

# Australian Standard<sup>®</sup>

## Food microbiology

### Method 9: Examination for specific organisms— Coliforms and *Escherichia coli* by the triplicate tube detection method

#### PREFACE

This Standard was prepared by the Standards Australia Committee FT-024, Food Products and Subcommittee FT-024-01, Food Microbiology (Constituted) to supersede, in part, AS 1766.2.3—1992, *Food microbiology*, Method 2.3: *Examination for specific organisms—Coliforms and Escherichia coli*.

The International Organization for Standardization (ISO) coliform and *E. coli* methods adopted as Australian Standards contain a series of procedures for the enumeration of these bacteria. There is no ISO qualitative procedure equivalent to the existing triplicate tube method in AS 1766.2.3 and this procedure is retained as an Australian Standard for use in food analyses.

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

#### METHOD

##### 1 SCOPE

This Standard sets out procedures for the examination of foods for coliforms and *Escherichia coli* by the triplicate tube method. This is a qualitative test and is suitable for determining the presence or absence of coliforms and *E. coli* in a stated quantity of material under test.

##### NOTES:

- 1 A flow diagram of the procedures is shown in Appendix A.
- 2 The method describes a general method and modifications may be required according to the material under test. Such modifications, if required, are prescribed in the method dealing with particular products.
- 3 Where the test is to estimate the level of coliforms only as an index of potential spoilage of the product and of hygiene, an incubation temperature of 30°C is specified. However, where the test is to be extended to include the estimation or detection of *E. coli*, incubation at 37°C is specified. Cultures which have been incubated initially at 30°C cannot be used for further tests for *E. coli*.
- 4 Methods for the examination of rinse fluids and filterable liquid products for coliforms and *E. coli* by membrane filtration are given in AS 5013.
- 5 Quantitative coliform and *E. coli* methods are given in AS 5013.3 and AS 5013.15 respectively.

## 2 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS

5013 Food microbiology series

5013.3 Method 3: Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of coliforms—Most probable number technique

5013.11.1 Method 11.1: Microbiology of food animal feeding stuff—Preparation of test sample, initial suspension for microbiological examination—General rules for the preparation of the initial suspension and decimal dilutions

5013.14 Method 14: Microbiology of food and animal feeding stuffs—General rules for microbiological examination

5013.15 Method 15: Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of presumptive *Escherichia coli*—Most probable number technique

## 3 DEFINITIONS

For the purpose of this Standard, coliforms and *E. coli* are organisms that are isolated and identified by the triplicate tube detection technique.

## 4 REFERENCE CULTURES

### 4.1 *Enterobacter aerogenes*

NCTC 10006 or ATCC 13048 or UQM 1976—this organism gives positive reactions for coliforms and negative reactions for *E. coli*.

### 4.2 *Escherichia coli*

NCTC 9001 or ATCC 11775 or UQM 1803—this organism gives positive reactions for coliforms and *E. coli*.

### 4.3 Use of reference cultures

The reference cultures are used as instructed in these methods. They are subjected to the test procedures at the same time as test samples in order to demonstrate that appropriate positive and negative reactions are obtained in the tests. A test shall be regarded as invalid unless the reference cultures give appropriate results.

## 5 CULTURE MEDIA, DILUENTS AND REAGENTS

### 5.1 Culture media and reagent (see Appendix B)

#### 5.1.1 *EC broth*

#### 5.1.2 *Eosin methylene blue (EMB) agar*

#### 5.1.3 *Indole reagent (Kovac's)*

#### 5.1.4 *Lauryl tryptose (LT) broth*

#### 5.1.5 *Tryptone water*

## 6 PROCEDURE

### 6.1 Presumptive test for coliforms

The procedure shall be as follows:

- (a) Prepare the sample for microbiological examination in accordance with the instructions given in the relevant AS 5013 methods for the examination of specific product.
- (b) Using the technique described in AS 5013.11.1, prepare dilutions, as required, of the sample.
- (c) Inoculate 1 mL of the appropriate dilution to be examined, or, where appropriate, 1 mL of the original sample, into each of three tubes containing 10 mL of LT broth (5.1.4).

NOTE: The amount of material inoculated into each tube is calculated using the volume (1 mL) and the dilution used.

- (d) If the test is for coliforms, prepare a control tube by inoculating 10 mL of LT broth (5.1.4) with either of the reference cultures (4.1 or 4.2).

If testing for *E. coli* prepare control tubes of each reference culture by inoculating two 10 mL portions of LT broth (5.1.4) respectively with the two reference cultures.

- (e) Mix the tubes by gentle rotation.
- (f) Incubate at  $30 \pm 1^\circ\text{C}$  or  $37 \pm 1^\circ\text{C}$  (see Note 3 in Clause 1) for up to 48 h, and examine for gas production after 24 h and 48 h. Approximately 30 min before each examination, gently tap all tubes to guard against false negative results due to gas supersaturation.
- (g) A presumptive positive reaction is indicated by the production of sufficient gas to fill the concavity of the Durham tube.
- (h) Record the result of the presumptive test as positive if at least two of the set of three tubes are positive.

### 6.2 Confirmatory test for coliforms

The procedure shall be as follows:

- (a) Subculture all cultures as they become positive in the presumptive test by streaking onto plates of EMB agar (5.1.2) and incubate at  $30 \pm 1^\circ\text{C}$  or  $37 \pm 1^\circ\text{C}$  for up to 18 h to 24 h.

- (b) Colonies of typical coliform appearance shall be regarded as coliforms.

NOTE: Typical coliform colonies may show any of the following characteristics:

- (a) Green metallic sheen.
  - (b) Dark purple centres.
  - (c) Opaque, unnucleated, mucoid and pink coloration.
- (c) Record the number of tubes from each dilution that yields plates containing typical coliform colonies.

### 6.3 Tests for *E. coli*

The procedure shall be as follows:

- (a) Using a straight wire, subculture all cultures as they become positive at 24 h and at 48 h (see Clause 6.1(f)) into EC broth (5.1.1) previously warmed to approximately  $44^\circ\text{C}$ .