

**INSTRUCTIONS FOR USE****MAC CONKEY AGAR**

Ready-to-use flasks

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 \***

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	0.003 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<sup>1</sup> and later modified by Albert Grunbaum ed Edward Hume in 1902<sup>2</sup> 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.<sup>3</sup>

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.<sup>4</sup> Mac Conkey Agar is used for the microbiological examination of human clinical specimens<sup>5,6</sup>, is included in the FDA-BAM<sup>7</sup> for the primary isolation of Enteropathogenic *E.coli* in food, meets harmonized EP, USP, JP specifications<sup>8</sup> for *E.coli* detection in non-sterile pharmaceutical products and is recommended by ISO 21150 for *E.coli* detection in cosmetics<sup>9</sup>.

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.<sup>4</sup> 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.<sup>4</sup> 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- METHOD OF PREPARATION**

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

**5 - PHYSICAL CHARACTERISTICS**

Medium 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	Ready-to-use flasks	5116702	6 x 100 mL; 6 glass bottles with flat bottom and aluminium screw-cap; packaging: cardboard box.
		5116703	6 x 200 mL; 6 glass bottles with flat bottom and aluminium screw-cap; packaging: cardboard box.

**7 - MATERIALS REQUIRED BUT NOT PROVIDED**

Autoclave, water bath or hot plate, incubator and laboratory equipment as required, sterile plastic Petri dishes, sterile loops, needles and swabs, 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.)<sup>5,6</sup> and non-clinical specimens, as food, non-sterile pharmaceutical products,





cosmetics<sup>7,8,9</sup>. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, storage and transport of the specimens to the Laboratory should be applied.

### 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*).<sup>4</sup>

For the detection of *E.coli* in non-sterile pharmaceuticals products, the technique recommended by European Pharmacopoeia<sup>8</sup> 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.<sup>10</sup>

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>E.coli</i> ATCC 8739	35-37°C / 18-24 h / A	red-violet colonies with red opaque halo
<i>P.mirabilis</i> ATCC 12453	35-37°C / 18-24 h / A	non-swarming colourless colonies
<i>S.Typhimurium</i> ATCC 14028	35-37°C / 18-24 h / A	colourless colonies
<i>E.faecalis</i> 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 ready-to-use flasks and of the raw material used for the production, 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  $\geq 0.7$  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, *Y.enterocolitica* ATCC 23715, and lactose non-fermenting strains: *S.Typhimurium* ATCC 14028, *S.flexneri* ATCC 12022, *P.mirabilis* ATCC 12453, *P.vulgaris* ATCC 6380, *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.<sup>4</sup>
- 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.<sup>4</sup>
- Some enterococci strains may exhibit growth after prolonged incubation.<sup>4</sup>
- 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.<sup>11</sup>
- 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.
- 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 product 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.

- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass.
- When using a hot plate and/or a water bath, boil sufficiently long to dissolve the whole medium.
- Wear heat-protective gloves during medium liquefaction. Do not place the hot flasks into an ice bath or in cold water to accelerate cooling as this might cause cracks of the glass.
- The time required for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device, its wattage, the size and volume of the bottle.
- Once the bottled medium is liquefied, it cannot be solidified and dissolved a second time.
- Ready-to-use flasks are subject to terminal sterilization by autoclaving.
- 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](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 flasks in their original pack at 2-8°C away from direct light. If properly stored, the flasks may be used up to the expiration date. Do not use the flasks beyond this date. Flasks from opened secondary packages can be used up to the expiration date. Opened flasks must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks with signs of deterioration (e.g. microbial contamination, abnormal turbidity, precipitate, atypical colour).

The user is responsible of the correctness of plates preparation. The user is responsible of the validation of plates shelf-life, according to the method of storage (temperature and packaging).

### 16 - REFERENCES

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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.
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8. European Pharmacopoeia, current edition.
9. ISO 21150:2015. Cosmetics- Microbiology -Detection of *Escherichia coli*.
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### TABLE OF APPLICABLE SYMBOLS

<b>REF</b> or <b>REF</b> Catalogue number	<b>LOT</b> Batch code	<b>IVD</b> <i>In vitro</i> 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
Revision 2	Updated layout and content in compliance with IVDR 2017/746	2021/09
Revision 3	Removal of obsolete classification	2023/04

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

