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

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**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 (heliobacter) 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 aid 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:**

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.

**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.

**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.

**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.

**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.





**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: \_\_\_\_\_

**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.

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: \_\_\_\_\_