Technical Information

GC Agar Base

Product Code: DM 1434

Application: - GC Agar Base, with added blood or haemoglobin and other supplements is recommended for selective isolation and cultivation of Gonococci.

Composition**

Ingredients	Gms / Litre
Peptone, special	15.000
Corn starch	1.000
Dipotassium phosphate	4.000
Monopotassium phosphate	1.000
Sodium chloride	5.000
Agar	10.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Majority of gonococcal infections effect lower genital tract in which infection is caused by infecting the columnar epithelium of mucosal membranes. *Neisseria gonorrhoeae* is the causative agent of gonococcal infections. Most *Neisseria* strains have complex growth requirements; some strains may be exquisitely sensitive to fatty acids, requiring the incorporation of soluble starch in the growth media [1]. Johnston developed a medium that could obtain the growth of *Neisseria* within 24 hours rather that the usual 48 hours [2]. This medium was later modified by Carpenter and Morton [3], by the addition of haemoglobin. Thayer and Martin improved the selectivity of GC Medium by the adding the antibiotics namely colistin, vancomycin and nystatin (V.C.N.) (MS2023) [4, 5]. An additional antibiotic trimethoprim lactate [7] was later coupled with V.C.N. to further increase the selectivity of the medium (MS2024) [6]. For the cultivation of fastidious organisms the medium should be supplemented with essential growth factors supplied mainly by yeast extract (MS2027). This can be replaced with a chemically defined supplement containing essential growth factors available from yeast extract (MS2025). X - factor needed for the growth of fastidious *Haemophilus* species is provided by haemoglobin (MS2022). GC Medium Base can be used for the preparation of Thayer Martin Medium by the adding MS2027, which contains yeast autolysate as a source of essential growth factors and V.C.N.T. antibiotics as selective agents [6, 7]. Vancomycin (3 mg/lit) in V.C.N.T. Supplement (MS2024) was replaced with lincomycin, since the later was found to be less inhibitory to gonococci [8, 9]. Also nystatin was replaced by amphotericin B (in MS2024) to improve the selectivity of the medium to yeast contaminants, regularly found in vaginal specimens [10]. This modified supplement is the Linco T Supplement (MS026). Certain strains of gonococci were found sensitive to 3 mg/ of lt vancomycin [8]. Therefore the concentration of vancomycin was reduced to 2 mg/lit to obtain the growth of these sensitive strains [10]. This modified supplement with reduced vancomycin concentrations and amphotericin B is the Vanclo T Supplement (MS2028).

GC Agar contains special peptone, which supplies essential nutrients to the organisms. The presence of starch ensures that the toxic metabolites produced by *Neisseria* are neutralized. Phosphates prevent changes in the pH due to amine production that can affect the survival of the organisms. Factor-X (hemin) needed for *Haemophilus* species is provided by haemoglobin. The other supplements added provide factor-V i.e. NAD (Nicotinamide Adenine dinucleotide) for Haemophilus species and amino acids, coenzymes, ferric ions etc, which improve the growth of pathogenic *Neisseria.*

Avoid cotton wool for specimen collection. Inoculate immediately after specimen collection. Specimens should be streaked on the surface of plates so as to get some areas heavily seeded and other areas lightly seeded. Incubation is done at 37°C in an atmosphere of 70% humidity and 5-10% carbon dioxide. All presumptive *Neisseria* must be confirmed by carbohydrate fermentation tests and other serological tests.

Methodology

Suspend 7.2 grams of powder media in 100 ml distilled water, to make a double strength base. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add separately prepared Haemoglobin (MS2022) (100 ml sterile 2% solution) and GC Supplement w/ Antibiotics (MS2021). Mix well and pour into sterile Petri plates. To increase the selectivity of medium antibiotic supplements such as V.C.N. Supplement (MS2023), V.C.N.T. Supplement (MS2024), Linco T Supplement (MS2026) or Vanclo T Supplement (MS2028) may be added. To enhance the nutritional properties of medium, Vitamino Growth Supplement (MS2025) or Yeast Autolysate Supplement (MS2027) may be added. For Chocolate Blood Agar, prepare single-strength medium using 3.6 grams in 100 ml of distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes and add 5% v/v defibrinated blood. Mix well and heat at 80°C for 10 minutes.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium

Basal medium: Light yellow coloured clear to slightly opalescent gel. After addition of 2% Haemoglobin: Chocolate brown coloured opaque gel forms in Petri plates.

Reaction

Reaction of 3.6% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH Range: 7.00-7.40

Cultural Response/Characteristics

DM 1434: Cultural characteristics observed in presence of 5-10% Carbon dioxide (CO_2) and 70% humidity with added sterile 2% Haemoglobin (MS2022) and GC Supplement with antibiotics (MS2021), after an incubation at 35-37°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Haemophilus influenzae ATCC 19418	50-100	good-luxuriant	>=50%
Neisseria gonorrhoeae ATCC19424	50-100	good-luxuriant	>=50%
Neisseria meningitidis ATCC 13090	50-100	good-luxuriant	>=50%
Streptococcus pyogenes ATCC19615	50-100	good-luxuriant	>=50%
Streptococcus pneumoniae ATCC 6303	50-100	good-luxuriant	>=50%

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. Murray P. R., Baron E. J., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology , 8th Ed., American Society for Microbiology, Washington, D.C 2.Johnston J., 1945, J. Vener. Dis. Inform., 26:239.

3. Carpenter C. M. and Morton H. E., 1947, Proc. N.Y. State Assoc. Public Hlth. Lab., 27:58.

4.Thayer J. D. and Martin J. E., 1964, Public Health Rep., 79:49 5.Thayer J. D. and Martin J. E., 1966, Public Health Rep., 8 1:559 6. Martin J. E. and Lester A., 1971, HSMHA Health Rep., 86:30 7.Seth A., 1970, Brit. J. Vener. Dis., 46, 201-202

8. Reyn A. and Bentzon M. W., 1972, Brit. J. Vener. Dis., 48, 363-368

9.Mirrett S., Reller L. B. and Knapp J. S., 1981, J. Clin. Microbiol., 14. 94-99

10. Faur Y. C., Wilsburd M. H., Wilson M. E. and May P. S.,1973, Health Lab Sci., 10. 44-54

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