

## Violet Red Bile Agar, Granulated

**GM049**

Violet Red Bile Agar, granulated is selective medium used for the isolation, detection and enumeration of coli-aerogenes bacteria in water, milk, other dairy food products and also from clinical samples.

### Composition\*\*

<b>Ingredients</b>	<b>Gms / Litre</b>
Peptic digest of animal tissue	7.000
Yeast extract	3.000
Sodium chloride	5.000
Bile salts mixture	1.500
Lactose	10.000
Neutral red	0.030
Crystal violet	0.002
Agar	15.000
Final pH ( at 25°C)	7.4±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 41.53 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and pour into sterile Petri plates containing the inoculum.

If desired, the medium can be sterilized by autoclaving at 15 lbs pressure at 15lbs pressure (121°C) for 15 minutes.

### Principle And Interpretation

The coliform group consists of several genera of bacteria belonging to the family *Enterobacteriaceae*. The historical definition of this group has been based on the method used for detection i.e. lactose fermentation. This group is defined as all aerobic and facultative anaerobic, gram-negative, non-spore-forming rod shaped bacteria that ferment lactose with gas and acid formation within 48 hour at 35°C (1, 2). Examination of foods, ingredients and raw materials, for the presence of marker groups such as coliforms is the one of the common tests.

Violet Red Bile Agar, a modification of MacConkeys original formulation (3) is used for the enumeration of coli-aerogenes 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. Organisms, which rapidly attack lactose, produce purple colonies surrounded by purple halos. Non-fermenters or late lactose-fermenters produce pale colonies with greenish zones (4). VRBA is recommended by APHA (2,5).

Selectivity of VRBA can be increased by incubation under anaerobic conditions and/ or at elevated temperature, i.e. equal to or above 42°C (6-8).

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. Violet Red Bile Agar is not completely specific for enteric; other accompanying bacteria may give the same reaction. Further biochemical tests are necessary for positive identification (7).

### Quality Control

#### Appearance

Light yellow to pink coloured granular medium

#### Gelling

Firm, comparable with 1.5% Agar gel.

#### Colour and Clarity of prepared medium

Reddish purple coloured clear to slightly opalescent gel forms in Petri plates.

**Reaction**

Reaction of 4.15% w/v aqueous solution at 25°C. pH : 7.4±0.2

**pH**

7.20-7.60

**Cultural Response**

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<b>Cultural Response</b>				
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	≥50%	pink to pinkish red
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	≥50%	pinkish red with bile precipitate
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	luxuriant	≥50%	Colourless to orangish yellow
<i>Staphylococcus aureus</i> ATCC 25923	≥10 <sup>3</sup>	inhibited	0%	

**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**

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3. MacConkey A., 1905, J. Hyg., 5, 333-379
4. Corry J. E. L., Curtis G. D. W. and Baird R. M., (Ed.), 1995, Culture Media for Food Microbiology, Vol. 34, Progress in Industrial Microbiology, Elsevier, Amsterdam.
5. Marshall R. T., (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th Ed., APHA, Washington, D. C.
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7. Mossel D. A. A., Eelderink I., Koopmans M. and Van Rossem F., 1979, Food Protect., 42 : 470
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Revision : 00 / 2014

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