Campylobacter Agar Base Blood Free (CCDA) ISO

Cat. 1129

Selective medium for the isolation of Campylobacter spp.

Practical information

Aplications	Categories
Selective enumeration	Campylobacter
Selective isolation	Campylobacter

Industry: Water / Food

Regulations: ISO 10272 / ISO 11133

Principles and uses

Campylobacter Agar Base Blood Free (CCDA) is a modified formula described by Bolton et al., replacing blood by charcoal, sodium pyruvate and ferrous sulfate. This medium supports the growth of most enteric Campylobacter and it is recommended for the selective isolation of Campylobacter jejuni, Campylobacter coli and thermophilic Campylobacter, in foods and in clinical and non-clinical specimens. It is recommended by ISO 10272.

Campylobacter is considered the main cause of enteric illnesses. Campylobacter spp. can cause mild to severe diarrhea, with loose, watery stools frequently followed by bloody diarrhea. These pathogens are very infective and are transmitted by contaminated food or water.

The medium contains ferrous sulfate, sodium pyruvate and charcoal to promote the growth of Campylobacter species, as they quench the toxic forms of oxygen (hydrogen peroxide) increasing the aerotolerance and enabling the oxygen sensitive strains to be readily isolated. Sodium desoxycholate partially or completely inhibits Gram positive organisms, coliforms and Proteus. Enzymatic digest of animal tissue and casein, and beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological agar is the solidifying agent. Cefoperazone increases selectivity and inhibits the growth of Gram negative enteric bacilli and some Gram-positive species, whilst Amphotericin B suppresses yeasts and fungi growth. The increment of temperature has shown an increase in selectivity.

Formula in g/L

Enzymatic digest of casein	3	Activated charcoal	4
Bacteriological agar	12	Ferrous sulfate	0,25
Beef extract	10	Sodium chloride	5
Sodium deoxycholate	1	Sodium pyruvate	0,25
Enzymatic digest of animal tissues	10		

Preparation

Suspend 22,75 grams of the medium in 500 ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. Cool to 44-47 ºC and aseptically add one vial of CCDA Supplement (Campylobacter Blood Free) (Cat. 6053), previously reconstituted in 5 ml of sterile distilled water. Homogenize gently and dispense into Petri dishes.

Instructions for use

For the detection and enumeration of Campylobacter spp. according to ISO 10272:

For samples with low numbers of campylobacters and low level of background microflora and/or stressed campylobacters:
- Inoculate 10 g or 10 ml of the test portion into 90 ml of the enrichment medium Bolton Broth, so as to obtain a 1 in 10 dilution.
- Incubate in a microaerobic atmosphere at 37 ºC for 4 to 6 hours, then at 41,5 ºC for 44±4 hours.
- Using the culture obtained from enrichment broth (Bolton Broth), inoculate the CCDA medium and other Campylobacter selective medium with a sterile loop.
- Incubate the CCDA plates at 41,5 ºC in a microaerobic atmosphere for 44±4 hours.
- Confirm the suspect colonies.

For samples with low numbers of campylobacters and high level of background microflora:
- Inoculate 10 g or 10 ml of the test portion into 90 ml of the enrichment medium Preston Broth, so as to obtain a 1 in 10 dilution.
- Incubate in a microaerobic atmosphere at 41,5 ºC for 14±2 hours.

- Using the culture obtained from enrichment broth (Preston Broth), inoculate the CCDA medium with a sterile loop.
- Incubate the CCDA plates at 41,5 ºC in a microaerobic atmosphere for 44±4 hours.
- Confirm the suspect colonies.

For samples with high numbers of campylobacters:
- Inoculate the test portion if the product is liquid or the initial suspension in the case of other products (direct plating) onto CCDA plates.
- Incubate the plates at 41,5 ºC in a microaerobic atmosphere for 44±4 hours.
- Confirm the suspect colonies.
- The direct plating method is the adequate procedure to count the colonies of Campylobacter.

If little information is available about the test samples, use one of the two enrichment methods in parallel with the direct plating method.

Typical colonies are greyish, often with a metallic sheen, and are flat and moist, with tendency to spread.

In order to confirm the suspect colonies examine the Campylobacter colonies for morphology and motility using a microscope and sub-cultured on a non-selective blood agar, and then confirm by detection of oxidase activity and an aerobic growth test at 25 ºC.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Black	Black	7,4±0,2

Microbiological test

According to ISO 10273:
Incubation conditions: (41,5±1 ºC, microaerobic atmosphere /44±4 h).
Inoculation conditions: Productivity quantitative (100±20. Min.50 CFU) / Productivity quantitative (10^3-10^4 CFU) / Selectivity (10^4-10^6 CFU).
Reference media: TSA

Microrganisms	Specification	Characteristic reaction
Escherichia coli ATCC 25922	Total or partial inhibition (0-1)	
Staphylococcus aureus ATCC 25923	Total inhibition (0)	
Campylobacter jejuni ATCC 29428	Good growth (2) >50%	Greyish, flat and moist, sometimes with metallic sheen
Campylobacter coli ATCC 43478	Good growth (2) >50%	Greyish, flat and moist, sometimes with metallic sheen

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Bolton F.J. Hutchinson D.N. y Cioste D. (1984) clin. Microbiol. 19,169-171 Bolton E.J., Roberstson L. (1982) J. Clin Parth 35, 462-67
ISO 10272-1:2017. Microbiology of the food chain -- Horizontal method for detection and enumeration of Campylobacter spp. -- Part 1: Detection method