Violet Red Bile Glucose Agar (Harmonized)

Intended Use
Violet Red Bile Glucose Agar (Harmonized) is recommended for isolation and cultivation of *Enterobacteriaceae* from pharmaceutical products in accordance with microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

Summary
Mossel *et al.,* added glucose and excluded lactose from the media observing improved detection of coliforms. Incubation can be carried out at different temperature and incubation time depending upon the group of *Enterobacteriaceae* to be recovered.

Principle
Pancreatic digest of gelatin and yeast extract provide nitrogenous compounds, vitamin B complex and other nutrients essential for the bacterial metabolism while Glucose Monohydrate and neutral red helps to detect glucose fermentation. Bile salts and crystal violet select the growth of Gram-negative intestinal bacteria inhibiting Gram-positive bacteria & unrelated flora. Sodium chloride maintains osmotic equilibrium.

Formula*

Ingredients	**g/L**
Pancreatic Digest of Gelatin	7.0
Yeast Extract	3.0
Bile Salts	1.5
Sodium Chloride	5.0
Glucose monohydrate	10.0
Crystal Violet	0.002
Neutral Red	0.03
Agar	15.0
Final pH (at 25°C)	7.4 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 40.62 g of (the equivalent weight of dehydrated medium per litre) powder in 1000 mL purified water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. DO NOT AUTOCLAVE.

Quality Control
Dehydrated Appearance: Light yellow to pinkish beige coloured, homogeneous, free flowing powder.
Prepared Appearance: Light pinkish purple coloured, clear to slightly opalescent gel forms in petridishes.
Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP/BP and growth is observed after an incubation at 30°C-35°C for 18 to 24 hours.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism at 30°C-35°C for 18 hours.
Indicative Properties: The test results observed are within the specified temperature and time, inoculating ≤100 cfu of appropriate microorganism.

Inhibitory Properties: No growth of the test microorganism occurs for the specified temperature and not less than the longest period of the time specified, inoculating > 100 cfu of the appropriate microorganism at 30°C-35°C for ≥ 24 hours.

Organism (ATCC)	Growth	Colour of Colony
Escherichia coli (8739)	Good	Pinkish red with bile precipitate
Pseudomonas aeruginosa (9027)	Good	Pink
Inhibitory		
Staphylococcus aureus subsp. *aureus* (6538)	Inhibited	-

Note:
For good growth – Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum.
For inhibition no growth of test microorganism should occur.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. The United States Pharmacopoeia, 2023, The United States Pharmacopoeial Convention. Rockville, MD.
2. British Pharmacopoeia, 2023, The Stationery Office British Pharmacopoeia.
3. Indian Pharmacopoeia, 2018, Ministry of Health and Family Welfare, Government of India.
4. European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
5. Japanese Pharmacopoeia, 2008.
6. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
201220040500	Dehydrated Culture Media	500 g
203220090100	Bottle Media	100 mL
205220140100	Ready Prepared Plates (90 mm)	100 Plates