Also known as

mCCDA

Specification

Selective plating medium used for the detection and enumeration of *Campylobacter spp* according to the ISO 10272 standard.

Formula * in g/L

Meat extract..... 10,00
Peptone..... 10,00
Sodium chloride..... 5,00
Bacteriological charcoal..... 4,00
Casein hidrolysate..... 3,00
Sodium deoxycholate..... 1,00
Iron (II) sulfate..... 0,25
Sodium pyruvate..... 0,25
Agar..... 15,00

Final pH 7,4 ± 0,2 at 25 ºC
* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 24.2 g of powder in 500 mL of distilled water and bring to the boil to dissolve. Sterilize in an autoclave at 121ºC for 15 minutes. Cool to 47-50ºC and aseptically add one vial of the Campylobacter CCDA Selective Supplement (Art. No. DSHB3110). Mix carefully and pour into sterile Petri dishes.
Note: If the plates are prepared in advance, they should be kept for not more than 4 hours at ambient temperature or for no more than 7 days in the dark at 3 ± 2ºC.

Description

CCD Modified Agar is formulated according to the ISO Standard 10272-1:2006 and is intended to detect and enumerate *Campylobacter spp* from food and animal feeding stuffs.
After determining that *Campylobacter* species grow best on solidified Nutrient Broth No. 2 compared to other media workers (1983) carried out a systematic survey of alternatives to blood for neutralizing oxygen toxicity. A combination of 0,4% charcoal, 0,25% ferrous sulfate and 0,25% sodium pyruvate proved best.
A further study surveyed the suppressive effect of several inhibitors on the undesirable microbiota showing deoxycholate and cefazolin as the most effective inhibitory agents. Later, in 1984, Hutchinson and Bolton replaced cefazolin (10 mg/L) with cefoperazone (32 mg/L). This allowed fewer contaminants to grow, and permitted the modified medium (modified CCD Agar or mCCDA) to be used at 37ºC. However amphotericin B was needed to prevent overgrowth by yeast able to grow at 37ºC but not at 41,5 ± 1ºC.
In 1993 Aspinall *et al.* developed a modification of mCCDA designed for use at 37ºC to isolate *C. upsaliensis* as well as the other thermophilic *campylobacter* species. This medium contains 8 mg/L cefoperazone and 4 mg/L teicoplanin replacing 32 mg/L cefoperazone in mCCDA. Teicoplanin has an antimicrobial spectrum similar to that of vancomycin, active mainly against Gram positive bacteria. By comparison with mCCDA the final formulation of this medium, called CAT Agar, isolated the same numbers of *Campylobacter spp* other than *C. upsaliensis* from faeces and is superior to mCCDA for *C. upsaliensis* with slightly higher growth of competing microflora.

Necessary supplements

Campylobacter CCDA Selective Suplement (Art. No. DSHB3110)
Vial Contents:
Necessary amount for 500 mL of complete medium.
Amphotericin B..... 5,00 mg
Cefoperazone..... 16,00 mg

Distilled water (Solvent)

Technique

Immediately before use, carefully dry the agar plates, preferably with the lids off and the agar surface downwards, in a drying cabinet, until the agar surface is free of visible moisture (maximum 30 minutes).
Using the culture obtained from enrichment broth (Bolton Broth), inoculate the mCCDA with a sterile loop. Incubate plates at 41,5ºC in a microaerobic atmosphere (approximately 5% O2, 10% CO2 and 85% N2 or H2), for 44 ± 4 hours.

- Campylobacter jejuni strains produce grey, moist flat and occasionally spreading growth which may be accompanied with a green hue and/or a metallic sheen.
- Campylobacter coli strains tend to be creamy-grey in colour, moist and often produce a more discrete type of colony.
- Campylobacter lari strains are more varied and produce both types of colonial morphology.
- Occasionally contaminating organisms may grow on this medium. These include cefoperazone-resistant Pseudomonas spp, Enterobacteriaceae, and some streptococci and yeasts.

Quality control

Incubation temperature: 41,5 ± 1ºC **Incubation time:** 44 ± 4h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (productivity) / 10^4-10^6 CFU (selectivity) according to ISO 11133:2014/Amd 1:2018 .

Microorganism	Growth	Remarks
Campylobacter jejuni ATCC® 29428	Productivity > 0.50	Under microaerophilic atmosphere
Campylobacter coli ATCC® 43478	Productivity > 0.50	Under microaerophilic atmosphere
Escherichia coli ATCC® 8739	Partial Inhibition	-
Staphylococcus aureus ATCC® 25923	Total inhibition	-

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· ISO 10272-2 Standard (2017) Microbiology of the food chain - Horizontal Method for detection and enumeration of Campylobacter spp. - Part 2:Colony count-tecnique.
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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).