

## Double Sugar HiVeg<sup>®</sup> Agar (Russell Double Sugar HiVeg<sup>®</sup> Agar)

MV057

### Intended Use:

Recommended for differentiation of gram-negative enteric bacilli on the basis of their ability to ferment dextrose and lactose with or without gas formation from various samples.

### Composition\*\*

Ingredients	g / L
HiVeg <sup>®</sup> peptone	2.500
HiVeg <sup>®</sup> hydrolysate	7.500
HiVeg <sup>®</sup> extract	3.000
Lactose	10.000
Dextrose (Glucose)	1.000
Sodium chloride	5.000
Phenol red	0.025
Agar	15.000
Final pH ( at 25°C)	7.3±0.2

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

### Directions

Suspend 44.02 grams in 1000 ml purified/distilled water. Mix thoroughly. Heat to boiling to dissolve the medium completely. Dispense in tubes or as desired and sterilize by autoclaving at  $\Delta$ 118-121°C for 15 minutes. Allow the tubes to solidify in slanting position to form generous butt.

$\Delta$  corresponds to 12-15 lbs pressure.

### Principle And Interpretation

Gram-negative bacilli belonging to *Enterobacteriaceae* are the most frequently encountered bacterial isolates recovered from clinical specimens. Definitive identification of the members of *Enterobacteriaceae* requires a battery of biochemical tests (1). Double Sugar Agar, Russell is used for the differentiation of gram-negative enteric bacilli on the basis of their ability to ferment dextrose and lactose with or without gas formation. This medium was originally formulated by Russell (2) using litmus indicator. It was later modified by Nichols (3) and Nichols and Wood (4) by replacing the litmus indicator with phenol red. This medium is used for differentiating gram-negative enteric bacilli especially the colon-typhoid-salmonellae-dysentery groups based on the fermentation of the double sugars incorporated namely, dextrose and lactose. Double Sugar HiVeg<sup>®</sup> Agar is same as Double Sugar Agar, Russell except that the animal based peptones are completely replaced with vegetable peptones to avoid the BSE/TSE risks associated with animal peptones.

On incubation of inoculated tubed medium, acid production under aerobic condition (on the slant) and under anaerobic condition (in the butt) can be detected by the change in colour of the indicator. Phenol red is the pH indicator in the medium. Gaseous fermentation is indicated by splitting of the agar or by bubble formation in the butt. Organism like *Salmonella* Typhi capable of fermenting dextrose but not lactose will show an initial acid slant in short incubation period. Over a period of time as the dextrose gets consumed the reaction under aerobic condition reverts and becomes alkaline due to the oxidation of acids. Under anaerobic condition (in the butt), the same organism fails to revert the reaction and remains acidic. HiVeg<sup>®</sup> peptone, HiVeg<sup>®</sup>hydrolysate and HiVeg<sup>®</sup> extract serve as sources of carbon, nitrogen, vitamins and other essential nutrients. Lactose and dextrose serve as sources of energy by being the fermentable carbohydrates. Phenol red is the pH indicator in the medium that is pink under alkaline conditions and yellow under acidic conditions. Sodium chloride helps to maintain the osmotic equilibrium of the medium. Pure cultures are used to inoculate the tubed medium (5).

### Type of specimen

Isolated Microorganism

### Specimen Collection and Handling:

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. Results should be recorded at specified time or it may result in erroneous results.
2. Other biochemicals must be carried out in conjunction for confirmation.

## 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 pink homogeneous free flowing powder

### Gelling

Firm, comparable with 1.5% Agar gel

### Colour and Clarity of prepared medium

Red coloured, clear to slightly opalescent gel forms in tubes as slants

### Reaction

Reaction of 4.4% w/v aqueous solution at 25°C. pH : 7.3±0.2

### pH

7.10-7.50

### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-40 hours.

Organism	Growth	Slant	Butt	Gas
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction
<i>Escherichia coli</i> ATCC 25922 (00013*)	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction
## <i>Proteus hauseri</i> ATCC 13315	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	luxuriant	alkaline reaction, red colour of the medium	alkaline reaction, red colour of the medium	negative reaction
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction
<i>Shigella dysenteriae</i> ATCC 13313	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction

Key : (\*) Corresponding WDCM numbers.

(#) Formerly known as *Enterobacter aerogenes*

(##) Formerly known as *Proteus vulgaris*

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippincott Company
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3. Nichols H. J., 1921, J. Infect. Dis., 2982
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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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