CHOCOLATE AGAR

INTENDED USE

Remel Chocolate Agar is a solid medium recommended for use in qualitative procedures for the isolation and cultivation of fastidious organisms, especially *Neisseria* and *Haemophilus* species.

SUMMARY AND EXPLANATION

Chocolate Agar was formulated in 1927 using peptones and yeast extract.[1] Carpenter and Morton improved the recovery time for gonococci to 24 hours using GC Agar Base enriched with hemoglobin and yeast concentrate.[2] The medium was further improved by replacing yeast concentrate with GCHI Enrichment, a chemically defined supplement formulated specifically to facilitate the growth of gonococci.[3]

PRINCIPLE

This medium contains GC Agar Base which supplies casein and meat peptones necessary for bacterial growth, a phosphate buffer to maintain pH, and cornstarch to neutralize toxic fatty acids that may be present. Hemoglobin, when heated, releases hemin, the X factor required by fastidious organisms such as *Haemophilus* spp. GCHI Enrichment is a defined supplement which provides the V factor (NAD), vitamins, amino acids, coenzymes, dextrose, and ferric ions which promote growth of *Neisseria* spp.

REAGENTS (CLASSICAL FORMULAE)*

Hemoglobin	10.0 g	Cornstarch	1.0 g
Casein Peptone	7.5 g	Monopotassium Phosphate	1.0 g
Meat Peptone	7.5 g	●GCHI Enrichment	10.0 ml
Sodium Chloride	5.0 g	Agar	10.0 g
Dipotassium Phosphate	4.0 g	Demineralized Water	1000.0 ml

pH 7.2 +/- 0.2 @ 25°C

●GCHI Enrichment:

Glucose	100.0 g	Cocarboxylase	0.1 g
Cysteine Hydrochloride	25.9 g	Guanine Hydrochloride	0.03 g
L-Glutamine	10.0 g	Ferric Nitrate	0.02 g
L-Cystine	1.1 g	ρ-Aminobenzoic Acid	0.013 g
Adenine	1.0 g	Vitamin B12	0.01 g
NAD	0.25 g	Thiamine Hydrochloride	0.003 g
		Demineralized Water	1000.0 ml

*Adjusted as required to meet performance standards.

PROCEDURE

1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory.
2. If material is being cultured directly from a swab, roll the swab over a small area of the agar surface and streak for isolation.
3. Incubate plate in 5-10% CO_2 (3-7% for *Neisseria**) for 24-48 hours at 33-37°C. Extended incubation may be necessary.
4. Subcultures of *Neisseria gonorrhoeae* should be made within 18-24 hours.

***Note:** CO_2 concentrations higher than 7% may be inhibitory to some strains of *Neisseria*.[4]

QUALITY CONTROL

All lot numbers of Chocolate Agar have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing meets or exceeds CLSI standards.[5] Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
**Haemophilus influenzae* ATCC® 10211	CO_2, 18-24 h @ 33-37°C	Growth
**Neisseria gonorrhoeae* ATCC® 43069	CO_2, 18-24 h @ 33-37°C	Growth
Neisseria meningitidis ATCC® 13090	CO_2, 18-24 h @ 33-37°C	Growth
Streptococcus pneumoniae ATCC® 6305	CO_2, 18-24 h @ 33-37°C	Growth

*CLSI recommended organism

LIMITATIONS

1. Chocolate Agar is an enriched medium on which pathogenic bacteria may be overgrown with other nonpathogenic bacteria. Improved recovery of *N. gonorrhoeae* may be achieved using a selective medium, such as Thayer Martin Agar.

BIBLIOGRAPHY

1. McLeod, J.W., B. Wheatley, and H.V. Phelon. 1927. Br. J. Exp. Pathol. 8:25.
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3. Martin, J.E., Jr., T.E. Billings, J.F. Hackney, and J.D. Thayer. 1967. Public Health Rep. 82:361.
4. Versalovic, J., K.C. Carroll, G. Funke, J.H. Jorgensen, M.L. Landry, and D.W. Warnock. 2011. Manual of Clinical Microbiology. 10th ed. ASM Press, Washington, D.C.
5. Clinical and Laboratory Standards Institute (CLSI). 2004. Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard, 3rd ed. M22-A3. CLSI, Wayne, PA.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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