Australian Standard™

Food microbiology

Method 4: Microbiology—General guidance for the enumeration of coliforms—Colony count 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 Eschenchia coli*.

This Standard is identical with and reproduced from ISO 4832:1991, *Microbiology—General guidance for the enumeration of coliforms—Colony count 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 provide general guidelines for the enumeration of coliforms present in products intended for human consumption or feeding of animals, by means of the techniques of counting colonies on a solid medium after incubation at 30°C, 35°C or 37°C, this temperature forming the subject of agreement between the parties concerned.

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

(a) Its number appears on the cover and title page while the International Standard dumber appears only the cover.

(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 to equivalent 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
7218	Microbiology—General guidance for microbiological examination	5013.14	Method 14: Microbiology of food and animal feeding stuffs—General rules for microbiological examination

STANDARDS AUSTRALIA

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 the enumeration of coliforms.

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

0.1 This International Standard is intended to provide general guidance for the examination of products not dealt with by existing International Standards and for reference for bodies 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 for some products in every detail, 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 guidelines provided 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 deviation 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.

0.2 The technique described in this International Standard is more precise than that described in ISO 4831:1990, *Microbiology — General guidance for the enumeration of coliforms — Most probable number technique*, but does not allow a microbiological examination to be carried out on such a large test portion. It is therefore the preferred method when large numbers of coliforms are present. Moreover, since the definition of "coliforms" adopted in the two documents is different, the micro-organisms enumerated are not necessarily the same.

For any particular product, the method to be chosen will be specified in the International Standard dealing with that product.

0.3 For the purposes of a practicable test method, the definition of "coliforms" given in clause 3 and used as the basis for the procedure is not necessarily identical with corresponding definitions given in other published texts. The method described in this International Standard will, on average, detect only about 90 % of strains of the micro-organisms referred to in other publications as "(presumptive) coliforms" (e.g. certain strains of *Citrobacter*, *Enterobacter*, *Klebsiella)*. (See Edwards, P.R. and Ewing, H.W. *Identification of Enterobacteriaceae,* 3rd edition, Burgess Publishing Company, Minneapolis, Minnesota, USA, 1972.)

NOTES

1 Scope

This International Standard gives general guidelines for the enumeration of coliforms present in products intended for human consumption or feeding of animals, by means of the technique of counting colonies on a solid medium, after incubation at 30 °C, 35 °C or 37 °C, this temperature forming the subject of agreement between the parties concerned.

NOTE 1 The incubation temperature of 30 °C is used when the aim of the enumeration is technological; the temperature of 35 °C or 37 °C is used when the aim of the enumeration is more in the field of public health.

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.

coliforms: Bacteria which, at the specified temperature (i.e. 30 °C, 35 °C or 37 °C, as agreed) form characteristic colonies in crystal violet neutral red bile lactose agar under the test conditions specified in this International Standard.

4 Principle

4.1 Preparation of two poured plates, using a solid selective culture medium and using a specified quantity of the test sample if the initial product is liquid, or using a specified quantity of an initial suspension in the case of other products.

Preparation of other pairs of poured plates, under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

4.2 Incubation of the plates at 30 °C, 35 °C or 37 °C (as agreed) for 24 h.

4.3 Calculation of the number of coliforms per millilitre or per gram of sample from the number of characteristic colonies obtained in the plates chosen (see 10.1).

5 Culture medium and dilution fluid

5.1 General

For current laboratory practice, see ISO 7218.

5.2 Dilution fluid

See ISO 6887 and the specific International Standard dealing with the product under examination.