microgen
Ready Prepared Media

Technical Information

Violet Red Bile Agar Plate

Product Code: PM 1049

Application: Recommended for selective isolation, detection and enumeration of coli-aerogenes bacteria in water, milk and other dairy, food products and clinical samples .

Composition**

Ingredients	Gms / Litre
Peptone	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

Principle & 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 aerobicand 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 markergroups such as coliforms is the one of the common tests.

Violet Red Bile Agar, a modification of MacConkey's original formulation (1) 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 (3). VRBA is recommended by APHA (4,5). 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). It is also recommended by ISO (9).

Peptone 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 (5).

Type of specimen

Clinical samples - Stool; Food and dairy samples; Water samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4,5).For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (10). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (11,12).After use, contaminated materials must be sterilized by autoclaving before discarding.

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Warning and Precautions

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. It is recommended to store the plates at 24-30°C to avoid minimum condensation.
4. Though the medium is recommended for selective isolation, further biochemical and serological testing must be carried out for further confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Methodology

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Quality Control

Appearance
Sterile Violet Red Bile Agar in 90 mm disposable plate with smooth surface and absence of black particles/cracks/bubbles
Colour of medium
Reddish purple coloured medium.
Quantity of medium
25 ml of medium in 90 mm plates
pH
7.20-7.60
Sterility Test
Passes release criteria
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours

Oragnism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	good-luxuriant	>=50 %	Pink to red
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=50 %	Pinkish red with bile ppt
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	>=10^3	Inhibited	0%	----
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	>=50 %	Colourless to orangish yellow

Key : (*) Corresponding WDCM numbers.

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Storage and Shelf Life

On receipt store between 20-30°C. Use before expiry date on the label. Product performance is best if used within stated expiry period

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Further Reading

1. MacConkey A., 1905, J. Hyg., 5, 333-3
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3. 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
4. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed.,Washington D.C.
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Mossel D. A. A. and Vega C. L., 1973, Hlth. Lab. Sci., 11:3
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10. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24thed. Washington DC:APHA Press; 2023.
11. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Editio
12. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter,(2015) S.S and Warnock., D.W. Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate
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