

## Giemsa Stain - Technical Memo

**SOLUTION:**  
Giemsa Stock Stain                      500 ml                      1 Liter  
Part 1120A                                      Part 1120B

**Additionally Needed:**  
Alcohol, Methanol Anhydrous, ACS                      Part 12236

*For storage requirements and expiration date refer to individual bottle labels.*

**APPLICATION:**

Newcomer Supply Giemsa Stain is a simple one-step method designed to demonstrate differential staining of cells types in peripheral blood smears and bone marrow smears/films as well as a method for detecting rickettsia, bacteria and parasites.

**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 Methanol for 3-5 minutes.
4. Air-dry slides in a vertical position.
5. Prepare Working Giemsa Stain; combine, mix well and filter with high quality filter paper.
  - a. *Giemsa Stock Stain*                                      6 ml
  - b. *Distilled Water*    30 ml
  - c. *See Procedure Note #1.*
6. Stain in filtered Working Giemsa Stain for 30-45 minutes.
  - a. *See Procedure Notes #2 and #3.*
7. Wash in distilled water.
8. Air-dry slides in a vertical position, then examine microscopically.
9. If coverslip is preferred, allow slides to air-dry and coverslip with compatible mounting medium.

**RESULTS:**

Erythrocytes                      Orange - pink to rose  
Platelets                              Red to purple granules with 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  
Rickettsia                              Bluish purple  
Bacteria                                      Blue

**PROCEDURE NOTES:**

1. For more intense staining, substitute a 1% Sodium Carbonate solution for the distilled water component in preparing the Working Giemsa Stain.
2. The timings provided in this procedure are suggested ranges. Optimal staining times will depend upon preference of stain intensity.
3. Smears containing primarily normal cell populations require minimum staining time, while immature cells may require a longer staining time. Bone marrow smears/films may also require a longer staining time.

**REFERENCES:**

1. Bailey, W. Robert, and Elvyn Scott. *Diagnostic Microbiology*. 4th ed. St Louis: C. V. Mosby Company, 1974. 394.
2. Luna, Lee G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: Blakiston Division, McGraw-Hill, 1968. 127-128.
3. McPherson, Richard and Matthew Pincus. *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 22nd ed. Philadelphia: Elsevier Saunders, 2011. 522-531.
4. Modifications developed by Newcomer Supply Laboratory.