



# PRODUCT SPECIFICATION SHEET

## Violet Red Bile Glucose Agar (DM287H)

### Intended Use

Violet Red Bile Glucose Agar (DM287H) is recommended for detection and enumeration of *Enterobacteriaceae* from pharmaceutical products using the microbial limit testing in compliance with the harmonized methodology of USP/EP/BP/JP.

### Product Summary and Explanation

*Enterobacteriaceae* are glucose-fermenting bacteria group comprising of lactose-fermenting coliform bacteria, lactose-nonfermenting strains of *E. coli*, and lactose-nonfermenting species, such as *Salmonella* and *Shigella*. It is desirable to detect *Enterobacteriaceae* rather than the coliform bacteria while examining some foods.<sup>(1)</sup> Mossel et al.<sup>(2)</sup> modified lactose-containing Violet Red Bile Agar by adding glucose to improve the recovery of *Enterobacteriaceae*. Later work by Mossel et al.<sup>(3,4)</sup> demonstrated that lactose could be omitted, resulting in the formulation known as Violet Red Bile Glucose Agar (VRBGA). 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 in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.<sup>(5,6,7,8,9)</sup>

### Principles of the Procedure

Violet Red Bile Glucose Agar contains pancreatic digest of gelatin and yeast extract which provide nitrogenous compounds, vitamins and other nutrients essential for bacterial metabolism. Bile salts and crystal violet are inhibitors which make the media selective. 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. As the pH of medium falls below 6.8 due to absorption of neutral red dye, the colour of the medium changes to red colour.

### Formula / Liter

Ingredients	Gms / Liter
Yeast extract	3.00
Pancreatic digest of gelatin	7.00
Bile salts	1.50
Sodium chloride	5.00
Glucose monohydrate	10.00
Agar	15.00
Neutral red	0.03
Crystal violet	0.002
Final pH: 7.4 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

### Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

### Directions

1. Suspend 40.62 grams (the equivalent weight) of the medium in one litre of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. DO NOT HEAT IN AN AUTOCLAVE.
4. Mix well and pour into sterile Petri plates.

### Quality Control Specifications

Dehydrated Appearance	Light yellow to pinkish beige homogeneous free flowing powder
Prepared Medium	Reddish purple coloured clear to slightly opalescent gel forms in Petri plates
Reaction of % solution	Not Applicable
Gel Strength	Firm, comparable with 1.5% Agar gel





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### 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  $\leq 100$  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).

### Expected Cultural Response:

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation Temperature
1.	<b>Growth Promoting + Indicative</b>						
2.	<i>Escherichia coli</i> ATCC 8739	50-100	good-luxuriant	25 -100	$\geq 50$ %	pink-red with bile precipitate	18 -24 hrs
3.	<i>Pseudomonas aeruginosa</i> ATCC 9027	50-100	good-luxuriant	25 -100	$\geq 50$ %	pink to red	18 -24 hrs
4.	<b>Additional Microbiological Testing</b>						
5.	<i>Escherichia coli</i> NCTC 9002	50-100	good-luxuriant	25 -100	$\geq 50$ %	pink-red with bile precipitate	18 -24 hrs
6.	<i>Escherichia coli</i> ATCC 25922	50-100	good-luxuriant	25 -100	$\geq 50$ %	pink-red with bile precipitate	18 -24 hrs
7.	<i>Salmonella enteritidis</i> ATCC 13076	50-100	good-luxuriant	25 -100	$\geq 50$ %	light pink	18 -24 hrs
8.	<i>Enterobacter aerogenes</i> ATCC 13048	50-100	good-luxuriant	25 -100	$\geq 50$ %	pink-red	18 -24 hrs
9.	<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	inhibited	0	0 %	--	$\geq 24$ hrs
10.	<i>Staphylococcus aureus</i> ATCC 6538	$\geq 10^3$	inhibited	0	0 %	--	$\geq 24$ hrs

The organisms listed are the minimum that should be used for quality control testing.

### Test Procedure

Refer to appropriate references for standard test procedures. This medium can be used in spread or pour plate procedures, with or without an overlay. In addition, this medium can be used as an over layer for spread plates to both prevent swarming colonies and to provide semi-anaerobic conditions that suppress the growth of non-fermentative gram-negative organisms. Stab inoculation procedures can also be used with this medium.

### Results

*Enterobacteriaceae* ferment glucose, produce acid products and form red to dark purple colonies surrounded by red-purple halos. Refer to appropriate references and standard test procedures for interpretation of results.

### Storage



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Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

### Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

### Limitations of the Procedure

1. When used in the pour plate procedure, the medium should be freshly prepared, tempered to 47°C, and used within 3 hours.
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

### Packaging

**Product Name : Violet Red Bile Glucose Agar**

**Product Code : DM287H**

**Available Pack sizes : 100gm / 500gm**

### References

1. Mossel. 1985. Int. J. Food Microbiol. 2:27.
2. Mossel, Mengerink and Scholts. 1962. J. Bacteriol. 84:381.
3. Mossel, Eelderink, Koopmans and van Rossem. 1978. Lab Practice 27:1049.
4. Mossel, Eelderink, Koopmans and van Rossem. 1979. J. Food Protect. 42:470.
5. The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
6. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia.
7. European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
8. Japanese Pharmacopoeia, 2008.
9. Indian Pharmacopoeia, 2010 Ministry of Health and Family Welfare, Govt. of India.

### Further Information

For further information please contact your local MICROMASTER Representative.



**MICROMASTER LABORATORIES PRIVATE LIMITED**

DM287HPSS,QAD/FR/024,Rev.00

Unit 38/39, Kalpataru Industrial Estate,

Off G.B. Road, Near 'R-Mall' , Thane (W) - 400607. M.S. INDIA.

Ph: +91-9320126789/9833630009/9819991103

Email: [sales@micromasterlab.com](mailto:sales@micromasterlab.com)

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