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

pH 6.9 +/- 0.2 @ 25°C

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

*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  CO2, 18-24 h @ 35°C  Positive
Haemophilus parahaemolyticus ATCC® 10014  CO2, 18-24 h @ 35°C  Positive
Haemophilus parainfluenzae ATCC® 7901  CO2, 18-24 h @ 35°C  Positive
Haemophilus influenzae ATCC® 10211  CO2, 18-24 h @ 35°C  Negative
BIBLIOGRAPHY

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.

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