Our Porphyrin (ALA Substrate) tubes are used to differentiate *Haemophilus* species based on their ability to synthesize heme and its precursors. Porphyrin tubes can replace conventional satellite X-factor tests with the added benefit of increased accuracy and rapidity of results.

Our Porphyrin tubes contain δ-aminolevulinic acid (ALA) which is the precursor for porphobilinogen, porphyrins, and heme. Most *Haemophilus* species require exogenous X factor (hemin) for growth, but certain strains of *Haemophilus* which possess the enzyme porphobilinogen synthase are hemin-independent and can synthesize their own heme. *Haemophilus* species with the enzyme will excrete different by-products including porphobilinogen and porphyrins. Detection of these by-products is possible since many porphyrins (i.e. uroporphyrin, copro-porphyrin, protoporphyrin) emit a strong red fluorescence when illuminated by ultraviolet light. An alternative method for detection of porphyrins is the addition of Kovac’s Reagent, which produces a pink end-product if porphyrins are present.

**Formula per Litre of Medium**

- Disodium phosphate ........................................ 7.9 g
- Monopotassium Phosphate ...................... 6.1 g
- δ-Aminolevulinic acid ............................... 0.34 g
- Magnesium sulfate ..................................... 0.096 g

pH 6.9 ± 0.2

**Recommended Procedure**

1. Allow the medium to adjust to room temperature.
2. Using a very heavy inoculum taken from an 18-24 hour pure culture of suspected *Haemophilus* species inoculate the Porphyrin tube.
3. Incubate tube aerobically at 35°C for 4-6 hours.
4. After incubation, expose the inoculated substrate tube to an UV (360nm) source in a darkened room or black box and observe for fluorescence.
5. If an UV source is not available, add 0.5 mL of Kovac’s Reagent (Dalynn RK75) to the tube and mix vigorously. Wait 5 minutes to allow phases to separate and interpret.

**Interpretation of Results**

Positive: Red fluorescence under UV light or the development of a pink color after the addition of Kovac’s Reagent indicates that porphyrins are present and that the *Haemophilus* strain **does not** require exogenous X factor

Negative: No fluorescence under UV light or no color change (yellow) after the addition of Kovac’s Reagent indicates that porphyrins are not present and that the *Haemophilus* strain **does** require exogenous X factor

- **Cultures being tested must not be older than 24 hours**
- **The inoculum must be heavy otherwise the 4-6 hour incubation period may be inadequate and result in false negatives**
• Test only Haemophilus species since other bacteria commonly found in the oropharynx can make heme and yield false-positive results

• The fluorescence test has been shown to be superior to the use of Kovac’s reagent due to its increased sensitivity

Quality Control

After checking the medium for correct pH, colour, depth, slant and sterility, the following organisms are used to determine the performance of the completed medium.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Expected results</th>
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<tbody>
<tr>
<td><strong>Haemophilus parainfluenzae</strong> ATCC 7901</td>
<td>+ Red fluorescence</td>
</tr>
<tr>
<td><strong>Haemophilus influenzae</strong> ATCC 10211</td>
<td>– No fluorescence</td>
</tr>
</tbody>
</table>

Storage and Shelf Life

Our Porphyrin (ALA Substrate) tubes should be stored in an upright position at -20°C and protected from light. Under these conditions the substrate has a shelf life of 26 weeks from the date of manufacture.

References