VIOLET RED BILE GLUCOSE (VRBG) AGAR

Dehydrated and ready-to-use culture media

1 - INTENDED USE

For the detection and enumeration of *Enterobacteriaceae* in food, animal feed and environmental samples.

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

DEHYDRATED AND READY-TO-USE MEDIUM

Peptone	7.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Bile salts No.3	1.5 g
Glucose	10.0 g
Neutral red	30.0 mg
Crystal violet	2.0 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

Enterobacteriaceae are usually considered by food manufacturers as hygiene indicators and thus used to monitor the effectiveness of preventive measures taken. This is also reflected in several national and international Standards or criteria in which *Enterobacteriaceae* are included as hygiene indicators.

Violet Red Bile Glucose (VRBG) Agar was designed by Mossel[1] for the enumeration of *Enterobacteriaceae,* by adding glucose to Violet Red Bile Lactose Agar. Later works of Mossel *et al.*[2,3] demonstrated that lactose could be omitted, resulting in the formulation known as VRBG Agar. Violet Red Bile Glucose Agar is recommended by ISO 21528-1[4] for the detection and the enumeration with a pre-enrichment step and with the MPN technique of *Enterobacteriaceae,* when the microorganisms sought are expected to need resuscitation, and when the number sought is expected to be below 100 per millilitre or per gram of test sample.

It is recommended by ISO 21528-2[5] for the enumeration of *Enterobacteriaceae* with pour plate technique, when the number of colonies sought is expected to be more than 100 per millilitre or per gram of the test sample.

Peptone provides essential growth factors for bacterial growth; yeast extract is a source of B-vitamins complex for growth stimulation; 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 glucose and neutral red. The dissimilation of glucose causes acidification of the medium, with the consequent precipitation of bile salts and neutral red uptake. The *Enterobacteriaceae* grow with red-pink to red-violet colonies surrounded by a red precipitation zone. Non-glucose fermenters (e.g., *Pseudomonas, Acinetobacter, Alcaligenes* etc.) exhibit transparent, colourless colonies. Some Gram-negative bacteria other than *Enterobacteriaceae* may grow but may be limited by the overlay procedure.

4A - DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

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.

4B - DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS/TUBES)

Liquefy the contents of the flask/tube in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle or the tube 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

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 Glucose (VRBG) Agar	Dehydrated medium	4021882 4021884	500 g (12 L) 5 Kg (120 L)
Violet Red Bile Glucose (VRBG) Agar	Ready-to-use medium in plates	542188	2 x 10 plates ø 90 mm
Violet Red Bile Glucose (VRBG) Agar	Ready-to-use medium in tubes	552188	20 x 15 mL
Violet Red Bile Glucose (VRBG) Agar	Ready-to-use medium in flasks	5121882 5121883	6 x 100 mL 6 x 200 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, ancillary culture media and reagents.

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. Consult the appropriate references for sample collection, storage and preparation.[4,5]

9 - TEST PROCEDURE

Detection of *Enterobacteriaceae* after pre-enrichment.[4]

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Inoculate VRBG Agar plates with a loop from each of the incubated cultures obtained after enrichment in Buffered Peptone Water.
Incubate the plates at 37 ± 1 °C for 24 h ± 2 h

Poured plates enumeration of *Enterobacteriaceae*.[5]

1. Using a sterile pipette, transfer to the Petri dish 1 mL of the test sample if the product is liquid, or 1 mL of the initial suspension in case of other products.
2. Repeat the procedure described with the further dilutions.
3. Add into each Petri dish approximately 15 mL of VRBG Agar.
4. Carefully mix the inoculum with the medium and allow the medium to solidify, with the Petri dishes standing on a cool horizontal surface.
5. After complete solidification of the mixture, add a covering layer of approximately 5 mL to 10 mL of VRBG Agar to prevent spreading growth and to achieve semi-anaerobic conditions. Allow to solidify.
6. Invert the prepared dishes and incubate them at 37 ± 1 °C for 24 h ± 2 h.
7. Count the colonies in the Petri dishes with less than 150 colonies.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical *Enterobacteriaceae* colonies are pink to red or purple (with or without precipitation haloes).
Select well-isolated colonies from each of the incubated plates for the biochemical confirmation tests: oxidase reaction (-) and glucose fermentation (+).

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.[4,5]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	37°C/24H-A	good growth, pink-red colonies with red halo
E. faecalis ATCC 19433	37°C/24H-A	inhibited

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

12 – PERFORMANCE CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated and Ready-to-use VRBG Agar are tested for productivity, 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 S. Typhimurium ATCC 14028. The plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 37°C for 24 hours. The colonies are enumerated on both media and the productivity ratio (Pr:CFU_{VRBG}/CGU_{TSA}) is calculated. If Pr is ≥ 0.5 and if the colonies morphology and colour are typical (pink-red colonies with red halo) the results are considered acceptable and conform to the specifications.
Moreover, the productivity characteristics are tested by semi-quantitative ecometric technique with *Y. enterocolitica* ATCC 23715. After incubation, the target strain exhibits good growth with red-violet 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 *S. aureus* ATCC 25923 and *E. faecalis* ATCC 19433. The growth non-target strains 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).[6]
- Medium is not completely specific for enterics; other accompanying bacteria may give the same reactions. Further biochemical tests are necessary for positive identification.[6]
- Medium selectivity diminishes after 24 hours of incubation and organisms previously suppressed may exhibit growth.[6]

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.
- Be careful when opening screw cap flasks or tubes 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 in 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.
- Each ready-to-use 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.
- 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 Sheets 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

Ready-to-use plates

Upon receipt, store plates in their original pack at +2°C /+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).

Ready-to-use medium in flasks and tubes

Upon receipt, store flasks/tubes in their original pack at +2°C /+8°C away from direct light. If properly stored, the flasks/tubes may be used up to the expiration date. Do not use the flasks/tubes beyond this date. Flasks/tubes from opened secondary packages can be used up to the expiration date. Opened flasks/tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks/tubes with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

Dehydrated medium

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/tubes/bottles) and the applied storage conditions (temperature and packaging). According to ISO 21528-1, self-prepared plates to be inoculated on the surface can be stored at +2 °C to +8 °C in the dark and protected against evaporation for up 2 weeks.[4] According to ISO 21528-2 medium for the poured plate technique must be used within 4 hours after its preparation.[5]

16 - REFERENCES

1. Mossel DAA, Mengerink WH, Scholts HH. Use of a modified MacConkey agar medium for the selective growth and enumeration of Enterobacteriaceae. J Bacteriol. 1962 Aug;84(2):381.
2. Mossel DAA, Eelderink I, Koopmans M, Van Rossem F. Lab Practice 1978; 27:1049.
3. Mossel DAA, Eelderink I, Koopmans M, Van Rossem F. Influence of Carbon Source, Bile Salts and Incubation Temperature on Recovery of Enterobacteriaceae from Foods Using MacConkey-type Agars. J Food Prot 1979 Jun;42(6):470-475.
4. ISO 21528-1:2017 Microbiology of the food chain —Horizontal method for the detection and enumeration of Enterobacteriaceae — Part 1: Detection of Enterobacteriaceae.
5. ISO 21528-2:2017 Microbiology of the food chain —Horizontal method for the detection and enumeration of Enterobacteriaceae —Part 2: Colony-count technique.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 3	Updated layout and content	2022/08

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

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