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Beaumont

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Blood Smear: Manual Slide Preparation and Staining-RO

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This procedure provides instructions on smear preparation and staining.

II. PRINCIPLE:

Wright's stain is a complex of dye made up of a mixture of basic polychrome methylene blue (oxidized by heating or with alkali to form a methylene azure) and acidic eosin. The resulting dye complex, thiazine eosinate, is neutral in reaction and soluble in methyl alcohol. By addition of a slightly acid buffer, partial dissociation of the dye yields the products of methylene azure which is blue-violet and stains the acidic (or basophilic) components of a cell (nuclear DNA, cytoplasmic RNA) and Eosin which is red and stains the basic (or acidophilic) components of the cell. The undissociated neutral portion of the dye complex stains the neutrophilic elements.

III. SPECIMEN COLLECTION AND HANDLING:

Type	A. Whole blood collected in a 4 mL vacutainer. This is the preferred sample. -OR- B. Capillary blood collected in a microtainer. -OR- C. Smears made directly from fingerstick blood at time of collection.
Anticoagulant	K_2EDTA (See VII, NOTE A.)
Amount:	A. Whole blood

	1. Minimum sample size is 2.0 mL 2. Optimum sample size is 4.0 mL B. Capillary blood 1. Minimum sample size is 300 mcL. 2. Optimum sample size is 500 mcL.
Special Handling	A. Smears from peripheral blood collected directly from a fingerstick (using no anticoagulant) must be made **immediately.** B. Anticoagulated specimens must be well mixed for minimum of 2 minutes before preparing smears. C. Samples containing lipemia, smudge cells, cold agglutinins, or cryoglobulins may give spurious results. Smudge cells due to lipemia or Chronic Lymphocytic Leukemia (CLL) can be corrected by mixing blood with 22% albumin before preparing smear. See Albumin Smear For Smudge Cells- RO. Cold agglutinin / cryoglobulin interference can be corrected by warming blood to 37°C before preparing smear. D. Samples with extremely low WBC counts (less than 1.0 x 10^9/L) should have a maximum of 2 peripheral smears made. Count as many cells as possible. Repeat differentials are not performed when WBC <1.0.
Timing	For best results, blood smears from EDTA-anticoagulated specimens must be prepared within 2-3 hours after collection and storage at room temperature.
Criteria for Unacceptable Specimens	Anticoagulated specimens containing clots or inappropriate volumes are unacceptable and must be redrawn.

IV. REAGENTS:

Refer to Sysmex SP-1000*i* Smear Preparation and Staining Procedure or Blood Slide Preparation and Staining SP-50 Procedure-RO.

V. QUALITY CONTROL:

A. Slides are visually examined for quality of the smear and of the staining. Criteria for a well made, well stained blood smear are as follows:
    1. Smears should have a minimum length of one inch.
    2. Smears should be a smooth spread with gradual transition from thick to thin, terminating in a feathered edge.

3. The smear should be free of precipitate, holes, scratches and thick cell pile-up at the feathered end.
4. Stained smears should have an overall pink color when viewed with the naked eye.
5. Cells should be evenly distributed.
6. Red blood cells should be pink.
7. The nuclei of leukocytes are blue to purple.
8. Eosinophil granules are red-orange and each distinctly discernible, so that individual granules may be counted.
9. Basophils have dark blue to purple granules.
10. The cytoplasmic neutrophil granules are tan in color.
11. Platelets have dark lilac granules.
12. Lymphocyte cytoplasm is generally robin's egg blue; monocyte cytoplasm generally has a faint bluish tinge.

B. Document quality smear findings on designated maintenance log.

VI. PROCEDURE:

A. **Manual Preparation of Smears**

1. Check anticoagulated specimens with two applicator sticks to be sure no clots are present. If clots (any size, shape or form) are present, the specimen is unsatisfactory and must be redrawn.
2. Using applicator sticks, place a small drop of well-mixed blood on the surface of a clean glass slide near the frosted end. If blood is taken from the finger, care must be taken to avoid touching the slide to the skin.
3. Hold the slide between two fingers and the thumb of the left hand with the frosted end toward the right. (Reverse for the left-handed individual.)
4. Place the edge of the spreader slide on the first slide to the left of the drop of blood.
5. Pull spreader slide to the edge of the drop. (See VII, NOTE B.)
6. Allow the drop of blood to bank evenly behind the spreader.
7. Push to the left in a smooth, quick motion. (See VII, NOTE C.)
8. Label the smear on the frosted edge of the slide using indelible ink or pre-printed computer label. Label with the patient's last name, first initial, accession number and date if slide labels are not available.

B. **Staining of Smears:** Refer to Sysmex SP-1000i Smear Preparation & Staining Procedure, Quick Slide Plus Stainer Procedure-RO, or Blood Slide Preparation and Staining SP-50 Procedure-RO.

VII. NOTES:

A. Ethylenediaminetetraacetic acid (EDTA) is the preferred anticoagulant for blood cell counts and morphologic studies for the following reasons:

1. Artifacts form only on prolonged standing. Acceptable blood smears can be made after two to three hours of collection.
2. Cell counts are valid for 72 hours, if the blood is refrigerated. If morphology is indiscernible, do NOT report, regardless of age of specimen.
3. EDTA prevents platelet clumping and is the anticoagulant of choice for platelet counting.

B. The angle between the two slides will vary according to the size of the drop and the viscosity of the blood. The larger the drop or the lower the hematocrit of the blood, the greater the angle must be to avoid running off the slide when spreading. Blood with a high hematocrit must be spread with a lower angle or the smear will be short and too thick to allow differentiation of cells. The approximate angle for normal blood is 30 to 40 degrees.

C. The more rapid the motion, the shorter and thicker the smear. The smear should cover approximately half the slide with a gradual transition from thick to thin. No ridges should be present and the end (called the "feather edge") should be smooth and even without streaks or ridges.

D. The following method can be used on old unstained slides or previously Wright-stained slides that are poorly stained:

1. Stain slides by routine Wright's stain method.
2. Rinse stained slides with **two quick dips** in acetic water (approximately 50 mL distilled water in Coplin jar plus 1 drop glacial acetic acid).
3. Wash **immediately** with distilled water and air dry.

VIII. REFERENCES:

A. Henry JB, ed. Clinical diagnosis and management by laboratory methods. 18th Ed. Philadelphia: WB Saunders Co, 1991: 555, 583-585.

B. Spanjers E. Method for Wright's staining "old" slide preparations of blood, bone marrow and imprints. J Med Tech 1987; 4:139.

Approval Signatures

Step Description	Approver	Date
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