

Technical Data Sheet

MOLEQULE-ON®

Violet Red Bile Agar with Glucose (VRBG) EP/USP/ISO

Cat #: MM-M-092

For the cultivation and enumeration of Enterobacteriaceae.

Principles and 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. MM-M-052) 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 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 suppresses the growth of Gram-negative non-fermenting bacteria due to its semi-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).

Formula per Litre:

Bacteriological agar	15g	Bile salts	1.5g
Crystal violet	0.002g	Gelatin pancreatic digest	7g
Glucose monohydrate	10g	Neutral red	0.03g
Sodium chloride	5g	Yeast extract	3g

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 47-50°C and dispense immediately. DO NOT OVERHEAT.

Instructions for 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 1g of the product in Trypticasein Soy Broth (TSB) (Cat. MM-M-224) 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 (Cat. MM-M-202) 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 0.001g of the product to inoculate in Mossel EE Broth (Cat. MM-M-202) 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:

Technical Data Sheet

MOLEQULE-ON[®]

- Inoculate Buffered Peptone Water (Cat. MM-M-402) 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 Buffered Peptone Water, then incubate at 37 °C for 24 hours.
- Typical presumptive colonies of Enterobacteriaceae should be sub cultured in a non-selective medium, and confirmed by tests for glucose fermentation and the presence of oxidase.

Quality control:

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Beige reddish	Purple-red	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 (10³-10⁴ CFU) / Selectivity (10⁴-10⁶ CFU).

Reference media: TSA.

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

Storage:

Temperature: 2°C - 25°C

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