INTERNATIONAL STANDARD

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Microbiology — General guidance for the detection of presumptive pathogenic *Yersinia enterocolitica*

Microbiologie — Directives générales pour la recherche des Yersinia enterocolitica *présumées pathogènes*

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Contents

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Annexes

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

Annexes A and B form an integral part of this International Standard.

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.

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Microbiology — General guidance for the detection of presumptive pathogenic *Yersinia enterocolitica*

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).

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

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.

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 °C ± 1 °C or at 37 °C ± 1 °C.

6.3 Drying cabinet or **oven**, with ventilation by convection, capable of operating between 37 °C ± 1 °C and 55 °C ± 1 °C.

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

6.5 Water bath, capable of operating between 45 °C ± 0,5 °C and 50 °C ± 0,5 °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 approximate diameter 3 mm, a **straight wire** of platinum/iridium or nickel/chromium, and/or a **glass rod** (Pasteur pipettes).

NOTES

2 Sterile plastic disposable loops or needles may be used.

3 Nickel/chromium is not suitable for the oxidase test (see 9.4.2.4).

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 **microscope**

6.15 Peristaltic blender (stomacher)

7 Sampling

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

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 the diagram in annex A.

9.1 Test portion and initial suspensions

9.1.1 See ISO 6887 and 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 (5.2.1), so as to obtain a test portion/enrichment medium dilution of 1/10 (ratio by mass/volume or volume/volume). Homogenize the suspension using a peristaltic blender (6.15) for 120 s.

9.1.3 Prepare the second initial suspension in the same way with the ITC broth (5.2.2), so as to obtain a test portion/enrichment medium dilution of 1/100 (mass/volume or volume/volume ratio).

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

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

9.3.1 Using the PSB culture [9.2 a)], inoculate, by means of a loop (6.11), the surface of a CIN agar plate (5.3.1) and spread so as to obtain well-separated colonies.

9.3.2 Using a sterile pipette (6.9), transfer 0,5 ml of the PSB culture [9.2 a)] into 4,5 ml of potassium hydroxide solution (5.6) and mix. After 20 s, inoculate, by means of a loop (6.11), the surface of a CIN agar plate (5.3.1) to obtain well-separated colonies.

9.3.3 Using the ITC culture [9.2 b)], inoculate, by means of a loop (6.11), the surface of an SSDC agar plate (5.3.2) to obtain well-separated colonies.

9.3.4 Invert the dishes (9.3.1 to 9.3.3) and place them in the incubator (6.2) set at 30 °C.

9.3.5 After incubation for 24 h, examine the dishes with a magnifying glass (6.14) or oblique transillumination in order to detect the presence of characteristic colonies of *Yersinia enterocolitica* as follows.

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 oblique transillumination (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 oblique transillumination.

9.3.6 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.

9.4 Confirmation

NOTE 4 Miniaturized biochemical identification kits, currently available commercially and permitting the identification of *Yersinia enterocolitica*, may be used.

9.4.1 Selection of colonies for confirmation

For confirmation, take from each dish of each selective medium (see 9.3.1 to 9.3.3), five colonies considered to be typical or suspect.

If on one dish there are fewer than five typical or suspect colonies, take for confirmation all the typical or suspect colonies.

Streak the selected colonies onto the surface of nutrient agar plates (5.3.3), in a manner which will allow well-isolated colonies to develop.

Incubate the inoculated plates at 30 °C for 24 h.

Store the nutrient agars at between 0 °C and + 5 °C for the supplementary biochemical confirmation and pathogenicity tests.

Use pure cultures for the biochemical confirmations and pathogenicity tests.

9.4.2 Presumptive tests

By means of a wire or glass rod (6.11), inoculate the media specified in 9.4.2.1 to 9.4.2.3 and perform the detection of oxidase as described in 9.4.2.4 with each of the cultures obtained from the colonies selected in 9.4.1.

9.4.2.1 Detection of urease

Inoculate just below the surface of the liquid medium (5.4.1).

Incubate at 30 °C for 24 h.

A pink-violet colour indicates a positive urease reaction.

An orange-yellow colour indicates a negative urease reaction.

9.4.2.2 Detection of tryptophan deaminase

Add three drops of reagent (5.4.2) to the culture obtained in 9.4.2.1 for the detection of tryptophan deaminase.

A brown colour indicates a positive reaction.

9.4.2.3 Kligler agar (5.4.3)

Streak the slant surface (B.8.2) of the agar and stab the butt to the bottom of the agar.

Incubate at 30 °C for 24 h to 48 h.

Interpret the changes in the medium as follows.

Butt

yellow:	glucose positive (fermentation of glucose)
red or unchanged:	glucose negative (no fermentation of glucose)
black:	formation of hydrogen sulfide
bubbles or cracks:	gas formation from glucose

Slant surface

yellow:	lactose positive (utilization of lactose)
red or unchanged:	lactose negative (no utilization of lactose)

9.4.2.4 Detection of oxidase

Using the glass rod (6.11), take a portion of each characteristic colony chosen (9.4.1) and streak it on a filter paper moistened with the oxidase reagent (5.4.4) or on a commercially available disc. Do not use a nickel/chromium loop or wire (see 6.11, note 3).

Consider the test to be negative when the colour of the filter paper has not changed to mauve, violet or deep blue within 10 s.

9.4.3 Biochemical confirmation tests

9.4.3.1 Selection of colonies and procedure

9.4.3.1.1 Continue the identification of colonies having the following characteristics:

— detection of urease: positive,

— detection of tryptophan deaminase: negative,

— fermentation of glucose: positive,

— no formation of gas from the glucose,

— fermentation of lactose: negative,

— no formation of H_2S,

— detection of oxidase: negative.

NOTES

5 Some strains of *Yersinia enterocolitica* that are lactose-positive have been isolated, particularly from dairy products. In the current state of knowledge, they are generally non-pathogenic.

6 Urease negative strains have been reported but none are known to be pathogenic.

7 For the formation of gas from glucose, a few bubbles may be produced. Although *Yersinia* is usually considered to ferment carbohydrates without gas production, some strains of *Yersinia enterocolitica* (such as *Y. enterocolitica* biovar 3) may produce one or two bubbles (weak gas production).

9.4.3.1.2 Using a loop, wire or glass rod (6.11), inoculate the media specified in 9.4.3.2 to 9.4.3.5 with each of the cultures obtained from the colonies isolated (9.4.1) on nutrient agar and selected in 9.4.3.1.1.

9.4.3.2 Lysine decarboxylation medium (5.4.5.1)

Inoculate just below the surface of the liquid medium.

Incubate at 30 °C for 24 h.

A violet colour after incubation indicates a positive reaction.

A yellow colour indicates a negative reaction.

9.4.3.3 Ornithine decarboxylation medium (5.4.5.2)

Inoculate just below the surface of the liquid medium.

Incubate at 30 °C for 24 h.

A violet colour after incubation indicates a positive reaction.

A yellow colour indicates a negative reaction.

9.4.3.4 Media for the fermentation of sucrose and rhamnose (5.4.6)

Inoculate each medium just below the surface of the liquid.

Incubate at 30 °C for 24 h.

A yellow colour after incubation indicates a positive reaction.

A red colour indicates a negative reaction.