CHN64: ISOLATION AND IDENTIFICATION OF *Campylobacter spp* ISOLATES FROM STOOL SAMPLES

1.0 PURPOSE / INTRODUCTION:

Introduction:
Campylobacter jejuni and other Campylobacter species are among several causative agents of diarrhoea in humans. To identify this specific microorganism, stool samples collected from patients are cultured on Campylobacter blood-free agar under microaerophilic conditions.

Purpose:
To give guidance on correct identification of *Campylobacter jejuni* isolates from stool samples.

2.0 SCOPE / RESPONSIBILITY:
This SOP is applicable to all lab staff working on the CHAIN Study microbiology sections.

The Principal Investigator (through the study coordinator when applicable) retains the overall responsibility of implementation of these standard procedures.

The Study Laboratory Coordinator is responsible for answering questions you may have about the content of this SOP and any other relevant study documentation. Please contact that the Study Laboratory Coordinator through your site coordinator.

3.0 SAFETY/RISK ASSESSMENT:
Specimen should be in leak proof containers and good laboratory practices observed especially wearing of Personal Protective Equipment and hand washing is of paramount importance

4.0 DEFINITIONS:
4.1 CAMPY - Campylobacter.
4.2 O_2 - Oxygen/Aerobiasis.
4.3 ^0C - Degrees Celsius.
4.4 CLSI - Clinical Laboratory Standards Institute.
4.5 NA - Nalidixic Acid.
4.6 MICROAEROPHILIC- Reduced oxygen atmosphere.

5.0 SPECIMEN:
5.1 Stool specimen.
5.2 *Campylobacter species* obtained on campy culture plates.

6.0 EQUIPMENT / MATERIALS/ REAGENTS:

6.1 **Equipments:**
- 6.1.1 Microscope.
- 6.1.2 O_2 incubator.
- 6.1.3 Vacuum pump.
- 6.1.4 Oxoid Anaerobic jar

6.2 **Materials:**

6.2.1 Campy agar.
6.2.2 Blood agar plate(s).
6.2.3 Nichrome wire loop.
6.2.4 Bunsen burner.
6.2.5 Glass slides.
6.2.6 Applicator sticks.
6.2.7 Eppendorf tubes
6.2.8 CAMPY Gas packs Oxoid Campy Gen ™ Pack 3.5 L Cat # CN 0035A

6.3 Reagents

6.3.1 Acetone
6.3.2 Oxidase reagent
6.3.3 Sodium hippurate
6.3.4 Ninhydrin

7.0 METHODOLOGY:

7.1 **Procedure:**

7.1.1 Register the request form information into the database.
7.1.2 Obtain Campy agar plates that passed QC for culture from the fridge and place them on the working bench to attain room temperature.
7.1.3 Label the media plates for culture with the patient admission serial number, date of processing and sample type.
7.1.4 Pull out the swab from the transport medium, ready for inoculation.
7.1.5 Inoculate the surface of the media plate by rolling the swab onto one point of the Campy agar medium.
7.1.6 Streak the medium you have just made using a pre-flamed and cooled wire loop.
7.1.7 Put the plate in an Oxoid anaerobic jar alongside a positive control plate and close the jar tightly.
7.1.8 Generate a microaerophilic environment by:-connecting the vacuum pump nozzle to the valve labelled 'vacuum' and putting on the pump to evacuate for one minute, unplug the pump nozzle and connect the gas tube nozzle to the jar filling valve and turn on the gas tap to fill the jar until the jar pressure gauge registers a reading of 5 psi. Repeat this three times and label the jar with the date when the culture are supposed to be removed for interpretation. You can also use an Oxoid Campy Gen gas pack in 3.5 L Campy jar for generation of microaerophilic.
7.1.9 Incubate the jar into the aerobic incubator at 37 0C for 48 hours.
7.1.10 Remove the campy agar plates from the Oxoid anaerobic jar after 48 hours and examine the morphological characteristics of growth obtained. Colonies typical of *Campylobacter* are:

1. Grey or colourless.
2. Can be flat and water-like droplets with irregular edges or round and convex with regular edges.
   Can be small or spreading over the agar plates.
3. In case of mixed growth, subculture/ purity plate on BA and incubate under microaerophilic.

7.2 **Identification:**

Colonies presenting any of the above morphology or characteristics are identified as follows:

7.2.1 Prepare a film for gram stain (Ref. SOP No.) and examine under a microscope using the X100 oil immersion objective.
NB:Counter stain with basic carbol fuchsin instead of 1% safranin.
Presumptive *Campylobacters* are gram negative, spiral and curved appearing in S-shape.

7.2.2 Perform an oxidase test (Ref. SOP No.) on the test colonies.
NB: Campylobacters are oxidase positive.

An isolate can be identified as presumptive campylobacter if it is microaerophilic and oxidase positive with distinctive spiral morphology

7.2.3 Perform a hippurate hydrolysis test (Ref. SOP No. ....). Include a positive and a negative control i.e. *Campylobacter jejuni* NCTC 11322/*Campylobacter jejuni sub spp jejuni* ATCC 33291 and *Escherichia coli* ATCC 25922 respectively.

7.2.4 Perform susceptibility testing on the confirmed isolate with an appropriate antibiotic panel as per CLSI/EUCAST recommendations

EUCAST Clinical Breakpoint Table v. 5.0, valid from 2015-01-01

Campylobacter spp			
Antibiotic	**Disk Content (ug)**	**Zone diameter breakpoint(mm)**	
		S ≥	**R <**
Ciprofloxacin	**5**	**26**	**26**
Erythromycin, C. jejuni	**15**	**20**	**20**
Erythromycin, C. coli	**15**	**24**	**24**
Tetracyclin	**30**	**30**	**30**

7.2.5 Freeze the isolate in Tryptone Soy broth with 15% glycerol at –70^{o}C freezer

7.3 **Results:**

7.3.1 **Interpretation:**

	Gram reaction	Oxidase	Hippurate hydrolysis
C. jejuni	Gram negative	+	+
C. coli	Gram negative	+	-
C. festus	Gram negative-	+	-
C laridis	Gram negative	+	-

TABLE 7.3.1 does not differentiate *Campylobacter coli, Campylobacter laridis and Campylobacter fetus*, so more tests are required.

Differentiation of the principal Campylobacter species

Campylobacter species	Growth at		Hippurate Hydrolysis	Susceptibility to	
	25 °C	42 °C		Nalidixic acid	Cephalothin
C. fetus subspp venerealis	+	-	-	v	S
C. fetus subspp fetus	+	v	-	R	S
C. jejuni sbspp jejuni	-	+	+	S	R
C. coli	-	+	-	S	R
C. lari	-	+	-	v	R
C. jejuni sbspp doylei	-	-	+	S	S

v= Variable reaction

R= Resistant — **Resistant: Growth up to the edge of the disk**

S= susceptible — **Susceptible: Any zone of inhibition**

API 20 E Campy also used for identification of Campylobacter at species level.

7.3.2 **Reporting:**

Results are reported on patient's request form and logged into the computer database as per site practice.

7.3.3 **Sources of error:**

- Misinterpretation of the gram stained film.
- Improper generated microaerophilic condition during incubation.
- Improper prepared agar plate(s).

8.0 APPENDICES:
N/A

9.0 REFERENCES:

9.1 De Mol, P. et al. Enteropathogenic agents in children with diarrhoea in rural Zaire. Lancet, 1, pp 516-518, 1983.
9.2 Mackie and MacCartney. Practical medical microbiology, thirteenth edition.
9.3 Campylobacter jejuni infections: Update on emerging issues and trends: Clin. Infect. Dis 2001 Apr. 15; 32(8): 1201-6.

Document history

Version	Author	Approved by	Dated
1.01 **(MASTER) ISOLATION AND IDENTIFICATION OF *Campylobacter spp* ISOLATES FROM STOOL SAMPLES.CHN**	Katana Karisa	Caroline Tigoi	10/10/2016
1.02			

Site training record
All sites are required to maintain a master copy of this SOP that documents the site staff that have been trained on this SOP.

Document History				
Version No.	**Trained staff initials**	**Signature of trained staff**	**Date**	**Trainer's Initials**
1.01	KDT	Example row	1st Jan 2016	DM