

HPA STANDARD METHOD

DETECTION OF *CAMPYLOBACTER* SPECIES IN WATER

W 8

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

DETECTION OF *CAMPYLOBACTER* SPECIES IN WATER

Issue no: 3 Issue date: 24.10.07 Issued by Standards Unit, Evaluations & Standards Laboratory in conjunction with the Regional
Food, Water and Environmental Coordinators Forum Page 1 of 14

Reference no: W 8i3

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

STATUS OF NATIONAL STANDARD METHODS

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.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

The performance of standard methods 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, the Health Protection Agency or any supporting organisation 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. The Health Protection Agency (HPA) should at all times be acknowledged.

The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at www.hpa.org.uk.

The HPA aims to be a fully Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions¹.

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

Suggested citation for this document:

Health Protection Agency (2007). *Detection of Campylobacter species in water*. National Standard Method W 8 Issue 3. http://www.hpa-standardmethods.org.uk/pdf_sops.asp.

DETECTION OF CAMPYLOBACTER SPECIES IN WATER

Issue no: 3 Issue date: 24.10.07 Issued by Standards Unit, Evaluations & Standards Laboratory in conjunction with the Regional Food, Water and Environmental Coordinators Forum Page 2 of 14

Reference no: W 8i3

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

INDEX

- STATUS OF NATIONAL STANDARD METHODS 2**
- INDEX..... 3**
- AMENDMENT PROCEDURE 4**
- SCOPE OF DOCUMENT 5**
- INTRODUCTION 5**
- 1 DEFINITIONS..... 6**
- 2 PRINCIPLE 6**
- 3 SAFETY CONSIDERATIONS 6**
 - 3.1 SAMPLE TRANSPORT AND STORAGE 6
 - 3.2 SAMPLE PROCESSING 6
- 4 EQUIPMENT 7**
- 5 CULTURE MEDIA AND REAGENTS..... 7**
- 6 SAMPLE PROCESSING 9**
 - 6.1 SAMPLE PREPARATION 9
 - 6.2 SELECTIVE ENRICHMENT..... 9
 - 6.3 SUB-CULTURE TO SELECTIVE AGAR 9
 - 6.4 RECOGNITION OF COLONIES..... 9
 - 6.5 CONFIRMATORY TESTS 10
- 7 CALCULATION OF RESULTS 11**
- 8 REPORTING..... 11**
- 9 QUALITY CONTROL..... 11**
 - 9.1 MEMBRANE FILTRATION 11
 - 9.2 CONFIRMATORY TESTS 12
- 10 REFERENCE FACILITIES..... 12**
- 11 ACKNOWLEDGEMENTS AND CONTACTS 12**
- APPENDIX: FLOWCHART SHOWING THE DETECTION OF CAMPYLOBACTER SPECIES BY
MEMBRANE FILTRATION 13**
- REFERENCES 14**

DETECTION OF *CAMPYLOBACTER* SPECIES IN WATER

Issue no: 3 Issue date: 24.10.07 Issued by Standards Unit, Evaluations & Standards Laboratory in conjunction with the Regional Food, Water and Environmental Coordinators Forum Page 3 of 14

Reference no: W 8i3

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

AMENDMENT PROCEDURE

Controlled document reference	W 8
Controlled document title	Detection of <i>Campylobacter</i> species in water

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.

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/ 24.10.07	2.1	3		Title	Amended to a generic title to encompass both membrane filtration and direct inoculation.
				Introduction	Amended to include use of Preston broth for dirty waters. Terminology for Bolton and Preston broth is clarified. Use of MPN method is cited as a possibility.
				Definition	Re-worded for <i>Campylobacter</i> species.
				Principle	Amended to include two procedures for clean and dirty waters.
				Safety Considerations	Re-formatted and re-worded.
				Equipment	Updated
				Culture media and reagents	Amended
				Sample processing	Amended to give procedure for membrane filtration and direct inoculation. Removed alternative method for oxidase test.
				Reporting	Amended
				Quality Control	Re-formatted and amended to remove negative control.
				Reference facilities	Updated
				Acknowledgements and contacts	Added
				Flowchart	Updated
				References	Updated

DETECTION OF *CAMPYLOBACTER* SPECIES IN WATER

Issue no: 3 Issue date: 24.10.07 Issued by Standards Unit, Evaluations & Standards Laboratory in conjunction with the Regional Food, Water and Environmental Coordinators Forum Page 4 of 14

Reference no: W 8i3

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

DETECTION OF *CAMPYLOBACTER* SPECIES IN WATER

SCOPE OF DOCUMENT

The method described is applicable to the detection of thermotolerant *Campylobacter* species in all types of water.

INTRODUCTION

Background

Water is potentially an important reservoir of campylobacters and is an established vehicle for the transmission of these organisms to man and domestic animals. Waterborne outbreaks of *Campylobacter* enteritis have been reported from various countries². Potable water supplies can become contaminated with campylobacters, but natural waters including rivers and coastal waters may be used for recreational purposes and may present potential risks. As campylobacters are a major cause of gastroenteritis it may occasionally be necessary to examine samples of water as part of epidemiological investigations or as part of local authority surveillance programmes.

This method is based on that described in the *Microbiology of Drinking Water 2002*³ and ISO/DIS 17995 – *Water Quality – Detection and enumeration of thermotolerant Campylobacter species*⁴. Enrichment procedures using Bolton broth and Preston broth are included because studies within the HPA London Food Water and Environmental Laboratory have shown that Bolton broth permits the detection of *Campylobacter* species from clean waters but the more selective Preston broth is better for detection from dirty waters.

Information note 1: The selective supplements used in these media have been modified by some commercial suppliers by replacing cycloheximide with amphotericin and renaming them as 'Modified'. However the enrichment broths in principle are unchanged and the initial nomenclature of "Bolton broth" and "Preston broth" has been retained in this SOP. The terminology "Modified Preston broth" is also used for Preston broth with the addition of cefoperazone which is also known as Exeter broth. The formulation of Exeter broth with cefoperazone has not been used in this method.

Information note 2: This method can be used to obtain a semi-quantitative result by using multiple volumes. ISO 8199:2005 recommends the use of five or more parallel tubes (100mL volumes)⁵.

DETECTION OF *CAMPYLOBACTER* SPECIES IN WATER

Issue no: 3 Issue date: 24.10.07 Issued by Standards Unit, Evaluations & Standards Laboratory in conjunction with the Regional Food, Water and Environmental Coordinators Forum Page 5 of 14

Reference no: W 8i3

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

1 DEFINITIONS

For the purpose 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 a one litre or other specified volume, when tests are carried out in accordance with this method.

2 PRINCIPLE

Two enrichment procedures, membrane filtration and direct inoculation may be used for the detection of *Campylobacter* species in water as follows:

a) Clean waters – the sample is tested by the membrane filtration method (minimum of 1 litre sample following NSM W1 *General technique for the detection and enumeration of bacteria by negative pressure membrane filtration*) and direct inoculation. One volume of water is added to nine volumes of Bolton broth. Bolton broth is incubated at 37°C for 48 hours.

b) Dirty waters (high background flora eg river waters) – the sample is tested by direct inoculation only. One volume of water is added to nine volumes of Preston broth. Preston broth is incubated at 37°C for 24 hours followed by a further 24 hours at 41.5°C.

The enrichment cultures are inoculated onto blood free selective agar medium, which is incubated microaerobically at 41.5°C for 48 hours and examined for characteristic colonies. Confirmation of suspect colonies of *Campylobacter* species is by means of biochemical, morphological and physiological tests.

NB: Direct inoculation can be adapted for use by MPN method.

3 SAFETY CONSIDERATIONS⁶⁻¹⁵

Normal microbiology laboratory precautions apply.

3.1 SAMPLE TRANSPORT AND STORAGE

N/A

3.2 SAMPLE PROCESSING

Some *Campylobacter* species are pathogenic to man and therefore isolation and identification must be carried out 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 pre-enrichment and enrichment broths and handling of *Campylobacter* cultures must be performed in a designated area of the laboratory.

DETECTION OF *CAMPYLOBACTER* SPECIES IN WATER

Issue no: 3 Issue date: 24.10.07 Issued by Standards Unit, Evaluations & Standards Laboratory in conjunction with the Regional Food, Water and Environmental Coordinators Forum Page 6 of 14

Reference no: W 8i3

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

4 EQUIPMENT

Normal laboratory equipment and in addition:

- Modified atmosphere jars and gas generation sachets for microaerobic conditions (approx. 10% carbon dioxide, 5 - 7% oxygen) or equivalent eg modified atmosphere cabinet
- Incubators: 37°C ± 1°C
41.5°C ± 1°C
Cycling incubator 37°C / 41.5°C (optional)
- Microscope: phase contrast (optional)
dark ground illumination (optional)
light (optional)
- Cellulose ester filters, 47 mm, 0.2 µm pore size
- Sterile screw capped containers (nominal volume 100 mL)
- Membrane filtration apparatus for clean waters (as detailed in [NSM W1 – General technique for the detection and enumeration of bacteria by negative pressure membrane filtration](#))

5 CULTURE MEDIA AND REAGENTS

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

Campylobacter enrichment broths

Bolton broth (using Oxoid Modified Bolton broth selective supplement No.SR0208E)

Note: Supplements by other manufacturers may be used providing that the formulation is identified.

Enzymic digest of animal tissues	10.0 g
Lactalbumin hydrolysate	5.0 g
Yeast extract	5.0 g
Sodium chloride	5.0 g
Alpha-ketoglutaric acid	1.0 g
Sodium pyruvate	0.5 g
Sodium metabisulphite	0.5 g
Sodium carbonate	0.6 g
Haemin	0.01 g
Cefoperazone	20 mg
Vancomycin	20 mg
Trimethoprim	20 mg
Amphotericin B or Cycloheximide	10 mg
Lysed blood	50 mL
Water	1 L
pH 7.4 ± 0.2 at 25°C	

DETECTION OF *CAMPYLOBACTER* SPECIES IN WATER

Issue no: 3 Issue date: 24.10.07 Issued by Standards Unit, Evaluations & Standards Laboratory in conjunction with the Regional Food, Water and Environmental Coordinators Forum Page 7 of 14

Reference no: W 8i3

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

Preston broth (using Oxoid Modified Preston Campylobacter selective Supplement No.SR0204E)

Nutrient broth No. 2	25.0 g
Lysed horse blood	50 mL
Ferric sulphate	0.25 g
Sodium metabisulphite	0.25 g
Sodium pyruvate	0.25 g
Trimethoprim	10 mg
Polymixin B	5000 IU
Rifampicin	10 mg
Amphotericin B	10 mg
Water	1 L

pH 7.0 ± 0.2 at 25°C

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
Cefoperazone	32 mg
Amphotericin B	10 mg
Agar	12.0 g
Water	1 L

pH 7.4 ± 0.2 at 25°C

Blood agar

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

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 hydrogen 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 – p – phenylenediamine hydrochloride	0.1 g
Water	10 mL

Gram staining reagents (optional)

DETECTION OF CAMPYLOBACTER SPECIES IN WATER

Issue no: 3 Issue date: 24.10.07 Issued by Standards Unit, Evaluations & Standards Laboratory in conjunction with the Regional Food, Water and Environmental Coordinators Forum Page 8 of 14

Reference no: W 8i3

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

6 SAMPLE PROCESSING

6.1 SAMPLE PREPARATION

Water samples should be received and handled as described in NSM W1 Section 5. The nature of the request and condition of the sample should be noted on arrival. Samples should be protected from direct sunlight and transported at ambient temperature in an insulated container. Samples should be examined as soon as possible on the day of collection. In exceptional circumstances, if there is a delay, storage under the above conditions should not exceed 24 hours before the commencement of analysis.

6.1.1 CLEAN WATERS

Allow the *Campylobacter* enrichment broth to reach room temperature for both of the following methods.

For samples of clean water filter a portion through a 0.2µm membrane filter and inoculate a second portion directly into Bolton broth.

a) membrane filtration – filter a minimum of 1 litre of water through a membrane filter following the procedures laid down in NSM W1 Section 6. Transfer the filter/s to 90 mL Bolton broth. Top up with extra broth if necessary to leave no more than 20 mm headspace and close the container tightly.

b) direct inoculation – inoculate the water sample into Bolton broth. Add 10 mL water to 90 mL of enrichment broth (or multiples if MPN is required) leaving no more than 20 mm headspace and close the container tightly.

6.1.2 DIRTY WATERS

Dirty waters (high background flora eg river waters) – inoculate the water sample directly into Preston broth. Add 10 mL water to 90 mL of enrichment broth (or multiples if MPN is required) leaving no more than 20 mm headspace and close the container tightly. The membrane filtration method is not performed on dirty waters.

6.2 SELECTIVE ENRICHMENT

Bolton broth (for clean water)

Mix well and place the inoculated broth in an incubator at 37°C ± 1°C for 44 hours ± 4 hours.

Preston broth (for dirty water)

Mix well and place the inoculated broth in an incubator at 37°C ± 1°C for 22 hours ± 2 hours then at 41.5°C ± 1°C for a further 22 hours ± 2 hours.

6.3 SUB-CULTURE TO SELECTIVE AGAR

Sub-culture the broth onto mCCDA. Incubate the plates in microaerobic conditions (80% N₂, 10% H₂, 10% CO₂) in an incubator at 41.5°C for 44 ± 4 hours.

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

6.4 RECOGNITION OF COLONIES

After incubation examine the plates for *Campylobacter* colonies which have the 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,

DETECTION OF CAMPYLOBACTER SPECIES IN WATER

often convex colonies with the surface usually remaining shiny. Morphology is variable and different colonial forms may be present on the same plate.

Note: 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

Moisten a piece of filter paper in a Petri dish with 2 – 3 drops of freshly prepared oxidase reagent or equivalent. Using a stick, glass rod or platinum/plastic (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. No colour change or a purplish colour which develops later are both negative reactions.

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

Campylobacter colonies are oxidase positive.

Microaerobic growth

Sub-culture 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 $41.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 22 hours \pm 2 hours.

Campylobacter species grow in microaerobic conditions but not aerobic conditions.

Cell morphology and motility

Prepare a wet preparation as soon as possible after removal from microaerobic conditions. Emulsify some of the suspect colony in a drop of brain heart infusion broth 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 and microscope. *Campylobacter* species are highly motile slender rods with spiral morphology. Motility is characterised by darting or cork-screw like movements. Record the morphology and the motility result.

Note: Motility can also be determined by means of the hanging drop method and light microscopy¹⁶.

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

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.

DETECTION OF *CAMPYLOBACTER* SPECIES IN WATER

Issue no: 3 Issue date: 24.10.07 Issued by Standards Unit, Evaluations & Standards Laboratory in conjunction with the Regional Food, Water and Environmental Coordinators Forum Page 10 of 14

Reference no: W 8i3

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

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.

7 CALCULATION OF RESULTS

If the MPN method is used follow the procedures described in National Standard Method: W 1 Section 7¹⁷, calculate the number of confirmed *Campylobacter* species present in the specified volume of the original sample.

8 REPORTING

Report the results using the procedure described in NSM W 1 Section 9.

If *Campylobacter* species are not isolated report as follows:

'*Campylobacter* species not detected in v of water'

where **v** is the specified volume

If *Campylobacter* species are isolated report as follows:

'*Campylobacter* species detected in v of water'

where **v** is the specified volume

'Further identification to follow'.

9 QUALITY CONTROL

9.1 MEMBRANE FILTRATION

Internal quality controls should be carried out using suspensions of positive and negative control organisms known to contain less than 100 colony forming units in the volume filtered.

Positive control

Prepare a dilute suspension of *C. jejuni* NCTC 11322

Blank control

Filter 1 L of sterile distilled water using the same funnel as that used for the positive control, after decontamination

Incubate all tests and perform all procedures in parallel with routine test samples.

DETECTION OF *CAMPYLOBACTER* SPECIES IN WATER

Issue no: 3 Issue date: 24.10.07 Issued by Standards Unit, Evaluations & Standards Laboratory in conjunction with the Regional Food, Water and Environmental Coordinators Forum Page 11 of 14

Reference no: W 8i3

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

9.2 CONFIRMATORY TESTS

Morphology, motility and microaerobic growth

C. jejuni should be used to control tests for colonial morphology, motility, microscopic morphology and microaerobic growth.

Oxidase test

P. aeruginosa NCTC 10662 + (Purple)
E.coli NCTC 9001 - (Colourless)

10 REFERENCE FACILITIES

In certain circumstances such as outbreaks or national surveys it may be necessary to investigate isolates further. Reference facilities for biotyping, serotyping and phage typing are available at the Laboratory of Enteric Pathogens, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London, NW9 5EQ. Isolates should only be referred following discussion and agreement with the reference laboratory.

Two colonies are selected from the direct culture for purity cultures. The fresh growth of each whole purity plate is sent on a swab in Amies charcoal transport medium.

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

11 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the Water Working Group for National Standard Methods (http://www.hpa-standardmethods.org.uk/wg_water.asp). 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.

The National Standard Methods are issued by Standards Unit, Evaluations and Standards Laboratory, Centre for Infections, Health Protection Agency London.

For further information please contact us at:

Standards Unit
Evaluations and Standards Laboratory
Centre for Infections
Health Protection Agency
Colindale
London
NW9 5EQ
E-mail: standards@hpa.org.uk

DETECTION OF *CAMPYLOBACTER* SPECIES IN WATER

Issue no: 3 Issue date: 24.10.07 Issued by Standards Unit, Evaluations & Standards Laboratory in conjunction with the Regional Food, Water and Environmental Coordinators Forum Page 12 of 14

Reference no: W 8i3

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

APPENDIX: FLOWCHART SHOWING THE DETECTION OF CAMPYLOBACTER SPECIES BY MEMBRANE FILTRATION

Transport to laboratory at ambient temperature out of direct sunlight in a suitable container



Samples should be examined as soon as is possible on the day of collection



Mix well



Clean waters: Membrane filtration



Clean waters: Direct inoculation



Dirty waters: Direct inoculation

Transfer the filter/s to 90 mL Bolton broth

Add 10 mL water to 90 mL of Bolton broth

Add 10 mL water to 90 mL of Preston broth



37°C ± 1°C for 44 hours ± 4 hours



37°C ± 1°C for 44 hours ± 4 hours



Incubate at 37°C ± 1°C for 22 h ± 2 h then at 41.5°C ± 1°C for a further 22 h ± 2 h



Sub-culture broth(s) onto *Campylobacter* selective agar



Incubate microaerobically at 41.5°C for 44 ± 4 hours



Identify *Campylobacter* colonies using tests for oxidase production, presence of microaerobic growth but absence of aerobic growth, cell morphology and motility. Further confirmation if desired using latex agglutination

DETECTION OF *CAMPYLOBACTER* SPECIES IN WATER

Issue no: 3 Issue date: 24.10.07 Issued by Standards Unit, Evaluations & Standards Laboratory in conjunction with the Regional Food, Water and Environmental Coordinators Forum Page 13 of 14

Reference no: W 8i3

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

REFERENCES

1. Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient-identifiable information. London. December 1997.
2. Bolton FJ, Coates D, Hutchinson DN, Godfree AF. A study of thermophilic campylobacters in a river system. J Appl Bacteriol 1987;62:167-76.
3. Standing Committee of Analysts. Environment Agency. The Microbiology of Drinking Water (2002). Methods for the Examination of Waters and Associated Materials - Part 10: Methods for the isolation of *Yersinia*, *Vibrio* and *Campylobacter* by selective enrichment. London. <http://www.environment-agency.gov.uk/commodata/acrobat/mdwpart10.pdf#search='the%20microbiology%20of%20drinking%20water%20%20part%2010'>. p. 1-32.
4. ISO 17995:2005 Water quality - Detection and enumeration of thermotolerant *Campylobacter* species. International Organization for Standardization; 2005.
5. BS ISO 8199:2005 Water quality - General guidance on the enumeration of micro-organisms by culture. International Organisation for Standardisation; 2005.
6. Advisory Committee on Dangerous Pathogens. 2004 Approved List of Biological Agents. <http://www.hse.gov.uk/pubns/misc208.pdf>. p. 1-17.
7. Public Health Laboratory Service Standing Advisory Committee on Laboratory Safety. Safety Precautions: Notes for Guidance. 4th ed. London: Public Health Laboratory Service (PHLS); 1993.
8. Control of Substances Hazardous to Health Regulations 2002. General COSHH. Approved Code of Practice and Guidance, L5. Suffolk: HSE Books; 2002.
9. Health and Safety Executive. 5 steps to risk assessment: a step by step guide to a safer and healthier workplace, IND (G) 163 (REVL). Suffolk: HSE Books; 2002.
10. Health and Safety Executive. A guide to risk assessment requirements: common provisions in health and safety law, IND (G) 218 (L). Suffolk: HSE Books; 2002.
11. NHS Estates. Health Building Note 15. Facilities for pathology services. 2nd ed. London: The Stationary Office; 2005.
12. BS EN 12469: 2000. Biotechnology - performance criteria for microbiological safety cabinets. London: British Standards Institution (BSI); 2000.
13. BS 5726: 1992. Microbiological safety cabinets. Part 2. Recommendations for information to be exchanged between purchaser, vendor and installer and recommendations for installation. London: British Standards Institution (BSI); 1992.
14. BS 5726: 1992. Microbiological safety cabinets. Part 4. Recommendations for selection, use and maintenance. London: British Standards Institution (BSI); 1992.
15. Advisory Committee on Dangerous Pathogens. The management, design and operation of microbiological containment laboratories. Suffolk: HSE Books; 2001.
16. National Standard Method BSOP TP21 - Motility test. Health Protection Agency; 2005.
17. National Standard Method W 1 - General technique for the detection of bacteria by membrane filtration. London: Health Protection Agency; 2007.

DETECTION OF *CAMPYLOBACTER* SPECIES IN WATER

Issue no: 3 Issue date: 24.10.07 Issued by Standards Unit, Evaluations & Standards Laboratory in conjunction with the Regional Food, Water and Environmental Coordinators Forum Page 14 of 14

Reference no: W 8i3

This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk