Global Salm-Surv

A global *Salmonella* surveillance and laboratory support project
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Laboratory Protocols

Level 2 Training Course

Isolation of thermotolerant *Campylobacter* from faeces

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Contents:

1. Isolation of 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 poisoning occurs either sporadically, affecting individuals and small groups such as families, or 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 are expected to be one of the major sources of infection.

Campylobacter may also be present in faeces or food in low numbers and they may be injured. To diminish the risk of obtaining false negative results, non-selective pre-enrichment of a large food sample on selective enrichment media is performed:

- 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 faeces.

Materials

Equipment

- Cotton swabs
- Disposable inoculation loops (1 μl and 10 μl)
- Incubators at 42.0°C (microarobic)

Media

- CCD-agar plates
- Blood Colombia plates containing 5% cattle, sheep or horse blood.

Bacterial strains

- Faeces samples
- *Campylobacter Coli* CCUG 11283
- *Campylobacter jejunii* CCUG 11284

Safety

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

Procedure for faeces

Day 1: Selective enrichment with CCD agar plates
Pick faeces by a swab, and streak it onto CCD agar plate. Incubate the plate at 42°C for 1-5 days under microaerobic conditions. Appendix 1: Filtration technique

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°C.
Further identification follows in the manual "Introduction to identification of thermotolerant *Campylobacter* from food, faeces or water".

Theory / comments

CCDA: Charcoal, cefoperazone, desoxycholate agar.

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
If the gasses are mixed separately the conditions and the ratios could be of 6% O_2, 7% CO_2, 7% H_2 and 80% N_2.
Alternative method to obtain a microaerobic conditions: Appendix 2. (It´s not a very reliable alternative, however if nothing else is available it could be used).

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.

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:

Filtration technique

This technique is simular to that described by Steele and McDermott.

Sterile cellulose acetate membranes of 0.45µ pore size are placed on the surface of Mueller-Hinton agar with 5% blood. Stool samples are emulsified (1 g in 1 ml saline), and then 10-15 drops of faecal suspension are placed on top of a membrane and allowed to filter passively under ambient conditions for 30-40 min.
Following filtration, the filter membranes are removed and the culture plates incubated for 1-2 days at 37-41^{0}C (microarobic).

References

1. L. López, F. J Castillo, A. Clavel, M. C. Rubio: Use of a Selective Medium and a Membrane Filter Method for Isolation of *Campylobacter* Species from Spanish Paediatric Patients. Eur J Clin Microbiol Infect Dis (1998) 17:489-492.
2. Blaser Mj, Cody HJ.: Methods for isolating Campylobacter jejuni from low-turbidity water. Appl Environ Microbiol 1986 Feb,51(2):312-5

Appendix 2:

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 35-37^{0}C.

References

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