PRODUCT SPECIFICATION SHEET

GC Agar Base (DM116)

Intended Use
GC Agar Base is used for selective isolation and cultivation of *Gonococci*, with added blood or haemoglobin and other supplements.

Product Summary and Explanation
In 1945, Johnston described a medium that successfully grew colonies of *N. gonorrhoeae* in 24 rather than 48 hours.[1] GC Agar was introduced in 1947 with reduced agar content. While investigating the growth rate of gonococcal strains, a medium containing growth factors glutamine and cocarboxylase was found to improve recovery.[2] 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. *Neisseria* are fastidious Gram-negative cocci that require nutrient supplementation to grow in laboratory cultures. *N. gonorrhoeae* is naturally competent for DNA transformation as well as being capable of conjugation. These processes allow for the DNA of *N. gonorrhoeae* to acquire or spread new genes. Especially dangerous from the aspect of healthcare is the ability to conjugate, since this can lead to antibiotic resistance.

Thayer and Martin improved the selectivity of GC Medium by the incorporation of the antibiotics colistin, vancomycin and nystatin (VCN) (MS033)[3, 4]. An additional antibiotic trimethoprim lactate [6] was later coupled with VCN to further increase the selectivity of the medium (MS034)[5]. For the cultivation of fastidious organisms the medium should be supplemented with essential growth factors supplied predominantly by yeast extract (MS037). This can be replaced with a chemically defined supplement containing essential growth factors available from yeast extract (MS037). Xfactor needed for the growth of fastidious *Haemophilus* species is provided by haemoglobin (MS088). GC Medium Base can be used as a base for the preparation of Thayer Martin Medium by the addition of MS037, which contains yeast autolysate as a source of essential growth factors and V.C.N.T. antibiotics as selective agents [5,6]. Vancomycin (3 mg/lit) in V.C.N.T. Supplement (MS034) was replaced with lincomycin, since the later was found to be less inhibitory to gonococci [7,8]. Also nystatin was replaced by amphotericin B (in MS034) to improve the selectivity of the medium to yeast contaminants, regularly found in vaginal specimens [9]. This modified supplement is the LCAT Supplement (MS020). Certain strains of gonococci were Composition **found to be sensitive to 3 mg/lt vancomycin regularly used [7]. Therefore the concentration of vancomycin was reduced to 2 mg/lit to obtain the growth of these sensitive strains [9]. This modified supplement with reduced vancomycin concentrations and amphotericin B is the VCAT Supplement (MS032).

Principles of the Procedure
GC Agar is employed as a basal medium in the preparation of Chocolate Agar, Thayer-Martin Medium, Modified Thayer-Martin Medium, Martin-Lewis Agar, and Transgrow Agar.
The Special Peptone provides nitrogen, carbon, and minerals in GC Agar. 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. Sodium Chloride maintains osmotic balance of the medium. Agar is the solidifying agent. Chocolate Agar is prepared from GC Agar with the addition of 2% Hemoglobin. Hemoglobin provides hemin (X factor) required for growth of Haemophilus and enhanced growth of *Neisseria spp*. A chemical enrichment composed of cofactors, vitamins, and nicotinamide adenine dinucleotide (NAD) are also required for growth of *Haemophilus* and *Neisseria spp*. If required, antimicrobial supplements are added as inhibitors for improved selectivity of the medium.
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*. The presence of starch ensures that toxic metabolites produced by *Neisseria* are absorbed. Phosphate buffers are included to prevent changes in pH due to amine production that would affect the survival of the organism.

Formula / Liter

Ingredients	Gms / Liter
Peptone, special	15.00
Corn starch	1.00
Dipotassium phosphate	4.00
Monopotassium phosphate	1.00

Sodium chloride	5.00
Agar	10.00
Final pH: 7.2 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 7.2 grams in 100 ml distilled water, to make a double strength base. Heat to boiling to dissolve the medium completely.
2. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add separately prepared Haemoglobin (MS088) (100 ml sterile 2% solution) and GC Supplement w/ Antibiotics (MS016). Mix well and pour into sterile Petri plates.
3. To increase the selectivity of medium antibiotic supplements such as VCN Supplement (MS033), VCNT Supplement (MS034), LCAT Supplement (MS020) or VCAT Supplement (MS032) may be added. To enhance the nutritional properties of medium, Vitox Growth Supplement (MS035) or Yeast Autolysate Growth Supplement (MS037) may be added.
4. 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 Specifications

Dehydrated Appearance	Cream to yellow colored, homogeneous, free flowing powder
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 of 2.4% Solution	pH : 7.2±0.2 at 25°C
Gel Strength	Firm, comparable with 1.0% Agar gel

Expected Cultural Response:

Cultural characteristics observed in presence of 5-10% Carbon dioxide (CO_2) and 70% humidity with added sterile 2% Haemoglobin (MS088) and GC Supplement with antibiotics (MS016), after an incubation at 35-37°C for 40-48 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	*Haemophilus influenza ATCC 19418*	50 -100	Good-luxuriant	>=50%
2.	*Neisseria gonorrhoeae ATCC19424*	50 -100	Good-Luxuriant (with added Antibiotic supplements)	>=50%
3.	*Neisseria meningitidis ATCC 13090*	50-100	Good-Luxuriant (with added Antibiotic supplements)	>=50%
4.	*Streptococcus pyogenes ATCC19615*	50-100	Good-luxuriant	>=50%
5.	*Streptococcus pneumoniae ATCC 6303*	50-100	Good-luxuriant	>=50%

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. For a complete discussion on the isolation and identification of *Neisseria spp.* and *Haemophilus spp.* Consult procedures outlined in the references.[10,11]
2. Avoid cotton wool for specimen collection.
3. Inoculate immediately after specimen collection.

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

Results
Refer to appropriate references and procedures for results.

Storage
Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration
Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure
Although certain diagnostic tests may be performed directly on GC Agar, biochemical and immunological testing using pure cultures are recommended for complete identification.

Packaging
Product Name : GC Agar Base.
Product Code : DM116
Available Pack sizes : 100gm / 500gm

References

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5. Martin J. E. and Lester A., 1971, HSMHA Health Rep., 86:30
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11. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Further Information
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