



Violet Red Bile Broth

M458

Violet Red Bile Broth is used for the detection and enumeration of coliform organisms from water and food.

Composition**

| Ingredients | Gms / Litre |
|--------------------------------|-------------|
| Peptic digest of animal tissue | 7.000 |
| Yeast extract | 3.000 |
| Bile salts mixture | 1.500 |
| Lactose | 10.000 |
| Sodium chloride | 5.000 |
| Neutral red | 0.030 |
| Crystal violet | 0.002 |
| Final pH (at 25°C) | 7.4±0.2 |

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.53 grams in 1000 ml distilled water. Heat with stirring to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45°C and dispense into sterile tubes containing the inoculum.

Principle And Interpretation

Violet Red Bile Broth, a modification of MacConkeys original formulation (1) is used for the enumeration of *coli-aerogens* bacterial group. It relies on the use of the selective inhibitory components crystals 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. Lactose non-fermenters and late lactose fermenters produce pale coloured medium. Other related gram-negative bacteria can be suppressed by incubation at >42°C or by anaerobic incubation. Incubation may be carried out at >42°C for 18 hours, 32°C for 24-48 hours or 4°C for 10 days depending on the temperature characteristics of the organisms to be recovered (2). Violet red bile Broth is similar to VRBA (M049), except agar that is recommended by APHA (3, 4).

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. Further biochemical tests are necessary for positive identification (5).

Quality Control

Appearance

Light yellow to pinkish beige homogeneous free flowing powder

Colour and Clarity of prepared medium

Reddish purple coloured clear solution in tubes.

Reaction

Reaction of 2.65% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

M458: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

| Organism | Inoculum (CFU) | Growth | Colour of medium |
|------------------------------------|----------------|-----------|---------------------|
| <i>Escherichia coli</i> ATCC 25922 | 50-100 | luxuriant | pink to pinkish red |

| | | | |
|---|-------------|-----------|----------------------------------|
| <i>Enterobacter aerogenes</i> ATCC 13048 | 50-100 | luxuriant | pink to pinkish red |
| <i>Salmonella Enteritidis</i> ATCC 50-100 13076 | | luxuriant | colourless to orangish yellow |
| <i>Staphylococcus aureus</i> ATCC 25923 | $\geq 10^3$ | inhibited | |

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. MacConkey A., 1905, J. Hyg., 5, 333-379.
2. Mossel D. A. A. and Vega C. L., 1973, Hlth. Lab. Sci., 11:303
3. 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.
4. Marshall R. T., (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th Ed., APHA, Washington, D. C.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, 6. Williams and Wilkins, Baltimore.

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