

## Wright-Giemsa, Romanowsky Stain for Smears - Technical Memo

<b>SOLUTIONS:</b>	<b>500 ml</b>	<b>6 X 500 ml</b>
Giemsa Stock Stain, Romanowsky	Part 11215A	Part 11215A

<b>Additionally Needed:</b>	
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-Giemsa, Romanowsky Stain for Smears is the classic Wright-Giemsa stain for hematology. Designed to demonstrate differential staining of cell types in peripheral blood smears and bone marrow smears/films, it is also a method for detecting parasites, bacteria, and inclusion bodies.

Romanowsky-type stains refers to stains made from water-soluble eosin, methylene blue and methanol. Wright-Giemsa stains, comprised of polychrome methylene blue, azure B and eosin Y dyes, are classified as Romanowsky stains.

### METHOD:

**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 provided staining procedure.

### PRESTAINING PREPARATION:

- 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.
- Allow slides to thoroughly air-dry prior to staining.
- Prepare Wright-Giemsa, Romanowsky Working Stain Solution; combine and mix well. Filter if particulates are present.
  - For thin smears:*

Giemsa Stock Stain, Romanowsky	20 ml
Wright Stain Buffer, pH 6.8	20 ml
  - For thick smears:*

Giemsa Stock Stain, Romanowsky	4 ml
Wright Stain Buffer, pH 6.8	36 ml

### STAINING PROCEDURE:

- Fix smears in Methanol (12236) for 3-5 minutes.
- Air-dry slides in a vertical position.
- Stain in Wright-Giemsa, Romanowsky Working Stain Solution (Step #3) for 30-45 minutes.
  - See Procedure Notes #1 and #2.
- Wash in distilled water.
- Air-dry slides in a vertical position; examine microscopically.
- If coverslip is preferred, air-dry slides and coverslip with compatible mounting medium.

### RESULTS:

Erythrocytes	Orange-pink to rose
Platelets	Red to purple granules with light blue halo

### Granulocytes

Neutrophils	Nucleus - Dark blue to violet Cytoplasm - Pink Granules - Purple to lilac
Eosinophils	Nucleus - Blue Granules - Orange to pink
Basophils	Nucleus - Deep blue to violet Granules - Deep blue to violet

### RESULTS CONTINUED:

#### Mononuclear Cells

Lymphocytes	Nuclei - Deep blue to violet Cytoplasm - Light blue
Monocytes	Nuclei - Light blue/purple Cytoplasm - Pale gray/blue
Mast cells	Nuclei - Deep blue to violet Granules - Deep blue-violet
Malarial parasites	Nucleus - Red chromatin dot Cytoplasm - Blue
Bacteria	Blue

### PROCEDURE NOTES:

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

### REFERENCES:

- Bauer, John D. *Clinical Laboratory Methods*. 9th ed. St. Louis: Mosby, 1982. 111-112.
- Conn's Biological Stains*. Edited by Richard Horobin and John Kiernan. 10th ed. Oxford, UK: BIOS Scientific Publishers, 2002. 303-312.
- Lillie, R. D., and Harold Fullmer. *Histopathologic Technic and Practical Histochemistry*. 4th ed. New York: McGraw-Hill, 1976. 744-748.
- McPherson, Richard and Matthew Pincus. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 22nd ed. Philadelphia: Elsevier Saunders, 2011. 522-532.
- Modifications developed by Newcomer Supply Laboratory.