

Triple Sugar Iron HiVeg® Agar

MV021

Intended Use:

Recommended for identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production.

Composition**

Ingredients	g / L
HiVeg® peptone	10.000
HiVeg® hydrolysate	10.000
Yeast extract	3.000
HiVeg® extract	3.000
Lactose	10.000
Sucrose	10.000
Dextrose (Glucose)	1.000
Sodium chloride	5.000
Ferrous sulphate	0.200
Sodium thiosulphate	0.300
Phenol red	0.024
Agar	12.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 64.52 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Allow the medium to set in sloped form with a butt about 1 inch long.

Note: For better results, the medium can be sterilized by autoclaving at 10 lbs pressure (115°C) for 15 minutes.

Principle And Interpretation

Triple Sugar Iron HiVeg® Agar is prepared by completely replacing animal based peptones with vegetable peptones, making the medium free of BSE/TSE associated risks. It is the modification of Triple Sugar Iron Agar which was originally proposed by Sulkin and Willett (1) and modified by Hajna (2) for identifying *Enterobacteriaceae*. The medium is equivalent in performance with the animal based medium recommended by APHA for the examination of meat and food products (3) and milk and dairy products (4). This has also been used in microbial limit test for confirming the presence of *Salmonellae*(5,6) and in the identification of gram-negative bacilli (5,7).

HiVeg® hydrolysate, HiVeg® peptone, yeast extract and HiVeg® extract provide nitrogenous, carbonaceous compounds, long chain amino acids, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose, sucrose and dextrose are the fermentable carbohydrates. Sodium thiosulphate and ferrous ions make H₂S (hydrogen sulphide) indicator system. Phenol red is the pH indicator. Organisms that ferment glucose produce a variety of acids, turning the colour of the medium from red to yellow. More amounts of acids are liberated in butt (fermentation) than in the slant (respiration). Growing bacteria also form alkaline products from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amounts of acid present in the butt. Thus the appearance of an alkaline (red) slant and an acid (yellow) butt after incubation indicates that the organism is a glucose fermenter but is unable to ferment lactose and/or sucrose. To enhance the alkaline condition of the slant, free exchange of air must be permitted by closing the tube cap loosely. If the tube is tightly closed, an acid reaction caused solely by dextrose fermentation will also involve the slant. Bacteria that ferment lactose or sucrose (or both), in addition to glucose, produce large amounts of acid enables no reversion of pH in that region and thus bacteria exhibit an acid slant and acid butt. Thiosulphate is reduced to hydrogen sulphide by several species of bacteria and H₂S (hydrogen sulphide) combines with ferric ions of ferric salts to produce the insoluble black precipitate of ferrous sulphide. Reduction of thiosulphate proceeds only in an acid environment and blackening usually occurs in the butt of the tube.

Acid environment and blackening usually occurs in the butt of the tube. Gas production (CO₂) is detected by the presence of cracks or bubbles in the medium, when the accumulated gas escapes. Do not use an inoculating loop to inoculate a tube of TSI, while stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production. Triple Sugar Iron Agar should be used in parallel with Urea Agar/ Broth (MV112/MV111) to distinguish between *Salmonella* and *Proteus* species.

The reactions can be summarized as follows:

Alkaline slant / acid butt-only glucose fermented

Acid slant / acid butt-glucose and sucrose fermented or glucose and lactose fermented or all the three sugars, glucose, lactose and sucrose fermented.

Bubbles or cracks present-gas production

Black precipitate present-H₂S gas production

Some members of the *Enterobacteriaceae* and H₂S (hydrogen sulphide) producing *Salmonella* may not be H₂S (hydrogen sulphide) positive on TSI Agar. Some bacteria may show H₂S (hydrogen sulphide) production on Kligler Iron Agar but not on TSI Agar. This can happen because utilization of sucrose in TSI Agar suppresses the enzymic pathway that result in H₂S (hydrogen sulphide) production.

Type of specimen

Pure bacterial isolate from water, food sample.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7, 9).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(1)

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 members of the *Enterobacteriaceae* and H₂S producing *Salmonella* may not be H₂S positive on TSI HiVeg[®] Agar.
2. Some bacteria may show H₂S production on Kligler Iron HiVeg[®] Agar but not on TSI HiVeg[®] Agar. This can happen because utilization of sucrose in TSI HiVeg[®] Agar suppresses the enzymic pathway that result in H₂S production.

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 1.2% Agar gel.

Colour and Clarity of prepared medium

Pinkish red coloured clear to slightly opalescent gel forms in tubes as slants.

Reaction

Reaction of 6.45% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Slant	Butt	Gas	H ₂ S
<i>Citrobacter freundii</i> ATCC 8090	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	positive, blackening of medium
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium

<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
<i>Klebsiella pneumoniae</i> ATCC 13883 (00097*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
## <i>Proteus hauseri</i> ATCC 13315	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	positive, blackening of medium
<i>Salmonella</i> Paratyphi A ATCC 9150	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
<i>Salmonella</i> Typhi ATCC 6539	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	positive, blackening of medium
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	positive, blackening of medium
<i>Shigella flexneri</i> ATCC 12022 (00126*)	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	negative, no blackening of medium
<i>Escherichia coli</i> ATCC 8739 (00012*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium

Key : (*) Corresponding WDCM numbers.

(##) Formerly known as *Proteus vulgaris*

Formerly known as *Enterobacter aerogenes*

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 (4,5).

Reference

1. Sulkin E.S. and Willett J.C., 1940, J. Lab. Clin. Med., 25:649.
2. Hajna A.A., 1945, J. Bacteriol, 49:516.
3. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

5. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
6. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.
7. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
9. 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.

Revision : 02/ 2025

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.