Technical Data

Modified Charcoal Cefoperazone Deoxycholate Agar Plate

MP887I

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.

Composition**

ISO 10272-1 and ISO 10272-2 Specification - mCCD agar

Ingredients	Gms / Litre
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	Gms / Litre
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

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Principle And Interpretation

Campylobacters 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) 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 (5). *Campylobacter* species are highly resistant to cefoperazone, an antibiotic which effectively suppresses growth of *Pseudomonas* and *Enterobacteriaceae* (6,7,8). 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 (8). 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

Specimen Collection and Handling:

Processesing : (3,4)

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. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate themedium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. 3.It is recommended to store the plates at 24-30°C to avoid minimum condensation.
4. 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

Sterile Modified Charcoal Cefoperazone Deoxycholate Agar in 90mm disposable plates with smooth surface and absence of black particles/ cracks/ bubbles.

Colour of medium

Black coloured medium

Quantity of medium

25ml of medium in 90mm plate

pH

7.20-7.60

Sterility Check

Passes release criteria

Cultural Response

Cultural characteristics observed after an incubation at 41.5°C ± 1°C for 40 hours under microaerobic atmosphere. Recovery is considered as 100% on Blood Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Productivity				
Campylobacter coli ATCC 33559 (00004*)	50-100	good-luxuriant	>=50%	greyish,flat colonies, may have metallic sheen
Campylobacter jejuni ATCC 29428 (00005*)	50-100	good-luxuriant	>=50%	greyish,flat colonies, may have metallic sheen
Selectivity				
Escherichia coli ATCC 25922 (00013)*	50-100	total or partial inhibition	<=10%	

Escherichia coli ATCC 8739 (00012*)	50-100	total or partial inhibition	<=10%
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

On receipt store between 2-8°C Use before expiry date on the label. 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 (9,10).

Reference

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