

Australian Standard[®]

Food microbiology

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

AS 5013.3—2009

PREFACE

This Standard was prepared by the Standards Australia Committee FT-024, Food Products and Constituted Subcommittee FT-024-01, Food Microbiology, to supersede AS 5013.3—2004, *Food microbiology, Method 3: Microbiology—General guidance for the enumeration of coliforms—Most probable number technique*.

This Standard is identical with and reproduced from ISO 4831:2006, *Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of coliforms—Most probable number technique*.

The objective of this Standard is to provide general guidance for the detection and the enumeration of coliforms present in products intended for human consumption or feeding of animals, by means of calculation of the most probable number (MPN) after incubation at 30°C or 37°C in a liquid medium, this temperature forming the subject of agreement between parties concerned.

Clauses 4 (Principle), 9 (Procedure) and 10 (Expression of results) of AS 5013.3—2004 have been technically revised. The main changes are as follows:

- (a) The alternative procedure of incubation at 35°C has been deleted.
- (b) Detection and enumeration of coliforms are covered (Clauses 4 and 9).
- (c) Description of the MPN and the CCT have been omitted (Clause 10) and reference is given to AS 5013.14 (ISO 7218).

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

- (i) Its number appears on the cover and title page while the international standard number appears only on the cover.
- (ii) In the source text ‘this International Standard’ should read ‘this Australian Standard’.
- (iii) A full point substitutes for a comma when referring to a decimal marker.
- (iv) Substitute ‘mL’ for ‘ml’ wherever it appears.

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

<i>Reference to International Standard</i>	<i>Australia Standard</i>
ISO	AS
6887 Microbiology of food and animal feeding stuffs—Preparation of test samples, initial suspension and decimal dilutions for microbiological examinations	5013 Food microbiology
6887-1 Part 1: General rules for the preparation of the initial suspension and decimal dilutions	5013.11.1 Method 11.1: Microbiology of food and animal feeding stuffs—Preparation of test samples initial suspension and decimal dilutions for microbiological examination—General rules for the preparation of the initial suspension and decimal dilutions
6887-2 Part 2: Specific rules for the preparation of meat and meat products	5013.11.2 Method 11.2: Microbiology of food and animal feeding stuffs—Preparation of test samples initial suspension and decimal dilutions for microbiological examination—Specific rules for the preparation of meat and meat products
6887-3 Part 3: Specific rules for the preparation of fish and fishery products	5013.11.3 Method 11.3: Microbiology of food and animal feeding stuffs—Preparation of test samples initial suspension and decimal dilutions for microbiological examination—Specific rules for the preparation of fish and fishery products
6887-4 Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products and fish and fishery products	5013.11.4 Method 11.4: Microbiology of food and animal feeding stuffs—Preparation of test sample initial suspension and decimal dilutions for microbiological examination—Specific rules for the preparation of products other than milk and milk products, meat and meat products and fish and fishery products
7218 Microbiology of food and animal feeding stuffs—General requirements and guidance for microbiological examinations	5013.14 Method 14: Microbiology of food and animal feeding stuffs—General rules for microbiological examinations
8261 Milk and milk products—General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination	5013.17 Method 17: Milk and milk products—General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination

Only international references that have been adopted as Australian Standards have been listed.

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

Because of the large variety of food and feed products, this horizontal method may not be appropriate in every detail for certain products. In this case, different methods, which are specific to these products, may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt should be made to apply this horizontal method as far as possible.

When this International Standard is next reviewed, account will be taken of all information then available regarding the extent to which this horizontal method has been followed and the reasons for deviations from this method 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 this horizontal method. 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 this horizontal method will be those necessary for well-established technical reasons.

The technique described in this International Standard is less precise than that described in ISO 4832 [1], but allows a microbiological examination to be carried out on a larger test portion, thus permitting a lower number of coliforms per gram or per millilitre of product to be detected. Moreover, since the definition of "coliforms" adopted in the two documents is different, the microorganisms 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.

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 to corresponding definitions given in other published texts. A proportion of strains of the microorganisms described in other published texts as "coliforms" (including *Escherichia coli*) fail to produce enough gas to be detectable by use of a Durham tube (i.e. "anaerogenic strains"). Therefore the method described in this International Standard will not detect all strains of the microorganisms referred to in other publications as "(presumptive) coliforms" (e.g. certain strains of *Citrobacter*, *Enterobacter*, *Klebsiella*) (see Reference [2]).

1 Scope

This International Standard gives general guidelines for the detection and the enumeration of coliforms. It is applicable to

- products intended for human consumption and for the feeding of animals, and
- environmental samples in the area of food production and food handling.

Enumeration is carried out by calculation of the most probable number (MPN) after incubation in a liquid medium at 30 °C or 37 °C.

NOTE The temperature is subject to agreement between the parties concerned. In the case of milk and milk products, the temperature of incubation is 30 °C.

This enumeration method is applicable when the number sought is expected to be in the range 1 to 100 per millilitre or per gram of test sample.

A limitation on the applicability of this International Standard is imposed by the susceptibility of the method to a large degree of variability. The information given in Clause 11 provides guidance on the applicability of the method and on the interpretation of the results.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887 (all parts), *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

ISO/TS 11133-2:2003, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on performance testing of culture media*