# Wright Stain, Buffered for Smears - Technical Memo

## SOLUTION:

<table>
<thead>
<tr>
<th>SOLVENT</th>
<th>500 ml</th>
<th>1 Liter</th>
<th>1 Gallon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wright Stain, Buffered</td>
<td>Part 1422A</td>
<td>Part 1422B</td>
<td>Part 1422C</td>
</tr>
</tbody>
</table>

## 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, Buffered for Smears provides a quick staining technique for differential staining of cell types in peripheral blood smears as well as bone marrow smears/films.

## METHOD:

**Technique**: Coplin jar or 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, Buffered prior to use with high quality filter paper.  
4. Prepare 25% Aqueous Methanol Rinse; combine and mix well.  
   a. Distilled Water  
   b. Methanol (12236)  
5. **Coplin Jar Method**: See Procedure Notes #1 and #2.  
   a. Fix smears in pure Methanol for 15 seconds.  
   b. Stain in filtered Wright Stain, Buffered for 1-2 minutes.  
   c. Place smear directly into Wright Stain Buffer, pH 6.8 (1430), for 1-4 minutes. **Do Not Agitate!**  
   d. Dip smears in 25% Aqueous Methanol Rinse (Step #4) for 1 second.  
   e. Rinse in distilled water.  
   f. Air-dry slides in a vertical position; examine microscopically.  
   g. If coverslip is preferred, allow slides to air-dry and coverslip with compatible mounting medium.  
6. **Flat Staining Rack Method**: See Procedure Notes #1 and #2.  
   a. Place slides on flat staining rack suspended over sink.  
   b. Fix smear by flooding slide with pure Methanol for 15 seconds.  
   c. Shake off excess Methanol; apply 1 ml of filtered Wright Stain, Buffered to each slide for 1 minute.  
   d. Retain Wright Stain, Buffered on slides. Directly add 2 ml of Wright Stain Buffer, pH 6.8 to each slide; gently agitate to completely mix with retained Wright Stain, Buffered.  
   e. Stain for an additional 3 minutes.  
   f. Flood smears with 25% Aqueous Methanol Rinse (Step #4) for 1 second.  
   g. Rinse in distilled water.  
   h. Air-dry slides in a vertical position; examine microscopically.  
   i. If coverslip is preferred, allow slides to air-dry and coverslip with compatible mounting medium.

## RESULTS:

- Erythrocytes  
- Neutrophils  
- Eosinophils  
- White blood cells  
- Lymphocytes  
- Monocytes  
- Bacteria

<table>
<thead>
<tr>
<th>TYPE</th>
<th>COLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>Pink</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Granules - Purple</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Granules - Pink</td>
</tr>
<tr>
<td>White blood cells</td>
<td>Chromatin - Purple</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Cytoplasm - Blue</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Cytoplasm - Blue</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Deep Blue</td>
</tr>
</tbody>
</table>

## 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:

4. Modifications developed by Newcomer Supply Laboratory.

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