

## TECHNICAL DATA SHEET

### M-CP Agar Base

#### Principle

M-CP agar base is described by the Armon and payment (1988). The media is composed of tryptose, yeast extract, sucrose, L-cysteine, magnesium sulphate, bromocresol purple, ferric chloride, indoxyl- $\beta$ -D-glucoside and agar. Tryptose, provide nitrogen, long chain amino acids and other essential minerals. Yeast extract provide nitrogenous, carbonaceous compounds, long chain amino acids, vitamins, minerals and other essential growth factors. Sucrose is the fermentable carbohydrate. L-cystine lower the oxidation-reduction potential of the medium by removing oxygen to maintain a low Eh. Magnesium sulphate and ferric chloride are essential ions and help to maintain osmotic balance. Bromocresol purple serves as a pH indicator. Indoxyl- $\beta$ -D-glucoside is a chromogenic substrate for  $\beta$ -D-glucosidase. Agar is solidifying agent. For more selectivity the media can be fortified with D-cycloserine, polymyxin B sulphate and phenolphthalein diphosphate. The addition of D-cycloserine and polymyxin B makes the medium inhibitory to associated non-clostridial microflora. The phenolphthalein diphosphate is indicator of acid phosphatase. The selectivity can be increase by incubation under anaerobic conditions. After incubation the colonies are exposed to ammonia fumes for 30seconds, if the colonies remain yellow, then they are  $\beta$ -D-glucosidase negative. If the colonies becoming old rose to pink-red they are  $\beta$ -D-glucosidase positive and may be *C. perfringens*. The green colored colonies are need to be confirmed by sulphite reduction, gram-positive, sporulating rods, nonmotile, reduction of nitrate, gelatine liquefaction, lactose fermentation and other biochemical tests.

**Use:** For the isolation and enumeration of *Clostridium perfringens* from water samples using membrane filtration technique.

#### Contents\*

Ingredients	Gram/Litre
Tryptose	30.00
Yeast Extract	20.00
Sucrose	5.00
L-Cysteine	1.00
Magnesium Sulphate	0.10
Bromocresol Purple	0.04
Ferric Chloride	0.09
Indoxyl-B-D-Glucoside	0.06
Agar	15.00
pH at 25°C	7.6 $\pm$ 0.2

\* Formula adjusted for optimum performance and parameters

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**Directions** Dissolve 35.60 grams in 485 ml distilled water, boil to dissolve the medium completely and sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 min, cool it to 42-45 °C, aseptically add sterile 5.0 ml of D-cycloserine (200mg) solution and Polymyxin B sulphate (12.500mg) and 10 ml Phenolphthalein diphosphate (0.05g) solution. Mix well and distribute aseptically in petri plates. Ensure complete solidification and inoculate test sample aseptically.

## Specimens' types analyzed

Pharmaceutical samples, clinical and non-clinical samples etc.

## Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

## Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

## Quality Control

<b>Appearance</b>	<b>Light greenish yellow colored free flowing, homogeneous powder</b>
<b>Reaction of 7.12% solution</b>	<b>7.6 ±0.2 at 25 °C</b>
<b>pH</b>	<b>7.40- 7.80</b>
<b>Gelling</b>	<b>Firm comparable with 1.5% agar gel</b>
<b>Color and clarity of ready medium</b>	<b>Purple colored clear to slightly opalescent gel</b>
<b>Growth Promotion properties</b>	<b>Best at ≤ 100 CFU at 32-37 °C for 18-72 h</b>
<b>Indicative properties</b>	<b>Optimum at ≤ 100 CFU at 32-37 °C for 18-48 h</b>
<b>Negative control</b>	<b>Performed using sterile distilled water</b>

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**Different Microbial Response: Cultural characteristics observed after an incubation at 44°C for 24-48 hours under anaerobic conditions. Inoculum 50-100 CFU.**

Organism	Growth	$\beta$ -D-glucosidase test	Phosphatase test
<i>Clostridium perfringens</i> (ATCC 3624)	Luxuriant	Positive (Colonies become old rose to light pink-red on exposure to ammonia fumes for 30 seconds)	Positive, yellowcolored colonies
<i>Staphylococcus aureus</i> (ATCC 25923)	Inhibited	--	--
<i>Escherichia coli</i> (ATCC 8739)	Inhibited	--	--
<i>Escherichia coli</i> (ATCC 25922)	Inhibited	--	--

**Storage and Shelf Life:** The product is highly hygroscopic; keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Note: Sterilize media immediately after reconstitution.

**Disposal:** To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

## Reference

1. Armon, R., and P. Payment. (1988). A modified M-CP medium for the enumeration of *Clostridium perfringens* from water samples. *Can. J. Microbiol.* 34:78-79.
2. Atlas, R. M. (2005). *Handbook of media for environmental microbiology.* CRC press.
3. British Pharmacopoeia, (2011), The Stationery office British Pharmacopoeia
4. Difco Manual (1998). 11<sup>th</sup> Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.

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