CHOCOLATE AGAR w/ BACITRACIN

INTENDED USE
Remel Chocolate Agar w/ Bacitracin is a solid medium recommended for use in qualitative procedures for the isolation of *Haemophilus* species from respiratory specimens containing mixed flora.

SUMMARY AND EXPLANATION
Haemophilus influenzae is a common cause of infections of the upper respiratory tract (e.g., epiglottitis, otitis media, sinusitis).[1] While culture remains the primary means of microbiological diagnosis, isolation and identification of *Haemophilus* spp. is often hindered by overgrowth with commensal microbial flora. Finland and Wilcox investigated the action of antibiotics on strains of *H. influenzae* and found all strains tested to be resistant to 750 µg/ml of bacitracin.[2] Del Love and Finland confirmed their results using 1000 µg/ml of bacitracin.[3] Hovig and Aandahl used chocolate agar with 300 µg/ml of bacitracin and reported improved recovery of *Haemophilus* when compared to isolation on sheep blood agar with a streak of *Staphylococcus aureus*.[4]

PRINCIPLE
Casein and meat peptones supply nitrogenous compounds and amino acids necessary for the growth of fastidious organisms, including *Haemophilus* spp. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. Dipotassium and monopotassium phosphates are buffers to maintain pH. Cornstarch neutralizes toxic fatty acids that may be present in the medium. Hemoglobin, when heated, releases hemin, the X factor required by certain fastidious organisms. GCHI Enrichment is a defined supplement which supplies the V factor (NAD), vitamins, amino acids, coenzymes, dextrose, and ferric ions which promote growth. Bacitracin is a selective agent which is inhibitory to most strains of streptococci, staphylococci, *Neisseria* spp., and *Micrococcus* spp. Agar is a solidifying agent.

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	Bacitracin	11,000 U
Dipotassium Phosphate	4.0 g	Agar	10.0 g
		Demineralized Water	1000.0 ml

pH 7.2 ± 0.2 @ 25°C

•GCHI Enrichment:

Dextrose	100.0 g	NAD	0.25 g
Cysteine Hydrochloride	25.9 g	Cocarboxylase	0.1 g
L-Glutamine	10.0 g	Ferric Nitrate	0.02 g
L-Cystine	1.1 g	Vitamin B12	0.01 g
Adenine	1.0 g	ρ-Aminobenzoic Acid	13.0mg
Guanine Hydrochloride	0.3 g	Thiamine Hydrochloride	3.0mg
		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 plates in 5-10% CO_2 for 24-48 hours at 33-37°C. Extended incubation may be required for some strains of *Haemophilus.*
4. Examine plate for typical colony morphology. On Chocolate Agar w/ Bacitracin, colonies of *Haemophilus* spp. are convex, smooth, entire, and 1-2 mm in diameter. Encapsulated strains may appear mucoid.

QUALITY CONTROL
All lot numbers of Chocolate Agar w/ Bacitracin have been tested using the following quality control organisms and have been found to be acceptable. 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
Staphylococcus aureus ATCC® 25923	CO_2, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Streptococcus sanguis ATCC® 10556	CO_2, 18-24 h @ 33-37°C	Inhibition (partial to complete)

BIBLIOGRAPHY

1. Winn, C.W., S.D. Allen, W.M. Janda, E.W. Koneman, G.W. Procop, P.C. Schreckenberger, and G.L. Woods. 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott Williams & Wilkins, Philadelphia, PA.
2. Finland, M. and C. Wilcox. 1950. Am. J. Clin. Pathol. 20:335-340.
3. Del Love, B. and M. Finland. 1950. J. Pediatr. 45:531-537.
4. Hovig, B. and E.H. Aandahl. 1969. Acta. Pathol. Microbiol. Scand. 77:676-684.

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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