Violet Red Bile Agar (Crystal Violet Neutral Red Bile Lactose Agar)

Intended Use
Violet Red Bile Agar (Crystal Violet Neutral Red Bile Lactose Agar) is a selective medium used for the detection and enumeration of coliforms.

Summary
Violet Red Bile Agar, a modification of MacConkey's original formulation is used for the enumeration of coli-aerogenes bacterial group. It relies on the use of the selective inhibitory components crystal violet and bile salts and the indicator system lactose, and neutral red. Thus, the growth of many unwanted organisms is suppressed, while tentative identification of sought bacteria can be made. Organisms, which rapidly attack lactose, produce purple colonies surrounded by purple halos. Non-fermenters or late lactose-fermenters produce pale colonies with greenish zones. VRBA is recommended by APHA.

Principle
Peptic digest of animal tissue and yeast extract serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Lactose is the fermentable carbohydrate, utilization of which leads to the production of acids. Neutral red indicator detects the acidity so formed. Crystal violet and bile salts mixture help to inhibit the accompanying Gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium.

Formula*

Ingredients	**g/L**
Lactose	10.0
Peptic Digest of Animal Tissue	7.0
Sodium Chloride	5.0
Yeast Extract	3.0
Bile Salts Mixture	1.5
Neutral Red	0.03
Crystal Violet	0.002
Agar	15.0
Final pH (at 25°C)	7.4 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Clinical samples; Food and Dairy samples; Water samples.

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 41.53 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely. DO NOT AUTOCLAVE.
4. Cool to 45°C and pour into sterile petridishes containing the inoculum.

Quality Control

Dehydrated Appearance: Light yellow to pinkish beige coloured, homogenous, free flowing powder.
Prepared Appearance: Light reddish purple to reddish purple coloured, clear to slightly opalescent gel forms in petridishes.
Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after an incubation at 30°C-35°C for 18 to 24 hours.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism at 30°C-35°C for 18 hours.
Indicative Properties: The test results observed are within the specified temperature and time, inoculating ≤100 cfu of appropriate microorganism.
Inhibitory Properties: No growth of the test microorganism occurs for the specified temperature ant not less than the longest period of the time specified, inoculating > 100 cfu of the appropriate microorganism at 30°C-35°C for ≥ 24 hours.

Organism (ATCC)	**Growth**	**Colour of Colony**
Escherichia coli (25922)	Good	Pinkish red with bile precipitate
Escherichia coli (8739)	Good	Pinkish red with bile precipitate
Klebsiella aerogenes (13048)	Good	Pink
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (14028)	Good	Colourless
Inhibitory		
Staphylococcus aureus subsp. *aureus* (6538)	Inhibited	-

Interpretation of Results

1. Lactose fermenters including coliforms form purple red colonies, with or without a zone of precipitate around the colonies. (Generally surrounded by a reddish zone of precipitated bile)
2. Non-lactose fermenters form colourless to transparent colonies.
3. Gram-positive cocci, if present, form colourless, pinpoint colonies.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. Boiling the medium for more than 2 minutes may decrease the ability to support growth. Do not reheat the medium after the addition of Urea 40% as urea decomposes very easily.
2. Do not incubate inoculated plates for more than 24 hours because microorganisms that are only slightly inhibited may grow after extended incubation.
3. Prepare and use the medium within 24 hours for optimum performance. Variations in the size of the inoculum can affect the time required to reach positive results.
4. This medium may not be completely inhibitory to Gram-positive organisms. Enterococci may grow as pinpoint colonies. Perform Gram-stain and biochemical tests to identify isolates.
5. Gram-negative bacilli other than *Enterobacteriaceae* may also grow. Perform biochemical tests to identify isolates to genus and species.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Eaton A. D., Clesceri L. S. and Greenberg A. E., (Ed.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., American Public Health Association, Washington, D.C.
2. Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
3. MacConkey A., 1905, J. Hyg., 5, 333-379.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product Description	Pack Size
201220010100	Dehydrated Culture Media	100 g
201220010500	Dehydrated Culture Media	500 g