

Fermentation HiVeg™ Medium for Neisseriae

MV825

Intended Use:

Recommended for studying fermentation reaction of fastidious microorganism such as *Neisseriae* species.

Composition**

Ingredients	g / L
HiVeg™ hydrolysate	20.000
L-Cystine	0.500
Sodium chloride	5.000
Sodium sulphate	0.500
Phenol red	0.017
Agar	3.500
Final pH (at 25°C)	7.5±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.52 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense and sterilize by autoclaving at 118°C for 15 minutes. The pressure should not exceed 12lbs. Cool to around 40-45°C and add membrane filter sterilized sugar solutions to final concentration of 1%. (i.e. 5 ml of 20% sugar solution per 100 ml of medium).

Principle And Interpretation

Neisseria species are oxidative i.e. they produce acid from carbohydrate by oxidation. Because these species are oxidative and produce less acid from carbohydrates than do fermentative organisms and because they also produce ammonia from peptones which may neutralize any acid produced from carbohydrates, acid production is determined in a medium with a low protein/carbohydrate ratio and a sensitive indicator such as phenol red (1,2). Fermentation Medium for Neisseriae is recommended for studying the fermentation reactions of fastidious organisms such as *Neisseria* (3). This medium is the modification of the medium originally formulated by Vera (4). *Neisseria* species oxidize the added carbohydrates to yield acids. The acids thus formed change the colour of the pH indicator, phenol red from orange to yellow. The organism also degrades the peptone source to yield ammonia. The alkalinity thus formed causes the phenol red to change to pink. However, if the acidity formed by carbohydrate metabolism is greater than the alkalinity formed by peptone degradation, the medium remains yellow in colour. Fermentation HiVeg™ Medium for Neisseriae is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks.

HiVeg™ hydrolysate supplies the necessary nitrogenous nutrients to the organisms. L-Cystine acts as an amino acid source as well as a reducing agent, which can remove (bind) molecular oxygen thereby preventing the accumulation of peroxides which are lethal to certain microorganisms (5). Small amount of agar in the medium reduces convection currents in the medium and hence contributes to maintaining anaerobic conditions in the depth of the tubes. Sodium chloride maintains the osmotic equilibrium in the medium. Phenol red is the pH indicator, which turns yellow at acidic pH. Observe the inoculated tubes after every 4 hours. Development of yellow colour throughout the medium indicates that the carbohydrate has been oxidized leading to the production of acids. Development of pink colour indicates that carbohydrates have not been oxidized and only the peptones have been degraded. *Neisseria* species tend to produce acids from carbohydrate in the vicinity of inoculated (stab) area. If accompanying contaminating organisms are present the entire medium may turn yellow.

Type of specimen

Food samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6). 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. *Neisseria* species should be further confirmed by gram staining and oxidase test.

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 light pink homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.35% Agar gel.

Colour and Clarity of prepared medium

Straw coloured, clear to slightly opalescent gel forms in tubes as butts

Reaction

Reaction of 2.95% w/v aqueous solution at 25°C. pH : 7.5±0.1

pH

7.40-7.60

Cultural Response

Cultural characteristics observed with added 1% dextrose after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Acid with added dextrose	Motility
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	positive reaction, yellow colour	positive, growth away from stabline causing turbidity
<i>Neisseria gonorrhoeae</i> ATCC 19424	50-100	luxuriant	positive reaction, yellow colour	negative, growth along the stabline, surrounding medium remains clear
<i>Streptococcus pneumoniae</i> ATCC 6303	50-100	luxuriant	positive reaction, yellow colour	negative, growth along the stabline, surrounding medium remains clear

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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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3. Atlas R. M., 2004, Handbook of Microbiological Media, 3rd Ed., CRC Press.
4. Vera, 1948, J. Bacteriol., 55:531
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol.1, Williams and Wilkins, Baltimore
6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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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