Nerve

Jacqueline Brooks, HTL, SCT, MB (ASCP) Q IHC
Housekeeping

To receive credits you must complete the following:

- View complete presentation
- Pass the Quiz with a 70% or higher score
- Fill out the Evaluation Form for the presentation
- Sign the hard copy roster provided by your laboratory manager

Visit [www.auroradx.com/edutraining](http://www.auroradx.com/edutraining) to access this presentation again, download a PDF of it, take your quiz and fill out your evaluation form as well as obtain instructions on how to report credits to NSH.

Encourage your teammates to participate and become the top-notch facility in the Aurora Diagnostics family to show case the best well-trained laboratorians and recognized for excellence in performance.
IHCs (Neural & Neuroendocrine Markers)

- Calcitonin
- Chromogranin A
- Gastrin
- Glial Fibrillary Acidic Protein (GFAP)
- Glucagon
- Insulin
- Myelin Basic Protein
- Neurofilament
- Neuron Specific Enolase (NSE)
- Neuroblastoma
- Somatostatin
- Synaptophysin
GFAP (Glial Fibrillary Acidic Protein)

Anti-GFAP Antibody detects astrocytes, Schwann cells, Satellite cells, enteric glia cells and some groups of Ependymal cells. This marker is mainly used to Distinguish neoplasms of astrocytic origin from other Neoplasms in the central nervous system.
NSE (Neuron Specific Enolase)

- NSE is present in high concentration in neurons and in central and peripheral neuroendocrine cells, therefore, anti-NSE reacts with cells of neural and neuroendocrine lineage. If neoplastic cells co-express keratins and NSE, neuroendocrine differentiation is probable, however, neural tumors that do not express keratin, and show no staining with NSE, would not exclude neural or neuroendocrine differentiation. Thus detection of neural and neuroendocrine lineage requires the use of panels that include NSE and other markers such as keratin, chromograinin, synaptophysin and neurofilament.
Synaptophysin

- Anti-Synaptophysin reacts with neuroendocrine cells of human adrenal medulla, carotid body, skin, pituitary, thyroid, lung, pancreas and gastrointestinal mucosa. Positive staining is seen in neurons of the brain, spinal cord, retina, and Paneth’s cells in the gastrointestinal tract and gastric parietal cells. This antibody identified normal neuroendocrine cells and neuroendocrine neoplasms. Diffuse, finely granular cytoplasmic staining is observed, which probably correlates with the distribution of the antigen within neurosecretory vesicles. The expression of synaptophysin is independent of the presence of NSE or other neuroendocrine markers. Anti-Synaptophysin is an independent broad range marker of neural and neuroendocrine differentiation.
CPT Code

- 88342 – Immunohistochemistry (including tissue immunoperoxidase), each antibody
- 88312 – Special stain including interpretation and report; Group 1 for microorganisms
- 88313- Special stain, Group II, all other, except stain for microorganisms, stains for enzyme constituents, or immunocytochemistry and immunohistochemistry.
Nervous System
Anatomically

1. Central Nervous System (CNS)
   - Brain
   - Spinal Cord

2. Peripheral Nervous System (PNS)
   - All other nervous tissue
Peripheral nervous System
Nervous System Functionally

1. Somatic
   • Voluntary

2. Autonomic
   • Involuntary
Nervous System
Histologically

1. Neuronal cell bodies and processes
2. Glial cells and processes
3. The myelin sheath
Neuronal cell bodies and processes

• **Neurons**
  Cell body- Nucleus and 1 or more cell processes (axon and dendrites)

• **Nissl Substances**
  RNA

• **Nerve Cell Processes**
  Dendrites – Information input, branched
  Axons – Carry information to other nerve cells or effector organ such as muscle
Neuroglia

1. Oligodendroglia
2. Astrocytes
3. Microglia
4. Ependymal Cells
Schwann Cells
Myelin
Nissl substance Stains

- Cresyl Echt Violet Method I
- Cresyl Echt Violet Method II
- Thionin stain
- Galloacyanine stain
Nissl Substance
Cresyl Echt Violet Method I

- **Purpose:** Identification of neurons in sections, or demonstration of the loss of Nissl substance
- **Principle:** RNA – Basophilic
- **Fixation:** 10% NBF
- **Technique:** 6-8 microns
- **Quality Control:** Normal Spinal Cord
Cresyl Echt Violet Method I
Procedure

1. Deparaffinize and hydrate to distilled water
2. Stain 3-5 min
3. Rinse
4. 95% Alcohol
5. 100% Alcohol
6. Xylene
7. Balsam-xylene
8. Differentiate in 100% alcohol (check microscopically)
9. Xylene
10. Repeat steps 7 through 9 until Nissl substance are well demonstrated
11. Mount with synthetic resin
Cresyl Echt Violet Method I Results

- Nissl Substance – Blue to purple
- Nuclei – Blue to purple
- Background - colorless
Cresyl Echt Violet Method I
Technical Notes

• Differentiation should be monitored carefully
• Solutions should be fresh
• The alcohol after the balsam-xylene may become cloudy and should be changed
Cresyl Echt Violet Method II for Nissl Substance

• Purpose –
• Identification of neurons or the demonstration of loss of Nissl substance
• Principle – Because of the RNA content nissl substance is very basophilic, this stain uses acidic Cresyl Echt violet
• Fixative – 10% NBF
• Technique – 6-8 microns
• Quality Control – Normal Spinal Cord
Cresyl Echt Violet Method II

1. Deparaffinize and hydrate to distilled water
2. Stain for 8 min
3. Dehydrate in 95% and 100% alcohol
4. Clear in Xylene

Note: Does not require Differentiation
Cresyl Echt Violet II
Results

• Nissl Substance and nuclei – Blue-purple
• Background - Colorless
Cresyl echt Violet II Technical Notes

• Slides must be checked microscopically using control on same slide is recommended
• Use only: Cresyl fast violet, cresyl echt violet or cresyl violet acetate dyes
• The Luxol fast blue-cresyl echt violet method can also be used
Nerve Fibers (processes) and Neurofibrils

- Gros-Bielschowskki technic
- Bielschowskki’s method for neurofibrils
- Rio-Hortega method for neurofibrils
- Nonidez’s method (block method)
- Bodian Method
- Holmes Method
- Sevier-Munger modification
Nerve Fibers, Nerve Endings, Neurofibrils: Bodian Method

- **Purpose:** Staining nerve fibers
- **Principle:** Uses a silver proteinate (protargol) compound to impregnate tissue sections
- **Fixative:** 10% NBF
- **Quality Control:** Peripheral nerve or cerebral cortex, do not use spinal cord and as with all silver stains glassware must be chemically cleaned
Bodian Method

1. Deparaffinize and hydrate to distilled water
2. For every 100 mL of Protargol solution, add 4-6 g of clean copper shot (cleaned with aqua regia and rinsed very well with distilled water). Place slides in solution and let stand at 37 degrees for 48 hours.
3. Rinse in distilled water
4. Reducing solution 10 min (Hydroquinone, formaldehyde, water)
5. Rinse in distilled water
6. Develop in oxalic acid
7. Rinse in distilled water
8. Sodium thiosulfate 5 minutes
9. Rinse in distilled water
10. Counterstain (aniline blue)
11. Dehydrate in alcohol
12. Clear in xylene
13. Mount in synthetic resin
Bodian Method Results

- Nerve fibers – black
- Background – Light gray or blue
- Nuclei - black
Bodian Method
Technical Notes

• Special precautions in discarding aqua regia
• Special precautions in making 1% Protargol
• Use chemically clean glassware
• Do not use metallic forceps
• Do not over counterstain
• If having difficulty differentiating blue from black use nuclear-fast red as a counterstain
Nerve Fibers and Neurofibriles: Holmes Silver Nitrate Method

• **Purpose:** Demonstration of nerve fibers and neurofibrils
• **Principle:** This is an argyrophil silver method, requiring that chemical reduction be used
• **Fixative:** 10% NBF
• **Technique:** 10-15 microns
• **Quality Control:** Cerebral cortex, chemical clean glassware, nonmetallic forceps
Holmes silver nitrate method - Reagents

- Silver Nitrate
- Boric Acid
- Borax Solution
- Pyridine solution
- Hydroquinone
- Sodium sulfite
- Gold Chloride
- Oxalic Acid
- Sodium Thiosulfate
Holmes Silver Nitrate - Procedure

1. Deparafinize and hydrate
2. 20% silver nitrate in dark room – 1 hr
3. Prepare impregnating solution
4. Rinse in distilled water
5. Impregnating solution – overnight
6. Reducer – 2 minutes
7. Wash in running water
8. Rinse in distilled water
9. Tone in gold chloride – 3 minutes
10. Rinse in distilled water
11. Oxalic acid – 3-10 minutes
12. Rinse in distilled water
13. 5% sodium thiosulfate
14. Rinse in tap water – 10 minutes – then counterstain
15. Dehydrate in alcohol
16. Clear in xylene and mount in synthetic resin
Holmes Silver Nitrate Results and Technical Notes

- Axons and nerve fibers – Black
- Neurofibrils – Black

Pyridine is very toxic and should be used under a hood
Nerve Fibers, Neurofibrillary Tangles, and Senile Plaques: Bielschowsky-PAS Stain

- **Purpose:** Demonstration of nerve fibers and the presence of neurofibrillary tangles and senile plaques in Alzheimer disease.
- **Principle:** The silver is deposited on neurofibrils and axons and the PAS stains both basement membranes and amyloid in the plaques.
- **Fixative:** 10% NBF
- **Technique:** 8-10 microns
- **Quality Control:** CNS tissue that contains senile plaques and neurofibrillary tangles.
Bielschowsky-PAS Stain Reagents

- Ammoniacal Silver Solution (Silver nitrate, ammonium hydroxide)
- Developer (Formaldehyde, Nitric acid, Citric acid)
- Gold Chloride
- Sodium thiosulfate
- Periodic Acid
- Schiff Reagent
Bielschowsky-PAS Stain Procedure

1. Prepare ammoniacal silver solution fresh before beginning stain
2. Deparaffinize and hydrate
3. 20% silver – 20 minutes
4. Rinse in distilled water
5. Ammoniacal silver – 20 minutes
6. Ammonia water wash
7. Place 2 drops of developer to the ammoniacal silver stain at this point
8. Place slides in mixed developer-ammoniacal silver solution – 3 minutes
9. Wash in ammonia water then in distilled water
10. Tone in Gold chloride – 30 seconds
11. Wash in ammonia water then in distilled water
12. 5% sodium thiosulfate – 30 seconds
13. Running tap water – 5 minutes
14. Rinse in distilled water
15. 1% periodic acid – 5 minutes
16. Rinse in two changes of distilled water
17. Schiff reagent – 5 minutes
18. Running water – 5 minutes
19. Dehydrate in alcohol
20. Clear in xylene and mount with a synthetic resin
Bielschowsky method
Results

• Neurofibrillar tangles – Dark black
• Peripheral neurites of neuritic plaques – Dark black
• Axons – Black
• Amyloid (plaque cores and vascular) – Magenta
• Lipofuscin - Magenta
Bielschowskly-PAS StainTechnical Notes

- Use chemically cleaned glassware
- Do not use metal forceps
Microwave Modification

- Nerve Fibers, Neurofibrillary Tangles, and Senile Plaques: Microwave Modification of Bielschowsky Method
Microwave Modification

- **Purpose:** Demonstration of nerve fibers and the presence of neurofibrillary tangles and senile plaques in Alzheimer disease.
- **Principle:** Impregnated with ammoniacal silver, reduced by formaldehyde, NOT Toned with gold chloride, unreduced silver removed by sodium thiosulfate
- **Fixative:** 10% NBF
- **Technique:** 8 microns
- **Quality Control:** CNS tissue that contain senile plaques and neurofibrillary tangles
Microwave modification Reagents

- Silver Nitrate
- Nitric Acid
- Formaldehyde
- Citric acid
- Ammonium Hydroxide
- Sodium thiosulfate
Microwave Procedure

1. Deparaffinize and hydrate
2. 1% silver nitrate microwave 1 minute
3. Distilled water
4. Make Ammoniacal Silver Solution
5. Microwave slides in ammoniacal solution for 15 minutes
6. 1% ammonium hydroxide 20 seconds
7. Develop for 3 minutes
8. 1% ammonium hydroxide 15 seconds
9. Rinse in distilled water
10. Wipe off excessive stain from slide
11. 2% sodium thiosulfate 30 seconds
12. Distilled water
13. Dehydrate in alcohol
14. Clear in xylene and mount with synthetic resin
Microwave modification

Results

• Axons Brown to black
• Cytoplasmic neurofibrils Brown to black
• Neurofibrillary tangles and plaques of Alzheimer disease Dark brown or black
• Neuromelanin Black
• Lipofuscin Brown or black
Microwave Modification
Nerve Fibers, Neurofibrillary Tangles, and Senile Plaques: The Sevier-Munger Modification of Bielschowsky Method
Sevier-Munger Method

• Purpose: Demonstration of nerve fibers and the presence of neurofibrillary tangles and senile plaques in Alzheimer disease
• Principle: Impregnated with ammoniacal silver, reduced with formaldehyde, NOT toned, unreduced silver removed with sodium thiosulfate
• Technique: 6-8 microns
• Quality Control: CNS tissue with neurofibrillary tangles and senile plaques
Sevier-Munger Reagents

- Silver Nitrate
- Formaldehyde
- Sodium Carbonate Solution
- Sodium Thiosulfate
- Ammoniacal silver solution (silver nitrate, ammonium hydroxide)
Sevier-Munger Stain Procedure

1. Deparaffinize and hydrate
2. 20% silver nitrate at 60 degrees for 15 minutes
3. Rinse in distilled water
4. Develop for 5 to 30 minutes in ammoniacal silver
5. Rinse in distilled water
6. Sodium thiosulfate 2 minutes
7. Wash in running water
8. Dehydrate, clear and mount with synthetic resin
Sevier-Munger Results

- Nerve endings and neurofibrils
- Neurofibrillary tangle and peripheral neurites of neuritic plaques

Black
Sevier-Munger Technical Notes

• Very reliable and reproducible technique
• Concentrations of chemicals critical
• Follow directions exactly
• Can be used to demonstrate carcinoid tumor cells
Neurofibrillary Tangles and Senile Plaques: Thioflavin’s (Modified)
Thioflavin’s Method

• Purpose: Demonstration of the presence of neurofibrillary degeneration and vascular and parenchymal amyloid deposition in Alzheimer disease
• Principle: Fluorescent dye, more sensitive than silver techniques
• Fixative: 10% to 20% NBF
• Technique: 6 micron (special drying instructions)
• Quality Control: CNS containing senile plaques and neurofibrillay tangles
Thioflavin’s Method
Reagents

- Potassium Permanganate
- Potassium metabisulfite
- Oxalic Acid
- Sodium Hydroxide
- Hydrogen peroxide
- Acetic Acid
- Thioflavin S
- 50% alcohol
Thioflavin Procedure

1. Deparaffinize and hydrate to distilled water
2. Distilled water 5 minutes
3. 0.25% potassium permanganate 20 minutes
4. Running water 5 minutes
5. 0.25% acetic acid 1 minute
6. Running water 5 minutes
7. Sodium hydroxide-peroxide 20 minutes
8. Running water 5 minutes
9. 50% alcohol
10. 0.0125% thioflavin-S for 7 minutes
11. 95% alcohol
12. Dehydrate in absolute alcohol and clean in xylene, mount with nonfluorescent mounting medium
13. View slides on fluorescent microscope
Thioflavin Results

- Alzheimer neurofibrillary tangles, senile plaque neurites, neuropil threads, senile plaque amyloid, and cerebrovascular amyloid
- BIRGHT GREEN
- PSP tangles and Pick bodies – not well demonstrated
Thioflavin Technical Notes

• Water bath must be chemically cleaned with no additives
• Use superfrost slides
• Slides must be completely dried
• Use Cytoseal 60 for mounting
• Stain only stable for a few months
Neuroglia

- Toluidine blue
- Cresyl echt Violet
- Trichrome stain for astrocytes
- Cajal’s gold sublimate method for astrocytes
- Holzer’s stain for glia fibers
- Phosphotungstic acid – Hematoxylin
Glial Fibers: Mallory Phosphotungstic Acid Hematoxylin (PTAH) Stain
PTAH

• Purpose: Demonstration of glial fibers
• Fixative: 10% NBF
• Technique: 6-8 microns
• Quality control: Cerebral Cortex (not spinal cord)
PTAH Solutions

- PTAH Stain (phosphotungstic acid, Hematoxylin, distilled water)
- Lugol Iodine (iodine, potassium iodide, distilled water)
- Potassium Permanganate
- Oxalic Acid
- Zenker solution
PTAH Procedure

1. Deparaffinize and hydrate to distilled water
2. Mordant overnight in Zenker solution
3. Running water 15 minutes
4. Lugol iodine 15 minutes
5. 95% alcohol 1 hour
6. Distilled water
7. 1% potassium permanganate
8. Running water 10 minutes
9. 5% oxalic acid 5 minutes
10. Running water 10 minutes
11. PTAH stain overnight
12. Dehydrate with alcohol, clear with xylene, and mount with synthetic resin
PTAH Results and Technical Notes

- Glial fibers: Blue
- Nuclei: Blue
- Neurons: Salmon
- Myelin: Blue

Largely replaced by IHC’s
Glial Fibers: Holzer Method

• Purpose: Demonstration of glial fibers and areas of gliosis
• Principle: Glial fibers stained with crystal violet
• Fixative: 10% NBF
• Technique: 6-8 microns
• Quality Control: Cerebral Cortex (not spinal cord)
Holzer Method
Reagents

- Phosphomolybdic Acid
- Absolute Alcohol
- Chloroform
- Potassium Bromide
- Crystal Violet Stain (crystal violet, absolute alcohol, chloroform)
- Differentiating solution (Aniline oil, chloroform, ammonium hydroxide)
Holzer Method Procedure

1. Deparaffinize and hydrate to distilled water
2. Phosphomolybdic acid-alcohol for 3 minutes
3. Absolute alcohol-chloroform
4. Crystal violet 30 seconds
5. 10% potassium bromide 1 minute
6. Blot dry
7. Differentiate 30 seconds (aniline-chloroform)
8. Xylene
9. Mount with synthetic resin
Holzer Method Results

- Glial fibers
- Background

Blue
Very pale blue to colorless
Holzer Method
Technical Notes

• Aniline oil is toxic
• Chloroform is toxic
Astrocytes: Cajal Stain

- **Purpose:** Demonstration of astrocytes
- **Principle:** Stained with Cajal gold on frozen sections
- **Fixative:** Formalin ammonium bromide
- **Technique:** 20 – 30 microns
- **Quality Control:** Cerebral Cortex (not spinal cord)
Cajal Stain Reagents

- Formalin Ammonium Bromide (ammonium bromide, formaldehyde, distilled water)
- Gold Sublimate (gold chloride, mercuric chloride, distilled water)
- Sodium Thiosulfate
Cajal Method Procedure

1. Wash in distilled water
2. Gold sublimate 4 hours
3. Wash in distilled water
4. 5% sodium thiosulfate 2 minutes
5. Wash in distilled water
6. Mount sections on slides and blot, dehydrate in alcohol
7. Clear in xylene, and mount in synthetic resin
Cajal Stain Results

- Astrocytes with perivascular feet - Black
Cajal Method
Technical Notes

• Chemical must be of the utmost purity
• Prolonged fixation must be avoided
• Staining temp must not exceed 30 degrees C.
• Follow directions carefully
• Sections must be kept flat and not overlap while floating sections in gold sublimate
• Mercuric chloride is extremely toxic
Myelin

- Weil’s Method
- Pal-Weigert Method
- Marchi’s method for degenerating myelin
- Luxol fast blue method
Myelin Sheath: Weil Method

- **Purpose:** Demonstration of myelin in tissue
- **Principle:** The mordant-hematoxylin solution attaches to the phospholipid component of the myelin sheath
- **Fixative:** 10% NBF
- **Technique:** 10-15 microns
- **Quality control:** Spinal Cord
Weil Method Reagents

• Ferric Ammonium sulfate 4%
• Alcoholic Hematoxylin 10%
• Staining Solution (alcoholic hematoxylin, ferric ammonium sulfate solution)
• Differentiating solution (sodium borate, potassium ferricyanide, distilled water)
Weil Method
Procedure

1. Deparaffinize and hydrate to distilled water
2. Staining solution 30 minutes
3. Wash in tap water
4. Differentiate in 4% ferric ammonium sulfate
5. Wash in tap water
6. Complete differentiation in sodium borate-potassium ferricyanide solution
7. Wash in tap water
8. Ammoniawater
9. Wash in distilled water
10. Dehydrate in alcohol
11. Clear in xylene, and mount with synthetic resin
Weil Method Results

- Myelin sheath: Blue to blue-black
- Background: Light tan
Weil Method
Technical Notes

• There should be a sharp contrast between demyelinated and myelinated white matter
• The quality of the myelin stain can be determined macroscopically with both gray and white matter easily distinguished.
• Hematoxylin should ripen before use
Myelin sheath: Luxol Fast Blue Method

• Purpose: Demonstration of myelin in tissue section
• Principle: Staining is caused by lipoproteins, and the mechanism is that of an acid-base reaction with salt formation
• Fixative: 10% NBF
• Technique: 10-15 microns
• Quality control: Spinal Cord or medulla
Luxol Fast Blue Procedure

- Deparaffinize and hydrate to 95% alcohol
- Luxol fast blue overnight at 56 to 58 degrees C
- 95% alcohol to remove excess stain
- Distilled water
- Begin differentiation in lithium carbonate
- Continue differentiation in 70% alcohol
- Wash in distilled water
- Finish differentiation in lithium carbonate then 70% alcohol
- Rinse in distilled water
- Dehydrate in alcohol
- Clear in xylene, mount with synthetic resin
Luxol Fast Blue Results

- Myelin: Blue to blue-green
- Background: Colorless
Luxol Fast Blue Technical Notes

• Gray matter and demyelinated white matter should be almost colorless and contrast sharply with the blue-stained myelinated white matter.

• The quality of a myelin stain can be determined macroscopically with the gray and white matter easily distinguished; on a good myelin stain, the areas of demyelination frequently are more easily identified macroscopically than microscopically.

• Differentiation is a key step.
Combination Methods

• Luxol Fast Blue-Periodic Acid Schiff-Hematoxylin
• Luxol Fast Blue-Holmes Silver Nitrate
• Luxol Falst Blue-Phosphotungstic Acid Hematoxylin
• Luxol Fast Blue-Oil Red O
Myelin Sheath and Nissl Substance Combined: Luxol Fast Blue-Cresyl Echt Violet Stain
Luxol Fast Blue-Cresyl Echt Violet

- **Purpose:** Demonstration of both myelin and Nissl substance
- **Fixation:** 10% NBF
- **Technique:** 10-15 microns
- **Quality Control:** Spinal Cord or medulla
Luxol Fast Blue-Cresyl Echt Violet Reagents

- Acetic Acid
- Luxol fast blue solution (luxol fast blue MBSN, 95% alcohol, 10% acetic acid)
- Cresyl Echt Violet 0.1%
- Lithium Carbonate, 0.05%
- Alcohol, 70%
Luxol Fast Blue-Cresyl Echt Violet Procedure

1. Deparafinize and hydrate to 95% alcohol
2. Luxol fast blue – overnight at 56 to 58 degree C
3. 95% alcohol to remove excess stain
4. Rinse in distilled water
5. Begin differentiation in lithium carbonate
6. Continue differentiation in 70% alcohol
7. Wash in distilled water
8. Finish differentiation by rinsing in lithium carbonate then several changes of 70% alcohol
9. Rinse thoroughly in distilled water
10. Cresyl echt violet for 6 minutes
11. Differentiate in 95% alcohol
12. Dehydrate in absolute alcohol, clear in xylene, and mount with synthetic resin
Luxol Fast blue-Cresyl Echt Violet Results

- Myelin Blue
- Nissl substance Violet
- Nuclei Violet
Luxol Fast Blue-Cresyl Echt Violet

Technical Notes

• You must add acetic acid to cresyl echt violet just before use or you will have background staining
• You must heat the cresyl echt violet prior to adding the slides or Nissl substance will have decreased staining
• The cresyl echt violet intensifies the staining of the myelin sheath
Myelin Sheaths and Nerve fibers Combined: Luxol Fast Blue-Holmes Silver Nitrate Method
Luxol Fast Blue-Holmes Silver

- **Purpose**: Demonstration of both myelin and nerve fibers
- **Fixative**: 10% NBF
- **Technique**: 10-15 microns
- **Quality Control**: Cerebral Cortex
Luxol Fast Blue-Holmes Silver Reagents

- Silver Nitrate 1%
- Boric Acid Solution
- Borax solution
- Pyridine, 10% solution
- Impregnating Solution (Boric acid, borax solution, distilled water, silver nitrate 1%, Pyridine 10%)
- Reducing Solution (Hydroquinone, Sodium sulfate, distilled water)
- Gold chloride, 0.2%
- Oxalic Acid, 2%
- Sodium Thiosulfate, 5%
- Luxol fast Blue. 0.1% solution (luxol fast blue MBSN, 95% alcohol, Acetic acid 10%)
- Lithium Carbonate, 0.05%
Luxol Fast Blue-Holmes Silver Procedure

1. Deparaffinize and hydrate to distilled water
2. 20% silver nitrate in dark room =1 hr
3. Prepare impregnating solution
4. 20% silver nitrate 10 minutes, then three changes of distilled water
5. Impregnating solution – 37 degrees overnight
6. Drain slides and place in reducer for 2 min
7. Running water 3 min
8. Tone in gold chloride for 3 min
9. Rinse in distilled water
10. 2% oxalic acid 3-10 min (the axons are thoroughly blue-black)
11. Rinse in distilled water
12. 5% sodium thiosulfate for 5 min
13. Wash in tap water for 10 min
14. 95% alcohol briefly
15. Luxol fast blue overnight at 60 degrees
16. Rinse in 95% alcohol
17. Place in distilled water
18. 0.05% lithium carbonate for 15 seconds
19. Differentiate 70% alcohol for 20-30 seconds
20. Rinse in Distilled water (repeat steps 18-20 if Luxol fast blue needs more differentiation)
21. Dehydrate in alcohol
22. Clear in xylene, and mount with synthetic resin
Luxol Fast Blue-Holmes Silver Results

Myelin Sheaths - Blue to green
Axons and nerve fibers - Black
Luxol Fast Blue-Holmes Silver Technical Notes

• A section showing complete degeneration of both the axon and myelin sheath can be visualized

• Pridine is toxic and must be used under a chemical fume hood
Luxol Fast Blue-PAS-Hematoxylin

• Purpose: The demonstration of the myelin sheath, basement membrane, senile plaques, fungi, and corpora amylacea.
• Fixative: 10% NBF
• Technique: 10-15 microns
• Quality control: Cerebral Cortex
Luxol Fast Blue-PAS-Hematoxylin Reagents

- Luxol fast blue solution
- Lithium Carbonate solution
- Schiff solution
- Periodic acid solution
Luxol Fast Blue-PAS-Hematoxylin Procedure

1. Deparaffinize and hydrate to 95% alcohol
2. Luxol fast blue overnight at 56 to 58 degrees
3. 95% alcohol to rinse off excessive stain
4. Rinse in distilled water
5. Begin differentiation in lithium carbonate
6. Continue differentiation in 70% alcohol
7. Wash in distilled water
8. Finish differentiation in lithium carbonate, then several changes of 70% alcohol
9. Rinse in 70% alcohol
10. Periodic acid 5 min
11. Rinse in 2 changes of distilled water
12. Schiff solution 15 minutes
13. Tap water 5 min
14. Harris hematoxylin 30 seconds
15. Tap water 5 minutes
16. Dehydrate in alcohol
17. Clear in xylene and mount in synthetic resin
Luxol Fast Blue-PAS-Hematoxylin Results

- Capillary basement membrane: Rose
- Fungi: rose
- Corpora amylacea: rose
- Senile plaques: rose
- Myelin sheath: Blue to blue-green
- Nuclei: Purple
The End