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**GMS - METHENAMINE SILVER - GROCOTT'S, MODIFIED**

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**PURPOSE:** To identify fungi.

**PRINCIPLE:** The mucopolysaccharide components of the fungal cell wall are oxidized to release aldehyde groups. The aldehyde groups then react with the silver nitrate, reducing it to a metallic silver, rendering them visible.

**CONTROL:** Any tissue containing fungus. A control block made up from tissue containing aspergillosis and pneumocystis is preferred.

**FIXATIVE:** 10% formalin

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

**EQUIPMENT:** Acid cleaned glassware, coplin jars, microwave oven.

**REAGENTS:**

**2% Chromic Acid:**

Chromium trioxide            10.0 gm  
Distilled water                500.0 ml

Mix, solution is stable for 6 months.

**CAUTION:** Corrosive acid, possible carcinogen, avoid contact and inhalation.

**1% Sodium Metabisulfite:**

Sodium metabisulfite        5.0 gm  
Distilled water                500.0 ml

Mix, solution is stable for 6 months.

**5% Borax:**

Sodium borate                5.0 gm  
Distilled water                100.0 ml

Solution is stable for 3 months.

**Methenamine Silver**

**Stock Solution:**

3% Methenamine  
(hexamethylenetetramine) 100.0 ml  
5% Silver nitrate                5.0 ml

Mix, pour into an acid cleaned brown bottle. Store in the refrigerator. Solution is stable for 3 months.

**CAUTION:** Corrosive, possible carcinogen.

**0.5% Gold Chloride:**

Gold chloride                0.5 gm  
Distilled water                100.0 ml

Mix, store in acid cleaned bottle, refrigerate. Stable for 1 year.

**CAUTION:** Avoid contact and inhalation.

**0.2% Light Green:**

Light green SF yellow.	0.2 gm
Distilled water	100.0 ml
Glacial acetic acid	0.2 ml

Mix. Stable for 6 months.

**CAUTION:** Avoid contact and inhalation.

**SAFETY:** Wear gloves, goggles and lab coat. Keep hot uncapped solutions under the fume hood. Avoid contact and inhalation of dyes and chemicals.

Chromic acid; corrosive to skin and mucous membranes. Highly toxic. Target organ effects on kidney. Carcinogen.

Sodium metabisulfite; Toxic on ingestion. Can cause GI distress. Irritant to skin, eyes and mucous membranes.

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

Sodium thiosulfate: Toxic on ingestion. Can irritate the stomach. Irritant to skin, eyes and respiratory tract.

Light Green SF Yellowish; possible carcinogen.

**PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. \*2% Chromic acid, microwave Hi power, 45 seconds, allow to stand for 5 minutes.
3. Wash in tap water, rinse in distilled.
4. 1% Sodium metabisulfite, 1 minute, room temperature.
5. Wash in tap water, rinse in distilled 3 changes.
6. \*Working methenamine silver solution, microwave Hi power, 70 seconds, tissue should be the color of a brown paper bag. Agitate the slides in the hot solution.
7. Rinse in distilled water, 2 changes.
8. 0.5% Gold chloride, 1 minute or until gray.
9. Wash in distilled water.
10. 5% Hypo, 3 minutes.
11. Wash in tap water, rinse in distilled.
12. Working Light green, 1 minute.
13. Rinse in distilled water.
14. Dehydrate, clear, and coverslip.

\*Conventional Method: 5% chromic acid, 60°C waterbath for 1 hour.  
Silver solution, 60°C water bath for 1 hour or until brown.

**RESULTS:**

Fungi	black
Background	green

**NOTES:**

1. If the chromic acid turns brown it needs to be changed. Note the 2% rather than 5% for the conventional method, the stronger chromic acid causes the tissue to fall off the slide when using the microwave technique.
2. If the organisms are not turning black, check:
  - a. fresh chromic acid.
  - b. fresh sodium metabisulfite.
  - c. fresh borax.
3. If the Working silver solution turns cloudy or mirrors on the sides of the coplin jar, make a new solution.

**REFERENCES:**

- Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp243-246, Battelle Press, Ohio
- Luna L, Manual of Histologic Staining Methods of the AFIP, 3rd Ed, 1968, pp 230-231, McGraw-Hill, NY
- Crookham, J, Dapson, R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: \_\_\_\_\_ By: \_\_\_\_\_

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## PROCEDURE CARD

### GMS - METHENAMINE SILVER, GROCOTT'S, MODIFIED

Page 1 of 2

**CONTROL:** Any tissue containing fungus.

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

#### PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. \*Chromic acid, microwave Hi power, 45 seconds, allow to stand for 5 minutes.
3. Wash in tap water, rinse in distilled.
4. 1% Sodium metabisulfite, 1 minute, room temperature.
5. Wash in tap water, rinse in distilled 3 changes.
6. \*Working methenamine silver solution, microwave Hi power, 70 seconds, tissue should be the color of a brown paper bag. Agitate the slides in the hot solution.
7. Rinse in distilled water, 2 changes.
8. 0.5% Gold chloride, 1 minute or until gray.
9. Wash in distilled water.
10. 5% Hypo, 3 minutes.
11. Wash in tap water, rinse in distilled.
12. Working Light green, 1 minute.
13. Rinse in distilled water.
14. Dehydrate, clear, and coverslip.

\*Conventional Method: 60°C waterbath for 1 hour for both the chromic acid and the silver solution. Leave the slides in the hot silver solution until they are brown.

#### RESULTS:

Fungi: black  
Background: green

**SAFETY:** Keep hot solutions in fume hood. Avoid contact and inhalation.

#### 2% Chromic Acid:

Chromium trioxide 10.0 gm  
Distilled water 500.0 ml

Mix, solution is stable for 6 months.

**CAUTION:** Corrosive acid, possible carcinogen, avoid contact and inhalation.

#### 1% Sodium Metabisulfite:

Sodium metabisulfite 5.0 gm  
Distilled water 500.0 ml

Mix, solution is stable for 6 months.

#### 5% Borax:

Sodium borate 5.0 gm  
Distilled water 100.0 ml

Solution is stable for 3 months.

#### Methenamine Silver

##### Stock Solution:

3 % M e t h e n a m i n e  
(hexamethylenetetramine) 100.0 ml  
5% Silver nitrate 5.0 ml

Mix, pour into an acid cleaned brown bottle. Store in the refrigerator. Solution is stable for 3 months.

**CAUTION:** Corrosive, possible carcinogen.

##### Working Solution:

Stock Silver Methen. 25.0 ml  
Distilled water 25.0 ml  
5% Borax 2.5 ml

Mix just before use, discard.

PROCEDURE CARD

**GMS - METHENAMINE SILVER, GROCOTT'S, MODIFIED**

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**0.5% Gold Chloride:**

Gold chloride 0.5 gm  
Distilled water 100.0 ml

Mix, store in acid cleaned bottle, refrigerate.  
Stable for 1 year.

**CAUTION: Avoid contact and inhalation.**

**0.2% Light Green**

Light green SF yellow. 0.2 gm  
Distilled water 100.0 ml  
Glacial acetic acid 0.2 ml

Mix. Stable for 6 months.

**CAUTION: Avoid contact and inhalation.**

**STOCK 5% SILVER NITRATE:**

Silver nitrate 25.0 gm  
Distilled water 500.0 ml

Use acid clean glassware. Store in refrigerator, solution is stable for 6 months.

CAUTION: Corrosive, possible carcinogen.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**3% METHENAMINE:**

Methenamine 30.0 gm  
Distilled water 1000.0 ml

Use acid clean glassware. Store in refrigerator, solution is stable for 6 months.

CAUTION: Avoid contact and inhalation.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**1% SODIUM METABISULFITE:**

Sodium metabisulfite 5.0 gm  
Distilled water 500.0 ml

Mix, solution is stable for 6 months.

CAUTION: Avoid contact and inhalation.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**1% SODIUM METABISULFITE**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

**METHENAMINE SILVER STOCK SOLUTION:**

3% Methenamine 400.0 ml  
5% Silver nitrate 20.0 ml

Use acid clean glassware. Store in the refrigerator, solution is stable for 6 months.

CAUTION: Corrosive, avoid contact and inhalation.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**5% BORAX/SODIUM BORATE**

Sodium borate 5.0 gm  
Distilled water 100.0 ml

Mix well, label with initials and date. Solution is stable for 6 months.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**2% CHROMIC ACID:**

Chromium trioxide 10.0 gm  
Distilled water 500.0 ml

Mix, solution is stable for 6 months.

CAUTION: Corrosive acid, possible carcinogen, avoid contact and inhalation.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**2% CHROMIC ACID**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

**0.5% GOLD CHLORIDE:**

1% Gold Chloride 2.5 ml  
Distilled water 50.0 ml

Use acid clean glassware. Store in the refrigerator, solution is stable for 6 months.

CAUTION: Avoid contact and inhalation.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**0.5% GOLD CHLORIDE**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

**0.2% LIGHT GREEN:**

Light green SF yellow. 0.2 gm  
Distilled water 100.0 ml  
Glacial acetic acid 0.2 ml

Mix. Stable for 6 months.

CAUTION: Avoid contact and inhalation.

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_

EXPIRATION: \_\_\_\_\_

**0.2% LIGHT GREEN**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_