

These procedures do not replace the methods given in Section 8.31 and should be used with discretion. The only purpose is to possibly accelerate recovery of *L. monocytogenes* by earlier plating. The steps given in this section do not in anyway eliminate the enrichment steps presented in Section 8.31 a, b and c until one or more colonies are isolated and identified from the MOX plate streaked in this method.

This section also presents a plan for starting on different days in order to maximize the use of laboratory time.

b. Accelerated isolation

- i. In order to accelerate the isolation of *L. monocytogenes* from samples suspected of containing *L. monocytogenes*, the UVM enrichment culture may be plated onto MOX agar (see Fig. 1).
- ii. Sample treatment is as described in the standard method (Section 8.31 a-d). The UVM enrichment culture is then streaked onto a MOX plate.
- iii. Even if the UVM culture is streaked onto MOX, a portion must still be transferred into Fraser broth and processed as per the standard qualitative method (Section 8.31 b).

c. Multiple start days

- i. It is recognized that analyses may begin on any weekday, meaning that they may not be completed within a standard work week.
- ii. In the current recovery protocol, the only step which MUST be carried out at the specified time interval is transferring 0.1 ml of UVM enrichment broth culture to Fraser broth after 20-24 h incubation. Fraser broth enrichment cultures, MOX cultures, and HL cultures may all be incubated at 25°C over the weekend, and analysis continued on Monday.

- iii. The use of programmable incubators (with refrigeration capabilities) is also recommended in order to minimize weekend work. If the optional miniaturized identification systems are used, however, it is important that the time intervals specified in the manufacturer's instructions are observed.

8.6 Method Quality Control Procedures

- a. It is recommended that a minimum of three method controls be analyzed whenever meat or poultry products are being examined for the presence of *L. monocytogenes*. These controls are: *L. monocytogenes* ATCC 19111 or NCTC 7973; *L. innocua* ATCC 33090; and an uninoculated media control.
- b. Prepare control cultures of *L. monocytogenes* and *L. innocua* by inoculating a tube of BHI broth and incubating at 35°C for 18 h. In order to provide ca. 30-300 cfu listeriae, it will be necessary to prepare a 10^{-6} dilution of each of the cultures. For the *L. monocytogenes* control, add 0.1 ml of the 10^{-6} dilution of the *L. monocytogenes* culture to a labelled tube containing 50 ml UVM broth; repeat the procedure to prepare the *L. innocua* control. Prepare an uninoculated media control consisting of 50 ml UVM broth in a sterile tube. Incubate the three media controls along with the samples, and analyze them in the same manner as the samples. Confirm at least one isolate recovered from each positive control and investigate the source of any colonies on the negative control.

8.7 Storage of *L. monocytogenes* Cultures

- a. For short-term storage of several months, *L. monocytogenes* cultures may be maintained in the motility test medium at 4°C.
- b. For extended storage, the organism may be grown on BHI agar plates, the growth scraped off the agar and suspended in sterile fetal calf serum (of the type commonly used for tissue culture), and stored at $\leq -60^{\circ}\text{C}$.

8.8 Precautions

- a. *Listeria monocytogenes* is a highly virulent microorganism causing a high mortality rate. Strict adherence must be given to good and safe laboratory practices when working with this microorganism.

- b. Isolation and identification steps should be done in an area isolated from general laboratory work.
- c. Pregnant women and immunocompromised personnel should be prohibited from entering the *L. monocytogenes* work area.

8.9 Selected References

Broome, C. V. 1993. Listeriosis: can we prevent it? ASM News 59:444-446.

Cassiday, P. K., L. M. Graves, and B. Swaminathan. 1990. Replica plating of colonies from *Listeria*-selective agars to blood agar to improve the isolation of *Listeria monocytogenes* from foods. Appl. Environ. Microbiol. 56:2274-2275.

Conner, D. E., V. N. Scott, S. S. Sumner, and D. T. Bernard. 1989. Pathogenicity of foodborne, environmental and clinical isolates of *Listeria monocytogenes* in mice. J. Food Sci. 54:1553-1556.

Del Coral, F., R. L. Buchanan, M. M. Bencivengo, and P. H. Cooke. 1990. Quantitative comparison of selected virulence associated characteristics in food and clinical isolates of *Listeria*. J. Food Prot. 53:1003-1009.

Farber, J. M., G. W. Sanders, and M. A. Johnston. 1989. A survey of various foods for the presence of *Listeria* species. J. Food Prot. 52:456-458.

Johnson, J. L., and C. P. Lattuada. 1993. Comparison of nucleic acid hybridization assays and biochemical characterization tests for the confirmation of *Listeria monocytogenes*. J. Food Prot. 56:834-840.

Kornacki, J. L., D. J. Evanson, W. Reid, K. Rowe, and R. S. Flowers. 1993. Evaluation of the USDA protocol for detection of *Listeria monocytogenes*. J. Food Prot. 56:441-443.

McClain, D., and W. H. Lee. 1989. FSIS method for the isolation and identification of *Listeria monocytogenes* from processed meat and poultry products. Laboratory Communication No. 57. USDA, FSIS, Microbiology Division, Beltsville, MD.

Nicolas, J.-A., E.-P. Espaze, B. Catimel, N. Vidaud, J. Rocourt, and A.-L. Courtieu. 1989. Isolation of *Listeria* from French meat products. Zbl. Bakt. 272:242-247.

Qvist, S., D. J. A. Liberski, and S. Mukherji. 1989. Occurrence of *Listeria monocytogenes* in meat products. Proc. Int. Cong. Meat Sci. and Technol.

Rocourt, J. 1994. *Listeria monocytogenes*: the state of the science. Dairy, Food, Environ. Sanitation 14(2):70-82.

Schiemann, D. A., S. R. Shope, and M. J. Brown. 1990. Development of new enrichment broths and plating agars for isolation of hemolytic species of *Listeria*. J. Food Safety 10:233-252.

Schuchat, A., B. Swaminathan, and C. V. Broome. 1991. *Listeria monocytogenes* CAMP reaction. Clin. Microbiol. Rev. 4:396.

Seeliger, H. P. R., and D. Jones. 1986. Genus *Listeria*, p. 1235-1245 In Bergey's Manual of Systematic Bacteriology, volume 2. Williams & Wilkins. Baltimore, MD.

Skalka, B., J. Smola, and K. Elischerová. 1983. Hemolytic phenomenon under the cultivation of *Listeria innocua*. Zbl. Bakt. Hyg., I. Abt. Orig. A 253: 559-565.

Skovgaard, N., and C.-A. Morgen. 1988. Detection of *Listeria* spp. in faeces from animals, in feeds, and in raw foods of animal origin. Int. J. Food Microbiol. 6:229-242.

Smola, J. 1989. Possibilities of differentiation of listerial hemolysins by synergistic hemolytic reactions (CAMP reactions). Int. J. Food Microbiol. 8:265-267.

Vázquez-Boland, J. A., L. Domínguez, J. F. Fernández, E. F. Rodríguez-Ferri, V. Briones, M. Blanco, and G. Suárez. 1990. Revision of the validity of CAMP tests for *Listeria* identification. Proposal of an alternative method for the determination of haemolytic activity by *Listeria* strains. Acta Microbiol. Hung. 37:201-206.

Vázquez-Boland, J. A., L. Domínguez, J. F. Fernández-Garayzabal, and G. Suárez. 1992. *Listeria monocytogenes* CAMP reaction. Clin. Microbiol. 5:343.

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. 54:669-676.

Ziegler, N. R. and H. O. Halvorson. 1935. Application of statistics to problems in bacteriology. IV. Experimental comparison of the dilution method, the plate count, and the direct count for the determination of bacterial populations. J. Bact. 29:609-634.

Table 1. Characteristics differentiating *L. monocytogenes* from other *Listeria* spp. which may be recovered from meat and poultry ^{a,b}

Organism	β -Hemolysis on HL	CAMP Test (t°) ^c	L-Rhamnose	D-Xylose
<i>L. monocytogenes</i>	+ ^d	+	+	-
<i>L. innocua</i>	-	-	D	-
<i>L. seeligeri</i>	+	+	-	+
<i>L. welshimeri</i>	-	-	D	+
<i>L. ivanovii</i>	+	-	-	+

^a Adapted from Bergey's Manual of Systematic Bacteriology (1986)

^b Standard Symbols: + indicates 90% or more of strains are positive; - indicates 90% or more of strains are negative; D indicates 11-89% of the strains are positive.

^c Reaction with either the β -lysin disc (Remel) or *S. aureus* ATCC 25923.

^d Not all strains of *L. monocytogenes* exhibit β -hemolysis (e.g. the type strain, ATCC 15313, is nonhemolytic on horse, sheep, and bovine blood).

Figure 1. Isolation scheme for *L. monocytogenes*, including standard procedure and optional additional procedures

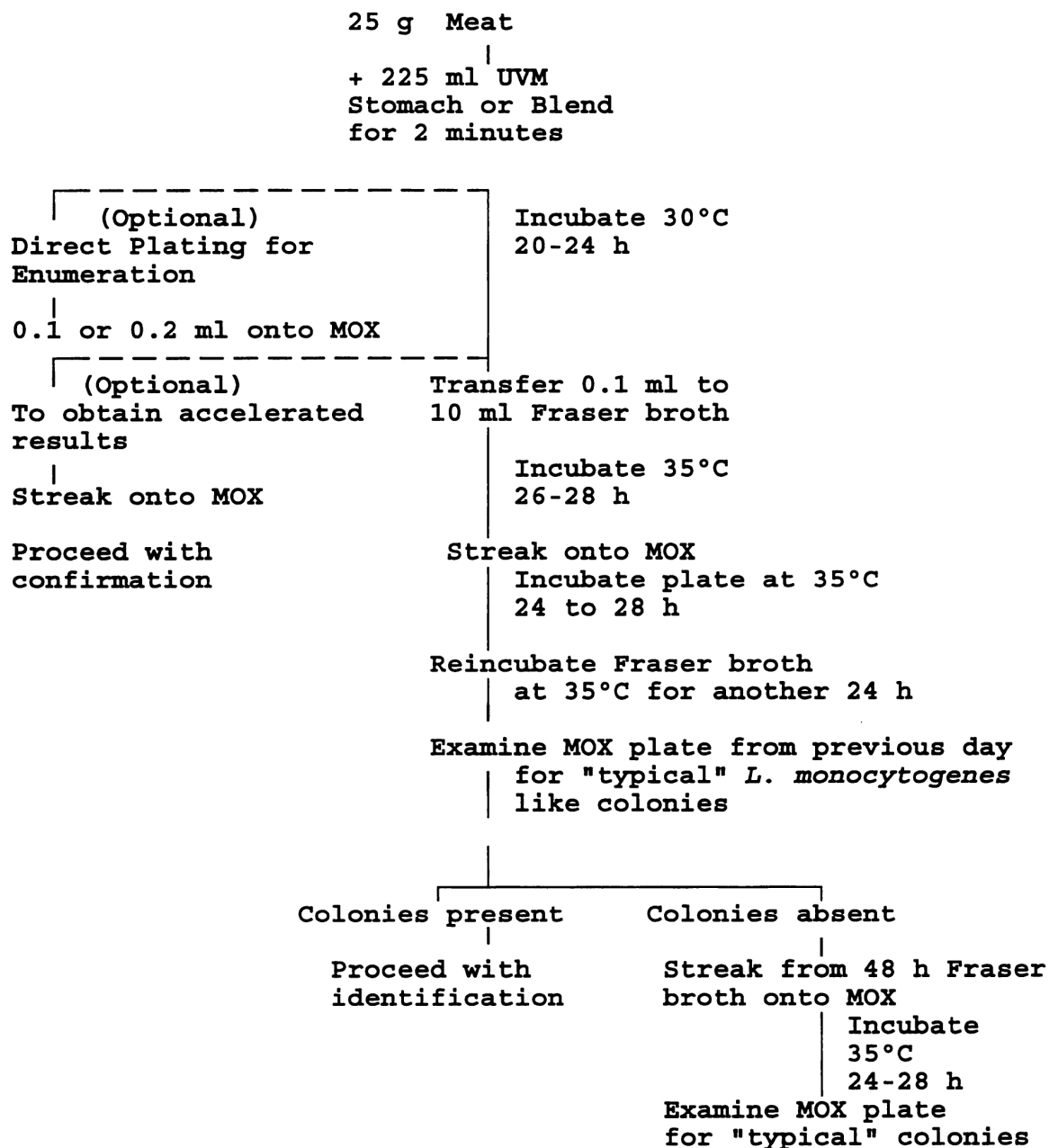
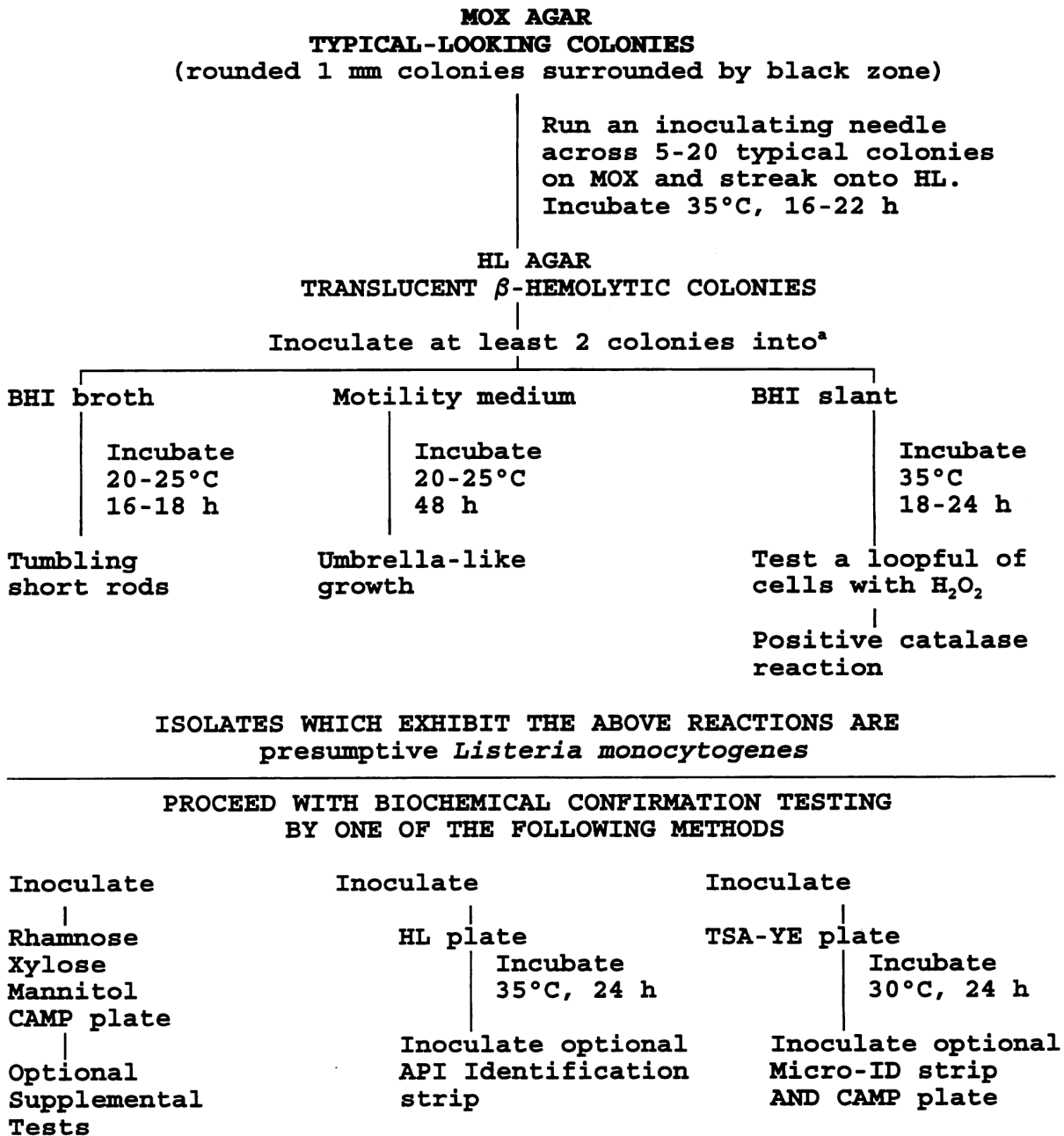


Figure 2. Screening and Identification Scheme for *L. monocytogenes*

* It is recommended that the tumbling motility determination be done because it will allow a visual check for culture purity. The umbrella-motility determination should be done by laboratories lacking a phase microscope; this test can also be done if the tumbling motility determination did not give clear results.

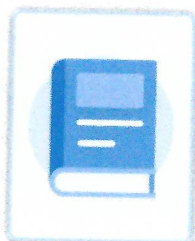
Exhibit 1059
Mullins Decl. Appendix O-2
(WorldCat Entry for MLG 1998)



Items ▾

Search for books, articles, and more

[Home](#) [Libraries](#) [Topics](#) [Lists](#) [About](#) [For Librarians](#)



Microbiology laboratory guidebook

Author: [United States](#)

Continuously Updated Resource, English, 1998-

Edition: 3rd ed., 1998 [View all formats and editions](#)

Publisher: U.S. Dept. of Agriculture, Food Safety and Inspection Service, Office of Public Health and Science, Microbiology Division : For sale by the U.S. G.P.O., Supt. of Docs., Washington, D.C., 1998-

Genre:

Laboratory manuals

Physical Description:

2 volumes in 1 (loose-leaf) : illustrations ; 28 cm

OCLC Number / Unique Identifier:

39555329

Subjects:

[Agricultural microbiology](#)

[Agricultural microbiology United States Laboratory manuals](#)

[Food Microbiology](#)

[Food Microbiology United States Laboratory manuals](#)

[Show more ▾](#)

Additional Physical Form Entry:

Online version:

[Microbiology laboratory guidebook](#)

[United States. Food Safety and Inspection Service.](#)

[Microbiology Division.](#)

[622672964](#)

Notes:

Shipping list no.: 98-0284-P

 2 editions in 121 libraries

Featured libraries All libraries

Showing 104 libraries near [Minnesota, United States](#)

☐ Only public libraries



Academic

Concordia College Library

Carl B. Ylvisaker Library



[99 miles](#) from your current location.

901 8th Street South, Moorhead, MN, 56562, United States

Borrow 



Favorite
Library



Academic

North Dakota State University Libraries



[101 miles](#) from your current location.

1201 Albrecht Blvd N, Fargo, ND, 58105, United States

Borrow 



Favorite
Library



Public

Hennepin County Library



[136 miles](#) from your current location.

12601 Ridgedale Drive, Minnetonka, MN, 55305, United States

Borrow 



Favorite
Library



Academic

University of Minnesota, Twin Cities



[140 miles](#) from your current location.

499 Wilson Libr, 309 19th Ave S, Minneapolis, MN, 55455-0414, United States

Borrow 



Favorite
Library

Exhibit 1059
Mullins Decl. Appendix O-3
(OPAC Record for MLG 1998)

Screen capture completed by J L Mullins from University of Minnesota OPAC at URL:
https://primo.lib.umn.edu/discovery/search?vid=01UMN_INST:TWINCITIES&lang=en&search_scope=TwinCitiesCampus_and_CI&query=any,contains,Microbiology%20Laboratory%20Guid ebook





BOOK

[Microbiology laboratory guidebook.](#)

United States. Food Safety and Inspection Service. [Microbiology Division](#).
3rd ed., 1998.; Washington, D.C. : U.S. Dept. of Agriculture, Food Safety and Inspection Service, Office of Public Health and Science, [Microbiology Division](#) : For sale by the U.S. G.P.O., Supt. of Docs.; 1998-

 [Available at TC Magrath Library Gov Pub \(US Docs\) \(A 110.8:M 58/V.1-2\)](#) >

 [Full Text Available at HathiTrust](#) 

☆

Get It

Please sign in to see request options and to place requests.  [Sign in](#)

< [BACK TO LOCATIONS](#)

LOCATION ITEMS

TC Magrath Library
[Available](#), Gov Pub (US Docs) ; A 110.8:M 58/V.1-2
(1 copy, 1 available, 0 requests)

Item in place 31951D00363011E
Loanable

≡

▼

Details	
Title	Microbiology laboratory guidebook.
Author/Creator	United States. Food Safety and Inspection Service. Microbiology Division. >
Edition	3rd ed., 1998.
Publisher	Washington, D.C. : U.S. Dept. of Agriculture, Food Safety and Inspection Service, Office of Public Health and Science, Microbiology Division : For sale by the U.S. G.P.O., Supt. of Docs.
Date	1998-
Format	2 volumes in 1 (loose-leaf) : illustrations ; 28 cm
Language	English
Description	Shipping list no.: 98-0284-P. Includes bibliographical references.
Subjects	Agricultural microbiology -- United States -- Laboratory manuals > Food -- Microbiology -- United States -- Laboratory manuals > Microbiology -- United States -- Laboratory manuals >
Identifier	OCLC : 39555329 GOVDOC : A 110.8:M 58/V.1-2
Source	University of Minnesota Catalog 9934079610001701