Detection and enumeration of *Campylobacter* species

National Infection Service
Food Water and Environmental Microbiology
Standard Method
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Detection and enumeration of Campylobacter species

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Status of National Infection Service, 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.

Whereas every care has been taken in the preparation of this publication, Public Health England (PHE) cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. PHE should at all times be acknowledged.

Citation for this document:
Amendment history

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<td>6</td>
<td>Background</td>
<td>Updated to include reference to commission regulation (EU) 2017/1495 (CR11945) Updated to include reference to ISO10272-1:2017 and ISO10272-2:2017 and table of difference updated to include use of 25 g sample weights as a difference to these ISO documents (CR12061)</td>
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<td>8.0 Calculation of results</td>
<td>Section 8.1 Calculation of results from routine samples removed. Section 8.2 and 8.3 renumber as 8.1 and 8.2.</td>
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<td>19</td>
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<td>Information note added requiring that any counts less than 40 CFU per g or mL include the comment “count estimated due to low numbers” (CR12061)</td>
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Introduction

Scope

The method described is applicable to the detection and enumeration of *Campylobacter* species in all types of food samples (including carcase rinse) and environmental samples including 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 samples.

Background

*Campylobacter* species are the most frequently identified bacterial agents of acute infective diarrhoea in most developed countries. The detection of *Campylobacter* in a 25g sample of ready to eat food is considered unsatisfactory and potentially injurious to health\(^1\). 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\(^2\). There is also a new commission regulation (EU) 2017/1495 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs as regards to *Campylobacter* in broiler carcases\(^3\). This provides process hygiene criteria where \(n = 50\), \(c = 20\) and \(M\) limit = 1000cfu/g

The method described is based on BS EN ISO 10272-1:2017\(^4\) (detection) and BS EN ISO 10272-2:2017\(^5\) (enumeration).

These are internationally recognised methods for the detection and enumeration of *Campylobacter* species. A *Campylobacter* isolation medium, *Modified Cefoperazone Charcoal Deoxycholate Agar* (mCCDA) is used and on this medium *Campylobacter* species form greyish, flat and moist colonies, often with a metallic sheen, and sometimes 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. This method will detect strains of Campylobacter that are capable of growth at 41.5°C; these include \textit{C. jejuni}, \textit{C. coli} and \textit{C. lari} but not usually \textit{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\textsuperscript{4} and 10272-2\textsuperscript{5}.

<table>
<thead>
<tr>
<th>Section</th>
<th>PHE FNES15 (F21)</th>
<th>BS EN ISO 10272-1:2017 and BS EN ISO 10272-2:2017</th>
<th>Justification for variation</th>
</tr>
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<tbody>
<tr>
<td>6.1 Sample Preparation, Inoculation and Incubation for Detection</td>
<td>25g used as standard sample size</td>
<td>Performance criteria based on 10g sample for enrichment detection</td>
<td>25g maintained as sample size to maximise sensitivity e.g. during public health investigations. No significant difference detected in sensitivity in EQA/IQC data.</td>
</tr>
<tr>
<td>Media</td>
<td>Brain Hearth Infusion Broth</td>
<td>Brucella Broth used for motility confirmatory test</td>
<td>Only affecting labs where confirmation is using motility as part of confirmation. BHIB used due to commercial availability</td>
</tr>
<tr>
<td></td>
<td>Preston broth not included in accredited method</td>
<td>Preston broth recommended for samples where interference from 3rd generation beta-lactams hamper detection</td>
<td>No interference noted in routine sample types and IQC and EQA data show no interference from such bacteria</td>
</tr>
<tr>
<td></td>
<td>One isolation medium is used.</td>
<td>Two plating media are used following enrichment culture</td>
<td>Single medium used and IQC and EQA supports effective use of a single plating medium</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 5h followed by microaerobic incubation at 41.5°C for 44h 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 40-48 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\(^5\)\(^6\). All laboratory activities associated with this SOP must be risk assessed to identify hazards\(^7\)\(^8\). 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 0.1g
- gravimetric diluter (optional)
- stomacher or pulsifier
- vortex mixer
- incubator: 37 ± 1°C
- incubator: 41.5 ± 1°C
- apparatus suitable for achieving a microaerobic atmosphere with oxygen content of approx. 5%, carbon dioxide 10%, optional hydrogen ≤10%, with the balance nitrogen - gas generation sachets for microaerobic conditions may be used
- stomacher bags with closures (sterile)
- microscope
- glass slides
- sterile 10µL 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.

5.0 Culture media and reagents

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

*Peptone saline diluent (Maximum recovery diluent)*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>8.5 g</td>
</tr>
<tr>
<td>Water</td>
<td>1 L</td>
</tr>
<tr>
<td>pH 7.0 ± 0.2 at 25 ºC</td>
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</tbody>
</table>

*Buffered peptone water (ISO formulation)*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymatic digest of casein</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate dodecahydrate</td>
<td>9.0 g</td>
</tr>
<tr>
<td>or anhydrous disodium hydrogen phosphate</td>
<td>3.5 g</td>
</tr>
<tr>
<td>Potassium di-hydrogen phosphate</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Water</td>
<td>1 L</td>
</tr>
<tr>
<td>pH 7.0 ± 0.2 at 25°C</td>
<td></td>
</tr>
</tbody>
</table>
Detection and enumeration of *Campylobacter* species

*Bolton Broth*

Enzymatic digest of animal tissues 10.0 g  
Lactalbumin hydrosylate 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)*

Enzymatic digest of animal tissues 10.0 g  
Meat extract 10.0 g  
Sodium chloride 5.0 g  
Bacteriological charcoal 4.0 g  
Casein hydrosylate 3.0 g  
Sodium deoxycholate 1.0 g  
Ferrous sulphate 0.25 g  
Sodium pyruvate 0.25 g  
Agar 12.0 g  
Water 1 L  
Cefperazone 0.032 g  
Amphotericin B 0.01 g  
Water 1 L  

pH 7.4 ± 0.2 at 25 °C

*Columbia agar base with 5% horse blood*
Detection and enumeration of *Campylobacter* species

**Brain Heart Infusion 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**
- Tetramethyl-p-phenelenediamine 0.1 g
- Water 10 mL

**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 FNES43 (M2)\(^1\) and FNES44 M3\(^\text{11}\) are used*
6.0 Sample processing

6.1 Sample preparation, inoculation and incubation for detection

Since Campylobacter is very sensitive to freezing but survives best at low temperatures, samples to be tested should not be frozen, but refrigerated and subjected to examination as rapidly as possible. Also, take care to prevent the samples from drying.

Prepare the sample using the procedure described in Standard Method FNES26 (F2) – Preparation of samples and dilutions, plating and sub-culture. Using sterile instruments and aseptic technique, weigh a representative 25 g (or less if not available) 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 Sample.

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

Allow prepared samples to equilibrate to room temperature before incubation. Place the enrichment cultures in an appropriate micro-aerobic atmosphere (5 % ± 2 % oxygen; carbon dioxide 10 % ± 3 %, optional hydrogen ≤10 %, with the balance nitrogen) using gastight jars and gas-generating kits (following the manufacturer’s instructions). Alternatively, the jar or incubator may be filled the appropriate gas mixture prior to the incubation at 37 ± 1 °C for 5 ± 1 h followed by incubation 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 mCCDA 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 FNES26 (F2) – Preparation of Samples and Dilutions, plating and sub-culture 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 swabs refer to Standard Method FNES4 (E1) - Detection and Enumeration of Bacteria in Swabs and Other Environmental Samples. Homogenise for between 30 seconds and 3 minutes in a
stomacher or pulsifier. The homogenisation time required will depend on the manufacturer instructions and the type of sample being examined.

**Chicken carcase rinses**

Transfer the carcase to a sterile bag and add 250 mL of BPW. Rock the carcase with media for 1 minute or alternatively on a rocker for 1 minute turning the carcase over once. Transfer a minimum of 3 mL of carcase washing to a sterile universal.

Prepare the mCCDA agar for use by removing any condensation from the lid and drying the plates to ensure that the inoculum can be readily adsorbed. 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 3 agar plates.

Incubate the plates at 41.5 ± 1 °C for 44 ± 4 h 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 may resemble droplets of fluid and thin spreading growth may also occur. 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 and/or suspect colonies; count these colonies. If more than one of these colonial type is present on enumeration plates perform a differential count. Choose 5 colonies of each type for the confirmation tests.

6.4 Confirmatory tests

Typical/suspect colonies (see above) from each plate must be subjected to physiological confirmation. For enumeration at least 5 (or, if fewer suspect colonies present, all) colonies must be confirmed while for enrichment the extent of confirmatory testing can be reduced once the presence of Campylobacter is established.

Microaerobic growth
Using a 1 µL disposable plastic loop, streak each of the suspect colonies to a segment on each of 2 blood agar plates. Incubate one plate microaerobically at 41.5 ± 1 °C and one aerobically at 25 ± 1 °C – both for 44 ± 4 h. Campylobacter species will grow microaerobically at 41.5 °C but will fail to grow aerobically at 25 °C.

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.

Information note: All isolates must be assessed to determine their oxidase reaction and their requirement for a microaerobic atmosphere for growth. Then further confirmation is made using at least one of the 3 methods below.

Colony confirmation using Latex test (optional)
Perform latex test according to the manufacturer’s instruction. If latex negative, examine motility and cell morphology as described below.
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*Colony confirmation using PCR (optional)*
If confirming using PCR for enumeration tests carefully emulsify each colony in a separate vial with 0.5 mL of PCR grade water.

If confirming using PCR for detection tests up to 5 colonies can be emulsified in the same 0.5 mL of PCR grade water.

Following method FNES44 (M3)\(^{10}\) heat treat the PCR grade water with emulsified colonie(s) at 95 °C for 15 minutes allow to cool and add 30 µL of heat-treated bacterial suspension to PCR assay tubes as described in Standard Method FNES43 (M2)\(^{12}\). The positive control described in Standard Method FNES45 (M4)\(^{13}\) should be included in each real time PCR assays.

*Information note:* If colonies with typical morphology (that are not able to grow aerobically) fail to confirm using the latex test or PCR further examination of cell morphology and motility is required to confirm if the isolates maybe Campylobacter spp.

*Cell morphology and motility*
If growth is only evident after microaerobic incubation at 41.5 °C examine a colony from the blood agar plate for cell morphology and motility. Motility and cell morphology can be observed using a wet preparation on a slide in BHIB or prepare a hanging drop slide. This test must be performed as soon as the plates are removed from microaerobic conditions. The motility test uses live cultures. 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.
7.0 Quality control

Further quality control of media and internal quality assurance checks including quantitative and qualitative assessments should be performed according to in-house procedures using the following test strains (or equivalent):

Positive control:
- *Campylobacter jejuni* NCTC 11322 (WDCM 00156)
- *Campylobacter coli* NCTC 13638 (WDCM 00004)
- *Pseudomonas aeruginosa* NCTC 10662 (WDCM 00114) (positive control oxidase test only)

Negative control:
- *Escherichia coli* NCTC 12923 (WDCM 00012) (negative control oxidase test and media)
- *Staphylococcus aureus* NCTC 12981 (WDCM 00034)

Information note: A positive control plate must be place in each microaerobic jar prior to incubation.

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 described in Method FNES6 (Q12) Sample processing and result entry in StarLims. Calculations are performed as described below.

8.1 Calculation of results from samples

For a result to be valid, it is considered necessary to count at least one dish containing a minimum of 10 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 2 successive dilutions using the following equation:

\[ N = \frac{\sum a}{V (n_1 + 0.1n_2)} \]
when:

\[ \Sigma a \] is the sum of the confirmed colonies counted on all the plates retained from 2 successive dilutions, at least one of which contains a minimum of 10 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 \([d = 1 \text{ in the case (liquid products) where the directly inoculated test sample is retained}, d = 0.1 \text{ for } 10^{-1} \text{ dilution etc}]\)

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

Round off the result to 2 significant figures.

8.2 Estimation of counts in samples with low numbers

If the plate contains less than 10 colonies, calculate the result using the formula:

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

(the report comment stating the number is estimated)

when:

\[ \Sigma a \] is the sum of the confirmed colonies counted on the plate(s)

\[ n \] is the number of plates retained

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

\[ V \] is the volume of the inoculum, in millilitres, applied to each plate
9.0 Reporting of results

All results are reported using the StarLims system as described in method FNES17 (Q13) Technical Validation and release of result in StarLims\textsuperscript{15}. 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 \textit{Campylobacter} species are not isolated by detection report as:

\textit{Campylobacter species Not Detected in 25 g or 25 mL or swab}

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

\textit{Campylobacter species DETECTED in 25 g or 25mL or swab/cloth}

If \textit{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 PHE FW&E Microbiology Laboratory Staff in the Investigation of Outbreaks of Food and Waterborne Disease-FNES18 (Q4)\textsuperscript{15}.

If the presence of \textit{Campylobacter} species has been confirmed by morphological biochemical and growth property testing and the lead microbiologist is satisfied with the procedural review, report can go ahead.

\textbf{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 \textit{Campylobacter} species are not detected by enumeration report as follows:

\textit{Liquid samples}

Where plates have been prepared from the undiluted (10\textsuperscript{0}) product are found to contain no colonies, report the result as

\textit{Campylobacter species Not Detected CFU / mL}
Solids samples
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 / g or mL or swab/cloth**
(1.0 mL surface spread using a $10^{-1}$ dilution)

**Information note**: Counts of less than 40 CFU/g or mL are calculated using the method described in section 8.2 and must therefore include the comment “Count Estimated due to low numbers” as plate counts of 3 or less give a less precise estimate of the true value.

OR

**Campylobacter species Less than $1 \times 10^2$ CFU / g or mL or swab/cloth**
(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:

$$a \times 10^b \text{ CFU per g or mL or swab/cloth}$$

where a is never less than 1.0 or greater than 9.9 and b represents the appropriate power of 10. Round counts up if the last figure is 5 or more, round counts down if the last figure is 4 or less.

e.g. 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\(^\text{11}\).
9.3 Detection and enumeration

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

*Campylobacter* species DETECTED in 25 g or 25 mL or swab/cloth.

Also report the limit of the enumeration test used *eg* Campylobacter species DETECTED in 25 g (Less than 10 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 whole genome sequencing-based 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:

11.0 Acknowledgements and contacts

This Standard Method has been developed, reviewed and revised by National Infection Service, 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: Flowchart for detection of *Campylobacter* species

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

DidEnterCellDiagram

Homogenise by stomaching or pulsifying

Ensure the correct microaerobic atmosphere is achieved either using appropriate sachets for generating the microaerobic atmosphere or using a cabinet flushed with the correct atmosphere - incubate at 37 °C for 5 h.

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

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

Incubate plates micro-aerobically 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 micro-aerobically at 41.5 °C but not aerobically at 25 °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 GBRU for further characterisation
Appendix: Flowchart for enumeration of *Campylobacter* species

If the sample is liquid test neat, for solid samples prepare a $10^{-1}$ dilution of the sample in BPW or MRD - for carcase washings transfer the carcase to a sterile bag, add 250 mL of BPW through the vent.

- If required homogenise by stomaching, pulsify or rock for the required period

- 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 dilutions as necessary

- Incubate plates micro-aerobically at 41.5 °C for 44 h

- Examine for typical and less typical colonies

Perform confirmatory tests. *Campylobacter* species are Oxidase positive, micro-organisms that grow microaerobically at 41.5 °C but fail to grow at aerobically at 25 °C. They are motile, Gram negative with a curved or spiral cell morphology, give a typical reaction in the latex test or confirm using PCR

- If required refer to GRBU for further characterisation