INSTRUCTIONS FOR USE

.

MAC CONKEY AGAR

Dehydrated culture medium

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

1 - INTENDED USE

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

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

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

*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 Mac Conkey agar; 120 years later, Mac Conkey 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 Mac Conkey 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 inhibits 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 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 50 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	pale pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid or slightly opalescent
Final pH at 25 °C	7.1 ± 0.2

6 - MATERIALS PROVIDED

Product	Type	REF	Pack
Mac Conkey Agar	Dehydrated medium	4016702 4016704	500 g (10 L) 5 kg (100 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 for the identification of the colonies.

8 - 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.

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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. Alternatively, if the 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.
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.

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 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*).

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.[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	non-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

12-PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Mac Conkey Agar is 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 ($Pr=CFU_{TB}/CFU_{RB}$) 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 the non-target strain is inhibited at the dilution 10^{-1} in both batches the results are considered acceptable and conform to the specifications.

13-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 enteric bacteria fail to grow or grow poorly; similarly some Gram-positive organisms may not be inhibited or are partially inhibited.[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 with 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 diseases; 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.

14 - 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.
- 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 preparation process of plated or bottled 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 plates 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.
- 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.

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 for the validation of the period of validity of the finished products, according to the type (plates/bottles), and the storage method applied (temperature and packaging).

16 - REFERENCES

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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	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2020/05
Revision 3	Modification of “precautions and warnings”, “storage conditions and shelf life”.	2022/01
Revision 4	Removal of obsolete classification	2023/04

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

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