VIOLET RED BILE AGAR WITH GLUCOSE (VRBG)

ABVG-IEP-500

- **Principle**

It is based on MacConkey Medium (AGMC-IEP-500) for the detection and enumeration of bile-tolerant Gram-negative Enterobacteriaceae in dairy products and foods. In this medium, the lactose is replaced by glucose as the carbohydrate. VRBG agar is becoming the preferred medium for use in investigations into raw materials, processed foods and plant hygiene. The Enterobacteriaceae group includes lactose-fermenting coliforms bacteria and non-lactose fermenting species like Salmonella and Shigella.

Pancreatic digest of gelatine provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is the source of vitamins, particularly of the B-group. Glucose is the fermentable carbohydrate providing carbon and energy. Glucose fermenters form red colonies in the presence of the pH indicator neutral red. Bile salts and crystal violet inhibit Gram-positive bacteria. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

The European Pharmacopoeia, USP in paragraph 2.6.13: “Microbiological examination of non-sterile products: test for specified microorganisms” recommends this medium for the testing of bile-tolerant Gram-negative bacteria in products. ISO 21528 proposes VRBG Agar for the detection and enumeration of Enterobacteriaceae.

The pour plate method creates semi-anaerobic conditions that inhibit Gram-negative non-fermenters while promoting glucose fermentation, producing purple-red colonies with a surrounding zone of the same colour. Coliforms ferment glucose, generating acid with or without gas. More heat-resistant genera such as Klebsiella and Citrobacter can also grow, indicating insufficient heating during production.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of USP, ISO 21528 as well as the European Pharmacopoeia.

- **Composition**

Ingredients	**g/L**
Bacteriological agar	15.00
Crystal violet	0.002
Neutral red	0.03
Yeast extract	3.00
Bile salts	1.50
Glucose monohydrate	10.00
Sodium chloride	5.00
Enzymatic digest of animal tissues	7.00

- **Preparation**

Suspend 41.5 grams of the medium in one litre of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Cool to 47-50 °C and dispense immediately. DO NOT OVERHEAT.

- **Applications and use**

For the test of specified microorganisms (Bile-tolerant Gram-negative bacteria) according to European Pharmacopoeia:

- Prepare the sample using a 1 in 10 dilution of not less than 1 g of the product in Trypticasein Soy Broth (TSB) (STRB-0EP-500) and incubate at 20-25 °C for 2-5 hours.
- For the absence test, use the dilution made previously corresponding to 1g of the product to inoculate in Mossel EE Broth and incubate at 30-35 °C for 24-48 hours.
- Subculture on plates of Violet Red Bile Agar with Glucose (VRBG) and incubate at 30-35 °C for 18-24 hours. The product complies with the test if there is no growth of colonies.
- For the quantitative test, use the dilution made previously corresponding to 0.1, 0.01 and 0.001 g of the product to inoculate in Mossel EE Broth and incubate at 30-35 °C for 24-48 hours.
- Subculture on plates of Violet Red Bile Agar with Glucose (VRBG) and incubate at 30-35 °C for 18-24 hours.
- Growth of colonies constitutes a positive result.

According to ISO 21528 for the detection and enumeration of Enterobacteriaceae:

- Inoculate Buffered Peptone Water (BPW) (AGPT-0DI-500) with the portion to be tested and incubate at 37 °C for 48 hours.
- Inoculate Violet Red Bile Agar with Glucose (VRBG) with the culture obtained after enrichment in BPW, then incubate at 37 °C for 24 hours.
- Typical presumptive colonies of Enterobacteriaceae should be subcultured in a non-selective medium and confirmed by tests for glucose fermentation and the presence of oxidase.

- **Quality control**

Solubility	w/o rests
Appearance	Fine powder
Colour of the dehydrated medium	Beige reddish
Colour of the prepared medium	Purple-red
Final pH (25°C)	7.4±0.2

- **Microbiological test**

According to Pharmacopoeia; *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027:

- Incubation conditions: 30-35 °C / 18-24 h.

- Inoculation conditions: ≤100 CFU.

According to ISO 11133; *Escherichia coli* ATCC 8739, *Escherichia coli* ATCC 25922, Salmonella STM ATCC 14028 and *Enterococcus faecalis* ATCC 29212:

- Incubation conditions: Productivity and Selectivity (37±1 °C/ 24±2 h).
- Inoculation conditions: Productivity quantitative (100±20. Min.50 CFU) / Productivity qualitative (10^3-10^4 CFU) / Selectivity (10^4-10^6 CFU).

Reference media: TSA.

Microorganisms	ATCC	Specification	Characteristic reaction
Salmonella typhimurium	14028	Good growth >50%	Pink to red colonies with or without precipitation halo
Escherichia coli	25922	Good growth >50%	Pink to red colonies with or without precipitation halo
Enterococcus faecalis	29212	Total inhibition	-
Escherichia coli	8739	Good growth >50%	Pink to red colonies with or without precipitation halo
Pseudomonas aeruginosa	9027	Good growth	-

- **Storage**

The product is highly hygroscopic; keep the container always closed and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

Note: Sterilize media immediately after reconstitution.

- **Bibliography**

D.A. Mossel, (1985) Media for Enterobacteriaceae (Inst. J. Food Microbiol 2:27).

ISO 21528. Microbiology of food and animal feeding stuffs -- Horizontal methods for the detection and enumeration of Enterobacteriaceae.

ISO 7402 Microbiology -- General guidance for the enumeration of Enterobacteriaceae without resuscitation -- MPN technique and colony-count technique.

ISO 8523 Microbiology -- General guidance for the detection of Enterobacteriaceae with pre-enrichment. European Pharmacopoeia 9.3

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.