

## Tryptose Blood Agar Base, HiVeg®

MV097

### Intended Use:

Recommended for the isolation of fastidious organisms and determining the haemolytic reactions.

### Composition\*\*

Ingredients	g / L
HiVeg® hydrolysate No. 1	10.000
HiVeg® extract#	3.000
Sodium chloride	5.000
Agar	15.000
Final pH ( at 25°C)	7.2±0.2

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

### Directions

Suspend 33.0 grams in 950 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the autoclaved medium to 45 - 50°C and aseptically add 5% v/v sterile defibrinated blood. Mix thoroughly, avoiding air bubbles and pour into sterile Petri plates.

### Principle And Interpretation

Tryptose Blood Agar Base is a tryptose based medium that can be used for the cultivation of fastidious organisms (1,2), on supplementation with blood. This medium is devoid of dextrose and therefore useful in determining the haemolytic reactions. Tryptose Blood Agar Base is recommended by FDA (3) and APHA (4). Tryptose Blood Agar Base can be used as a general-purpose medium without supplementation of blood. These media can be used to determine the haemolytic reactions of fastidious organisms. The four different types of haemolysis observed are as follows:

- Alpha haemolysis: partial lysis of the erythrocytes surrounding colony, causing a grey green or brownish discoloration in the media.
- Beta haemolysis: complete lysis of the red blood cells surrounding a colony, causing a clearing of blood from the medium.
- Gamma haemolysis: no haemolysis and consequently, no colour change of the medium surrounding a colony. Organisms showing no haemolysis are generally termed non-hemolytic rather than gamma haemolytic.
- Alpha-prime or wide zone alpha: a small zone of intact erythrocytes immediately adjacent to the colony, with a zone of complete red cell haemolysis surrounding the zone of intact erythrocytes. This type of haemolysis may be confused with beta haemolysis (5).

Tryptose Blood Agar Base, HiVeg™ is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks.

HiVeg™ hydrolysate No. 1 and HiVeg™ extract provide nitrogenous and carbonaceous compounds, sulphur, vitamin B complex and trace elements essential for bacterial metabolism. Blood provides additional nutrients and serves as a base to study haemolytic reactions. This medium not only keeps the blood cells in a good state but also help in forming distinct haemolysis. Perform biochemical test for further identification (6).

### Type of specimen

Food samples

### Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (3).

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. Some fastidious organisms may not grow.
2. Inoculum density affects inhibition zone. Heavy inoculum may result in smaller zones while scanty growth may result in enlarged zones.

## 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

Cream to yellow homogeneous free flowing powder

### Gelling

Firm, comparable with 1.5% Agar gel

### Colour and Clarity of prepared medium

Basal medium : Yellow coloured clear to slightly opalescent gel forms in Petri plates. After addition of 5% v/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates.

### Reaction

Reaction of 3.3% w/v aqueous solution at 25°C. pH : 7.2±0.2

### pH

7.00-7.40

### Cultural Response

Cultural characteristics observed with added 5% v/v sterile defibrinated blood, after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth w/o blood	Recovery w/o blood	Growth w/ blood	Haemolysis	Recovery w/ blood
<i>Neisseria meningitidis</i> ATCC 13090	50-100	good-luxuriant	≥70%	luxuriant	none	≥70%
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50-100	good-luxuriant	≥70 %	luxuriant	beta/gamma	≥70%
<i>Staphylococcus epidermidis</i> ATCC 12228 (00036*)	50-100	good-luxuriant	≥70%	luxuriant	gamma	≥70%
<i>Streptococcus pneumoniae</i> ATCC 6303	50-100	fair-good	40-50%	good	alpha	50-70%
<i>Streptococcus pyogenes</i> ATCC 19615	50-100	fair-good	40-50%	good	beta	50-70%

Key : \*Corresponding WDCM numbers.

## 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 (7,8).

## Reference

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2. Casman E. P., 1947, Am. J. Clin. Pathol., 17: 281.
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4. American Public Health Association, 1970, Diagnostic Procedures and Reagents, 5th Ed., APHA Inc., New York.
5. 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.
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
8. 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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