HAEMOPHILUS IDENTIFICATION TEST KIT

INTENDED USE
Remel Haemophilus Identification Test Kit contains two reagent-impregnated disks recommended for use in qualitative procedures to test for the growth requirements of various Haemophilus species with simultaneous testing for production of porphyrins.

SUMMARY AND EXPLANATION
Haemophilus spp. require hemin (X factor) and/or nicotinamide adenine dinucleotide (NAD or V factor) for growth. Parker and Hoeprich developed the “disk method” which used paper disks impregnated with hemin or NAD. The disks were placed on an agar medium devoid of the growth factors, which had been inoculated with the test organism. Doern and Chapin reported tryptic soy agar to be the medium of choice for demonstration of growth factor requirements. Biberstein et al. studied the action of Haemophilus strains on delta-aminolevulinic acid (ALA) and found a perfect correlation between the absence of hemin requirement (X factor) and the ability to convert ALA to porphyrins. White and Granick reported some species of Haemophilus lack the enzymes to convert ALA to protoporphyrin, which accounts for their dependence on hemin for growth.

PRINCIPLE
The H disk contains hemin (X factor) and the VP disk contains NAD (V factor) and ALA. The pattern of growth between and/or around the disks demonstrates the requirements of growth for Haemophilus spp. Those able to use ALA as a porphyrin precursor to synthesize protoporphyrin IX, will exhibit an orange fluorescence under a long wave ultraviolet light.

REAGENTS
Reactive Ingredients:
H Disk - Hemin
VP Disk - NAD and delta-aminolevulinic acid (ALA)

PRECAUTIONS
This product is for In Vitro diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE
This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use. Protect product from light, as it is light sensitive.

PRODUCT DETERIORATION
This product should not be used if (1) the color has changed from white on the VP Disk or black/brown with residue on the Hemin Disk, (2) the expiration date has passed, (3) the desiccant has changed from blue to pink, or (4) there are other signs of deterioration. Protect disks from moisture by removing from the vial only those disks necessary for testing. Promptly replace the cap and return the vial to 2-8°C.

SPECIMEN COLLECTION, STORAGE, TRANSPORT
Specimens should be collected and handled following recommended guidelines.

MATERIALS REQUIRED BUT NOT SUPPLIED

PROCEDURE
1. Inoculate 5 ml of sterile Tryptic Soy Broth (REF R07222) or other sterile broth with 3-5 colonies from a fresh, pure culture of the test isolate and mix well.

2. Using a sterile swab, inoculate a plate of Tryptic Soy Agar (REF R01920) by swabbing the entire agar surface to achieve a confluent, even lawn of growth.

3. Allow the inoculum to dry. Place the H and VP disks on the plate approximately 20 mm apart.

4. Incubate in 5-10% CO₂ for 18-24 hours at 35-37°C.

5. Observe growth patterns around the disks.

Porphyrin Test Method A:
1. Using a wooden applicator stick, inoculate the top of the VP disk with 3-5 colonies.

2. In a darkened room, shine a long wave ultraviolet light on the inoculated area of the VP disk and observe for a buff colored area indicating sufficient inoculum has been attained.

3. After overnight incubation, observe the disk for orange fluorescence in a darkened room with a long wave ultraviolet light.

Porphyrin Test Method B:
1. Following overnight incubation, remove the VP disk from the plate and smear the disk across the colony growth around the disks.

2. Place the VP disk back on the plate and incubate at 35-37°C in 5-10% CO₂ for up to 4 hours.

3. Observe the disk for orange fluorescence in a darkened room with a long wave ultraviolet light.
INTERPRETATION

_Haemophilus_ species requiring both X and V factors exhibit growth between the H and VP disks. Species requiring only V factor will exhibit confluent growth on the agar surface.

Porphyrin Test:
- Positive Test - Orange fluorescence on the VP disk
- Negative Test - No fluorescence on the VP disk

QUALITY CONTROL

All lot numbers of Haemophilus Identification Test Kit 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  | 5-10% CO₂, 24 h @ 35-37°C  | Growth between H and VP disks, porphyrin negative

_Haemophilus parainfluenzae_ ATCC® 7901  | 5-10% CO₂, 24 h @ 35-37°C  | Growth around VP disk, porphyrin positive

_Haemophilus parahaemolyticus_ ATCC® 10014  | 5-10% CO₂, 24 h @ 35-37°C  | Growth around VP disk, porphyrin positive

LIMITATIONS

1. Exercise careful colony selection to avoid carryover of X factor from primary media.
2. Clinical specimens may contain more than one species of _Haemophilus_. Strict attention to colony morphology and hemolytic reaction is necessary when selecting colonies from primary isolation media.
3. _Haemophilus_ identification methods requiring growth of the test isolate around X-factor-containing disks, are erroneous 18% of the time. This is due to the fact that most complex media is not totally free of X factor and carryover is difficult to avoid. Therefore, the porphyrin test provides a more accurate means of determining the X factor requirement.³

4. This test is only part of the overall scheme for identification of _Haemophilus_ spp. Further biochemical testing may be required for definitive identification. Consult appropriate references.⁴

BIBLIOGRAPHY


PACKAGING

_Haemophilus_ Identification Test Kit:
REF R21125.................................25 Disks/Vial

Symbol Legend

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