Detection and enumeration of *Campylobacter* species

Microbiology Services
Food Water and Environmental Microbiology
Standard Method

FNES15 [F21]
About Public Health England

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Status of Microbiology Services Food, Water and Environmental Microbiology Methods

These methods are well referenced and represent a good minimum standard for food, water and environmental microbiology. However, in using Standard Methods, laboratories should take account of local requirements and it may be necessary to undertake additional investigations.

The performance of a standard method depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

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Citation for this document:
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<th>Page</th>
<th>Section(s) involved</th>
<th>Amendment</th>
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<td>PHE FNES15 2</td>
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<td></td>
<td>Revised to remove requirement for assessing growth at 25°C. Update to include option of Latex and PCR for confirmation</td>
</tr>
<tr>
<td>12</td>
<td>8.0 Calculation of results</td>
<td></td>
<td></td>
<td></td>
<td>Updated to include calculation method for weighted means and reference to Starlims</td>
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<td>9.0 Reporting of results</td>
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<td>Flowcharts</td>
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The amendment history is shown below. On issue of revised or new documents each controlled document should be updated by the copyholder in the laboratory.
Introduction

Scope

The method described is applicable to the detection and enumeration of Campylobacter species in all types of food samples and other environmental samples (eg carcase washings). It is also applicable to the detection of Campylobacter species in swabs.

The mathematical lower limit of enumeration using this method is 1 or 2 colony forming units (CFU) per millilitre (mL) of liquid samples, or 10 or 20 CFU per gram (g) of other food products.

Background

Campylobacter species are the most frequently identified bacterial agents of acute infective diarrhoea in most developed countries. Consequently, the detection of Campylobacter in a 25 g sample of ready to eat food is considered unsatisfactory and potentially injurious to health. Poultry is a common source of infection and the UK Food Standards Agency have as part of their Campylobacter intervention strategy recommended to reduce the number of fresh raw chicken samples containing greater than $10^3$ CFU per g of skin.

The method described is based on BS EN ISO 10272-1:2006 (detection) and BS EN ISO/TS 10272-2:2006 (enumeration).

These are internationally recognised methods for the detection and enumeration of Campylobacter species. A Campylobacter isolation medium, Modified Cefperazone Charcoal Deoxycholate Agar (mCCDA) is used and on this medium Campylobacter species form greyish, flat and moist colonies, often with a metallic sheen, and with a tendency to spread.

Information 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). Speciation of the Campylobacter species is not carried out as part of this method and significant isolates are sent to a reference laboratory for speciation and definitive typing.

Food manufacturing processes such as heating, freezing or chilling can cause sub-lethal injury to Campylobacter species, resulting in increased sensitivity to antibiotics and lower resistance to elevated temperatures. The enrichment culture method described uses Bolton Broth which allows resuscitation and recovery of injured organisms. The method is based on BS EN ISO 10272-1:2006. This method will detect strains of Campylobacter species.
that are capable of growth at 41.5 °C; these include *C. jejuni*, *C. coli* and *C. lari* but not usually *C. fetus* (an organism associated with abortion in cattle and sheep).

The table below gives details of the difference between this method and ISO 10272-1\(^3\) and 10272-2\(^4\).

<table>
<thead>
<tr>
<th>Section</th>
<th>PHE method F21</th>
<th>BS EN ISO 10272-1:2006 and BS EN ISO/TS 10272-2:2006</th>
<th>Justification for variation</th>
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<tr>
<td>Inoculation</td>
<td>One isolation medium is used.</td>
<td>Two plating media are used</td>
<td>In ISO the second plating medium is poorly defined. Single medium used and IQC and EQA supports effective use of a single plating medium.</td>
</tr>
<tr>
<td>Media</td>
<td>Brain Heart Infusion Broth</td>
<td>Brucella Broth</td>
<td>BHIB used due to commercial availability</td>
</tr>
</tbody>
</table>

1.0 Principle

The detection of *Campylobacter* species in food and environmental samples involves enrichment in a selective liquid medium at 37°C for 5 h followed by microaerobic incubation at 41.5°C for 44 h to allow recovery and growth, sub-culture onto selective solid media, and examination for colonies considered to be typical of *Campylobacter* species. Confirmation of the colonies as *Campylobacter* species is performed using morphological, biochemical and growth property tests.

The enumeration of *Campylobacter* species by this method involves inoculation of the surface of a selective agar media with a defined volume of an appropriate decimal dilution of the test sample. Agar plates are incubated microaerobically at 41.5 °C for up to 48 h. Calculation of the number of colony forming units (CFU) per gram (g) or millilitre (mL) of sample for *Campylobacter* species is determined from the number of typical colonies obtained on the selective media, and subsequently confirmed by morphological, biochemical and growth property tests.
2.0 Definitions

For the purposes of this method the following definitions apply:

**Campylobacter species**
Micro-organisms which form typical or less typical colonies on solid selective agar media incubated at 41.5°C and which display the morphological, biochemical and growth properties described in this method.

**Detection of Campylobacter species**
Determination of the presence or absence of these micro-organisms in a defined weight or volume of food or in a swab, cloth or other environmental sample.

**Enumeration of Campylobacter species**
Determination of the number of these micro-organisms per g or mL in a food product or environmental sample.

3.0 Safety considerations

3.1 General safety considerations

Normal microbiology laboratory precautions apply. All laboratory activities associated with this SOP must be risk assessed to identify hazards. Appropriate controls must be in place to reduce the risk to staff or other groups. Staff must be trained to perform the activities described and must be provided with any personal protective equipment (PPE) specified in this method. Review of this method must also include a review of the associated risk assessment to ensure that controls are still appropriate and effective.

*Information Note:* Throughout this method hazards are identified using red text. Where a means of controlling a hazard has been identified this is shown in green text.

3.2 Specific Safety Considerations

**Campylobacter** are pathogenic to man and 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 sub-culturing from enrichment broths and handling of Campylobacter cultures during identification procedures must be performed in a designated area of the laboratory.
3.3 Laboratory Containment

All samples and cultures are handled in a containment level 2 (CL2) laboratory.

4.0 Equipment

Usual laboratory equipment and in addition:

- Top pan balance capable of weighing to 0.1 g
- Gravimetric diluter (optional)
- Stomacher
- Vortex mixer
- Incubator: 37 ± 1 °C
- Incubator: 41.5 ± 1 °C
- Modified atmosphere jars and gas generation sachets for microaerobic conditions (approx. 10 % CO₂, and 5-7 % O₂)
- Apparatus suitable for achieving a microaerobic atmosphere with oxygen content of approx. 5 %, carbon dioxide 10 %, optional hydrogen ≤10 %, with the balance nitrogen. Appropriate gastight containers or equivalent (e.g., use of a microaerobic cabinet) to hold Petri dishes and/or flasks or bottles used for the enrichment broths
- Stomacher bags with closures (sterile)
- Microscope
- Glass slides
- Sterile 10 µL loops

**Information Note** 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 may be filled with an appropriate gas mixture prior to incubation. As an alternative to incubation in a microaerobic atmosphere, the enrichment can be done in tightly closed containers filled with enrichment broth, leaving a headspace of less than 20%.
5.0 Culture media and reagents

Equivalent commercial dehydrated media may be used; follow the manufacturer’s instructions.

**Peptone saline diluent (Maximum recovery diluent)**

- Peptone: 1.0 g
- Sodium chloride: 8.5 g
- Water: 1 L
- pH 7.0 ± 0.2 at 25 °C

**Buffered peptone water (ISO formulation)**

- Enzymatic digest of casein: 10.0 g
- Sodium chloride: 5.0 g
- Disodium hydrogen phosphate dodecahydrate: 9.0 g
  or anhydrous disodium hydrogen phosphate: 3.5 g
- Potassium di-hydrogen phosphate: 1.5 g
- Water: 1 L
- pH 7.0 ± 0.2 at 25°C

**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 metabisulphate: 0.5 g
- Sodium pyruvate: 0.5 g
- Sodium carbonate: 0.6 g
- Haemin: 0.01 g
- Water: 1 L
- pH 7.4 ± 0.2 at 25 °C

**Supplements**

- Laked Horse Blood: 50 mL
- Cefperazone: 0.02 g
- Vancomycin: 0.02 g
- Trimethoprim: 0.02 g
- Amphotericin B: 0.01 g
Information note: This medium is very sensitive to heat and is best produced in a preparator. Antimicrobial supplement and laked blood should be added after sterilisation.

Campylobacter Selective Agar (Modified Cefperazone Charcoal Deoxycholate Agar, mCCDA)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic digest of animal tissues</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Meat extract</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Bacteriological charcoal</td>
<td>4.0 g</td>
</tr>
<tr>
<td>Casein hydrolysate</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Sodium deoxycholate</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Agar</td>
<td>12.0 g</td>
</tr>
<tr>
<td>Water</td>
<td>1 L</td>
</tr>
<tr>
<td>Cefperazone</td>
<td>0.032 g</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.01 g</td>
</tr>
<tr>
<td>Water</td>
<td>1 L</td>
</tr>
</tbody>
</table>

pH 7.4 ± 0.2 at 25 °C

Columbia agar base with 5 % horse blood

Brain Heart Infusion broth

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf brain infusion solids</td>
<td>12.5 g</td>
</tr>
<tr>
<td>Beef heart infusion solids</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Proteose peptone</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Disodium phosphate</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Water</td>
<td>1 L</td>
</tr>
</tbody>
</table>

pH 7.4 ± 0.2 at 25 °C

Oxidase Reagent

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetramethyl-p-phenelenediamine dyhydrochloride</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Water</td>
<td>10 mL</td>
</tr>
</tbody>
</table>
Information Note: Equivalent commercial reagents and kits may be used; follow the manufacturer's instruction.

Gram stain reagents

Isopropyl alcohol

Campylobacter latex confirmation assay
Oxoid DRYSPOT CAMPYLOBACTER TEST KIT (Product Code: DR0150), or Microgen Bioproducts Campylobacter latex confirmation assay Product Code: M46 or equivalent if validated

PCR testing reagents
Reagents as specified in M2\(^{13}\) and M3\(^{12}\) are used

6.0 Sample processing

6.1 Sample preparation, inoculation and incubation for detection and dilutions

Prepare the sample using the procedure described in Standard Method F2 – Preparation of Samples and Dilutions\(^5\). Using sterile instruments and aseptic technique, weigh a representative 25 g sample of each food into a sterile stomacher bag with closures. Prepare a 10\(^{-1}\) homogenate of the sample in Bolton broth. For swabs refer to Standard Method FNES4 (E1) - Detection and Enumeration of Bacteria in Swabs and Other Environmental Samples\(^{10}\).

Homogenise for between 30 seconds and 3 minutes in a stomacher. The homogenisation time required will depend on the manufacturer instructions and the type of sample being examined.

Information note: When carrying out enrichment culture to detect Campylobacter it is essential that the culture vessel head space does not exceed 10 mm. This can be achieved by expelling air from a stomacher bag with closure and rolling the top down to achieve this or using an appropriately sized rigid container topping up with Bolton broth to reduce the head space if necessary.

Allow prepared samples to equilibrate to room temperature before incubation. Place the enrichment cultures in an incubator at 37 ± 1 °C for 5 ± 1 h to allow for resuscitation of injured organisms. Transfer the broths to an incubator at 41.5 ± 1 °C for a further 44 ± 4 h.

After incubation remove the enrichment cultures from the incubator, do not mix. Using a 10 μL loop sub-culture from just beneath the surface of the broth to mCCDC and spread to
achieve single colonies. Transfer the plates to a gas jar and incubate micro-aerobically at 41.5 ± 1 °C for 44 ± 4 h.

6.2 Sample preparation, inoculation and incubation for enumeration

Following the procedure described in Standard Method F2 – Preparation of Samples and Dilutions\(^9\) prepare a 10\(^{-1}\) homogenate of the sample in either peptone saline diluent (PSD) or buffered peptone water (BPW) and further decimal dilutions as required in PSD. For dairy products, appropriate diluents for the preparation of a 10\(^{-1}\) homogenate are given in Standard Method D1 – Preparation of Samples and Decimal Dilutions\(^1\). For swabs refer to Standard Method FNES4 (E1) - Detection and Enumeration of Bacteria in Swabs and Other Environmental Samples\(^10\).

Homogenise for between 30 seconds and 3 minutes in a stomacher. The homogenisation time required will depend on the manufacturer instructions and the type of sample being examined.

Prepare the mCCDA agar for use by removing any condensation from the lid of the plates and store at room temperature in the dark overnight. Using a sterile pipette, transfer 0.1 mL of the initial suspension to a mCCDA plate. Using a sterile hockey stick, carefully spread the inoculum uniformly and as quickly as possible over the surface of the agar plate, without touching the sides of the dish, until there is no longer any liquid visible on the agar surface. If high counts are expected, repeat the procedure using further decimal dilutions.

When it is necessary to estimate low numbers of Campylobacter species, the limit of enumeration may be lowered by examining a total of 1.0 mL of the sample by plating onto the surface of three agar plates and plating further dilutions in duplicate.

Incubate the plates at 41.5 °C for 44 ± 4h in a microaerobic atmosphere.

6.3 Recognition and counting of colonies

6.3.1 Recognition

Remove plates from the incubator and examine the plates for colonies typical of Campylobacter species. Typically, 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. If plates are wet a thin spreading film may be seen. With continued incubation the colonies become low and convex with a dull surface and a metallic sheen often develops. C. coli often 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.

Information note: Cultures will rapidly deteriorate in air and colony confirmations must be carried out immediately after the plates have been examined.
6.3.2 Counting of colonies from the enumeration method

Select plates containing up to 150 typical or suspect colonies; count these colonies. If more than one suspect colonial type is present on enumeration plates perform a differential count. Choose five colonies of each type for the confirmation tests.

6.4 Confirmation tests

Typical colonies (see above) from each plate must be subjected to physiological confirmation or colony confirmation by PCR. The extent of testing may be reduced once the presence of Campylobacter is established (see below).

**Oxidase test**

Wearing gloves and safety glasses prepare fresh solution of the oxidase reagent for each time of use. Positive and negative control of this solution must be performed for each batch prepared.

Wearing gloves and safety glasses moisten a piece of filter paper in a Petri dish with 2-3 drops of freshly prepared oxidase reagent. Using a stick, glass rod or plastic loop transfer a colony of the organism of interest to the filter paper and rub it on the moistened area. The appearance of a dark purple colour within 10 seconds at the point of contact denotes a positive result. No colour change or a delayed colour change denotes a negative reaction. Campylobacter species are oxidase positive.

If using a commercial reagent or kit follow the manufacture instructions.

**Microaerobic growth**

Using a 1 µL disposable plastic loop, streak each of the five suspect colonies (or all suspect colonies if less than five are present) to a segment on each of two blood agar plates. If confirming using PCR the loop is then carefully emulsified in 0.5 mL of PCR grade water. All picks (up to five colonies) from a single sample should be emulsified in the same tube.

Incubate one plate microaerobically at 41.5 ± 1 °C for 44 ± 4 h, and one aerobically at 41.5 ± 1 °C for 44 ± 4 h. Campylobacter species will grow micro-aerobically at 41.5 °C but will fail to growth aerobically at 41.5°C.

**Information note:** All isolates must be assessed to determine their oxidase reaction and their requirement for a microaerobic atmosphere for growth. Further confirmation is made using at least one of the three methods below.

**Colony confirmation using PCR (optional)**

Following method M312 heat treat the PCR grade water with emulsified colonies at 95°C for 15 minutes allow to cool and add 30 µL of heat-treated bacterial suspension to lyophilised real time PCR assay tubes as described in Standard Method M213. The positive control described in Standard Method M414 should be included in each real time PCR assays.
Examination of morphology and motility (optional)

If growth is only evident after microaerobic incubation at 41.5°C examine a colony from the blood agar plate for morphology and motility. This test must be performed as soon as the plates are removed from microaerobic conditions. The motility test uses live cultures. Motility and morphology can be observed using a wet preparation on a slide in BHIB or prepare a hanging drop slide. Using a microscope view under dark ground, phase contrast or light illumination. Campylobacter are usually motile, slender rods, with curved or spiral morphology. Motility is characterised by darting or corkscrew movements. Gloves must be worn and great care must be taken to avoid cross contamination of the microscope or surrounding areas. The microscope should be wiped down with Isopropyl alcohol after use. Cell morphology can also be determined using a Gram stain. Gloves and safety glasses must be worn during this procedure. Campylobacter species are Gram negative, slender rods usually curved or spiral in shape.

Latex test (optional)

Perform latex test according to the manufacturers instruction.

7.0 Quality control

Further quality control of media and internal quality assurance checks should be performed according to in-house procedures using the following test strains:

Positive control:

- Campylobacter jejuni NCTC 11322
- Pseudomonas aeruginosa NCTC 10662 (positive control oxidase test),

Negative control:

- Escherichia coli NCTC 9001

8.0 Calculation of results

No calculations are required for the detection test as this is a presence absence tests. For enumeration calculations occur automatically in the STARLIMS system as describe in Method Q12 Sample processing and result entry in STARLIMS\textsuperscript{15}. Calculations are performed as described below.

8.1 Calculation of results from routine samples

Calculate the number Campylobacter per g as follows:
8.2 Calculation of results from samples expected to have low numbers

For a result to be valid, it is considered necessary to count at least one dish containing a minimum of 15 colonies. Use the plate counts to calculate $N$, the *Campylobacters* present in the test sample per millilitre or per g, as the weighted mean from two successive dilutions using the following equation:

$$ N = \frac{\Sigma a}{V \left(n_1 + 0.1n_2\right) d} $$

when:

$\Sigma a$ is the sum of the colonies counted on all the plates retained from two successive dilutions, at least one of which contains a minimum of 15 CFU

$n_1$ is the number of plates counted at the first dilution

$n_2$ is the number of plates counted at the second dilution

$d$ is the dilution from which the first counts were obtained [d = 1 in the case (liquid products) where the directly inoculated test sample is retained, d = 0.1 for $10^{-1}$ dilution etc]

$V$ is the volume of the inoculum, in millilitres, applied to each plate

Round off the result to two significant figures.

8.3 Estimation of counts in samples expected to have low numbers

If both dishes at the level of the first retained dilution contain less than 15 confirmed colonies, calculate $N_E$, the estimated number of *Campylobacters* present in the test sample, as the arithmetical mean from two parallel plates using the following equation:

$$ N_E = \frac{\Sigma a}{V \cdot n \cdot d} $$

when:
Detection and enumeration of *Campylobacter* species

Σa  is the sum of the confirmed colonies counted on the two plates

n  is the number of plates retained

d  is the dilution from which the first counts were obtained [d = 1 in the case (liquid products) where the directly inoculated test sample is retained, d = 0.1 for 10⁻¹ dilution etc]

V  is the volume of the inoculum, in millilitres, applied to each plate

*Information Note:* Estimated counts (8.3) should be reported with the comment “Count Estimated due to low number”

### 9.0 Reporting of results

All results are reported using the STARLIMS system as described in method Q13 Technical Validation and release of result in STARLIMS. The test report specifies the method used, all details necessary for complete identification of the sample and details of any incidents that may have influenced the result.

#### 9.1 Detection

If *Campylobacter* species are recovered from a RTE food the laboratory must review its procedures in accordance with the advice given in: Public Health Response: Involvement of HPA FW&E Microbiology Laboratory Staff in the Investigation of Outbreaks of Food and Waterborne Disease-Q417 If the presence of *Campylobacter* species has been confirmed by morphological biochemical and growth property testing and the lead microbiologist is satisfied with the procedural review, report as follows:

If *Campylobacter* species are not isolated by detection report as:

*Campylobacter* species Not Detected in 25 g or 25 mL or item.

If *Campylobacter* species are isolated by detection but enumeration has not been performed report as:

*Campylobacter* species DETECTED in 25 g or 25 mL or item

*Information Note:* Where enrichment culture has been performed the actual weight of sample examined must be reported, for example, 10 g or mL, 25 g or mL or 100 g or mL.
9.2 Enumeration

If *Campylobacter* species are not detected by enumeration report as follows:

**Liquid products**
Where plates have been prepared from the undiluted \((10^0)\) product are found to contain no colonies, report the result as

*Campylobacter* species Not Detected CFU per mL.

**Solid food products**
Where plates have been prepared from the \(10^{-1}\) dilution of the product contain no colonies report the result as

*Campylobacter* species Less than 10 CFU per g or mL
(1.0 mL surface spread using a \(10^{-1}\) dilution)

OR

*Campylobacter* species Less than \(1 \times 10^2\) CFU per g or mL
(0.1 mL surface spread using a \(10^{-1}\) dilution)

If *Campylobacter* species are found by enumeration, report the total count as *Campylobacter* species CFU per g or mL or sample. If the count is 100 or more, report counts with one figure before and one figure after the decimal point in the form of:

\[a \times 10^b\text{ CFU per g or mL}\]

where a is never less than 1.0 or greater than 9.9 and b represents the appropriate power of ten. Round counts up if the last figure is 5 or more, round counts down if the last figure is 4 or less.

eg: 1920 CFU per g = 1.9 \(\times 10^3\) CFU per g
235,000 CFU per g = 2.4 \(\times 10^5\) CFU per g

If there are only plates containing more than 150 typical *Campylobacter* colonies report as greater than the upper limit for the test dilution used with the comment “Count too high to be estimated at the dilution used”.
Environmental Samples
Guidance on the calculation for results from environmental samples can be obtained from Standard Method FNES4 (E1)- Detection and Enumeration of Bacteria in Swabs and other Environmental Samples\textsuperscript{10}.

9.4 Detection and enumeration

If \textit{Campylobacter} species are not isolated by enumeration but are isolated by detection report as:

\textit{Campylobacter} species DETECTED in 25 g or 25 mL or sample.

Also report the limit of the enumeration test used \textit{eg} \textit{Campylobacter} species DETECTED in 25 g (Less than $1 \times 10^2$ CFU per g).

10.0 Reference facilities and referral of cultures

Isolates associated with outbreak investigations or detected in ready to eat food (including raw drinking milk) should be referred to a reference laboratory for further characterisation. Isolates obtained from samples submitted as part of national surveys may also require further investigation. Reference facilities for serotyping, bio-typing and phage typing are available from the Gastrointestinal Bacteria Reference Unit (GBRU), PHE, Colindale. Fresh growth from a purity plate collected into Amies charcoal transport medium must be sent.

A request form for referral to reference facilities can be obtained using the following link:

http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1194947325929
11.0 Acknowledgements and contacts

This Standard Method has been developed, reviewed and revised by Microbiology Services, Food, Water and Environmental Microbiology Methods Working Group.

The contributions of many individuals in Food, Water and Environmental laboratories, reference laboratories and specialist organisations who have provided information and comment during the development of this document are acknowledged.

For further information please contact us at:

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References


Appendix 1: Flowchart showing the process to perform the detection of *Campylobacter* species

1. Prepare a $10^{-1}$ dilution of sample in Bolton broth. For swabs and other environmental samples cover with broth so as to achieve an approximate $10^{-1}$ dilution

   ↓

2. Homogenise by stomaching

   ↓

3. Ensure the head space is less than 20% and allow the homogenised sample to equilibrate to room temperature before incubating at 37°C for 5 h.

   ↓

4. Transfer the broth to an incubator at 41.5°C and incubate for a further 44 h.

   ↓

5. Remove the broth from the incubator and using a 10µL loop sub-culture to mCCDA and spread for single colonies

   ↓

6. Incubate plates microaerobically at 41.5°C for 44h

   ↓

7. Examine for typical and atypical colonies

   ↓

8. Perform confirmatory tests. *Campylobacter* species are Oxidase positive, micro-organisms that grow microaerobically at 41.5 °C. They are motile, Gram negative with a curved or spiral cell morphology or give a typical reaction in the latex test or confirm using PCR.

   ↓

9. If required refer to GBRU for further characterisation
Appendix 2: Flowchart showing the process to perform enumeration of *Campylobacter* species

If the sample is liquid test neat, for solid samples prepare a 10⁻¹ dilution of the sample in MRD or BPW.

- Homogenise by stomaching

- Sub-culture 0.1 mL or 1.0 mL (over 3 plates) of the neat or 10⁻¹ dilution to mCCDA and spread

- Perform further decimal dilution if necessary

- Incubate microaerobically at 41.5 °C for 44 h

- Examine for typical and atypical colonies

Perform confirmatory tests. *Campylobacter* species are Oxidase positive, micro-organisms that grow microaerobically at 41.5 °C. They are motile, Gram negative with a curved or spiral cell morphology or give a typical reaction in the latex test or confirm using PCR

- If required refer to GRBU for further characterisation