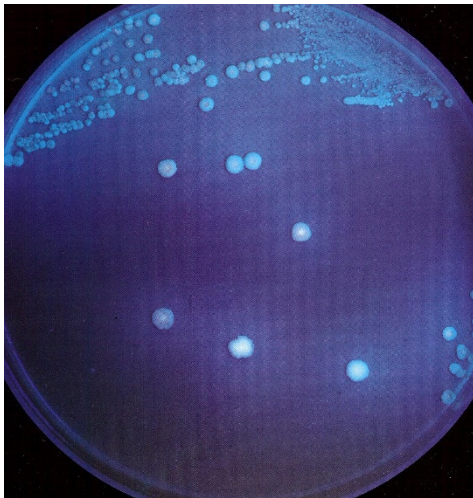




# MAC CONKEY AGAR MUG

## Ready-to-use plates



Mac Conkey Agar MUG: *E.coli* colonies, fluorescent under Wood's lamp

### 1 - INTENDED USE

*In vitro* diagnostic device. A selective fluorogenic medium for the isolation and differentiation of Enterobacteriaceae and other non-fastidious, aerobic, Gram-negative bacilli from clinical and nonclinical specimens and for the presumptive differentiation of *Escherichia coli*.

### 2 - COMPOSITION -TYPICAL FORMULA \*

Gelatin peptone	17.000 g
Peptocomplex	3.000 g
Lactose monohydrate	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
4-Methylumbelliferyl- β-D-glucuronide (MUG)	0.100 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 MUG is a selective and differential medium based on the formula described by Trepeta and Edberg<sup>1</sup>, who modified classical MacConkey Agar by incorporating the fluorogenic compound 4-methylumbelliferyl-β-D-glucuronide (MUG), according to the preliminary studies of Dahlen and Linden<sup>2</sup> and Kilian and Bülow<sup>3</sup>. The inclusion of MUG does not alter the selectivity or lactose fermentation characteristics of MacConkey Agar. <sup>1</sup>

Mac Conkey Agar MUG is intended for the isolation of *Enterobacteriaceae* and other Gram-negative bacilli from clinical specimens, for the differentiation of lactose-fermenting and non-lactose-fermenting Gram-negative enteric bacilli, and for the rapid presumptive identification of *Escherichia coli* through the detection of β-glucuronidase activity. <sup>1,4</sup>

MUG is cleaved by β-D-glucuronidase produced by *E. coli* into 4-methylumbelliferone and glucuronide. The fluorogenic 4-methylumbelliferone can be detected directly using long-wave ultraviolet light.

The selective action of Mac Conkey Agar MUG 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. Peptones provide carbon, nitrogen, and trace elements for bacterial growth, while sodium chloride maintains osmotic balance. Lactose fermentation by coliforms causes acidification of the medium, resulting in precipitation of bile salts and uptake of neutral red.<sup>4</sup> Coliform bacteria produce red-pink to red-violet colonies surrounded by a zone of bile precipitation. Non-lactose-fermenting strains (e.g., *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas*, *Alcaligenes*, etc.) develop transparent, colourless colonies without a precipitation zone. Swarming of *Proteus* spp. is partially inhibited through the use of selected raw materials. β-glucuronidase-positive, lactose-positive *E. coli* strains produce red-pink to red-violet colonies surrounded by a zone of bile precipitation and show a slight blue fluorescence under long-wave ultraviolet light.

### 4 - PHYSICAL CHARACTERISTICS

Medium appearance	red-violet, limpid or slightly opalescent
Final pH at 20-25 °C	7.1 ± 0.2

### 5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Mac Conkey Agar MUG	Ready-to-use plates	541672	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

### 6 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave and water-batch, sterile loops and swabs, incubator and laboratory equipment as required, Wood's Lamp, ancillary culture media and reagents for the identification of the colonies.

### 7 - SPECIMENS

Mac Conkey Agar MUG is intended for the bacteriological processing of the same specimens examinable with Mac Conkey Agar: urine and several human clinical specimens with mixed flora (e.g. stool, materials from respiratory tract, wounds and abscesses etc.).<sup>5,6,7</sup> Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.<sup>5</sup>

### 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.

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. Incubate in aerobic conditions at 35-37°C for 18-24 hours or longer if necessary (up to 48 h for late lactose fermenters: *Citrobacter*, *Providencia*, *Serratia*, *Hafnia*).<sup>4</sup>





### 9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies at the normal light. Check the plates by using a hand-held 366-nm light or by placing the plate under a long-wave UV light (Wood's Lamp).

Colonies of lactose fermenters are red-pink to red-violet and may be surrounded by red zones of precipitated bile.

Colonies of *E. coli* develop a slightly blue fluorescence under Wood's lamp.

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.<sup>8</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, fluorescent under Wood's lamp
<i>P. mirabilis</i> ATCC 12453	35-37°C / 18-24 h / A	not 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 / 44-48 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 MUG and of the raw material used for the production of prepared plates (dehydrated Mac Conkey Agar MUG REF 401672) are tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity and specificity characteristics are tested by semi-quantitative ecometric technique, by incubating at 35-37°C for 18-24 hours, with 2 lactose fermenting and  $\beta$ -glucuronidase positive strains (*E. coli* ATCC 25922, *E. coli* ATCC 8739), with 3 lactose fermenting and  $\beta$ -glucuronidase negative strains (*E. aerogenes* ATCC 13048, *K. pneumoniae* ATCC 27736, *Y. enterocolitica* ATCC 23715), and with 5 lactose non-fermenting strains (*S. Typhimurium* ATCC 14028, *S. flexneri* ATCC 12022, *P. mirabilis* ATCC 12453, *P. vulgaris* ATCC 8427, *P. aeruginosa* ATCC 9027). Typical colonies of *E. coli* are pink-red to red violet in colour with red precipitation zones, fluorescent under Wood's Lamp: typical colonies of lactose fermenters and  $\beta$ -glucuronidase negative strains are pink-red to red violet in colour with or without precipitation zones with no fluorescence under Wood's lamp; typical colonies of lactose non fermenters are colourless or green for *P. aeruginosa*. The amount of growth on the plates is evaluated and shall be comparable in both batches.

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.

Biolife Mac Conkey Agar MUG was evaluated by Goglio et al.<sup>9</sup> using 1534 urine samples and this product was compared with the routine method of calibrated loop with Mac Conkey Agar and blood agar for screening for uropathogens. Combining positivity to  $\beta$ -glucuronidase, positivity to lactose fermentation and the indole test, the identification of *E. coli* colonies has a sensitivity of 100% and a specificity of 85%. The authors' conclusion was: the detection of  $\beta$ -glucuronidase with Mac Conkey Agar MUG allows to anticipate the identification of *E. coli* and the information to the clinician by 24 hours with a possible impact on the therapeutic strategy.

According to the data of Trepeta and Edberg<sup>1</sup> MUG supplemented MacConkey agar (MCM) proved to be sensitive in elucidating  $\beta$ -glucuronidase positive microbes directly from clinical specimens. Compared to MacConkey agar (MCA), MCM showed enhanced recovery of *E. coli* organisms: 255 clinical specimens were processed with both media and *E. coli* has been isolated in 82 specimens with MCM and in 77 specimens with MCA. MCM proved especially useful in establishing the presence of *E. coli* mixed with other pathogens.

Accuracy was assessed by reviewing the Quality Control data. The results of 14 batches produced from 01/01/2018 to 24/04/2020 were evaluated. 100% of the batches showed conformity to defined acceptance criteria in terms of productivity and differential properties with target strains and selectivity with non-target strains.

### 12 - LIMITATIONS OF THE METHOD

- It has been reported that approximately 40% of *Shigella* species, various bioserotypes of *Salmonella* (13% of *Salmonella* subgenus I) may be  $\beta$ -glucuronidase positive and fluorescent under Wood's Lamp; only exceptionally this test is positive with *Providencia*, *Enterobacter* and *Yersinia* strains (1-5%).<sup>1,10,11</sup>
- Approximately 3-4% of *E. coli* are  $\beta$ -glucuronidase negative, notably *E. coli* O157 strains<sup>10</sup>.
- Up to 10% of clinical *E. coli* isolates have been reported to be slow or non-lactose fermenting.<sup>12</sup>
- Prolonged incubation may lead to confusion of results<sup>4</sup>; do not incubate plates longer than 48 hours.
- Due to selective properties of this medium some strains of Gram-negative enteric bacteria fail to grow or grow poorly.<sup>4</sup> A lack of growth or the absence of typical colonies does not preclude the presence of Gram-negative bacilli in the sample.
- Some enterococci strains may exhibit growth after prolonged incubation.<sup>4</sup>
- A single medium is only rarely useful to recover the target-strains contained in a specimen, so concomitant cultures are necessary to recover organisms for typing.
- Appropriate tests are required for complete identification and epidemiological typing of colonies; if necessary, perform antimicrobial susceptibility tests using recommended methods.
- The device is not intended to diagnose infections or to guide the antimicrobial therapy. It is used in a diagnostic set of investigations to provide microbial colonies isolated from clinical samples of patients with suspected bacterial infection..

### 13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative in vitro diagnostic device intended for professional use only, is not automated and is not a companion diagnostic tool. It must be used by adequately trained and qualified laboratory personnel, observing 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. 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](http://www.biolifeitaliana.it), describing the measures implemented 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](http://www.biolifeitaliana.it).
- Notify the Manufacturer ([complaint@biolifeitaliana.it](mailto:complaint@biolifeitaliana.it)) and the relevant Authorities of any serious incident occurring in connection with the use of the in vitro diagnostics.
- The Manufacturer may not be held responsible for any loss or damage in any way resulting from or related to use of the product in manners not compliant with the instructions provided.















#### 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

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12. Gokul Yaratha, MD, Sarah Perloff, DO, Kinesh Changala, MBBS. Lactose vs non-lactose fermenting E. coli: Epidemiology, Clinical Outcomes, and Resistance. Open Forum Infect Dis 2017; V4 (Suppl 1)

**TABLE OF APPLICABLE SYMBOLS**

 Catalogue number	 Batch code	 <i>In vitro</i> diagnostic medical device	 Manufacturer	 This way up	 For single use only	 European conformity mark
 Temperature limitations	 Contents sufficient for <n> tests	 Consult electronic instructions for use	 Use by	 Keep away from sunlight	 Fragile, handle with care	 Unique device identifier

#### REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content in compliance with IVDR 2017/746	2020/05
Revision 2	Removal of obsolete classification	2023/03
Revision 3	Modified sections: intended use, specimens, performances characteristics, limitations of the method, precautions and warning, references, table of applicable symbols	2026/02

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

