

Violet Red Bile Glucose Agar

MH581

Intended use

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	g / L
Yeast extract	3.000
Gelatin peptone #	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

Pancreatic digest of gelatin

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.

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 test for specified organisms by harmonized methodology of USP/EP/BP/JP/IP (1-5) from non-sterile products and pharmaceutical preparations. Gelatin peptone and yeast extract provide nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other nutrients essential for bacterial metabolism. This media is selective due to presence of the inhibitors; bile salts and crystal violet for inhibiting gram positive organisms especially Staphylococci and gram negative organisms respectively. Neutral red is an indicator that helps to detect glucose fermentation. Glucose fermenting 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.

Type of specimen

Pharmaceutical samples.

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (1-5). 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. Though the medium is for selective isolation of *Enterobacteriaceae*, further biochemical and serological testing must be carried out for further confirmation.
2. Over incubation may result in reverting of reaction.

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 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	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature
Growth Promoting + Indicative						
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	luxuriant	25 -100	≥ 50 %	pink-red with bile precipitate	18 -24 hrs
<i>^Pseudomonas paraaeruginosa</i> ATCC 9027 (00026*)	50 -100	luxuriant	25 -100	≥ 50 %	pink to red	18 -24 hrs

Additional Microbiological Testing

<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	good-luxuriant	25 -100	≥ 50 %	pink-red with bile precipitate	18 -24 hrs
<i>Salmonella Enteritidis</i> ATCC 13076 (00030*)	50 -100	good-luxuriant	25 -100	≥ 50 %	light pink	18 -24 hrs
<i># Klebsiella aerogenes</i> ATCC 13048 (00175*)	50 -100	good-luxuriant	25 -100	≥ 50 %	pink-red	18 -24 hrs
<i>Staphylococcus aureus subsp. aureus</i> ATCC 25923 (00034*)	$\geq 10^3$	inhibited	0	0%		≥ 24 hrs
<i>Staphylococcus aureus subsp. aureus</i> ATCC 6538 (00032*)	$\geq 10^3$	inhibited	0	0%		≥ 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 (6,7).

Reference

1. The United States Pharmacopoeia-National Formulary (USP-NF), 2022.
2. The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
3. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
4. The Japanese Pharmacopoeia, 17th edition, 2016, The Ministry of Health, Labour and welfare.
5. Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. 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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