

INTERNATIONAL  
STANDARD

**ISO**  
**7251**

Second edition  
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**Microbiology — General guidance for  
enumeration of presumptive *Escherichia  
coli* — Most probable number technique**

*Microbiologie — Directives générales pour le dénombrement  
d'Escherichia coli présumés — Technique du nombre le plus probable*



Reference number  
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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.

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 7251 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Sub-Committee SC 9, *Microbiology*.

This second edition cancels and replaces the first edition (ISO 7251:1984), which has been technically revised.

Annex A forms an integral part of this International Standard.

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## Introduction

This International Standard is intended to provide general guidance for the examination of products not dealt with by existing International Standards and to be taken into account by organizations 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 in every detail for certain products, 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 provided guidelines 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 deviations 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.

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# Microbiology — General guidance for enumeration of presumptive *Escherichia coli* — Most probable number technique

## 1 Scope

This International Standard gives general guidelines for the enumeration of presumptive *Escherichia coli* in products intended for human consumption or feeding of animals, by means of the liquid-medium culture technique and calculation of the most probable number (MPN) after incubation at 35 °C or 37 °C (this temperature forming the subject of agreement between the parties concerned), then incubation at 45 °C.

**CAUTION — Some *Escherichia coli* pathogenic species do not grow at 45 °C.**

A limitation of the applicability of this International Standard is imposed by the susceptibility of the method 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 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.

**3.1 presumptive *Escherichia coli*:** Bacteria which, at 45 °C, cause fermentation of lactose with the production of gas, and which, at 45 °C, produce indole from tryptophan, when the test is carried out in accordance with the method specified in this International Standard.

## 4 Principle

**4.1** Inoculation of three tubes of double-strength liquid selective enrichment medium [5.3.1 a)]<sup>1)</sup> with a specified quantity of the test sample if the initial product is liquid, or with a specified quantity of the initial suspension in the case of other products.

**4.2** Inoculation of three tubes of single-strength liquid enrichment medium [5.3.1 b)]<sup>1)</sup> with a specified quantity of test sample if the initial product is liquid, or with a specified quantity of the initial suspension in the case of other products.

Then, under the same conditions, inoculation of the medium [5.3.1 b)] with decimal dilutions of the test sample or of the initial suspension.

**4.3** Incubation of the tubes of double- and single-strength medium at 35 °C or 37 °C (as agreed) for 24 h to 48 h. Examination of the tubes for gas formation.

**4.4** Inoculation, from the tubes of double- and single-strength medium which have given rise to gas formation, of a new series of tubes containing a liquid selective medium.

1) If necessary, another liquid enrichment medium may be used prior to inoculation of the selective medium.