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Part 1120
Revised September 2019

Giemsa Stain for Smears/Films - Technical Memo

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

Additionally Needed:

Alcohol, Methanol Anhydrous, ACS	Part 12236
Phosphate Buffer, pH 7.0	Part 1331

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 provided staining procedure.

STAINING PROCEDURE:

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.

a. *Proceed with either the thin or thick smear/film staining method.*

Thin Smear/Film Staining Method: *See Procedure Notes #1 and #2.*

1. Allow smear to thoroughly air-dry prior to staining.
2. Fix smear in Methanol: 1-2 minutes.
3. Air-dry slides in a vertical position.
4. Prepare fresh 1:20 Working Giemsa Stain; combine and mix well.
   a. *Giemsa Stock Stain 2 ml*
   b. *Phosphate Buffer, pH 7.0 (1331) 40 ml*
5. Stain in Working Giemsa Stain for 20-30 minutes.
6. Rinse briefly in Phosphate Buffer, pH 7.0 or distilled water.
7. Air-dry slides in a vertical position.
8. If coverslip is preferred, allow slides to air-dry; coverslip with compatible mounting medium.

Thick Smear/Film Staining Method: *See Procedure Notes #1 and #2.*

1. Allow smear to thoroughly air-dry prior to staining; several hours or overnight.
2. Proceed directly to stain; do not place in fixative.
3. Prepare fresh 1:50 Working Giemsa Stain; combine and mix well.
   a. *Giemsa Stock Stain 1 ml*
   b. *Phosphate Buffer, pH 7.0 (1331) 50 ml*
4. Stain in Working Giemsa Stain for 50 minutes.
5. Rinse briefly in Phosphate Buffer, pH 7.0 or distilled water.
6. Air-dry slides in a vertical position.
7. If coverslip is preferred, allow slides to air-dry; 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
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. The timings provided are suggested ranges. Optimal staining times will depend upon smear/film thickness and preference of stain intensity.
2. Smears/films containing primarily normal cell populations require minimum staining time.
   a. *Immature cells may require a longer staining time.*
   b. *Bone marrow smears/films may 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. Garcia, Lynne Shore. *Diagnostic Medical Parasitology*. 5th ed. Washington DC: ASM Press, 2007. 888-889.
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.
