



## m-FAECAL COLIFORM BROTH (m-FC BROTH) Dehydrated culture medium

### 1 - INTENDED USE

m-Faecal Coliform Broth is used with rosolic acid for the cultivation and enumeration of faecal coliforms by the membrane filter technique.

### 2 - COMPOSITION - TYPICAL FORMULA \*

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	10.0 g
Peptocomplex	5.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Lactose	12.5 g
Bile salts N° 3	1.5 g
Aniline blue	0.1 g

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

### 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Faecal (thermotolerant) coliforms are found in the gastro intestinal tracts and faeces of warm-blooded animals and may be differentiated from coliforms from environmental sources by their ability to grow at 44.5 °C.

m-Faecal Coliform Broth is prepared according to the formulation reported by Geldrich et al. in 1965<sup>1</sup> for the enumeration of faecal coliforms using the membrane filter technique without prior enrichment. Faecal Coliform Media are recommended by many International Authorities for the enumeration of faecal coliforms using the membrane filter technique.<sup>2-4</sup> Faecal coliforms (or thermotolerant coliform bacteria) are frequently used as indicators of faecal pollution although they are less specific indicators of faecal contamination than *Escherichia coli*, since they may sometimes arise from nonfecal sources, especially in tropical climates.<sup>5</sup> Tryptose and peptocomplex provide nitrogen and minerals for microbial growth, yeast extract is a source of B-vitamins complex for growth stimulation, lactose is a fermentable carbohydrate and a source of carbon and energy, sodium chloride maintains the osmotic balance. Bile salts N° 3 and aniline blue inhibit growth of Gram-positive bacteria. The high incubation temperature makes the medium more selective. Aniline blue and rosolic acid form the indicator system of the medium.

### 4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 37 g in 1000 mL of cold purified water. Add 10 mL of Rosolic Acid (REF 4211901) 1% solution in NaOH 0.2 N, heat to boiling with frequent agitation and continue to boil for 1 minute. Do not sterilize in the autoclave. Cool and use for absorbing the suitable sterile absorbent pads.

### 5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	grey-violet, limpid
Final pH at 20-25 °C	7.4 ± 0.1

### 6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
m-Faecal Coliform Broth (m-FC Broth)	Dehydrated medium	4014862	500 g (13.5 L)

### 7 - MATERIALS REQUIRED BUT NOT PROVIDED

Water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, absorbent pads, membrane filters, plastic bags, Rosolic Acid (REF 4211901), ancillary culture media and reagents.

### 8 - SPECIMENS

Water samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

### 9 - TEST PROCEDURE

1. Filter an appropriate volume of water onto the membrane depending on the expected faecal coliforms number. When the sample's bacterial density is unknown, filter several volumes or dilutions to achieve a countable plate (20-60 CFU/dish).
2. Using aseptic technique, place a sterile absorbent pad in each culture dish and pipet at least 2 mL of broth. Carefully remove any excess of liquid from culture dish by decanting plate.
3. Roll the membrane filter used to collect the water sample onto the surface of the pad, so as to avoid the formation of air bubbles between the filter and the pad.
4. Within 30 minutes place the dishes in plastic bags and incubate, by immersion, in a water bath at 44.5 ± 0.2°C for 24 ± 2 hours.

### 10 - READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies. Count and record colonies with various shades of blue as faecal coliforms. Colonies of non-faecal coliforms are grey or cream or pink.

### 11 - USER QUALITY CONTROL

All manufactured lots of the product 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
<i>E. coli</i> ATCC 25922	44.5° / 24 H-A	growth with blue colonies
<i>E. faecalis</i> ATCC 19433	44.5° / 24 H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection



**12 – PERFORMANCES CHARACTERISTICS**

Prior to release for sale, a representative sample of all lots of dehydrated m-Faecal Coliform Broth (Test Batch) is assessed for productivity and selectivity by comparing the results with a previously approved Reference Batch (RB). Productivity is tested by a quantitative method with the target strains *E. coli* ATCC 25922 and *E. coli* ATCC 8739: the filters rolled on pads are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 44°C for 24 hours. The colonies are enumerated on both batches and the productivity ratio (Pr: CFU<sub>TB</sub>/CFU<sub>RB</sub>) is calculated. If Pr is  $\geq 0.7$  and if the colonies morphology and colour are typical (blue colonies) the results are considered acceptable and conform to the specifications.

Selectivity and specificity are evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following non-target strains: *S. aureus* ATCC 25923, *E. faecalis* ATCC 19433, *K. pneumoniae* ATCC 27736, *C. freundii* ATCC 8090, *S. Typhimurium* ATCC 14024. The growth of Gram-positive strains is totally inhibited while the growth of *K. pneumoniae* and *C. freundii* is partially inhibited and the growth of *S. Typhimurium* is not inhibited and the strain grows with pink colonies.

**13 – LIMITATIONS OF THE METHOD**

- Since the incubation temperature is critical, the use of submerged waterproofed MF culture is recommended or the use of an incubator that is documented to hold the temperature at  $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$  throughout the chamber over a 24 hours period.<sup>2</sup>
- There are limitations to the interpretation of a thermotolerant coliform result from thermal waters and pulp and paper mill effluent samples where thermotolerant *Klebsiella* have predominated and not been indicative of a sewerage source. Approximately 60% to 80% of all *Klebsiella* from faeces and clinical samples are positive in the thermotolerant coliform test and are *K. pneumoniae*.<sup>2</sup>

**14 - PRECAUTIONS AND WARNINGS**

- The medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- 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 this 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](http://www.biolifeitaliana.it), describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet are available on the website [www.biolifeitaliana.it](http://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.

**15 - STORAGE CONDITIONS AND SHELF LIFE**

Upon receipt, store at  $+10^{\circ}\text{C} / +30^{\circ}\text{C}$  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). 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/flasks) and the applied storage conditions (temperature and packaging).

**16 – REFERENCES**

- Geldreich EE, Clark HF, Huff CB, Best LC. Faecal coliform organisms medium for membrane filtration technique. J Am Water Works Assoc 1965; 57:208.
- APHA Standard methods for the examination of water and wastewater, 23rd ed. American Public Health Association, Washington, D.C., 2017
- AOAC Official methods of analysis, 18th ed., AOAC International. Gaithersburg, Md. 2007
- U.S. Environmental Protection Agency. Manual for the certification of laboratories analysing drinking water. EPA-814B-92-002. Office of Ground Water and Technical Support Division, USEPA, Cincinnati, Ohio.
- Cisneros BJ, in Treatise on Water Science, 4.06.4.1.5 Biological indicators, 2011.

**TABLE OF APPLICABLE SYMBOLS**

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

**REVISION HISTORY**

Version	Description of changes	Date
Revision 1	Updated layout and content	2023/01

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

