Hugh Leifson Glucose Medium

M871

Hugh Leifson Glucose Medium is recommended for the differentiation of Staphylococci from Micrococci on the basis of their ability to ferment glucose anaerobically.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	2.000
Yeast extract	0.500
Sodium chloride	30.000
Glucose	10.000
Bromocresol purple	0.015
Agar	3.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 45.52 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 Glucose Medium is formulated by Hugh and Leifson (2). Hugh Leifson Glucose Medium is prepared as described by FDA (1) for differentiation of Staphylococci from Micrococci. 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 peptic digest of animal tissue to avoid the possibility of an aerobic organism utilizing peptic digest of animal tissue and producing an alkaline condition which would neutralize slight acidity produced by an oxidative organism (3, 4). 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 purple 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.

Hugh Leifson Glucose Medium contains high salt concentration thus it is used for the identification of pathogenic and halophilic organisms and for testing aerobic and anaerobic breakdown of glucose by Staphylococci and Micrococci (5). Inoculate the culture under test into two tubes of the medium by stabbing throughout their length with a long wire loop. Cover one tube of the pair with layer of sterile liquid paraffin and incubate at 37°C. Read yellow colouration as acid production from glucose. Staphylococci produce acid by fermentation throughout the depth of the medium both in the anaerobic tubes sealed with paraffin and the aerobic unsealed tube. Micrococci either fail to produce acid in either tube or produce it only by oxidation in the upper part of the aerobic tube.

Quality Control

Appearance

Light yellow to bluish grey homogeneous free flowing powder

Gelling

Semisolid,comparable with 0.3% Agar gel.

Colour and Clarity of prepared medium
Purple coloured, clear to slightly opalescent gel forms in tubes as butts

Reaction
Reaction of 4.55% 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 .

Cultural Response

Organism	Inoculum (CFU)	Growth	Colour of Medium (Aerobic)	Colour of Medium (Anaerobic)
Cultural Response				
Micrococcus luteus ATCC 10240	50-100	good	yellow	pink-purple
Staphylococcus aureus ATCC 25923	50-100	good	yellow	yellow

Storage and Shelf Life
Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference
1.Bacteriological Analytical Manual, 1995, 8th Ed., Food & Drug Administration, AOAC International, USA.
2.Hugh and Leifson, 1953, J. Bacteriol., 66:24.
3.MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol.I, Williams and Wilkins, Baltimore.
4.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.
5.Baird Parker, 1966, International subcommittee on Staphylococci and Micrococci.

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