

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.

Intended Use: To verify histological techniques and reagent reactivity.

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

CONTROL SLIDE VALIDATION:

With Urate Stain, Gomori Methenamine Silver Method:	Individual Stain Solution
Methenamine 3%, Aqueous	Part 12239
Silver Nitrate 5%, Aqueous	Part 13805
Sodium Borate 5%, Aqueous	Part 13826
Gold Chloride 0.25%, Aqueous	Part 11287
Sodium Thiosulfate 2.5%, Aqueous	Part 13889
Light Green SF Yellowish Stain 0.2%, Aqueous	Part 12202

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:

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

NEWCOMER SUPPLY VALIDATION PROCEDURE:

- Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Rinse in two changes of 100% ethyl alcohol, 10 dips each.
 - Do not use 95% ethyl alcohol or distilled water steps.
 - See Procedure Notes #3 and #4 (page 2).

- Incubate slides in preheated Methenamine Silver Working Solution (Step #5) in a 60°C water bath for 30 minutes.
 - Remove control slide, rinse in warm distilled water, check microscopically for adequate silver development. Crystals should be dark brown/black.
 - If structures are not sufficiently dark, place slides back in warm silver solution.
 - Recheck at 2-3 minute intervals until desired intensity is achieved.
- Rinse well in distilled water.
- Tone in Gold Chloride 0.25%, Aqueous until brown colorization disappears; 5 to 30 seconds.
- Rinse well in distilled water.
- Place in Sodium Thiosulfate 2.5%, Aqueous; 2 to 3 minutes.
- Wash well in running tap water for 3 minutes; rinse in distilled water.
- Counterstain in Light Green SF Yellowish Stain 0.2%, Aqueous for 1 to 2 minutes, depending on preference of counterstain intensity.
- 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.

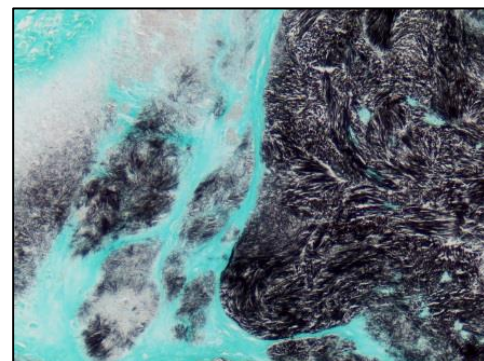
RESULTS:

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:

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 255-256, 267-268.
2. Luna, Lee G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: Blakiston Division, McGraw-Hill, 1968. 187-188.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 225-226.
4. Modifications developed by Newcomer Supply Laboratory.