Australian Standard™

Food microbiology

Method 15: Microbiology—General guidance for enumeration of presumptive *Escherichia coli*—Most probable number technique

PREFACE

This Standard was prepared by the Joint Standards Australia/Standards New Zealand Committee FT-004, Food Microbiology to supersede (in part) AS 1766.2.3:1992, *Food microbiology*, Method 2.3: *Examination for specific organisms—Coliforms and Escherichia coli*.

This Standard is identical with and reproduced from ISO 7251:1993, *Microbiology—General guidance for enumeration of presumptive Escherichia coli—Most probable number technique.*

This Standard was prepared by the Australian members of the Joint Standards Australia/Standards New Zealand Committee FT-004. After consultation with stakeholders in both countries, Standards Australia and Standards New Zealand decided to develop this Standard as an Australian Standard rather than an Australian/New Zealand Standard.

The objective of this Standard is to specify general guidelines for the enumeration of presumptive *Escherichia coli* in products intended for human consumption or feeding of animals by means of the liquid-medium culture technique and calculation of the most probable number (MPN) after incubation at 35°C or 37°C (this temperature forming the subject of agreement between the parties concerned), then incubation at 45°C.

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 Standard'.

(c) A full point substitutes for a comma when referring to a decimal marker.

(d) Substitute 'mL' for 'ml' wherever it appears.

References to International Standards should be replaced by references equivalent to Australian Standards, as follows:

Reference to International Standard	*Australian Standard*	
ISO	AS	
6887	Microbiology—General guidance for the preparation of dilutions for microbiological examination	5013 Food microbiology 5013.11.1 Method 11.1: Microbiology of food animal feeding stuff—Preparation of test sample initial suspension and decimal dilutions for microbiological examination—General rules for the preparation of the initial suspension and decimal dilutions

STANDARDS AUSTRALIA

ISO		AS	
7218	Microbiology—General guidance for microbiological examination	5013.14	Method 14: Microbiology of food and animal feeding stuffs—General rules for microbiological examination

The laboratory should have a clearly defined quality control system to ensure that the apparatus, culture media reagents and technique are suitable for the test. The use of positive controls is part of this system.

The method given in this Standard is for use for all foods for enumeration of presumptive *Escherichia coli*.

The term 'normative' has been used in this Standard to define the application of the annex to which it applies. A 'normative' annex is an integral part of a Standard.

INTRODUCTION

This International Standard is intended to provide general guidance for the examination of products not dealt with by existing International Standards and to be taken into account by organizations preparing microbiological methods of test for application to foods or to animal feeding stuffs. Because of the large variety of products within this field of application, these guidelines may not be appropriate in every detail for certain products, and for some other products it may be necessary to use different methods. Nevertheless, it is hoped that in all cases every attempt will be made to apply the provided guidelines as far as possible and that deviations from them will only be made if absolutely necessary for technical reasons.

When this International Standard is next reviewed, account will be taken of all information then available regarding the extent to which the guidelines have been followed and the reasons for deviations from them in the case of particular products.

The harmonization of test methods cannot be immediate and for certain groups of products International Standards and/or national standards may already exist that do not comply with these guidelines. In cases where International Standards already exist for the product to be tested, they should be followed, but it is hoped that when such standards are reviewed they will be changed to comply with this International Standard so that eventually the only remaining departures from these guidelines will be those necessary for well-established technical reasons.

NOTES

1 Scope

This International Standard gives general guidelines for the enumeration of presumptive *Escherichia coli* in products intended for human consumption or feeding of animals, by means of the liquid-medium culture technique and calculation of the most probable number (MPN) after incubation at 35 °C or 37 °C (this temperature forming the subject of agreement between the parties concerned), then incubation at 45 °C.

CAUTION — Some *Escherichia coli* pathogenic species do not grow at 45 °C.

A limitation of the applicability of this International Standard is imposed by the susceptibility of the method to a large degree of variability. The method should be applied and the results interpreted in the light of the information given in 10.4.

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 6887:1983, *Microbiology — General guidance for the preparation of dilutions for microbiological examination.*

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

3 Definition

For the purposes of this International Standard the following definition applies.

3.1 presumptive *Escherichia coli*: Bacteria which, at 45 °C, cause fermentation of lactose with the production of gas, and which, at 45 °C produce indole from tryptophan, when the test is carried out in accordance with the method specified in this International Standard.

4 Principle

4.1 Inoculation of three tubes of double-strength liquid selective enrichment medium [5.3.1 a)][1] with a specified quantity of the test sample if the initial product is liquid, or with a specified quantity of the initial suspension in the case of other products.

4.2 Inoculation of three tubes of single-strength liquid enrichment medium [5.3.1 b)][1] with a specified quantity of test sample if the initial product is liquid, or with a specified quantity of the initial suspension in the case of other products.

Then, under the same conditions, inoculation of the medium [5.3.1 b)] with decimal dilutions of the test sample or of the initial suspension.

4.3 Incubation of the tubes of double- and single-strength medium at 35 °C or 37 °C (as agreed) for 24 h to 48 h. Examination of the tubes for gas formation.

4.4 Inoculation, from the tubes of double- and single-strength medium which have given rise to gas formation, of a new series of tubes containing a liquid selective medium.

1) If necessary, another liquid enrichment medium may be used prior to inoculation of the selective medium.