

Australian Standard®

AS 5013.6:2015

Food microbiology

Method 6: Examination for specific organisms—*Campylobacter*

PREFACE

This Standard was prepared by the Standards Australia Committee FT-035, Food Microbiology, to supersede AS 5013.6—2004.

The objectives of this revision are—

- (a) to update the references;
- (b) to incorporate culture media, reagents and to remove references to AS 1766.5;
- (c) to update reference cultures; and
- (d) to incorporate minor technical variations on the apparatus used in the test technique.

During the review, the Committee considered ISO 10272-1:2006, *Microbiology of food and animal feeding stuffs—Horizontal method for detection and enumeration of Campylobacter spp.*, Part 1: *Detection method*, and ISO/TS 10272-2:2006, *Microbiology of food and animal feeding stuffs—Horizontal method for detection and enumeration of Campylobacter spp.*, Part 2: *Colony-count technique*, for adoption.

ISO 10272-1 and ISO 10272-2 have not been adopted due to the age of these methods and because work has commenced to technically revise the first editions. The ISO drafts are in the very early stages of drafting.

The terms ‘normative’ and ‘informative’ have been used in this Standard to define the application of the appendices to which they apply. A ‘normative’ appendix is an integral part of a Standard, whereas an ‘informative’ appendix is only for information and guidance.

METHOD

1 SCOPE

This Standard sets out reference methods for the detection and enumeration of *Campylobacter jejuni* and *Campylobacter coli* in meat and poultry. Two procedures are described as follows:

- (a) A qualitative test using enrichment culture.
- (b) A quantitative test using a surface spread technique.

NOTE: A flow diagram of the examination procedures is shown in Appendix A.

2 SAFETY PRECAUTIONS

The safety precautions described in AS/NZS 2243.3 shall be observed.

3 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS

- | | |
|-----------|--|
| 5013 | Food microbiology (series) |
| 5013.11.2 | Method 11.2: Microbiology of food and animal feeding stuffs—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—Specific rules for the preparation of meat and meat products |
| 5013.14.3 | Method 14.3: Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of microorganisms—Colony count at 30°C by the surface plating technique |
| 5013.20 | Method 20: Preparation of test samples for microbiological examination—Poultry and poultry products |

AS/NZS

- | | |
|--------|--|
| 2243 | Safety in laboratories |
| 2243.3 | Part 3: Microbiological aspects and containment facilities |

4 CULTURE MEDIA, REAGENTS AND REFERENCE CULTURES

4.1 Media (refer to Appendix B)

4.1.1 *Blood agar*

4.1.2 *Preston broth*

4.1.3 *Preston agar*

4.1.4 *Skirrow agar*

4.1.5 *Nalidixic acid antimicrobial sensitivity discs, 30 µg*

4.1.6 *Cephalothin antimicrobial sensitivity discs, 30 µg*

4.1.7 *Nutrient broth*

4.2 Reagents

4.2.1 *Sodium hippurate*

10 g/L aqueous solution.

4.2.2 *Ninhydrin solution*

3.5 percent in 1:1 mixture of acetone and butanol.

4.2.3 *Oxidase reagent (Kovacs)*

4.2.4 *Saline solution*

8.5 g/L aqueous solution.

4.3 Reference cultures

The following cultures acquired from recognized culture collections should be used as reference cultures:

Campylobacter jejuni NCTC 13367 or WDCM 00005.

Campylobacter coli NCTC 11366 or WDCM 00072.

NOTE:

WDCM—World Data Centre for Microorganisms.

NCTC—National Collection of Type Cultures.

5 APPARATUS

5.1 Blender

Capable of producing a homogeneous fine slurry of sample and medium as required in Clause 6.3.1. A peristaltic-type blender is preferable.

5.2 Gas cylinders or kits

Capable of producing a microaerobic incubation atmosphere (see Clause 6.2).

5.3 Incubators

Capable of operating at $25 \pm 1^\circ\text{C}$, $37 \pm 1^\circ\text{C}$ and $42 \pm 1^\circ\text{C}$.

5.4 Water bath

Capable of operating at $37 \pm 1^\circ\text{C}$.

5.5 Microscope

Preferably with phase contrast (for observing the characteristic motility of *Campylobacter*).

6 TEST PROCEDURES

6.1 General

The sample is mixed thoroughly with enrichment broth and 0.1 mL is used for the surface spread method (Clause 6.6, Quantitative test). The remainder is used for the enrichment culture method (Clause 6.5, Qualitative test).

6.2 Microaerobic incubation conditions

Growth of campylobacters is enhanced by incubation in a special microaerobic atmosphere.

Apparatus suitable for achieving a microaerobic atmosphere with oxygen content of $5 \pm 2\%$, carbon dioxide $10 \pm 3\%$, optional hydrogen $\leq 10\%$, with the balance nitrogen shall be used. The appropriate microaerobic atmosphere can be obtained using gastight jars and commercially available gas-generating kits, following precisely the manufacturer's instructions. Alternatively, the jar or incubator may be filled with an appropriate gas mixture prior to incubation using commercially available gas cylinders.

6.3 Preparation of first dilution

6.3.1 Solid samples

Solid samples taken in accordance with AS 5013.11.2 and AS 5013.20 shall be treated as follows:

- Weigh aseptically between 24.8 g and 25.0 g (maximum) of test sample into a blender bag or container. Record the mass of the test portion.
- Add 100 mL of the Preston broth (without antibiotic supplement).
- Homogenize for 30 s or until finely divided.
- Take 0.1 mL portions of this dilution for the surface spread method (Clause 6.6) and use the remainder for the qualitative test (Clause 6.5).

6.3.2 Rinse fluids

Rinse fluids from poultry obtained by the rinse technique in AS 5013.20 shall be treated as follows:

- For the surface spread method (Clause 6.6), take 0.1 mL portions of the rinse fluid.
- For enrichment culture (Clause 6.5), add 50 mL of rinse fluid to 50 mL of double strength Preston broth (without antibiotic supplement) and mix well.

6.4 Controls for broth culture

Separate tubes of Preston broth (without antibiotic supplement) shall be inoculated with the reference cultures specified in Clause 4.3.

6.5 Qualitative test—Enrichment culture method

6.5.1 Resuscitation

The first dilutions (Clause 6.3) and controls (Clause 6.4) shall be incubated at $37 \pm 1^\circ\text{C}$ for 2 h.

6.5.2 Selective enrichment

After the two hours incubation, 0.4 mL of antibiotic supplement shall be added to each 100 mL of broth culture. The selective broths shall be incubated under microaerobic conditions (see Clause 6.2) at $42 \pm 1^\circ\text{C}$ for 44 ± 4 h.

6.5.3 Plating out on selective agar media

Each enrichment broth culture shall be inoculated onto plates of Preston agar and Skirrow agar as follows:

- Using a 5 mm loop, streak the surface of each agar plate to obtain isolated colonies.
- Incubate the plates at $42 \pm 1^\circ\text{C}$ in a microaerobic atmosphere (see Clause 6.2).
- Examine the plates at 21 ± 3 h and at 44 ± 4 h incubation. If suspect colonies have formed, select three well-isolated colonies for the confirmation tests in Clause 7.

NOTES:

- Campylobacter* colonies are smooth, flat, translucent and colourless to grey, with an irregular edge, pinpoint to 4 mm in diameter, often spreading along the streak line.
- Microscopic examination (dark field or phase contrast) of wet mounts from colonies of campylobacter will show rapidly mobile, darting, curved, S-shaped or spiral cells. Non-motile, non-culturable coccal forms may also be present.
- It is not necessary to confirm the presence of *Campylobacter* if confirmed *C. jejuni*/*C. coli* have been isolated from the same sample (see Clause 6.6).

6.6 Quantitative test—Surface spread method

The procedure shall be as follows:

- Using the surface spread method described in AS 5013.14.3, inoculate the 0.1 mL portions of unincubated first dilution (Clause 6.3) onto plates of Preston agar and plates of Skirrow agar.

NOTE: Two culture media are used in order to improve the recovery of campylobacter according to the serotypes present and the type of food being examined.

- As controls, inoculate half plates of Preston agar and of Skirrow agar with each of the reference cultures specified in Clause 4.3.
- Incubate the plates at $42 \pm 1^\circ\text{C}$ for 44 ± 4 h under microaerobic conditions (see Clause 6.2).
- Examine the plates at 44 ± 4 h and count the number of suspect colonies [see Notes to Clause 6.5.3(c)] per plate.
- For colony counts exceeding 100, select at random, 10 of the typical colonies on the presumptive plates; for colony counts below 100, select a number which approximates the square root of the count. Confirm the selected colonies by the methods described in Clause 7.
- Estimate the counts per unit mass or per unit surface area of product by the methods set out in AS 5013.11.2 and AS 5013.20 on both the Preston and Skirrow agars and record the higher of the two counts and the agar medium from which it was obtained.

7 CONFIRMATION TESTS

7.1 Culture of selected colonies

The procedure shall be as follows:

- (a) Subculture each selected colony onto a separate blood agar plate.
- (b) Incubate the inoculated plates in a microaerobic atmosphere (see Clause 6.2) at $42 \pm 1^\circ\text{C}$ for 21 ± 3 h or until growth is visible.

7.2 Oxidase test and Gram stain

The procedure shall be as follows:

- (a) Check each blood agar culture for oxidase production.
NOTE: Campylobacters are oxidase-positive.
- (b) Perform Gram stains on all oxidase-positive cultures, using dilute carbol fuchsin rather than safranin as the counterstain.
NOTE: Typical campylobacter cells are Gram-negative, small ($0.2\ \mu\text{m}$ to $0.8\ \mu\text{m}$ by $1.5\ \mu\text{m}$ to $5\ \mu\text{m}$), curved, S-shaped or spiral.

7.3 Further confirmation tests

The procedure shall be as follows:

- (a) Inoculate the following media with the cultures that are Gram-negative and oxidase-positive as follows:
 - (i) A tube of nutrient broth. Incubate under microaerobic conditions at $42 \pm 1^\circ\text{C}$ for 21 ± 3 h.
 - (ii) Two blood agar plates. Incubate one *aerobically* at $37 \pm 1^\circ\text{C}$ and the other under microaerobic conditions at $25 \pm 1^\circ\text{C}$. Incubate the plates for up to 5 days or until growth occurs if before then.
 - (iii) One blood agar plate with a heavy suspension in saline solution spread over the surface of the plate (lawn culture). Place a nalidixic acid disc and a cephalothin disc separately on the dried surface of the plate. Incubate at $42 \pm 1^\circ\text{C}$ in a microaerobic atmosphere for up to two days.
 - (iv) 0.5 mL of sterile sodium hippurate solution (see Appendix C for hippurate test).
- (b) Inspect the incubated cultures and record the reactions observed in the following tests:
 - (i) Motility—campylobacters are motile in nutrient broth with a corkscrew or darting motion.
 - (ii) Conditions of growth—*C. jejuni*/*C. coli* do not grow at 37°C aerobically or at 25°C under microaerobic conditions.
 - (iii) Nalidixic acid sensitivity—*C. jejuni*/*C. coli* are sensitive to nalidixic acid.
 - (iv) Cephalothin sensitivity—*C. jejuni*/*C. coli* are resistant to cephalothin.
NOTE: Sensitivity to nalidixic acid or cephalothin is indicated if the annular radius of the zone of inhibition is 6 mm or more.
 - (v) Hippurate hydrolysis test—*C. jejuni* hydrolyses hippurate; *C. coli* does not.

8 REPORT

The report shall provide the following information:

- (a) All details necessary for the complete identification of the sample.
- (b) Reference to this Australian Standard, i.e. AS 5013.6.
- (c) The results obtained as follows:
 - (i) For the enrichment method—the presence or absence of campylobacters in the quantity of sample examined.
 - (ii) For the surface spread method—the highest estimated number of campylobacters per unit mass or per unit surface area of the sample, stating whether the count was obtained on Preston agar or on Skirrow agar.
- (d) The results of further confirmation tests identifying *C. jejuni* and *C. coli*.
- (e) Any circumstances or conditions that may have influenced the results.