

Modified charcoal cefoperazone desoxycholate (mCCD) medium base (ISO)

Code 84695.0500

Also known as

mCCD agar, Bolton CCDA, Campylobacter blood free agar Bolton.

Intended use

Blood free medium base for the detection and enumeration of *Campylobacter* spp. in food and animal feeding stuffs.

Formula* - Composition in g/L

Meat extract	10.00
Enzymatic digest of animal tissues....	10.00
Enzymatic digest of casein	3.00
Charcoal	4.00
Sodium chloride	5.00
Sodium desoxycholate	1.00
Iron(II) sulfate.....	0.25
Sodium pyruvate	0.25
Agar.....	15.00

* Adjusted and/or supplemented as required to meet performance criteria

Final pH 7.4 ± 0.2 at 25 °C.

Instructions for preparation

Dissolve 24.5 g in 500 ml of purified water by bringing to the boil with frequent shaking. Sterilise in the autoclave at 121 °C for 15 minutes and cool to 45-50 °C. Dissolve the contents of one vial of CCD selective supplement (Art.: 84742.0001) with 5 ml of sterile purified water and add to the cooled medium. Mix well and pour into sterile Petri dishes.

Principle of the method and general information

Modified charcoal cefoperazone desoxycholate (mCCD) medium base, with the addition of the CCD selective supplement, corresponds to a modification of the original formulation proposed by Bolton, Hutchinson and Coates and complies with the formulation described by ISO Standard 10272 parts 1, 2 and 3. The inclusion of charcoal in the medium base neutralizes toxic oxygen derivatives, and the antimicrobials compounds supplied by the freeze-dried supplement (cefoperazone and amphotericin B), provide for an excellent medium for the recovery of *C. jejuni*, *C. coli*, and *C. lariidis* from faeces and other specimens. ISO Standards recommend the use of mCCDA agar for the detection and enumeration of *Campylobacter*, from food and animal feeding stuffs.

Instruction for use

For laboratory use only.

For the detection of *Campylobacter* in food and animal feeding stuffs, ISO 10272 recommends the method summarized below.

- For preparing the initial suspension, introduce a quantity x (g or ml - mass or volume) of the test portion into nine times its volume of Bolton broth (Art. N° 84697.0500) so as to obtain a test portion/enrichment medium ratio of 1:10 and homogenize.
- Incubate the initial suspension in a microaerophilic atmosphere at 37 °C for 4 h to 6 h, then at 41.5°C for 44 h ± 4 h.
- Using the culture obtained in the enrichment medium, inoculate with a sterile loop the surface of the first selective medium: Modified charcoal cefoperazone (CCD) agar (Art. N° 84695.0500)
- Proceed in the same manner for the second isolation medium (e.g. Karmali agar, Art. N° 84696-0500)
- Incubate the plates at 41,5°C for 44 h ± 4 h in a microaerophilic atmosphere

- After 44 h \pm 4 h of incubation, examine the plates for typical and/or suspected colonies of *Campylobacter*. The typical colonies on Modified charcoal cefoperazone (CCD) agar are greyish often with a metallic sheen and are flat and moist with a tendency to spread. Colonies spread less on dried agar surfaces. Other forms of colonies may occur.
- For confirmation tests, take from each plate of selective medium at least one colony considered to be typical or suspected and a further four colonies if the first is negative. Streak a plate of Columbia blood agar in order to allow the development of well isolate colonies. Incubate the plates at 41.5°C for 24-48 h in a microaerophilic atmosphere and perform the confirmatory tests and, if required, the identification tests.

Limitations

- It is recommended that biochemical and/or serological tests be performed on pure culture for complete identification.

Quality Control

Physical characteristics:

Appearance of powder	Black, fine, homogeneous, hygroscopic powder
Appearance of prepared medium	Black, opaque
pH (25°C)	7.4 \pm 0.2

Microbiological characteristics:

Test strains	Incubation T° / t / At.	Inoculation method	Growth characteristics	Productivity rate
<i>C. jejuni</i> ATCC 29428	41,5 °C / 44 h / M	QT	good growth, grey colonies	PR \geq 0.7
<i>C. coli</i> ATCC 43478	41,5 °C / 44 h / M	QT	good growth, grey colonies	PR \geq 0.7
<i>E. coli</i> ATCC 8739	41,5 °C / 44 h / M	MM	Totally inhibited	
<i>S. aureus</i> ATCC 25923	41,5 °C / 44 h / M	MM	Totally inhibited	
<i>C. albicans</i> ATCC 18804	41,5 °C / 44 h / M	MM	Partially inhibited	
<i>E. faecalis</i> ATCC 19433	41,5 °C / 44 h / M	MM	Totally inhibited	

Notes

Medium supplementation: CCD selective supplement (REF 84742.0001)
 PR (Productivity Ratio): CFU obtained on the culture medium under test / CFU obtained on the Reference Batch
 Incubation atmosphere M: microaerophilic atmosphere
 Inoculation method QT : quantitative surface plating method; MM: modified Miles-Misra surface drop method
 Microbiological characteristics tested in accordance to ISO/TS 11133-2
 ATCC is a registered trade mark of American Type Culture Collection;

References

- Bolton, F.J. Hutchinson, D.N. and D. Coates. 1984. J. Clin. Microbiol. 19, 167-171.
- ISO 10272-1:2006 - Microbiology of food and animal feeding stuffs - Horizontal method for detection and enumeration of *Campylobacter* spp. -- Part 1: Detection method.
- ISO/TS 10272-2:2006-Microbiology of food and animal feeding stuffs -- Horizontal method for detection and enumeration of *Campylobacter* spp. -- Part 2: Colony-count technique
- ISO/TS 10272-3:2010-Microbiology of food and animal feeding stuffs -- Horizontal method for detection and enumeration of *Campylobacter* spp. -- Part 3: Semi-quantitative method

Storage conditions

For laboratory use only. Keep tightly closed, away from bright light at +2°C to 8°C and <60% RH.

Ordering information

Dehydrated medium:

84695.0500 Modified charcoal cefoperazone desoxycholate (mCCD) medium base (ISO) Bottle of 500

Supplement:

84742.0001 CCD selective supplement 10 vials, each for 500 ml of complete medium