

## VIOLET RED BILE GLUCOSE (VRBG) AGAR EP

### Dehydrated culture medium

#### 1 - INTENDED USE

For the detection and enumeration of bile-tolerant Gram-negative bacteria in pharmaceutical products.

#### 2 – COMPOSITION\*

##### TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Yeast extract	3.0 g
Pancreatic digest of gelatin	7.0 g
Bile salts No.3	1.5 g
Sodium chloride	5.0 g
Glucose monohydrate	10.0 g
Agar	15.0 g
Neutral red	30.0 mg
Crystal violet	2.0 mg

\*The formula may be adjusted and/or supplemented to meet the required performances criteria.

#### 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Violet Red Bile Glucose Agar was designed by Mossel<sup>1</sup> for the enumeration of *Enterobacteriaceae*, by adding glucose to Violet Red Bile Lactose Agar. Later works of Mossel *et al.*<sup>2,3</sup> demonstrated that lactose could be omitted, resulting in the formulation known as VRBG.

Violet Red Bile Glucose (VRBG) Agar EP meets the requirement of European Pharmacopoeia<sup>4</sup> and is recommended for presence/absence test and for the enumeration of bile-tolerant Gram-negative bacteria in non-sterile pharmaceutical products.

Pancreatic digest of casein provides carbon, nitrogen and trace elements for bacterial growth; sodium chloride maintains the osmotic balance. The selective agents of the medium are crystal violet and bile salts n° 3 which inhibits the growth of Gram-positive bacteria. 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 often surrounded by a red precipitation zone. Non-glucose fermenters (e.g. *Pseudomonas*, *Acinetobacter*, *Alcaligenes* etc.) exhibit transparent, colourless colonies.

#### 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 Glucose (VRBG) Agar EP	Dehydrated medium	4021892	500 g (12 L)
Violet Red Bile Glucose (VRBG) Agar EP	Ready-to-use plates	542189	2 x 10 plates ø 90 mm

#### 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

Non-sterile pharmaceutical products. Consult the appropriate reference for sample collection, storage and preparation.<sup>4</sup>

#### 9 - TEST PROCEDURE

Prepare the sample suspension in Tryptic Soy Broth using at least 1 g or 1 mL of sample.

Incubate this suspension at 20°C / 25°C for 2-5 hours to ensure revitalisation but not multiplication of bacteria.

##### a- Test for absence

Inoculate a quantity of the initial suspension into Enterobacteriaceae Enrichment Broth Mossel EP (REF 401467) to ensure an inoculum of 1 g of sample and incubate at 30°C / 35°C for 24-48 hours

Subculture on plates of VRBG Agar EP and incubate 30°C / 35°C for 18-24 hours.

##### b- Quantitative test

Inoculate suitable quantities of Enterobacteriaceae Enrichment Broth Mossel EP (REF 401467) with the initial suspension and/or dilution of sample containing respectively 1 g, 0.1 g, 0.01 g and 0.001 g of the product to be examined.

Incubate at 30°C / 35°C for 24-48 hours.

Subculture each of the cultures on a plate of VRBG Agar EP (REF 402189) and incubate 30°C / 35°C for 18-24 hours

#### 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).

Typical Gram-negative non-glucose fermenters colonies are transparent and colourless.

Test for absence: according to European Pharmacopoeia the product complies with the test if there is no growth of colonies on VRBG Agar EP plates.

Quantitative test: refer to European Pharmacopoeia for the interpretation criteria of probable number of bacteria per g or mL of product.



### 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>4,5</sup>

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
<i>E. coli</i> ATCC 8739	30-35°C/18-24H-A	good growth, pink-red colonies with red halo
<i>P. aeruginosa</i> ATCC 9027	30-35°C/18-24H-A	good growth, colourless colonies
<i>E. faecalis</i> ATCC 19433	30-35°C/18-24H-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 and ready to use VRBG Agar EP is tested for productivity, and selectivity by comparing the results with a previously approved Reference Batch.

The productivity is tested by a quantitative method with the target strains *E. coli* ATCC 8739, *S. Typhimurium* ATCC 14028 and *P. aeruginosa* ATCC 9027. The plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 30-35°C for 18 hours. The colonies are enumerated on Test Batch (TB) and on Reference Batch (RB) and the productivity ratio (Pr: CFU<sub>TB</sub>/CFU<sub>RB</sub>) is calculated. If Pr is ≥ 0.7 and if the colonies morphology and colour are typical, the results are considered acceptable and conform to the specifications.

Moreover, the productivity characteristics are tested by semi-quantitative ecometric technique with the target strain *Y. enterocolitica* ATCC 23715. After incubation, the amount of growth and the colony characteristics are evaluated: the target strain exhibits good growth with pink-red colonies.

The 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 6538 and *E. faecalis* ATCC 19433. The non-target strains are 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).<sup>5</sup>
- Medium is not completely specific for enterics; other accompanying bacteria may give the same reactions. Further biochemical tests are necessary for positive identification.<sup>5</sup>
- Medium selectivity diminishes after 24 hours of incubation and organisms previously suppressed may exhibit growth.<sup>5</sup>

### 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](http://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.
- 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](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

#### Ready to use plates

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).

#### 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 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.<sup>6</sup>

### 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. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. ISO 21528-1:2017 Microbiology of the food chain —Horizontal method for the detection and enumeration of Enterobacteriaceae — Part 1: Detection of Enterobacteriaceae.

### TABLE OF APPLICABLE SYMBOLS

<b>REF</b> or <b>REF</b> Catalogue number	<b>LOT</b> 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 1	Updated layout and content	2022/11

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

