STAINING MANUAL - MICROORGANISMS Page: 1 of 3

## STEINER & STEINER METHOD - SPIROCHETES & LEGIONELLA

Date:

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: 0.5% Silver Nitrate:

Uranyl nitrate 0.5 gm Silver nitrate 0.5 gm Distilled water 100.0 ml Distilled water 100.0 ml

Mix well, pour into an acid cleaned

bottle, store in the refrigerator.

CAUTION: Severe irritant, corrosive,

Solution is stable for 1 year.

possible carcinogen.

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

CAUTION: Severe irritant, corrosive, possible carcinogen.

2% Hydroquinone:

Hydroqinone 0.5 gm Distilled water 25.0 ml

Make fresh.

CAUTION: Irritant, possible carcinogen.

#### **MICROORGANISMS**

## STEINER & STEINER

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:

15.0 ml
25.0 ml
0.6 ml

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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.

### **MICROORGANISMS**

## STEINER & STEINER METHOD

- 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:

- 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

Prepared:	By:
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### PROCEDURE CARD

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Uranyl nitrate 0.5 gm Hydroqinone 0.5 gm Distilled water 100.0 ml Distilled water 25.0 ml

CAUTION:

Irritant,

possible

15.0 ml

Mix well, pour into acid cleaned bottle. Stable Make fresh.

for 1 year.

CAUTION: Severe irritant, corrosive, carcinogen.

possible carcinogen.

Reducing Solution: 0.5% Silver Nitrate: 2.5% gum mastic

Silver nitrate 0.5 gm 2% hydroquinone 25.0 ml Distilled water 100.0 ml 0.5% silver nitrate 0.6 ml

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

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

CAUTION: Severe irritant, corrosive,

possible carcinogen. CAUTION: Irritant, possible carcinogen.

Discard.

10% Gum Mastic Stock	2.5% Gum Mastic:
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Gum mastic 10.0 ml	Absolute alcohol 75.0 ml
Absolute alcohol 100.0 ml	Filter through Whatman #4 filter
Allere de discolus commissis Eller	Filter through Whatman #4 filter
Allow to dissolve overnight. Filter	paper. Solution is stable for 6
through #1 filter paper, into an	months.
airtight bottle.	CAUTION: Flammable.
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	DATE:
DATE:	
	TECH:
TECH:	
	EXPIRATION:
EXPIRATION:	
	0.5% Silver Nitrate:
0.5% Uranyl Nitrate:	Silver nitrate 0.5 gm
Uranyl nitrate 0.5 gm	Distilled water 100.0 ml
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•	· ·
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DATE:	DATE:
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