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Part 11215
Revised July 2020

Wright-Giemsa, Romanowsky Stain for Smears - Technical Memo

SOLUTIONS:	**500 ml**	**6 X 500 ml**
Giemsa Stock Stain, Romanowsky	Part 11215A	Part 11215A

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-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:

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. Prepare Wright-Giemsa, Romanowsky Working Stain Solution; combine and mix well. Filter if particulates are present.
    a. *For thin smears:*
    Giemsa Stock Stain, Romanowsky *20 ml*
    Wright Stain Buffer, pH 6.8 *20 ml*
    b. *For thick smears:*
    Giemsa Stock Stain, Romanowsky *4 ml*
    Wright Stain Buffer, pH 6.8 *36 ml*

STAINING PROCEDURE:

4. Fix smears in Methanol (12236) for 3-5 minutes.
5. Air-dry slides in a vertical position.
6. Stain in Wright-Giemsa, Romanowsky Working Stain Solution (Step #3) for 30-45 minutes.
    a. *See Procedure Notes #1 and #2.*
7. Wash in distilled water.
8. Air-dry slides in a vertical position; examine microscopically.
9. 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:

1. Timings provided are suggested ranges. Optimal times will depend upon staining intensity preference.
2. Smears containing primarily normal cell populations require minimum staining time; immature cells and bone marrow smears/films may require longer staining time.
3. The color range of stained cells may vary depending on buffer pH and pH of rinse water.
    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. Bauer, John D. *Clinical Laboratory Methods*. 9th ed. St. Louis: Mosby, 1982. 111-112.
2. *Conn's Biological Stains*. Edited by Richard Horobin and John Kiernan. 10th ed. Oxford, UK: BIOS Scientific Publishers, 2002. 303-312.
3. Lillie, R. D., and Harold Fullmer. *Histopathologic Technic and Practical Histochemistry*. 4th ed. New York: McGraw-Hill, 1976. 744-748.
4. McPherson, Richard and Matthew Pincus. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 22nd ed. Philadelphia: Elsevier Saunders, 2011. 522-532.
5. Modifications developed by Newcomer Supply Laboratory.
