Hugh Leifson Medium

M826

Intended use

Hugh Leifson Medium is used to distinguish between anaerobic and aerobic breakdown of carbohydrate (glucose).

Composition**

Ingredients	Gms / Litre
Peptone	2.000
Sodium chloride	5.000
Dipotassium phosphate	0.300
Glucose	10.000
Bromothymol blue	0.050
Agar	2.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 19.35 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense into test tubes in duplicate for aerobic and anaerobic fermentation. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubed medium in an upright position

Principle And Interpretation

Hugh Leifson Medium was formulated by Hugh and Leifson (1). They described the taxonomic significance of fermentative and oxidative metabolism of carbohydrates in gram-negative intestinal bacteria.

There are two ways of utilizing carbohydrates by microorganisms, namely fermentation and oxidation. This property may be frequently used for the differentiation of some bacteria.

The medium contains a high concentration of carbohydrate and low concentration of peptone to avoid the possibility of an aerobic organism utilizing peptone and producing an alkaline condition which would neutralize slight acidity produced by an oxidative organism (2,3). Dipotassium phosphate promotes fermentation and acts as pH controlling buffer. Agar concentration enables the determination of motility and aids in distribution of acid throughout the tube produced at the surface of medium. The tubes for aerobic and anaerobic fermentation are inoculated and the agar surface of one tube of duplicate is covered with layer of sterile paraffin oil, about 25 mm thickness and incubated at 37°C. Oxidative organisms produce acid in unsealed tube with little or no growth and no acid formation in sealed tube while fermentative organisms produce acid in both sealed and unsealed tubes. If acid is produced, it is indicated by change in colour from greenish blue to yellow throughout the medium. Liquid paraffin tube used should be dry sterilized at 160-170°C for 2 hours. Wet sterilization with high pressure is not sufficient for the purpose.

Type of specimen

Clinical samples - Blood ; Food and dairy samples ; Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,5,9).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(6)

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. 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 clincal specimens. Saftey guidelines may be referred in individual safety data sheets.

Limitations :

This medium is general purpose medium and may not support the growth of fastidious organisms.

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 bluish green homogeneous free flowing powder

Gelling

Semisolid,comparable with 0.2% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

Reaction of 1.94% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Motility	Aerobic fermentation	Anaerobic fermentatoin
Cultural Response				
Enterobacter aerogenes ATCC 13048	50-100	positive,growth away from stabline causing turbidity	acid (yellow) and gas production	acid (yellow) and gas production
Escherichia coli ATCC 25922	50-100	positive, growth away from stabline causing turbidity	acid (yellow) and gas production,	acid (yellow) and gas production
Pseudomonas aeruginosa ATCC 27853	50-100	positive,growth away from stabline causing turbidity	acid (yellow) production	unchanged (green) or alkaline (blue)
Salmonella Typhi ATCC 6539	50-100	positive, growth away from stabline causing turbidity	acid (yellow) and gas production	acid (yellow) and gas production
Shigella sonnei ATCC 25931	50-100	negative, growth along the stabline, surrounding medium	acid (yellow) production	acid (yellow) and gas production

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 inorder 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. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1.Hugh and Leifson, 1953, J. Bacteriol., 66:24.
2.MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification- Maintenance of Medical Bacteria,Vol. I, Williams and Wilkins, Baltimore.
3.Finegold S. M., Martin W. J., and Scott E. G., 1978, Bailey and Scotts Diagnostic Microbiology, 5th Ed., The C.V. Mosby Co., St. Louis.
4. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
5. Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
6. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington, D.C.
7.Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 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.
9. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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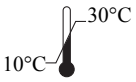
IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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