



PRODUCT SPECIFICATION SHEET

Violet Red Bile Agar (1.2%) (DM285)

Intended Use

Violet Red Bile Agar (1.2%) (DM285) is recommended for selective isolation, detection and enumeration of *coli-aerogenes* in water, milk and other dairy products.

Product Summary and Explanation

The coliform group of bacteria includes aerobic and facultatively anaerobic gram-negative non-sporeforming bacilli that ferment lactose and form acid and gas at 35°C within 48 hours. *Enterobacteriaceae* members comprise the majority of the group but other lactose fermenting organisms may also be included. Procedures to detect, enumerate and presumptively identify coliforms are used in testing foods and dairy products.⁽¹⁻³⁾ One method for performing the presumptive test for coliforms uses Violet Red Bile Agar. Violet Red Bile Agar, a modification of MacConkeys original formulation⁽⁴⁾ is used for the enumeration of *coli-aerogenes* bacterial group. The selective inhibitory components crystals violet and bile salts and the indicator system lactose, and neutral red constitutes to reliable elements of this medium. Thus, the growth of many unwanted organisms is suppressed, while tentative identification of sought bacteria can be made. VRBA is recommended by APHA.^(5, 6) Violet Red Bile Agar (1.2 % Agar) (DM285) is prepared, in accordance with the ISO Committee.⁽⁷⁾ Selectivity of VRBA can be increased by incubation under anaerobic conditions and/ or at elevated temperature, i.e. equal to or above 42°C.⁽⁸⁻¹⁰⁾

Principles of the Procedure

Violet Red Bile Agar (1.2%) contains peptic digest of animal tissue and yeast extract which acts as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Lactose is the fermentable carbohydrate, utilization of which leads to acid production. Neutral red is a pH indicator which 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 / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	7.00
Yeast extract	3.00
Lactose	10.00
Bile salts mixture	1.50
Sodium chloride	5.00
Neutral red	0.03
Crystal violet	0.002
Agar	12.00
Final pH: 7.4 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.





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Directions

1. Suspend 38.53 grams of medium in one liter of distilled water.
2. Heat with stirring to boiling, to dissolve the medium completely.
3. DO NOT AUTOCLAVE. Cool to 45 - 50°C.
4. Mix well and pour into sterile Petri plates containing sample.

Quality Control Specifications

Dehydrated Appearance	Light yellow to pink homogeneous free flowing powder
Prepared Medium	Reddish purple coloured clear to slightly opalescent gel forms in Petri plates.
Reaction of 3.85% Solution	pH : 7.4 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.2% Agar gel.

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

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Colour of colony
1.	<i>Enterobacter aerogenes ATCC 13048</i>	50 - 100	good- luxuriant	≥50%	pink to pinkish red
2.	<i>Escherichia coli ATCC 25922</i>	50 - 100	good- luxuriant	≥50%	pinkish red with bile
3.	<i>Salmonella Enteritidis ATCC 13076</i>	50 - 100	good- luxuriant	≥50%	Colourless to orangish yellow
4.	<i>Staphylococcus aureus ATCC 25923</i>	≥10 ³	inhibited	0%	--

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for standard test procedures.

Results

1. Lactose fermenters produce pink to pink-red colonies, with or without a zone of precipitate around the colonies.
2. Lactose nonfermenters produce colorless to orangish yellow colonies.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Violet Red Bile Agar is not completely specific for enterics; other accompanying bacteria may give the same reaction.





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2. The medium will grow gram-negative bacilli other than members of the *Enterobacteriaceae*. Perform biochemical tests to identify isolates to genus and species.
3. Boiling the medium for longer than 2 minutes can decrease the ability to support growth.
4. Plates of Violet Red Bile Agar should not be incubated longer than 24 hours because microorganisms that are only partially inhibited may grow after extended incubation.
5. For optimum performance, prepare and use the medium within 24 hours.

Packaging

Product Name : Violet Red Bile Agar 1.2%

Product Code : DM285

Available Pack sizes : 500gm

References

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4. MacConkey A., 1905, *J. Hyg.*, 5, 333-379
5. 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.
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9. Mossel D. A. A., Eclerink I., Koopmans M. and Van Rossem F., 1979, *Food Protect.*, 42 : 470
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Further Information

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