



Fieldwork phase 2

## Sample culture-based testing

### Standard Operating Procedure

v2

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**Project title:** Feasibility study with a non-blinded, pilot, randomised baseline trial to evaluate the fidelity of a play space intervention model to reduce infant *Campylobacter* infection in rural households in Sidama Zone, Ethiopia

**PhD researcher (Cranfield):** Sophie Budge

**PhD supervisors (Cranfield):** Dr Alison Parker, Dr Paul Hutchings

**Sponsored by and in collaboration with:** People in Need (Camila Garbutt)

**Ethiopian collaboration partners:** Mathewos Moges (Hawassa University, College of Medicine and Health Sciences)

This SOP is adapted from the CAGED study SOP with approval from the CAGED study Primary Investigators.

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## 1. Campylobacter testing: Safety considerations<sup>1</sup>

- Campylobacter species are Hazard Group 2 organisms and their infectious dose is 500 organisms by ingestion. The most effective method for preventing laboratory-acquired infections is the adoption of safe working practices
- Appropriate personal protective equipment and techniques designed to minimise exposure of the laboratory workers should be worn and adhered to at all times
- This includes a laboratory coat, goggles, gloves
- Good occupational hygiene practices should be followed, especially washing with warm water and soap
- All use of *C. jejuni* should be undertaken within the CAT 2 microbiology hood
- This protocol should be supplemented with approved COSHH and risk assessments.

<sup>1</sup>: Public Health England. (2018). Identification of Campylobacter species. UK Standards for Microbiology Investigations. ID 23 Issue 3.1.  
[https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\\_data/file/685065/ID\\_23i3.1.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/685065/ID_23i3.1.pdf)

## 1. Media preparation

### 1.1 Preparation of blood-free broth for sample enrichment

**Broth medium:** Blood Free Campylobacter Broth

**Selective supplement:** Thermo Scientific™ Oxoid™ CCDA Selective Supplement

**Directions:**

1. Suspend 16.75 grams in 500 mL distilled water
2. Heat to dissolve the medium completely
3. Sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 minutes
4. Cool to 45–50°C
5. Aseptically add 1 vial of CCDA-Selective-Supplement
6. Mix well before dispensing into **sterile** containers

### 1.2 Preparation of CHROMagar plates – calculation for 1 L

Campylobacter growth is on CHROMagar™ at 42°C for 40–48 hrs. Campylobacter growth is on CHROMagar™. Preliminary identification of Campylobacter species from primary culture is by colonial appearance. On CHROMagar™, colonies appear as intense red coloured colonies on a translucent agar.

#### COMPOSITION

The product is composed of a powder base (B) and 1 supplement (S).

Product	=	Base (B)	+	Supplement (S)
Total g/L		51.2 g/L		0.21 g/L
Composition g/L		Agar 15.0 Peptone and yeast extract 25.0 Salts 9.0 Chromogenic and selective mix 2.2		Chromogenic and selective mix 0.21
Aspect		Powder Form		Powder Form
STORAGE		15/30 °C		2/8 °C
FINAL MEDIA pH		7.4 +/- 0.2		

Final Media	HELPING CALCULATION
1 L	0.21 g into 10 ml of purified water
5 L	1.05 g into 50 ml of purified water

#### Step 1: Preparation of the base

- Disperse slowly 51.2 g of powder base in 1L of purified water
- Stir until agar is well thickened
- Heat and bring to high temp (90-95°C) while swirling or stirring regularly

**DO NOT HEAT TO MORE THAN 95 °C. DO NOT AUTOCLAVE AT 121°C**

- Cool in a water bath to 45-50 °C. Swirl or stir gently to homogenize

#### Step 2: Preparation of the supplement

- In a transparent vessel, add 210 mg of supplement in 10 ml of purified water
- Swirl well until complete dissolution
- Filter to sterilize at 0.45 µm

#### Step 3: Base + supplement

- Add the 10 ml of the supplement solution to the melted base (Step1) when the base has reached 45-50 °C
- Swirl or stir gently to homogenize

#### Step 4: Pouring

- Pour into sterile Petri dishes
- Let it solidify and dry

#### Step 5: Storage

- Store in the dark before use
- Prepared media plates can be kept for one day at room temperature
- Plates can be stored for up to 1 month under refrigeration (2/8 °C) if properly prepared and protected from light and dehydration
- Note: If not fully used, rehydrated CHROMagar Campylobacter supplement can be stored one month at 2-8°C or at -20°C

If the agar plate has been refrigerated, allow to warm to room temperature before inoculation.

## 2. Plating samples

**Note: all plating should take place under a laminar flow cabinet.**

### 2.1 Direct plating of faecal samples

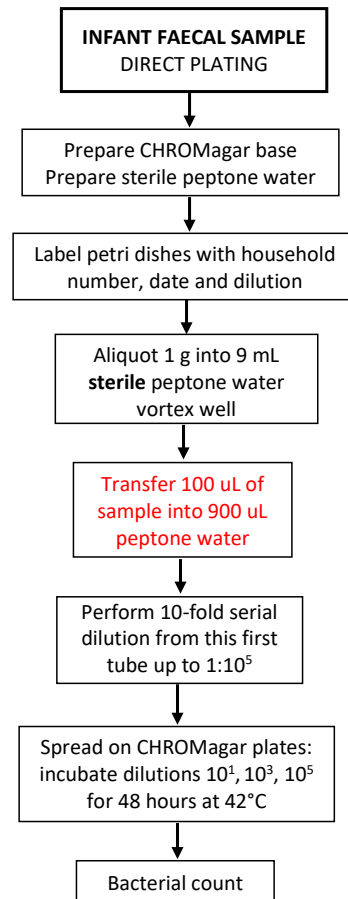
1. Label CHROMagar petri dishes with the household number, the date of the incubation and the dilution series
2. Collect 1 g of infant faeces. Record the **exact** faecal weight collected
3. Remove the air from the bag and return the bag to store the bag at 4°C until needed
4. Transfer the 1 g of faecal sample into a 15 mL sterile tube containing 9 mL of peptone water and vortex well with a vortex mixer
5. Take 100 uL of sample using pipette into a sterile centrifuge tube containing 900 uL of peptone water and perform 10-fold serial solution up to 1:10<sup>5</sup> dilution
6. Vortex very well each tube before transferring the 100 uL into a new tube. Change pipette tips between dilutions
7. Take 100 uL of the diluted samples and spread the dilution series as below on **pre-plated/pre-labelled** CHROMagar petri dishes (10<sup>1</sup>, 10<sup>3</sup>, 10<sup>5</sup>)
8. **Do not change the pipette tip if you go from a higher to a lower dilution but you must change tips between samples**
9. Incubate at **42°C for 48 hours** in microaerophilic conditions. This is done by placing the stack of petri dishes into the microaerophilic jar with 1 sachet of CampyGen per stack of 10 petri dishes in each 2.5 L jar  
(e.g. in a 2.5 litre jar, 1 sachet is required, in a 7.5 L jar, 3 sachets are required).
10. As soon as the sachets are opened, the contents activate, so place the sachets in the jar and seal the jar quickly
11. **Use the log sheet** to note the time the plate is cultured, infant ID number and the time the plate must come out

**Commented [SB1]:** Note: I can't remember if we did this step or if we just dispensed 100 uL of the vortexed sample and did a serial from that – I expect so as that would've been the first concentration. This is highlighted in red on the diagram below, so see if you need to skip this step

**Note: all reading of colonies should take place under a laminar flow cabinet. Be careful when removing the lid of the jar and taking stacks of plates out – high contamination risk!**

12. After 48 hours, count the colonies on each dilution plate and **record the data on the log sheet**

Figure 1. Testing flowchart for *Campylobacter* isolation, growth and enumeration



Whirl-Pak™ sterile bag with scoop	<a href="https://www.sigmaaldrich.com/catalog/product/sigma/wpb01478wa?lang=en&amp;region=GB">https://www.sigmaaldrich.com/catalog/product/sigma/wpb01478wa?lang=en&amp;region=GB</a>
Buffered dehydrated peptone water	<a href="https://www.fishersci.co.uk/shop/products/oxoid-buffered-peptone-water/p-4524834#?keyword=buffered+peptone+water">https://www.fishersci.co.uk/shop/products/oxoid-buffered-peptone-water/p-4524834#?keyword=buffered+peptone+water</a>
CHROMagar™ powder	Quoted from BioConnections: <a href="https://www.bioconnections.net/chromagar.html">https://www.bioconnections.net/chromagar.html</a>
CHROMagar supplement	Quoted from BioConnections: <a href="https://www.bioconnections.net/chromagar.html">https://www.bioconnections.net/chromagar.html</a> . Chromagar website for US customers is: <a href="https://drg-international.com/products/chromagar/">https://drg-international.com/products/chromagar/</a>
0.33 um filters, syringe	From university
Blood Free Campy Broth 500g	<a href="https://www.sigmaaldrich.com/catalog/product/sial/59751?lang=en&amp;region=GB">https://www.sigmaaldrich.com/catalog/product/sial/59751?lang=en&amp;region=GB</a>
CCDA Selective Supplement	<a href="https://www.sigmaaldrich.com/catalog/product/sial/77093?lang=en&amp;region=GB">https://www.sigmaaldrich.com/catalog/product/sial/77093?lang=en&amp;region=GB</a>
Oxoid™ CampyGen™ 2.5L sachet (packs of 10)	<a href="https://www.fishersci.co.uk/shop/products/oxoid-campygen-2-5l-sachet/10108012#?keyword=campygen">https://www.fishersci.co.uk/shop/products/oxoid-campygen-2-5l-sachet/10108012#?keyword=campygen</a>
Diamond shaped weigh boats	<a href="https://www.sigmaaldrich.com/catalog/product/sigma/hs1424aa?lang=en&amp;region=GB">https://www.sigmaaldrich.com/catalog/product/sigma/hs1424aa?lang=en&amp;region=GB</a>
Petri dishes (90 mm)	From university
15 ml polypropylene centrifuge tubes (flat top cap, conical bottom)	From university
Microaerophilic jar (2.5 L, 7.5 L)	From university
Pocket digital weighing scale	<a href="https://tinyurl.com/y8np6jks">https://tinyurl.com/y8np6jks</a>
500 mL glass jar for prepared broth storage	From university
1000ul pipette	From university
200ul pipette	From university
L-Shaped spreaders	<a href="https://tinyurl.com/y3onc2wh">https://tinyurl.com/y3onc2wh</a>
Incubator capable of 42°C	Lab
Laminar flow cabinet	Lab
Vortex mixer	Lab
Hot plate for agar prep	Lab
Nitrile gloves	Lab