

# 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, Weigert & Lugol, 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** is a naturally occurring pigment produced by melanocytes and provides skin, hair and eyes with color. If requested or when melanin pigment obscures cellular detail, melanin pigment can be bleached with potassium permanganate and oxalic acid solutions.

Artifact pigments are a result of chemical action and are produced/deposited in tissues during processing, often a result of fixation. These pigments, when viewed microscopically, commonly appear to lie on top of the tissue and not within the cell.

- **Mercury pigment** is deposited in tissues from immersion in any fixative containing mercuric chloride, such as Zenker and Helly solutions. The use of mercury in fixatives has been discouraged due to environmental concerns and costly disposal requirements. Any tissue previously or currently fixed in a mercuric chloride containing fixative must be treated for mercury pigment removal prior to any staining procedure.
- **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 substances that are birefringent. The use of Formalin 10%, Phosphate Buffered (Part 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 positive melanin control slides and two patient slides are recommended. Label one control slide and one patient slide "with"; label the other control slide and patient slide "without". (Set "without" aside for Step f).
  - Treat "with" sections with Potassium Permanganate 0.25% Aqueous (133931) for 5 to 20 minutes.
  - Rinse in several changes of distilled water.
  - Place 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 slide and untreated patient slide labeled "without".
  - See Procedure Notes #2, #3 and #4.

## 3. **Mercury Pigment Removal:**

- Treat sections with Iodine, Gram, Aqueous (1140) or Iodine, Weigert & Lugol, 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 #5.

## PROCEDURE NOTES:

- All pigment removal solutions for provided procedures are harsh on tissues sections. The use of adhesive slides (Part 5070, 5079 or 6203) is highly recommended to ensure tissue remains adhered during the treatment process.
- The darker the melanin pigment the longer the bleach will take to decolorize the pigment.
- Oxalic Acid 5%, Aqueous may take less time to whiten the sections than the suggested 1-2 minutes.
- Melanin bleaching can be performed on free floating frozen or paraffin sections. Concentration of solutions and timings remain the same.
- The time necessary to remove formalin pigment will vary and be dependent upon the amount of pigment present in the tissue.

## 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.