Global Salm-Surv

A global *Salmonella* surveillance and laboratory support project
of the World Health Organization

Laboratory Protocols

Step 2 Training Course

Isolation of thermotolerant *Campylobacter* from water

4th Ed. March. 2003

EDITED BY: RENE S. HENDRIKSEN (DFVF), JAAP WAGENAAR (ASG), MARCEL VAN BERGEN (ASG)

Contents:

1. Isolation thermotolerant *Campylobacte*r from faeces, food or water

Introduction

The following procedures will guide you through the steps that are necessary to isolate *Campylobacter* from faeces, food or water

Isolation of thermotolerant *Campylobacter* from faeces, food or water

Campylobacter food-borne infections occurs either sporadically, affecting individuals and small groups such as families, or (less frequently) as larger community outbreaks. In large outbreaks a cause may generally be determined but identification of the infective vehicle in sporadic cases is often much less successful. *Campylobacter jejuni* is generally the most common cause of human enteritis but *Campylobacter coli* may also be responsible.

Pigs commonly carry *Campylobacter coli*, but serological studies have shown differences between isolates from pigs and humans indicating that pigs do not appear to be a major source of infection. However, in some countries where large quantities of pork are consumed *Campylobacter coli* infections frequently occur.

Campylobacter jejuni are commonly isolated from chicken and cattle, and chicken is expected to be one of the major sources of infection.

Campylobacter may also be present in faeces (in faeces often in large numbers) or food in low numbers and they may be injured. To diminish the risk of obtaining false-negative results, selective enrichment of a large food sample is performed before isolation on selective agar:

- Enrichment in selective enrichment broth (Preston).
- Isolation on selective CCD-agar plates.

References

1. Nachamkin I. and M. J. Blaser (eds) (2000). *Campylobacter 2nd ed.* ASM Press, Washington, D.C

2. Jacobs-Reitsma, W.F., 2000. Campylobacter in the food supply. In: Campylobacter, 2nd Edition. I. Nachamkin and M.J. Blaser (eds.), ASM, Washinton DC.

3. Hunt, J.M., and C. Abeyta. 1995. Campylobacter. Bacteriological Analytical Manual. 8th Ed. 7.01-7.27.

4. Post, D.E. Food-borne pathogens monograph number 3 *Campylobacter*. Oxoid Limited, wade Road, Basingstoke, Hampshire RG24, UK.

2. Isolation of thermotolerant *Campylobacter* from water

Materials

Equipment

- Measuring glass
- Disposable inoculation loops (1 μl and 10 μl)
- Incubators at 42°C (microaerobic)
- Erlenmeyer flasks (500 ml) etc. sterile (for enrichment)
- Bunsen burner
- Laboratory coats
- Waste containers
- Bottle of 70% ethanol

Media

- Preston broth
- CCD-agar plates
- Blood Columbia agar plates containing 5% cattle, sheep or horse blood.

Bacterial strains

- Water sample
- *Campylobacter coli* CCUG 11283
- *Campylobacter jejuni* CCUG 11284

Safety

Carry out all procedures in accordance with the local codes of safe practice.

Procedure for water

Day 1: Enrichment in selective medium
Transfer 10 ml of the water to a flask containing 90 ml of Preston broth or more in general to filtrate a portion of water, and enrich the filter in 10-25 ml Preston Incubate the enrichment broth at 42 ^{o}C for (24-) 48 h. The flask must be equipped with a cotton plug and incubated under microaerobic conditions (not neccesary when a flask with very little head-space is used!).

Day 2: Isolation on solid selective medium, CCD-agar
Using a 10 µl loop, transfer material from the incubated enrichment broth to a CCD agar plate. Incubate under microaerobic conditions at 42^{o}C for 1-5 days.

Day 3: Spreading on Columbia agar plates containing 5% cattle blood
Characteristic growth from CCD-agar plates is transferred to a blood plate in a way that single colonies can be expected. Incubate under microaerobic conditions overnight at 42^{o}C. Further identification follows in the manual "Introduction to identification of thermotolerant *Campylobacter* from food, faeces or water".

Theory / comments

The amount of water sampled, depends on the quality of the water (eg poultry slaughterhouse). Waste water can be tested in 10 ml portions, surface water in 100 ml portions and drinking water in 1000 ml portions (1 ml in 9 ml single strenght, depending on the quality of the water sample). Large volumes need filtration, but 100 ml and 10 ml portions might also be added to the same volume of double strength Preston enrichment broth (when filtration systems are difficult to obtain)

Microaerobic conditions: CO2 and N_2. Depending of the kind of Campy gas-generating envelopes or pouches that are used or even a pump system, like Anoxomat, replacing air from an anaerobic jar by a defined gas-mixture
Conditions containing gasses in the ratio of 6% O_2, 7% CO_2, 7% H_2 and 80% N_2 could be used if mixed separately.
Alternative method to obtain a microaerobic conditions: Appendix 1.
(It´s not a very reliable alternative, however if nothing else is available it could be used).

CCD-agar: Charcoal, cefoperazone, desoxycholate agar.

A typical *Campylobacter* on CCD-agar has a gray, moistening and effuse appearance**.** *Campylobacter jejuni* will have a green or gray appearance that can be very dry. At the same time the appearance can be with or without a shine of metal.
A creamy grey, moistening and raised colony is typical a *Campylobacter coli.* but it will not be possible to determine the species only on basis of colony appearcance.

3. Composition and preparation of culture media and reagents

The media and reagents are available from companies like Oxoid, Merck and Difco. The composition of the dehydrated media given below is an example and may vary a little among the different manufacturers. Also the media should be prepared according to the manufacturers description if it differs from the description given here.

Preston Broth

Lab-Lemco meat extract[b]	10.0 g
Peptone[b]	10.0 g
Sodium chloride[b]	5.0 g
Sodium pyruvate[a]	0.25 g
Sodium metabisulphite[a]	0.25 g
Ferrous sulphate[a]	0.25 g
Water	1000 ml

2 vials of Preston selective supplement (e.g. Oxoid SR204 or?!)
consisting of: (per liter)

Polymyxin B	5000 i.u.
Trimethroprim	10.0 mg
Rifampicin	10.0 mg
Cycloheximide (instead: Amphotericine-B)	100.0 mg
Lysed horse blood	50 ml

[a] Also available as so-called Campylobacter growth-supplement or FBP (e.g. Oxoid SR84)
[b] Also available a dehydrated powder: Nutrient broth no.2. (eg Oxoid ..)
Preparation:
Dissolve the dehydrated medium in the water by heating if necessary. Transfer into a bottle and autoclave at 121°C for 15 min. Allow the media to cool to below 50°C before adding the selective (and growth) supplements and the lysed horse blood as appropriate.

CCD-agar

Campylobacter Blood-Free Selective Agar Base (Oxoid, CM739) 45,5 g

Meat extract	10,0 g
Enzymatic digest of animal tissues	10,0 g
Sodium chloride	5,0 g
Charcoal	4,0 g
Casein hydrolysate	3,0 g
Sodium deoxycholate	1,0 g
Ferrous sulphate	0,25 g
Sodium pyruvate	0,25 g
Agar	8,0 g to 18,0 g[1)]
Water	1 000 ml

2 vials of CCDA Selective Supplement (Oxoid, SR 155E)
consisting of: (per liter)

Cefoperazone	32 mg
Amphotericin-B	10 mg (check this amount)
Water	1000 ml

Dissolve Campylobacter Agar Base in water by heating if necessary. Autoclave at 121^{o}C for 15 minutes.Add to each of the 2 vials 2 ml of sterile water. Dissolve gently. Add the selective supplement to the
50^{o}C warm Campylobacter Agar Base. Pour plates with about 15-20 ml melted medium in each petri-dish (preferably with “nocks”).

Columbia-agar

Columbia agar base (Oxoid CM331)	45 g
Water	1000 ml

Dissolve the Agar Base in water, and let it stand for 15 min. Boil the solution for 15 min., and adjust pH~7,1-7,5. The medium is poured into 1000 ml flasks and autoclaved at 121^{o}C for 15 min.

Columbia-agar with cattle blood

Columbia agar	950 ml
Cattle blood	50 ml

Melt the agar and bring to a temperature of about 50°C and add the cattle blood. Pour plates with about 15-20 ml melted medium in each petri dish (preferably with “nocks”).

Appendix 1:

Candle jar

Purpose:
The candle jar creates an atmosphere with reduced oxygen and elevated levels of carbon dioxide. These conditions enhance the growth of microaerophiles.

Principle:
The flame of the candle within a closed environment will use up a certain percentage of the oxygen. When the available oxygen is reduced and elevated carbon dioxide created by the flame is increased, the flame will be extinguished. The plated medium within this atmosphere will show enchanced growth of certain bacteria. The candle jar will usually be incubated at 42^0C.

References

1. ANAEROBIC JAR & CANDLE JAR
   Lab Index, Photo Atlas Reference: p.7 Lab Text Ref: Ex. 2-5

Appendix 2:

Water filtration technique

Collect 2-4 liters for the analysis. When collected, 5 ml of 1 M sodium thiosulfate should be added per liter of chlorinated water sample.

Filter smaller volume samples through 45 µm Zetapor filters, 47 mm diameter. These filters have a positive charge. The negatively charged Gram-negative organisms are more effectively retained in the filter. Filter larger volumes, especially those that are turbid, through 90 mm or larger diameter filters.

Place filter unit into autoclavable pan. If filter clogs, wear sterile gloves and open filter holder unit to aseptically remove filter with sterile forceps. Place filter into enrichment broth (**see** below). Place another sterile filter in unit, reassemble, and continue filtering. Use as many filters as needed per subsample. When analyzing sea or other salt water, flush excess salt off filter by running 100-1,000 ml (depending on filter size) sterile phosphate buffer through the filter as the last of the sample is going through the filter. Do this with every filter used for salt water analyses.

Do not let filter become completely dry. Immediately transfer finished filter to broth. Campylobacters are very sensitive to drying and high salt concentrations.

Place filter(s) in broth in the enrichment container. When using large filters, fragment with a sterile pipet. Be sure the broth covers the filter(s).

Enrichments incubated in Campy gas in anaerobe jars should be 125 ml or less. Larger volumes should be divided into smaller amounts, aseptically dividing the filters.

References

1. FDA Bacteriological Analytical Manual :(see website FDA, BAM chapter 7)