PORPHYRIN TEST AGAR

INTENDED USE
REMEL's Porphyrin Test Agar is a solid medium recommended for use in qualitative procedures to speciate the genus *Haemophilus* based on their ability to convert delta-aminolevulinic acid (ALA) to porphyrins.

SUMMARY AND EXPLANATION
Members of the genus *Haemophilus* require hemin (X factor) and/or nicotinamide adenine dinucleotide (NAD - V factor). The need for either one or both factors provides the main means of differentiation of these organisms. Biberstein et al. studied the action of *Haemophilus* strains on ALA and found a perfect correlation between absence of hemin requirement and the ability to convert ALA to porphyrins.[1] White and Granick found that strains of the species *H. influenzae*, *H. aegyptius* and *H. haemoglobinophilus* all lack the enzyme capacities to convert ALA to protoporphyrin.[2] This accounts for their dependence on hemin for growth. In 1974, Kilian described two rapid tests used to detect the ability of non-hemin requiring *Haemophilus* species to synthesize heme precursors from ALA.[3] Lund and Blazevic determined the porphyrin test to be more rapid and accurate than the satellite test used previously to speciate *Haemophilus* organisms.[4]

PRINCIPLE
Porphyrin Test Agar contains hemoglobin as a source of hemin (X factor). GCHI Enrichment is a defined supplement which provides NAD (V factor), vitamins, amino acids, coenzymes, dextrose, and other nutrients for *Haemophilus* species. This medium provides an accurate means of determining X factor requirement based on the observation that X-independent *Haemophilus* strains excrete porphobilinogen and porphyrins, all of which are intermediates in the hemin biosynthetic pathway when supplied with ALA. After incubation, exposure to longwave ultraviolet light will cause an orange-red fluorescence to appear from the bacterial cells that contain porphyrins.

REAGENTS (CLASSICAL FORMULAE)*
Base Medium

Casein Peptone	7.5	g	Corn Starch	1.0	g
Meat Peptone	7.5	g	Delta-aminolevulinic Acid	Q.S.	
Monopotassium Phosphate	1.0	g	Hemoglobin Solution	333.0	ml
Dipotassium Phosphate	4.0	g	Agar	10.0	g
Sodium Chloride	5.0	g	Demineralized Water	667.0	ml
*GCHI Enrichment	10.0	ml			

pH 6.9 +/- 0.2 @ 25°C

*GCHI Enrichment:

Vitamin B-12	0.01	g	Glucose	100.0	g
L-Glutamine	10.0	g	NAD	0.25	g
Adenine	1.0	g	Cocarboxylase	0.1	g
Guanine Hydrochloride	0.03	g	Ferric Nitrate	0.02	g
P-Aminobenzoic Acid	13.0	mg	Thiamine Hydrochloride	3.0	mg
L-Cystine	1.1	g	Cysteine Hydrochloride	25.9	g
Demineralized Water	1000.0	ml			

*Adjusted as required to meet performance standards.

PROCEDURE
1. Inoculate the medium by making a pencil streak across the plate with the suspected *Haemophilus* isolate.
2. Incubate in 5-10% CO2 at 35-37°C for 18-24 hours.

INTERPRETATION OF THE TEST:
Positive Test - orange red fluorescence
Negative Test - no fluorescence

QUALITY CONTROL
All lot numbers of Porphyrin Test Agar 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 ORGANISM	INCUBATION	RESULTS
Haemophilus aphrophilus ATCC® 19415	CO_2, 18-24 h @ 35°C	Positive
Haemophilus parahaemolyticus ATCC® 10014	CO_2, 18-24 h @ 35°C	Positive
Haemophilus parainfluenzae ATCC® 7901	CO_2, 18-24 h @ 35°C	Positive
Haemophilus influenzae ATCC® 10211	CO_2, 18-24 h @ 35°C	Negative

BIBLIOGRAPHY

1. Biberstein, E.L., P.D. Mini, and M.G. Gills, 1963, J. Bacteriol. 86:814-819.
2. White, D.C. and S. Granick. 1963. J. Bacteriol. 85:842-850.
3. Kilian, M. 1974. Acta. Pathol. Microbiol. Scand. Sect. B. 82:835-842.
4. Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken. 1999. Manual of Clinical Microbiology. 7th ed. ASM, Washington, D.C.

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

IFU 1688, Revised January 16, 2003 Printed in U.S.A.

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