Sero: Takayama Confirmatory Test for Blood

Principle

The Takayama (Hemochromogen) test is specific for hemoglobin and is based on the formation of insoluble, pink, needle-shaped crystals of hemochromogen, resulting from a series of reactions involving the components of the Takayama reagent.

Sodium hydroxide releases the heme prosthetic groups from the globin through alkaline hydrolysis. Glucose reduces the heme iron, and pyridine combines with it to form the product, pyridine ferroprotoporphyrin.

Equipment and supplies

This procedure uses the following laboratory equipment and supplies:
- hotplate
- compound microscope
- glass slides
- coverslips
- disposable pipets.

Reagents

This procedure uses the following reagents:

**Saturated Aqueous Glucose Solution** (100 mL)

Using heat, dissolve 100 grams of glucose in enough water to make 100 mL.

**10% Aqueous Sodium Hydroxide** (100 mL)

Dissolve 10 grams of sodium hydroxide in enough water to make 100 mL.

**Takayama Reagent** (50 mL)

10 mL Saturated Aqueous Glucose Solution
10 mL 10% Aqueous Sodium Hydroxide
10 mL pyridine
20 mL deionized water

Combine and store refrigerated in a dark bottle.
Sero: Takayama Confirmatory Test for Blood, Continued

Quality testing

The Takayama Reagent must be tested against a positive control (bloodstain) before each use. The results of these tests are recorded in the case notes.

Procedure

Use the following procedure to perform the Takayama test.

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<th>Step</th>
<th>Action</th>
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<tbody>
<tr>
<td>1</td>
<td>Place a small portion of suspected blood (scraped, teased apart thread, concentrated extract) on a microscope slide.</td>
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<tr>
<td>2</td>
<td>Add a drop of Takayama reagent and cover with a coverslip.</td>
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</table>
| 3    | Warm the slide on a hotplate (a characteristic reddish color will appear around the sample).  
|      | NOTE: This step is optional. |
| 4    | Observe microscopically for pink needle or rhomboid shaped crystals. |

Interpretation

A positive reaction, the formation of pink needle or rhomboid-shaped crystals with the Takayama reagent, confirms the presence of blood in suspected stains.

However, the absence of the characteristic crystals does not confirm the absence of blood.

References

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