

VIOLET RED BILE AGAR WITH GLUCOSE (VRBG) ISO 21528, EUROPEAN PHARMACOPEIA

CAT N°: 1092

For the cultivation and enumeration of Enterobacteria in water, foods and other materials

FORMULA IN g/l

Glucose Monohydrate	10.00	Bile Salts	1.50
Pancreatic Digest of Gelatin	7.00	Neutral Red	0.03
Sodium Chloride	5.00	Crystal Violet	0.002
Yeast Extract	3.00	Bacteriological Agar	15.00

Final pH 7.4 ± 0.2 at 25°C

PREPARATION

Suspend 41.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Cool to 45°C and dispense immediately. Alternatively, sterilize in autoclave at 118°C for 15 minutes. DO NOT OVERHEAT. The prepared medium should be stored at 8-15°C. The color is Purple-red.

The dehydrated medium should be homogeneous, free-flowing and beige-reddish in color. If there are any physical changes, discard the medium.

USES

VIOLET RED BILE AGAR WITH GLUCOSE (VRBG) is a selective medium, containing Bile and Violet Red dye, for the isolation and enumeration of enterobacteria. It is based on MacConkey Medium (Cat. 1052) 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 gelatin provide 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.

When testing raw vegetables, for example, VRBL agar (cat. 1093) is a more practical choice for a hygienic state because certain non-lactose fermenting but glucose utilizing organisms, e.g. *Pseudomonas* species, predominate amongst the naturally occurring flora and may easily overgrow the indicator on VRBG Agar.

The European Pharmacopoeia 7.0 recommends in Paragraph 2.6.13: "Microbiological examination of non-Sterile products: test for specified microorganisms" the testing of the product for bile-tolerant gram-negative bacteria. Use the volume of 1gr corresponding to product to inoculate with Mossel EE Broth (Cat. 1202) at 30-35°C for 24-48 hours. Subculture on plates of Violet Red Bile Agar with Glucose. Incubate at 30-35°C for 18-24 hours. The product complies with the test if there is no growth of colonies.

The pour plate method suppresses the growth of Gram-negative non-fermenting bacteria due to its anaerobic conditions. The fermentation of glucose is likewise stimulated and results in the formation of purple-red colonies, clearly visible, surrounded by a zone of the same color. Note that coliforms will ferment the glucose and produce acid with or without gas. *Klebsiella* and *Citrobacter*, which are more heat-resistant than coliforms, also grow in this medium and can indicate a production process defect (insufficient heating).

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30-35°C and observed after 18-24 hours. According to European Pharmacopoeia 7.0.

Microorganisms	Growth	Colony Color
* <i>Escherichia coli</i> ATCC 11775	Good	Red
* <i>Escherichia coli</i> ATCC 8739	Good	Red
* <i>Salmonella gallinarum</i> NCTC 9240	Good	Red
<i>Staphylococcus aureus</i> ATCC 6538	Inhibited	-
* <i>Shigella flexneri</i> ATCC 29903	Good	Red
<i>Pseudomonas aeruginosa</i> ATCC 9027	Good	-
<i>Enterococcus faecalis</i> ATCC 29122	Inhibited	-

* According to ISO 21528. Incubation at 37°C during 24 ± 2 hours.

According to 11133(24 h/37°C) Productivity and Selectivity

Microorganisms	Inoculum (cfu/ml)	Productivity Quantitative	Selectivity Qualitative	Specificity Qualitative
<i>Escherichia coli</i> ATCC 25922	10 ²	pr ≥ 0.9		Pink to red colonies with or without precipitation
<i>Salmonella STM</i> ATCC 14028	10 ²	pr ≥ 0.9		
<i>Enterococcus faecalis</i> ATCC 29212	10 ⁴ / 10 ⁶		Inhibited	

Reference Media Productivity: TSA

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 7.0



STORAGE

Once opened keep powdered medium closed to avoid hydration.

