Technical Information

Hugh Leifson Medium

Product Code: DM 1826

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

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.

Hugh and Leifson [1] formulated Hugh Leifson medium and described the taxonomic significance of fermentative and oxidative metabolism of carbohydrates in gram-negative intestinal 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 [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 3 7°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.

Methodology

Suspend 19.35 grams of powder media in 1000 ml distilled water. Shake well & heat 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

Quality Control

Physical 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 range: 6.60-7.00

Cultural Response/Characteristics
DM 1826: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Motility	Aerobic fermentation	Anaerobic fermentation
Enterobacter aerogenes ATCC 13048	50-100	Positive growth away from stabline causing turbidity	Acid and gas production positive reaction	Acid and gas production positive reaction
Escherichia coli ATCC 25922	50-100	Positive growth away From stabline causing turbidity	acid and gas production, positive reaction	acid and gas production, positive reaction
Pseudomonas aeruginosa ATCC 27853	50-100	positive, growth away from stabline causing turbidity	acid production, negative reaction, no colour change	acid production,positive reaction, yellow colour
Salmonella Typhi ATCC 6539	50-100	positive, growth away from stabline causing turbidity	acid and gas production, positive reaction	acid and gas production ,positive reaction
Shigella sonnei ATCC 25931	50-100	negative, growth along the stabline, surrounding medium remains clear	acid production, positive reaction, yellow colour	acid production, positive reaction, yellow colour

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

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.

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