

## Wolbach Giemsa Stain - Technical Memo

### SOLUTIONS:

Giemsa Stock Stain, Wolbach  
Rosin 10%, Alcoholic

**500 ml**  
Part 1121A  
Part 13398A

**1 Liter**  
Part 1121B

### Additionally Needed:

Giemsa Control Slides  
Xylene, ACS  
Alcohol, Ethyl Denatured, 100%  
Alcohol, Ethyl Denatured, 95%  
Acid Alcohol 1%  
Alcohol, Methanol Anhydrous, ACS

Part 4240  
Part 1445  
Part 10841  
Part 10842  
Part 10011  
Part 12236

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

### APPLICATION:

Newcomer Supply Wolbach Giemsa Stain is used for differential staining of hematopoietic tissue and for the identification of bacteria and rickettsia that may be present in tissue sections.

### METHOD:

**Fixation:** According to protocol for hematopoietic tissue

a. See Procedure Note #1.

**Technique:** Paraffin sections cut at 5 microns

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

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

### STAINING PROCEDURE:

- 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.
  - See Procedure Notes #2 and #3.
- Treat slides with Acid Alcohol 1% (10011) Solution for 5 minutes.
  - See Procedure Note #4.
- Wash in running tap water for 5 minutes.
- Prepare fresh Working Giemsa Stain Solution; combine and mix well.
 

a. Distilled water	50 ml
b. Giemsa Stock Stain, Wolbach	2 ml
c. Methanol (12236)	2 ml
- Stain slides in fresh Working Giemsa Stain Solution for 60 minutes at room temperature.
  - See Procedure Note #5.
- Differentiate each slide individually in Rosin 10%, Alcoholic until sections are purplish-pink; 5-10 dips. Check microscopically.
  - Rinse in 100% ethyl alcohol to stop differentiation.
- Dehydrate in two changes of 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.
  - Do not use a 95% ethyl alcohol dehydration step.

### RESULTS:

Nuclei	Blue/violet
Cytoplasm	Pink/rose to lighter blue shades
Bacteria	Blue
Rickettsia, inclusions	Reddish-purple

### PROCEDURE NOTES:

- Zenker Fixative, Modified, Zinc Chloride (Part 1461) and B-5 Fixative Modified, Zinc Chloride (Part 1015) are preferred fixatives for hematopoietic tissue; Formalin 10%, Phosphate Buffered (Part 1090) or other well fixed tissue is acceptable.
- Drain staining rack/slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during staining procedure.
- The Wolbach Giemsa Stain is more effective in tissue with an acid pH. Treatment of sections with Acid Alcohol 1% will ensure an acid pH and improve staining.
- For more intense staining, stain slides in fresh Working Giemsa Stain Solution in a 60°C water bath for 60 minutes. Differentiation in Step #6 may require additional dips in Rosin 10%, Alcoholic.
- The color range of the stained cells may vary depending upon fixation and degree of differentiation.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

### REFERENCES:

- Luna, Lee G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: Blakiston Division, McGraw-Hill, 1968. 119-120.
- Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 156-157.
- Wolbach, S. Burt, and John Todd. *The Etiology and Pathology of Typhus*. S.I.: Harvard University Press, 1922. 13-14.
- Modifications developed by Newcomer Supply Laboratory.