INSTRUCTIONS FOR USE

MAC CONKEY AGAR

Ready-to-use plates

Mac Conkey Agar: *E.coli* (colonies with red halo) and *Pseudomonas aeruginosa* (greenish colonies)

1-INTENDED USE

In vitro diagnostic device. Selective and differential medium for the isolation and differentiation of *Enterobacteriaceae* and other Gram negative bacilli from clinical and nonclinical specimens.

2-COMPOSITION - TYPICAL FORMULA *

Gelatin peptone	17.000 g
Peptones (meat and casein)	3.000 g
Lactose	10.000 g
Bile salts n°3	1.500 g
Sodium chloride	5.000 g
Neutral red	0.030 g
Crystal violet	0.001 g
Agar	13.500 g
Purified water	1000 mL

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

3-PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Mac Conkey Agar is a selective, differential medium based on the formulation described by Alfred Theodore MacConkey in 1900[1] and later modified by Albert Grunbaum ed Edward Hume in 1902[2] with the inclusion of neutral red and crystal violet.
By 1930, ten modifications of "MacConkey's Basal Bile Salt Peptone" agar were published in a compendium of microbiological media, but among all of these, it was Grunbaum and Hume's formula that stood the test of time and is (with minor modifications) the basis of modern MacConkey agar; 120 years later, MacConkey agar remains ubiquitous in clinical and industrial laboratories, where it is used routinely to detect non-fastidious Gram-negative organisms in a variety of human specimens and non clinical materials.[3]
Mac Conkey Agar is intended for the isolation of *Enterobacteriaceae* and other Gram negative bacilli and for the differentiation of lactose-fermenting from lactose-nonfermenting Gram-negative enteric bacilli. Mac Conkey Agar is used for the microbiological examination of human clinical specimens[5,6], is included in the FDA-BAM[7] for the primary isolation of Enteropathogenic *E.coli* in food, meets harmonized EP, USP, JP specifications[8] for *E.coli* detection in non sterile pharmaceutical products and is recommended by ISO 21150 for *E.coli* detection in cosmetics[9].
The original MacConkey medium has been modified in the present preparation: the agar content is lower, 5g/L of sodium chloride have been added, the concentration of bile salts and neutral red has been modified.[4] These modifications support excellent growth of most strains of *Salmonella* and *Shigella*, and permit better differentiation of these pathogens from coliform bacteria. The selective action of Mac Conkey Agar is due to the presence of bile salts no. 3, which inhibit the growth of Gram-positive bacteria; this inhibitory activity is enhanced by the addition of crystal violet. 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, with the consequent precipitation of the bile salts and absorption of the neutral red.[4] The coliform bacteria grow with red-pink to red-violet colonies surrounded by a red precipitation zone. Lactose non-fermenters strains (e.g. *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas*, *Alkaligenes* etc.) develop transparent, colourless colonies without precipitation zone. The swarming of *Proteus* spp. is partially controlled on Mac Conkey Agar by using selected raw materials.

4-PHYSICAL CHARACTERISTICS

Medium appearance	red-violet, limpid or slightly opalescent
Final pH at 25 °C	7,1 ± 0,2

5-MATERIALS PROVIDED

Product	Type	REF	Pack
Mac Conkey Agar	Ready-to-use plates	541670	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6-MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

7-SPECIMENS

Mac Conkey Agar is intended for the bacteriological examination of several human clinical specimens with mixed flora (e.g. urine, stool, materials from respiratory tract, wounds and abscesses etc.)[5,6] and non-clinical specimens, as food, non sterile pharmaceutical products, cosmetics[7,8,9]. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, storage and transport of the specimens to the Laboratory should be applied.

8-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. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

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

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*).[4]
For the detection of *E.coli* in non sterile pharmaceuticals products the technique recommended by European Pharmacopoeia[8] and summarized below, should be followed:

- Prepare a sample using a 1:10 dilution of not less than 1 g of the product to be examined and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate the suitable amount of Tryptic Soy Broth. Mix and incubate at 30-35°C for 18-24 h.
- Shake the container, transfer 1 mL of Tryptic Soy Broth to 100 mL of Mac Conkey Broth EP and incubate at 42-44 °C for 24-48 h.
- Subculture on a plate of Mac Conkey Agar and incubate at 30-35 °C for 18-72 h.

Growth of colonies indicates the possible presence of *E. coli*. This is confirmed by identification tests.

9-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 and may be surrounded by red zones of precipitated bile.
Colonies of lactose non-fermenters are colourless or white or light yellow or with a natural pigmentation (e.g. green for *P.aeruginosa*).

10-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.[10]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E.coli ATCC 8739	35-37°C / 18-24 h / A	red violet colonies with red opaque halo
P.mirabilis ATCC 12453	35-37°C / 18-24 h / A	not swarming colourless colonies
*S.*Typhimurium ATCC 14028	35-37°C / 18-24 h / A	colourless colonies
E.faecalis ATCC 29212	35-37°C / 18-24 h / A	inhibited

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

11-PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of ready-to use plates of Mac Conkey Agar and of the raw material used for the production of prepared plates (dehydrated Mac Conkey Agar REF 401670) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by a quantitative test with the target strain *E.coli* ATCC 8739; Mac Conkey Agar plates are inoculated with decimal dilutions in saline of a suspension of colonies and incubated at 35-37°C for 18-24 hours. The colonies are enumerated on Test Batch (TB) and Reference Batch (RB) and the productivity ratio (*Pr*=UFC_{TB}/UFC_{RB}) is calculated. If *Pr* is ≥ 0.7 and if the colonies' morphology and colour are typical (red-pink to red-violet colonies with red opaque halo) the results are considered acceptable and conform to the specifications. Furthermore 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, and lactose non-fermenting strains: *S.*Typhimurium ATCC 14028, *S.flexneri* ATCC 12022, *P.mirabilis* ATCC 12453, *P.vulgaris* ATCC 6380, *Y.enterocolitica* ATCC 23715, *P.aeruginosa* ATCC 9027. Typical colonies of lactose fermenters are pink-red to red violet in colour with or without precipitation zones; typical colonies of lactose non fermenters are colourless or green for *P.aeruginosa*. The amount of growth on the plates after incubation 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 decimal dilutions in saline from 10^{-1} to 10^{-4} of a 0.5 McFarland suspension of the non-target Gram positive strain *E.faecalis* ATCC 29212. If the growth of non-target strain is inhibited at the dilution 10^{-1} in both batches the results are considered acceptable and conform to the specifications.

12-LIMITATIONS OF THE METHOD

- Prolonged incubation may lead to confusion of results; do not incubate longer than 48 hours.[4]
- Due to selective properties of this medium some strains of Gram negative enterics fail to grow or grow poorly; similarly some Gram positive organisms may not be inhibited or are partially inibited.[4]
- Some enterococci strains may exhibit growth after prolonged incubation.[4]
- Mac Conkey agar is not a satisfactory medium for the detection and enumeration of coliform organisms in food. One of the most reliable methods uses violet red bile agar in pour plate counts.[11]
- 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. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 ready-to use plates 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- 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.
- Sterilize all biohazard waste before disposal and dispose of the unused medium and the sterilized plates inoculated with samples or microbial strains, in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- 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 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).

15 - 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. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. McElvania E, Singh K. Specimen Collection, Transport and Processing: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
6. Vandepitte J, Verhaegen J, P. Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd edition Geneve: World Health Organization Geneva; 2003.
7. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Bacteriological Analytical Manual Chapter 4A Diarrheagenic *Escherichia coli.* Content current as of:11/03/2017
8. European Pharmacopoeia, current edition.
9. ISO 21150:2015. Cosmetics- Microbiology -Detection of Escherichia coli.
10. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
11. Harrigan WF,. McCance ME. The Microbiological examination of foods. in Laboratory Methods in Microbiology, Elsevier B.V. 1966.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 2	Updated layout and content in compliance with IVDR 2017/746	2020/05
Instructions for Use (IFU) - Revision 3	Removal of obsolete classification	2023/03

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

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