AS 5013.4:2021

Food microbiology

Method 4: Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique (ISO 4832:2006, MOD)

AS 5013.4:2021

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Preface

This Standard was prepared by the Standards Australia Committee FT-035, Food Microbiology, to supersede AS 5013.4:2009, *Food microbiology, Method 4: Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique.*

The objective of this document is to set out general guidelines for the enumeration of coliforms. It is applicable to:

(a) products intended for human consumption and for the feeding of animals; and

(b) environmental samples in the area of food production and food handling,

by means of the technique of counting colonies after incubation on a solid medium at 30 °C or at 37 °C.

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 technique is recommended when the number of colonies sought is expected to be more than 100 per mL or per gram of the test sample.

This document is an adoption with national modifications, and has been reproduced from, ISO 4832:2006, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique*. The modifications are additional requirements and are set out in Appendix ZZ, which has been added at the end of the source text.

Appendix ZZ lists the variations to ISO 4832:2006 for the application of this document in Australia.

As this document has been reproduced from an International Standard, the following applies:

(i) In the source text “this International Standard” should read “this document”.

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

Australian or Australian/New Zealand Standards that are identical adoptions of international normative references may be used interchangeably. Refer to the online catalogue for information on specific Standards.

The terms “normative” and “informative” are used in Standards to define the application of the appendices or annexes to which they apply. A “normative” appendix or annex is an integral part of a Standard, whereas an “informative” appendix or annex is only for information and guidance

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 4832 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This third edition of ISO 4832 cancels and replaces ISO 4832:1991 and ISO 5541-1:1986. The main changes are follows:

— the alternative procedure of incubation at 35 °C has been deleted (see 4.2);

— a confirmation test in brilliant green lactose bile broth has been introduced (see 5.4 and 9.4).

Considering the nature of the changes to the previous edition of this International Standard, it is considered that the validation of alternative methods based on ISO 4832:1991 is not affected by this revision.

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 more precise than that described in ISO 4831[1], 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 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. The method described in this International Standard will, on average, detect only about 90 % of strains of the microorganisms referred to in other publications as "(presumptive) coliforms" (e.g. certain strains of *Citrobacter, Enterobacter, Klebsiella*) (see Reference [2]).

NOTES

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1 Scope

This International Standard gives general guidelines for 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,

by means of the technique of counting colonies after incubation on a solid medium at 30 °C or at 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 technique is recommended when the number of colonies sought is expected to be more than 100 per millilitre or per gram of the test sample.

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:—[1)], *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*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
coliforms
bacteria which, at the specified temperature (i.e. 30 °C or 37 °C, as agreed) form characteristic colonies in crystal violet neutral red bile lactose agar, and which in the confirmation test cause fermentation of lactose with the production of gas under the test conditions specified in this International Standard

1) To be published.