

Methods for

Microbiological examination of food and animal feeding stuffs —

Part 17: Detection of thermotolerant
Campylobacter

ICS 07.100; 65.120; 67.040

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 and is identical with ISO 10272:1995 *Microbiology of food and animal feeding stuffs — Horizontal method for detection of thermotolerant Campylobacter* (including its Technical Corrigenda 1:1996 and 2:1997), published by the International Organization for Standardization (ISO) and in the preparation of which the United Kingdom played a full part.

The revision of ISO 7218 mentioned in clause 2 of this standard has now been published as ISO 7218:1996.

Cross-references

International standard	Corresponding British Standard
	BS 5763 <i>Methods for microbiological examination of food and animal feeding stuffs</i>
ISO 6887:1983	Part 6:1983 <i>Preparation of dilutions</i> (Identical)
ISO 7218:1996	Part 0:1996 <i>General laboratory practices</i> (Identical)

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Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, pages 1 to 12, 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

Because of the large variety of food and feed products, this horizontal method 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 this horizontal method as far as possible and that deviations from this 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 this horizontal method has been followed and the reasons for deviations from this in the case of particular products.

The harmonization of test methods cannot be immediate and, for certain group 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.

1 Scope

This International Standard describes a horizontal method for the detection of thermotolerant *Campylobacter*.

It is applicable to products intended for human consumption or for the feeding of animals, subject to the limitations stated in the introduction.

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: — *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*¹⁾.

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

3 Definitions

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

3.1

thermotolerant *Campylobacter*

microorganisms forming characteristic colonies on solid selective media when incubated at 42 °C and which possess the characteristic motility and biochemical properties described when the test is conducted in accordance with this International Standard

3.2

detection of thermotolerant *Campylobacter*

determination of the presence or absence of these microorganisms in a defined quantity of product, when the test is conducted in accordance with this International Standard

4 Principle

In general, the detection of thermotolerant *Campylobacter* requires the following stages (see Annex A for a diagram of the procedure):

4.1 Enrichment in the liquid medium

Inoculation of the test portion in one of two liquid enrichment media:

- Preston broth, or
- Park and Sanders broth when the sample or the product it comes from has received some physical treatment, for example freezing, which could entail bacterial stress.

Incubation in a microaerophilic atmosphere (5 % oxygen and, for example, 10 % carbon dioxide and 85 % nitrogen):

- at 42 °C for 18 h for the Preston broth;
- at 32 °C for 4 h, then at 37 °C for 2 h and, finally, at 42 °C for 40 h to 42 h for the Park and Sanders broth.

NOTE 1 Since *Campylobacter* spp. are very sensitive, it is recommended that they should not be frozen, that they should be stored at + 3 °C ± 2 °C, and subjected to analysis as rapidly as possible.

4.2 Plating out and identification

From the cultures obtained in 4.1, inoculation of solid selective media:

- Karmali agar and another solid selective medium [modified Butzler agar, Skirrow agar, charcoal cefoperazone deoxycholate agar (CCDA) or Preston agar].

Incubation at 42 °C in a microaerophilic atmosphere (5 % oxygen and, for example, 10 % carbon dioxide and 85 % nitrogen) and inspection after 48 h, 72 h and, if necessary, 5 days, to detect the presence of colonies presumed to be thermotolerant *Campylobacter*.

4.3 Confirmation

Subculturing of the colonies presumed to be thermotolerant *Campylobacter* and which were plated out in 4.2, then confirmation by means of appropriate biochemical tests.

5 Culture media and reagents

5.1 General

For current laboratory practice, see ISO 7218.

NOTE 2 Because of the large number of culture media and reagents, it is considered preferable for the clarity of the text to give their compositions and preparations in Annex B.

5.2 Liquid enrichment media

5.2.1 Preston broth

See B.1.

5.2.2 Park and Sanders broth

See B.2.

5.3 Selective plating-out media

5.3.1 Karmali agar

See B.3.

5.3.2 Modified Butzler agar

See B.4.

5.3.3 Skirrow agar

See B.5.

5.3.4 Charcoal cefoperazone deoxycholate agar (CCDA)

See B.6.

5.3.5 Preston agar

See B.7.

5.4 Identification media and reagents

5.4.1 Brucella broth

See B.8.

5.4.2 Columbia blood agar

See B.9.

5.4.3 Reagent for the detection of oxidase

See B.10.

5.4.4 Triple sugar/iron(III) agar (TSI agar)

See B.11.

5.4.5 Hydrogen peroxide solution, 3 %

5.4.6 Reagents for the detection of the hydrolysis of hippurate

See B.12.

5.4.7 Mueller Hinton blood agar

See B.13.

6 Apparatus and glassware

NOTE 3 Disposable apparatus is an acceptable alternative to re-useable glassware, if it has similar specifications.

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

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

See ISO 7218.

6.2 Drying cabinet or oven, ventilated by convection, capable of operating between 37 °C ± 1 °C and 55 °C ± 1 °C.

6.3 Incubator, capable of operating at 42 °C ± 1 °C.

6.4 Water baths, capable of operating at 25 °C ± 1 °C, 32 °C ± 1 °C, 37 °C ± 1 °C and 42 °C ± 1 °C, or *incubators* capable of operating at 25 °C ± 1 °C, 32 °C ± 1 °C and 37 °C ± 1 °C.

6.5 Water bath, capable of operating at 47 °C ± 2 °C.

6.6 pH-meter, capable of being read to the nearest 0,01 pH unit at 25 °C.

6.7 Containers, in particular *culture tubes* of dimensions 18 mm × 180 mm and 9 mm × 180 mm, *haemolysis tubes* of dimensions 13 mm × 75 mm, *bottles* with non-toxic metal closures and/or *flasks* of appropriate capacity with appropriate covers or plugged with cotton wool to allow gases to pass so that a microaerophilic atmosphere can be achieved when needed.

6.8 Petri dishes, in glass or plastic, with a diameter between 90 mm and 100 mm.

6.9 Total-delivery graduated pipettes, with a wide opening, and a nominal capacity of 1 ml and 10 ml, graduated respectively in 0,1 ml and 0,5 ml divisions, and *Pasteur pipettes*.

6.10 Rubber teats, or any other safety system capable of being adapted to the graduated pipettes.

6.11 Loops, of platinum/iridium or nickel/chromium, approximately 3 mm in diameter, and *wires* of the same material, or a *glass rod*.

NOTE 4 A nickel/chromium loop is not suitable for use in the oxidase test (see 9.5.5.1).

6.12 Forceps, fine, round-ended, of stainless steel.

6.13 Microscope, if possible with phase contrast (for observing the characteristic movement of *Campylobacter*).