GIEMSA - SHEEHAN'S MODIFIED MAY-GRUNWALD

PURPOSE To permit differentiation of cells present in hematopoietic tissue. The stain is also used for the demonstration of some microorganisms.

PRINCIPLE: The "neutral" dyes combining the basic dye methylene blue and the acid dye eosin, give a wide color range when staining. The pH of the staining solution is critical and ideally should be adjusted for different fixatives. More acid pH levels give more selective chromatin staining and less cytoplasmic basophilia; less acid pH levels give denser nuclei and increased cytoplasmic basophilia. The pH range should be between 6.4 and 6.9.

CONTROL: Spleen

FIXATIVE: 10% formalin, B-5 fixative.

TECHNIQUE: Cut paraffin sections 4-5 microns.

EQUIPMENT: Rinse glassware in Dl water. Coplin jars, filter paper, staining rack, pipettes.

SAFETY/PPE: Wear gloves, goggles and lab coat. Avoid contact and inhalation of dyes and chemicals.

REAGENTS:

WRIGHT STAIN:
Commercial

GIEMSA STAIN:
Commercial

PHOSPHATE BUFFER, pH 6.8:

Sodium phosphate, di	0.3 gm
Sodium phosphate, mono	0.7 gm
Distilled water	100.0 ml

Store in the refrigerator, stable for 1 year.

GIEMSA STAIN:

Phosphate buffer	50.0 ml
Giemsa stain	2.5 ml
Methanol, acetone free	2.5 ml

Make fresh, filter, discard after use.

ACETIC WATER:

Acetic acid	1.0 ml
Distilled water	400.0 ml

Stable for 1 year.

PROCEDURE:

1. Deparaffinize, bring to absolute alcohol.
2. Methanol, three changes.
3. Place slide on staining rack, cover with Wright stain, 5 minutes.
4. Do not drain off stain, add an equal amount of distilled water until a metallic sheen appears. Leave for 5 minutes.
5. Place slides directly into the Giemsa solution, for 45 minutes, room temperature.
6. Differentiate and dehydrate in the following:

acetic water	3 dips
distilled water	2 dips
95% alcohol	3 dips
100% alcohol	3 dips
100% alcohol	3 dips
xylene	3 changes

7. Coverslip

RESULTS:

Nuclei	blue	Rickettsias	reddish purple
Cytoplasm	pale blue	Erythrocytes	yellowish pink

REFERENCE:

Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp155-156, Battelle Press, Ohio

Carson F, Histotechnology: A Self-Instructional Text, 1990, pp110-112, ASCP, Ill

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