

## Pigment and Artifact Pigment Removal - Technical Memo

### SOLUTIONS:

	250 ml	500 ml	1 Liter	4 Liters
<b>Melanin Pigment Removal</b>				
Potassium Permanganate 0.25%, Aqueous	Part 133931A	Part 133931B		
Oxalic Acid 5%, Aqueous	Part 1293A	Part 1293B		
<b>Mercury Pigment Removal</b>				
Melanin Control Slides	Part 4430			
Iodine, Gram, Aqueous		Part 1140A	Part 1140C	Part 1140E
or				
Iodine, Lugol's, Aqueous		Part 12092A	Part 12092B	
Sodium Thiosulfate 5%, Aqueous		Part 1389A	Part 1389B	
<b>Formalin Pigment Removal</b>				
Picric Acid, Saturated Alcoholic		Part 1337A	Part 1337B	

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

### APPLICATIONS:

Newcomer Supply Pigment and Artifact Pigment Removal Technical Memo provides procedures for removal of pigments, both naturally occurring and artifact, from tissue sections.

**Melanin pigment** naturally occurs and is produced by melanocytes that provides skin, hair and eyes with color. When melanin pigment obscures cellular detail, it can be bleached with potassium permanganate and oxalic acid solutions.

Artifact pigments are produced in tissues during processing, often a result of fixation. Microscopically, these pigments usually appear to lie on top of the tissue and not within the cell.

- **Mercury pigment** is deposited after exposure to fixatives containing mercuric chloride. Sections must be treated for mercury pigment removal prior to staining.
- **Formalin pigment** results when acidic formalin solutions react with blood rich tissues such as spleen and areas of hemorrhage, forming brown or brownish-black crystalline birefringent substances. The use of Formalin 10%, Phosphate Buffered (1090) assists in minimizing formalin pigment deposition.

### METHOD:

**Technique:** Paraffin sections on adhesive slides

- See Procedure Note #1.

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

### PROCEDURES:

- 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.
- Melanin Pigment Removal:**
  - Two Melanin Control Slides (4430) and two patient slides are needed.
  - Label one control slide and one patient slide "with".
  - Label the other control slide and patient slide "without". Set aside for Step h.
  - Bleach "with" sections in Potassium Permanganate 0.25% Aqueous (133931) for 5 to 20 minutes.
  - Rinse in several changes of distilled water.
  - Clear in Oxalic Acid 5%, Aqueous (1293) for 1-2 minutes or until sections turn white.
  - Wash in gently running tap water for 10 minutes.
  - Stain as desired; including untreated melanin control and untreated patient slides labeled "without".
  - See Procedure Note #2.

### 3. Mercury Pigment Removal:

- Treat sections with Iodine, Gram, Aqueous (1140) or Iodine, Lugol's, Aqueous (12092) for 10 minutes.
- Wash briefly in running tap water.
- Place in Sodium Thiosulfate 5%, Aqueous (1389) for 3 minutes.
- Wash in gently running tap water for 10 minutes.
- Stain as desired.

### 4. Formalin Pigment Removal:

- Treat sections with Picric Acid, Saturated Alcoholic (1337) for 10 minutes to 3 hours.
- Wash in gently running tap water for 10 minutes.
- Stain as desired.
- See Procedure Note #3.

### PROCEDURE NOTES:

- Pigment removal procedures are harsh on tissues sections. The use of adhesive slides (Part 5070, 5079 or 6203) is recommended to ensure tissue adherence.
- The darker the melanin pigment the longer bleach will take to decolorize the pigment.
- Timing to remove formalin pigment will vary and will depend on the amount of pigment present in the sections.

### REFERENCES:

- Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 252-253.
- Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 23-24, 254-255.
- Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 130, 214, 220-221.
- Modifications developed by Newcomer Supply Laboratory.