Australian Standard®

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 ±1°C, 37 ±1°C and 42 ±1°C.

5.4 Water bath

Capable of operating at 37 ±1°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 ±2%, carbon dioxide 10 ±3%, optional hydrogen ≤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:

(a) 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.

(b) Add 100 mL of the Preston broth (without antibiotic supplement).

(c) Homogenize for 30 s or until finely divided.

(d) 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:

(a) For the surface spread method (Clause 6.6), take 0.1 mL portions of the rinse fluid.

(b) 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.