M-CP Agar

Intended Use

M-CP Agar is recommended for isolation and enumeration of *Clostridium perfringens* from water samples using membrane filtration technique.

Summary

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. 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. 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. It is also 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.

Principle

Tryptose, yeast extract provide nitrogenous, carbonaceous compounds, long chain amino acids, vitamins, minerals and other essential growth factors while sucrose is the fermentable carbohydrate. Bromocresol purple serves as a pH indicator. Indoxyl ß-D-glucoside is a chromogenic substrate for ß-D-glucosidase or cellobiose and phenolphthalein diphosphate for the detection of acid phosphatase. The addition of D-cycloserine and polymyxin B makes the medium inhibitory to accompanying nonclostridial microflora and thus allows analysis of both clostridial vegetative cells and spores.

Formula*

Ingredients	**g/L**
Tryptose	30.0
Yeast Extract	20.0
Sucrose	5.0
L-Cysteine Hydrochloride	1.0
Magnesium Sulphate.7H_2O	0.1
Bromocresol Purple	0.04
Ferric Chloride.6H_2O	0.09
Indoxyl ß-D-glucoside	0.06
Agar	15.0
Final pH (at 25°C)	7.6 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability

Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen

Water samples; Food samples

Specimen Collection and Handling

Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 35.64 g of the powder in 485 mL distilled water.
2. Heat to boiling to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle. Cool to 45°C-50°C.
4. Aseptically add the rehydrated contents of 1 vial of MCP Selective Supplement I and 1 vial of M-CP Selective Supplement II or rehydrated contents of 1 vial of M-CP Selective Supplement II, Modified.
5. Mix well and pour into sterile petriplates.

Quality Control

Dehydrated Appearance: Light yellow to light green homogeneous free flowing powder.
Prepared Appearance: Purple coloured clear to slightly opalescent gel forms in petriplates.
Cultural Response: Cultural characteristics observed after an incubation at 44°C for 24-48 hours.

Organism (ATCC)	**Growth**	**Phosphatase Test (on Exposure to Ammonia)**
Clostridium perfringens (12924)	Good	Positive, yellow coloured colonies (colonies become old rose to lightpink-red on exposure to ammonia fumes for 30 seconds)
Clostridium perfringens (13124)	Good	Positive, yellow coloured colonies (colonies become old rose to lightpink-red on exposure to ammonia fumes for 30 seconds)
Clostridium perfringens (10543)	Good	Positive, yellow coloured colonies (colonies become old rose to lightpink-red on exposure to ammonia fumes for 30 seconds)
Clostridium perfringens (12916)	Good	Positive, yellow coloured colonies (colonies become old rose to lightpink-red on exposure to ammonia fumes for 30 seconds)
Clostridium bifermentans (NCTC 506)	Good	Negative, blue coloured colonies
Escherichia coli (8739)	Inhibited	-
Escherichia coli (25922)	Inhibited	-
Salmonella Typhi(6539)	Inhibited	-
Staphylococcus aureus (25923)	Inhibited	-

Interpretation of Results

1. Yellow (cellobiase-negative) colonies becoming old rose to pink-red upon exposure to ammonia fumes for 30 seconds are considered to be presumptive *C. perfringens*.
2. Colour differentiation on M-CP Agar is sometimes difficult, so typical colonies (yellow turning into pink) as well as atypical colonies (green or those that remain yellow upon exposure to ammonia fumes) are picked for confirmation.
3. 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.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions / Limitations

Acid phosphatase reaction is reversible and hence read within 30 seconds of exposure to ammonia fumes.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Czeczulin J. R., Hanna P. C., Mcclane B. A., 1993, Infect. Immun., 61: 3429-3439.
2. Armon R. and Payment P., 1988, Can. J. Microbiol., 34:78-79.
3. Directive of the Council of the European Union 98/83/EC
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201130040500	Dehydrated Culture Media	500 g