Wright-Giemsa, Romanowsky Stain for Smears - Technical Memo

**SOLUTIONS:**

<table>
<thead>
<tr>
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<th>500 ml</th>
<th>6 X 500 ml</th>
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<tr>
<td>Giemsa Stock Stain, Romanowsky</td>
<td>Part 11215A</td>
<td>Part 11215A</td>
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**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 deemed the classic Wright-Giemsa stain for hematology. It is designed to demonstrate differential staining of cell types in peripheral blood smears and bone marrow smears/films as well as a method for detecting parasites, bacteria, and inclusion bodies.

A Romanowsky-type stain refers to a stain 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 staining procedure provided below.

**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 smear to thoroughly air-dry prior to staining.
3. Fix smear in Alcohol, Methanol Anhydrous (12236) for 3-5 minutes.
5. Prepare Wright-Giemsa, Romanowsky Working Stain Solution; combine, mix well and 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
   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 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. 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:**

5. Modifications developed by Newcomer Supply Laboratory.