Sero: Brentamine Presumptive Test for Acid Phosphatase

Principle
Acid phosphatase is found in high concentrations in semen.

A reaction that results in the formation of a purple azo dye is used to indicate the presence of acid phosphatase.

Sodium α-napthyl phosphate is cleaved by the acid phosphatase from the stain, releasing sodium phosphate and naphthol. Naphthol couples with brentamine (o-dianisidine tetrazotized) to create the purple azo dye.

Supplies
The following supplies are used in this procedure:
- filter paper
- cotton swabs
- glass plates.

Reagents
This procedure uses the following reagents:

**Brentamine Test Reagent (10 mL)**
Dissolve 0.26 grams of SERI Acid Phosphatase Spot Test PMR in 10 mL of deionized water. This solution must be made fresh prior to use.

Alternate preparation:
- 10 mg α-naphthyl acid phosphate, monosodium salt
- 10 mg o-dianisidine tetrazotized
Dissolve the chemicals in 10 mL of Brentamine Buffer. This reagent must be made fresh prior to use.

**Brentamine Buffer (100 mL)**
Dissolve 1.2 grams of sodium acetate (anhydrous) in 100 mL of deionized water. Adjust pH to 5.0 with glacial acetic acid.

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Quality control
The Brentamine Test Reagent must be tested against a positive control (semen stain) and a negative control (water) before each use. The results of these tests are recorded in the case notes.

Overlay procedure
Use the following procedure to perform the Brentamine Spot Test on filter paper overlays.

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisten a suitably sized piece of filter paper with deionized water.</td>
</tr>
<tr>
<td>2</td>
<td>Lay the filter paper over the area to be tested (for example, swab or area on clothing) and maintain good contact for at least 15 seconds. Mark seams or other reference points for orientation.</td>
</tr>
<tr>
<td>3</td>
<td>After removing the filter paper from the evidence item, apply the Brentamine Test Reagent until the filter paper is saturated.</td>
</tr>
<tr>
<td></td>
<td>• The development of a purple color within 60 seconds indicates a positive (+) test for the presence of acid phosphatase.</td>
</tr>
<tr>
<td></td>
<td>• The absence of a color reaction within 60 seconds indicates a negative (-) test for the presence of acid phosphatase.</td>
</tr>
</tbody>
</table>

Swab procedure
Use the following alternate procedure to perform the Brentamine Spot Test on swabs.

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisten a swab with deionized water.</td>
</tr>
<tr>
<td>2</td>
<td>Rub the swab over the area to be tested.</td>
</tr>
<tr>
<td>3</td>
<td>Add a drop of Brentamine Test Reagent to the swab.</td>
</tr>
<tr>
<td></td>
<td>• The development of a purple color within 60 seconds indicates a positive (+) test for the presence of acid phosphatase.</td>
</tr>
<tr>
<td></td>
<td>• The absence of a color reaction within 60 seconds indicates a negative (-) test for acid phosphatase.</td>
</tr>
</tbody>
</table>

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Interpretation

The following information must be considered when interpreting results of this analysis.

Acid phosphatase is present in semen in high concentrations; however, it is also present in other body fluids at lower concentrations and in plants, fungi and bacteria. Semen stains tend to give a faster and stronger reaction than other sources.

Since acid phosphatase is not unique to semen, the brentamine spot test is a presumptive test only. The presence of semen must be confirmed by microscopic examination for spermatozoa, the identification of p30 or the identification of semenogelin.

References

The following references were used in the development of this procedure:


Forensic Examination of Sexual Assault Evidence Training Manual, California Criminalistics Institute, California Department of Justice, 1992.