

HPA STANDARD METHOD

# DETECTION OF *CAMPYLOBACTER* SPECIES

F 21

Issued by Standards Unit, Evaluations and Standards Laboratory  
Centre for Infections

**DETECTION OF *CAMPYLOBACTER* SPECIES**

Issue no: 2.1 Issue date: 08.08.08 Issued by: Standards Unit, Evaluations and Standards Laboratory in conjunction with the  
Regional Food, Water and Environmental Coordinators Forum Page 1 of 12

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National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

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## **Suggested citation for this document:**

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# AMENDMENT PROCEDURE

<b>Controlled document reference</b>	<b>F 21</b>
<b>Controlled document title</b>	<b>Detection of <i>Campylobacter</i> species</b>

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no	Page	Section(s) involved	Amendment
6/08.08.08	2	2.1	5	<b>Introduction</b>	Scope expanded to include swabs and environmental samples Background expanded to explain that only strains capable of growth at 41.5°C will be recovered. Comparison with ISO 10272-1 inserted
			6	<b>Principle</b>	Pre-incubation of enrichment changed to 4-6 h Incubation of plates changed to 44 h
			6	<b>Definitions</b>	Definition of <i>Campylobacter</i> species expanded to specify growth under microaerobic conditions at 41.5°C
			6	<b>Safety considerations</b>	Information included about infectious dose and laboratory advised to perform risk assessment
			6	<b>Equipment</b>	Pastettes included
			7	<b>Culture media &amp; reagents</b>	Bolton broth: terminology of meat peptone changed; sodium chloride added mCCDA: nutrient broth no 2 replaced by components; cycloheximide added as alternative to amphotericin. Broth for motility and <i>Campylobacter</i> latex agglutination kit added
			8	<b>6.1 Sample preparation</b>	Instructions for swabs and environmental samples added
			8	<b>6.2 Selective enrichment</b>	Time tolerances changed for both stages of incubation
			9	<b>6.3 Subculture to selective agar</b>	Time tolerance changed
			9	<b>6.5 Confirmatory tests</b>	Time tolerances updated. Option given to perform either the cell morphology and motility test or a latex agglutination test Criteria for identification changed
			10	<b>Reporting of results</b>	Updated to include swabs
			10	<b>Reference facilities</b>	Updated
			11	<b>Appendix</b>	Updated in line with text
12	<b>References</b>	Updated; reference 5,6 and 7 inserted			

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# DETECTION OF *CAMPYLOBACTER* SPECIES

## INTRODUCTION

### Scope

The method described is applicable to the detection of thermotolerant *Campylobacter* species in all food types and environmental samples including swabs.

### Background

*Campylobacter* species (*C. jejuni*, *C. coli* and *C. lari*,) are the most frequently identified agents of acute infective diarrhoea in most developed countries. Consequently, the detection of *Campylobacter* species in a 25 g sample of a ready-to-eat food is considered to be significant<sup>2</sup>. Poultry and raw milk are common sources of infection. Food treatments, such as heating, freezing or chilling can cause sub-lethal injury to *Campylobacter* species, resulting in increased sensitivity to some antibiotics and lowered resistance to elevated incubation temperatures. The enrichment culture method described uses Bolton broth which allows resuscitation and recovery of injured organisms. The method is based on ISO 10272-1<sup>3</sup> and is also recommended by the Food and Drugs Administration<sup>4</sup>. It will detect strains of *Campylobacter* that are capable of growth at 41.5°C; these include *C. jejuni*, *C. coli* and *C. lari* but usually not *C. fetus*, the organism associated with abortion in cattle and sheep.

This method follows ISO 10272-1 for isolation of *Campylobacter* but does not use a second isolation medium. The confirmation tests used are similar to those specified in ISO 10272-1 but include an optional latex agglutination test. The test for growth at 25°C has not been included.

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# 1 PRINCIPLE

The detection of *Campylobacter* species in food involves enrichment in a selective liquid medium that is incubated at 37°C for 4 - 6 hours followed by 41.5°C for 44 ± 4 hours. The enrichment culture is inoculated onto a blood free selective agar medium, which is incubated microaerobically at 41.5°C for 44 ± 4 hours and examined for characteristic colonies.

Confirmation of suspect colonies of *Campylobacter* species is by means of biochemical, morphological and physiological tests.

# 2 DEFINITIONS

For the purposes of this method the following definitions apply:

*Campylobacter species*

These include *C. jejuni*, *C. coli*, and *C. lari*. These are microorganisms which grow under microaerobic conditions at 41.5°C to form typical colonies on solid selective media and which display the biochemical, morphological and physiological characteristics described when tests are performed in accordance with this method.

*Detection of Campylobacter species:*

Determination of the presence or absence of these microorganisms in the sample or in 25g or other defined weight or volume of sample.

# 3 SAFETY CONSIDERATIONS

Normal microbiology laboratory precautions apply. In addition, campylobacters are pathogenic to man and only low levels are needed to cause infection. Therefore isolation and identification must be performed by trained laboratory personnel in a properly equipped laboratory and under the supervision of a qualified microbiologist. Care must be taken in the disposal and sterilisation of all test materials. Procedures involving subculturing from pre-enrichment and enrichment broths and handling of *Campylobacter* cultures must be performed in a designated area of the laboratory. The laboratory should perform a risk assessment to determine whether the entire method, but particularly the motility test, can be performed safely.

# 4 EQUIPMENT

Usual laboratory equipment and in addition:

- Top pan balance capable of weighing to 0.1g
- Gravimetric diluter (optional)
- Stomacher
- Stomacher bags (sterile)
- Modified atmosphere jars and gas generation sachets for microaerobic conditions (approx. 10% carbon dioxide, 5-7% oxygen) or equivalent
- Incubators:- 37°C ± 1°C  
41.5°C ± 1°C
- Microscope:- phase contrast (optional)  
dark ground illumination (optional)  
light (optional)
- Sterile screw capped containers or plastic closure bags (nominal volume 250 mL)
- Pastettes

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## 5 CULTURE MEDIA AND REAGENTS

Equivalent commercial dehydrated media can be used; follow the manufacturer's instructions.

### *Bolton broth*

Enzymatic digest of animal tissues	10.0 g
Lactalbumin hydrolysate	5.0 g
Yeast extract	5.0 g
α- ketoglutaric acid	1.0 g
Sodium chloride	5.0 g
Sodium metabisulphite	0.5 g
Sodium pyruvate	0.5 g
Sodium carbonate	0.6 g
Haemin	0.01 g
Laked horse blood	50 mL
Cefoperazone	20 mg
Vancomycin	20 mg
Trimethoprim	20 mg
Amphotericin B	10 mg
or Cycloheximide	50 mg
Water	1 L

pH of basal broth  $7.4 \pm 0.2$  at  $25^{\circ}\text{C}$

Warning: This medium is very heat sensitive and is best produced in a preparator (if available)

### *Campylobacter selective agar (modified Cefoperazone Charcoal Deoxycholate Agar (mCCDA) or equivalent)*

Enzymatic digest of animal tissues	10.0 g
Meat extract	10.0 g
Sodium chloride	5.0 g
Bacteriological charcoal	4.0 g
Casein hydrolysate	3.0 g
Sodium deoxycholate	1.0 g
Ferrous sulphate	0.25 g
Sodium pyruvate	0.25 g
Agar	12.0 g
Cefoperazone	32 mg
Amphotericin B	10 mg
or Cycloheximide	50 mg
Water	1 L

pH  $7.4 \pm 0.2$  at  $25^{\circ}\text{C}$

### *Blood agar*

Columbia agar or any other suitable base with 5 % horse blood

### *Broth medium (for motility testing)*

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*Brain heart infusion broth, nutrient broth or Brucella broth*

Calf brain infusion solids	12.5 g
Beef heart infusion solids	5.0 g
Proteose peptone	10.0 g
Glucose	2.0 g
Sodium chloride	5.0 g
Disodium phosphate	2.5 g
Water	1 L
pH 7.4 ± 0.2 at 25°C	

*Oxidase reagent (Prepare fresh as required or use commercial equivalent)*

Tetramethyl- <i>p</i> -phenylenediamine dihydrochloride	0.1 g
Water	10 mL

*Gram staining reagents (optional)*

*Campylobacter latex agglutination kit (optional)*

## 6 SAMPLE PROCESSING

### 6.1 SAMPLE PREPARATION

*Food*

Using sterile instruments and aseptic techniques weigh a representative 25 g sample of each food into a sterile stomacher bag or plastic closure bag<sup>5</sup>. Using a stomacher, homogenise with nine times that weight or volume of Bolton broth. Avoid touching the inside of the bag with the hands.

Note: The Bolton broth should be allowed to equilibrate to room temperature prior to inoculation.

If a stomacher bag has been used transfer the suspension to a sterile screw capped container or plastic closure bag, leaving no more than 20 mm headspace; add extra Bolton broth if necessary and close the container tightly. If the amount of food available is less than 25 g maintain a 1:9 sample to Bolton broth ratio then top up if necessary to ensure minimal headspace in the container. If a plastic closure bag has been used carefully expel air and roll down to leave no more than 20 mm headspace.

*Swabs*

Transfer the swab to a stomacher bag or plastic closure bag and add sufficient Bolton broth to ensure that the swab is well covered. Then follow the instructions for food samples.

*Environmental samples*

Depending on the type of sample, either weigh a 25 g portion and follow the instructions for food samples or ensure that the sample is well covered with Bolton broth and close the container to ensure a minimal headspace. Note: special precautions should be taken to prevent contamination of the environment with dusty samples or cross-contamination of equipment.

Note: For further details on how to minimise the risk of cross-contamination in the FWE laboratory refer to NSM QSOP 12 - Avoiding cross-contamination risks in the food laboratory<sup>6</sup>

### 6.2 SELECTIVE ENRICHMENT

Place the enrichment broth in an incubator at 37°C for 4-6 hours. Transfer to an incubator at 41.5°C and incubate for a further 44 ± 4 hours.

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### 6.3 SUBCULTURE TO SELECTIVE AGAR

Subculture the broth onto *Campylobacter* selective agar (mCCDA). Incubate the plates in microaerobic conditions in an incubator at 41.5°C for 44 hours ± 4 hours.

Note: If required, such as in an outbreak investigation, plates can be examined at 18 - 24 hours and reincubated if no growth is present.

### 6.4 RECOGNITION OF COLONIES

After incubation examine the plates for *Campylobacter* colonies which have typical characteristics. *C. jejuni* and *C. lari* have flat, glossy, effuse colonies with a tendency to spread along the inoculation track. Well-spaced colonies resemble droplets of fluid. On moist agar a thin, spreading film may be seen. With continued incubation colonies become low and convex with a dull surface. A metallic sheen will eventually develop. *C.coli* has less effuse, often convex colonies with the surface usually remaining shiny. Morphology is variable and different colonial forms may be present on the same plate.

The culture will quickly deteriorate in air; plates should be examined immediately after removal from the microaerobic environment and the following tests performed on suspect growth immediately after reading the plates.

### 6.5 CONFIRMATORY TESTS

#### *Oxidase*

Immerse a swab in freshly prepared oxidase reagent and touch lightly to the surface of the colony to be tested. The immediate appearance (within 10 seconds) of a dark purple colour at the point of contact denotes a positive reaction. No colour change or a purplish colour that develops later are both considered to be negative reactions. Alternatively, moisten a piece of filter paper in a Petri dish with 2 - 3 drops of oxidase reagent. Using a stick, glass rod, disposable or platinum (not nichrome) loop transfer a colony of the test organism to the filter paper and rub it on the moistened area. A positive reaction is indicated by the appearance of a dark purple colour within 10 seconds. If using a commercial preparation follow the manufacturer's instructions.

*Campylobacter* species are oxidase positive.

Note: Oxidase negative colonies do not require further confirmatory tests.

#### *Microaerobic growth*

Subculture suspect colonies from the *Campylobacter* selective agar to two blood agar plates. Incubate one plate in microaerobic conditions and the other plate aerobically in an incubator at 41.5°C ± 1°C for 22 ± 2 hours.

*Campylobacter* species grow in microaerobic conditions but not aerobic conditions.

To complete the confirmation stage of the method, either perform the cell morphology and motility test (with Gram stain if necessary) or perform the latex agglutination test as described below.

#### *Cell morphology and motility (optional)*

Prepare a wet preparation as soon as possible after removal of the culture from microaerobic conditions. Emulsify some of the suspect colony in a drop of broth medium on a slide and cover with a coverslip. Examine immediately, using either phase contrast or dark ground microscopy; take appropriate precautions to avoid cross contamination of self or microscope. *Campylobacter* species are highly motile slender rods with curved or spiral morphology. Motility is characterised by darting or corkscrew like movements. Record the morphology and the motility result.

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Note: Motility can also be determined by means of the hanging drop method and light microscopy<sup>7</sup>.

An isolate that is oxidase positive, grows in microaerobic but not aerobic conditions, appears as a slender, curved or spiral rod and is typically motile is confirmed as *Campylobacter* species and reported as such.

*Gram stain* (optional)

If cell morphology cannot be determined from the motility test then perform a Gram stain. Young cultures appear as small curved Gram negative bacilli; older cultures may appear coccal.

In addition to the tests described above, the following test may be performed.

*Latex agglutination test* (optional)

Verify that the kit is able to detect strains of *C. jejuni*, *C. coli* and *C. lari*.

Perform a latex agglutination test for *Campylobacter* according to the manufacturer's instructions. *Campylobacter* strains will give a positive result.

Note: For safety reasons and to help prevent cross-contamination, avoid the use of conventional long-bodied loops. Either use the applicators supplied in the kit or use short-bodied applicators.

*Control cultures*

Positive control: - *C. jejuni* NCTC 11322

Negative control: - *E. coli* NCTC 9001

## 7 REPORTING OF RESULTS

If the presence of *Campylobacter* has not been confirmed, report in the following way:

*Campylobacter* species not detected in 25 g (or weight examined) or in the sample/swab

If the presence of *Campylobacter* is confirmed, report:

*Campylobacter* species detected in 25 g (or weight examined) or in the sample/swab

In both instances, the amount of food examined must also be reported and pre-printed forms amended as necessary.

## 8 REFERENCE FACILITIES

Isolates associated with outbreaks or obtained from ready to eat food (including raw drinking milk) should be referred for confirmation and further characterisation; isolates from national surveys may also require further investigation. Reference facilities for biotyping, serotyping and phage typing are available at the Laboratory of Enteric Pathogens, HPA Centre for Infections, Colindale. The fresh growth of a whole purity plate should be sent on a swab in Amies charcoal transport medium.

Centre for Infections: tel: 0208 200 4400 or 0870 084 2000

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# APPENDIX: FLOWCHART SHOWING THE DETECTION OF CAMPYLOBACTER SPECIES

Prepare a  $10^{-1}$  dilution of 25g sample in 225mL of Bolton broth (at room temperature)  
For swabs, completely cover with Bolton Broth  
For other environmental samples use an approximate 1:9 ratio of sample: broth or completely cover



Homogenise by stomaching



Transfer to an appropriate container leaving a small headspace; close securely



Incubate at 37°C for 4-6 hours followed by 41.5 °C for 44 ± 4 hours



Subculture to *Campylobacter* selective agar (mCCDA)



Incubate microaerobically at 41.5°C for 44 ± 4hours



Identify *Campylobacter* colonies using tests for oxidase production, presence of microaerobic growth but absence of aerobic growth, and either cell morphology and motility (plus Gram stain if necessary) or latex agglutination

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