

Technical Specification Sheet



Violet Red Bile Glucose Agar (VRBGA) (NCM0022)

Intended Use

Violet Red Bile Glucose Agar is used for the enumeration of *Enterobacteriaceae* in foods. Violet Red Bile Glucose Agar conforms to Harmonized USP/EP/JP requirements and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

A medium recommended by the Harmonized USP/EP/JP for isolation and identification of bile-tolerant Gram-negative bacteria from non-sterile products. Conforms to Harmonized USP/EP/JP performance specification. Yeast extract provides vitamins and gelatin serves as source of carbon and nitrogen. Glucose is a fermentable carbohydrate and sodium chloride maintains the osmotic balance. Bile salts and crystal violet act as selective agents inhibiting many Gram-positive bacteria. The formulation is a modification of Violet Red Bile Agar by Mossel which substitutes lactose for glucose. *Enterobacteriaceae*, such as *Escherichia coli* and *Salmonella* spp., are able to ferment glucose. This produces acid which results in a pH drop indicated by neutral red resulting in pink colonies. Enough acid production will cause the precipitation of bile salts resulting in a bile precipitate or halo around glucose fermenting bacteria. Non-glucose fermenting bile tolerant bacteria such as *Pseudomonas aeruginosa* grow but remain colorless with no bile precipitate. According to the Harmonized USP/EP/JP, Enterobacteria Enrichment Broth-Mossel is used as a selective enrichment broth, with subculture performed onto Violet Red Bile Glucose Agar (VRBGA).

Typical Formulation

| | |
|------------------------------|-----------|
| Yeast Extract | 3.0 g/L |
| Pancreatic Digest of Gelatin | 7.0 g/L |
| Bile Salts | 1.5 g/L |
| Sodium chloride | 5.0 g/L |
| Glucose Monohydrate | 10.0 g/L |
| Agar | 15.0 g/L |
| Neutral Red | 0.03 g/L |
| Crystal Violet | 0.002 g/L |

pH: 7.4 ± 0.2 at 25°C.

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 41.5 grams of the medium in 1 liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE. Cool to 45-50°C.

Test Procedures

Inoculate directly on Violet Red Bile Glucose Agar by the streak method from selective or nonselective medium or from the sample itself.



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Quality Control Specifications

Powder: Fine, free-flowing, homogeneous, light beige to reddish purple.

Finished medium: Reddish purple, clear.

Expected Cultural Response and USP/EP/JP Growth Promotion Testing: Cultural response on Violet Red Bile Glucose Agar incubated at Harmonized USP/EP/JP specified temperatures and incubation times.

| Microorganism | Approx. inoculum (CFU) | Expected Results | |
|---|------------------------|------------------|----------------------------------|
| | | Growth | Reaction |
| <i>Escherichia coli</i> ATCC® 8739 | 10-100 | Growth | Pink colonies w/ red precipitate |
| <i>Escherichia coli</i> ATCC® 25922 | 10-100 | Growth | Pink colonies w/ red precipitate |
| <i>Pseudomonas aeruginosa</i> ATCC® 9027 | 10-100 | Growth | Colorless to grey |
| <i>Salmonella typhimurium</i> ATCC® 14028 | 10-100 | Growth | Pink colonies w/ red precipitate |
| <i>Staphylococcus aureus</i> ATCC® 6538 | >10 ³ | Inhibited | N/A |
| <i>Enterococcus faecalis</i> ATCC® 29212 | >10 ³ | Inhibited | N/A |

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

Results

Enterobacteriaceae ferment dextrose, produce acid products, and form pink to reddish colonies with reddish precipitate.

Expiration

Refer to 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 Procedures

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. Mossel, D.A.A. Media for Enterobacteriaceae (1985) International Journal of Food Microbiology, 2 (1-2), pp. 27-32.
2. European Pharmacopoeia 10th Edition (2020)
3. United States Pharmacopeia National Formulary 2018: USP 41 NF 36
4. Japanese Pharmacopeia 17th Edition (2017)