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Porphyrin Reagent

1. INTENDED USE

Remel Porphyrin Reagent is a biochemical single substrate test recommended for use in the determination of the X factor (hemin) requirement for *Haemophilus* spp.

2. SUMMARY AND EXPLANATION

Biberstein et al. studied the action of *Haemophilus* strains with δ-aminolevulinic acid (ALA) and found a correlation between the absence of hemin requirement (X factor) and the ability to convert ALA to protoporphyrin.[1] Non-hemin requiring strains (X-independent) can use ALA as a substrate for the synthesis of porphyrins (another name for hemin). White and Granick found certain species of *Haemophilus* lack the enzyme to convert ALA to protoporphyrin, and are therefore X-dependent.[2] Kilian developed a rapid test for demonstrating porphyrin synthesis using ALA as the substrate.[3]

The substrate used is a combination of δ-aminolevulinic acid (ALA), a hemin precursor, and magnesium in a phosphate buffer. Hemin-independent *Haemophilus* spp. can utilize δ-aminolevulinic acid in the biosynthesis of hemin according to the following pathway:

δ-aminolevulinic acid
↓
porphyrins
↓
porphobilinogen
↓
hemin

Hemin-dependent *Haemophilus* spp. are enzymatically incapable of completing this biosynthesis and therefore, will not convert the ALA substrate.[1-4]

3. PRINCIPLE

Determination of X factor requirement is accomplished by detection of porphyrin synthesis from ALA as indicated by a red-pink fluorescence under long-wave (360 nm) ultraviolet light (Wood's Lamp).

4. REAGENTS (CLASSICAL FORMULA)*

5-aminolevulinic acid.....0.6 g

$MgSO_4 \bullet 7H2O$.....0.2 g

$KH2PO_4$ Buffer ..... 1000.0 ml

*Adjusted as required to meet performance standards.

5. 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.

6. 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 come to room temperature before use. Do not incubate prior to use.

7. PRODUCT DETERIORATION

This product should not be used if (1) the color has changed from colorless, (2) the expiration date has passed, (3) particulate matter is observed, or (4) there are other signs of deterioration.

8. SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.[5]

9. MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swab, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Small glass tubes or filter paper, (7) Pasteur pipettes.

10. PROCEDURE

10.1. Preparation of inoculum: Remove test organisms from solid non-selective media that has been incubated for 18-24 hours (preferably), but not more than 48 hours.

Glass Tube Method:

a. Dispense 2-3 drops of Porphyrin Reagent into a clean, glass tube.

b. Suspend sufficient growth in the reagent to achieve a dense or "milky" suspension.

Filter Paper Method:

a. Saturate a section of filter paper with 2-3 drops of Porphyrin Reagent.

b. Rub test organisms onto the reagent saturated filter paper in an empty Petri dish.

10.2. Incubate the inoculated test at 35-37°C in a non-CO_2 atmosphere for up to 4 hours.

10.3. Expose the test suspension or filter paper to a long-wave ultraviolet light (Wood's Lamp) and observe for the presence or absence of a red-pink fluorescence.

11. INTERPRETATION

Positive test	Red-pink fluorescence (Not X factor dependent)
Negative test	No red-pink fluorescence (X factor dependent)

12. QUALITY CONTROL

All lot numbers of Porphyrin Reagent 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
H. parainfluenzae ATCC® 7901	Aerobic, 4h @ 35°C	Positive
H. influenzae ATCCÆ 9006	Aerobic, 4h @ 35°C	Negative

13. LIMITATIONS

The determination of X factor requirement is only one step in the overall scheme for the identification of *Haemophilus* spp. and should be used in conjunction with other tests.

14. EXPECTED VALUES

The following table lists the expected results for *Haemophilus* spp. using Porphyrin Reagent.

ORGANISM	FLUORESCENCE	REQUIRES X FACTOR
H. aegyptius	Negative	Yes
H. ducreyi	Negative	Yes
H. haemolyticus	Negative	Yes
H. influenzae	Negative	Yes
H. parainfluenzae	Positive	No
H. segnis	Positive	No
H. parahaemolyticus	Positive	No
H. paraphrophilus	Positive	No
H. aphrophilus	Positive	No

15. 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. Section B 82:835-842.
4. Washington, J.A. 1981. Laboratory Procedures in Clinical Microbiology. Springer-Verlag, New York, NY.
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16. PACKAGING

REF R8388001.....5 ml/Dropper Tube (50-75 determinations)

17. SYMBOL LEGEND

REF	Catalogue Number
IVD	*In Vitro* Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp.)
LAB	For Laboratory Use Only
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufactured by

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