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Area	Laboratory-Histology
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Histology Special Stain - Giemsa - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of different cell types in hematopoietic and lymphoid tissue. It can also be used to non-differentially stain bacteria and for the demonstration of some parasites.

II. PRINCIPLE:

All the Romanowsky stains use a polychromatic dye (Giemsa) which contains methylene blue (basic dye) and eosin (acid dye). The methylene blue is impure, and will oxidize into azure A, azure B, and methylene violet, thereby giving a wide range of colors. The acetic acid is a weak dye used to differentiate the colors of the cells.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well-fixed tissue.
    2. Zenker or B5 preferred.

B. Processing
    1. Standard, overnight processing.

C. Section Thickness
    1. Cut routine paraffin sections at 5µ.
    2. Cut bone marrow or lymph nodes at 4µ.

D. Slide Drying

1. 30 minutes at 60°C.

E. Type of Slide

1. Plain slides

IV. REAGENTS:

A. **Stock Giemsa Solution**

Giemsa dye	**1.0 gm**
Glycerin	**66.0 mL**
Absolute methanol	**66.0 mL**

Mix together glycerin and Giemsa dye. Place in 60°C. oven for 30 minutes to 2 hours, until dissolved. Add methanol and mix.
Store at room temperature in a closed, dark brown bottle. Keep away from heat and sunlight, which will shorten the life of the solution. Stable about 1 year.

B. **Working Giemsa Solutions**

Stock Giemsa solution	**10.0 mL**
Distilled Water	**40.0 mL**

JUST BEFORE USE, mix together; stable for one day only.

C. **0.5% Acetic Acid Water**

Acetic Acid	**0.5 mL**
Distilled water	**99.5 mL**

JUST BEFORE USED, mix together; stable for one day only.

V. EQUIPMENT:

A. Balance

B. 60°C oven

C. Magnetic stirrer

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Coplin jars

D. Forceps

VII. SPECIAL SAFETY PRECAUTIONS:

A. Giemsa

1. Toxicological properties have not been fully investigated.

B. Glycerin

1. Low hazard for recommended handling.

C. Methanol

1. Is poisonous.
2. May be fatal or cause blindness if swallowed.
3. Cannot be made nonpoisonous.
4. Liquid and vapor are flammable.
5. Harmful if inhaled or absorbed through skin.
6. May cause skin and eye irritation.

D. Acetic Acid

1. Is an acid.
2. Add drop by drop to solution.
3. May cause skin or eye burns.

VIII. QUALITY CONTROL(QC):

A. None needed, as every tissue has nuclei.

B. If required, use a section of spleen, lymph node, or bone marrow.

IX. LIMITATIONS:

A. Isopropyl alcohol is used, as ethanol would continue to extract the dye.

B. Working Giemsa solution is good for one day only.

X. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate sections through graded alcohol to distilled water.		
2	Place in WORKING Giemsa solution.	2 hours	Make just before use.
3	Differentiate in 0.5% acetic acid.	1-3 seconds	
4	Complete differentiation of chromatin pattern until crisp and clear in 95% ethanol, 2 changes.	1-10 seconds	Differentiate on slide at a time. Use a microscope to determine the endpoint of differentiation. Chromatin material of nuclei should be readily distinguished for good differentiation. If over differentiated, slides may be placed back into the Giemsa solution and re-stained.
5	Dehydrate in absolute isopropyl	5-15	

	alcohol, 3 changes each.	seconds	
6	Clear in xylene, 2 changes.	10 seconds each	
7	Coverslip.		

XI. RESULTS:

A. Nuclei - **blue**
B. Cytoplasm, collagen, muscle - **pink/blue/gray**
C. Eosinophilic granules - **pink**
D. Neutrophilic granules - **blue**
E. Bacteria - **blue**
F. Nuclei of parasitic protozoa - **red**
G. Cytoplasm of parasitic protozoa - **blue**
H. Red blood cells **- pinkish/bluish**
I. Bone spicules - **pink**

XII. REFERENCES:

A. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd ed. New York, NY, Churchill Livingstone, 1990.
B. Carson FL: Histotechnology: A Self-Instructional Text, Chicago, IL, ASCP Press, 1990.
C. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak