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

Revised October 2015

Wright-Giemsa Stain, Modified for Tissue Sections - Technical Memo

SOLUTIONS:	500 ml	1 Liter	1 Gallon
Wright Stain, Modified	Part 1421A	Part 1421B	Part 1421C
Giemsa Stock Stain	Part 1120A	Part 1120B	

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

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

APPLICATION:

Newcomer Supply Wright-Giemsa Stain, Modified for Tissue Sections combines a modified Wright's formula with a Giemsa Stain Solution for differential staining of hematopoietic tissue and demonstration of bacteria that may be present in the sections. This procedure is applicable for either hand or automated staining processes.

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. Stop at 95% ethyl alcohol.
   a. *See Procedure Notes #2 and #3.*
2. Treat slides in two changes of Methanol (12236); 3 minutes each.
3. Stain in Wright Stain, Modified for 6 minutes.
4. Prepare fresh Working Giemsa Stain:
   a. *Distilled Water* *40 ml*
   b. *Giemsa Stock Stain* *5 ml*
5. Stain in fresh Working Giemsa Stain in a 60°C oven for 60 minutes.
6. 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
Cytoplasm	Pink to red
Bacteria	Blue

PROCEDURE NOTES:

1. 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.
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.
5. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Shapiro, Stanley H., and Hilda Laufer. "Observations on Fixation and Staining of Bone Marrow Biopsies." *The Journal of Histotechnology* 11.3 (1988): 145-47.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 155-156.
3. Modifications developed by Newcomer Supply Laboratory.

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

Part 1421
Revised October 2015

Wright Stain, Modified for Smears - Technical Memo

SOLUTION:	**500 ml**	**1 Liter**	**1 Gallon**
Wright Stain, Modified	Part 1421A	Part 1421B	Part 1421C

Additionally Needed:

Alcohol, Methanol Anhydrous, ACS	Part 12236
Wright Stain Buffer, pH 6.8	Part 1430

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

APPLICATION:

Newcomer Supply Wright Stain, Modified for Smears, provides a concentrated Wright's formula for differential staining of cell types in peripheral blood smears as well as bone marrow smears/films. This procedure is applicable for either hand or automated staining processes.

METHOD:

Technique: Flat staining rack method
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

STAINING PROCEDURE:

1. Prepare within an accepted time frame, a well-made blood smear or bone marrow smear/film per your laboratories protocol, with a focus on uniform cell distribution.
2. Allow slides to thoroughly air-dry prior to staining.
3. Filter Wright Stain, Modified prior to use with high quality filter paper.
4. Place slides on flat staining rack suspended over sink.
5. Fix smears by flooding slides with Methanol (12236) for 10-30 seconds.
6. Shake off excess Methanol; flood each slide with 1 ml of filtered Wright Stain, Modified for 3-5 minutes.
    a. *See Procedure Notes #1 and #2.*
7. Retain Wright Stain, Modified on slides. Directly add 1 ml of Wright Stain Buffer, pH 6.8 (1430) to each slide; gently agitate to completely mix with retained Wright Stain, Modified.
8. Stain for an additional 6-10 minutes.
9. Wash well in distilled water; rinse thoroughly in running tap water.
10. Air-dry slides in a vertical position; examine microscopically.
11. If coverslip is preferred, allow slides to air-dry and coverslip with compatible mounting medium.

RESULTS:

Erythrocytes	Pink
Neutrophils	Granules - Purple
Eosinophils	Granules - Pink
White blood cells	Chromatin - Purple
Lymphocytes	Cytoplasm - Blue
Monocytes	Cytoplasm- Blue
Bacteria	Deep Blue

PROCEDURE NOTES:

1. The timings provided in this procedure are suggested ranges. Optimal staining times will depend upon staining intensity preference.
2. Smears containing primarily normal cell populations require minimum staining time; immature cells may require a longer staining time. Bone marrow smears/films may also require a longer staining time.
3. The color range of the stained cells may vary depending upon the pH of the buffer and the pH of the rinse water used.
    a. *Alkalinity is indicated by red blood cells being blue-grey and white blood cells only blue.*
    b. *Acidity is indicated by red blood cells being bright red or pink and lack of proper staining in white blood cells.*
    c. *If necessary adjust buffer pH accordingly to 6.8 +/ - 0.2.*

REFERENCES:

1. Lillie, R. D., and Harold Fullmer. *Histopathologic Technic and Practical Histochemistry*. 4th ed. New York: McGraw-Hill, 1976. 747-748.
2. McPherson, Richard and Matthew Pincus. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 22nd ed. Philadelphia: Elsevier Saunders, 2011. 522-532.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 154-155.
4. Modifications developed by Newcomer Supply Laboratory.
