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Section: Technical Manual	Subject Title: ALA (Rapid Porphyrin Test)	
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ALA (RAPID PORPHYRIN TEST)

Principle

This test is used for rapidly detecting porphyrin as a means of speciating *Haemophilus* species. Enzymes which convert ALA (delta - aminolevulinic acid) to porphyrins in the biosynthesis of hemin (X factor) are produced by *Haemophilus parainfluenzae* but not by *H. influenzae*. The production of porphyrins is detected by examination with an ultra-violet (UV) light.

Reagents

BBL TAXO Differentiation Disks ALA. (Store refrigerated in the dark. Allow 10-15 minutes for the container to reach room temperature before opening).

Sterile distilled water

Other Materials

Petri dish
Inoculating loop
Gauze
Long-wave UV lamp
Forceps

Procedure

1. Place one ALA disk for each organism to be tested on the inside of a Petri dish using forceps.
2. Moisten each disk with one drop of sterile water.
3. Rub a loopful of the test organism onto the moistened disk holding it in place with sterile forceps.
4. Saturate gauze with water, squeeze out any excess and place it in the petri dish as far away from the disks as possible.
5. Incubate at 35°C.
6. Examine at hourly intervals for 6 hours by removing the top of the petri dish and exposing the disks to UV light in a darkened room. **NB:** Wear UV safety goggles when using the UV light.

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Interpretation

- A. Positive: Orange-red fluorescence
- B. Negative: No fluorescence observed

Precautions

- 1. Use for differentiating *Haemophilus* spp. only.
- 2. Best results are obtained when a heavy inoculum is used.
- 3. ALA is light sensitive. Disks must be protected from light.

Quality Control

Test the following positive and negative controls each time an unknown is tested:

Positive: *H. parainfluenza* (ATCC 7901)
Negative: *H. influenzae* (ATCC 35056)

Reference

BBL TAXO Differentiation Disks ALA package insert, 1999. Becton Dickinson Microbiology Systems.