2505 Parview Road • Middleton, WI 53562-2579 • 800-383-7799 • www.newcomersupply.com • newly@newcomersupply.com

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May-Grunwald Giemsa Stain - Technical Memo

SOLUTIONS:	**500 ml**	**1 Liter**
Jenner Stock Stain	Part 1210A	Part 1210B
Giemsa Stock Stain, Wolbach	Part 1121A	Part 1121B

Additionally Needed:	
Giemsa Control Slides	Part 4240
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Alcohol, Methanol Anhydrous, ACS	Part 12236
Acetic Acid 1%, Aqueous	Part 10012

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

APPLICATION:

Newcomer Supply May-Grunwald Giemsa Stain procedure, employing both Jenner Stock Stain and Giemsa Stock Stain, Wolbach for intense colorization results, is used for differential staining of hematopoietic tissue as well as for demonstration of some microorganisms.

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:

1. 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 #2 and #3.*
2. Rinse in two changes of Methanol (12236); 3 minutes each.
3. Prepare fresh Working Jenner Stain just prior to use; combine and mix well.
   - *a. Jenner Stock Stain 20 ml*
   - *b. Distilled Water 20 ml*
4. Place slides in fresh Working Jenner Stain for 6 minutes.
5. Prepare fresh Working Giemsa Stain just prior to use; combine and mix well.
   - *a. Giemsa Stock Stain, Wolbach 3 ml*
   - *b. Distilled Water 47 ml*
6. Place slides, without rinsing, directly into fresh Working Giemsa Stain for 45 minutes.
7. Rinse quickly in distilled water.
8. Differentiate each slide individually in Acetic Acid 1%, Aqueous (10012); 6-10 dips.
   - *a. Check microscopically for well differentiated nuclei.*
9. Rinse in distilled water.
10. 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:

Nuclei	Blue/violet
Cytoplasm	Pink/rose to lighter blue shades
Bacteria	Blue

PROCEDURE NOTES:

1. Zenker Fixative, Modified, Zinc Chloride (Part 1461) and B-5 Fixative Modified, Zinc Chloride (Part 1015) are the preferred fixatives for hematopoietic tissue; Formalin 10%, Phosphate Buffered (Part 1090) or other well fixed tissue is acceptable.
2. Drain staining rack/slides after each step to prevent solution carry over.
3. Do not allow sections to dry out at any point during staining procedure.
4. The color range of the stained cells may vary depending upon fixation and degree of differentiation.
5. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida. *Histotechnology: A Self-Instructional Text.* 2nd ed. Chicago: ASCP Press, 1997. 106-107.
2. Luna, Lee G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: Blakiston Division, McGraw-Hill, 1968. 121-122.
3. Shapiro, Stanley H., and Hilda Laufer. "Observations on Fixation and Staining of Bone Marrow Biopsies." *The Journal of Histotechnology* 11.3 (1988): 145-47.
4. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 157.
5. Modifications developed by Newcomer Supply Laboratory.
