

Australian/New Zealand Standard™

AS/NZS 1766.2.12

Food microbiology

Method 2.12: Examination for specific organisms—*Escherichia coli* in bivalve molluscs— Rapid method

PREFACE

This Standard was prepared by the Joint Standards Australia/Standards New Zealand Committee FT-004, Food Microbiology, to supersede AS 1766.2.12—1994.

The objective of this revision is—

- (a) to stipulate the resuscitation step as an integral part of the method;
- (b) to delete the note given in relation to its applicability for regulatory purposes; and
- (c) to bring the Standard in line with current Standards Australia style.

Experience has shown that the membrane plate method is a reliable, rapid method for checking oysters and other bivalve molluscs for *E. coli* contamination, but may give erroneous results for other types of foods. Therefore, the method has been confined to the examination of bivalve molluscs only.

This Standard is one of a series of methods for the microbiological examination of foods for quality control and investigative purposes and, where applicable, for checking that foods comply with regulatory specifications.

METHOD

1 SCOPE

This Standard sets out a rapid method for enumerating *Escherichia coli* in bivalve molluscs using a membrane plate technique. This method will detect both typical *E. coli* and lactose non-fermenting or anaerogenic variants.

NOTES:

- 1 Other methods for examination of foods, including molluscs, for coliforms and *E.coli* are given in AS 1766.2.3.
- 2 A flow diagram of the examination procedure is shown in Figure 1.

2 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS

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| 1766 | Food microbiology |
| 1766.1.4 | Method 1.4: General procedures and techniques—Colony count—Surface spread method |
| 1766.2.3 | Method 2.3: Examination for specific organisms—Coliforms and <i>Escherichia coli</i> |
| 1766.5 | Method 5: Preparation of culture media, diluents and reagents |

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| 1766.3.5 | Method 3.5: Examination of specific products—Molluscs, crustaceans and fish, and products thereof |
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3 DEFINITION

For the purpose of this Standard the definition below applies.

3.1 *Escherichia coli*

Bacteria which grow at 44.0°C to 44.5°C to form characteristic indole-positive colonies on cellulose ester membranes overlaid on tryptone bile agar, under the operational conditions described.

4 CULTURE MEDIA, DILUENTS AND REAGENTS

4.1 General

Formulations and instructions for preparing culture media, diluents and reagents are given in AS 1766.5.

4.2 Peptone solution, 0.5%—as specified for molluscs in AS/NZS 1766.3.5.

4.3 Tryptone bile agar (TBA)

4.4 Minerals modified glutamate agar (MMGA)

4.5 Indole reagent (Vracko and Sherris)

5 REFERENCE CULTURES

5.1 *Escherichia coli*—NCTC 9001 or ATCC 11775 or NZRM 3309 or ACM 1803.

5.2 *Enterobacter aerogenes*—NCTC 10006 or ATCC 13048 or NZRM 798 or ACM 4982.

NOTES:

- 1 NCTC—National Collection of Type Cultures
ATCC—American Type Culture Collection
ACM—Australian Collection of Microorganisms
NZRM—New Zealand Reference Culture Collection, Medical Section
- 2 These cultures are obtainable from the following Laboratories:
In Australia:
Australian Collection of Microorganisms (ACM)
Department of Microbiology
The University of Queensland
BRISBANE QLD 4072

In New Zealand:

New Zealand Reference Culture Collection (Medical Section) (NZRM)
ESR Kenepuru Science Centre
Kenepuru Drive
PORIRUA

6 APPARATUS

6.1 Cellulose ester membranes—85 mm dia., 0.45 μm pore size.

NOTE: Some types of membranes are unsuitable for this method so a laboratory should test the membranes to ensure they are satisfactory.

6.2 Ultraviolet lamp— wavelength 365 nm.

7 PROCEDURE

7.1 General

A resuscitation step is incorporated as an integral part of this method, as it is specifically for use with bivalve molluscs. In particular, resuscitation is used in the examination of foods containing high concentration of carbohydrates which may have an inhibitory effect on indole production. This permits the diffusion of carbohydrates present in the test sample during the resuscitation step. The resuscitation procedure is also recommended for use when examining foods in which *E. coli* may be damaged by freezing, cold storage, drying or by heat or chemical processes.

7.2 Resuscitation

The procedure shall be as follows:

- (a) Prepare duplicate Petri dishes of MMGA using the method described in AS 1766.1.4, for preparing and drying plates for determining colony count by the surface spread method.
- (b) Using forceps, aseptically place cellulose ester membranes onto the surface of the MMGA, taking care to avoid trapping air bubbles beneath the membranes. Gently flatten membranes with a sterile spreader.
NOTE: Some membranes require to be placed with a particular side up.
- (c) An undiluted sample may be plated to achieve increased sensitivity in the test. However, appropriate controls shall be set up to check for matrix effects. If the increased sensitivity is not required, prepare dilutions of the sample in accordance with AS/NZS 1766.3.5, using 0.5% peptone solution as the diluent. Several dilutions may be required.
- (d) Using the procedure described for the surface spread method in AS 1766.1.4, inoculate the MMGA plates with either 0.5 mL or 1.0 mL of each dilution being tested or the undiluted suspension where appropriate. Leave the periphery of the membrane free of inoculum so that fluid does not run under the membrane. Leave inoculated plates in a horizontal position at room temperature until the inocula have soaked into the membranes (approximately 15 min).
NOTE: The volume of inoculum is governed by the type of food and the anticipated count.
- (e) Prepare control plates for each series of tests by streaking the reference cultures specified in Clause 5 directly onto the membranes.
- (f) Incubate Petri dishes upright at $37 \pm 1^\circ\text{C}$ for 4 h.