MAC CONKEY BROTH (PURPLE)

Dehydrated culture medium

1 - INTENDED USE

Liquid medium for the detection of coliform organisms in foodstuffs and water samples.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	20.00 g
Lactose	10.00 g
Bile salts	5.00 g
Sodium chloride	5.00 g
Bromocresol purple	0.01 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

Alfred Theodore MacConkey[1] in 1901 devised a liquid medium for the cultivation of "*Bacillus coli",* containing sodium taurocholate as a selective agent and litmus as an indicator. The medium was later modified by MacConkey[2,3] by replacing litmus with phenol red and by Childs and Allen[4] who introduced the less inhibitory bromocresol purple as a pH indicator.
Mac Conkey Broth (Purple), in its current formulation, has been used for decades for the presumptive determination of coliforms by the MPN method in foods, water and dairy products.[5-7]
Essential growth factors are provided by peptone which is a source of nitrogen, carbon and minerals. Lactose is the fermentable carbohydrate and a source of carbon and energy. Bromocresol purple is the pH indicator. Bile salts inhibit the growth of Gram-positive organisms. Compared to the formula included in the European Pharmacopoeia, Mac Conkey Broth (Purple) additionally contains sodium chloride. Acids and gas are produced from lactose fermentation: the acidity of the medium is detected by the pH indicator, which turns yellow, whereas the gas is evidenced by the formation of bubbles that are collected in the Durham tubes.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 40 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 10 mL into test tubes containing inverted Durham tube. Sterilise by autoclaving at 121°C for 15 minutes. The Durham tubes shall not contain air bubbles after sterilization. If required, prepare the double strength medium by weighing 80 g/L.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	purple, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	purple, limpid
Final pH at 20-25 °C	7.4 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Mac Conkey Broth (Purple)	Dehydrated medium	4016752	500 g (12.5 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, microbiological tubes, Durham tubes, ancillary culture media and reagents.

8 – SPECIMENS

Food and water samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

1. Transfer 1 mL of the test sample and its serial decimal dilutions into the tubes.
2. Alternatively transfer 10 mL of the test sample into the tubes with 10 mL of double strength medium.
3. For coliform bacteria, incubate for 24-48 hours at 30 or 37°C, depending on the analytical protocol.
4. For faecal coliform bacteria, incubate at 44.5°C for 24-48 hours.

10 - READING AND INTERPRETATION

After incubation the microbial growth is evidenced by turbidity in the broth. The yellowing of the broth and the production of gas suggest the presence of *E. coli* and possibly of other coliform bacteria. Yellowing alone suggests the presence of coliforms other than *E. coli.*
Bacteria in gas-positive tubes can be identified by subculturing on suitable media such as Levine EMB Agar (REF 401595).

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
E. coli ATCC 8739	37°/ 24 H-A	good growth with gas, the medium turns yellow
S. aureus ATCC 6538	37°/ 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 Mac Conkey Broth Purple is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

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Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 37°C for 24 hours and recording the highest dilution showing growth/gas/yellow colour in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following strains *E. coli* ATCC 25922, *E. aerogenes* ATCC 13048, *K. pneumoniae* ATCC 27736, *C. freundii* ATCC 43864, S. Enteritidis ATCC 13076. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Selectivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of Gram-positive organisms in test tubes, incubating at 37°C for 24 hours and recording the highest dilution showing growth. Selectivity is tested with the following strains: *S. aureus* ATCC 25923 and *E.faecalis* ATCC 19433. *S.aureus* is totally inhibited whereas *E. faecalis* is partially inhibited.

13 - PRECAUTIONS AND WARNINGS

- This culture 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, 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 of the products 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

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).
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 and the applied storage conditions (temperature and packaging).

15 – REFERENCES

1. MacConkey A. Zentralbl Bakteriol 1901; 29:740.
2. MacConkey A. Lactose-Fermenting Bacteria in Faeces. J Hyg (Lond) 1905; Jul;5(3):333-79
3. MacConkey A. Bile Salt Media and their advantages in some Bacteriological Examinations. J Hyg (Lond) 1905; 8:322.
4. Childs E, Allen LA. Improved methods for determining the most probable number of Bacterium coli and of Streptococcus faecalis. J Hyg Camb 1953; 51:468
5. Departments of the Environment, Health, Social Security and Public Health Laboratory Service. The Bacteriological Examination of Drinking Water Supplies. Report No. 71. 1982. HMSO London.
6. World Health Organization. International Standards for Drinking Water' 2nd ed., 1963. WHO, Geneva.
7. Davis JG. `Milk Testing' 2nd ed. Dairy Industries Ltd., London,1959.

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 2	Updated layout and content	2022/09

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

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