TM 2130 – M-CP AGAR BASE

INTENDED USE

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

PRODUCT SUMMARY AND EXPLANATION

Clostridial species are gram-positive, spore-forming rods and one of the major causes of food poisoning/ gastro-intestinal illnesses. They naturally occur in the soil. Among the family there are many species like *Clostridium tetani*, which is causative agent of tetanus; *Clostridium botulinum* produces one of the most potent toxins and *Clostridium perfringens* responsible of wound infections and diarrhea cases. Many bacterial pathogens possess toxins as virulence factor to damage the host. *C. perfringens* produces CPE enterotoxin as major virulence factor, which is secreted upon invasion of the host gut leads to food poisoning and other gastrointestinal illnesses. There are several solid media devised for quantitation of *C. perfringens*. Due to incorporation of the one or more antibiotics leads to media more selective that inhibit certain anaerobes or facultative anaerobes. Armon and Payment formulated M-CP Agar Base. Membrane filtration technique used for water sample. Directive of the Council of the European Union 98/83/EC recommended M-CP Agar Base for isolation and enumeration of *Clostridium perfringens.*

COMPOSITION

Ingredients	Gms / Ltr
Tryptose	30.000
Sucrose	5.000
Bromocresol purple	0.040
Yeast extract	20.000
Magnesium sulphate, 7 H_2O	0.100
Indoxyl β-D-glucoside	0.060
Ferric chloride, 6H_2O	0.090
L-Cysteine hydrochloride	1.000
Agar	15.000

PRINCIPLE

Yeast extract and tryptose serve as carbonaceous compounds, nitrogenous, long chain amino acids, minerals, vitamins and other essential growth factors. Sucrose act as fermentable carbohydrate. Bromocresol purple is a pH indicator. Indoxyl-ß-D-glucoside is used for the detection of the acid phosphatase, as it is chromogenic substrate for ß-D-glucosidase or cellobiase and phenolphthalein diphosphate. For analysis of the clostridial vegetative cells and spores D-cycloserine and polymyxin B are added to make the medium inhibitory to accompanying non-clostridial microflora. Further selectivity is provided by incubation under anaerobic conditions. Upon exposure of ammonia fumes for 30 seconds yellow (cellobiase-negative) colonies becoming old roseto pink-red, considered to be *presumptive C. perfringens*. Color differentiation on M-CP Agar Base is difficult sometimes, so typical colonies characterized yellow turning into pink and atypical colonies will show green or those that remain yellow upon exposure to ammonia fumes, this observation leads to confirmation. Sulphite reduction, lactose fermentation, gram positive, non-motile, sporulating rods, reduction of nitrate, gelatine liquefaction and other biochemical tests can also confirm presumptive *C. perfringens*.

INSTRUCTION FOR USE

- Dissolve 35.60 grams in 485 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 psi 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 and 1 vial of M-CP Selective Supplement II or rehydrated contents of 1 vial of M-CP Selective Supplement II, Modified.
- Mix well and pour into sterile Petri plates

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Light yellow to light green homogeneous free flowing powder
Appearance of prepared medium : Purple colored clear to slightly opalescent gel forms in Petri plates
pH (at 25°C) : 7.6 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation with added contents of 1 vial of M-CP 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 under anaerobic conditions.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Phosphatase test (on exposure of ammonia)	Incubation Temperature	Incubation Period
Clostridium perfringes	12924	50-100	Good-luxuriant	>=50%	Positive yellow colored colonies (colonies become old rose to light pink-red on exposure to ammonium fumes for 30 seconds)	44°C	24-48 Hours
Clostridium perfringes	13124	50-100	Good-luxuriant	>=50%	Positive yellow colored colonies (colonies become old rose to light pink-red on exposure to ammonium fumes for 30 seconds)	44°C	24-48 Hours
Clostridium perfringes	10543	50-100	Good-luxuriant	>=50%	Positive yellow colored colonies (colonies become old rose to light pink-red on exposure to ammonium fumes for 30 seconds)	44°C	24-48 Hours
Clostridium perfringes	12916	50-100	Good-luxuriant	>=50%	Positive yellow color colonies (colonies become old rose to light pink-red on exposure to ammonium fumes for 30 seconds)	44°C	24-48 Hours
Escherichia coli	8739	50-100	Good-luxuriant	>=50%	Positive yellow color colonies (colonies become old rose to light pink-red on exposure to ammonium fumes for 30 seconds)	44°C	24-48 Hours
Escherichia coli	25922	>=10^3	Inhibited	0%	-	44°C	24-48 Hours

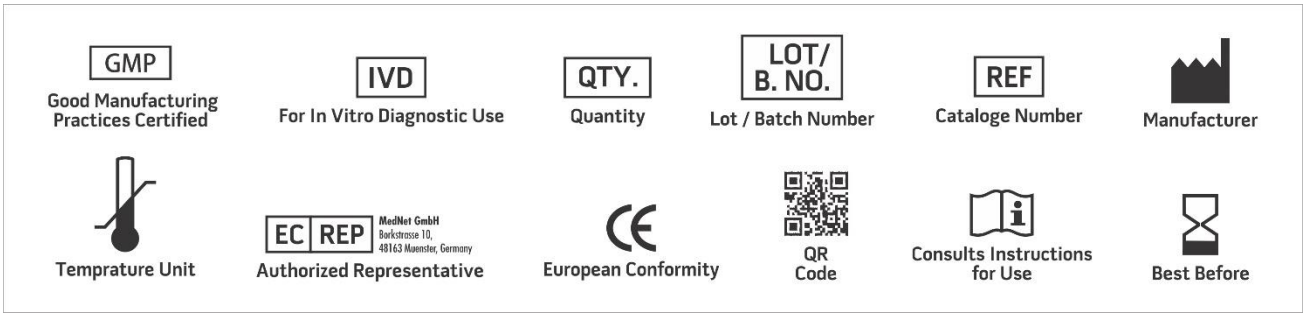
Salmonella Typhi	6539	>=10^3	Inhibited	0%	-	44°C	24-48 Hours
Staphylococcus aureus subsp. aureus	25923	>=10^3	Inhibited	0%	-	44°C	24-48 Hours

PACKAGING:
In pack size of 100 gm and 500 gm bottles.

STORAGE
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 15-25°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES
1. Armon R. and Payment P., 1988, Can. J. Microbiol., 34:78-79
2. Czeczulin J. R., Hanna P. C., Mcclane B. A., 1993, Infect. Immun., 61: 3429-3439.
3. Directive of the Council of the European Union 98/83/EC.
4. Sartory D. P., Field M., Curbishley S. M., Pritchard A. M., 1998, Lett. Appl. Microbiol., 27:323-327.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.
***For Lab Use Only**
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