

Methods for

Microbiological examination of food and animal feeding stuffs —

Part 16: Detection of presumptive
pathogenic *Yersinia enterocolitica*

Committees responsible for this British Standard

The preparation of this British Standard was entrusted to Technical Committee AW/9, Microbiology, upon which the following bodies were represented:

- AFRC Institute of Food Research
- Association of Public Analysts
- BLWA Ltd. (the Association of the Laboratory Supply Industry)
- British Food Manufacturing Industries Research Association
- Campden and Chorleywood Food Research Association
- Dairy Industry Federation
- Department of Agriculture Northern Ireland
- Department of Health
- Department of Trade and Industry (Laboratory of the Government Chemist)
- Food and Drink Federation
- Institute of Brewing
- Meat and Livestock Commission
- Milk Marketing Board for Northern Ireland
- Ministry of Agriculture, Fisheries and Food
- Natural Resources Institute
- Preserved Milk Association
- Public Health Laboratory Service
- Scottish Milk Marketing Board
- Society for Applied Bacteriology
- University of Bristol
- University of Reading

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National foreword

This Part of BS 5763 has been prepared by Technical Committee AW/9. It is identical with ISO 10273:1994, *Microbiology — General guidance for the detection of presumptive pathogenic Yersinia enterocolitica*, published by the International Organization for Standardization (ISO) and in the preparation of which the United Kingdom played a full part.

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)

It is envisaged that the revision of ISO 7218:1985 will be implemented as a dual-numbered revision of BS 5763 *Methods for microbiological examination of food and animal feeding stuffs Part 0. General laboratory practices*. Until the revision has been published, however, it is suggested that reference be made to BS 5763-0:1986.

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 14, 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 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.

1 Scope

This International Standard gives general guidance on the detection of *Yersinia enterocolitica* presumed to be pathogenic to man.

This International Standard is applicable to products for human or animal consumption.

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:—¹⁾, *Microbiology — General rules for microbiological examinations*.

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1 presumptive pathogenic *Yersinia enterocolitica*

psychrotrophic bacteria forming characteristic colonies on solid selective media and having the biochemical properties meeting the pathogenicity criteria described when the test is carried out in accordance with this International Standard

3.2 detection of presumptive pathogenic *Yersinia enterocolitica*

determination of the presence or absence of these bacteria in a predetermined quantity of product, when the test is carried out in accordance with this International Standard

4 Principle

Detection of presumptive pathogenic *Yersinia enterocolitica* by the following three successive stages.

4.1 Enrichment in selective liquid media

Inoculation of the test portion in two enrichment media:

- peptone, sorbitol and bile salts (PSB) broth, and
- irgasan, ticarcillin and potassium chlorate (ITC) broth.

Incubation at between 22 °C and 25 °C for 48 h for the ITC broth and for 3 to 5 days for the PSB broth.

4.2 Plating out and identification

Using the cultures obtained in 4.1, surface plating of two solid selective culture media:

- agar with cefsulodin, irgasan and novobiocin (CIN), and
- *Salmonella/Shigella* agar, with sodium desoxycholate and calcium chloride (SSDC).

Incubation at 30 °C, followed by examination after 24 h and, if necessary, after 48 h depending on the medium, to check if any characteristic colonies of *Yersinia enterocolitica* are present.

¹⁾ To be published. (Revision of ISO 7218:1985)

4.3 Confirmation

On plated-out colonies, testing for presumptive *Yersinia enterocolitica* followed by biochemical confirmation tests, tests of the appropriate pathogenicity criteria, and possibly serological tests.

5 Culture media and reagents

5.1 General

For current laboratory practice, see ISO 7218.

In view of the large number of culture media and reagents, for the clarity of the text, their compositions are given in Annex B, which also includes details of dispensing, storage, etc.

5.2 Enrichment media

5.2.1 *Peptone, sorbitol and bile salts (PSB) broth*

See B.1.

5.2.2 *Irgasan, ticarcillin and potassium chlorate (ITC) broth*

See B.2.

5.3 Plating-out media

5.3.1 *Cefsulodin, irgasan and novobiocin (CIN) agar*

See B.3.

5.3.2 *Salmonella/Shigella agar, with sodium desoxycholate and calcium chloride (SSDC)*

See B.4.

5.3.3 *Nutrient agar*

See B.5.

5.4 Identification media and reagents

5.4.1 *Urea tryptophan medium*

See B.6.

5.4.2 *Reagent for detection of tryptophan deaminase*

See B.7.

5.4.3 *Kligler agar*

See B.8.

5.4.4 *Reagent for detection of oxidase*

See B.9.

5.4.5 *Decarboxylation media*

5.4.5.1 *Lysine decarboxylation medium*

See B.10.

5.4.5.2 *Ornithine decarboxylation medium*

See B.11.

5.4.6 *Media for fermentation of carbohydrates (sucrose, rhamnose or salicin)*

See B.12.

5.4.7 *Simmons citrate medium*

See B.13.

5.4.8 *Bile and aesculin agar*

See B.14.

5.4.9 *Casein-soya agar*

See B.15.

5.4.10 *Casein-soya agar for detection of pyrazinamidase*

See B.16.

5.4.11 *Ammonium iron (II) sulfate solution for detection of pyrazinamidase*

See B.17.

5.4.12 *Casein-soya agar with magnesium and oxalate*

See B.18.

5.5 Saline solution

See B.19.

5.6 Potassium hydroxide in saline solution

See B.20.

6 Apparatus and glassware

NOTE 1 Disposable apparatus is an acceptable alternative to reusable glassware, if it has suitable specifications.

Usual microbiology laboratory equipment and, in particular, the following.

6.1 *Apparatus for dry sterilization (oven) or wet sterilization (autoclave)*

See ISO 7218.

6.2 *Incubator*, capable of operating at $30\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ or at $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$.

6.3 *Drying cabinet or oven*, with ventilation by convection, capable of operating between $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ and $55\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$.

6.4 *Water baths or incubators*, capable of operating between $22\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ and $25\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$, possibly with a suitable agitation device.

6.5 *Water bath*, capable of operating between $45\text{ }^{\circ}\text{C} \pm 0,5\text{ }^{\circ}\text{C}$ and $50\text{ }^{\circ}\text{C} \pm 0,5\text{ }^{\circ}\text{C}$.

6.6 *Test tubes*, of dimensions $18\text{ mm} \times 180\text{ mm}$, $9\text{ mm} \times 180\text{ mm}$ and $12\text{ mm} \times 50\text{ mm}$.

6.7 *Bottles and/or flasks*, of suitable capacity.

6.8 *Petri dishes*, made of glass or plastics, of diameter 90 mm to 100 mm.