



## HiCombi™ VRBGA - VRBGA Agar Plate

MPH581

### Intended use

Recommended for the selection and subculture of bile tolerant organisms in accordance with the harmonized method of USP/EP/BP/JP/IP.

### Composition\*\*

Ingredients	Gms / Litre
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

Streak the test inoculum (50-100 CFU) aseptically.

### 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 (7,1,2,3,4) 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 positive organisms especially Staphylococci. Neutral red indicator helps to detect glucose fermentation. Glucose is the fermentable carbohydrate, utilization of which leads to the production of acids. Neutral red indicator detects the acidity so formed. Crystal violet and bile salts mixture help to inhibit the accompanying gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium. Further biochemical tests are necessary for positive identification (7).

### Type of specimen

Pharmaceutical samples

### Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (8,1,2,3,4).

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

Sterile VRBGA - VRBGA Agar in 90 mm disposable plate.

### Colour

Red with purplish tinge coloured medium

### Quantity of Medium

10 ml of medium in each side of 90mm disposable Biplate.

### pH

7.20 - 7.60

### Sterility Test

Passes release criteria

### Cultural Response

Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP. Cultural response was observed after 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 100 cfu (at 30-35°C for 18-24hours).

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature
<b>Growth Promoting + Indicative</b>						
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	luxuriant	25 -100	$\geq 50$ %	pink-red with bile precipitate	18 -24 hrs
<i>Pseudomonas aeruginosa</i> ATCC 9027 (00026*)	50 -100	luxuriant	25 -100	$\geq 50$ %	pink to red	18 -24 hrs
<b>Additional Microbiological Testing</b>						
<i>Escherichia coli</i> NCTC 9002	50 -100	good-luxuriant	25 -100	$\geq 50$ %	pink-red with bile precipitate	18 -24 hrs
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	good-luxuriant	25 -100	$\geq 50$ %	pink-red with bile precipitate	18 -24 hrs
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	good-luxuriant	25 -100	$\geq 50$ %	light pink	18 -24 hrs
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50 -100	good-luxuriant	25 -100	$\geq 50$ %	pink-red	18 -24 hrs
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	$\geq 10^3$	inhibited	0	0%		$\geq 24$ hrs
<i>Staphylococcus aureus</i>	$\geq 10^3$	inhibited	0	0%		$\geq 24$ hrs

*subsp. aureus ATCC*  
*ATCC 6538 (00032\*)*

**Key :- (# )** Formerly known as *Enterobacter aerogenes* (\*) Corresponding WDCM numbers

### Storage and Shelf Life

Store between 20-30°C. Use before expiry date on the label. 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 (5,6).

### Reference

1. British Pharmacopoeia, 2017, The Stationery office British Pharmacopoeia
2. European Pharmacopoeia, 2016, European Dept. for the quality of Medicines.
3. Japanese Pharmacopoeia, 2016.
4. Indian Pharmacopoeia, 2018 Ministry of Health and Family Welfare, Govt. of India.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. 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.
7. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
8. The United States Pharmacopoeia, 2019 The United States Pharmacopoeial Convention. Rockville, MD.

Revision : 00 / 2019

### Disclaimer :

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