TM 116 - GC AGAR BASE

INTENDED USE

For selective isolation and cultivation of Gonococci.

SUMMARY AND EXPLANATION

Majority of gonococcal infections are uncomplicated lower genital tract infection caused by direct infection of 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, necessitating the incorporation of soluble starch in the growth media. Johnston developed a medium that could obtain the growth of *Neisseria* within 24 hours rather than the usual 48 hours. This medium was later modified by Carpenter and Morton, by the addition of haemoglobin. Thayer and Martin improved the selectivity of GC Medium by the incorporation of the antibiotics colistin, vancomycin and nystatin. An additional antibiotic trimethoprim lactate was later coupled with V.C.N. to further increase the selectivity of the medium. For the cultivation of fastidious organisms, the medium should be supplemented with essential growth factors supplied predominantly by yeast extract. This can be replaced with a chemically defined supplement containing essential growth factors available from yeast extract in Vitamino Growth Supplement (Twin Pack). X-factor needed for the growth of fastidious *Haemophilus* species is provided by haemoglobin.

GC Medium Base can be used as a base for the preparation of Thayer Martin Medium by the addition of Yeast Autolysate Supplement, which contains yeast auto lysate as a source of essential growth factors and V.C.N.T. antibiotics as selective agents. Vancomycin (3 mg/L) in V.C.N.T. Supplement was replaced with lincomycin, since the later was found to be less inhibitory to gonococci. Also nystatin was replaced by amphotericin B to improve the selectivity of the medium to yeast contaminants, regularly found in vaginal specimens. This modified supplement is the Linco T Supplement. Certain strains of gonococci were found to be sensitive to 3 mg/lt vancomycin regularly used. Therefore, the concentration of vancomycin was reduced to 2 mg/lit to obtain the growth of these sensitive strains. This modified supplement with reduced vancomycin concentrations and amphotericin B is the Vanclo T Supplement.

COMPOSITION

Ingredients	Gms / Ltr
Peptone, special	15.000
Corn Starch	1.000
Dipotassium hydrogen phosphate	4.000
Potassium dihydrogen phospahte	1.000
Agar	10.000
Sodium chloride	5.000

PRINCIPLE

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.

INSTRUCTION FOR USE

- Dissolve the 36.0 grams in 500 ml purified / distilled water, to make a double strength base.
- Heat to boiling to dissolve the medium completely.
- Separately suspend 10 grams of Haemoglobin powder in 500 ml purified / distilled water (2% solution) to make a uniform solution. Separately sterilize both the solutions by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 45-50°C.
- Mix both the solutions uniformly and aseptically add the rehydrated contents of two vials of GC Supplement w/ Antibiotics. Mix well and pour into sterile Petri plates.
- To increase the selectivity of medium antibiotic supplements such as V.C.N. Supplement or V.C.N.T. Supplement or Linco T Supplement or Vanclo T Supplement (two vials per litre) may be added.
- To enhance the nutritional properties of medium, Vitamins amino growth supplement or Yeast Autolysate Supplement 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 psi 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 SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder
Appearance 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.
pH (at 25°C) : 7.6 ± 0.2

INTERPRETATION

Cultural characteristics observed in presence of 5-10% Carbon dioxide (CO_2) and 70% humidity with added sterile 2% Haemoglobin and GC Supplement with antibiotics, after an incubation.

Microorganism	ATCC	Inoculum (CFU)	Growth	Recovery	Incubation Temperature	Incubation Period
Haemophilus influenzae	19418	50-100	Good-luxuriant	>=50%	35-37°C	40-48 Hours
Neisseria gonorrhoeae	19424	50-100	Good-luxuriant (with added antibiotic supplements)	>=50%	35-37°C	40-48 Hours
Neisseria meningitidis	13090	50-100	Good-luxuriant (with added antibiotic supplements)	>=50%	35-37°C	40-48 Hours
Streptococcus pyogenes	19615	50-100	Good-luxuriant	>=50%	35-37°C	40-48 Hours

Streptococcus pneumoniae	6303	50-100	Good-luxuriant	>=50%	35-37°C	40-48 Hours

PACKAGING:
In pack size of 100 gm and 500 gm bottles.

STORAGE
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES
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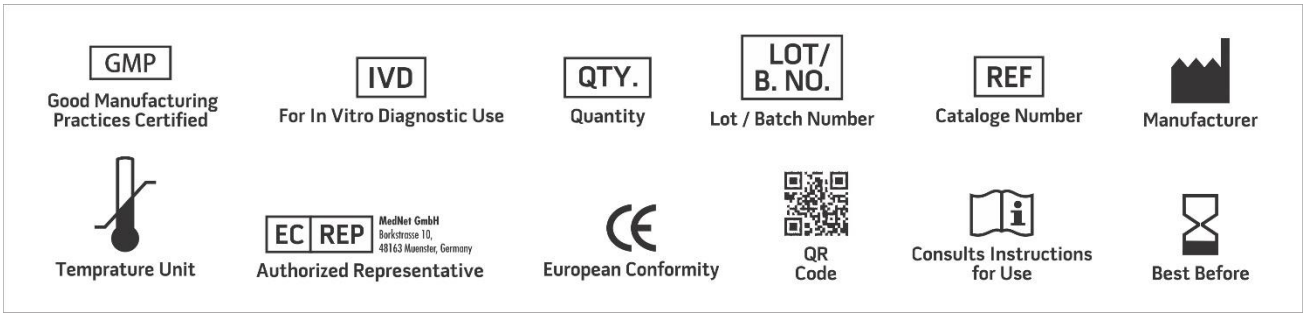

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.
***For Lab Use Only**
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