Fermentation Medium for Neisseria

M825

Fermentation Medium for Neisseriae is recommended for studying fermentation reaction of fastidious microorganism such as Neisseriae.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Cystine	0.500
Sodium chloride	5.000
Sodium sulphate	0.500
Phenol red	0.017
Agar	3.500
Final pH (at 25°C)	7.5±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.52 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense and sterilize by autoclaving at 118°C for 15 minutes. The pressure should not exceed 12lbs. Cool to around 40-45°C and add membrane filter sterilized sugar solutions to final concentration of 1%. (i.e. 5 ml of 20% sugar solution per 100 ml of medium).

Principle And Interpretation

Neisseria species are oxidative i.e. they produce acid from carbohydrate by oxidation. Because these species are oxidative and produce less acid from carbohydrates than do fermentative organisms and because they also produce ammonia from peptones which may neutralize any acid produced from carbohydrates, acid production is determined in a medium with a low protein/ carbohydrate ratio and a sensitive indicator such as phenol red (1, 2). Fermentation Medium for Neisseriae is recommended for studying the fermentation reactions of fastidious organisms such as *Neisseria* (3). This medium is the modification of the medium originally formulated by Vera (4).

Neisseria species oxidize the added carbohydrates to yield acids. The acids thus formed change the colour of the pH indicator, phenol red form orange to yellow. The organism also degrades the peptone source to yield ammonia. The alkalinity thus formed causes the phenol red to change to pink. However, if the acidity formed by carbohydrate metabolism is greater than the alkalinity formed by peptone degradation, the medium remains yellow in colour.

Casein enzymic hydrolysate supplies the necessary nitrogenous nutrients to the organisms. Cystine acts as an amino acid source as well as a reducing agent, which can remove (bind) molecular oxygen thereby preventing the accumulation of peroxides which are lethal to certain microorganisms (5). Small amount of agar in the medium reduces convection currents in the medium and hence contributes to maintaining anaerobic conditions in the depth of the tubes. Sodium chloride maintains the osmotic equilibrium in the medium. Phenol red is the pH indicator, which turns yellow at acidic pH. Observe the inoculated tubes after every 4 hours.

Development of yellow colour throughout the medium indicates that the carbohydrate has been oxidized leading to the production of acids. Development of pink colour indicates that carbohydrates have not been oxidized and only the peptones have been degraded. *Neisseria* species tend to produce acids from carbohydrate in the vicinity of inoculated (stab) area. If accompanying contaminating organisms are present the entire medium may turn yellow. *Neisseria* species should be further confirmed by gram staining and oxidase test.

Quality Control

Appearance

Light yellow to light pink homogeneous free flowing powder

Gelling

Semisolid,comparable with 0.35% Agar gel.

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

Reaction
Reaction of 2.95% w/v aqueous solution at 25°C. pH : 7.5±0.1

pH
7.40-7.60

Cultural Response
M825: Cultural characteristics observed with added 1% dextrose after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Acid with added dextrose	Motility
Escherichia coli ATCC 25922	50-100	luxuriant	positive reaction ,yellow colour	positive,growth away from stabline causing turbidity
Neisseria gonorrhoeae ATCC 19424	50-100	luxuriant	positive reaction, yellow colour	negative,growth along the stabline, surrounding medium remains clear
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant	positive reaction, yellow colour	negative,growth along the stabline, surrounding medium remains clear

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

Reference
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2. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
3. Atlas R. M., 2004, Handbook of Microbiological Media, 3rd Ed., CRC Press.
4. Vera, 1948, J. Bacteriol., 55:531
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol.1, Williams and Wilkins, Baltimore

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