MAC CONKEY AGAR OMS W/O CRYSTAL VIOLET (CV)

Dehydrated culture medium

1 - INTENDED USE

A moderately selective medium used for the isolation and differentiation of Gram-negative organisms from food and water samples.

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

Peptone	17.00 g
Peptocomplex	3.00 g
Lactose	10.00 g
Bile salts	5.00 g
Sodium chloride	5.00 g
Neutral red	0.05 g
Agar	15.00 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, working at the University of Liverpool under the auspices of the Royal Commission on Sewage Disposal, in 1900[1] published in Lancet the formulation of MacConkey Agar. The use of the medium caught on rapidly amongst those interested in water microbiology. Later in 1902, Albert Grunbaum ed Edward Hume[2] modified the MacConkey's formulation with the inclusion of neutral red and crystal violet. By 1930, ten modifications of "MacConkey's Basal Bile Salt Peptone" agar were published and among these MacConkey Agar without crystal violet.[3]

Mac Conkey Agar OMS w/o CV compared to the classic formula of Mac Conkey Agar (REF 401670) does not contain crystal violet, includes bile salts instead of bile salts n° 3 and has lower selective properties.

Mac Conkey Agar OMS w/o CV is a moderately selective medium used for the isolation and differentiation of Gram-negative organisms from a variety of food, water samples and industrial sources; the lack of crystal violet permits the growth of enterococci and mycobacteria. This formula has been recommended by WHO and Windle-Taylor for the examination of waters.[4,5]

The moderate selective action of the medium is due to the presence of bile salts, which inhibits the growth of some Gram-positive bacteria. The peptones provide carbon, nitrogen and trace elements for bacterial growth; sodium chloride maintains the osmotic balance. The fermentation of lactose by coliforms causes acidification of the medium and the formation of red-pink to red-violet colonies. Non-lactose fermenter strains (e.g., *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas*, *Alkaligenes* etc.) develop transparent, colourless colonies. Staphylococci are partially inhibited and produce pale pink to red colonies and enterococci produce compact tiny red colonies.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 55 g in 1000 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, mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey-pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red, limpid or slightly opalescent
Final pH at 25 °C	7.3 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Mac Conkey Agar OMS w/o Crystal Violet	Dehydrated medium	4016712	500 g (9.1 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and swabs, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents.

8 - SPECIMENS

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

9 -TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap.

Incubate in aerobic atmosphere at 35-37°C for 18-24 hours or longer if necessary (maybe up to 48 h for late lactose fermenters: *Citrobacter, Providencia*, *Serratia*, *Hafnia*).[6]

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Colonies of lactose fermenters are red-pink to red-violet.

Colonies of non-lactose fermenters are colourless or white or light yellow or with a natural pigmentation (e.g. green for *P.aeruginosa*).

Enterococci produce compact tiny red colonies.

Staphylococci are partially inhibited and produce pale pink to red colonies.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

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	35-37°C / 18-24 h / A	red-violet colonies
S. Typhimurium ATCC 14028	35-37°C / 18-24 h / A	colourless colonies
E.f aecalis ATCC 29212	35-37°C / 18-24 h / A	small red colonies

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 Agar OMS w/o CV is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
The productivity characteristics are tested by semi-quantitative ecometric technique with the following lactose fermenting strains *E. coli* ATCC 25922, *E. aerogenes* ATCC 13048, *K. pneumoniae* ATCC 27736, *Y. enterocolitica* ATCC 23715, and non-lactose fermenting strains: *S.* Typhimurium ATCC 14028, *P. vulgaris* ATCC 8427, *P. aeruginosa* ATCC 14207. After incubation at 37°C for 24 hours, typical colonies of lactose fermenters are red in colour; typical colonies of non-lactose fermenters are colourless or green for *P. aeruginosa*. The amount of growth on the plates is evaluated and shall be comparable in both batches.
The selectivity is 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 non-target Gram-positive strains *E. faecalis* ATCC 29212 and *S. aureus* ATCC 25923. The growth of *S. aureus* is partially inhibited whereas *E. faeacalis* grows with small red colonies.

13-LIMITATIONS OF THE METHOD

- Prolonged incubation may lead to confusion of results; do not incubate longer than 48 hours.[6]
- Due to selective properties of this medium some strains of Gram-negative enteric bacteria fail to grow or grow poorly; similarly, some Gram-positive organisms may not be inhibited or are partially inhibited.[6]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

14 - PRECAUTIONS AND WARNINGS

- This product 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 culture medium 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 product 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.

15 - 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 (plates/bottles) and the applied storage conditions (temperature and packaging). According to MacFaddin the self-prepared plates can be stored at +2°C /+8°C in the dark and protected against evaporation for up to 6-8 weeks.[6]

16 - REFERENCES

1. MacConkey AT. Note on a new medium for the growth and differentiation of the Bacillus coli communis and the Bacillus Typhi abdominalis. The Lancet, July 07, 1900; vol 156, Issue 4010, P20.
2. Grunbaum AS, Hume EH. Note on media for distinguishing B.coli, B.typhosus and related species. Brit Med J, June 14 1902; p 1473-1474
3. Smith KP. The origin of MacConkey Agar. American Society for Microbiology: Articles, Oct. 14, 2019.
4. World Health Organisation. International Standards for Drinking Water, 2nd Ed. 1963. WHO, Geneve.
5. Windle-Taylor E. The examination of waters and water supplies. 7th Ed. 1958. Churchill Ud., London.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

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	2022/09

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it