**STEINER & STEINER METHOD - SPIROCHETES & LEGIONELLA**

**PURPOSE:** Spirochetes are a spiral-shaped bacteria. Their size, shape, and flagella vary according to the genera. Treponema, cause: syphilis, Yaws, and Pinta diseases. Borrelia, includes: Lyme disease. The third infection to humans is Leptospira. Campylobacter pyloridis (heliobactor) and Legionella pneumophila bacteria. Cat Scratch disease bacteria are also stained with this technique.

**PRINCIPLE:** The bacteria absorb the silver from the silver solution, with the aide of a reducing agent, the silver is then transformed to a visible metallic state.

**CONTROL:** Tissue containing known positive spirochetes or legionella.

**FIXATIVE:** 10% formalin

**TECHNIQUE:** Cut paraffin sections 4-5m.

**EQUIPMENT:** Acid cleaned glassware, coplin jars, Whatman #4 filter paper, and microwave oven.

**REAGENTS:**

**0.5% Uranyl Nitrate:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uranyl nitrate</td>
<td>0.5 gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100.0 ml</td>
</tr>
</tbody>
</table>

Mix well, pour into acid cleaned bottle. Stable for 1 year.

**CAUTION:** Severe irritant, corrosive, possible carcinogen.

**0.5% Silver Nitrate:**

<table>
<thead>
<tr>
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<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver nitrate</td>
<td>0.5 gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100.0 ml</td>
</tr>
</tbody>
</table>

Mix well, pour into an acid cleaned bottle, store in the refrigerator. Solution is stable for 1 year.

**CAUTION:** Severe irritant, corrosive, possible carcinogen.

**2% Hydroquinone:**

<table>
<thead>
<tr>
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<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroquinone</td>
<td>0.5 gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>25.0 ml</td>
</tr>
</tbody>
</table>

Make fresh.

**CAUTION:** Irritant, possible carcinogen.
**10% Gum Mastic Stock Solution:**

<table>
<thead>
<tr>
<th></th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gum mastic</td>
<td>10.0 ml</td>
</tr>
<tr>
<td>Absolute alcohol</td>
<td>100.0 ml</td>
</tr>
</tbody>
</table>

Allow to dissolve overnight. Filter through #1 filter paper, into an airtight bottle.

**CAUTION:** Flammable.

**2.5% Gum Mastic:**

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>10% Gum mastic stock</td>
<td>15.0 ml</td>
</tr>
<tr>
<td>Absolute alcohol</td>
<td>75.0 ml</td>
</tr>
</tbody>
</table>

Filter through Whatman #4 filter paper. Solution is stable for 6 months.

**CAUTION:** Flammable.

**Reducing Solution:**

<table>
<thead>
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<tbody>
<tr>
<td>2.5% gum mastic</td>
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</tr>
<tr>
<td>2% hydroquinone</td>
<td>25.0 ml</td>
</tr>
<tr>
<td>0.5% silver nitrate</td>
<td>0.6 ml</td>
</tr>
</tbody>
</table>

Add gum mastic to the hydroquinone, then the silver nitrate. Prepare just before use. The solution will appear milky. Do not filter. Discard.

**CAUTION:** Irritant, possible carcinogen.

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**SAFETY:** Wear gloves, goggles and lab coat, especially when working with dry chemicals. Keep hot, uncapped solutions under the fume hood.

Uranyl nitrate; severe irritant, highly toxic. Target organ effects on liver, urinary, circulatory and respiratory systems, radiation hazard from inhalation of fine particles.

Silver nitrate: severe skin and eye irritant. Oxidizer. Ingestion will produce violent gastrointestinal discomfort. Possible carcinogen: equivocal tumorigenic agent.

Hydroquinone; irritant to skin and eyes. Toxic by inhalation and ingestion. Chronic exposure has caused bladder cancer in mice.

**PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. 0.5% uranyl nitrate, microwave 70 power, 30 seconds.
3. Rinse in several changes of hot distilled water.
4. 0.5% silver nitrate, microwave 70 power, 40 seconds. Allow slide to stand in solution for 90 seconds, agitate.
5. Rinse in several changes of hot distilled water.
6. 95% alcohol, 2 changes.
7. 100% alcohol, 2 changes.
8. 2.5% gum mastic, 1 minute.
9. Reducing solution, microwave 40 power, 30 seconds. Agitate slides, microwave 40 power, 30 seconds. Allow slides to stand in solution for 2 minutes, or until tissue sections turn yellow or brownish yellow.
10. Rinse in 3 changes of distilled water.
11. Dehydrate, clear, and coverslip.

RESULTS:
Spirochetes, legionella, and campylobactor black
Background yellow

NOTES:
1. Use acid cleaned glassware, be sure it is all well rinsed in distilled water.
2. Microwave 1000 ml of distilled water, Hi power, 5 minutes, for the hot distilled rinse water.

REFERENCES:
Churukian C, Dept Patho, University of Rochester Medical Center, Rochester, NY 14642, 1989
Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech
PROCEDURE CARD

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0.5% Uranyl Nitrate:
Uranyl nitrate 0.5 gm
Distilled water 100.0 ml
Mix well, pour into acid cleaned bottle. Stable for 1 year.

CAUTION: Severe irritant, corrosive, possible carcinogen.

2% Hydroquinone:
Hydroquinone 0.5 gm
Distilled water 25.0 ml
Make fresh.

CAUTION: Irritant, possible carcinogen.

0.5% Silver Nitrate:
Silver nitrate 0.5 gm
Distilled water 100.0 ml
Mix well, pour into an acid cleaned bottle, store in the refrigerator. Solution is stable for 1 year.

CAUTION: Severe irritant, corrosive, possible carcinogen.

Reducing Solution:
2.5% gum mastic 15.0 ml
2% hydroquinone 25.0 ml
0.5% silver nitrate 0.6 ml
Add gum mastic to the hydroquinone, then the silver nitrate. Prepare just before use. The solution will appear milky. Do not filter. Discard.

CAUTION: Irritant, possible carcinogen.
10% Gum Mastic Stock Solution:
Gum mastic 10.0 ml
Absolute alcohol 100.0 ml
Allow to dissolve overnight. Filter through #1 filter paper, into an airtight bottle.
CAUTION: Flammable.

DATE:__________________________
TECH:__________________________
EXPIRATION:____________________

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Distilled water 100.0 ml
Mix well, pour into acid cleaned bottle. Stable for 1 year.
CAUTION: Severe irritant, corrosive, possible carcinogen.

DATE:__________________________
TECH:__________________________
EXPIRATION:____________________

2.5% Gum Mastic:
10% Gum mastic stock 15.0 ml
Absolute alcohol 75.0 ml
Filter through Whatman #4 filter paper. Solution is stable for 6 months.
CAUTION: Flammable.

DATE:__________________________
TECH:__________________________
EXPIRATION:____________________

0.5% Silver Nitrate:
Silver nitrate 0.5 gm
Distilled water 100.0 ml
Mix well, pour into an acid cleaned bottle, store in the refrigerator. Solution is stable for 1 year.
CAUTION: Severe irritant, corrosive, possible carcinogen.

DATE:__________________________
TECH:__________________________
EXPIRATION:____________________