Preparation of Blood Films

A blood film or **peripheral blood smear:** is a thin layer of blood smear on a microscope slide and then stained in such a way to allow the various blood cells to be examined microscopically.

Aim of the study blood film: Blood films are usually examined to investigate hematological problems (disorders of the blood like anemia, polycythemia and leukemia).

Principle:

Blood film enables us to evaluate WBC, RBC, and PLT morphology, also, allows us to perform differential WBC count, furthermore estimation of WBC and platelets counts can be done on blood films. Blood films are made on glass microscopic slides.

Sample:

- Finger blood may be used. Films of peripheral blood must be made immediately. All specimens should be free of clots.

Procedure:

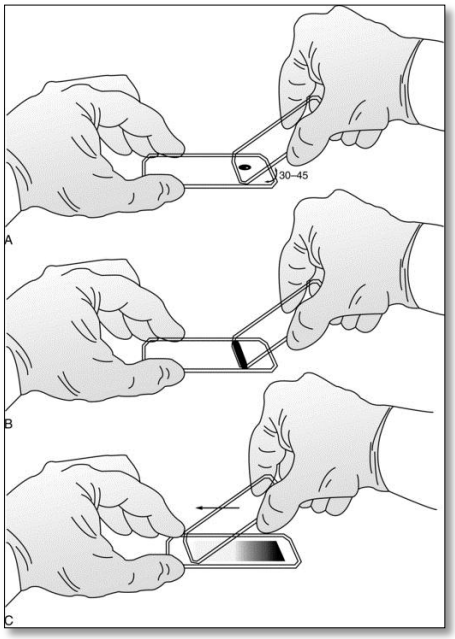
1- Use clean standard size glass slides (3 inch x 1 inch = 7.5 cm x 2.5 cm), wiped from dust just immediately before use.

2- Clean the finger with alcohol, allow it dry & then prick it with a disposable lancet to obtain a drop of blood.

3- Place a small drop of well mixed whole blood, in the center line of the slide, about 1.5 to 2 cm from one end, with the aid of a capillary tube.

4- At once, without delay, with the aid of a second clean slide with uniform smooth edges (spreader slide), with a 30 –45 degrees angle, move back so blood drop will spread along the edge of the spreader

slide spread, or smear the film by a quick, uniform forward motion of the spreader.



Common faults in blood smear

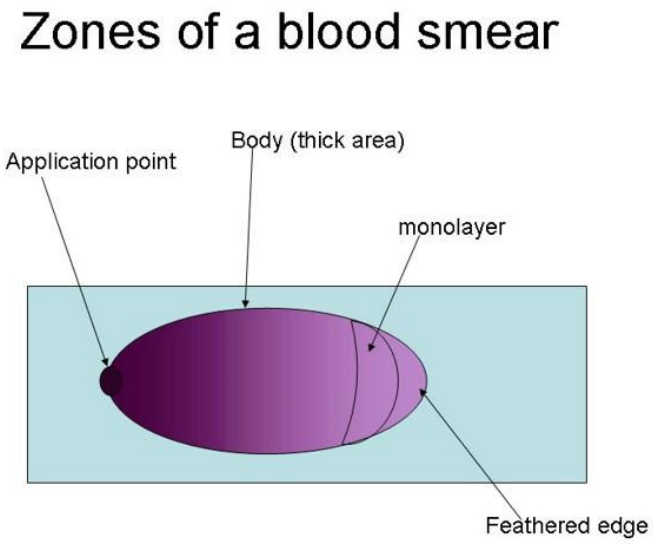
1. Too thick or too large drop of blood or wide angle of spread.
2. Too thin or too small drop of blood or spread too slowly or narrow angle of spread.
3. Alternate thin and thick bands, spreading done with a jerky motion, usually due to hesitation.
4. Streaks throughout the length of the smear an irregular edge to the spreader or dried blood on the edge of the spreader .
5. Very narrow thick smear.
6. The slides are not clean.

Smear examination

Scan the smear at low magnification 10 X, then use a higher magnification (40X, 100X) to perform Blood smear analysis allows quantitation of the different types of leukocytes (called the differential count). Blood smear examination requires a well-prepared, well-stained blood smear and some basic skills in the methods of assessment.

Components of a blood smear

A well-made blood smear consists of several areas: The feathered edge, the monolayer, and the body of the smear. All parts of the smear should be examined; however the monolayer is the area where the cells are examined in close detail and differential cell counts performed.



Differential white blood cells count

A differential white cell count (leukocyte formula) consists of an examination of blood to determine the presence and the number of different types of white blood cells.

Normal distribution of different white blood cell types:

W.B.C.	Normal ratio	Increase	Decrease
Neutrophils	60-70 %	Neutrophilia	Neutropenia
Eosinophils	2-4 %	Eosinophilia	Eosinopenia
Basophils	0.5-1 %	Basophilia	Basopenia
Lymphocytes	20-25 %	Lymphocytosis	Lymphopenia
Monocytes	3-8 %	Monocytosis	Monocytopenia

WBC type	high count may indicate	low count may indicate
neutrophils	bacterial infection, burns, stress, inflammation	radiation exposure, drug toxicity, vitami B12 deficiency, systemic lupus erythematosus (SLE)
eosinophils	allergic reactions, parasitic infectio autoimmune diseases	drug toxicity, stress
basophils	allergic reactions, leukemias, cance hypothyroidism	pregnancy, ovulation, stress, hyperthyroidism
lymphocytes	viral infections, some leukemias	prolonged illness,immunosuppression, treatment with cortisol
monocytes	viral or fungal infections, tuberculosis, some leukemias, othe chronic diseases	bone marrow suppression, treatment wit cortisol

Leishman's stain: Contain

- Eosin.
- Acetone (free absolute methyl alcohol), acting as a solvent for these stain, it is also a fixative.
- Methylene blue.

Leishman's Stain Procedure:

1- Let the films be air dried.

2- Put the films on a staining rack.

3- Flood the slides with the stain.

4- After 2 minutes (or more, if the stain in newly prepared), add double volume of water, and blow to mix the stain with water, until a shiny layer is seen.

5- After 5-7 minutes, wash with a stream of water.

6- Wipe the back of the slides with gauze.

7- Set the films in upright position on a filter paper to dry.

8- Read the blood films microscopically.

Materials & instruments:

1. Whole blood
2. Glass slides
3. Microscope
4. Leishman's stain
5. Alcohol 70%
6. Lancet

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