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Beaumont

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Histology Special Stain - Jenner-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 and also Jenner in this procedure) 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 specimens 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 Jenner Solution**

Jenner dye	**1.0 gm**
Absolute methanol	**400.0 ml**

Dissolve together. Store at room temperature; stable for months.

B. **Working Jenner Solution**

Stock Jenner solution	**25.0 ml**
Distilled water	**25.0 ml**

JUST BEFORE USE, mix together. Good for 1 day only.

C. **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 for about 1 year.

D. **Working Giemsa Solutions**

Stock Giemsa solution	**1.5 ml**
Distilled water	**50.0 ml**

JUST BEFORE USE, mix together. Good for one day only.

E. **0.5% Acetic Acid Water**

Acetic acid	**0.5 ml**
Distilled water	**99.5 ml**

JUST BEFORE USE, 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. Methanol
    1. Is a poison and may be fatal or cause blindness if swallowed.
    2. Cannot be made nonpoisonous.
    3. Harmful if inhaled or absorbed through skin.
    4. Can cause skin and eye irritation.

B. Giemsa
    1. This chemical is not considered hazardous.

C. Jenner
    1. Oral toxicity.
    2. Toxic to skin.
    3. Toxic to respiratory system.
    4. Irritant to eyes.

D. Glycerin
    1. Eye, skin and respiratory tract irritant.

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

VIII. QUALITY CONTROL:

None required, as all tissue contains nuclei. 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 2 changes of absolute solution.	3 minutes each	
3	Stain in WORKING Jenner solution.	6 minutes	Make Just Before Use.
4	Transfer directly into WORKING Giemsa solution.	45 minutes	
The following steps will be done one slide at a time			
5	Rinse quickly in distilled water.	3 seconds	
6	Differentiate in 0.5% acetic acid.	1-3 seconds	
7	Rinse in distilled water.	2-3 seconds	
8	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.
9	Dehydrate in absolute alcohol, 2 changes each.	5-10 seconds	
10	Clear in xylene, 2 changes.	10 seconds each	
11	Coverslip.		

XI. RESULTS:

A. Nuclei - **blue**
B. Cytoplasm, collagen, muscle, bone spicules - **pink/blue/gray**
C. Eosinophilic granules - **pink**
D. Basophilic granules - **blue**
E. Bacteria, parasitic protozoa cytoplasm - **blue**
F. Red blood cells - **yellowish-pink**
G. Eosinophilic granules - **pink**
H. Neutrophilic granules - **blue**
I. Bacteria - **blue**

J. Nuclei of parasitic protozoa - **red**

K. Cytoplasm of parasitic protozoa - **blue**

L. Red blood cells - **pinkish/bluish**

M. 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