VIOLET RED BILE AGAR MUG

Dehydrated culture medium

1 - INTENDED USE

For the detection and enumeration of coliform bacteria and *Escherichia coli* in food, animal feed and environmental samples.

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

Peptone	7.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Bile salts No.3	1.5 g
Lactose	10.0 g
Neutral red	30.0 mg
Crystal violet	2.0 mg
4-methylumbelliferyl- β-D-glucuronide (MUG)	100.0 mg
Agar	15.0 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

The presence of *E. coli* in food or water is accepted as indicative of recent faecal contamination and the possible presence of pathogens, while coliforms are used as an indicator of sanitary condition in the food-processing environment.
Violet Red Bile (VRBL) Agar, designed for the enumeration of bacteria of the coli-aerogenes group, is derived from MacConkey's[1] original formula. Trepeta and Edberg[2] modified the classical MacConkey Agar by incorporating the fluorogenic compound 4-methylumbelliferyl- β-D-glucuronide (MUG), for the rapid detection of *E. coli*, according to the preliminary studies of Dahlen and Linde[3] and of Kilian and Bulow[4]. VRBL Agar supplemented with MUG is recommended by FDA-BAM[5] for the enumeration of coliforms and *E.coli* in foodstuffs, with the poured plate technique.
In VRBL Agar MUG, essential growth factors are provided by peptone and yeast extracts which are sources of nitrogen, carbon, vitamins and minerals; sodium chloride maintains the osmotic balance. The medium relies on the use of the selective inhibitory components crystal violet and bile salts which suppress the growth of Gram-positive bacteria and the indicator system lactose and neutral red.[6] Organisms which rapidly attack lactose produce purple colonies often surrounded by purple halos. Non-fermenters and late lactose fermenters exhibit pale or colourless colonies. The presence of MUG enables the enumeration of *E. coli* colonies by distinguishing them within the typical coliform colonies. MUG is cleaved by β-D-glucuronidase produced by *E. coli* to 4-methylumbelliferone and glucuronide; the fluorogenic 4-methylumbelliferone can be detected directly by using a long-wave ultraviolet light.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Do not autoclave and do not overheat. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green-violet, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	violet, clear
Final pH at 20-25 °C	7.4 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Violet Red Bile Agar MUG	Dehydrated medium	4021862	500 g (12 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, ancillary culture media and reagents, Wood's lamp.

8 – SPECIMENS

Materials of sanitary importance such as products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[5]

9 - TEST PROCEDURE

1. Prepare the test portion, initial suspension (primary dilution) and further dilutions in accordance with the specific International Standard appropriate to the product concerned.
2. Using a sterile pipette, transfer in the centre of two Petri dishes 1 mL of the test sample if the product is liquid, or 1 mL of the initial suspension in case of other products. Repeat the procedure described with the further dilutions.
3. Pour approximately 15 mL of the VRBL Agar MUG into each Petri dish.
4. Carefully mix the inoculum with the medium and allow the medium to solidify, with the Petri dishes standing on a cool horizontal surface. If required, add a covering layer of approximately 5 mL to 10 mL of VRBL Agar MUG to prevent spreading growth and to achieve semi-anaerobic conditions. Allow to solidify
5. Invert the prepared dishes and incubate them at 35°C for 24 h ± 2 h.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical coliforms colonies are pink to red or purple (with or without precipitation haloes).

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Typical *E. coli* colonies are pink to red or purple, with precipitation haloes and exhibit a bluish fluorescence under a long-wave ultraviolet light (Wood's lamp) .
Perform the confirmation tests in accordance with the specific International Standard appropriate to the product concerned.

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 25922	35°C/24H-A	good growth, pink-red colonies with red halo, fluorescent under Wood's Lamp
E. aerogenes ATCC 13048	35°C/24H/A	good growth, pink-red colonies, not fluorescent under Wood's lamp
E. faecalis ATCC 19433	35°C/24H-A	inhibited

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

12 – PERFORMANCE CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated VRBL Agar MUG is tested for productivity, specificity and selectivity by comparing the results with Tryptic Soy Agar.
Productivity is tested by a quantitative method with the target strains *E. coli* ATCC 25922 and *E. coli* ATCC 8738. The plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 35°C for 24 hours. The colonies are enumerated on both media and the productivity ratio ($Pr:CFU_{VRBL-MUG}/CFU_{TSA}$) is calculated. If Pr is ≥ 0.5 and if the colonies morphology and colour are typical (pink-red colonies with red halo, fluorescent under Wood's lamp) the results are considered acceptable and conform to the specifications.
Moreover, the productivity characteristics are tested by semi-quantitative ecometric technique with *E. aerogenes* ATCC 13048. After incubation, the target strain exhibits good growth with pink-red colonies without fluorescence under Wood's lamp.
Specificity is assessed by semi-quantitative ecometric technique with the non-target strains *P.aeruginosa* ATCC 27853. After incubation, *P.aeruginosa* exhibits good growth with colourless colonies.
Selectivity is assessed with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of *E. faecalis* ATCC 19433. The growth of the non-target strain is totally inhibited.

13-LIMITATIONS OF THE METHODS

- Occasionally enterococci grow on this medium; however, the colonies are pinpoint. If in doubt perform a Gram staining and a catalase test (Gram-positive cocci, catalase-negative).[7]
- Medium is not completely specific for enterics; other accompanying bacteria may give the same reactions. Further biochemical tests are necessary for positive identification.[7]
- Medium selectivity diminishes after 24 hours of incubation and organisms previously suppressed may exhibit growth.[7]
- Colonies of dubious colour can be expected on the medium in particular when dairy products containing sugars other than lactose are examined; in this case, the conversion of these sugars can give rise to colonies with an appearance similar to that of typical coliforms.[8]
- 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%).[9]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[9]
- Up to 10% of *E. coli* isolates have been reported to be slow or non-lactose fermenting.[10]

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and 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 production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium 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 products 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 +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 the validation of their shelf life, according to the type (plates/bottles) and the applied storage conditions (temperature and packaging). According to ISO 4832, the medium for the poured plate technique must be used within 4 hours after its preparation.[8]

16 - REFERENCES

1. MacConkey A. Lactose fermenting bacteria in faeces. J Hyg 1905; 5:333-379
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3. Dahlén G, Linde A. Screening plate method for detection of bacterial beta-glucuronidase. Appl Microbiol 1973;26 (6): 863-6
4. Killian M, Bulow P. 1976. Rapid diagnosis of Enterobacteriaceae. Detection of bacterial glycosidases. Acta Pathol Microbiol Scand Sec B 1976; 84:245-251.
5. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria Rev 10/2020
6. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:282-284.
7. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
8. ISO 4832-1:2006. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique
9. Robison, B.J. 1984. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 48:285-288
10. 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

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/08

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