Service contract for the provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in *Salmonella* species and *Campylobacter* species in human samples

SC 2019 74 09

Identification of thermotolerant Campylobacter

May 2022

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1. BACKGROUND

The following procedures will guide you through the steps that are necessary to carry out a biochemical identification of Campylobacter.

Campylobacter are generally identified by the following traits:
• Slender helical or curved gram-negative rods.
• Highly motile by means of a single polar flagellum. Characteristic darting and corkscrew like motility can be observed using phase contrast microscopy.
• Optimal oxygen concentration for growth 5-10%.
• Do not ferment or oxidize sugars.
• Do not produce indole (mind the different with hydrolysis of indoxyl acetate!).

According to ISO 10272 (Microbiology of food and animal feeding stuffs – Horizontal method for detection of thermotolerant Campylobacter) Campylobacter is identified by the following characteristics:
• Morphology and motility
• Gram staining
• Oxidase
• Fermentation of
  glucose
  lactose
• sucrose

In this course identification and differentiation of Campylobcater is performed by:
• Hippurate hydrolysis test
• Test for hydrolysis of indoxyl acetate
• PCR (There is a protocol for PCR)

2. MATERIAL AND METHODS

Equipment

• Disposable inoculation loops (1 µl and 10 µl)• Incubators at 37°C/42 °C
• Pipette 200 µl

Media
• 1%-hippurate solution
• 3.5%-ninhydrin solution
• 10%-indoxyl acetat solution

Bacterial strains:
Campylobacter jejuni ATCC 33560
Campylobacter coli ATCC 33559
Campylobacter lari ATTC 23947

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

PROCEDURE

Hippurate hydrolysis

Suspend a loopful of a culture from an 18-24 hour Columbia agar plate containing 5% e ba in 400 µl of a 1%-hippurate solution (take care not to incorporate agar!). Incubate at 37°C for 2 hours. Then slowly add 200 µl 3.5%-ninhydrin solution to the side of the tube to form an overlay. Reincubate at 37°C for 10 min, and read the reaction.

Positive reaction: dark purple/blue.
Negative reaction: clear or grey.

Hippurate hydrolysis test is used to detect the ability of bacteria to hydrolyse substrate hippurate into glycine and benzoic acid by action of hippuricase enzyme present in bacteria. Hippuricase is a constitutive enzyme that hydrolyzes the substrate hippurate to produce the amino acid glycine. Glycine is detected by oxidation with Ninhydrin reagent, which results in the production of a deep purple color. Hippurate hydrolysis test is used in the presumptive of Campylobacterjejun.

1%-hippurate solution can be stored at -20 ^{o}C for six month.

3.5%-ninhydrin solution: Stable for about one month when stored at room temperatur in a dark bottle.

Hydrolysis of indoxyl acetate

Add 50 µl of a 10% (w/v) solution of indoxyl acetate in acetone to an absorbent paper disc 6 mm in diameter and allow to dry in air or use commercial disc. Apply material from a culture from an 18-24 hour Columbia agar plate containing 5% e directly to disc and then wet with a drop of sterile distilled water.
Appearance of a blue-green color within 5-10 minutes indicates a positive result.

The bacterial enzyme esterase releases indoxyl from indoxyl acetate which spontaneously forms indigo in the presence of oxygen.

Dried discs are stable for at least 12 months if stored at 4ºC in a dark glass bottle with silica gel. Discs should not be used if the color has changed from white, or if the expiration date has passed

3. COMPOSITION AND PREPARATION OF CULTURE MEDIA AND REAGENTS

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

Saline solution

Sodium chloride 8.5 g
Water 1000 ml
Preparation:
Dissolve sodium chloride in water, by heating if necessary. Adjust pH ~ 7.0 after sterilisation. Dispense the solution into tubes of 4 ml and autoclave at 121°C for 20 min.

3,5% Ninhydrin solution
Ninhydrin ($C_9H_6O_4$) 3,5 g
Acetone (C_3H_6O) 50 ml °
Butanol ($C_4H_{10}O$) 50 ml

Dissolve/mix the chemical in the solutions. Stored at +5 °C in dark bottles of 20 ml.

1% Hippurat solution
Natriumhippurat ($C_9H_8NNaO_3$) 1 g
PBS 99 ml

Dissolve the chemical with the solutions. Stored at -20 °C in tubes of 15 ml.

10% (wt/vol) indoxylacetat solution
Indoxylacetat ($C_{10}H_9NO_2$) 10 g
Acetone (C_3H_6O) 90 ml

Dissolve the chemical in acetone. Stored at +4 °C in a dark bottle.

4. REFERENCES

- Nachamkin I. and M. J. Blaser (eds) (2000). Campylobacter 2nd ed. ASM Press, Washington, D.C.
- BARROW & FELTHAM (eds.): Cowan and Steel´s Manual for the Identification of Medical Bacteria, 3 rd edn.
- NMKL method no. 119, 2nd ed, Campylobacter Jejuni/Coli detection in foods. Nordic committee on food analysis.

IDENTIFICATION OF CAMPYLOBACTER

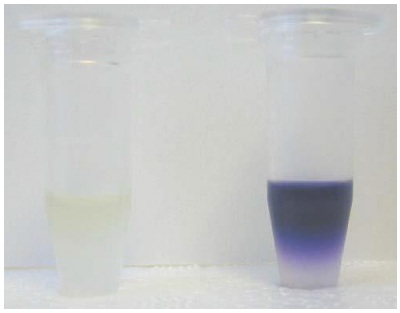
Biochemical tests

Dato: _____ Initialer:_____

	Strain #	**Strain** #	**Strain** #	**Strain** #	**Strain** #	**Strain** #
Hippurate Hydrolusis						
Hydrolysis of indoxyl acetate						
Species:						

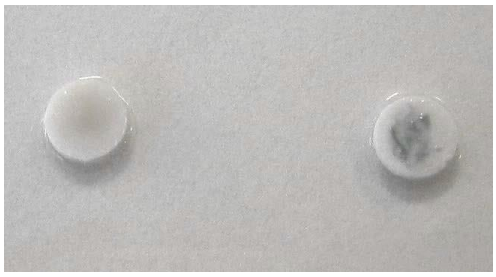
5. IDENTIFICATION HIPPURATE AND INDOXYL ACETATE

Hippurate hydrolysis:



Left: Negative reaction: C. coli ATCC 33559
Right: Positive reaction: C. jejuni ATCC 33560

Hydrolysis of Indoxyl acetate:



Left: Negative reaction: C. lari ATCC 23947
Right: Positive reaction: C. jejuni ATCC 33560

6. RESULT SHEET HIPPURATE AND INDOXYL ACETATE

Result sheet for identification of Campylobacter

	Campylobacter jejuni	*Campylobacter lari*	*Campylobacter coli*
Hippurate hydrolysis	+	-	-
Hydrolysis of indoxyl acetate	+	-	+

Comment:_____

_____

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