



## Violet Red Bile Agar with Glucose and Lactose

MU1684

### Intended Use:

For selective isolation and enumeration of bile tolerant Gram negative bacteria in accordance with USP.

### Composition\*\*

Ingredients	g / L
Gelatin peptone	7.000
Yeast extract	3.000
Lactose	10.000
Bile salts	1.500
D-Glucose monohydrate	10.000
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.002
Agar	15.000
pH after sterilization ( at 25°C)	7.4±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 50.62 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 AUTOCLAVE**. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

This medium is a selective medium, recommended for detection and enumeration of gram negative bile-tolerant bacteria in accordance with United States Pharmacopoeia, 2008 from food and dietary supplement preparations (1).

Gelatin peptone 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 monohydrate fermentation. Lactose and glucose monohydrate fermenting strains grow as red or pink and may be surrounded by a zone of acid precipitated bile. 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.

### Type of specimen

Pharmaceutical samples

### Specimen Collection and Handling:

For pharmaceutical samples, follow appropriate techniques for handling specimens as per established guidelines (1,2). After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

### Limitations :

1. Further biochemical tests must be carried out for confirmation.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

### Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

### 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 USP. 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.

**Cultural Response**

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature	Incubation period
<b>Test for <i>Enterobacteriaceae</i></b>						
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	25 -100	≥50 %	pink-red with bile precipitate	30 -35 °C	18 -24 hrs
^ <i>Pseudomonas paraeruginosa</i> ATCC 9027 (00026*)	50 -100	25 -100	≥50 %	pink to purple	30 -35 °C	18 -24 hrs
<b>Additional Microbiological Testing</b>						
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	25 -100	≥50 %	pink-red	30 -35 °C	18 -24 hrs
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	25 -100	≥50 %	light pink	30 -35 °C	18 -24 hrs
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50 -100	25 -100	≥50 %	pink-red	30 -35 °C	18 -24 hrs
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	≥10 <sup>3</sup>	0	0%		30 -35 °C	18 -24 hrs
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	≥10 <sup>3</sup>	0	0%		30 -35 °C	18 -24 hrs

Key : (\*) Corresponding WDCM numbers.

(#) Formerly known as *Enterobacter aerogenes* ^ Formerly known as *Pseudomonas aeruginosa*

**Storage and Shelf Life**

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

**Disposal**

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

**Reference**

1. Davis J.G., 1951, Milk Testing, dairy Industries Limited, London; pg.131.
2. The United States Pharmacopoeia-National Formulary (USP-NF), 2022.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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**Disclaimer :**

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