

Australian/New Zealand Standard®

Food microbiology

Method 2.15: Examination for specific
organisms—*Listeria monocytogenes* in dairy
products

AS/NZS 1766.2.15

PREFACE

This Standard was prepared by the Joint Standards Australia/Standards New Zealand Committee FT/4, Food Microbiology, to supersede AS 1766.2.15(Int)—1991.

This Standard is technically equivalent to and has been reproduced from ISO 10560:1993, *Milk and milk products—Detection of Listeria monocytogenes* including Technical Corrigendum 1.

A number of variations have been made in this Standard to the requirements of ISO 10560:1993. These variations, which are necessary for Australian/New Zealand requirements, are listed in Annex ZZ and are indicated in the text by a marginal bar set adjacent to the varied text.

The objective of this Standard is to specify a method for the detection of *Listeria monocytogenes* in milk and milk products.

As this Standard is reproduced from an international Standard, the following applies:

- (a) Its number does not appear on each page of text and its identity is shown only on the cover and title page.
- (b) In the source text 'this International Standard' should read 'this Australian/New Zealand Standard'.
- (c) A full point substitutes for a comma when referring to a decimal marker.
- (d) References to International Standards should be replaced by references to the following Australian Standards:

<i>Reference to International Standard or other publication</i>		<i>Australian Standard</i>	
ISO		AS	
7218	Microbiology—General guidance for microbiological examinations	1766 1766.1	Food microbiology Part 1: General procedures and techniques
8261	Milk and milk products—Preparation of test samples and dilutions for microbiological examination	1766.3	Part 3: Examination of specific products

The terms 'normative' and 'informative' have been used in this Standard to define the application of the annex or appendix to which they apply. A 'normative' annex or appendix is an integral part of a Standard, whereas an 'informative' annex or appendix is only for information and guidance.



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METHOD

1 Scope

This International Standard specifies methods for the detection of *Listeria monocytogenes* in milk and milk products.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 7218:1985, *Microbiology — General guidance for microbiological examinations*.

ISO 8261:1989, *Milk and milk products — Preparation of test samples and dilutions for microbiological examination*.

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1 *Listeria* spp.: Microorganisms which form typical colonies on a solid selective medium and which display the morphological, physiological and biochemical characteristics described when tests are carried out in accordance with this International Standard.

3.1.1 *Listeria monocytogenes*: A *Listeria* species which is considered as pathogenic and which can be differentiated by specific biochemical characteristics from other, non-pathogenic species occurring in milk and milk products.

3.2 *detection of Listeria monocytogenes*: Determination of the presence or absence of this microorganism, in a specified mass or volume, when tests are carried out in accordance with this International Standard.

4 Principle

In general, the detection of *Listeria* spp. necessitates at least three successive stages as in 4.1 to 4.3. See also the diagram of procedure in figure 1.

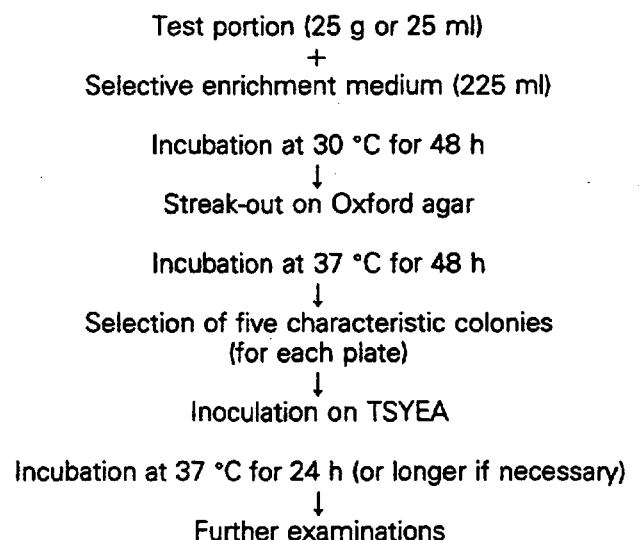


Figure 1 — Diagram of procedure

4.1 Enrichment in selective liquid medium

Inoculation of the selective medium with the test portion of the sample and incubation at 30 °C for 48 h.

4.2 Isolation and presumptive identification

Inoculation of the isolation medium with the culture obtained in the enrichment medium (4.1), incubation at 37 °C and examination after 48 h to check for the presence of colonies which, from their appearance, are considered to be presumptive *Listeria* spp.

4.3 Confirmation

Subculturing of colonies of presumptive *Listeria* spp. (4.2) on a non-selective solid medium, and confirmation by means of appropriate morphological, physiological and biochemical tests.

5 Culture media and reagent

5.1 General

For current laboratory practice, see ISO 7218.

5.2 Culture media

5.2.1 Selective medium: Enrichment medium

5.2.1.1 Base

5.2.1.1.1 Composition

Tryptone soya broth ¹⁾	30 g
Yeast extract	6 g
Water	1 000 ml

1) Composition of tryptone soya broth

Tryptone (pancreatic digest of casein): 17 g
Soytone (papaic digest of soyabean meal): 3 g
Dextrose: 2,5 g
Sodium chloride: 5 g
Dipotassium phosphate: 2,5 g

5.2.1.1.2 Preparation

Dissolve the dehydrated components or dehydrated complete base in the water by boiling.

Adjust the pH, if necessary, so that after sterilization it is 7,3 °C at 25 °C.

Dispense the base in quantities of 225 ml into flasks of 500 ml capacity (or multiples of 225 ml into flasks of suitable capacity). Sterilize for 15 min in the autoclave (6.1.1.2) set at 121 °C.

5.2.1.2 Supplement 1

5.2.1.2.1 Composition

Acriflavine hydrochloride	23 mg
Water	10 ml

5.2.1.2.2 Preparation

Dissolve the acriflavine hydrochloride in the water. Sterilize by filtration.

NOTE 1 For details of the technique of sterilization by filtration, reference may be made to any appropriate textbook on microbiology.

5.2.1.3 Supplement 2

5.2.1.3.1 Composition

Nalidixic acid (sodium salt)	46 mg
Sodium hydroxide (0,05 mol/l)	10 ml

5.2.1.3.2 Preparation

Dissolve the nalidixic acid in the sodium hydroxide solution. Sterilize by filtration.

5.2.1.4 Supplement 3

5.2.1.4.1 Composition

Cycloheximide	57,5 mg
Ethanol	4 ml
Water	6 ml

5.2.1.4.2 Preparation

Dissolve the cycloheximide in the ethanol/water mixture. Sterilize by filtration.

5.2.1.5 Complete medium

Store the base (5.2.1.1) and the prepared supplements (5.2.1.2 to 5.2.1.4) separately in the dark at a temperature between 2 °C and 5 °C. Prepare the complete medium by adding 1 ml of supplement 1, 2 ml of supplement 2 and 2 ml of supplement 3 to 225 ml of the base medium (5.2.1.1).

NOTE 2 Multiples of 1 ml, 2 ml and 225 ml, respectively, in flasks of suitable capacity may be used where appropriate.



5.2.2 Isolation medium (Oxford agar)**5.2.2.1 Agar base****5.2.2.1.1 Composition**

Columbia agar base	39 g
Aesculin	1 g
Ammonium iron(III) citrate	0,5 g
Lithium chloride	15 g
Water	1 000 ml

5.2.2.1.2 Preparation

Dissolve the solid ingredients in the water by boiling.

5.2.2.2 Supplement for 500 ml medium**5.2.2.2.1 Composition**

Cycloheximide	200 mg
Colistin sulfate	10 mg
Acriflavin	2,5 mg
Cefotetan	1 mg
Fosfomycin	5 mg
Ethanol	2,5 ml
Water	2,5 ml

5.2.2.2.2 Preparation

Dissolve the solid ingredients in the ethanol/water mixture. Sterilize by filtration.

5.2.2.3 Preparation of complete medium

Take 500 ml of the agar base (5.2.2.1). Sterilize for 15 min in the autoclave (6.1.1.2) set at 121 °C. Cool to 50 °C and aseptically add the supplement (5.2.2.2). The pH of the final medium should be 7,0 °C at 25 °C.

Dispense the medium into sterile Petri dishes in quantities of about 15 ml and allow to solidify.

5.2.3 Solid culture medium: Tryptone soya yeast extract agar (TSYEA)**5.2.3.1 Composition**

Tryptone soya broth	30 g
Yeast extract	6 g
Agar ¹⁾	12 g to 18 g
Water	1 000 ml

1) According to the gel strength of the agar.

5.2.3.2 Preparation

Dissolve the components or dehydrated complete medium in the water by boiling.

Adjust the pH, if necessary, so that after sterilization it is 7,3 °C at 25 °C.

Dispense quantities of about 6 ml of the solid culture medium into tubes (6.2.2).

Sterilize the tubes for 15 min in the autoclave (6.1.1.2) set at 121 °C.

Allow to set in a sloping position.

For the preparation of agar plates, sterilize the solid culture medium in flasks or bottles of suitable capacity. Dispense the medium while still liquid in quantities of about 15 ml into sterile Petri dishes and allow to solidify.

5.2.4 Liquid culture medium: Tryptone soya yeast extract broth (TSYEB)**5.2.4.1 Composition**

The formulation of the medium is described in 5.2.1.1.1. Use 6 g of yeast extract.

5.2.4.2 Preparation

Prepare the medium as described in 5.2.1.1.2.

Dispense quantities of about 6 ml into tubes before sterilizing in the autoclave.

5.2.5 Blood agar

5.2.5.1 Composition

Blood agar base No. 21)	40 g
Water	1 000 ml
Horse or sheep defibrinated blood	70 ml

1) Composition of blood agar base No. 2

Proteose peptone: 15 g
 Liver digest: 2,5 g
 Yeast extract: 5 g
 Sodium chloride: 5 g
 Agar (according to the gel strength of the agar):
 12 g to 18 g

5.2.5.2 Preparation

Dissolve the dehydrated blood agar base in the water by boiling.

Adjust the pH, if necessary, so that after sterilization it is 7,0 °C at 25 °C. Dispense the medium into tubes or flasks of not more than 500 ml capacity.

Sterilize the blood agar base for 15 min in the autoclave (6.1.1.2) set at 121 °C.

Cool the medium to 45 °C. Add the defibrinated blood and mix well.

Dispense the medium in quantities of about 20 ml into sterile Petri dishes and allow to solidify.

5.2.6 Carbohydrate utilization broth

5.2.6.1 Base

5.2.6.1.1 Composition

Proteose peptone	10 g
Beef extract	1 g
Sodium chloride	5 g
Bromocresol purple	0,02 g
Water	1 000 ml

5.2.6.1.2 Preparation

Dissolve the components in the water by boiling.

Adjust the pH, if necessary, so that after sterilization it is 6,8 °C at 25 °C.

Dispense the medium into tubes in quantities such that after sterilization 9 ml will remain.

Sterilize for 15 min in the autoclave (6.1.1.2) set at 121 °C.

5.2.6.2 Carbohydrate solutions

5.2.6.2.1 Composition

Carbohydrate ¹⁾	5 g
Water	100 ml

1) 100 ml of L-rhamnose solution and 100 ml of D-xylose solution are required.

5.2.6.2.2 Preparation

Dissolve separately each carbohydrate in 100 ml of water. Sterilize by filtration.

5.2.6.3 Complete media

For each carbohydrate, add aseptically 1 ml of solution (5.2.6.2) to 9 ml of the base medium (5.2.6.1). If smaller volumes of base medium are prepared, add accordingly smaller volumes of the carbohydrate solution.

5.2.7 Motility medium

5.2.7.1 Composition

Casein peptone	20,0 g
Meat peptone	6,1 g
Agar	3,5 g
Water	1 000 ml

5.2.7.2 Preparation

Dissolve the components in the water by boiling.

Adjust the pH, if necessary, so that after sterilization it is 7,3 °C at 25 °C.

Dispense the medium into tubes in quantities of about 5 ml.

Sterilize for 15 min in the autoclave (6.1.1.2) set at 121 °C.

NOTE 3 Commercially available media for the examination of motility may be used.

5.2.8 CAMP (Christie, Atkins, Munch-Petersen) test

Blood agar plates (5.2.5) may be used for this test, but