Violet Red Bile Glucose Agar, Granulated

GMH581

Violet Red Bile Glucose Agar, granulated is recommended for detection and enumeration of *Enterobacteriaceae* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.62 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. DO NOT HEAT IN AN AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates or as desired.

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 by harmonized methodology of USP/EP/BP/JP/IP (1,2,3,4,5) from non-sterile products and pharmaceutical preparations.

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 glucose 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 coloured granular medium

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 USP/EP/BP/JP. 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	Recovery	Colour of colony	Incubation temperature
Growth Promoting + Indicative					
Escherichia coli ATCC 8739	50 -100	luxuriant	>=50 %	pink-red with bile precipitate	18 -24 hrs
Pseudomonas aeruginosa ATCC 9027	50 -100	luxuriant	>=50 %	pink to red	18 -24 hrs
Additional Microbiological Testing					
Escherichia coli NCTC 9002	50 -100	good-luxuriant	>=50 %	pink-red with bile precipitate	18 -24 hrs
Escherichia coli ATCC 25922	50 -100	good-luxuriant	>=50 %	pink-red with bile precipitate	18 -24 hrs
Salmonella Enteritidis *ATCC 13076*	50 -100	good-luxuriant	>=50 %	light pink	18 -24 hrs
Enterobacter aerogenes ATCC 13048	50 -100	good-luxuriant	>=50 %	pink-red	18 -24 hrs
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%		>=24 hrs
Staphylococcus aureus ATCC 6538	>=10^3	inhibited	0%		>=24 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.The United States Pharmacopeia, 2014, The United States Pharmacopoeial Convention,Rockville, MD.
2.British Pharmacopoeia, 2014, The Stationery Office British Pharmacopoeia.
3.European Pharmacopoeia, 2014, European Department for the Quality of Medicines of Council of Europe.
4.Japanese Pharmacopoeia, 2008, Published by Society of Japanese Pharmacopoeia,Tokyo, Japan.
5.Indian Pharmocoepoeia, 2014 Ministry of Health and Family Welfare, Govt. of India.

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