Urates Control Slides – Technical Memo

CONTROL SLIDES: Part 4700A
10 Slide/Set

PRODUCT SPECIFICATIONS:
- Tissue: Positive staining gouty tophi tissue.
- Fixation: Formalin 10%, Phosphate Buffered (Part 1090).
- Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.
- Quality Control Stain: Gomori Methenamine Silver quality control stained slide(s) included.
- Reactivity: Guaranteed product specific reactivity for one year from date of receipt. Revalidate after one year to verify continued reactivity.
- Storage: 15-30°C in a light deprived and humidity controlled environment.

Before using unstained control slides, review the enclosed stained slide(s) to ensure that this tissue source is acceptable for testing needs.

PRODUCT DESCRIPTION:
The enclosed positive control slides are intended to verify histological techniques and reagent reactivity. The intended use is for the qualitative purpose of determining positive or negative results, and not intended for any quantitative purpose. These positive control slides are produced from human surgical or autopsy tissues under carefully controlled conditions. Quality control measures are used to deliver control slides that are as consistent as possible.

CONTROL SLIDE VALIDATION:

<table>
<thead>
<tr>
<th>With Urate Stain, Gomori Methenamine Silver Method</th>
<th>Individual Stain Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methenamine 3%, Aqueous</td>
<td>Part 12239</td>
</tr>
<tr>
<td>Silver Nitrate 5%, Aqueous</td>
<td>Part 13805</td>
</tr>
<tr>
<td>Sodium Borate 5%, Aqueous</td>
<td>Part 13826</td>
</tr>
<tr>
<td>Gold Chloride 0.25%, Aqueous</td>
<td>Part 11287</td>
</tr>
<tr>
<td>Sodium Thiourea 2.5%, Aqueous</td>
<td>Part 13889</td>
</tr>
<tr>
<td>Light Green SF Yellowish Stain 0.2%, Aqueous</td>
<td>Part 12202</td>
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APPLICATION:
Newcomer Supply Urates Control Slides are for the positive histochemical staining of urates in tissue sections. With abnormal accumulations found around joints and in soft tissues, this disturbance in uric acid metabolism is known as gout, with collections of urate crystals referred to as gouty tophi.

Calcium pyrophosphate crystals or pseudogout may also be demonstrated in this procedure. When viewed with a polarizing filter and red compensator filter, gout and pseudogout can be distinguished.

Fixation: Urate crystals are soluble in aqueous solutions. Fix in 100% ethyl alcohol; a minimum of two changes, 4 hours each.

Processing: Transfer from 100% ethyl alcohol fixative to xylene for 1 hour; proceed with equal parts xylene/paraffin at 58°C for 2 hours. Infiltrate with paraffin for a minimum of 1 hour; embed.

Technique: Chill paraffin blocks in 100% ethyl alcohol; cut paraffin sections with minimal water bath exposure.

PRESTAINING PREPARATION:
1. Heat dry sections in oven according to your laboratory protocol.
2. All glassware/plasticware must be acid cleaned prior to use.
   a. See Procedure Notes #1 and #2 (page 2).
3. Prepare Methenamine Silver Stock Solution.
   a. Methenamine 3%, Aqueous 50 ml
   b. Silver Nitrate 5%, Aqueous 2.5 ml
   c. Slowly add silver nitrate; mix to clear milky precipitate.
   d. Store clear solution at 2°C-8°C for up to 2 months.
4. Prepare fresh Methenamine Silver Working Solution; combine and mix well.
   a. Methenamine Silver Stock Solution 25 ml
   b. Distilled Water 25 ml
   c. Sodium Borate 5%, Aqueous 3 ml
5. Preheat fresh Methenamine Silver Working Solution to 60°C in a water bath.

NEWCOMER SUPPLY VALIDATION PROCEDURE:
6. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Rinse in two changes of 100% ethyl alcohol, 10 dips each.
   a. Do not use 95% ethyl alcohol or distilled water steps.
   b. See Procedure Notes #3 and #4 (page 2).
7. Incubate slides in preheated Methenamine Silver Working Solution (Step #5) in a 60°C water bath for 30 minutes.
   a. Remove control slide, rinse in warm distilled water, check microscopically for adequate silver development.
   b. If structures are not sufficiently dark, place slides back in warm silver solution.
   c. Recheck at 2-3 minute intervals until desired intensity is achieved.
8. Rinse well in distilled water.
9. Tone in Gold Chloride 0.25%, Aqueous until brown colorization disappears; 5 to 30 seconds.
10. Rinse in distilled water.
11. Place in Sodium Thiourea 2.5%, Aqueous; 2 to 3 minutes.
12. Wash well in running tap water for 3 minutes; rinse in distilled water.
13. Counterstain in Light Green SF Yellowish Stain 0.2%, Aqueous for 1 to 2 minutes, depending on preference of counterstain intensity.
14. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.
RESULTS:

Light Field Microscopy:
- Gout/urate crystals: Black
- Background: Green

Polarized/Red Compensator Filter: (long axes aligned parallel)
- Gout/urate crystals: Yellow, long & needle shaped
- Pseudogout crystals: Blue, shorter & rhomboidal

PROCEDURE NOTES:

1. Acid clean all glassware/plasticware (12086) and rinse thoroughly in several changes of distilled water.
2. Plastic (5500), plastic-tipped or paraffin coated metal forceps must be used with any silver solution to prevent precipitation of silver salts. No metals of any kind should be in contact with any silver solution. Only glass thermometers should be used.
3. Drain slides after each step to prevent solution carry over.
4. Do not allow sections to dry out at any point during procedure.
5. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

REFERENCES:

4. Modifications developed by Newcomer Supply Laboratory.