HUGH LEIFSON MEDIUM (as per BIS) TM 742

For detecting aerobic and anaerobic breakdown of glucose

Composition

Ingredients	Gms/Ltr.
Glucose	10.0
Sodium chloride	5.00
Agar	3.00
Peptic digest of animal tissue	2.00
Dipotassium phosphate	0.30
Bromothymol blue	0.03

***** Dehydrated powder store, in a dry place in tightly- sealed containers below 25°C and protected from direct sunlight.

Instructions for Use

Dissolve 20.33gms in 1000ml of distilled water. Gently heat to dissolve the medium completely. The glucose was prepared as a 10% stock solution, sterilized by filtration through a 0.22-µm-pore-size membrane, and added aseptically to the semisolid basal medium to give a 1% final concentration (or if preferred, add 1 g of carbohydrate directly to 100 ml of medium and sterilize at 118°C for 10 minutes). The complete medium was dispensed in 4-ml portions in sterile culture tubes (12 by 100 mm). Mix well. Sterilize them in an autoclave at 15 psi (at 118^{0}C) for 10 minutes

Appearance: Green in colour, Semi – solid, opalescent gel
pH (at 25^{0}C): 6.8 ± 0.2

Principle

HUGH LEIFSON MEDIUM is used for detecting aerobic and anaerobic breakdown of glucose. To investigate the suitability of Hugh and Leifson's medium (HLM) as the basis of a simple screening test to differentiate between contaminants and *Arcobacter* spp. during their isolation from foodstuffs. The medium contains a high concentration of added carbohydrates relative to the Peptic digest of animal tissue concentration to avoid the utilization of peptone by an aerobic organism and the resultant production of an alkaline reaction which would neutralize slight acidity produced by an oxidative organism. The dipotassium phosphate adds buffering capacity to the medium. The agar permits the property of semi solid media, which helps in determination of motility and aids in the even distribution of any acid produced at the surface of the medium. Growth in the oxidation-fermentation medium of Hugh and Leifson with two tubes, one open (aerobic) and another closed (anaerobic), is a common test to determine this character. Inoculate duplicate tubes by stabbing and pour 1 - 2 ml of sterile mineral oil on top of the medium in one tube. Cover one tube of each pair with sterile liquid paraffin and place them in vertical position. Loosen caps and boil the medium in a boiling water bath* for approximately 2 min prior to use. A positive reaction (anaerobic growth) is indicated by a yellow color in both tubes in 24 hours at 27°C. A green color indicates a negative reaction even if growth is visible.

Interpretation

Cultural characteristics observed after inoculating (10^3CFU / ml), on incubation at 35 ± 2°C for 18 – 48 hours.

Microorganisms	ATCC	With glucose	
		⬭	⬬
Enterobacter aerogenes	13048	AG	AG
Pseudomonas aeruginosa	27853	A	K
Salmonella Typhi	6539	AG	AG

⬭ = Aerobic; ⬬ = Anaerobic; A= production of acid; G= production of gas, sometimes perceptible; K= alkaline, green (without change).

References

1. MacFaddin, J.F. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkens, Baltimore. (1985).
2. Shigei, J. Test methods used in the identification of commonly isolated aerobic gram-negative bacteria. Oxidation-fermentation test, p. (1992).
3. Hugh, R., and E. Leifson. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol. 66:24-26. (1953).