



Violet Red Bile Agar

M049S

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

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	7.000
Yeast Extract	3.000
Lactose	10.000
Bile salts mixture	1.500
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.002
Agar	15.000
Final pH (at 25°C)	7.4±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 41.53 grams in 1000 ml distilled water. Heat with stirring to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45°C and pour into sterile Petri plates. Overheating is detrimental to the medium.

Principle And Interpretation

Violet Red Bile Agar is recommended by APHA for the detection and enumeration of coliform organisms in water, milk, dairy and other food products (1,2). It is also recommended by BIS as a presumptive test solid medium for detection and estimation of coliform bacteria in food stuff (3). Druce et al (4) found this medium equally good as the indicator of coli-aerogenes in milk as MacConkey Broth. Recently, the agar formulation is recommended by ISO committee for the enumeration of coliforms (5).

The medium is selective due to the presence of the inhibitors - bile salts and crystal violet. Crystal violet inhibits gram-positive microorganisms especially Staphylococci. Organisms which rapidly ferment lactose produce red colonies surrounded by red-purple halo (6). Lactose non-fermenters and late lactose fermenters produce pale colonies. Other related gram-negative bacteria can be suppressed by incubation at > 42°C or by anaerobic incubation. An overlay method is helpful to improve the specificity of the medium. 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 (7).

Quality Control

Appearance

Light yellow to pink coloured homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 4.15% w/v aqueous solution at 25°C. pH : 7.4±0.1

pH

7.30-7.50

Cultural Response

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
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Cultural Response

<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	>=50%	pink
<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
<i>Staphylococcus aureus</i> ATCC 25923	>=10 ³	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

1. Speck M. (Ed.), 1984, Compendium of Methods For The Microbiological Examination of Foods, 2nd ed. APHA, Washington, D.C.
2. Richardson G. (Ed.), 1985, Standard Methods for the Microbiological Examination of Dairy Products, 15th ed., APHA, Washington, D.C.
3. Bureau of Indian Standards, IS: 5401, 1969 (Second reprint - June 1990). ,
4. Druce R.G. et al, 1957, J. Appl. Bact., 20: 1.
5. International Organization for Standardization (ISO), 1991, Draft ISO/DIS 4832.
6. Davis J.G., 1951, Milk Testing, Dairy Industries Limited, London; pg 131
7. Mossel D.A.A. and Vega C.L., 1973, Hlth. Lab. Sci., 11:303.

Revision : 02 / 2015

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