

## Bielschowsky Control Slides – Technical Memo

<b>CONTROL SLIDES:</b>	<b>Part 4058A</b>	<b>Part 4058B</b>
	10 Slide/Set	98 Slide/Set

Bielschowsky Control Slides contain a section of positive staining brain.

### 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:

<b>With Bielschowsky, Lester King Modified Stain Kit:</b>	<b>Part 9154A</b>	<b>Individual Stain Solution</b>
Solution A: Silver Nitrate 20%, Aqueous	250 ml	Part 13807
Solution B: Ammonium Hydroxide 28-30%, ACS	100 ml	Part 1006
Solution C: Developer	25 ml	
Solution D: Sodium Thiosulfate 5%, Aqueous	250 ml	Part 1389

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

### APPLICATION:

Newcomer Supply Bielschowsky Control Slides are for the positive histochemical staining of nerve fibers, neurofibrils/tangles, senile plaques and axons, instrumental in assisting in the diagnosis of Alzheimer disease and other neurological disorders.

### METHOD:

**Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)

**Technique:** Paraffin sections cut at 8 microns on Superfrost® Plus

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

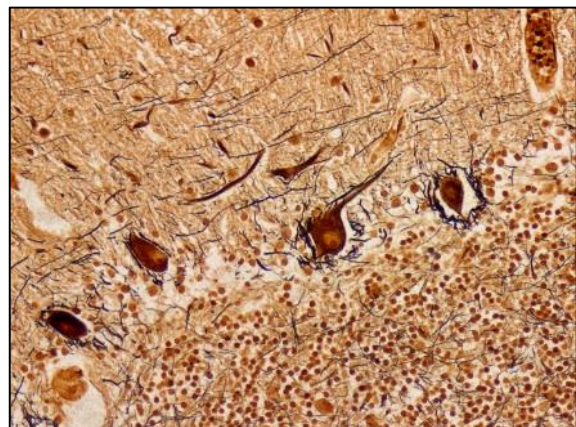
### NEWCOMER SUPPLY VALIDATION PROCEDURE:

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. Preheat container of Solution A: Silver Nitrate 20%, Aqueous in water bath to 37°C.
  - a. Warm 80 ml of distilled water in a separate container in water bath to 37°C for slide rinsing/holding steps.
4. Place slides in 37°C Solution A: Silver Nitrate 20%, Aqueous for 15 minutes.
  - a. See Procedure Note #5 (page 2).
5. Remove slides from Solution A: Silver Nitrate 20%, Aqueous (**do not discard**); hold slides in warmed distilled water.
6. Add Solution B: Ammonium Hydroxide 28-30%, ACS drop by drop into heated Solution A: Silver Nitrate 20%, Aqueous swirling or stirring with a glass/plastic rod until precipitate disappears. **Do not go past this point.**
  - a. Approximately 10 ml of Solution B: Ammonium Hydroxide 28-30%, ACS will be required. More than 10 ml indicates ammonia is old and deteriorating.
7. Place slides back into the Silver Nitrate Solution with added Ammonium Hydroxide in water bath at 37°C for 10 minutes.
8. Remove slides and hold in warmed distilled water. (**Do not discard Ammoniacal Silver Solution.**)

9. Add 1 drop of Solution C: Developer to the Ammoniacal Silver Solution, while swirling or stirring with a glass/plastic rod.
10. Return slides to heated Ammoniacal Silver Solution with added Developer, in water bath at 37°C for 5-15 minutes; average time of 6 minutes. Check slides microscopically at 3 minutes for development of neurons to dark brown. Follow with checks at 1 minute intervals to avoid silver over-development.
11. Rinse thoroughly in distilled water for 5 minutes.
12. Place in Solution D: Sodium Thiosulfate 5%, Aqueous for 5 minutes.
13. Rinse thoroughly in tap water.
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:

Senile plaques, neurofibrils/tangles	Dark brown to black
Neurons	Dark brown
White and gray matter	Yellowish brown
Nerve fibers, axons	Brown to black



**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. Do not overuse the silver solution. A maximum number of 8 slides per 40 ml of Solution A: Silver Nitrate 20%, Aqueous is recommended.
6. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

**REFERENCES:**

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 368-370.
2. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 202-205.
3. King, Lester. "The Impregnation of Neurofibrils". *Yale Journal of Biology and Medicine* 14.1 (1941). 59-68.
4. Modifications developed by Newcomer Supply Laboratory.