

## Basement Membrane Control Slides – Technical Memo

<b>CONTROL SLIDES:</b>	<b>Part 4055A</b>	<b>Part 4055B</b>
	10 Slide/Set	98 Slide/Set

Basement Membrane Control Slides contain a section of positive staining kidney.

### PRODUCT DESCRIPTION:

The enclosed positive control slides are intended to be used to verify histological techniques and reagent reactivity. These slides are to be used for the qualitative purpose of determining positive or negative results, and are not intended to be used for any quantitative purpose. The first serial section within the control box is stained and provided for your reference. **Before using the unstained slides, review the enclosed stained slide with your pathologist to ensure that this tissue source is acceptable. Newcomer Supply will not accept a return with missing slides in the series. Newcomer Supply guarantees reactivity of these control slides for one year from the date of receipt. Revalidate after one year to verify continued reactivity. Store at 15-30°C in a light deprived and humidity controlled environment.**

These positive control slides were produced from human surgical or autopsy tissues under carefully controlled conditions. Reasonable measures are used to deliver quality control slides that are as consistent as possible. However, characteristics of quality control slides may be dissimilar due to variations in the reagents, stains, techniques, laboratory conditions, and tissue sources used. Newcomer Supply Laboratory uses a manual method of performing quality control procedures, specifically avoiding automation, in order to provide reactive control slides for even less aggressive methods of staining that our customers may be using.

### CONTROL SLIDE VALIDATION:

With Basement Membrane, Gomori Stain Kit	Part 9167A	Individual Stain Solution
Solution A: Methenamine 3%, Aqueous	250 ml	Part 12239
Solution B: Silver Nitrate 5%, Aqueous	50 ml	Part 13805
Solution C: Sodium Borate 5%, Aqueous	50 ml	Part 13826
Solution D: Periodic Acid 0.5%, Aqueous	250 ml	Part 13308
Solution E: Gold Chloride 0.25%, Aqueous	250 ml	Part 11287
Solution F: Sodium Thiosulfate 2.5%, Aqueous	250 ml	Part 13889
Solution G: Light Green SF Yellowish Stain 0.1%, Aqueous	250 ml	Part 12203

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

### APPLICATION:

The Newcomer Supply Basement Membrane Control Slides are for the positive histochemical staining of basement membranes in tissue sections.

### METHOD:

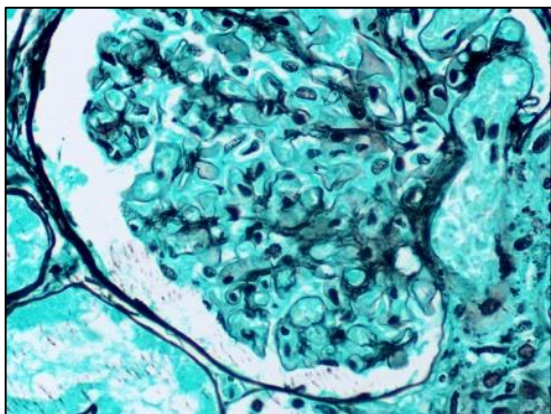
**Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)  
**Technique:** Paraffin sections cut at 4 microns on Superfrost® Plus  
**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

### NEWCOMER SUPPLY VALIDATION PROCEDURE:

1. All glassware/plasticware must be acid cleaned prior to use.
  - a. See Procedure Notes #1 and #2 (page 2).
2. 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 #3 and #4 (page 2).
3. Place slides in Solution D: Periodic Acid 0.5%, Aqueous for 15 minutes.
4. Wash in gently running tap water for 5 minutes; rinse in distilled water.
5. Prepare Silver-Methenamine Working Solution 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.
6. Heat Silver-Methenamine Working Solution to 45°C-60°C.
  - a. See Procedure Notes #5 and #6 (page 2).
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 the warm silver solution. Recheck at 2-3 minute intervals until desired intensity is achieved.
  - a. Staining at room temperature will require an overall longer incubation time.
8. **Microwave Modification:** See Procedure Note #7 (page 2).
  - a. Place slides in a plastic Coplin jar containing prepared Silver-Methenamine Working Solution (Step #5) and microwave at 70°C for 3 minutes.
  - b. Check microscopically for adequate development. If additional incubation is required, return slides to the warm 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
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. Drain staining rack/slides after each step to prevent solution carry over.
4. Do not allow sections to dry out at any point during staining procedure.
5. Preheating Silver-Methenamine Working Solution to 45°C-60°C prior to incubation is suggested for timely silver development. A water bath can be used for preheating if a microwave is unavailable. Begin preheating the silver solution approximately 20-30 minutes before use.
6. 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.
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.