AURAMINE- RHODAMINE FLUORESCENCE - ACID FAST BACTERIA

PURPOSE: To demonstrate acid fast bacteria, mycobacterium tuberculosis.

PRINCIPLE: Both dyes are basic dyes that fluoresce at short wavelengths.

CONTROL: Tissue containing acid-fast organisms. Millipore™-filtered water only should be used in the floatation bath, in stain preparation, and procedure.

FIXATIVE: 10% formalin

TECHNIQUE: Cut paraffin sections 4-5m

EQUIPMENT: Coplin jars (all glassware must be rinsed with DI water) microwave oven, fluorescence microscope.

REAGENTS:

Auramine- Rhodamine Solution:
Auramine O 10.5 gm
Rhodamine B 5.25 gm
Glycerol 525.0 ml
Phenol 70.0 ml
Millipore™ water 350.0 ml

Rinse all glassware in distilled water. Place the solution in a 60°C oven overnight, mix on stir plate. Filter amount needed prior to use, discard after use. Label with initials and date, solution is stable for 1 year.

CAUTION: Possible carcinogen.

0.5% Acid Alcohol:
Hydrochloric acid 5.0 ml
70% alcohol 995.0 ml
Mix well, label with date and initials. Solution is stable for 1 year.

CAUTION: Flammable, corrosive.

0.5% Potassium Permanganate:
Potassium permanganate 0.25 gm
Distilled water 50.0 ml
Make fresh, discard after use.

CAUTION: Corrosive.
SAFETY: Wear gloves, lab coat and goggles. Work in a well ventilated area, preferable under a hood. Avoid contact and inhalation of chemicals.

Auramine O: possible carcinogen to humans. Animal studies; causes tumors, neoplastic.

Rhodamine B: possible carcinogen, equivocal tumorigenic.

Phenol: toxic by ingestion, inhalation and skin absorption. Readily absorbed through skin, causing increased heart rate, convulsions and death. Will burn eyes and skin, analgesic action may cause loss of pain. Target organ effects in digestive, nervous and urinary systems.

Hydrochloric acid: strong irritant to skin, eyes and respiratory system. Target organ effects via inhalation on skin, respiratory, reproductive and fetal systems.

Potassium permanganate: skin and eye irritant, ingestion will lead to severe gastrointestinal distress. Oxidant.

PROCEDURE:
1. Deparaffinize and bring to distilled water.
2. *Auramine-rhodamine solution, microwave 80 power, 45 seconds, allow to stand 5 minutes.
3. Differentiate sections in acid alcohol until colorless.
4. Wash in running tap water.
5. Potassium permanganate, 2 minutes.
6. Rinse in distilled water.
7. Dehydrate, clear, and coverslip.
   * Conventional Method: 60°C oven for 1 hour.

RESULTS:
Acid-fast organisms: reddish-yellow fluorescence
Background: black
MICROORGANISMS

AURAMINE - RHODAMINE

REFERENCES:
Carson F, Histotechnology: A Self-Instructional Text, 1990, pp192-193, ASCP, ILL
Crookham J, Dapson R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: ____________________________ By: ________________

Approved: ____________________________ By: ________________

Downloaded from WebPath: Internet Pathology Laboratory
http://www-medlib.med.utah.edu/WebPath/webpath.html
PROCEDURE CARD

ACID FAST BACTERIA - AURAMINE- RHODAMINE FLUORESCENCE

CONTROL: Tissue containing acid-fast organisms. Millipore™-filtered water only should be used in the floatation bath, in stain preparation, and the staining procedure.

PROCEDURE:
1. Deparaffinize and bring to distilled water.
2. *Auramine-rhodamine solution, microwave 80 power, 45 seconds, allow to stand 5 minutes.
3. Differentiate sections in acid alcohol until colorless.
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RESULTS:
Acid-fast organisms reddish-yellow fluorescence
Background black

SAFETY: Wear gloves, lab coat and goggles. Work in a well ventilated area, preferrable under a hood.

Auromine and Rhodamine: carconogen.

Open hot solutions within exhaust hood. Phenol will burn the skin wear nitrile gloves.

Auramine- Rhodamine Solution:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auramine O</td>
<td>10.5 gm</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>5.25 gm</td>
</tr>
<tr>
<td>Glycerol</td>
<td>525.0 ml</td>
</tr>
<tr>
<td>Phenol</td>
<td>70.0 ml</td>
</tr>
<tr>
<td>Millipore™ water</td>
<td>350.0 ml</td>
</tr>
</tbody>
</table>

Rinse all glassware in distilled water. Place the solution in a 60°C oven overnight, mix on stir plate. Filter amount needed prior to use, discard after use. Label with initials and date, solution is stable for 1 year.

CAUTION: Carcinogen.

0.5% Acid Alcohol:

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<tr>
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<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrochloric acid</td>
<td>5.0 ml</td>
</tr>
<tr>
<td>70% alcohol</td>
<td>995.0 ml</td>
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</tbody>
</table>

Mix well, label with date and initials. Solution is stable for 1 year.

CAUTION: Flammable, corrosive.

0.5% Potassium Permanganate:

<table>
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<tr>
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<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium permanganate</td>
<td>0.25 gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>50.0 ml</td>
</tr>
</tbody>
</table>

Make fresh, discard after use.

CAUTION: Corrosive.
**Auramine-Rhodamine Solution:**

Auramine O 10.5 gm  
Rhodamine B 5.25 gm  
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TECH: ________________________  
DATE: ________________________  
EXPIRATION: __________________

**0.5% Acid Alcohol:**

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