

Methods for

Microbiological examination of food and animal feeding stuffs —

Part 8: Enumeration of presumptive
Escherichia coli. Most probable number
technique

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Committees responsible for this British Standard

The preparation of this British Standard was entrusted by the Agriculture and Food Standards Policy Committee (AFC/-) to Technical Committee AFC/9, upon which the following bodies were represented:

- Association of Public Analysts
- BLWA Ltd. (Association of the Laboratory Supply Industry)
- British Food Manufacturing Industries Research Association
- Campden Food and Drink Research Association
- Department of Agriculture Northern Ireland
- Department of Health
- Department of Trade and Industry (Laboratory of the Government Chemist)
- Flour Milling and Baking Research Association
- Food and Drink Federation
- Fruit and Vegetable Canners' Association
- Institute of Brewing
- Joint Committee of the Milk Marketing Board and the Dairy Trade Federation
- Ministry of Agriculture, Fisheries and Food
- Natural Resources Institute
- Public Health Laboratory Service
- Society for Applied Bacteriology
- United Kingdom Dairy Association
- University of Bristol
- University of Reading

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National foreword

This British Standard has been prepared under the direction of the Agriculture and Food Standards Policy Committee. It is identical with ISO 7251:1993 *Microbiology — General guidance for the enumeration of presumptive Escherichia coli — Most probable number technique*, published by the International Organization for Standardization (ISO), and in the preparation of which the United Kingdom played a full part. This Part of BS 5763 supersedes BS 5763-8:1985 which is withdrawn. The revised standard differs from the previous edition in that the technical requirements have been updated in line with current microbiological practice.

Cross-references

International standard	Corresponding British Standard
ISO 6887:1983	BS 5763 <i>Methods for microbiological examination of food and animal feeding stuffs</i> Part 6:1983 <i>Preparation of dilutions</i> (Identical)
ISO 7218:1985	Part 0:1986 <i>General laboratory practices</i> (Identical)

Textual error. When adopting the text of the international standard the textual error given below was discovered. It has been marked in the text and has been reported to ISO in a proposal to amend the text of the international standard.

In 6.5, line 3, “sterile disposable bags” should read “sterile disposable loops”.

A British Standard does not purport to include all the necessary provisions of a contract. Users of British Standards are responsible for their correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, pages 1 to 8, an inside back cover and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

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.

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)]¹⁾ 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)]¹⁾ 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.