

Lab : 2/ Second semester (Medical Biology)

Dep: Clinical laboratories / first stage

Blood smear (film):

I- Preparation of blood smear

* There are three types of blood smears:

1-The cover glass smear.

2-The wedge smear .

3-The spun smear.

* There are two additional types of blood smear used for specific purposes

4-Buffy coat smear for WBCs $< 1.0 \times 10^9/L$

5-Thick blood smears for blood parasites .

*** Procedure**

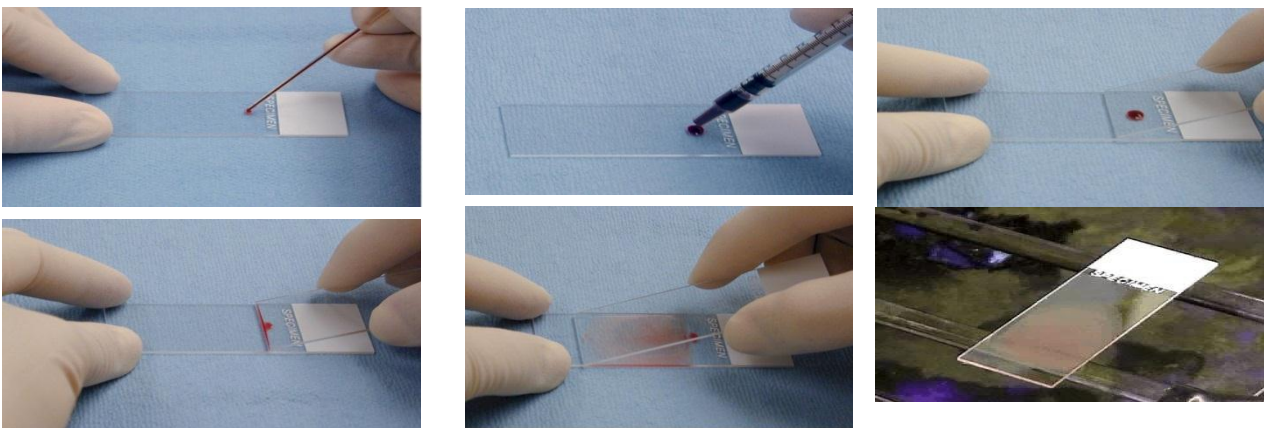
a-placing a drop of blood *from mixed sample* on a clean glass slide.

b-Spreader slide using another clean glass slide at 30-40 degree angle.

c-Control thickness of the smear by changing the angle of spreader slide

d-Allow the blood film to air-dry completely before staining. (Do not blow to dry. The moisture from your breath will cause RBC artifact.)

*** STEPS FOR BLOOD FILM**



*** The thickness of the spread**

Notes:

1-If the hematocrit is increased, the angle of the spreader slide should be decreased.

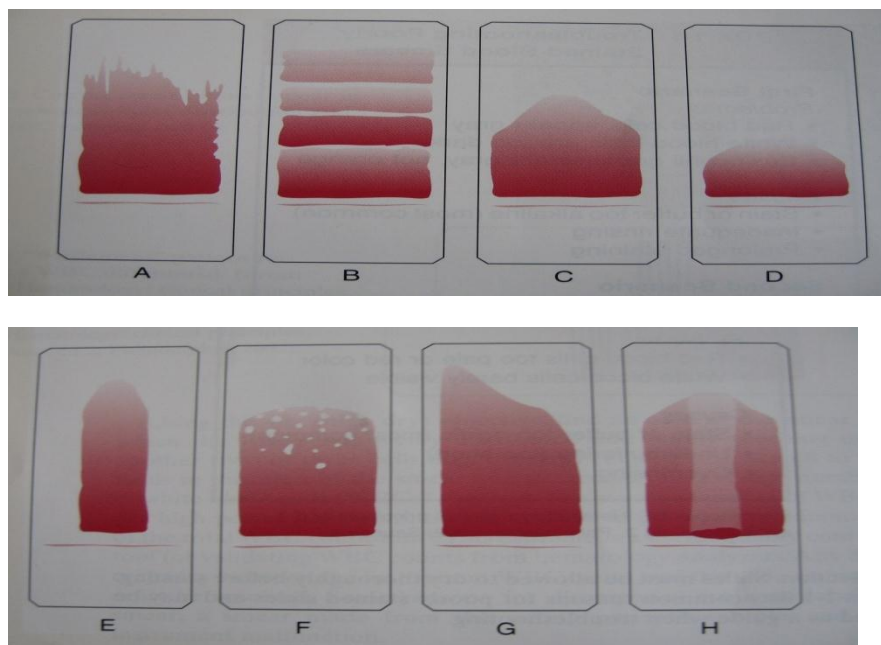
2-If the hematocrit is decreased, the angle of the spreader slide should be increased.

* Characteristics of a Good Smear

- 1-Thick at one end, thinning out to a smooth rounded feather edge.
- 2-Should occupy 2/3 of the total slide area.
- 3-Should not touch any edge of the slide.
- 4-Should be margin free, except for point of application.

Note: As soon as the drop of blood is placed on the glass slide, the smear should be made without delay. Any delay results in an abnormal distribution of the white blood cells, with many of the large white cells accumulating at the thin edge of the smear.

* Examples of unacceptable smears



II- Fixing the films

- 1-To preserve the morphology of the cells, films must be fixed as soon as possible after they have dried.
- 2-It is important to prevent contact with water before fixation is complete.
- 3-Methyl alcohol (methanol) is the choice, although ethyl alcohol ("absolute alcohol") can be used.

4-Methylated spirit (95% ethanol) must not be used as it contains water.

5-To fix the films, place them in a covered staining jar or tray containing the alcohol for 2-3 minutes. In humid climates it might be necessary to replace the methanol 2-3 times per day; the old portions can be used for storing clean slides.

III. Staining the film

*** Staining procedure**

a-Thin smear are air dried.

b-Flood the smear with stain.

c-Stain for 1-5 min. Experience will indicate the optimum time.

d-Add an equal amount of buffer solution and mix the stain by blowing an eddy in the fluid.

e-Leave the mixture on the slide for 10-15 min.

f-Wash off by running water directly to the centre of the slide to prevent a residue of precipitated stain.

g-Stand slide on end, and let dry in air.

*** normal peripheral blood smear**

