CHOCOLATE AGAR BASE TM 064

For isolation and cultivation of fastidious microorganisms like *Neisseria gonorrhoeae*

Composition

Ingredients	Gms/Ltr.
Proteose peptone	20.0
Agar	15.0
Sodium chloride	5.00
Disodium phosphate	5.00
Dextrose	0.50

* Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct Sunlight.

Instructions for Use

Dissolve 45gms in 445 ml distilled water. Gently heat to boiling with gentle swirling and dissolve the medium completely. Sterilize by autoclaving at 15 psi (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add equal amount (445ml) of sterile 2% Haemoglobin powder (TS 021) and add 10ml of Vitamins growth supplement (TS 022) or Yeast autolysate supplement (TS 023). Mix well. Dispense into sterile Petri plates or sterile culture tubes.

Appearance: Light amber color, clear to slightly opalescent gel. On addition of Haemoglobin: Chocolate brown colour appears, opaque gel
pH (at 25°C): 7.3 ± 0.2

Principle

CHOCHOLATE AGAR BASE is used for isolation and cultivation of fastidious microorganisms like *Neisseria gonorrhoeae*. It can also be used for cultivation of aerobic, anaerobic and microaerophillic microorganisms. Medium contains Proteose peptone is a nitrogen source required for the growth of wide variety of organisms. Dextrose acts a carbon energy source. Disodium phosphate buffers the medium whereas sodium chloride maintains the osmotic equilibrium. Agar is the solidifying agent. This medium is supplemented with cofactor, which provides NAD to facilitate the growth of *Haemophilus influenzae*, *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Heated sheep blood is added to give the medium its "chocolate" appearance. This medium is prepared, stored and dispensed under oxygen-free conditions to prevent the formation of oxidized products prior to use.

Interpretation

Cultural characteristics observed after inoculating (10^3CFU / ml), on incubation at 35 ± 2°C for 48 hours with CO_2.

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth
Neisseria meningitidis	13090	10^3	Good
Streptococcus pneumoniae	6303	10^3	Good
Haemophilus influenza	19418	10^3	Good

References

1. Lennette, E.H., Ballows, A., Hausler, W.J.Jr., and Shadomy, H.J. Manual of Clinical Microbiology. 4th ed. Washington D.C.: American society for Microbiology. (1985).
2. N.C.C.L.S. Quality Assurance for Commercially Prepared Microbiological Culture Media. Approved Standard. Vol.10, No.14. NCCLS Document M22-A. (1990).
3. Mac Faddin, Jean F., Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol.1. Baltimore, MD.: Williams & Wilkins. (1985).