

# INTERNATIONAL STANDARD

**ISO**  
**4831**

Second edition  
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## **Microbiology — General guidance for the enumeration of coliforms — Most probable number technique**

*Microbiologie — Directives générales pour le dénombrement des  
coliformes — Technique du nombre le plus probable*



Reference number  
ISO 4831:1991(E)

## 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.

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.

International Standard ISO 4831 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*.

This second edition cancels and replaces the first edition (ISO 4831:1978), of which it constitutes a technical revision.

Annexes A and B form an integral part of this International Standard.

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International Organization for Standardization  
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## 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 less precise than that described in ISO 4832:1990, *Microbiology — General guidance for the enumeration of coliforms — Colony count technique*, 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 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. A proportion of strains of the micro-organisms 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 micro-organisms referred to in other publications as "(presumptive) coliforms" (e.g. certain strains of *Citrobacter*, *Enterobacter*, *Klebsiella*).

(See Edwards, P.R. and Ewing, W.H. *Identification of Enterobacteriaceae*, 3rd edition, Burgess Publishing Company, Minneapolis, Minnesota, USA, 1972.)

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# Microbiology — General guidance for the enumeration of coliforms — Most probable number technique

## 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 calculation of the most probable number (MPN) after incubation at 30 °C, 35 °C or 37 °C in a liquid medium, 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.

A limitation on the applicability of this International Standard is imposed by the method's susceptibility 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 6579:1990, *Microbiology — General guidance on methods for the detection of Salmonella*.

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) cause fermentation of lactose with the production of gas under the test conditions specified in this International Standard.

## 4 Principle

**4.1** Inoculation of three tubes of double-strength liquid selective enrichment medium [see 5.3 a)] with a specified quantity of the test sample if the initial product is liquid, or with a specified quantity of an initial suspension in the case of other products.

**4.2** Inoculation of three tubes of single-strength liquid selective enrichment medium [see 5.3 b)] with a specified quantity of the test sample if the initial product is liquid, or with a specified quantity of an initial suspension in the case of other products.

Then, under the same conditions, inoculation of further tubes of medium 5.3 b) with decimal dilutions of the test sample or of the initial suspension.

**4.3** Incubation at 30 °C, 35 °C or 37 °C (as agreed) of the tubes containing double-strength medium [5.3 a)] for 24 h and of the tubes containing single-strength medium [5.3 b)] for 24 h or 48 h, and examination of these tubes.

**4.4** Inoculation of a series of tubes of the confirmation medium (5.4), with the cultures from the tubes of medium 5.3 a) and with those cultures from the first series of tubes of medium 5.3 b) in which opacity or gas formation has been noted.

**4.5** Incubation at 30 °C, 35 °C or 37 °C (as agreed) for 24 h or 48 h, and examination of the new series of tubes (4.4).