NEWCOMER SUPPLY®
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Part 9167
Revised July 2017

Basement Membrane, Gomori Stain Kit - Technical Memo

KIT INCLUDES:	**Part 9167A**
Solution A: Methenamine 3%, Aqueous	250 ml
Solution B: Silver Nitrate 5%, Aqueous	50 ml
Solution C: Sodium Borate 5%, Aqueous	50 ml
Solution D: Periodic Acid 1%, Aqueous	250 ml
Solution E: Gold Chloride 0.25%, Aqueous	250 ml
Solution F: Sodium Thiosulfate 2.5%, Aqueous	250 ml
Solution G: Light Green SF Yellowish Stain 0.1%, Aqueous	250 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:

Hydrochloric Acid 5%, Aqueous	Part 12086 (for acid cleaning glassware)
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Coplin Jar, Plastic	Part 5184 (for microwave modification)

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Basement Membrane, Gomori Stain Kit procedure, with included microwave modification, is a straightforward silver technique similar to the Jones Method for identification of glomerular and tubular basement membranes in renal tissue.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 2-3 microns
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

PRESTAINING PREPARATION:

1. All glassware/plasticware must be acid cleaned prior to use.
   a. *See Procedure Notes #1 and #2 (page 2).*
2. Prepare Silver-Methenamine Working Solution; combine and mix well:
   a. *Solution A: Methenamine 3%, Aqueous 40 ml*
   b. *Solution B: Silver Nitrate 5%, Aqueous 2 ml*
   c. *Solution C: Sodium Borate 5%, Aqueous 4 ml*
   d. ***Proceed to Step #8 for Microwave Modification.***
3. Preheat Silver-Methenamine Working Solution to 45°C-60°C.
   a. *See Procedure Notes #3 and #4 (page 2).*
   b. ***Do not preheat solution if using Microwave Modification.***

STAINING PROCEDURE:

4. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
   a. *See Procedure Notes #5 and #6 (page 2).*
5. Place in Solution D: Periodic Acid 1%, Aqueous for 15 minutes.
6. Wash in gently running tap water for 5 minutes; rinse in distilled water.
7. Place slides in preheated Silver-Methenamine Working Solution and incubate in 45°C-60°C oven or water bath, or bench top/room temperature for 12-18 minutes until sections appear paper-bag brown. Periodically remove the control, rinse in warm distilled water, check microscopically for adequate silver impregnation. Basement membranes should be dark brown. If the tissue structures are not sufficiently dark, place slides back in heated silver solution. Recheck at 2-3 minute intervals until desired intensity is achieved.
   a. *Staining at room temperature will require longer incubation times.*
8. **Microwave Modification:** *See Procedure Note #7 (page 2).*
   a. *Place sides in a plastic Coplin jar containing prepared Silver-Methenamine Working Solution (Step #2) and microwave at 70°C for 3 minutes.*
   b. *Check microscopically for adequate development.*
   c. *If additional incubation is required, return slides to heated Silver-Methenamine Working Solution.*
9. Rinse in three changes of distilled water.
10. Tone sections in Solution E: Gold Chloride 0.25%, Aqueous for 1 minute.
11. Rinse well in three changes of distilled water.
12. Place in Solution F: Sodium Thiosulfate 2.5%, Aqueous for 2 minutes.
13. Wash in gently running tap water for 5 minutes; rinse in distilled water.
14. Counterstain in Solution G: Light Green SF Yellowish Stain 0.1%, Aqueous for 1 minute.
15. Quickly rinse slides in two changes of distilled water.
16. 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:

Kidney glomerular basement membranes	Black
Intra-glomerular deposits	Black
Reticular fibers	Black
Nuclei	Outlined in black
Cytoplasm	Light green

PROCEDURE NOTES:

1. Acid clean all glassware/plasticware (12086) and rinse thoroughly in several changes of distilled water. Cleaning glassware with bleach is not equivalent to acid washing.
2. Plastic (5500), plastic-tipped or paraffin coated metal forceps must be used with silver solutions to prevent precipitation of silver salts. No metals of any kind should come in contact with silver solutions. Only glass thermometers should be used.
3. Preheating Silver-Methenamine Working Solution to 45°C-60°C prior to incubation is suggested for timely silver development. A water bath or oven can be used for preheating. Begin preheating the silver solution approximately 20-30 minutes before use.
4. Staining slides at higher temperatures will cause the development reaction to happen faster, but may also cause precipitate to form in the working silver solution and deposit on the slides. Maintaining the silver solution between 45°C-60°C will help to minimize precipitate.
5. Drain staining rack/slides after each step to prevent solution carry over.
6. Do not allow sections to dry out at any point during staining procedure.
7. The suggested microwave procedure has been tested at Newcomer Supply using an "EB Sciences", 850 watt microwave oven with temperature probe and agitation tubes. This procedure is reproducible in our laboratory. It is nonetheless a guideline and techniques should be developed for your laboratory which meet the requirements of your situation. Microwave devices should be placed in a fume hood or vented into a fume hood, according to manufacturer's instructions, to prevent exposure to chemical vapors.
8. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Luna, Lee G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: Blakiston Division, McGraw-Hill, 1968. 97-99.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 187-188.
3. Modifications developed by Newcomer Supply Laboratory.