m-CP AGAR BASE
m-CP ANTIMICROBIC SUPPLEMENT
m-CP SUPPLEMENTS A / B / C
m-CP AGAR

Dehydrated and ready-to-use culture medium, selective and differential supplements

1 - INTENDED USE

For the isolation, enumeration and presumptive identification of *Clostridium perfringens* from water samples.

2 – COMPOSITION*

M-CP AGAR BASE

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	30.0 g
Yeast extract	20.0 g
Sucrose	5.0 g
L-cysteine HCl	1.0 g
Magnesium sulphate. $7H_2O$	0.1 g
Agar	13.2 g
Bromocresol purple	0.04 g

M-CP ANTIMICROBIC SUPPLEMENT
(VIAL CONTENT FOR 500 ML OF MEDIUM BASE)

Polymyxin B sulphate	12.5 mg (105,000 IU)
D-cycloserine	200 mg

M-CP SUPPLEMENT A
(VIAL CONTENTS FOR 500 G OF MEDIUM BASE)

Phenolphthalein diphosphate	750 mg

M-CP SUPPLEMENT B
(VIAL CONTENTS FOR 500 G OF MEDIUM BASE)

Ferric chloride	700 mg

M-CP SUPPLEMENT C
(VIAL CONTENTS FOR 500 G OF MEDIUM BASE)

Indoxyl β-D glucoside	450 mg

M-CP AGAR
READY-TO-USE PLATES

M-CP Agar Base	69.7 g
Polymyxin B sulphate	25 mg (210,000 IU)
D-Cycloserine	400 mg
Phenolphthalein diphosphate	100 mg
Ferric chloride	90 mg
Indoxyl β-D glucoside	60 mg
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

m-CP Agar is a selective and chromogenic medium for the isolation, enumeration and presumptive identification of *Clostridium perfringens* from water samples. m-CP Agar was devised by Bisson and Cabelli[1] who included a number of compatible, differential tests in a selective medium to be used in a membrane filter procedure. The medium has been recommended by the European Council Directive 98/83/EC[2] in its original drafting for the examination of water intended for human consumption. The amendment of 6 October 2015[3] of the Directive refers to the EN ISO 14189[4] for the enumeration of *C. perfringens* including spores.
Tryptose provides nitrogen, carbon, minerals and amino acids for the microbial growth. The yeast extract is a source of vitamins particularly of the B-group. L-cysteine hydrochloride is a reducing agent. Magnesium and iron salts enhance the microbial growth. Sucrose is the fermentable carbohydrate and bromo cresol purple is a pH indicator: sucrose fermenting bacteria exhibit yellow colonies due to the colour change of the pH indicator. Indoxyl-β-D-glucoside is a chromogenic substrate for β-D-gucosidase which cleaves the substrate with the formation of a blue chromophore (*C. perfringens* does not cleave the chromogen). The presence of phosphatase results in the cleavage of phenolphthalein diphosphate, evidenced by the development of a pink-red colour by exposing the colonies to ammonium hydroxide vapour; no colour change will be seen with colonies of organisms that do not possess acid phosphatase. *C. perfringens* does not cleave indoxyl-β-D-glucoside, ferments sucrose and is positive to phosphatase. D-cycloserine and polymyxin B help in the selective isolation of *C.perfringens* by inhibiting accompanying flora.

4- DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 34.7 g in 500 mL of cold purified water, heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add and the contents of one vial of m-CP Antimicrobic Supplement (REF 4240070), reconstituted with 5 mL of sterile purified water. Aseptically add the following fresh filter sterilised solutions, prepared with the powdered compounds supplied with the vials of m-CP Supplements A, B and C dissolved in purified water:

Phenolphthalein diphosphate (REF 4240070A)	0.5% solution	10 mL in 500 mL of medium base
Ferric chloride hexahydrate (REF 4240070B)	4.5% solution	1 mL in 500 mL of medium base
Indoxyl β -D-glucoside (REF 4240070C)	0.75% solution	4 mL in 500 mL of medium base

Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

m-CP Agar Base

Dehydrated medium appearance	pale blue, fine, homogeneous, free-flowing powder
Solution appearance	violet, limpid
Prepared plates appearance	violet, limpid
Final pH at 20-25 °C	7.6 ± 0.2
m-CP Antimicrobic Supplement	
Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless limpid
m-CP Supplement A	
Powder appearance	white to faint yellow or tan powder
m-CP Supplement B	
Powder appearance	faint yellow to yellow-brown powder
m-CP Supplement C	
Powder appearance	almost white powder

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
m-CP Agar Base	Dehydrated medium	4013202	500 g (7.2 L)
m-CP Antimicrobic Supplement	Freeze-dried supplement	4240070	10 vials, each for 500 mL of medium
m-CP Supplement A (Phenolphthalein diphosphate	Powdered supplement	4240070A	1 vial (750 mg for 500 g of medium)
m-CP Supplement B (Ferric chloride)	Powdered supplement	4240070B	1 vial (700 mg for 500 g of medium)
m-CP Supplement C (Indoxyl β -D-glucoside)	Powdered supplement	4240070C	1 vial (450 mg for 500 g of medium)
m-CP Agar	Ready-to-use plates	491320	3 x 10 plates, ø 55 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, swabs and pipettes, membrane filters, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, controlled atmosphere generators and jars, ancillary culture media and reagents.

8 - SPECIMENS

Water samples. Refer to applicable International Standards for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

9 - TEST PROCEDURE

1. Filter a suitable volume of sample through a 0.45 µm cellulose acetate or cellulose nitrate membrane.
2. Using aseptic technique, roll the membrane filter onto the surface of the m-CP Agar prepared as described above, so as to avoid the formation of air bubbles between the filter and the agar surface.
3. Incubate in anaerobic conditions for 21 ± 3 hours at 44 ± 1° C.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
The following typical colonies can be observed on m-CP Agar:

Microorganism	Colony characteristics	Sucrose	β -D-glucosidase	Phosphatase
C. perfringens	Opaque yellow; pink/red after exposure to ammonium hydroxide vapours for 20-30 seconds	+	-	+
Other clostridia (*C.baratii, C.paraputrificum, C.tertium*)	Blue-green	+	+	
Other clostridia (*C.bifermentans, C.difficile, C.sporogenes*)	Purple	-	+ or -	
Other clostridia	Opaque yellow, negative to exposure to ammonium hydroxide vapours	+	-	-

11 - USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control:

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	44°C/ 18-24 H / AN	good growth, opaque yellow colonies, pink/red after exposure to ammonium
C. bifermentas NCTC 506	44°C/ 18-24 H / AN	growth, blue colonies
E. coli ATCC 25922	44°C/ 18-24 H / AN	totally inhibited

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Collection Type Cultures.

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated m-CP Agar Base supplemented with m-CP Antimicrobic Supplement and m-CP Supplement A, B, and C (Test Batch:TB), are tested for productivity, specificity and selectivity with target and non-target strains with incubation at 44°C for 18-24 hours, by comparing the results with Blood Agar plates (Reference Batch:RB).
Productivity is tested by a quantitative technique with the target strain *C. perfringens* ATCC 13124. The filters rolled on the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 44°C for 18-24 hours. The colonies are enumerated on both batches and the productivity ratio (Pr: CFU_{TB}/CFU_{RB}) is calculated. If Pr is ≥ 0.5 and if the colonies morphology and colour are typical (yellow colonies pink-red after exposure to ammonium hydroxide vapour) the results are considered acceptable and conform to the specifications.
Specificity is tested by semi-quantitative ecometric technique with *C. bifermentans* NCTC 506. After incubation *C. bifermentans* exhibits good growth with blue colonies, phosphatase negative.
Selectivity is tested with modified Miles-Misra method with the following non-target strains: *P. mirabilis* ATCC 10005, *P. vulgaris* ATCC 9484, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 18804 and *E. faecalis* ATCC 19433. After incubation, the growth of *P. mirabilis, P. vulgaris* and *E. coli* is totally inhibited while *P. aeruginosa, C. albicans* and *E. faecalis* are partially inhibited.

13 - PRECAUTIONS AND WARNINGS

- The medium base and the supplements are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplements shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. M-CP Supplement B is classified as dangerous. Before use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The ante and post mortem controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Be careful when opening the metal ring of the supplements vials to avoid injury.
- The selective supplement is sterilized by membrane filtration.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplements or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplements and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Ready to use plates
Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).
Dehydrated medium
Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
Freeze-dried supplement
Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).
Powdered supplements
m-CP Supplement A: upon receipt, store the product in the original package at -20°C away from direct light
m-CP Supplement B: upon receipt, store the product in the original package at +10/+30°C away from direct light
m-CP Supplement C: upon receipt, store the product in the original package at -20°C away from direct light

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/bottles) and the applied storage conditions (temperature and packaging).

15 - REFERENCES

1. Bisson JW, Cabelli VJ. Membrane filter enumeration method for *Clostridium perfringens*. Appl Environ Microbiol 1979; 37: 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
3. Commission Directive (EU) 2015/1787 of 6 October 2015 amending Annexes II and III to Council Directive 98/83/EC on the quality of water intended for human consumption. Off J Eur Commun L260: 6-17

4. ISO 14189:2013 Water quality - Enumeration of *Clostridium perfringens* - Method using membrane filtration

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/06

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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