TECHNICAL SPECIFICATION

ISO/TS 10272-3

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Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration of *Campylobacter* spp. —

Part 3: Semi-quantitative method

Microbiologie des aliments — Méthode horizontale pour la recherche et le dénombrement de Campylobacter *spp. —*

Partie 3: Méthode semi-quantitative

Reference number
ISO/TS 10272-3:2010(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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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/TS 10272-3 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

ISO 10272 consists of the following parts, under the general title *Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration of* Campylobacter *spp.*:

— *Part 1: Detection method*

— *Part 2: Colony count technique* [Technical Specification]

— *Part 3: Semi-quantitative method* [Technical Specification]

Introduction

Because of the large variety of food and feed products, ISO 10272 may not be appropriate in every detail for certain products, and for some other products it may be necessary to use different methods.

Nevertheless, in all cases, every attempt should be made to apply ISO 10272 as far as possible, any deviations being made only if absolutely necessary for technical reasons.

When ISO 10272 is next reviewed, account will be taken of all information then available regarding the extent to which the methods have been followed and the reasons for deviations from it 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 ISO 10272. It is hoped that, when such standards are reviewed, they will be changed to comply with ISO 10272, so that eventually the only remaining departures are those necessary for well-established technical reasons.

Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration of *Campylobacter* spp. —

Part 3: Semi-quantitative method

1 Scope

This part of ISO 10272 describes a horizontal method for the semi-quantitative determination of *Campylobacter* spp.

It is applicable to products intended for human consumption or for the feeding of animals, and to environmental samples in the area of food production and food handling. However, it is possible that this part of ISO 10272 is not appropriate in every detail for certain products, deviations from it being made necessary for technical reasons. It is possible that this part of ISO 10272 is not applicable at all to some other products.

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 requirements and guidance for microbiological examinations*

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*

ISO/TS 11133-2:2003, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on performance testing of culture media*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
Campylobacter
⟨food and feed *Campylobacter* microbiology⟩ genus of microorganisms forming characteristic colonies on solid selective media when incubated microaerobically at 41,5 °C, but not at 25 °C, and which possess the characteristic motility and biochemical and growth properties described when the tests are conducted in accordance with this part of ISO 10272

NOTE The most frequently encountered species are *Campylobacter jejuni* and *C. coli*. Other species have, however, been described (*C. lari*, *C. upsaliensis* and some others).

3.2
semi-quantitative determination
⟨food and feed *Campylobacter* microbiology⟩ determination of the level of *Campylobacter* contamination, when a low number is expected, or if the level of accompanying flora is relatively high

4 Principle

4.1 General. The semi-quantitative determination of *Campylobacter* spp. requires stages 4.2 to 4.4 (see Figure A.1).

4.2 Enrichment in selective liquid medium. The test portion and decimal dilutions thereof are inoculated or diluted in the liquid enrichment medium (Bolton broth) and homogenized.

The enrichment medium is incubated at 37 °C for 4 h to 6 h followed by incubation at 41,5 °C for (44 ± 4) h.

4.3 Isolation and selection for confirmation. From the cultures obtained in 4.2, the selective solid medium modified charcoal cefoperazone deoxycholate agar (mCCD agar) is inoculated, incubated at 41,5 °C in a microaerobic atmosphere and inspected after (44 ± 4) h to detect the presence of colonies presumed to be *Campylobacter* spp. because of their characteristics.

4.4 Confirmation. The colonies presumed to be *Campylobacter* spp. are subcultured on the non-selective Columbia blood agar, then confirmed by means of microscopic examination and appropriate biochemical and growth tests. Optionally, the *Campylobacter* spp. are identified by specific biochemical tests and antibiotic sensitivity tests.

5 Culture media and reagents

5.1 General. For current laboratory practice, see ISO 7218, ISO/TS 11133-1 and ISO/TS 11133-2.

NOTE Because of the large number of culture media and reagents and for the clarity of the text, their compositions and preparations are given in Annex B.

5.2 Liquid enrichment medium: Bolton broth. See B.1.

5.3 Selective plating medium: modified charcoal cefoperazone deoxycholate agar (mCCD agar). See B.2.

5.4 Confirmation and identification media and reagents.

5.4.1 Columbia blood agar. See B.3.

5.4.2 Brucella broth. See B.4.

5.4.3 Reagent for the detection of oxidase. See B.5.

5.4.4 Hydrogen peroxide solution, 3 % (volume fraction).

5.4.5 Reagents for the detection of hydrolysis of hippurate. See B.6.

5.4.6 Mueller Hinton blood agar. See B.7.

5.4.7 Nalidixic acid discs and **cephalothin discs**. Each type of disc contains 30 µg of reagent.

5.4.8 Indoxyl acetate discs. See B.8.

6 Apparatus

Usual microbiological laboratory equipment (see ISO 7218) and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) or **wet sterilization (autoclave)**. See ISO 7218.

6.2 Oven, **laminar flow cabinet** or **incubator**, capable of being maintained between 37 °C and 55 °C.

6.3 Incubators, capable of being maintained at (25 ± 1) °C, (37 ± 1) °C and (41,5 ± 1) °C.

6.4 Water bath, capable of being maintained at (37 ± 1) °C.

6.5 Water bath, capable of being maintained between 47 °C and 50 °C.

6.6 pH-meter, accurate to within 0,1 pH units 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.

6.8 Petri dishes, in glass or plastic, with diameters 90 mm to 100 mm.

6.9 Total-delivery graduated pipettes, with a wide opening, and a nominal capacity of 1 ml and 10 ml, graduated in 0,1 ml divisions, ISO 835 [1] class A, and **Pasteur pipettes**, ISO 7712 [2].

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

6.11 Sterile loops, of platinum-iridium alloy, nickel-chromium alloy or plastic, approximately 3 mm in diameter, and wires of the same material, or a **glass rod** or **plastic rod**.

A nickel-chromium alloy loop is not suitable for use in the oxidase test (see 9.5.6).

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

6.13 Microscope, preferably with phase contrast (for observing the characteristic motility of *Campylobacter* spp.).

6.14 Apparatus suitable for achieving a microaerobic atmosphere, with volume fractions of: oxygen, (5 ± 2) %; carbon dioxide, (10 ± 3) %; optionally hydrogen, ⩽ 10 %; the balance being nitrogen. Appropriate gastight containers are used to hold Petri dishes and/or flasks or bottles of about 350 ml capacity used for the enrichment broth, e.g. bacteriological anaerobic jars.

NOTE 1 The appropriate microaerobic atmosphere can be obtained using commercially available gas-generating kits, following precisely the manufacturer's instructions, particularly those relating to the volume of the jar and the capacity of the gas-generating kit. Alternatively, the jar can be filled with an appropriate gas mixture prior to incubation.

NOTE 2 As an alternative to incubation in a microaerobic atmosphere, the enrichment broth can be incubated in screw-capped bottles, flasks or tubes filled with enrichment broth, leaving a headspace of less than 20 mm and tightly screwing on the caps.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this part of ISO 10272. See the specific International Standard dealing with the product concerned. If no specific International Standard exists, it is recommended that the parties concerned come to an agreement on this subject.

Since *Campylobacter* spp. are very sensitive to freezing but survive best at low temperatures, it is recommended that samples be stored at (+ 3 ± 2) °C and subjected to analysis as rapidly as possible. Also take care to prevent the samples from drying.

8 Preparation of test sample

Prepare the test sample in accordance with the relevant part of ISO 6887 dealing with the product concerned. If ISO 6887 is not appropriate, it is recommended that the parties concerned come to an agreement on this subject.

9 Procedure

9.1 General

See Figure A.1.

9.2 Test portion, initial suspension and dilutions

9.2.1 Introduce a test portion of x g or x ml (minimum 15 g or 15 ml) from the test sample (Clause 8) into eight times (120 ml minimum) its volume of the enrichment medium Bolton broth (5.2), and homogenize.

This is the initial suspension.

9.2.2 Transfer an amount of 90 ml of the initial suspension (9.2.1) to a 100 ml bottle. This corresponds to 10 g of the test portion. When reading the results, this dilution corresponds to 10^1.

9.2.3 Transfer 10 ml of the initial suspension (9.2.1) to a culture tube. When reading the results, this dilution corresponds to 10^0. After the next dilution step (9.2.4), 9 ml of this dilution remains. This corresponds to 1 g of the test portion.

9.2.4 Make an ordinary 10-fold dilution series (e.g. to 10^{-4}) from the 10^0 dilution (9.2.3) by transferring 1,0 ml to tubes containing 9,0 ml Bolton broth. A quantity of 1,0 ml from the highest dilution is discarded, as all tubes must contain 9,0 ml. When reading the results, these dilutions correspond to 10^{-1}, 10^{-2}, etc.

9.3 Enrichment

Incubate the test portions and dilutions (9.2.2, 9.2.3 and 9.2.4) in a microaerobic atmosphere (6.14) at 37 °C for 4 h to 6 h and then at 41,5 °C for (44 ± 4) h.

9.4 Isolation

9.4.1 Using each of the cultures obtained in the enrichment media (9.3), inoculate with a sterile loop (6.11) the surface of plates of the selective isolation medium mCCD agar (5.3).

9.4.2 Incubate the plates (9.4.1) at 41,5 °C in a microaerobic atmosphere (6.14).

9.4.3 After (44 ± 4) h of incubation, examine the plates for typical and/or suspect colonies of *Campylobacter* spp.

The typical colonies are greyish on mCCD agar, often with a metallic sheen, and are flat and moist, with a tendency to spread. Colonies spread less on drier agar surfaces. Other forms of colonies may occur.

9.5 Confirmation of *Campylobacter* spp.

9.5.1 General

As the bacteria rapidly deteriorate in air, follow the procedure described in 9.5.2 to 9.5.6 without any delay.

9.5.2 Selection of colonies for confirmation

9.5.2.1 For confirmation, take from each plate (9.4.3) at least one colony considered to be typical or suspected as being *Campylobacter* spp., examine the morphology and motility by microscopic examination (9.5.3.1) and proceed in the same manner with up to a further four colonies if the first one is negative.

9.5.2.2 Streak each of the selected colonies on to a Columbia blood agar plate (5.4.1) in order to allow the development of well-isolated colonies. Incubate the plates in a microaerobic atmosphere at 41,5 °C for 24 h to 48 h. Use the pure cultures for examination of morphology, motility, microaerobic growth at 25 °C, aerobic growth at 41,5 °C and the presence of oxidase.

9.5.3 Examination of morphology and motility

9.5.3.1 Suspend one colony from the Columbia blood agar plate (9.5.2.2) in 1 ml of Brucella broth (5.4.2) or in peptone salt solution and examine for morphology and motility using a microscope (6.13).

9.5.3.2 Retain for further examination all cultures (9.5.2.2) in which curved bacilli with a spiralling “corkscrew” motility are found (9.5.3.1).

9.5.4 Study of growth at 25 °C (microaerobic)

Using the colonies isolated in 9.5.2.2, inoculate with the aid of a loop (6.11) the surface of a Columbia blood agar plate (5.4.1).

Incubate the plate at 25 °C in a microaerobic atmosphere (6.14) for (44 ± 4) h.

Examine the plate for visible growth of colonies of *Campylobacter* spp.

9.5.5 Study of growth at 41,5 °C (aerobic)

Using the colonies isolated in 9.5.2.2, inoculate with the aid of a loop (6.11) the surface of a Columbia blood agar plate (5.4.1).

Incubate the plate at 41,5 °C in an aerobic atmosphere for (44 ± 4) h.

Examine the plate for visible growth of colonies of *Campylobacter* spp.

9.5.6 Detection of oxidase

Using a platinum-iridium alloy or plastic loop or a glass rod (6.11), take a portion of a well-isolated colony from each individual plate (9.5.2.2) and streak it on to a filter paper moistened with the oxidase reagent (5.4.3); the appearance of a mauve, violet or deep blue colour within 10 s indicates a positive reaction. If a commercially available oxidase test kit is used, follow the manufacturer's instructions.

Confirm the results using positive and negative controls. Examples of suitable control strains are *Pseudomonas aeruginosa* NCTC 10662 (positive control), *Escherichia coli* NCTC 9001 (negative control).

9.5.7 Interpretation

Campylobacter spp. give results in accordance with Table 1.

Table 1 — Characteristics of *Campylobacter* spp.

Morphology (9.5.3)	small curved bacilli
Motility (9.5.3)	characteristic
Microaerobic growth at 25 °C (9.5.4)	–
Aerobic growth at 41,5 °C (9.5.5)	–
Oxidase (9.5.6)	+

Campylobacter spp. are present if at least one colony presents the above characteristics.

9.6 Identification of *Campylobacter* spp. (optional)

9.6.1 General

Among the *Campylobacter* spp. growing at 41,5 °C, those most frequently encountered are *C. jejuni* and *C. coli*. Other species have, however, been described (*C. lari*, *C. upsaliensis* and some others); the characteristics given in Table 2 permit their differentiation.

Table 2 — Characteristics of *Campylobacter* spp.

Characteristic	*C. jejuni*	*C. coli*	*C. lari*	*C. upsaliensis*
Catalase (9.6.2)	+	+	+	– or slight
Nalidixic acid (9.6.3)	S[a]	S[a]	R/S[b]	S
Cephalothin (9.6.3)	R	R	R	S
Hydrolysis of hippurate (9.6.4)	+[c]	–	–	–
Indoxyl acetate (9.6.5)	+	+	–	+
Key: + = positive; – = negative; S = sensitive; R = resistant.				

[a] An increase in the resistance to nalidixic acid of *C. jejuni* and *C. coli* strains has been shown.

[b] Both sensitive and resistant *C. lari* strains exist.

[c] Hippurate-negative *C. jejuni* strains exist.

9.6.2 Detection of catalase

For each colony selected in 9.5.2.2, deposit a loop of culture into a drop of hydrogen peroxide solution (5.4.4) on a clean microscope slide.

The test is positive if bubbles appear within 30 s.

Confirm the results using positive and negative controls. Examples of suitable control strains are *Staphylococcus aureus* NCTC 8532 (positive control), *Enterococcus faecalis* NCTC 775 (negative control).

9.6.3 Detection of sensitivity to nalidixic acid and to cephalothin

For each colony selected in 9.5.2.2, use a loop (6.11) to prepare a suspension in Brucella broth (5.4.2) of density 0,5 on the McFarland scale.

Dilute this suspension 1→10 with the same broth.

Flood the surface of a Mueller Hinton 5 % volume fraction blood agar plate (5.4.6) with the suspension.

Leave in contact for 5 min, then drain off excess suspension.

Dry the plates in a drying cabinet (6.2) maintained at 37 °C for 10 min.

Place a disc of nalidixic acid (5.4.7) and a disc of cephalothin (5.4.7) on the surface of the agar.

Incubate the plates, with lids uppermost, at 37 °C for (22 ± 2) h in a microaerobic atmosphere (6.14).

Interpret the bacterial growth in the following manner:

— growth that is in contact with the disc is classified as resistant;

— the presence of a zone of any size due to inhibition of growth is classified as susceptible.

9.6.4 Detection of hippurate hydrolysis

For each colony selected in 9.5.2.2, use a loop (6.11) with a heavy inoculum to prepare a suspension in a haemolysis tube (6.7) containing 0,4 ml of a sodium hippurate solution (5.4.5), taking care not to incorporate any agar.

Shake in order to mix thoroughly and incubate for 2 h in a water bath (6.4) maintained at 37 °C or 4 h in an incubator maintained at 37 °C.

Carefully add 0,2 ml of a ninhydrin solution (5.4.5) on the top of the sodium hippurate solution. Do not shake.

Interpret after an additional incubation of 10 min in the water bath (6.4) maintained at 37 °C or in an incubator maintained at 37 °C.

A dark violet colour indicates a positive reaction.

A pale violet colour or no colour change indicates a negative reaction.

Confirm the results using positive and negative controls. Examples of suitable control strains are *C. jejuni* NCTC 11351 (positive control), *C. coli* NCTC 11366 (negative control).

9.6.5 Detection of indoxyl acetate hydrolysis

Place a colony selected in 9.5.2.2 on an indoxyl acetate disc (5.4.8) and add a drop of sterile distilled water. A loopful of colony material is required for a clear reaction.

If the indoxyl acetate is hydrolysed, a colour change to dark blue occurs within 5 min to 10 min. No colour change indicates that hydrolysis has not taken place.

Confirm the results using positive and negative controls. Examples of suitable control strains are *C. jejuni* NCTC 11351 (positive control), *C. lari* NCTC 11352 (negative control).

9.6.6 Interpretation

Campylobacter spp. growing at 41,5 °C may be identified at a species level according to Table 2.

10 Calculation and expression of results

10.1 Method of calculation

The semi-quantitative method is based on qualitative detection in selected dilutions. The result is therefore given in intervals according to Table 3.

The Poisson distribution is used to estimate the range of concentrations that are consistent with a set of results. For example, if the true concentration is 0,005 cfu/g, then there is a 5 % chance of seeing a positive result for the 10^1 dilution. If the true concentration is 3 cfu/g, then there is a 95 % chance of seeing a positive result for the 10^0 dilution. Hence, if a positive result is observed for the 10^1 dilution and the 10^0 and higher dilutions are negative, then the true concentration probably lies between 0,005 cfu/g and 3 cfu/g.

Table 3 — Result intervals for dilution series

Dilution[a]	Growth of confirmed *Campylobacter* spp.						
10^1	–	+	+	+	+	+	+
10^0	–	–	+	+	+	+	+
10^{-1}	–	–	–	+	+	+	+
10^{-2}	–	–	–	–	+	+	+
10^{-3}	–	–	–	–	–	+	+
10^{-4}	–	–	–	–	–	–	+
Result, n, cfu/g	$n < 0{,}3$	$0{,}005 \leqslant n \leqslant 3$	$0{,}05 \leqslant n \leqslant 30$	$0{,}5 \leqslant n \leqslant 300$	$5 \leqslant n \leqslant 3\,000$	$50 \leqslant n \leqslant 30\,000$	$n > 500$

[a] Dilution 10^1 represents the analysis of 10 g of the test portion, 10^0 represents 1 g of the test portion, etc. (9.2).

10.2 Precision

An international collaborative study was organized in 2005 by the Nordic Committee on Food Analysis (NMKL), including semi-quantitative determination of *Campylobacter* spp. as described in this part of ISO 10272. The study involved 14 European laboratories and was carried out on raw chicken meat and milk. The food samples were each tested at different levels of contamination, plus a negative control. The specificity of the semi-quantitative method was 100 %. The overall sensitivity of the method was 82,1 %. The sensitivity for detecting *Campylobacter* spp. in milk was lower than for chicken meat. The quantitative performance of the method for *Campylobacter* spp. in chicken meat observed in the collaborative study was consistent with the concentration ranges given in Table 3.

11 Test report

The test report shall contain at least the following information:

a) the sampling method used, if known;

b) the method used, with reference to this part of ISO 10272 (ISO/TS 10272-3:2010);

c) the liquid enrichment medium used;

d) the isolation medium used;

e) the incubation temperature chosen;

f) the test results obtained;

g) all operating details not specified in this part of ISO 10272, or regarded as optional, together with details of any incidents which may have influenced the test results;

h) all information necessary for the complete identification of the sample.

Annex A
(normative)

Diagram of procedure

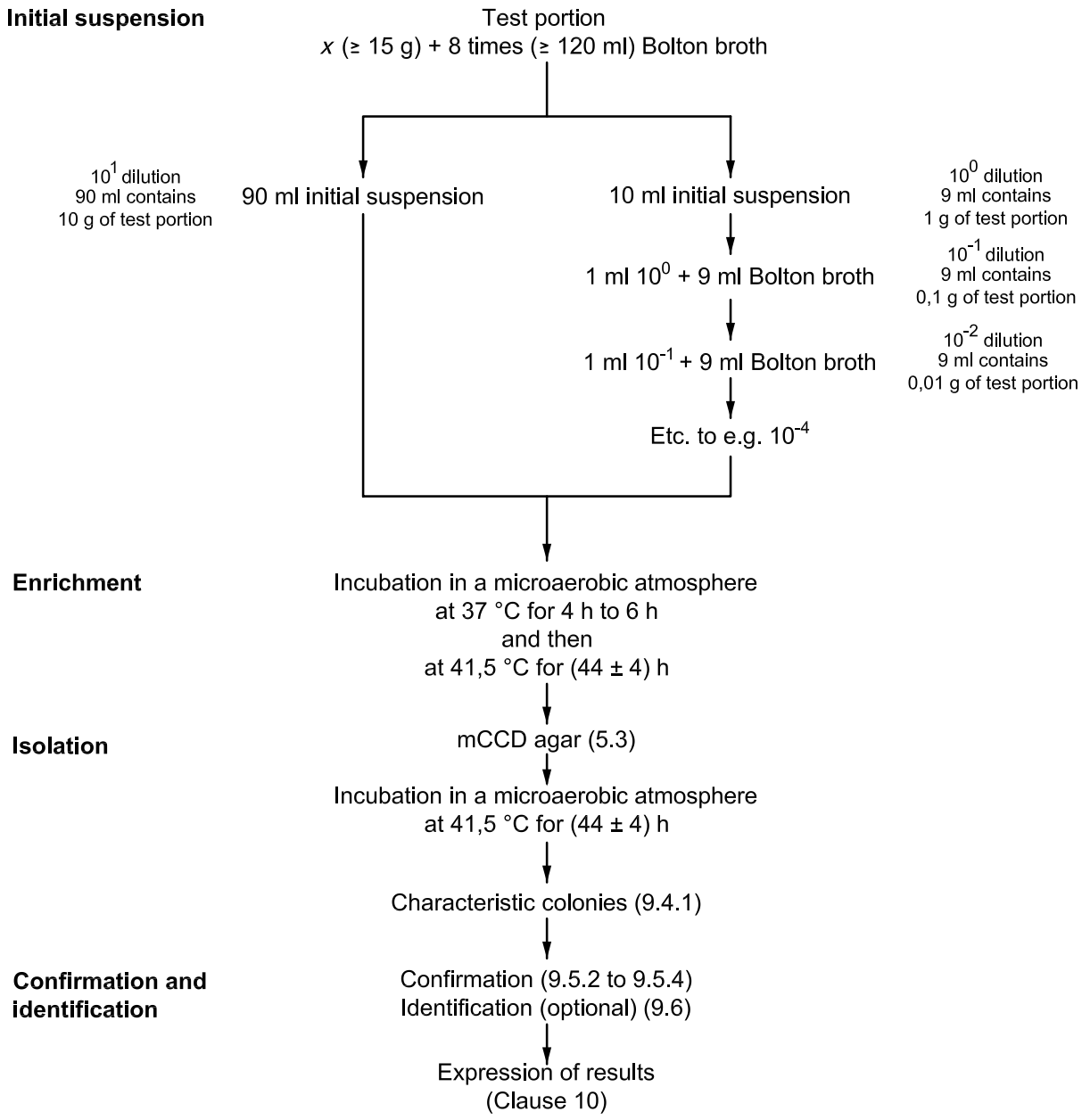

Figure A.1 — Diagram of procedure

Annex B
(normative)

Composition and preparation of culture media and reagents

B.1 Bolton broth

B.1.1 Basic medium

B.1.1.1 Composition

Enzymatic digest of animal tissues	10,0 g
Lactalbumin hydrolysate	5,0 g
Yeast extract	5,0 g
Sodium chloride	5,0 g
Sodium pyruvate	0,5 g
Sodium metabisulfite	0,5 g
Sodium carbonate	0,6 g
α-Ketoglutaric acid	1,0 g
Haemin (dissolved in 0,1 % mass fraction sodium hydroxide)	0,01 g
Water	1 000 ml

B.1.1.2 Preparation

Dissolve the basic components or the dehydrated complete basic medium in the water, heating if necessary. Adjust the pH, if necessary, so that after sterilization the pH of the complete medium is 7,4 $\pm$ 0,2 at 25 °C. Dispense the basic medium into flasks of suitable capacity. Sterilize in the autoclave (6.1) maintained at 121 °C for 15 min.

B.1.2 Sterile lysed defibrinated horse blood

Use saponin-lysed horse blood or horse blood lysed by freezing then thawing out.

B.1.3 Antibiotic solution composition

Cefoperazone	0,02 g
Vancomycin	0,02 g
Trimethoprim lactate	0,02 g
Amphotericin B	0,01 g
Ethanol and water mixture: 1 + 1 parts by volume	5 ml

B.1.4 Preparation

Dissolve the components in the 1 + 1 ethanol and water mixture.

B.1.5 Complete medium

B.1.5.1 Composition

Basic medium (B.1.1)	1 000 ml
Sterile lysed defibrinated horse blood (B.1.2)	50 ml
Antibiotic solution (B.1.3)	5 ml

B.1.5.2 Preparation

To the basic medium, at a temperature below 50 °C, add the blood aseptically, then the antibiotic solution and mix. Dispense the medium aseptically into tubes or flasks of suitable capacity (see 9.2.2) to obtain the portions necessary for the test. If the enrichment medium has been prepared in advance, do not keep it for more than 4 h at ambient temperature, or for more than 7 days in the dark at (3 ± 2) °C.

B.1.6 Performance testing

Test the performance of the Bolton broth (B.1.5.1) in accordance with the methods and criteria described in ISO/TS 11133-2. Examples of suitable control strains are *C. jejuni* NCTC 11351 or ATCC 33291 with the following criteria: > 10 colonies on mCCD agar (5.3) after microaerobic incubation at 41,5 °C for (44 ± 4) h.

B.2 Modified charcoal cefoperazone deoxycholate agar (mCCD agar)

B.2.1 Basic medium

B.2.1.1 Composition

Meat extract	10,0 g
Enzymatic digest of animal tissues	10,0 g
Sodium chloride	5,0 g
Charcoal	4,0 g
Enzymatic digest of casein	3,0 g
Sodium deoxycholate	1,0 g
Iron(II) sulfate	0,25 g
Sodium pyruvate	0,25 g
Agar	8,0 g to 18,0 g[a]
Water	1 000 ml
[a] Depending on the gel strength of the agar.	

B.2.1.2 Preparation

Dissolve the basic components or the dehydrated complete basic medium in the water by bringing to the boil. Adjust the pH, if necessary, so that after sterilization it is 7,4 ± 0,2 at 25 °C. Dispense the basic medium into flasks of suitable capacity. Sterilize in the autoclave (6.1) maintained at 121 °C for 15 min.

B.2.2 Antibiotic solution

B.2.2.1 Composition

Cefoperazone	0,032 g
Amphotericin B	0,01 g
Water	5 ml

B.2.2.2 Preparation

Dissolve the components in the water. Sterilize by filtration.

B.2.3 Complete medium

B.2.3.1 Composition

Basic medium (B.2.1)	1 000 ml
Antibiotic solution (B.2.2)	5 ml

B.2.3.2 Preparation

Add the antibiotic solution to the basic medium, cooled to 47 °C to 50 °C, then mix carefully. Pour about 15 ml of the complete medium into sterile Petri dishes. Allow to solidify. Immediately before use, carefully dry the agar plates, preferably with the lids off and the agar surface downwards, in a drying cabinet (6.2) for 30 min or until the agar surface is free of visible moisture. If they have been prepared in advance, do not keep the undried agar plates for more than 4 h at ambient temperature, or for more than 7 days in the dark at (3 ± 2) °C.

B.2.4 Performance testing

For the definition of selectivity and productivity, refer to ISO/TS 11133-1. For the performance criteria, refer to ISO/TS 11133-2:2003, Table B.5.

B.3 Columbia blood agar

B.3.1 Basic medium

B.3.1.1 Composition

Enzymatic digest of animal tissues	23,0 g
Starch	1,0 g
Sodium chloride	5,0 g
Agar	8,0 g to 18,0 g[a]
Water	1 000 ml
[a] Depending on the gel strength of the agar.	

B.3.1.2 Preparation

Dissolve the basic components or the dehydrated complete medium in the water, by heating. Adjust the pH, if necessary, so that after sterilization it is 7,3 ± 0,2 at 25 °C. Dispense the basic medium into flasks of suitable capacity. Sterilize in the autoclave (6.1) maintained at 121 °C for 15 min.

B.3.2 Sterile defibrinated sheep blood

B.3.3 Complete medium

B.3.3.1 Composition

Basic medium (B.3.1)	1 000 ml
Sterile defibrinated sheep blood (B.3.2)	50 ml

B.3.3.2 Preparation

Add the blood aseptically to the basic medium, cooled to 47 °C to 50 °C, then mix. Pour about 15 ml of the complete medium into sterile Petri dishes. Allow to solidify. Immediately before use, carefully dry the agar plates, preferably with the lids off and the agar surface downwards, in a drying cabinet (6.2) for 30 min or until the agar surface is free of visible moisture. If they have been prepared in advance, do not keep the undried agar plates for more than 4 h at ambient temperature, or for more than 7 days at (3 ± 2) °C.

B.4 Brucella broth

B.4.1 Composition

Enzymatic digest of casein	10,0 g
Enzymatic digest of animal tissues	10,0 g
Glucose	1,0 g
Yeast extract	2,0 g
Sodium chloride	5,0 g
Sodium hydrogen sulfite	0,1 g
Water	1 000 ml

B.4.2 Preparation

Dissolve the basic components or the dehydrated complete medium in the water, by heating if necessary. Adjust the pH, if necessary, so that after sterilization it is 7,0 ± 0,2 at 25 °C. Dispense the medium in quantities of 10 ml into tubes of suitable capacity. Sterilize in the autoclave (6.1) maintained at 121 °C for 15 min.

B.5 Reagent for the detection of oxidase

B.5.1 Composition

N,*N*,*N*′,*N*′-Tetramethyl-1,4-phenylenediamine dihydrochloride	1,0 g
Water	100 ml

B.5.2 Preparation

Dissolve the component in the water immediately prior to use.

B.6 Reagents for the detection of hydrolysis of hippurate

B.6.1 Sodium hippurate solution

B.6.1.1 Composition

Sodium hippurate	10 g
Phosphate-buffered saline (PBS)	
Sodium chloride	8,5 g
Disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$)	8,98 g
Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$)	2,71 g
Water	1 000 ml

B.6.1.2 Preparation

Dissolve the sodium hippurate in the PBS solution. Sterilize by filtration. Dispense the reagent aseptically in quantities of 0,4 ml into small tubes of suitable capacity (6.7). Store at about –20 °C.

B.6.2 Ninhydrin solution, 3,5 % (mass per volume)

B.6.2.1 Composition

Ninhydrin	1,75 g
Acetone	25 ml
Butanol	25 ml

B.6.2.2 Preparation

Dissolve the ninhydrin in the acetone and butanol mixture. Store the solution in the refrigerator for a maximum period of 1 week in the dark.

B.7 Mueller Hinton blood agar

B.7.1 Basic medium

B.7.1.1 Composition

Enzymatic digest of animal tissues	6,0 g
Enzymatic digest of casein	17,5 g
Starch, soluble	1,5 g
Agar	8,0 g to 18,0 g[a]
Water	1 000 ml
[a] Depending on the gel strength of the agar.	

B.7.1.2 Preparation

Dissolve the basic components or the dehydrated complete basic medium in the water, by bringing to the boil. Adjust the pH, if necessary, so that after sterilization it is 7,3 ± 0,2 at 25 °C. Dispense the basic medium into flasks of suitable capacity. Sterilize in the autoclave (6.1) maintained at 121 °C for 15 min.

B.7.2 Sterile defibrinated sheep blood

B.7.3 Complete medium

B.7.3.1 Composition

Basic medium (B.7.1)	1 000 ml
Sterile defibrinated sheep blood (B.7.2)	50 ml

B.7.3.2 Preparation

Add the blood aseptically to the basic medium, cooled to 47 °C to 50 °C, then mix. Pour about 15 ml of the complete medium into sterile Petri dishes. Allow to solidify. Immediately before use, carefully dry the agar plates, preferably with the lids off and the agar surface downwards, in a drying cabinet (6.2) for 30 min or until the agar surface is free of visible moisture. If they have been prepared in advance, do not keep the undried agar plates for more than 4 h at ambient temperature, or for more than 7 days at (3 ± 2) °C.

B.8 Indoxyl acetate discs

B.8.1 Composition

Indoxyl acetate	0,1 g
Acetone	1 ml

B.8.2 Preparation

Dissolve the indoxyl acetate in the acetone. Add 25 µl to 50 µl of this solution to blank paper discs (diameter 6 mm to 12 mm). After drying at room temperature, store the discs at (3 ± 2) °C in a brown tube or bottle in the presence of silica gel.

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