GC HiVeg® Agar Base

MV434

Intended Use:

With added blood or haemoglobin and other supplements it is recommended for selective isolation and cultivation of Gonococci.

Composition**

Ingredients	g / L
HiVeg® special peptone	15.000
Corn starch	1.000
Dipotassium hydrogen phosphate	4.000
Potassium dihydrogen 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

Directions

Suspend 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 FO Growth Supplement (FD022) in 500 ml purified / distilled water (2% solution) to make a uniform solution . Separately sterilize both the solutions by autoclaving at 15 lbs 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 Selective Supplement (FD021). Mix well and pour into sterile Petri plates. To increase the selectivity of medium antibiotic supplements such as V.C.N. Supplement (FD023- two vials per litre) or V.C.N.T. Supplement (FD024 - two vials per litre) or Linco T Supplement (FD026 - two vials per litre) or Vanclo T Supplement (FD028 - two vials per litre) may be added. To enhance the nutritional properties of medium, Vitamino Growth Supplement (FD025- two vials per litre) or Yeast Autolysate Supplement (FD027 - two vials per litre) 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.

Principle And Interpretation

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 (1). Johnston developed a medium that could obtain the growth of *Neisseria* within 24 hours rather than 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 incorporation of the antibiotics colistin, vancomycin and nystatin (V.C.N.) (FD023) (4,5). An additional antibiotic trimethoprim lactate (6) was later coupled with V.C.N. to further increase the selectivity of the medium (FD024) (7). For the cultivation of fastidious organisms the medium should be supplemented with essential growth factors supplied predominantly by yeast extract (FD027). This can be replaced with a chemically defined supplement containing essential growth factors available from yeast extract in Vitamino Growth Supplement (Twin Pack) (FD025). X-factor needed for the growth of fastidious *Haemophilus* species is provided by haemoglobin (FD022). GC HiVeg® Agar Base can be used as a base for the preparation of Thayer Martin Medium by the addition of FD027, which contains yeast auto lysate as a source of essential growth factors and V.C.N.T. antibiotics as selective agents (6,7). Vancomycin (3 mg/L) in V.C.N.T. Supplement (FD024) 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 FD024) to improve the selectivity of the medium to yeast contaminants, regularly found in vaginal specimens (10). This modified supplement is the Linco T Supplement (FD026). Certain strains of gonococci were found to be sensitive to 3 mg/lit vancomycin regularly used (9).

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 (FD028). GC HiVeg® Agar Base is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks. HiVeg® Agar Base 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 (Nicotinamid 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.

Type of specimen

Clinical samples : urine, respiratory exudates, etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (11,12).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Growth supplements like haemoglobin and Vitamino growth supplements must be added for growth of fastidious organisms like *Haemophilus* and Gonococci.
2.Carry out confirmatory tests of all the colonies

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

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

7.00-7.40

Cultural Response

Cultural characteristics observed in presence of 5-10% Carbon dioxide (CO2) and 70% humidity with added sterile FO Growth Supplement (FD022) and GC Selective Supplement (FD021), 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 ATCC 19424	50-100	good-luxuriant (with added antibiotic supplements)	>=50%

Neisseria meningitidis ATCC 13090	50-100	good-luxuriant >=50% (with added antibiotic supplements)
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant >=50%
Streptococcus pneumoniae ATCC 6303	50-100	good-luxuriant >=50%

Storage and Shelf Life

Store below 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (11,12).

Reference

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