Wright-Giemsa Stain Solution

Instructions for Use

**Intended Use:**
Solution specifically intended to stain human blood cells. For differential cell count. For in-vitro diagnostic use.

**Summary and Explanation of Tests:**
The use of polychrome methylene blue and eosin Y, which are now used in the Wright-Giemsa Stain Solution, was developed by Romanowsky in 1891. He observed that this combination of dyes gave excellent selective staining of blood films. Also in 1891, Giemsa modified Leishman’s stain to provide better stain intensity and fine cellular detail. The stain, however, required an extended staining process. The Wright-Giemsa Stain Solution has been developed to incorporate the exceptional brilliance and resolution of cellular details obtained from Giemsa Stain with the rapid staining time of Wright’s Stain.

**Reagents**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>g/L</th>
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<tbody>
<tr>
<td>Wright’s Stain, certified</td>
<td>1.53</td>
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<tr>
<td>Giemsa Stain, certified</td>
<td>2.50</td>
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<tr>
<td>Glycerin</td>
<td>10% (v/v)</td>
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In absolute methanol.
All stains are certified by the Biological Stain Commission.

**General Purpose Reagents Required:**
- Distilled or deionized water
- Phosphate buffer

**General Supplies:**
- Microscope slides
- Cover glasses
- Coplin jars
- 1000 ml volumetric flask
- Beakers
- Microscope

**Safety Precautions:**
**Danger!** Poison! May be fatal or cause blindness if swallowed. Flammable. Keep away from heat, sparks and open flames. In case of fire, use water spray, CO₂, foam or dry chemical.

**First Aid:** Call a physician. If the stain has been swallowed, induce vomiting. Repeat until vomit is clear.

**Storage Instructions:**
Solution should be kept in the original bottle, tightly closed, to prevent contamination with water. It should be stored at room temperature at 15-30°C (59-86°F). When stored in this manner, solution has good stability. To check usefulness of solution, stain a known specimen in the usual manner and compare with usual results.

**Specimen Preparations:**
Blood films must be made properly to ensure correct morphology and staining. In particular, blood films must be uniformly thin so as to obtain unvarying quality in staining from slide to slide.

**Procedure:**

a) Using any of the conventional techniques, smear a small drop of blood on a clean microscope slide. Allow to air dry.

b) Fix by immersing in methanol for at least 5 minutes.

c) Prepare pH 6.8 phosphate buffer solution consisting of potassium phosphate, monobasic 50.1% (w/w) and sodium phosphate, dibasic 49.9% (w/w). Weight out accurately 0.501 grams of potassium phosphate, monobasic and 0.499 grams of sodium phosphate, dibasic. Dissolve in 1000 ml of distilled water.

d) Mix equal parts of Wright-Giemsa Stain and buffer solution immediately before use.

e) Immerse blood films in Coplin jar or staining dish of solution prepared in Step “d” for 2 to 4 minutes, longer if preferred.

f) Rinse in pH 6.8 buffer, three changes, 10 dips each.

g) Wipe back of slide. Blot dry or stand on end and allow to dry.
h) Examine smear by microscope, with or without coverslip-  
ing. If using a coverslip, use the least amount of mount-  
ing medium and a No. 1 thickness coverglass if the blood  
is spread on the slide. If the blood is spread on a cover-  
glass, the thickness should be No. 1 1/2.

i) Discard the stain and the buffer rinses after each use.

Results:

- Erythrocytes appear yellowish-red to tawny.
- Polymorphonuclear neutrophils’ cytoplasm appears pale  
pink to tan; the granules, lilac; the nuclei, reddish-purple.
- Eosinophils’ granules appear red-orange; the nuclei,  
reddish-purple.
- Basophils’ granules appear purple to dark bluish-purple;  
the nuclei, reddish-purple.
- Lymphocytes’ cytoplasm appears sky blue; the nuclei, red  
dish-purple.
- Platelets appear violet to purple.

Limitations:

The information obtained from the blood smear is dependent on  
the method of specimen collection, the preparation of the film,  
the drying, the fixation and the final staining of the smear. To  
this end, slides should be free of all dirt and oily film. Care  
should be taken in the staining and washing, as it can result in  
a precipitate, the wrong colors or excessive coloring. This  
excessive staining can also be due to thick smears.

<table>
<thead>
<tr>
<th>Cat. #</th>
<th>Description</th>
<th>Size</th>
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</thead>
<tbody>
<tr>
<td>08711</td>
<td>Wright-Giemsa Stain</td>
<td>470ml</td>
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To Order:

In The U.S. Call: 1-800-523-2575 • 215-343-6484
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References:

1) Romanowsky, D.L., St. Petersburg Med. Wshr. 16,  
2) Lillie, R.O., Biological Stains, 8th Edition, Williams  
3) Ibid., pp. 355-360.