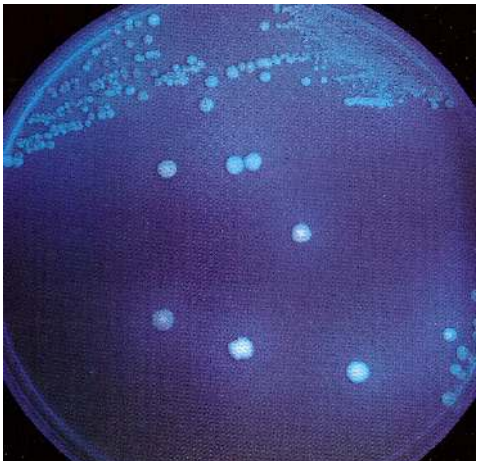
INSTRUCTIONS FOR USE

MAC CONKEY AGAR MUG

Dehydrated culture medium



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

1 - INTENDED USE

In vitro diagnostic. Selective and differential medium for the isolation and differentiation of *Enterobacteriaceae* and other Gram-negative bacilli and for the presumptive identification of *Escherichia coli*, from clinical specimens.

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

Gelatin peptone	17.000 g
Peptocomplex	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
4-Methylumbelliferyl- β-D-glucuronide (MUG)	0.100 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 MUG is a selective and differential medium based on the formula described by Trepeta and Edberg[1] who modified the classical Mac Conkey Agar [by] incorporation of the fluorogenic compound 4-methylumbelliferyl- β-D-glucuronide (MUG), according to the preliminary studies of Dahlen and Linden[2] and of Kilian and Bulow[3]. Inclusion of MUG doesn't change Mac Conkey Agar characteristics of selectivity or lactose fermentation.[1]

Mac Conkey Agar MUG is intended for the isolation of *Enterobacteriaceae* and other Gram negative bacilli from clinical specimens and for the differentiation of lactose-fermenting from lactose-nonfermenting Gram-negative enteric bacilli and for the rapid, presumptive identification of *E.coli* by the detection of β-glucuronidase enzyme.[1,4]

MUG is cleaved by β-D-glucuronidase produced by *E.coli* to 4-methylumbelliferone and glucuronide; the fluorogenic 4-methylumbelliferone can be determined directly by using a long-wave ultraviolet light.

The selective action of Mac Conkey Agar MUG 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 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. *Proteus* swarming is partially controlled by using selected raw materials. β-glucuronidase positive and lactose positive *E.coli* strains grow with red-pink to red-violet colonies surrounded by a red precipitation zone, with a slight blue fluorescence under long-wave ultraviolet light.

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	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid or slightly opalescent
Final pH at 20-25 °C	7.1 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Mac Conkey Agar MUG Medium	Dehydrated medium	4016722	500 g (10L)
Mac Conkey Agar MUG	Dehydrated medium	4016724	5 kg (100 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

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

8 - 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, abscesses, etc.)[5,6,7] . Good laboratory practices for collection, transport and storage of the specimens should be applied.[5]

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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 conditions at 35-37°C for 18-24 hours or longer if necessary (up to 48 h for late lactose fermenters: *Citrobacter, Serratia*, *Hafnia*).[4]

10 - 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 lightbox (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 slight 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*).
The confirmation of *E.coli* identification can be done by indole test (+), directly on the plate.

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 with red opaque halo, fluorescent under Wood's lamp
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 / 44-48 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 MUG is 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 β-glucuronisase positive strains (*E.coli* ATCC 25922, *E.coli* clinical isolate), with 3 lactose fermenting and β-glucuronisase 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 6380, *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 β-glucuronisase 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.[9] 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 β-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 β-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[1] MUG supplemented Mac Conkey agar (MCM) proved to be sensitive in elucidating β-glucuronidase positive microbes directly from clinical specimens. Compared to Mac Conkey 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.

13 - LIMITATIONS OF THE METHOD

- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of *Salmonella* subgenus I) may be β-glucuronidase positive and fluorescent under Wood's Lamp; only exceptionally this test is positive with *Providencia*, *Enterobacter* and *Yersinia* strains (1-5%).[1,10,11]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E.coli* O157 strains[10].
- Up to 10% of clinical *E.coli* isolates have been reported to be slow or non-lactose fermenting.[12]
- Prolonged incubation may lead to confusion of results[4]; 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.[4]
- Some enterococci strains may exhibit growth after prolonged incubation.[4]
- 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.
- 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 +2°C /+8°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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TABLE OF APPLICABLE SYMBOLS

REF o 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 1	Updated layout and content	2020/05
Revision 2	Update of "precautions and warnings" and "storage conditions and shelf life"	2022/01
Revision 3	Removal of obsolete classification	2023/04

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

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