ETHIOPIAN STANDARDS

ES ISO 10273:2006

First edition
2006-03-18

Microbiology- General guidance for the detection of presumptive pathogenic Yersinia enterocolitica

(Identical with ISO 10273:1994)

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Descriptors: bacteriology,foodproducts,animal feed, microbiological analysis, detection, bacteria enterobacteriaceae,general conditions

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Reference number
ES ISO 10273:2006

National foreword

This Ethiopian Standard has been prepared under the direction of the Agriculture and Food Technology Technical Committee and published by the Quality and Standards Authority of Ethiopia (QSAE).

It is identical with ISO 10273 First edition, 1994 " Microbiology- General guidance for the detection of presumptive pathogenic Yersinia enterocolitica", published by the International Organization for Standardization (ISO).

For the purpose of this Ethiopian Standard, the adopted ISO text shall be modified as follows:

a) the word "International Standard" shall be read as " Ethiopian Standard ".

b) a full point (.) shall substitute a comma (,) as a decimal marker.

c) reference to the ISO standard shall be read as reference to the following Ethiopian Standard

International Standard	**corresponding Ethiopian Standard**
ISO 6887:1983,Microbiology- General guidance for the preparation of dilutions for microbiological examination	ES ISO 6887:2001,Microbiology- General guidance for the preparation of dilutions for microbiological examination
ISO 7218 Microbiology - General rules for microbiological examinations	ES ISO 7218 :2001,Microbiology - General rules for microbiological examinations
ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*	ES ISO 8261:2001, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

In addition, the responsible technical committee has reviewed the provisions of the following international standard to which normative reference is made in the text, and has decided that it is acceptable for use in conjunction with this Ethiopian Standard.

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*

INTERNATIONAL STANDARD

ISO 10273

Second edition
2003-06-15

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*

Reference number
ISO 10273:2003(E)

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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*.

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

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.

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.

2 Normative references

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

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]).

4.3 Plating out and identification

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.

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

5.3.1 Urea indole medium

See B.6.

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.

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

See B.20.

5.5 Potassium hydroxide in saline solution

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.

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.

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.

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.

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 $\pm$ 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.

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.

a) On CIN agar, characteristic colonies of *Yersinia enterocolitica* are small ($\leqslant$ 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 ($\leqslant$ 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]).

9.4.2 Selection of colonies for confirmation

9.4.2.1 For confirmation, take from each dish of each selective medium (see 9.3.2 to 9.3.4), five colonies considered to be characteristic or suspect.

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

Streak the selected colonies onto the surface of nutrient agar plates (B.5), in a manner which will allow well-isolated colonies to develop.

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

Examine the incubated plates for purity of culture. If mixed cultures are present, subculture each individual colony type onto further nutrient agar plates and incubate as above.

Use pure cultures for the biochemical confirmations and pathogenicity tests.

9.4.2.2 Plasmids that determine traits related to the pathogenicity of *Yersinia* can be spontaneously lost during culture above 30 °C or with lengthy culture and passage below 30 °C in the laboratory.

Therefore preserve these as a frozen culture by immediately subculturing each pure culture into veal infusion broth (B.22).

Incubate at 22 °C to 25 °C for 24 h to 48 h.

Add 10 % sterile glycerol (B.23), mix well and freeze, preferably at −70 °C.

9.4.3 Presumptive tests

9.4.3.1 General

By means of a wire (6.10), inoculate the media specified in 9.4.3.2 to 9.4.3.4 and perform the detection of oxidase as described in 9.4.3.5 with each of the cultures obtained from the colonies selected in 9.4.2.

9.4.3.2 Detection of urease

Use a heavy inoculum to inoculate the broth just below the surface of the urease/indole medium (B.6).

Incubate at 30 °C for 24 h, preferably in a water bath.

Pink-violet or red-pink colours indicate a positive urease reaction. *Yersinia enterocolitica* mostly gives positive urease reaction within 1 min to 5 min. Recording of the speed of a positive reaction can be of diagnostic value.

An orange-yellow colour indicates a negative urease reaction. Possible false negative reactions can occur if the medium is not inoculated with sufficient microorganisms.

9.4.3.3 Detection of indole

Add 0,1 ml to 0,2 ml of the reagent (B.7) to the tubes (9.4.3.2) for the detection of indole.

A red ring at the surface of the culture indicates a positive reaction in 15 min.

9.4.3.4 Kligler's agar

Stab the butt to the bottom of the agar and streak over the slant surface (B.7.2).

Incubate at 30 °C for 24 h to 48 h.

Interpret the changes in the medium as follows.

a) 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.

b) Slant surface

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

9.4.3.5 Detection of oxidase

Using the glass rod or platinum/iridium loop (6.11), take a portion of each characteristic colony chosen (9.4.2) and streak onto a filter paper lightly moistened (one drop) with the oxidase reagent (B.9) or onto a commercially available disc. Do not use a nickel/chromium loop or wire (see 6.11).

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.

9.4.4 Biochemical confirmation test

9.4.4.1 Selection of colonies and procedure

9.4.4.1.1 Continue the identification of colonies having the following characteristics:

- detection of urease: positive;
- detection of indole: positive or negative;
- fermentation of glucose: positive;
- formation of gas from the glucose: negative;
- fermentation of lactose: negative;
- formation of H_2S: negative;
- detection of oxidase: negative.

NOTE 1 Urease-negative strains have been reported but none is known to be pathogenic.

NOTE 2 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 *Yersinia enterocolitica* biovar 3) may produce one or two bubbles (weak gas production).

NOTE 3 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.

9.4.4.1.2 Using a loop or a wire (6.10), inoculate the media specified in 9.4.4.2 to 9.4.4.5 with each of the cultures obtained from the colonies isolated (9.4.2) on nutrient agar and selected in 9.4.4.1.1.

9.4.4.2 Lysine decarboxylation medium

Inoculate just below the surface of the liquid medium (B.10). If the tubes are not full of medium and airtight, cover the surface with molten (heated then just cooled so that it remains still liquid) Vaseline® oil or sterile liquid paraffin.

Incubate at 30 °C for 24 h.

A violet colour after incubation indicates a positive reaction.

A yellow colour indicates a negative reaction.

9.4.4.3 Ornithine decarboxylation medium

Inoculate just below the surface of the liquid medium (B.11). If the tubes are not full of medium and airtight, cover the surface with molten (heated then just cooled so that it remains still liquid) Vaseline® oil or sterile liquid paraffin.

Incubate at 30 °C for 24 h.

A violet colour after incubation indicates a positive reaction.

A yellow colour indicates a negative reaction.

9.4.4.4 Media for the fermentation of sucrose, rhamnose, trehalose and xylose

Inoculate each medium (B.12) 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.

9.4.4.5 Simmons' medium for the hydrolysis of citrate

Streak the slant surface of the agar (B.13). Do not close the caps of the tubes tightly so that air can enter and aerobic growth conditions prevail.

Incubate at 30 °C for 24 h.

The reaction is positive if the medium turns blue.

9.4.4.6 Tween-esterase test

Streak the agar (B.14) slope surface.

Incubate at 25 °C for 5 days and examine at intervals.

The reaction is positive if an opaque zone of precipitate due to calcium oleate microcrystals appears.

9.4.5 Presumptive pathogenicity tests (see reference [8])

9.4.5.1 Selection of colonies and procedure

9.4.5.1.1 Use only colonies that have the following characteristics:

— detection of lysine decarboxylase: negative;

— detection of ornithine decarboxylase: positive;

— fermentation of rhamnose: negative;

— fermentation of sucrose: positive;

— hydrolysis of citrate: negative

NOTE 1 Rare strains of presumptive pathogenic *Yersinia enterocolitica* that are sucrose-negative have been isolated from pork.

NOTE 2 *Yersinia enterocolitica* biovars 4 and 5 have been reported to be ornithine decarboxylase negative.

9.4.5.1.2 Using a loop or wire (6.11), inoculate the media specified in 9.4.5.2 to 9.4.5.4 with each of the pure cultures on nutrient agar from the selected colonies (9.4.5.1).

9.4.5.2 Medium for fermentation of aesculin

Streak the slant surface (B.15) of the agar.

Incubate at 30 °C for 24 h.

A black halo around the colonies indicates a positive reaction.

NOTE This test for fermentation of aesculin is equivalent to the test for fermentation of salicin.

9.4.5.3 Medium for detection of pyrazinamidase

Inoculate a large area of the slant surface (B.17) of the medium.

Incubate at 30 °C for 48 h.

Add 1 ml of 1 % ammonium iron(III) sulfate solution (B.18).

The appearance after 15 min of a pinkish-brown colour indicates a positive reaction.

9.4.5.4 Test of calcium requirements at 37 °C

9.4.5.4.1 The test of calcium requirements at 37 °C may be replaced by sodium acetate utilization.

NOTE 1 Tests incubated at 37 °C can cause this characteristic to be lost because genes encoded for this character are carried on a plasmid.

9.4.5.4.2 For each pure culture isolated on nutrient agar (9.4.5.1), suspend a small portion of a colony in sodium chloride solution (B.20) to obtain a suspension of approximately 1 000 bacteria per millilitre.

Inoculate 0,1 ml of each suspension by spreading onto

— two plates of casein-soya agar (B.16) (reference dishes), and

— two plates of casein-soya agar with magnesium and oxalate (B.19).

Incubate one dish of each medium at 25 °C for 48 h and the other at 37 °C for 48 h.

9.4.5.4.3 The reaction is regarded as positive if at 25 °C the colonies are of uniform size, and if at 37 °C, in the presence of magnesium and oxalate, an inhibition of the culture is seen where $>$ 20 % of the colonies are smaller, being 0,1 mm in diameter, and the remainder are 0,5 mm to 1 mm in diameter.

Those colonies that are inhibited are calcium dependent and are presumed to be pathogenic.

9.4.6 Interpretation of biochemical and pathogenicity tests

9.4.6.1 The strains of presumptive pathogenic *Yersinia enterocolitica* generally show the reactions given in Table 1; additional test may be performed (see Table C.1).

Table 1 — Interpretation of biochemical and pathogenicity tests for *Yersinia enterocolitica*

Test	Reaction
Species determination	
Urea (9.4.3.2)	+
Indole (9.4.3.3)	– / + [a]
Glucose (9.4.3.4)	+
Gas formation from glucose (9.4.3.4)	–
Lactose (9.4.3.4)	–
Hydrogen sulfide (9.4.3.4)	–
Oxidase (9.4.3.5)	–
Lysine decarboxylase (9.4.4.2)	–
Ornithine decarboxylase (9.4.4.3)	+
Sucrose (9.4.4.4)	+
Trehalose (9.4.4.4)	+ / – [b]
Rhamnose (9.4.4.4)	+ / – [b]
Xylose (9.4.4.4)	+ / – [b]
Citrate (9.4.4.5)	–
Tween-esterase (9.4.4.6)	+ / – [b]
Pathogenicity determination	
Aesculin (9.4.5.2)	–
Pyrazinamidase (9.4.5.3)	–
Calcium dependence at 37 °C (9.4.5.4)[c]	+

[a] Biovar 1 and some serovars of biovar 2 are indole positive. Biovars 3, 4, 5 and some serovars of biovar 2 are indole negative.

[b] Depending on the biovar of *Yersinia enterocolitica* (Annex C).

[c] Pathogenicity character was encoded by a virulence plasmid.

9.4.6.2 Determination of the biovar of *Yersinia enterocolitica* according to the tests in Table D.1 (Annex D) should be carried out in order to confirm presumed pathogenicity (Tween-esterase, aesculin, pyrazinamidase, indole, xylose, trehalose). *Yersinia enterocolitica* biovars 1B, 2, 3, 4 and 5 are known to be pathogenic.

9.4.6.3 Aesculin and pyrazinamidase tests shall be established to determine presumed pathogenicity. An aesculin and/or pyrazinamidase positive and calcium dependence at 37 °C negative strain is not pathogenic. An aesculin and pyrazinamidase negative and calcium dependence at 37 °C positive strain is pathogenic. Pathogenicity tests should be routinely carried out (see reference [9]).

9.4.6.4 For epidemiological purposes, determination of the somatic antigens of *Yersinia enterocolitica* should be investigated. Presumptive pathogenic strains serotyped by use of appropriate antisera usually belong to serovar O:3, O:8, O:9 and O:5,27.

10 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard;

d) the incubation temperature used;

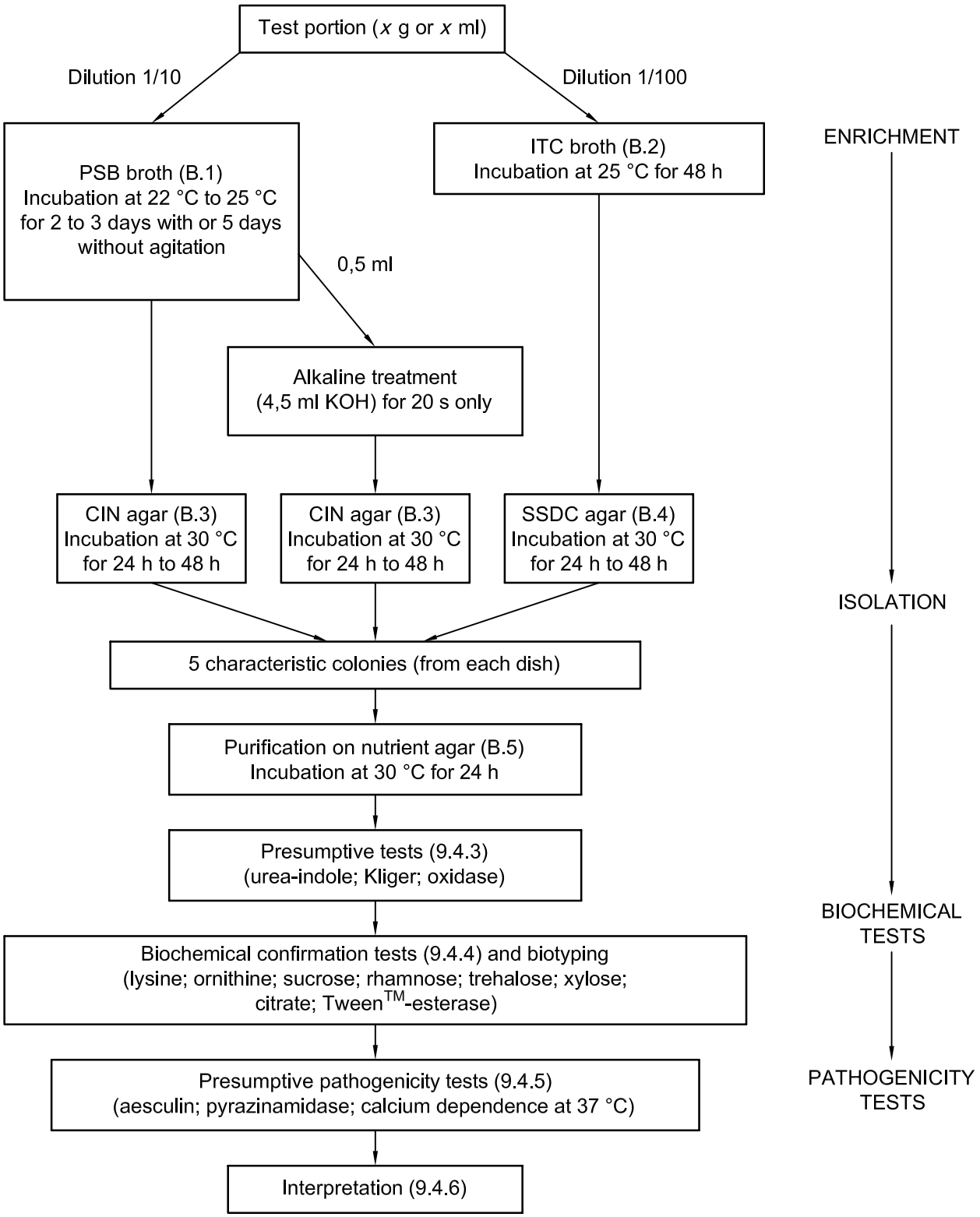
e) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

f) the test result(s) obtained, or, if the repeatability has been checked, the final quoted result obtained.

The test report shall also state if further tests are to be carried out by a reference laboratory and, if available, what those results were.

Annex A
(normative)

Diagram of procedure



Annex B
(normative)

Composition and preparation of culture media and reagents

B.1 Peptone, sorbitol and bile salts (PSB) broth

B.1.1 Composition

Enzymatic digest of casein	5,0 g
Sorbitol	10,0 g
Sodium chloride	5,0 g
Disodium hydrogen phosphate (Na_2HPO_4)	8,23 g
Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$)	1,2 g
Bile salts	1,5 g
Water	1 000 ml

B.1.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary.

Adjust the pH, if necessary, so that after sterilization it is 7,6 $\pm$ 0,2 at 25 °C.

Dispense the medium into tubes or flasks of suitable capacity to obtain portions appropriate for the test samples (see 9.1.2).

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

B.2 Irgasan™, ticarcillin and potassium chlorate (ITC) broth

B.2.1 Basic medium

B.2.1.1 Composition

Enzymatic digest of casein	10,0 g
Yeast extract	1,0 g
Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$)	60,0 g
Sodium chloride	5,0 g
Malachite green, 0,2 % aqueous solution	5,0 ml
Water	1 000 ml

B.2.1.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary.

Adjust the pH, if necessary, so that after sterilization it is 6,9 ± 0,2 at 25 °C.

Dispense the basic medium into flasks (6.7) of suitable capacity to obtain the portions necessary (e.g. 988 ml for 1 litre of complete medium).

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

B.2.2 Ticarcillin solution (1 mg/ml)

B.2.2.1 Composition

Ticarcillin	10,0 mg
Water	10 ml

B.2.2.2 Preparation

Dissolve the ticarcillin in the water. Sterilize by filtration.

B.2.3 Irgasan™ [5-chloro-2-(2,4-dichlorophenoxy)phenol], ethanolic solution (1 mg/ml)

B.2.3.1 Composition

Irgasan™	10,0 mg
Ethanol, 95 % (by volume)	10,0 ml

B.2.3.2 Preparation

Dissolve the Irgasan™ in the ethanol as and when required, or alternatively store the solution at about −20 °C for not more than 4 weeks.

B.2.4 Potassium chlorate solution (100 mg/ml)

B.2.4.1 Composition

Potassium chlorate ($KClO_3$)	10,0 g
Water	100 ml

B.2.4.2 Preparation

Dissolve the potassium chlorate in the water. Sterilize by filtration.

B.2.5 Complete medium

B.2.5.1 Composition

Basic medium (B.2.1)	988 ml
Ticarcillin solution (B.2.2)	1 ml
Irgasan™ solution (B.2.3)	1 ml
Potassium chlorate solution (B.2.4)	10 ml

B.2.5.2 Preparation

When required, add the ticarcillin, Irgasan™ and potassium chlorate solutions aseptically to the basic medium cooled to about 47 °C and mix.

Dispense the medium aseptically in 10 ml amounts into tubes, or in 100 ml amounts into flasks of suitable capacity (see 9.1.3), so as to obtain the minimum area/volume ratio (relative anaerobiosis).

B.3 Cefsulodin, Irgasan™ and novobiocin (CIN) agar

B.3.1 Basic medium

B.3.1.1 Composition

Enzymatic digest of gelatin	17,0 g
Enzymatic digest of casein and animal tissues	3,0 g
Yeast extract	2,0 g
Mannitol	20,0 g
Sodium pyruvate	2,0 g
Sodium chloride	1,0 g
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	0,01 g
Sodium desoxycholate	0,5 g
Neutral red	0,03 g
Crystal violet	0,001 g
Agar	9 to 18 g[1)]
Water	1 000 ml

B.3.1.2 Preparation

Dissolve the components or the dehydrated basic medium in the water by boiling.

Adjust the pH, if necessary, so that after sterilization it is 7,4 ± 0,2 at 25 °C.

Dispense the medium into flasks (6.7) of suitable capacity.

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

1) Depending on the gel strength of the agar.

B.3.2 Cefsulodin solution (15 mg/ml)

B.3.2.1 Composition

Cefsulodin	1,5 g
Water	100 ml

B.3.2.2 Preparation

Dissolve the cefsulodin in the water. Sterilize by filtration.

B.3.3 Irgasan™ [5-chloro-2-(2,4-dichlorophenoxy)phenol], ethanolic solution (4 mg/ml)

B.3.3.1 Composition

Irgasan™	0,4 g
Ethanol, 95 % (by volume)	100 ml

B.3.3.2 Preparation

Dissolve the Irgasan in the ethanol as and when required, or alternatively store the solution at about −20 °C for not more than 4 weeks.

B.3.4 Novobiocin solution (2,5 mg/ml)

B.3.4.1 Composition

Novobiocin	0,25 g
Water	100 ml

B.3.4.2 Preparation

Dissolve the novobiocin in the water. Sterilize by filtration.

B.3.5 Complete medium

B.3.5.1 Composition

Basic medium (B.3.1)	997 ml
Cefsulodin solution (B.3.2)	1 ml
Irgasan™ solution (B.3.3)	1 ml
Novobiocin solution (B.3.4)	1 ml

B.3.5.2 Preparation

Add each antibiotic solution aseptically to the basic medium cooled to about 45 °C and mix.

B.3.5.3 Preparation of CIN agar plates

Pour approximately 15 ml of the complete medium into sterile Petri dishes (6.8). Leave to set.

B.4 *Salmonella/Shigella* agar with sodium desoxycholate and calcium chloride (SSDC)

B.4.1 Composition

Yeast extract	5,0 g
Meat extract	5,0 g
Enzymatic digest of animal tissues	5,0
Lactose	10,0 g
Bile salts	8,5 g
Sodium desoxycholate	10,0 g
Calcium chloride	1,0 g
Sodium citrate	10 g
Sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$)	8,5 g
Iron(III) citrate	1,0 g
Brilliant green	0,000 3 g
Neutral red	0,025 g
Agar	9 g to 18 g[1)]
Water	1 000 ml

B.4.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling.

Adjust the pH, if necessary, so that it is 7,4 $\pm$ 0,2 at 25 °C.

Do not sterilize.

B.4.3 Preparation of SSDC agar plates

Pour approximately 20 ml of the medium, cooled to about 45 °C, into sterile Petri dishes (6.8). Leave to set.

If prepared in advance, the undried agar plates shall be kept in the dark for one week at 8 °C $\pm$ 2 °C in a plastic bag. Do not refrigerate at 3 °C $\pm$ 2 °C as a precipitate forms in the medium and decreases its performance.

B.5 Nutrient agar

B.5.1 Composition

Meat extract	3,0 g
Peptone	5,0 g
Agar	9 g to 18 g[1)]
Water	1 000 ml

B.5.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling.

Adjust the pH, if necessary, so that after sterilization it is 7,0 ± 0,2 at 25 °C.

Dispense the medium into flasks (6.7) of suitable capacity.

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

B.5.3 Preparation of nutrient agar plates

Pour approximately 15 ml of the medium, cooled to about 45 °C, into sterile Petri dishes (6.8). Leave to set.

B.6 Urea indole medium

B.6.1 Composition

L-Tryptophan, free from indole	3,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,0 g
Dipotassium hydrogen phosphate (K_2HPO_4)	1,0 g
Sodium chloride	5,0 g
Urea	20,0 g
Ethanol at 95 ° (volume concentration)	10 ml
Phenol red	0,025 g
Water	1 000 ml

B.6.2 Preparation

Dissolve the L-tryptophan in the water at 60 °C. Cool then dissolve the other components in the water by stirring.

Alternatively, dissolve the dehydrated complete medium in the water by stirring.

Adjust the pH, if necessary, so that it is 6,9 ± 0,2 at 25 °C.

Sterilize by filtration.

Dispense the medium aseptically in 0,5 ml amounts into sterile tubes of dimensions 12 mm × 50 mm (6.6). Store at 3 °C ± 2 °C in the dark.

B.7 Kovac's reagents

B.7.1 Composition

4-Dimethylaminobenzaldehyde	5,0 g
Hydrochloric acid, p = 1,18 g/ml to 1,19 g/ml	25 ml
2-Methylbutan-2-ol	75 ml

B.7.2 Preparation

Dissolve 4-dimethylaminobenzaldehyde in 2-methylbutan-2-ol in a water bath set at 60 °C.

Cool to room temperature and place the flask in an ice bath. Then add carefully the hydrochloric acid, mixing slowly.

Store at 3 °C ± 2 °C in an amber-coloured flask. Avoid the use of rubber bottle caps as they spoil the reagent.

B.8 Kligler's agar

B.8.1 Composition

Meat extract	3,0 g
Yeast extract	3,0 g
Casein pancreatic peptone	20,0 g
Sodium chloride	5,0 g
Lactose	10,0 g
Glucose	1,0 g
Iron(II) sulfate	0,2 g
Sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$)	0,3 g
Phenol red	0,025 g
Agar	9 g to 18 g[1)]
Water	1000 ml

B.8.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling.

Adjust the pH, if necessary, so that after sterilization it is 7,4 ± 0,2 at 25 °C.

Dispense the medium in 10 ml amounts into tubes (6.6) of suitable capacity.

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

Leave the tubes in a tilted position whilst the agar sets, so as to obtain a butt approximately 3 cm deep and slope 5 cm long.

B.9 Reagent for detection of oxidase

B.9.1 Composition

N,N,N′,N′-Tetramethyl-ρ-phenylene-diamine dichlorhydrate	1,0 g[2)]
Water	100 ml

2) Dihydrochloride may be replaced with oxalate but the shelf-life of the prepared solution is shorter.

B.9.2 Preparation

Dissolve the reagents in the water immediately before use.

Store at 3 °C ± 2 °C in the dark for no longer than one week.

B.10 Lysine decarboxylase medium

B.10.1 Composition

L-Lysine monohydrochloride	5,0 g
Yeast extract	3,0 g
Glucose	1,0 g
Bromocresol purple	0,015 g
Water	1 000 ml

B.10.2 Preparation

Dissolve the components in the water, by heating if necessary.

Adjust the pH, if necessary, so that after sterilization it is 6,8 ± 0,2 at 25 °C.

Dispense the medium in 5 ml amounts into tubes of dimensions 9 mm × 180 mm (6.6).

Sterilize for 15 min in an autoclave (6.12) set at 121 °C.

B.11 Ornithine decarboxylase medium

B.11.1 Composition

L-Ornithine monohydrochloride	5,0 g
Yeast extract	3,0 g
Glucose	1,0 g
Bromocresol purple	0,015 g
Water	1000 ml

B.11.2 Preparation

Dissolve the components in the water, by heating if necessary.

Adjust the pH, if necessary, so that after sterilization it is 6,8 ± 0,2 at 25 °C.

Dispense the medium in 5 ml amounts into tubes of dimensions 9 mm × 180 mm (6.6).

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

B.12 Media for fermentation of carbohydrates (peptone water with phenol red, rhamnose or sucrose or trehalose or xylose)

B.12.1 Basic medium

B.12.1.1 Composition

Peptone	10,0 g
Sodium chloride	5,0 g
Phenol red	0,02 g
Water	1 000 ml

B.12.1.2 Preparation

Dissolve the components or the dehydrated basic medium in the water, by heating if necessary.

Adjust the pH, if necessary, so that after sterilization it is 6,8 ± 0,2 at 25 °C.

Dispense the basic medium into flasks (6.7) of suitable capacity.

Sterilize for 10 min in an autoclave (6.1) set at 121 °C.

B.12.2 Carbohydrate solutions (rhamnose, sucrose, trehalose or xylose, 100 mg/ml)

B.12.2.1 Composition

Carbohydrate (rhamnose, sucrose, trehalose or xylose)	10,0 g
Water	100 ml

B.12.2.2 Preparation

Prepare separate solutions of each carbohydrate by adding it to the distilled water.

Sterilize by filtration.

B.12.3 Complete medium

B.12.3.1 Composition

Basic medium (B.12.1)	900 ml
Carbohydrate solution (B.12.2)	100 ml

B.12.3.2 Preparation

For each carbohydrate, add the carbohydrate solution aseptically to the basic medium cooled to about 45 °C and mix.

Dispense the complete medium aseptically in 10 ml amounts into tubes (6.6) or bottles (6.7) of suitable capacity.

B.13 Simmons' citrate medium

B.13.1 Composition

Sodium citrate	2,0 g
Sodium chloride	5,0 g
Dipotasium hydrogen phosphate (K_2HP0_4)	1,0 g
Bromothymol blue	0,08 g
Ammonium dihydrogen phosphate ($NH_4H_2PO_4$)	1,0 g
Magnesium sulfate	0,2 g
Agar	9 g to 18 g[1)]
Water	1 000 ml

B.13.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling.

Adjust the pH, if necessary, so that after sterilization it is 6,8 ± 0,2 at 25 °C.

Dispense the medium in 10 ml amounts into new tubes (6.6) of suitable capacity. If new tubes are not available, and before using cleaned tubes, it is essential to show that they are free of substances interfering with this test.

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

Leave to stand in a tilted position so as to obtain a slant 2,5 cm deep.

B.14 Media for Tween-esterase test

B.14.1 Base medium

B.14.1.1 Composition

Peptic digest of meat	10,0 g
Sodium chloride (NaCl)	5,0 g
Calcium chloride ($CaCl_2$)	0,1 g
Agar	9 g to 18 g[1)]
Water	1 000 ml

B.14.1.2 Preparation

Dissolve the components in the water, by heating if necessary.

Adjust the pH, if necessary, so that after sterilization it is 7,4 ± 0,2 at 25 °C.

Sterilize for 30 min in an autoclave (6.1) set at 121 °C.

B.14.2 Complete medium

B.14.2.1 Composition

Base (B.14.1)	990 ml
Tween 80™ (sorbitol mono-oleate)	10 ml

B.14.2.2 Preparation

Add the Tween 80™ solution to the liquid base, and homogenize.

Sterilize for 30 min at 110 °C.

Dispense the medium in 2,5 ml amounts into tubes (6.6) of suitable capacity.

Lay the tubes in an almost horizontal position so that there is a long slant with a minimal base.

B.15 Bile and aesculin agar

B.15.1 Composition

Meat extract	3,0 g
Meat peptone	5,0 g
Aesculin	1,0 g
Bile salts	40,0 g
Iron(III) citrate	0,5 g
Agar	9 g to 18 g[1)]
Water	1 000 ml

B.15.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by gentle boiling.

Adjust the pH, if necessary, so that after sterilization it is 6,6 ± 0,2 at 25 °C.

Dispense the medium in 10 ml amounts into tubes (6.6) of suitable capacity.

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

Leave to stand in a tilted position so as to obtain a butt 2,5 cm deep.

B.16 Casein-soya agar

B.16.1 Composition

Enzymatic digest of casein	15,0 g
Soya peptone	5,0 g
Sodium chloride	5,0g
Agar	9 g to 18 g[1)]
Water	1 000 ml

B.16.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling.

Adjust the pH, if necessary, so that after sterilization it is 7,3 ± 0,2 at 25 °C.

Dispense the medium into flasks (6.7) of suitable capacity and in 830 ml amounts (see B.19.5.1). This medium is required for use in B.19.5.1.

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

B.17 Casein-soya agar for detection of pyrazinamidase

B.17.1 Composition

Enzymatic digest of casein	15,0 g
Soya peptone	5,0 g
Pyrazinecarboxamide ($C_5H_5N_3O$)	1,0 g
Sodium chloride	5,0 g
Agar	9 g to 18 g[1)]
Tris-maleate buffer (0,2 mol/l, pH 6)	1 000 ml

B.17.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling.

Adjust the pH, if necessary, so that after sterilization it is 7,3 ± 0,2 at 25 °C.

Dispense the medium in 10 ml amounts into flasks (6.7) of suitable capacity.

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

After sterilization, leave to stand in a tilted position so as to obtain a long slope.

B.18 Ammonium iron(III) sulfate solution for detection of pyrazinamidase

B.18.1 Composition

Ammonium iron(II) sulfate	1,0 g
Water	100 ml

B.18.2 Preparation

Immediately prior to use, dissolve the ammonium iron(II) sulfate in the water.

B.19 Casein-soya agar with magnesium and oxalate

B.19.1 Basic medium (see B.16)

B.19.2 Magnesium chloride solution

B.19.2.1 Composition

Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) (0,25 mol/l)	5,09 g
Water	100 ml

B.19.2.2 Preparation

Dissolve the magnesium chloride in the water. Sterilize by filtration.

B.19.3 Sodium oxalate solution

B.19.3.1 Composition

Sodium oxalate	3,35 g
Water	100 ml

B.19.3.2 Preparation

Dissolve the sodium oxalate in the water. Sterilize by filtration.

B.19.4 Glucose solution

B.19.4.1 Composition

Glucose	18,0 g
Water	100 ml

B.19.4.2 Preparation

Dissolve the glucose in the water. Sterilize by filtration.

B.19.5 Complete medium

B.19.5.1 Composition

Basic medium (B.16)	830 ml
Magnesium chloride solution (B.18.2)	80 ml
Sodium oxalate solution (B.18.3)	80 ml
Glucose solution (B.18.4)	10 ml

B.19.5.2 Preparation

Add the magnesium chloride, sodium oxalate and glucose solutions aseptically to the basic medium, cooled to about 47 °C, and mix.

B.19.5.3 Preparation of agar plates

Pour approximately 15 ml of the complete medium into sterile Petri dishes (6.8). Leave to set.

B.20 Saline solution

B.20.1 Composition

Sodium chloride	5 g
Water	1000 ml

B.20.2 Preparation

Dissolve the sodium chloride in the water.

Dispense the solution into flasks (6.7) of suitable capacity.

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

B.21 Potassium hydroxide in saline solution

B.21.1 Composition

Potassium hydroxide (KOH)	0,5 g
Saline solution (B.20)	100 ml

B.21.2 Preparation

Dissolve the potassium hydroxide in the saline solution.

Dispense the solution into flasks (6.7) of suitable capacity.

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

NOTE A stock solution of 40 % potassium hydroxide may be prepared and stored at 3 °C ± 2 °C. A 1:80 dilution of this solution is made in 0,5 % NaCl solution to give 0,5 % KOH.

B.22 Veal infusion broth

B.22.1 Composition

Veal infusion (dehydrated)	500 g
Enzymatic digest of casein	10 g
Sodium chloride	5 g
Distilled water	1 000 ml

B.22.2 Preparation

Dissolve the components or the complete dehydrated medium in the water, by heating if necessary.

Adjust the pH if necessary so that after sterilization it is pH 7,4 ± 0,2 at 25 °C.

Dispense in 10 ml amounts into tubes (6.6) and close.

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

B.23 Sterile glycerol

Dispense the glycerol in 100 ml into flasks or bottles and sterilize for 15 min in an autoclave (6.1) set at 121 °C.

Annex C
(informative)

Biochemical characteristics at 30 °C of *Yersinia pseudotuberculosis*, *Yersinia enterocolitica* and biochemically related species

Table C.1 — Biochemical characteristics at 30 °C of *Yersinia*

Test	*Yersinia pseudotuberculosis*	*Yersinia enterocolitica*	Related species
Glucose	+ [a]	+	+
Gas from glucose	–	– (or some bubbles)	– (or some bubbles)
Lactose	–	–	–
ONPG	+	+/–	+/–
Adonitol	–	–	–
Cellobiose	–	+	D
Dulcitol	–	–	–
Mannitol	+	+	+
Melibiose	+/–	–	D
Rhamnose	+	–	D
Saccharose	–	+	D
Sorbitol	–	+/–	D
Trehalose	+	+/–	+
Xylose	+	D	+
Aesculin	+	D	D
Salicin	+	D	D
Urea	+	+	+
Indole	–	D	D
Voges Proskauer	–	+*/–	D
Hydrogen sulfide	–	–	–
Deaminase (APP)	–	–	–
Lysine	–	–	–
Ornithine	–	+/–	+
Citrate (Simmons)	–	–	D
Lipase (Tween 80)	–	D	D
Mucate	–	–	D

[a] + positive; – negative; +/– majority of strains positive; D divergent biochemical types.

* Strains are always almost negative at 37 °C.

Annex D
(informative)

Biovars (biotyping) of *Yersinia enterocolitica*

Table D.1 — Biovars of *Yersinia enterocolitica*

Biovar	Tween-esterase	Aesculine	Pyrazinamidase	Indole	Xylose	Trehalose
1A [a]	+	+	+	+	+	+
1B	+	–	–	+	+	+
2	–	–	–	(+) [b]	+	+
3	–	–	–	–	+	+
4	–	–	–	–	–	+
5	–	–	–	–	D [b]	–

[a] Non-pathogenic.

[b] Often weak or delayed.

Bibliography

[1] WAUTERS, G., GOOSSENS, V., JANSSENS, M. and VANDEPITTE, J. New enrichment method for isolation of pathogenic *Yersinia enterocolitica* serogroup O:3 from pork. *Appl. Environ. Microbiol*., **54**, 1988, pp. 851-854

[2] DE BOER, E. Isolation of *Yersinia enterocolitica* from foods. *Int. J. Food Microbiol.*, **17**, 1992, pp. 75-84

[3] DE ZUTTER, L., LE MORT, L., JANSSENS, M. and WAUTERS, G. Short-comings of irgasan ticarcillin chlorate broth for the enrichment of *Yersinia enterocolitica* biovar 2, serovar 9 from meat. *Int. J. Food. Microbiol*., **23**, 1994, pp. 231-237

[4] SCHIEMANN, D.A. Synthesis of selective agar medium for *Yersinia enterocolitica. Can. J. Microbiol*., **25**, 1979, pp. 1298-1304

[5] AULISIO, C.C.G., MEHLMAN, I.J. and SANDERS, A.C. Alkali method for rapid recovery of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from foods. *Appl. Environ. Microbiol*., **39**, 1980, pp. 135-140

[6] ARCHER, J.R., SCHELL, R.F., PENNELL, D.R. and WICK, P.D. Identification of *Yersinia* spp. with the API 20E system. *J. Clin. Microbiol*., **25**, 1987, pp. 2398-2399

[7] SHARMA, N.K., DOYLE, P.W., GERBASI, S.A. and JESSOP, J.H. Identification of *Yersinia* species by the API 20E. *J. Clin. Microbiol*., **28**, 1990, pp. 1443-1444

[8] FARMER III, J.J., CARTER, G.P., MILLER, V.L., FALKOW, S. and WACHSMUTH, I.K. Pyrazinamidase, CR-MOX Agar, Salicin Fermentation – Esculin Hydrolysis, and D-Xylose fermentation for identifying pathogenic serotypes of *Yersinia enterocolitica*. *J. Clin. Microbiol*., **30**, 1992, pp. 2589-2594

[9] Food and Drug Administration. Protocol in FDA. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. In: *Bacteriological Analytical Manual*, 8th edn., Washington, DC, 1998

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