Modified Charcoal Cefoperazone Deoxycholate Agar Base (mCCD agar)

M887I

Intended use

Recommended for selective detection and enumeration of *Campylobacter* species from food chain. The composition and performance criteria of this medium are as per the specifications laid down in ISO 10272-1:2017, 10272-2:2017, ISO 17995:2019 and ISO 11133:2014 (E) /Amd.: 2020

Composition**

ISO 10272-1 and ISO 10272-2 / ISO 17995:2019 (E)

Specification - mCCD agar

Ingredients	g / L
Meat extract	10.000
Enzymatic digest of animal tissues	10.000
Enzymatic digest of casein	3.000
Sodium chloride	5.000
Sodium deoxycholate	1.000
Iron (II) sulfate, hydrate	0.250
Sodium pyruvate	0.250
Activated charcoal	4.000
Agar	8.0-18.0
Supplements to be added after autoclaving	
Cefoperazone	0.032 g
Amphotericin B	0.01 g
Final pH (at 25°C)	7.4±0.2

mCCD agar **M887I**

Ingredients	g / L
HM Extract #	10.000
Peptone ##	10.000
Tryptone ###	3.000
Sodium chloride	5.000
Sodium deoxycholate	1.000
Iron (II) sulfate, hydrate	0.250
Sodium pyruvate	0.250
Activated charcoal	4.000
Agar	12.000
CCDA Selective Supplement-FD135 (2 vials)	
Cefoperazone	16 mg
Amphotericin B	5mg
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Meat extract ## Equivalent to Enzymatic digest of animal tissues,
Equivalent to Enzymatic digest of casein

Directions

Suspend 22.74 gram (the equivalent weight of dehydrated medium per litre) in 500 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of CCDA Selective Supplement (FD135). Mix well and pour into sterile Petri plates.

Principle And Interpretation

*Campylobacter*s are carried in the intestinal tract of animal and therefore contaminate foods of animal origin (1). *Campylobacter* causes intestinal upset or abortion in animals. It is also one of the most important causes of human gastroenteritis, particularly in children. Initially blood was used in the isolation of *Campylobacter*. But, later it was reported by Bolton et al (2) that charcoal can be effectively used in place of blood. This rules out the variability obtained due to the use of blood.Modified Charcoal Cefoperazone Deoxycholate Agar Base is formulated as per APHA (1) and is also recommended by the ISO Committee (3,4,5) for detection and enumeration of Campylobacter spp. from food and environmental samples in food production area.

Cephalothin in the original formulation was replaced by Cefoperazone as the selective agent since the latter gave better selectivity (7). Campylobacter species are highly resistant to cefoperazone, an antibiotic which effectively suppresses growth of Pseudomonas and Enterobacteriaceae (8-10). Addition of cefoperazone increases the selectivity of the medium. Due to this addition, the medium is also known as Campylobacter Charcoal Differential Agar (CCDA). Charcoal, sodium pyruvate and iron sulfate reduces the aero tolerance of medium by quenching photo chemically generated toxic oxygen derivatives (11). Peptone, Tryptone and HM extract serve as sources of carbon, nitrogen, long chain amino acids and essential nutrients. Sodium chloride maintains osmotic balance. Additional Amphotericin B suppresses the growth of yeast and mold contaminants.

Type of specimen

Food samples, Water samples

Specimen Collection and Handling:

Processesing : (3,4,5)

Test portion and initial suspension:

Selective Enrichment A : To prepare the initial suspension, combine a quantity of 10g or 10ml of the test portion with 90ml of the enrichment medium Bolton Broth (M1592) from cooked and frozen food. Incubate the initial suspension in a microaerobic atmosphere at 37°C for 4 to 6 hours and then at 41.5°C for 44 hours ± 4 hours.

Selective Enrichment B: To prepare the initial suspension, combine a quantity of 10g or 10ml of the test portion with 90ml of the enrichment medium Preston Broth (M899I) from raw meats or raw milk. Incubate the initial suspension in a microaerobic atmosphere at 41.5°C for 24 hours ± 2 hours.

Plating out : Using the culture obtained in the enrichment medium, inoculate with a sterile 10 µl loop on the surface of mCCD Agar. Incubate the plates at 41.5°C in a microaerobic atmosphere for 44 hours ± 4 hours.

Selective Enrichment C: Directly swab or streak on mCCD agar from caecal or faecal samples by using a loop or a sterile swab and second medium can be optional. Incubate the plates at 41.5°C in a microaerobic atmosphere for 44 hours ± 4 hours.

Confirmation : Biochemical and serological tests are performed for confirmation.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Further biochemical tests must be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Grey to black homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel

Colour and Clarity of prepared medium

Black coloured, opaque gel forms in Petri plates

Reaction

Reaction of 4.55% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Productivity : Cultural characteristics observed with added CCDA Selective Supplement V (FD135),after an incubation at 41.5°C±1°C for 44 ± 4 hours under micro-aerobic atmosphere. Recovery is considered as 100 % on Reference medium - Blood Agar.

Selectivity : Cultural characteristics observed with added CCDA Selective Supplement V (FD135),after an incubation at 41.5°C±1°C for 44 ± 4 hours under micro-aerobic atmosphere.

Organism	Inoculum (CFU)	Growth	Recovery	Characteristic reaction
Productivity				
Campylobacter coli ATCC 33559 (00004*)	50-100	good-luxuriant	>=50%	Greyish, flat and moist colonies, sometimes with metallic sheen
Campylobacter jejuni ATCC 33291 (00005*)	50-100	good-luxuriant	>=50%	Greyish, flat and moist colonies, sometimes with metallic sheen

Campylobacter jejuni ATCC 29428 (00156*)	50-100	good-luxuriant	>=50%	Greyish, flat and moist colonies, sometimes with metallic sheen
Selectivity				
Escherichia coli ATCC 25922 (00013*)	>=10^4	total or partial inhibition		No characteristic colonies
Escherichia coli ATCC 8739 (00012*)	>=10^4	total or partial inhibition		No characteristic colonies
Escherichia coli ATCC 11775 (00090*)	>=10^4	total or partial inhibition		No characteristic colonies
Escherichia coli (00179*)	>=10^4	total or partial inhibition		No characteristic colonies
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	total or partial inhibition		No characteristic colonies
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	>=10^4	total or partial inhibition		No characteristic colonies

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (11,12).

Reference

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