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Microbiology of food and animal feeding stuffs — Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica*

Microbiologie des aliments — Méthode horizontale pour la recherche de Yersinia enterocolitica *présumées pathogènes*

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 10273 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

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This second edition cancels and replaces the first edition (ISO 10273:1994), Subclause 9.4 of which has been technically revised.

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Introduction

Because of the large variety of food and feed products, this horizontal method may not be appropriate in every detail for certain products. In this case, different methods which are specific to these products may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt will be made to apply this horizontal method as far as possible.

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 method 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 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.

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Microbiology of food and animal feeding stuffs — Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica*

WARNING — The use of this standard may involve hazardous materials, operations and equipment. It is the responsibility of the user of this standard to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior the use.

1 Scope

This International Standard specifies a horizontal method for the detection of *Yersinia enterocolitica* presumed to be pathogenic to human subjects. This International Standard is applicable to

— products intended for human consumption and the feeding of animals, and

— environmental samples in the area of food production and food handling.

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2 Normative references

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The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887 (all parts), *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

ISO 7218, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*, and Amd.1:2001

ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

3 Terms and definitions

For the purposes of this document, the following terms and 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

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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

4.1 General

Presumptive pathogenic *Yersinia enterocolitica* are detected by the following three successive stages.

4.2 Enrichment in selective liquid media

The test portion is inoculated into two enrichment media

— peptone, sorbitol and bile salts (PSB) broth, and

— irgasan™, ticarcillin and potassium chlorate (ITC) broth.

The ITC broth is incubated at 25 °C for 48 h and the PSB broth for 3 to 5 days.

NOTE Enrichment in ITC broth has been proposed (see reference [1]) for the isolation of *Yersinia enterocolitica* biovar 4/serovar O:3 but not for biovar 1B serovar O:8, biovar 2/serovar O:9 (see reference [2]), or biovar 2 serovar O:5,27. Isolation of *Yersinia enterocolitica* biovar 2/serovar O:9 needs the use of an ITC medium without chlorate and which contains 80 % of the original concentration of magnesium chloride and malachite green (see reference [3]).

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4.3 Plating out and identification

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Using the cultures obtained in 4.2, surface plating of the following two solid selective culture media is carried out:

— agar with cefsulodin, irgasan™ and novobiocin (CIN) (see reference [7]);

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

The media are incubated at 30 °C, then examined after 24 h and, if necessary, after 48 h depending on the medium, to check if any characteristic colonies of *Yersinia enterocolitica* are present.

4.4 Confirmation

On plated-out colonies, tests for presumptive *Yersinia enterocolitica* are carried out, followed by biochemical confirmation tests, biotyping tests, tests to establish pathogenic criteria, and possibly serological tests.

5 Reagents and media

For current laboratory practice, see ISO 7218.

See ISO/TS 11133-1 for specific requirements about quality assurance and performance of media.

NOTE ISO/TS 11133-2 on practical guidelines on performance testing of culture media is under preparation.

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

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5.1 Enrichment media

5.1.1 Peptone, sorbitol and bile salts (PSB) broth

See B.1.

5.1.2 Irgasan™, ticarcillin and potassium chlorate (ITC) broth

See B.2.

5.2 Plating out media

5.2.1 Cefsulodin, Irgasan™ and novobiocin (CIN) agar (see reference [7])

See B.3.

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

See B.4.

5.2.3 Nutrient agar

See B.5.

5.3 Identification media and reagents

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5.3.1 Urea indole medium

See B.6.

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5.3.2 Reagent for indole detection

See B.7.

5.3.3 Kligler's agar

See B.8.

5.3.4 Reagent for detection of oxidase

See B.9.

5.3.5 Decarboxylation media

5.3.5.1 Lysine decarboxylation medium

See B.10.

5.3.5.2 Ornithine decarboxylation medium

See B.11.

5.3.6 Media for fermentation of carbohydrates (sucrose, rhamnose, trehalose and xylose)

See B.12.

5.3.7 Simmons' citrate medium

See B.13.

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5.3.8 Tween™-esterase medium

See B.14.

5.3.9 Bile and aesculin agar

See B.15.

5.3.10 Casein soya agar

See B.16.

5.3.11 Casein soya agar, for detection of pyrazinamidase.

See B.17.

5.3.12 Ammonium iron(II) sulfate solution, for detection of pyrazinamidase.

See B.18.

5.3.13 Casein-soya agar, with magnesium and oxalate.

See B.19.

5.4 Saline solution

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See B.20.

5.5 Potassium hydroxide in saline solution

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See B.21.

5.6 Veal infusion broth

See B.22.

5.7 Sterile glycerol

See B.23.

6 Apparatus and glassware

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 Incubators, capable of operating at 22 °C ± 1 °C, 25 °C ± 1 °C, 30 °C ± 1 °C and 37 °C ± 1 °C.

6.3 Drying cabinet or **oven**, with ventilation by convection, capable of operating between 37 °C ± 1 °C and 50 °C ± 1 °C.

6.4 Water baths or **incubators**, capable of operating between 22 °C ± 1 °C, 24 °C ± 2 °C and 25 °C ± 1 °C, preferably with a suitable agitation device.

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6.5 **Water bath**, capable of operating at 44 °C to 47 °C.

6.6 **Test tubes**, of dimensions 18 mm × 180 mm, 9 mm × 180 mm, and 12 mm × 50 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.

6.9 **Total-delivery pipettes**, of nominal capacities 10 ml and 1 ml, with large opening and 0,1 ml graduations.

6.10 **Rubber teats**, or other microbiologically safe pipetting systems.

6.11 **Loop**, of approximately 3 mm diameter, **straight wires** of platinum/iridium and/or nickel/chromium, **glass rods** and **Pasteur pipettes**.

Sterile plastic disposable loops or needles may be used. Nickel chromium is not suitable for the oxidase test (see 9.4.3.5).

6.12 **pH-meter**, accurate to within ± 0,1 pH units at 25 °C.

6.13 **Lighting**, appropriate for oblique illumination.

6.14 **Magnifying glass** or **stereomicroscope**.

6.15 **Peristaltic blender**.

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7 Sampling

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It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Freezing of samples before analysis is not recommended, despite *Yersinia* spp. being recovered from frozen products.

Sampling is not part of the method specified in this International Standard. If there is no specific International Standard dealing with sampling of the product concerned, it is recommended that the parties concerned come to an agreement on this subject.

8 Preparation of test sample

Prepare the test sample in accordance with the specific International Standard appropriate to the product concerned. If there is no specific International Standard available, it is recommended that the parties concerned come to an agreement on this subject.

9 Procedure (see Annex A)

9.1 Test portion and initial suspension

9.1.1 See the relevant part of ISO 6887, or ISO 8261, or any specific International Standard appropriate to the product concerned.

9.1.2 In general, for preparing the initial suspension, place a quantity (x) of the test portion (of known mass or volume) in a known volume of the PSB broth (B.1), to give a 1/10 dilution (by mass/volume or volume/volume). Homogenize the suspension using a peristaltic blender (6.15) for 2 min.

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9.1.3 Prepare the second initial suspension in the same way with the ITC broth (B.2), so as to obtain a test portion/enrichment medium dilution of 1/100 (mass/volume or volume/volume).

9.2 Enrichment

Incubate the two initial suspensions (9.1.2 and 9.1.3) as follows:

a) PSB medium at 22 °C to 25 °C for 48 h to 72 h with agitation, or for 5 days without agitation;

b) ITC medium at 25 °C for 48 h.

9.3 Plating out and identification

9.3.1 After incubation of the enrichment media (9.2), proceed as follows.

9.3.2 Using the PSB culture (9.2), inoculate, by means of a loop (6.11), the surface of a CIN agar plate (B.3) to obtain well-separated colonies.

9.3.3 Using a sterile pipette (6.9), transfer 0,5 ml of the PSB culture (9.2) into 4,5 ml of potassium hydroxide solution (B.21) and mix (see reference [5]). After 20 s ± 5 s, immediately inoculate, by means of a loop (6.11), the surface of a CIN agar plate (B.3) to obtain well-separated colonies.

9.3.4 Using the ITC culture (9.2), inoculate, by means of a loop (6.11), the surface of an SSDC agar plate (B.4) to obtain well-separated colonies.

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9.3.5 Invert the dishes (9.3.2 to 9.3.4) and place them in the incubator (6.2) set at 30 °C.

9.3.6 After incubation for 24 h, examine the dishes with a magnifying glass (6.14) preferably equipped with an obliquely transmitted light (6.13) in order to detect the presence of characteristic colonies of *Yersinia enterocolitica* as follows.

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a) On CIN agar, characteristic colonies of *Yersinia enterocolitica* are small (⩽ 1 mm) and smooth with a red centre and translucent rim and, when examined with obliquely transmitted light (6.13), are non-iridescent and finely granular.

b) On SSDC agar, characteristic colonies of *Yersinia enterocolitica* are small (⩽ 1 mm) and grey with an indistinct rim, non-iridescent and very finely granular when examined with obliquely transmitted light.

NOTE Obliquely transmitted light helps to distinguish characteristic colonies of *Yersinia enterocolitica* from very similar colonies of *Pseudomonas.*

9.3.7 If the development of colonies is slow, if coloration is weak, or if there are no characteristic colonies, continue incubation of the plates for up to 48 h, then re-examine them.

9.4 Confirmation

9.4.1 General

Miniaturized biochemical identification kits, currently available commercially and permitting the identification of *Yersinia enterocolitica*, may be used. Some miniaturized biochemical identification kits do not identify with accuracy *Yersinia* species such as *Yersinia mollaretii* and *Yersinia bercovieri* (previous biovars of *Yersinia enterocolitica* 3A and 3B) and *Yersinia intermedia* which are identified as *Yersinia enterocolitica*. In this last case, the Mucate test shall be performed to discriminate between these species. An improvement of the discriminatory powers of these miniaturized biochemical identification kits has been proposed (see references [6] and [7]).

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