Membrane Clostridium Perfringens (m-CP) Selective Supplement

PRODUCT INFORMATION

P007-1MU - Polymyxin B Sulfate, Powder, 1MU

P007-10MU - Polymyxin B Sulfate, Powder, 10MU

P007-100MU - Polymyxin B Sulfate, Powder, 100MU

C041-1g - D-Cycloserine, Crystal, 1g

C041-5g - D-Cycloserine, Crystal, 5g

DESCRIPTION

Membrane Clostridium Perfringens (m-CP) Medium with Membrane Clostridium Perfringens (m-CP) Selective Supplement is a medium for rapid isolation and presumptive identification of *Clostridium perfringens* from water samples.

BACKGROUND

APPLICATION IN MEMBRANE CLOSTRIDIUM PERFRINGENS (M-CP) MEDIUM

Membrane Clostridium perfringens (m-CP) Medium is a selective and chromogenic medium for the presumptive identification of *Clostridium perfringens* from water samples.

m-CP Medium was first described by Bisson and Cabelli for the rapid quantitation of *Clostridium perfringens* from a variety of water samples (seawater, potable water and sewage). The medium was shown to give better recovery of *Clostridium perfringens* from water and sewage samples than the Bonde pour tube method.

m-CP Medium has now been recommended in European Council Directive 98/83/EC for testing the quality of water intended for human consumption.

In m-CP Medium lack of b-D-glucosidase activity (an enzyme involved in cellobiose fermentation), fermentation of sucrose and production of acid phosphatase are used to differentiate presumptive *Clostridium perfringens* colonies from other *Clostridium* spp.

Lack of b-D glucosidase activity means that *Clostridium perfringens* does not cleave the chromogen, indoxyl b-D glucoside, in the medium. Furthermore, as the organisms ferment the sucrose in the medium, reducing the pH, bromocresol purple changes from purple to yellow. This results in characteristic opaque yellow *Clostridium perfringens* colonies.

Most other *Clostridium* spp. will appear as either purple colonies, due to the lack of sucrose fermentation, or blue/green colonies where the organism is still cleaving Indoxyl b-D glucoside and also fermenting sucrose (see table).

Presumptive positive *Clostridium perfringens* colonies can be further tested for acid phosphatase activity by exposure to ammonium hydroxide vapour for 20 to 30 seconds. *Clostridium perfringens* colonies turn pink or red as phenolphthalein diphosphate is cleaved by acid phosphatase. No colour change will be seen with colonies of organisms that do not posses acid phosphatase. It is important this further test is carried out as there are a very small number of non-perfringens clostridia that produce yellow colonies. However, these colonies will remain yellow after exposure to ammonium hydroxide as they are acid phosphatase negative.

D-cycloserine, polymyxin B and incubation at 44°C inhibit the growth of background flora such as Gram-negative organisms and staphylococci.

Content concentrations

Typical Formula*	**mg/litre**
Membrane Clostridium Perfringens (m-CP)	
Tryptose	30
Yeast Extract	20
Sucrose	5
L-cysteine hydrochloride	1
Magnesium sulphate.7H2O	0.1
Agar	15
Bromocresol purple	0.04
Final pH 7.6 ± 0.2 @ 25°C	
Membrane Clostridium Perfringens (m-CP) Selective Supplement	
Polymyxin B sulphate	25.0 (210,000IU)
D-Cycloserine	400.0
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Membrane Clostridium Perfringens (m-CP) Medium and Membrane Clostridium Perfringens (m-CP) Selective Supplement

METHOD

Preparation

Suspend appropriate amount of m-CP Agar Base in distilled water Mix well and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of m-CP Selective Supplement reconstituted as directed. Aseptically add the following sterile solutions dissolved in distilled water:

Component	Solution Strength	Volume
Phenolphthalein biphosphate tetrasodium salt	0.50%	10.0 ml
Ferric chloride hexahydrate	4.50%	1.0 ml
Indoxyl b-D-glucoside	0.75%†	4.0 ml

† equivalent to 30mg in 4ml

NOTE: Fresh solutions must be used. Mix well and pour into sterile Petri dishes.

To reconstitute m-CP Selective Supplement, aseptically add 2ml of sterile distilled water to 1 vial of supplement. Mix gently to dissolve.

Protocol

1. Filter the water sample using a 0.45 mm cellulose acetate filter,(cellulose acetate has been found to be the best performing filter type, however the filter quality may vary from brand to brand. It is advised to validate the filter type to be used, according to ISO 7706).

2. Place the filter onto the m-CP Medium. Incubate anaerobically for 21 ± 3 hours at 44 ± 1°C.

3. Examine the plates for presumptive positive opaque yellow colonies that turn pink or red after exposure to ammonium hydroxide vapours for 20-30 seconds.

On m-CP Medium typical colonies will appear as follows:

Organism	Typical Colony Colour
Clostridium perfringens	Opaque Yellow Sucrose positive/Glucosidase negative then pink/red after exposure to NH4OH
Other *Clostridium* spp.	Blue/Green Sucrose positve/Glucosidase positive(e.g. Cl. baratii, Cl. paraputrificum, Cl. tertium)
	Purple Sucrose negative/Glucosidase positive or negative (e.g. Cl. biferentans, Cl. difficle, Cl. sporogenes)
	Opaque Yellow Sucrose positive/Glucosidase negative remain yellow after exposure to NH4OH

Quality control

Positive control:

Clostridium perfringens ATCC® 13124: Yellow, then Pink/Red

Negative control:

Escherichia coli ATCC® 25922: Inhibited

Clostridium sporogenes ATCC® 19404: Purple

REFERENCES

1. Bisson, J. W. and Cabelli, V. J. (1979) Applied and Environmental Microbiology, Vol. 37, No. 1, pp 55-88.

2. E.U. (1998) 98/83/EC of Council of 3rd of November 1998 on the quality of water intended for human consumption. Off. J. Eur. Commun., L330, 32-54