M-CP Agar Base

Recommended by the Directive of the Council of the European Union 98/83/EC for isolation and enumeration of *Clostridium perfringens* from water sample using membrane filtration technique.

M1354

Composition **

Ingredients	Grams/Litre
Tryptose	30.00
Yeast extract	20.00
Sucrose	5.00
L-Cysteine hydrochloride	1.00
Magnesium sulphate, $7H_2O$	0.10
Bromo cresol purple	0.04
Ferric chloride, $6H_2O$	0.09
Indoxyl-β-D-glucoside	0.06
Agar	15.00

Final pH (at 25°C) 7.6 ± 0.2

** Formula adjusted, standardized to suit performance parameters

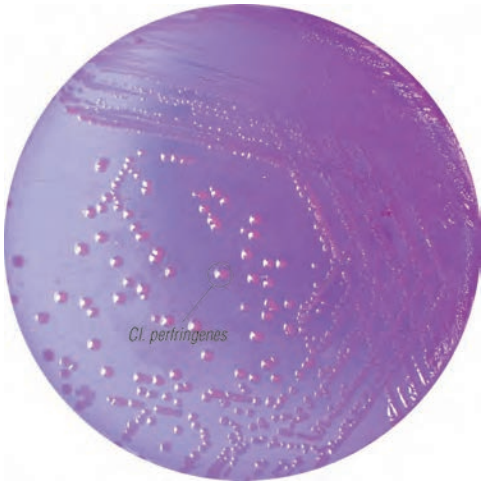
Directions

Suspend 35.60 grams (the equivalent weight of dehydrated powder per 485 ml) in 485 ml 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. Aseptically add the rehydrated contents of 1 vial of M-CP Selective Supplement I (FD153) and 1 vial of M-CP Selective Supplement II (FD154) or rehydrated contents of 1 vial of M-CP Selective Supplement II Modified (FD154A). Mix well and pour into sterile Petri plates.

Principle and Interpretation

Clostridial species are one of the major causes of food poisoning/ gastro-intestinal illnesses. They are gram-positive, spore-forming rods that occur naturally in the soil (2). Among the family are: *Clostridium botulinum* which produces one of the most potent toxins in existence; *Clostridium tetani*, causative agent of tetanus; and *Clostridium perfringens* commonly found in wound infections and diarrhoea cases. The use of toxins to damage the host is a method deployed by many bacterial pathogens. The major virulence factor of *C. perfringens* is the CPE enterotoxin, which is secreted upon invasion of the host gut, and contributes to food poisoning and other gastrointestinal illnesses (2). Several solid media have been devised for quantitation of *C. perfringens*. The selectivity of the media is achieved by incorporation of one or more antibiotics that inhibit certain anaerobes or facultative anaerobes. M-CP Agar Base is prepared as per the formula of Armon and Payment (1). It is also recommended by the Directive of the Council of the European Union 98/83/EC (3) for isolation and enumeration of Clostridium perfringens from water sample using membrane filtration technique.

Tryptose, yeast extract provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamin B complex and other essential growth nutrients compounds while sucrose is the fermentable carbohydrate. Bromocresol purple serves as a pH indicator. Indoxyl-β-D-glucoside is a chromogenic substrate for β-D-glucosidase or cellobiose and phenolphthalein diphosphate for the detection of acid phosphatase. The addition of D-cycloserine and polymyxin B (FD153) makes the medium inhibitory to accompanying non-clostridial microflora and thus allows analysis of both clostridial vegetative cells and spores. Further selectivity is provided by incubation under anaerobic conditions. Yellow (cellobiose-negative) colonies becoming old rose to pink-red upon exposure to ammonia fumes for 30 seconds are considered to be presumptive *C. perfringens*. Colour differentiation on M-CP Agar Base is sometimes difficult, so typical colonies (yellow turning into pink) as well as a typical colonies (green or those that remain yellow upon exposure to ammonia fumes) are picked for confirmation. Presumptive *C. perfringens* can be confirmed by sulphite reduction, gram-positive, sporulating rods, non-motile, reduction of nitrate, gelatine liquefaction, lactose fermentation and other biochemical tests (4).



M1354 M-CP Agar Base

HiCromeVeg™ Single Streak Rapid Differentiation Series *Freedom from BSE / TSE worries*

M-CP Agar Base (M1354) is also available as M-CP HiVeg™ Agar Base (MV1354) wherein all the animal origin nutrients have been replaced by vegetable based nutrients.

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Type of specimen

Food samples

Specimen Collection and Handling

For Food samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (5, 6). After use, contaminated materials must be sterilized by autoclaving before discarding.

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. Colour differentiation on M-CP Agar Base is sometimes difficult, so typical colonies (yellow turning into pink) as well as a typical colonies (green or those that remain yellow upon exposure to ammonia fumes) are picked for confirmation.
2. Due to variable nutritional requirements, some strains may show poor growth on this medium.

Performance and Evaluation

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

Quality Control

Appearance of Powder	:	Light yellow to light green coloured, homogeneous, free flowing powder.
Gelling	:	Firm, comparable with 1.5% Agar gel.
Colour and Clarity of prepared medium	:	Purple coloured, clear to slightly opalescent gel forms in Petri plates.
Reaction	:	Reaction of 7.12% w/v aqueous solution at 25°C. pH:7.6 ± 0.2.
Culture Response	:	Cultural characteristics observed after an incubation at 44°C for 24-48 hours with added contents of 1 vial of M-CP Selective Supplement I (FD153) and 1 vial of M-CP Selective Supplement II (FD154) or rehydrated contents of 1 vial of M-CP Selective Supplement II Modified (FD154A) under anaerobic conditions.

Organisms (ATCC)	Inoculum (CFU)	Growth	Colour of Colonies
Clostridium perfringens (12924)	50-100	good	yellow**
Staphylococcus aureus subsp *aureus* (25923) (00034*)	≥10^3	inhibited	-
Bacillus subtilis subsp. spizizenii (6633) (00003*)	≥10^3	inhibited	-
Salmonella Typhi (6539)	≥10^3	inhibited	-

Key : * : Corresponds to WDCM number

**: colonies becomes old rose to light pink-red upon exposure to ammonia fumes for 30 seconds.

Storage and Shelf-life

Store between 2-8°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 inorder 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. 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 (6, 7).

References

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5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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