



## Violet Red Bile Glucose Agar

M581BP

Violet Red Bile Glucose Agar is recommended for detection and enumeration of *Enterobacteriaceae* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of BP

### 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
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 from non-sterile products and pharmaceutical preparations. The medium is prepared as described in British Pharmacopoeia (2) and is in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP (1,2,3,4,5)

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 lactose and 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.

The sample is initially enriched in Enterobacteria Enrichment broth -Mossel (M287B) and then subcultured on Violet Red Bile Glucose Agar (M581BP).

### 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 BP. 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  $\leq 100$  cfu (at 30-35°C for  $\leq 18$  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  $\leq 100$  cfu (at 30-35°C for 18-24 hours).

**Cultural Response**

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature	Incubation period
<b>Growth Promoting + Indicative</b>						
<i>Escherichia coli</i> ATCC 8739	50 -100	25 -100	$\geq 50$ %	pink-red with bile precipitate	30 -35 °C	18 -24 hrs
<i>Pseudomonas aeruginosa</i> ATCC 9027	50 -100	25 -100	$\geq 50$ %	pink to purple	30 -35 °C	18 -24 hrs
<b>Additional Microbiological Testing</b>						
<i>Escherichia coli</i> NCTC 9002	50 -100	25 -100	$\geq 50$ %	pink-red with bile precipitate	30 -35 °C	18 -24 hrs
<i>Escherichia coli</i> ATCC 25922	50 -100	25 -100	$\geq 50$ %	pink-red with bile precipitate	30 -35 °C	18 -24 hrs
<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	25 -100	$\geq 50$ %	light pink	30 -35 °C	18 -24 hrs
<i>Enterobacter aerogenes</i> ATCC 13048	50 -100	25 -100	$\geq 50$ %	pink-red	30 -35 °C	18 -24 hrs
<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	0	0%		30 -35 °C	$\geq 24$ hrs
<i>Staphylococcus aureus</i> ATCC 6538	$\geq 10^3$	0	0%		30 -35 °C	$\geq 24$ 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 Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
2. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia
3. European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
4. Japanese Pharmacopoeia, 2008.
5. Indian Pharmacopoeia, 2010, Govt. of India, the controller of Publication, Delhi, India.

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