ETHIOPIAN STANDARD

ES ISO 10272:2006

First edition
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Microbiology of food and animal feeding stuffs- Horizontal method for detection of thermotolerant Campylobacter

(Identical with ISO 10272:1995)

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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 10272 First edition, 1995 " Microbiology of food and animal feeding stuffs- Horizontal method for detection of thermotolerant Campylobacter", 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 corresponding Ethiopian Standard

International Standard	**Corresponding Ethiopian Standard**
ISO 6887:1983,Microbiology- General guidance for the preparation of dilutions for microbiological examination	ES ISO 6887:1983,Microbiology- General guidance for the preparation of dilutions for microbiological examination
ISO 7218 Microbiology - General rules for microbiological examinations	ES ISO 7218 Microbiology - General rules for microbiological examinations

INTERNATIONAL STANDARD

ISO 10272

First edition
1995-10-15

Microbiology of food and animal feeding stuffs — Horizontal method for detection of thermotolerant *Campylobacter*

Microbiologie des aliments — Méthode horizontale pour la recherche de Campylobacter *thermotolérants*

Reference number
ISO 10272:1995(E)

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 10272 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.

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Introduction

Because of the large variety of food and feed products, this horizontal method may not be appropriate in every detail for certain products, and for some other products it may be necessary to use different methods. Nevertheless, it is hoped that in all cases every attempt will be made to apply this horizontal method as far as possible and that deviations from this will only be made if absolutely necessary for technical reasons.

When this International Standard is next reviewed, account will be taken of all information then available regarding the extent to which this horizontal method has been followed and the reasons for deviations from this in the case of particular products.

The harmonization of test methods cannot be immediate and, for certain group of products, International Standards and/or national standards may already exist that do not comply with this horizontal method. It is hoped that when such standards are reviewed they will be changed to comply with this International Standard so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

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Microbiology of food and animal feeding stuffs — Horizontal method for detection of thermotolerant *Campylobacter*

1 Scope

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

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

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 6887:1983, *Microbiology — General guidance for the preparation of dilutions for microbiological examination.*

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

3 Definitions

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

3.1 thermotolerant *Campylobacter*: Microorganisms forming characteristic colonies on solid selective media when incubated at 42 °C and which possess the characteristic motility and biochemical properties described when the test is conducted in accordance with this International Standard.

3.2 detection of thermotolerant *Campylobacter*: Determination of the presence or absence of these microorganisms in a defined quantity of product, when the test is conducted in accordance with this International Standard.

4 Principle

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

4.1 Enrichment in the liquid medium

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

— Preston broth, or

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

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

— at 42° C for 18 h for the Preston broth;

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

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

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

4.2 Plating out and identification

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

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

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

4.3 Confirmation

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

5 Culture media and reagents

5.1 General

For current laboratory practice, see ISO 7218.

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

5.2 Liquid enrichment media

5.2.1 Preston broth

See B.1.

5.2.2 Park and Sanders broth

See B.2.

5.3 Selective plating-out media

5.3.1 Karmali agar

See B.3.

5.3.2 Modified Butzler agar

See B.4.

5.3.3 Skirrow agar

See B.5.

5.3.4 Charcoal cefoperazone deoxycholate agar (CCDA)

See B.6.

5.3.5 Preston agar

See B.7.

5.4 Identification media and reagents

5.4.1 *Brucella* broth

See B.8.

5.4.2 Columbia blood agar

See B.9.

5.4.3 Reagent for the detection of oxidase

See B.10.

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

See B.11.

5.4.5 Hydrogen peroxide solution, 3 %

5.4.6 Reagents for the detection of the hydrolysis of hippurate

See B.12.

5.4.7 Mueller Hinton blood agar

See B.13.

6 Apparatus and glassware

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

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

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

See ISO 7218.

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

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

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

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

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

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

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

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

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

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

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

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

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

6.14 Appropriate apparatus for growth in a microaerophilic atmosphere and for allowing a constant oxygen content of 5 % and, for example, a carbon dioxide content of 10 % and nitrogen content of 85 % to be maintained throughout the incubation.

NOTE 5 Use appropriate gastight containers able to hold Petri dishes and/or flasks or bottles of about 350 ml capacity used for the enrichment broth; e.g. bacteriological anaerobic jars. A microaerophilic atmosphere with an oxygen content of between 3 % and 6 % may be obtained using commercially available gas-generating kits (follow precisely the manufacturers instructions, particularly those relating to the volume of the jar and the capacity of the gas-generating kit). Alternatively, sparging of the jar prior to incubation with a gas mixture containing 5 % oxygen in 10 % carbon dioxide and 85 % nitrogen may be used.

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 dealing with the product concerned. If there is no specific International Standard, it is recommended that the parties concerned come to an agreement on this subject.

9 Procedure

9.1 Test portion, initial suspension and dilutions

9.1.1 See ISO 6887 and the specific International Standard dealing with the product concerned.

9.1.2 In general, for preparing the initial suspension, introduce a quantity x of the test portion (mass or volume) into a $9x$ volume of the enrichment medium, Preston broth (5.2.1) or Park and Sanders broth

(5.2.2), so as to obtain a test portion/enrichment medium ratio of 1/10 (mass/volume or volume/volume ratio).

9.2 Direct plating out

For those products suspected of containing a large quantity of thermotolerant *Campylobacter*, use a loop (6.11) to plate the non-incubated initial suspension (9.1.2) onto the surface of the Karmali agar (5.3.1) and another medium (5.3.2, 5.3.3, 5.3.4 or 5.3.5).

Incubate at 42 °C in a microaerophilic atmosphere (6.14) with inspection after 48 h, 72 h and, if necessary, 5 days to detect the presence of colonies presumed to be thermotolerant *Campylobacter*.

Then proceed as described in 9.4.3 and 9.5.

9.3 Enrichment

9.3.1 If the Preston broth is used, incubate the initial suspension (9.1.2) at 42 °C in a microaerophilic atmosphere (6.14) for 18 h.

9.3.2 If the Park and Sanders broth is used, incubate in a microaerophilic atmosphere (6.14) the initial suspension (9.1.2) at 32 °C for 4 h, then add the antibiotic solution B (B.2.4) at a concentration of 5 % (*V/V*). Incubate at 37 °C for 2 h, then at 42 °C for 40 h to 42 h.

9.4 Plating out and identification

9.4.1 Using the culture obtained in the enrichment medium (9.3.1 or 9.3.2), inoculate with a loop (6.11) the surface of the first selective isolation medium, the Karmali agar (5.3.1).

Proceed in the same manner with the second selective isolation medium chosen (5.3.2, 5.3.3, 5.3.4 or 5.3.5).

9.4.2 Incubate the dishes (9.4.1) at 42 °C in a microaerophilic atmosphere (6.14).

9.4.3 After 24 h or, more generally, 48 h and even 3 to 5 days of incubation, examine the dishes to detect the presence of characteristic colonies of thermotolerant *Campylobacter*.

On the Karmali agar, the characteristic colonies are greyish, flat and moistened, with a tendency to spread. On the Butzler and Skirrow agars, the characteristic colonies have a greyish to brownish colour, and may be of different sizes on the two media.

9.5 Confirmation

As the bacteria need a microaerophilic atmosphere to survive, follow the procedure described in 9.5.1 to 9.5.2.4 without any delay.

9.5.1 Selection of colonies for confirmation

Select, for confirmation tests, a total of five typical and/or suspect colonies from all the inoculated dishes (9.2, 9.4.3). If there are less than five, select all the colonies.

9.5.2 Examination of morphology and motility

9.5.2.1 Using a well-isolated colony, conduct a Gram's stain on each of the dishes (9.4.3).

9.5.2.2 Emulsify separately each of the colonies selected in 9.5.1 in 1 ml of *Brucella* broth (5.4.1).

9.5.2.3 Examine each of the selected colonies for morphology and motility using the microscope (6.13).

9.5.2.4 Retain for further examination all suspensions (9.5.2.2) in which curved Gram-negative bacilli (9.5.2.1) with a spiralling "corkscrew" motility are found (9.5.2.3).

9.5.3 Study of morphology in a Gram's strain

Using each selected suspension (9.5.2.4), inoculate with a loop (6.11) the surface of a Columbia blood agar plate (5.4.2) in order to allow the development of well-isolated colonies.

Incubate the inoculated dishes in a microaerophilic atmosphere (6.14) at 42 °C for 24 h and use the pure cultures for the biochemical tests.

9.5.4 Study of growth at 25 °C

Using the colonies isolated in 9.5.3, inoculate, using a loop (6.11), a tube of *Brucella* broth (5.4.1).

Incubate at 25 °C in a microaerophilic atmosphere (6.14) for 2 to 5 days.

Examine to check whether or not there is growth.

9.5.5 Biochemical tests

9.5.5.1 Detection of oxidase

Using a platinum/iridum loop or glass rod (6.11), take a portion of a well-isolated colony from each individual plate (9.5.3) and streak onto a filter paper moistened with the oxidase reagent (5.4.3); the appearance of a mauve, violet or deep blue colour within 10 s is 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.

9.5.5.2 TSI agar (5.4.4)

Using the wire (6.11), inoculate the agar with each of the colonies selected in 9.5.3.

Inoculate the agar slant in longitudinal streaks and stab the butt.

Incubate at 42 °C for 24 h and extend the incubation to 5 days if necessary in a microaerophilic atmosphere (6.14).

Interpret the reactions in the following manner:

Butt	
Yellow	glucose positive (fermentation of glucose)
Red or unchanged	glucose negative (no fermentation of glucose)
Black	formation of hydrogen sulfide (H_2S)
Bubbles or cracks	gas formation from glucose
Slant surface	
Yellow	lactose and/or sucrose positive (one or both sugars used)
Red or unchanged	lactose and sucrose negative (no sugar used)

9.5.5.3 Detection of catalase

Deposit a loop of culture from the TSI agar (9.5.5.2) into a drop of hydrogen peroxide solution (5.4.5) on a clean microscope slide.

The test is positive if bubbles appear in 30 s.

9.5.5.4 Detection of sensitivity to nalidixic acid and to cephalothin[2)]

Inoculate, using a loop (6.11), the colonies selected in 9.5.2.4, in a suspension of density 0,5 on the MacFarland scale, into the *Brucella* broth.

Then dilute this suspension 1:10 with the same broth.

Flood the surface of a Mueller Hinton 5 % blood agar plate (5.4.7) with the suspension.

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

Dry the dishes in the oven (6.2) set at 37 °C for 10 min.

On the surface of the agar, place a disc of nalidixic acid (30 µg) and a disc of cephalothin (30 µg).

Incubate at 37 °C for 24 h in a microaerophilic 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 **sensitive**.

9.5.5.5 Detection of hydrolysis of hippurate[2)]

In a haemolysis tube (6.7) containing 0,4 ml of the sodium hippurate solution (5.4.6), inoculate, using a loop (6.11), the colonies selected in 9.5.3 taking care not to incorporate any agar.

Shake in order to mix thoroughly and incubate for 2 h in the water bath (6.4) set at 37 °C.

Carefully add 0,2 ml of the ninhydrin solution (B.12.2) 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) set at 37 °C.

The reaction is positive when the colour is dark violet.

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

2) These tests are useful for differentiating the species of thermotolerant *Campylobacter*.

9.6 Interpretation of results

Thermotolerant *Campylobacter* give the following results:

Campylobacter spp.	
Morphology (9.5.2.3)	small curved bacilli
Motility (9.5.2.3)	characteristic
Gram's strain (9.5.3)	–
Oxidase (9.5.5.1)	+
Glucose (9.5.5.2)	–
Lactose (9.5.5.2)	–
Sucrose (9.5.5.2)	–
Gas (9.5.5.2)	–

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

Among the *Campylobacter* spp. growing at 42 °C, certain species constitute the thermotolerant group, of which the most frequently encountered species are *Campylobacter jejuni* and *Campylobacter coli*. Other species have, however, been described (*Campylobacter lari* and *Campylobacter upsaliensis*); the characteristics given in table 1 allow differentiation of them.

10 Expression of results

According to the interpretation of the results, indicate the presence or absence of thermotolerant *Campylobacter* in a test portion of x g or x ml of product.

11 Test report

The test report shall indicate

— the method in accordance with which sampling was carried out, if known;

— the method used;

— the chosen incubation temperature, and

— the test results obtained.

It shall also mention 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 results.

The test report shall include all information necessary for the complete identification of the sample.

Table 1

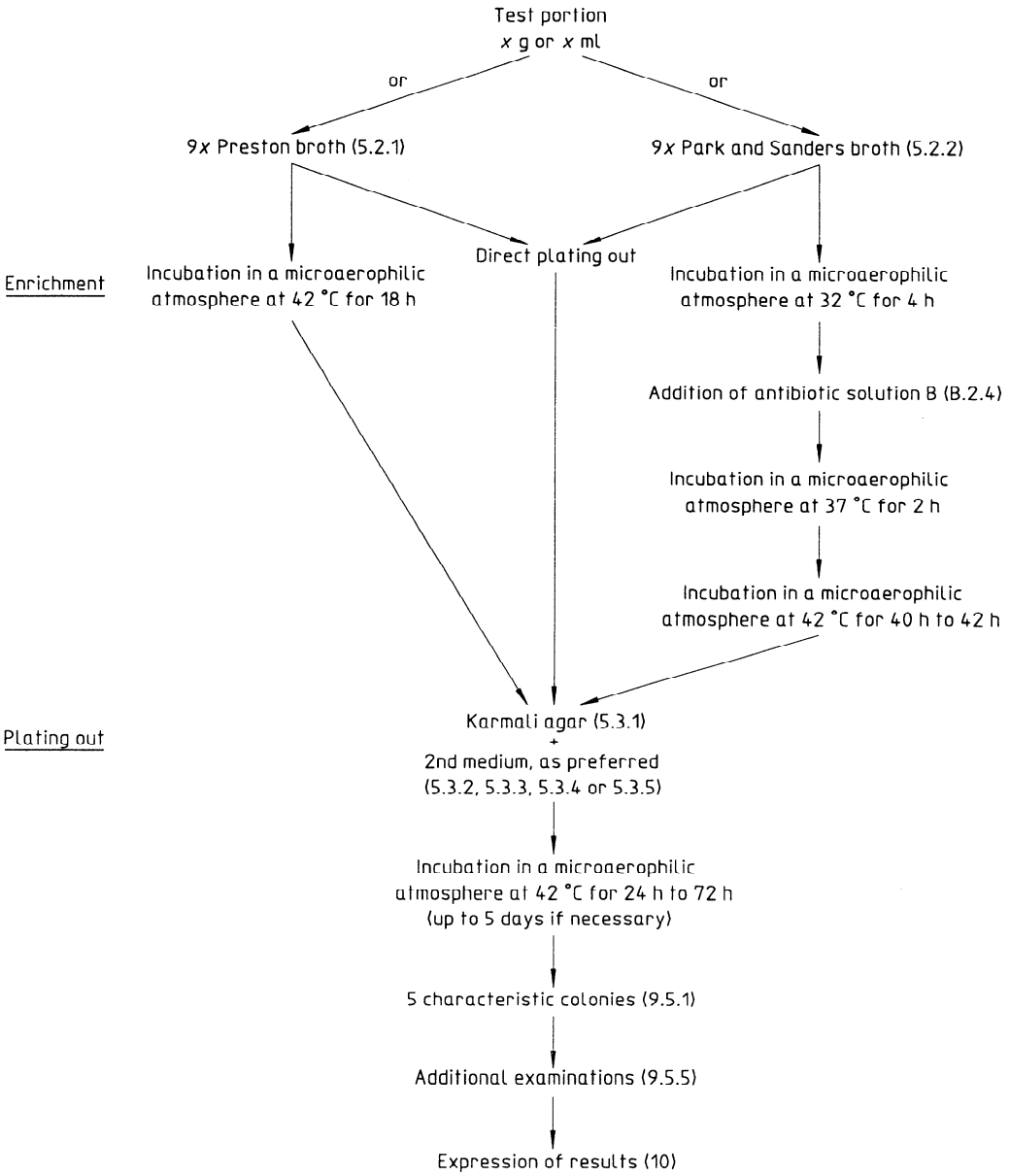
Characteristics	*C. jejuni*	*C. coli*	*C. lari*	*C. upsaliensis*
Growth at 25 °C (9.5.4)	–	–	–	–
H_2S (TSI) (9.5.5.2)[1]	–	(+)	–	–
Nalidixic acid (9.5.5.4)	S	S	R	S
Hydrolysis of hippurate (9.5.5.5)	+	–	–	–
Catalase (9.5.5.3)	+	+	+	– or slight
Cephalothin (9.5.5.4)	R	R	R	S

Key: + = positive; – = negative; (+) = slightly positive; S = sensitive; R = resistant.

1) Slight development of H_2S in the condensed water after 5 days.

Annex A
(normative)

Diagram of procedure



Annex B
(normative)

Composition and preparation of culture media and reagents

B.1 Preston broth

B.1.1 Basic medium

B.1.1.1 Composition

Meat extract	10,0 g
Peptone	10,0 g
Sodium chloride	5,0 g
Agar	1,0 g
Water	1 000 ml

B.1.1.2 Preparation

Dissolve the basic components or the dehydrated complete basic medium in the water, by heating until completely dissolved.

Adjust the pH, if necessary, so that after sterilization it is 7,5 ± 0,2 at 25 °C.

Dispense the basic medium into bottles of suitable capacity.

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

B.1.2 Sterile lysed defibrinated horse blood

Use blood lysed by freezing then thawing out.

B.1.3 Antibiotic solution

B.1.3.1 Composition

Polymyxin B	5 000 IU
Rifampicin	0,010 g
Trimethoprim lactate	0,010 g
Cycloheximide	0,100 g
Ethanol, 95 % (*V/V*)	10 ml

B.1.3.2 Preparation

Dissolve the components in the ethanol.

B.1.4 Complete medium

B.1.4.1 Composition

Basic medium (B.1.1)	940 ml
Sterile lysed defibrinated horse blood (B.1.2)	50 ml
Antibiotic solution (B.1.3)	10 ml

B.1.4.2 Preparation

Add the blood aseptically to the basic medium cooled down to about 45 °C, then add the antibiotic solution and mix.

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

B.2 Park and Sanders broth

B.2.1 Basic medium

B.2.1.1 Composition

Tryptone	10,0 g
Meat peptic peptone	10,0 g
Glucose	1,0 g
Yeast extract	2,0 g
Sodium citrate	1,0 g
Sodium chloride	5,0 g
Sodium hydrogen sulfite	0,1 g
Sodium pyruvate	0,25 g
Water	945 ml

B.2.1.2 Preparation

Dissolve the basic components or the dehydrated complete basic 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 basic medium into flasks of suitable capacity.

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

B.2.2 Sterile lysed defibrinated horse blood

Use blood lysed by freezing then thawed out.

B.2.3 Antibiotic solution A

B.2.3.1 Composition

Vancomycin	0,02 g
Trimethoprim lactate	0,02 g
Ethanol, 95 % (*V/V*)	5 ml
Water	5 ml

B.2.3.2 Preparation

Mix the water and the ethanol.

Dissolve the components in the water/ethanol.

Sterilize by filtration.

B.2.4 Antibiotic solution B

B.2.4.1 Composition

Cefoperazone	0,032 g
Cycloheximide	0,1 g
Water/acetone, 50/50 (*V/V*)	5 ml

B.2.4.2 Preparation

Dissolve the components in the water/acetone.

Sterilize by filtration.

This solution is subsequently added to the complete medium at the rate of 5 % (*V/V*) (see 9.3.2).

B.2.5 "Complete" medium

B.2.5.1 Composition

Basic medium (B.2.1)	945 ml
Sterile lysed defibrinated horse blood (B.2.2)	50 ml
Antibiotic solution A (B.2.3)	5 ml

B.2.5.2 Preparation

To the basic medium, at a temperature of 45 °C or less, add the blood aseptically, then the antibiotic solution A and mix.

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

B.3 Karmali agar (CSM)

B.3.1 Basic medium

B.3.1.1 Composition

Columbia agar (basic medium without water) (B.9.1)	39 g to 42 g [1]
Active charcoal	4 g
Water	1 000 ml

1) Depending on which manufacturer.

B.3.1.2 Preparation

Dissolve the 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) set at 121 °C for 15 min.

B.3.2 Haemin solution

B.3.2.1 Composition

Haemin	0,32 g
Water	100 ml

B.3.2.2 Preparation

Dissolve the haemin in the water.

Sterilize by filtration.

B.3.3 Supplemented antibiotic solution

B.3.3.1 Composition

Sodium pyruvate	0,2 g
Vancomycin	0,04 g
Cefoperazone	0,064 g
Cycloheximide	0,2 g
Ethanol, 95 % (*V/V*)	20 ml

B.3.3.2 Preparation

Dissolve the components in the ethanol.

Sterilize by filtration.

B.3.4 Complete medium

B.3.4.1 Composition

Basic medium (B.3.1)	980 ml
Haemin solution (B.3.2)	10 ml
Supplemented antibiotic solution (B.3.3)	10 ml

B.3.4.2 Preparation

Add, under sterile conditions, the haemin solution to the basic medium, melted then recooled to about 47 °C, then add the supplemented antibiotic solution and 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, the undried agar plates shall not be kept for more than 4 h at ambient temperature or more than 3 days at + 3 °C ± 2 °C.

B.4 Modified Butzler agar

B.4.1 Basic medium: Columbia agar

B.4.1.1 Composition

Peptone	23,0 g
Starch, soluble	1,0 g
Sodium chloride	5,0 g
Agar	8,0 g to 18,0 g [1]
Water	1 000 ml

1) Depending on the gel strength of the agar.

B.4.1.2 Preparation

Dissolve the basic components or the dehydrated complete basic 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 basic medium into flasks of suitable capacity.

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

B.4.2 Sterile defibrinated sheep blood

B.4.3 Antibiotic solution

B.4.3.1 Composition

Cefoperazone	0,03 g
Rifampicin	0,02 g
Colistin	20 000 IU
Amphotericin B	0,004 g
Water/ethanol, 50/50 (*V/V*)	20 ml

B.4.3.2 Preparation

Dissolve the components in the water/ethanol.

Sterilize by filtration.

B.4.4 Complete medium

B.4.4.1 Composition

Basic medium (B.4.1)	940 ml
Sterile defibrinated sheep blood (B.4.2)	50 ml
Antibiotic solution (B.4.3)	10 ml

B.4.4.2 Preparation

Add the blood then the antibiotic solution aseptically to the basic medium, melted and recooled to about 47 °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, the undried agar plates shall not be kept for more than 4 h at ambient temperature or more than 3 days at + 3 °C ± 2 °C.

B.5 Skirrow agar

B.5.1 Basic medium

B.5.1.1 Composition

Proteose peptone	15,0 g
Liver enzymatic extract	2,5 g
Yeast extract	5,0 g
Sodium chloride	5,0 g
Agar	8,0 g to 18,0 g [1]
Water	1 000 ml

1) Depending on the gel strength of the agar.

B.5.1.2 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) set at 121 °C for 15 min.

B.5.2 Sterile lysed defibrinated horse blood

Use blood lysed by freezing then thawing out.

B.5.3 Antibiotic solution

B.5.3.1 Composition

Vancomycin	0,02 g
Trimethoprin lactate	0,01 g
Polymyxin B	5 000 IU
Water	20 ml

B.5.3.2 Preparation

Dissolve the components in the water.

Sterilize by filtration.

B.5.4 Complete medium

B.5.4.1 Composition

Basic medium (B.5.1)	940 ml
Sterile lysed defibrinated horse blood (B.5.2)	50 ml
Antibiotic solution (B.5.3)	10 ml

B.5.4.2 Preparation

Add the blood then the antibiotic solution aseptically to the basic medium, melted and recooled to about 47 °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, the undried agar plates shall not be kept for more than 4 h at ambient temperature or more than 3 days at + 3 °C ± 2 °C.

INTERNATIONAL STANDARD ISO 10272:1995
TECHNICAL CORRIGENDUM 1

Published 1996-06-15

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Microbiology of food and animal feeding stuffs — Horizontal method for detection of thermotolerant *Campylobacter*

TECHNICAL CORRIGENDUM 1

Microbiologie des aliments — Méthode horizontale pour la recherche de Campylobacter *thermotolérants*

RECTIFICATIF TECHNIQUE 1

Technical corrigendum 1 to International Standard ISO 10272:1995 was prepared by Technical Committee ISO/TC 34, *Agricultural food products,* Subcommittee SC 9, *Microbiology.*

Page 1

Clause 1

In the last line, replace "started" with "stated".

Page 5

Subclause 9.5.5.1

In the first line, replace "platinum/iridum" with "platinum/iridium".

Page 6

Subclause 9.6

In line 6, replace "Gram's strain" with Gram's stain".

Page 9

Subclause B.2.4.1

Replace the amount "5 ml" with the amount "50 ml".

ICS 07.100 **Ref. No. ISO 10272:1995/Cor.1:1996(E)**

Descriptors : agricultural products, food products, animal feeding products, tests, microbiological analysis, detection, microorganisms, bacteria.

Page 11

Subclause B.5.3.1

In line 2, replace "Trimethoprin" with "Trimethoprim".

Page 13

Subclause B.7.3.1

Replace the unit "mg"with the unit "g".

B.6 Charcoal cefoperazone desoxycholate agar (CCDA)

B.6.1 Basic medium

B.6.1.1 Composition

Meat extract	10,0 g
Peptone	10,0 g
Sodium chloride	5,0 g
Charcoal	4,0 g
Casein hydrolysate	3,0 g
Sodium desoxycholate	1,0 g
Iron(II) sulfate	0,25 g
Sodium pyruvate	0,25 g
Agar	8,0 g to 18,0 g [1]
Water	1 000 ml

1) Depending on the gel strength of the agar.

B.6.1.2 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) set at 121 °C for 15 min.

B.6.2 Cefoperazone solution

B.6.2.1 Composition

Cefoperazone	0,064 g
Water	20 ml

B.6.2.2 Preparation

Dissolve the cefoperazone in the water.

Sterilize by filtration.

B.6.3 Complete medium

B.6.3.1 Composition

Basic medium (B.6.1)	990 ml
Cefoperazone solution (B.6.2)	10 ml

B.6.3.2 Preparation

Add the cefoperazone solution to the basic medium, melted and recooled to about 47 °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, the undried agar plates shall not be kept for more than 4 h at ambient temperature or more than 3 days at + 3 °C ± 2 °C.

B.7 Preston agar

B.7.1 Basic medium

B.7.1.1 Composition

Meat extract	10,0 g
Peptone	10,0 g
Sodium chloride	5,0 g
Agar	8,0 g to 18,0 g [1]
Water	1 000 ml

1) 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 7,4 ± 0,2 at 25 °C.

Dispense the basic medium into flasks of suitable capacity.

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TECHNICAL CORRIGENDUM 2

Published 1997-08-01

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Microbiology of food and animal feeding stuffs — Horizontal method for detection of thermotolerant *Campylobacter*

TECHNICAL CORRIGENDUM 2

Microbiologie des aliments — Méthode horizontale pour la recherche de Campylobacter *thermotolérants*

RECTIFICATIF TECHNIQUE 2

Technical Corrigendum 2 to International Standard ISO 10272:1995 was prepared by Technical Committee ISO/TC 34, *Agricultural food products,* Subcommittee SC 9, *Microbiology.*

Page 4

Subclause 9.3.2

Replace "5 % (*V/V*)" by "5 ‰ (*V/V*)".

Page 9

Subclause B.2.4.1

Technical Corrigendum 1 was incorrect. The volume of water/acetone should be 5 ml.

Subclause B.2.4.2

Replace "5 % (*V/V*)" by "5 ‰ (*V/V*)".

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

B.7.2 Sterile lysed defibrinated horse blood

Use blood lysed by freezing then thawing out.

B.7.3 Antibiotic solution

B.7.3.1 Composition

Polymyxin B	5 000 IU
Rifampicin	0,010 mg
Trimethoprim lactate	0,010 mg
Cycloheximide	0,100 mg
Ethanol, 95 % (*V/V*)	10 ml

Dissolve the components in the ethanol.

Sterilize by filtration.

B.7.4 Complete medium

B.7.4.1 Composition

Basic medium (B.7.1)	940 ml
Sterile lysed defibrinated horse blood (B.7.2)	50 ml
Antibiotic solution (B.7.3)	10 ml

B.7.4.2 Preparation

Add the blood then the antibiotic solution aseptically to the basic medium, melted and recooled to about 47 °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 oven (6.2) for 30 min or until the agar surface is free of visible moisture.

If they have been prepared in advance, the undried agar plates shall not be kept for more than 4 h at ambient temperature or more than 3 days at + 3 °C ± 2 °C.

B.8 *Brucella* broth

B.8.1 Composition

Tryptone	10,0 g
Meat peptic peptone	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.8.2 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) set at 121 °C for 15 min.

B.9 Columbia blood agar

B.9.1 Basic medium

B.9.1.1 Composition

Peptones	23,0 g
Starch	1,0 g
Sodium chloride	5,0 g
Agar	8,0 g to 18,0 g [1]
Water	1 000 ml

1) Depending on the gel strength of the agar.

B.9.1.2 Preparation

Dissolve the basic components or the dehydrated complete 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) set at 121 °C for 15 min.

B.9.2 Sterile defibrinated sheep blood

B.9.3 Complete medium

B.9.3.1 Composition

Basic medium (B.9.1)	950 ml
Sterile defibrinated sheep blood (B.9.2)	50 ml

B.9.3.2 Preparation

Add the blood aseptically to the basic medium, melted and recooled to about 47 °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, the undried agar plates shall not be kept for more than 4 h at ambient temperature or more than 7 days at + 3 °C ± 2 °C.

B.10 Reagent for detecting oxidase

B.10.1 Composition

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

B.10.2 Preparation

Dissolve the component in the water immediately prior to use.

B.11 Triple sugar/Iron agar (TSI)

B.11.1 Composition

Meat extract	3,0 g
Yeast extract	3,0 g
Peptone	20,0 g
Sodium chloride	5,0 g
Lactose	10,0 g
Sucrose	10,0 g
Glucose	1,0 g
Iron(III) citrate	0,3 g
Sodium thiosulfate	0,3 g
Phenol red	0,024 g
Agar	8,0 g to 18,0 g [1]
Water	1 000 ml

1) Depending on the gel strength of the agar.

B.11.2 Preparation

Dissolve the basic components or the dehydrated complete 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 medium in quantities of 10 ml into tubes of suitable capacity.

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

Allow to stand in the inclined position so as to obtain a butt of 2,5 cm in depth.

B.12 Reagents for detecting the hydrolysis of hippurate

B.12.1 Sodium hippurate solution

B.12.1.1 Composition

Sodium hippurate	10 g
Phosphate-buffered saline (PBS) consisting of:	
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, to a final volume of	1 000 ml

B.12.1.2 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.12.2 Ninhydrin solution, 3,5 %

B.12.2.1 Composition

Ninhydrin	1,75 g
Acetone	25 ml
Butanol	25 ml

B.12.2.2 Preparation

Dissolve the ninhydrin in the acetone/butanol mixture.

Store the solution in the refrigerator for a maximum period of 1 week away from light.

B.13 Mueller Hinton blood agar

B.13.1 Basic medium

B.13.1.1 Composition

Meat infusion	6,0 g
Casein hydrolysate	17,5 g
Starch, soluble	1,5 g
Agar	8,0 g to 18,0 g [1)]
Water	1 000 ml

1) Depending on the gel strength of the agar.

B.13.1.2 Preparation

Dissolve the basic components or the dehydrated complete basic medium in the water, 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) set at 121 °C for 15 min.

B.13.2 Sterile sheep blood

B.13.3 Complete medium

B.13.3.1 Composition

Basic medium (B.13.1)	950 ml
Sterile sheep blood (B.13.2)	50 ml

B.13.3.2 Preparation

Add the blood aseptically to the basic medium, melted and recooled to about 47 °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, the undried agar plates shall not be kept for more than 4 h at ambient temperature or more than 7 days at + 3 °C ± 2 °C.

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