Hugh Leifson Medium

M826

Intended use:

Recommended for detecting aerobic and anaerobic breakdown of glucose.

Composition**

Ingredients	g / L
Peptone	2.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	0.300
Dextrose (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 purified/distilled water. Heat with frequent stirring. Heat to boiling to dissolve the medium completely. Dispense into test tubes in duplicate for aerobic and anaerobic fermentations. 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. Bromthymol blue is the pH indicator.

Type of specimen

Isolated microorganism

Specimen Collection and Handling:

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.

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. Other biochemical tests must be performed 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 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.

Organism	Motility	Aerobic fermentation	Anaerobic fermentatoin
#*Klebsiella aerogenes* ATCC 13048 (00175*)	positive,growth away from stabline causing turbidity	acid (yellow) and gas production	acid (yellow) and gas production
Escherichia coli ATCC 25922 (00013*)	positive, growth away from stabline causing turbidity	acid (yellow) and gas production,	acid (yellow) and gas production
Pseudomonas aeruginosa ATCC 27853 (00025*)	positive,growth away from stabline causing turbidity	acid (yellow) production	unchanged (green) or alkaline (blue)
Salmonella Typhi ATCC 6539	positive, growth away from stabline causing turbidity	acid (yellow) and gas production	acid (yellow) and gas production
Shigella sonnei ATCC 25931	negative, growth along the stabline, surrounding medium	acid (yellow) production	acid (yellow) and gas production

Key : (*)Corresponding WDCM numbers. (#) Formerly known as*Enterobacteraerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°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. 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 (4,5).

Reference

1. Hugh and Leifson, 1953, J. Bacteriol., 66:24.
2. 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.
3. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification- Maintenance of Medical Bacteria,Vol. I, Williams and Wilkins, Baltimore.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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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