

Australian Standard[®]

Food microbiology

**Method 16: Microbiology of food and
animal feeding stuffs—Horizontal
method for the enumeration of
Clostridium perfringens—Colony-count
technique**



This Australian Standard® was prepared by Committee FT-024, Food Products. It was approved on behalf of the Council of Standards Australia on 15 November 2006. This Standard was published on 29 December 2006.

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- Australian Food and Grocery Council
- Australian Institute of Food Science and Technology Limited
- Australian Society for Microbiology Incorporated
- Consumers' Federation of Australia
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PREFACE

This Standard was prepared by the Standards Australia Committee FT-024, Food Products and Subcommittee FT-024-01, Food Microbiology to supersede AS 5013.16—2004, *Food microbiology, Method 16: Microbiology of food and animal feeding stuffs—Horizontal method for enumeration of Clostridium perfringens—Colony-count technique*.

This Standard is identical with, and has been reproduced from ISO 7937:2004, *Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of Clostridium perfringens—Colony-count technique*.

The objective of this Standard is to specify a horizontal method for the enumeration of viable *Clostridium perfringens*.

Clauses 1 (Scope), 3 (Definitions), 4 (Principle), 5 (Diluent culture media and reagents), 6 (Apparatus and glassware), 9 (Procedure) and 10 (Expression of results) of AS 5013.16—2004 have been technically revised. The main changes are as follows:

- (a) The scope of Standard is now applicable to environment samples in the area of food production and food handling (See Clause 1).
- (b) The definition of *C. perfringens* is now improved by describing the appearance and reactions of characteristic colonies and now two techniques are specified for confirmatory step (see Clause 3.1).
- (c) The temperature and time of the incubator is now 37°C for 20 h ±2 h (see Clause 4).
- (d) Clause 5 (Diluent, culture media and reagents) is changed by providing storage conditions for prepared media, shelf-life, performance testing for the quality assurance of SC medium and thioglycollate broth, listing Lactose sulfite medium (LS), nitrate motility medium, nitrite detection agent, zinc dust, lactose-gelatine medium as optional media and reagents (see Clause 5).
- (e) The use of equivalent sterile disposal loops and inoculating needles are covered (see Clause 6.5).
- (f) The temperature of water baths are now 44°C to 47°C (see Clause 6.10).
- (g) The use of rubber bulbs for use with the graduated pipettes when distributing the components of the nitrite detection reagent (if necessary) is now covered (see Clause 6.11).
- (h) The confirmation technique using the nitrate motility medium and the lactose-gelatine medium is now included under biochemical confirmation (see Clause 9.4.3).
- (i) The method of calculation is referenced to ISO 7218 (see Clause 10.1) and a separate subclause on precision is included, with Annex A giving the details of summarized interlaboratory test results (see Clause 10.2).

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

- (i) Its number does not appear on each page of text and its identity is shown only on the cover and the title page.
- (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>Australian 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 sample 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 sample 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 sample 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 rules 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

With the exception of the above ISO documents, which have been adopted as Australian Standards, the ISO documents listed as normative references in Clause 2 have not been adopted as Australian Standards.

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 *Clostridium perfringens*.

The term ‘informative’ has been used in this Standard to define the application of the annex to which it applies. An ‘informative’ annex is only for information and guidance.

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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 that 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.

AUSTRALIAN STANDARD

Food microbiology

Method 16:

Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of *Clostridium perfringens*—Colony-count technique

1 Scope

This International Standard describes a horizontal method for the enumeration of viable *Clostridium perfringens*. It is applicable to

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

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 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 6887-2, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

ISO 6887-3, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 6887-4, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of products other than milk and milk products meat and meat products, and fish and fishery products*

ISO 7218, *Microbiology of food and animal feeding stuffs — General rules 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*