



PRODUCT SPECIFICATION SHEET

Violet Red Bile Agar (DM286)

Intended Use

Violet Red Bile Agar (DM286) is used for the enumeration of coliforms in food and dairy product conforms to American Public Health Association (APHA).

Product Summary and Explanation

The coliform group of bacteria includes aerobic and facultative anaerobic, Gram-negative, non-spore forming bacilli. Coliforms ferment lactose and form acid and gas at 35°C within 48 hours. Members of *Enterobacteriaceae* 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⁾

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⁽⁶⁾ 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. Coliform colonies lower the pH of the medium, subsequently causing their colonies to look red to purple and to precipitate the bile salts. Non-fermenters or late lactose-fermenters produce pale colonies with greenish zones.⁽⁴⁾ VRBA is recommended by APHA.^(5,8)

Selectivity of VRBA can be increased by incubation under anaerobic conditions and/ or at elevated temperature, i.e. equal to or above 42°C.⁽⁷⁻¹⁰⁾

Principles of the Procedure

Enzymatic digest of gelatin is the source of carbon, nitrogen, vitamins, and minerals in Violet Red Bile Agar. Yeast Extract supplies B-complex vitamins that stimulate bacterial growth. Bile Salts Mixture and Crystal Violet inhibit most Gram-positive bacteria. Lactose is a carbohydrate source. Sodium chloride maintains the osmotic equilibrium. Neutral Red is a pH indicator. Agar is the solidifying agent.

Formula / Liter

| Ingredients | Gms / Litre |
|--|-------------|
| Yeast Extract | 3.00 |
| Peptic digest of animal tissue | 7.00 |
| Bile Salts Mixture | 1.50 |
| Lactose | 10.00 |
| Sodium Chloride | 5.00 |
| Neutral Red | 0.03 |
| Crystal Violet | 0.002 |
| Agar | 15.00 |
| Final pH: 7.4 ± 0.2 at 25°C | |
| Formula may be adjusted and/or supplemented as required to meet performance specifications | |





PRODUCT SPECIFICATION SHEET

Precautions

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

Directions

1. Suspend 41.53 grams of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for two minutes. DO NOT AUTOCLAVE.
3. Cool the medium to 45-46°C and dispense 15-20mL into 100mm petri dishes containing inoculum.
4. After solidification of the inoculated medium, evenly add a cover layer of 4 mL of the cooled (45 - 46°C) agar medium.

Quality Control Specifications

| | |
|-----------------------------------|---|
| Dehydrated Appearance | Light Yellow to pink colored, homogeneous, free flowing powder |
| Solution | 4.15% Solution in Distilled or deionized water is soluble on boiling, red-purple colored, and very slightly to slightly opalescent. |
| Prepared Medium | Reddish purple colored slightly opalescent gel. |
| Reaction of 4.15% Solution | pH 7.4 ± 0.2 at 25°C |
| Gel Strength | Firm, compared to 1.5% Agar Gel. |

Expected Cultural Response: Cultural response on Violet Red Bile Agar at 35-37°C after 18 - 24 hours incubation.

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

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





PRODUCT SPECIFICATION SHEET



Escherichia coli ATCC 25922

Test Procedure

Presumptive test for coliforms using solid medium:

1. Transfer a 1 mL aliquot of test sample to a petri dish.
2. Add 10 mL of Violet Red Bile Agar (at 48°C) and swirl to mix.
3. Allow medium to solidify before incubating at 35°C for 18 - 24 hours; use 32°C for dairy products.
4. Examine for purple-red colonies, 0.5 mm in diameter (or larger), surrounded by a zone of precipitate bile acids.
5. Continue with confirmatory testing of typical organisms colonies.⁽¹⁻³⁾

Results

Lactose fermenters are purple-red, with or without a zone of precipitate around the colonies. Lactose non-fermenters are colorless to transparent colonies. Gram-positive cocci are colorless, pinpoint 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. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.





PRODUCT SPECIFICATION SHEET

2. VRBA may not be completely inhibitory to Gram-positive organisms, and will grow Gram-negative bacilli other than members of *Enterobacteriaceae*. Perform a Gram stain and biochemical tests to identify isolates.⁽⁷⁾
3. Boiling the medium for longer than 2 minutes can decrease the ability to support growth.

Packaging

Product Name : Violet Red Bile Agar

Product Code : DM286

Available Pack sizes : 100gm / 500gm

References

1. Marshall, R. T. (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. Vanderzant, C., and D. F. Splittstoesser (eds.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed., AOAC International, Gaithersburg, MD.
4. 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.
5. MacConkey A., 1905, J. Hyg., 5, 333-379
6. Marshall R. T., (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th Ed., APHA, Washington, D. C.
7. International Organization for Standardization (ISO), 1991, Draft ISO/DIS 4382
8. Mossel D. A. A. and Vega C. L., 1973, Hlth. Lab. Sci., 11:303
9. Mossel D. A. A., Eclerink I., Koopmans M. and Van Rossem F., 1979, Food Protect., 42 : 470

Further Information

For further information please contact your local MICROMASTER Representative.



MICROMASTER LABORATORIES PRIVATE LIMITED

DM286PSS,QAD/FR/024,Rev.00/01.01.2018

Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall' , Thane (W) - 400607. M.S. INDIA.
Ph: +91-22-25895505, 4760, 4681. Cell: 9320126789.

Email: micromaster@micromasterlab.com
sales@micromasterlab.com

| Prepared By | Checked By | Approved By |
|---|---|---|
|  |  |  |
| Microbiologist | Head Quality Control | Head Quality Assurance |





PRODUCT SPECIFICATION SHEET

Disclaimer :

All Products conform exclusively to the information contained in this and other related Micromaster Publications. Users must ensure that the product(s) is appropriate for their application, prior to use. The information published in this publication is based on research and development work carried out in our laboratory and is to the best of our knowledge true and accurate. Micromaster Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are intended for laboratory, diagnostic, research or further manufacturing use only and not for human or animal or therapeutic use, unless otherwise specified. Statements included herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

