



Medium 5. Violet Red Bile Glucose Agar

MM581

Violet Red Bile Glucose Agar is recommended for detection and enumeration of bile tolerant gram negative bacteria from pharmaceutical products in accordance Indian pharmacopoeia 2010.

Composition**

Ingredients	Gms / Litre
Yeast extract	3.000
Pancreatic digest of gelatin	7.000
Bile salts	1.500
Sodium chloride	5.000
Dextrose monohydrate	10.000
Neutral red	0.030
Crystal violet	0.002
Agar	15.000
pH after heating (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.62 grams of dehydrated medium in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Violet Red Bile Glucose Agar is a selective medium recommended for detection and enumeration of *Enterobacteriaceae* especially the bile tolerant gram negative bacteria in accordance with the microbial limit testing of Indian pharmacopoeia (1) from non-sterile products and pharmaceutical preparations. This medium is also recommended by USP/EP/BP/JP/IP (1,2,3,4,5) under the harmonized methodology of microbial limit test.

Pancreatic digest of gelatin and yeast extract provide nitrogenous compounds and other nutrients essential for bacterial metabolism. This media is selective due to presence of the inhibitors; bile salts and crystal violet. Crystal violet inhibits gram-positive organisms especially Staphylococci. Neutral red indicator helps to detect dextrose fermentation. Glucose fermenting strains produce red colonies with pink-red halos in the presence of neutral red. Sodium chloride maintains the osmotic equilibrium in the medium. The red colour is due to absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8.

Quality Control

Appearance

Light yellow to pinkish beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Reddish purple coloured clear to slightly opalescent gel forms in Petri plates.

pH

7.20-7.60

Growth Promotion Test

Growth Promotion is carried out in accordance with the harmonized method of IP. Cultural response was observed after an incubation at 30-35°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Growth promoting properties

Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating ≤ 100 cfu (at 30-35°C for ≤ 18 hours).

Indicative properties

Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating ≤ 100 cfu (at 30-35°C for 18-24 hours).

Cultural Response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature
Growth Promoting + Indicative						
<i>Escherichia coli</i> ATCC 8739	50 -100	luxuriant	25 -100	≥ 50 %	pink-red with bile precipitate	18 -24 hrs
<i>Pseudomonas aeruginosa</i> ATCC 9027	50 -100	luxuriant	25 -100	≥ 50 %	pink to purple	18 -24 hrs
Additional Microbiological Testing						
<i>Escherichia coli</i> NCTC 9002	50 -100	good-luxuriant	25 -100	≥ 50 %	pink-red with bile precipitate	18 -24 hrs
<i>Escherichia coli</i> ATCC 25922	50 -100	good-luxuriant	25 -100	≥ 50 %	pink-red with bile precipitate	18 -24 hrs
<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	good-luxuriant	25 -100	≥ 50 %	light pink	18 -24 hrs
<i>Enterobacter aerogenes</i> ATCC 13048	50 -100	good-luxuriant	25 -100	≥ 50 %	pink-red	18 -24 hrs
<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	inhibited	0	0%		≥ 48 hrs
<i>Staphylococcus aureus</i> ATCC 6538	$\geq 10^3$	inhibited	0	0%		≥ 48 hrs

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Indian Pharmacopoeia 2010, Ministry of Health and Family welfare, Government of India, New Delhi.
2. The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
3. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia
4. European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
5. Japanese Pharmacopoeia, 2008.

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