

GC AGAR (7104)

Intended Use

GC Agar is used with hemoglobin and enrichment for the isolation and cultivation of *Neisseria gonorrhoeae* and other fastidious organisms in a laboratory setting. GC Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Product Summary and Explanation

In 1945, Johnston described a medium that grew *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 gonococcus, a medium containing growth factors glutamine and cocarboxylase improved recovery.² In 1964, Thayer and Martin formulated a medium incorporating the antibiotics Polymyxin B and Ristocetin, with added supplements, into GC Agar.³ Thayer and Martin improved their medium by replacing the antibiotics with a new solution of Colistin, Vancomycin, and Nystatin (CVN).⁴ In 1970, Martin and Lester improved the new Thayer-Martin Medium by increasing the agar and glucose, and adding Trimethoprim Lactate (T), calling it Modified Thayer-Martin (MTM) Medium.⁵ Martin and Lewis improved selectivity of MTM by increasing the concentration of Vancomycin and replacing Nystatin with Anisomycin for greater inhibition of yeasts, known as Martin Lewis (ML) Agar.⁶

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.

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, carbon, and minerals in GC Agar. Corn Starch absorbs any toxic metabolites produced. The Phosphates are buffering agents. 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.

Formula / Liter

Enzymatic Digest of Casein	7.5 g
Enzymatic Digest of Animal Tissue.....	7.5 g
Corn Starch.....	1 g
Dipotassium Phosphate	4 g
Monopotassium Phosphate	1 g
Sodium Chloride	5 g
Agar	10 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplements

Hemoglobin Solution, 2%, 100 mL
 Growth Enrichment, 2 mL
 Antimicrobials, if required

Precautions

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

Directions, Double Strength

1. Suspend 7.2 g of the medium in 100 mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to 45 – 50°C.
4. Prepare 100 mL of a 2% hemoglobin solution and autoclave at 121°C for 15 minutes.
5. Cool to 45 - 50°C and aseptically add to the molten GC Agar.
6. Add 2 mL of growth enrichment. Add antimicrobials, if desired. Mix thoroughly and dispense.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared GC Agar supplemented as Chocolate Agar is opaque and brown.

Expected Cultural Response: Cultural response on Chocolate Agar incubated at $35 \pm 2^\circ\text{C}$ aerobically or under 7 – 10% CO_2 as appropriate, and examined for growth after 18 – 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Growth
<i>Haemophilus influenzae</i> ATCC® 10211	10 - 300	Good to excellent
<i>Neisseria gonorrhoeae</i> ATCC® 43070	10 - 300	Good to excellent
<i>Neisseria meningitidis</i> ATCC® 13090	10 - 300	Good to excellent
<i>Streptococcus agalactiae</i> ATCC® 13813	10 - 300	Good to excellent
<i>Streptococcus pneumoniae</i> ATCC® 6303	10 - 300	Good to excellent

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

Test Procedure

For a complete discussion on the isolation and identification of *Neisseria* spp. and *Haemophilus* spp. consult procedures outlined in the references.^{7,8}

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

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

Limitation 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

GC Agar	Code No.	7104A	500 g
		7104B	2 kg
		7104C	10 kg

References

1. Johnson, J. 1945. Comparison of gonococcus cultures read at 24 and 48 hours. J. Venera. Dis. Inform. **26**:239.
2. Lankford, C. E., V. Scott, M. F. Cox, and W. R. Cooke. 1943. Some aspects of nutritional variation of the gonococcus. J. Bacteriol. **45**:321.
3. Thayer, J. D., and J. E. Martin, Jr. 1966. Improved medium selective for cultivation of *N. gonorrhoeae* and *N. meningitidis*. Public Health Rep. **81**:559.
4. Thayer, J. D., and A. Lester. 1971. Transgrow, a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. HSMHA Health Service Rep. **86**:30.
5. Martin, J. E., Jr., and R. L. Jackson. 1975. A biological environmental chamber for the culture of *N. gonorrhoeae* with a new commercial medium. Public Health Rep. **82**:361.
6. Martin, J. E., Jr., and J. S. Lewis. 1977. Anisomycin: improved anti-mycotic activity in modified Thayer-Martin Medium. Public Health Rep. **35**:53.
7. Isenberg, H. D. (ed.). 1992. Clinical microbiology procedures handbook. vol. 1. American Society for Microbiology, Washington, D.C.

8. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.



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