

Lab 08:

Blood Film Preparation and Staining

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- **Blood and bone marrow films** that are well prepared and properly stained have a great value in hematology. They are used in:
 - a) Viewing cell morphology for diagnosing anemias and leukemias
 - b) WBC differential counts and
 - c) Estimating the number of platelets.

Technique of Spreading

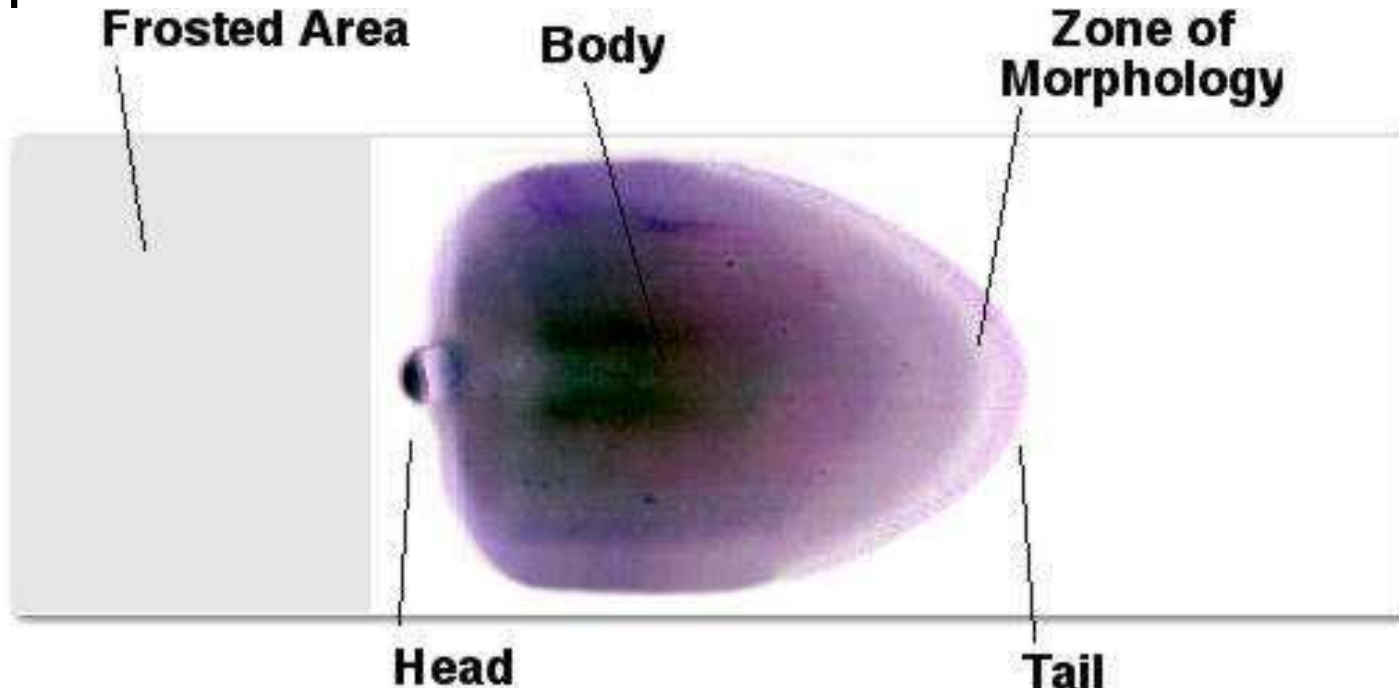
- Spreading is done on dust **free glass microscope** slides using a spreader, which is a microscope slide having one corner removed at each end.

Is determined by:

1. The **angle** of the spreader slide. (the greater the angle, the thicker and shorter the smear).
2. **Size** of the blood drop.
3. **Speed** of spreading

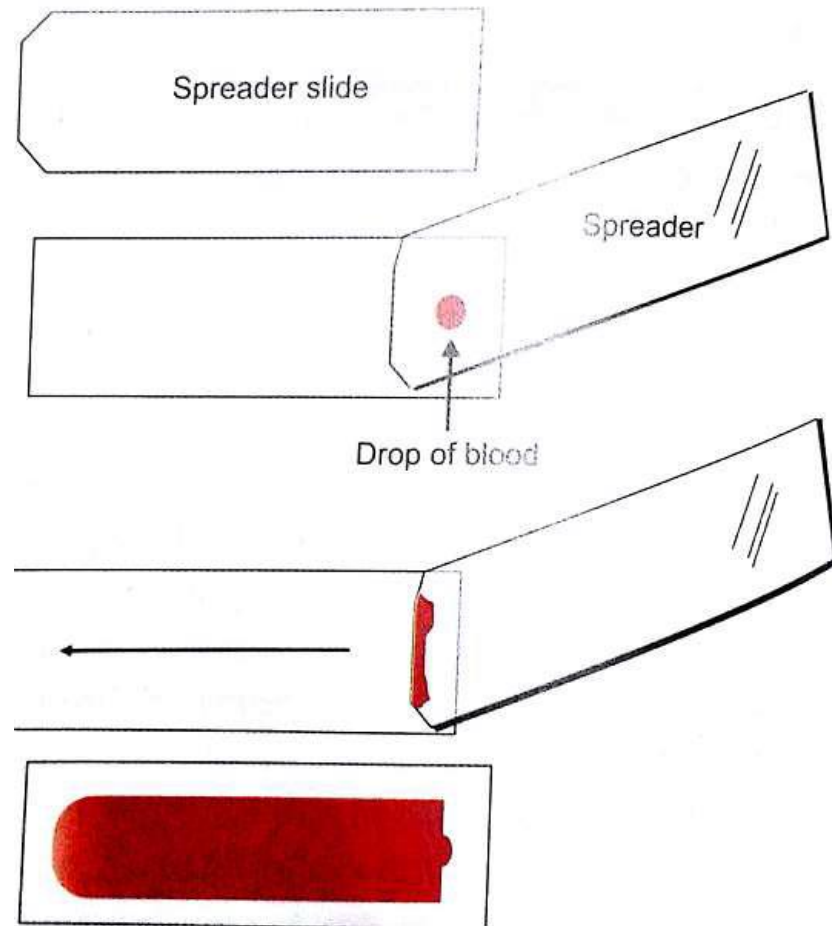
Types of Blood Films

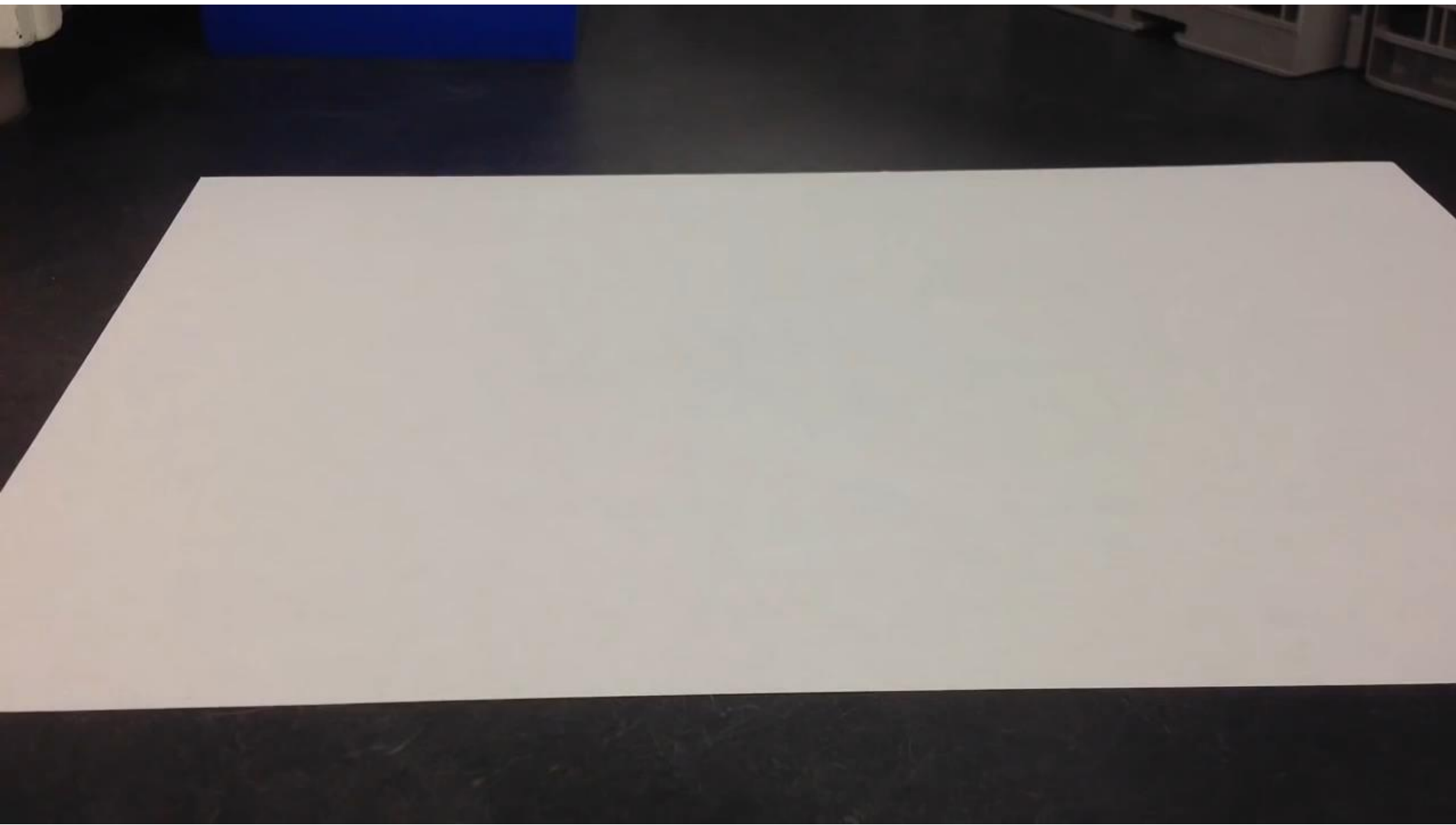
- (1) **Thick blood film** which is used for **parasitology** e.g. **malaria**. It is prepared by handling the spreader by the edge, using the corner to spread the blood in a circular form with 3-6 movements.
- (2) **Thin blood film** which is used in hematology and prepared as described below.



Blood Smear Preparation Method

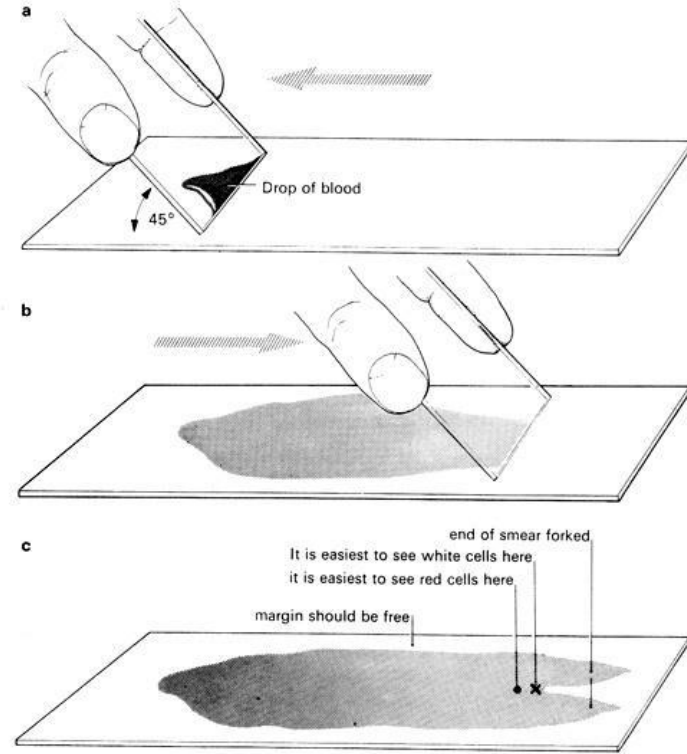
1. EDTA **anticoagulated** blood is used. Blood is taken using capillary tubes.
2. **Place** a drop of blood near the end of the slide, about **1-2 cm** from one end.
3. Place the **spreading** slide at an **angle of 45°** about 1 cm in front of the drop of blood.
4. **Back** the spreader into the **drop** of blood. The spreader catches the blood and it spreads by **capillary action** along its edge.
5. Maintaining the 45° angle, **push** the spreader **smoothly** across the slide. This pulls the blood across to make the smear. It should take about **one second** to make the smear.
6. Allow the film to air **dry**.





Notes on method:

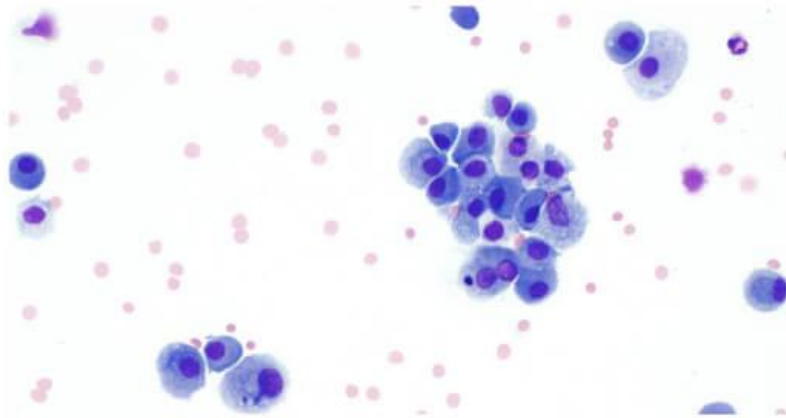
- The edge of the spreader must be very smooth, and narrower than that of the slide.
- The spreader must be cleaned and dried if it had been used for spreading more than five films.
- A smooth action is required, with the edge of the spreader held against the slide.
- Films may be prepared manually or automated slide spreaders.
- The blood film length shouldn't be too long nor should it be too short.
- All slides should be labeled for identification.



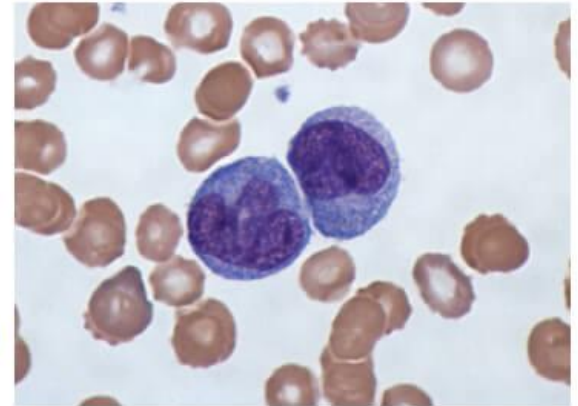
Types of Stains

- **Romanowsky dyes** are universally employed for routine staining of blood films. Romanowsky stains include **leishman's stain**, **write stain**, **may grunwald stain**, **jenner's stain** and **giemsa stain**. Romanowsky dyes consist of two components:
 - (1) **Basic Part:** azure blue or methylene blue which bind to the acidic part of the cell (**nucleus**) and stain it **blue**.

Romanowsky Stains



Bronchoalveolar lavage specimen stained with Diff-Quik, a commercial Romanowsky stain variant widely used in cytopathology



Blood film with Giemsa stain. White blood cells (center) surrounded by red blood cells.

Types of Stains

- (2) **Acidic Part:** eosin Y which binds to the basic parts of the cell (**proteins, cytoplasm and Hb**) giving them a **red** color.
- Azure B(basic) → nuclei (containing nucleic acid) → **Blue**
- Azure B(basic) → Basophilic granules (containing heparin which is acidic) → **Violet**
- Eosin Y(acidic) → Cytoplasm and Hb (basic) → **Red**
- Eosin Y(acidic) → Eosinophilic granules (alkaline) → **Red orange granules**

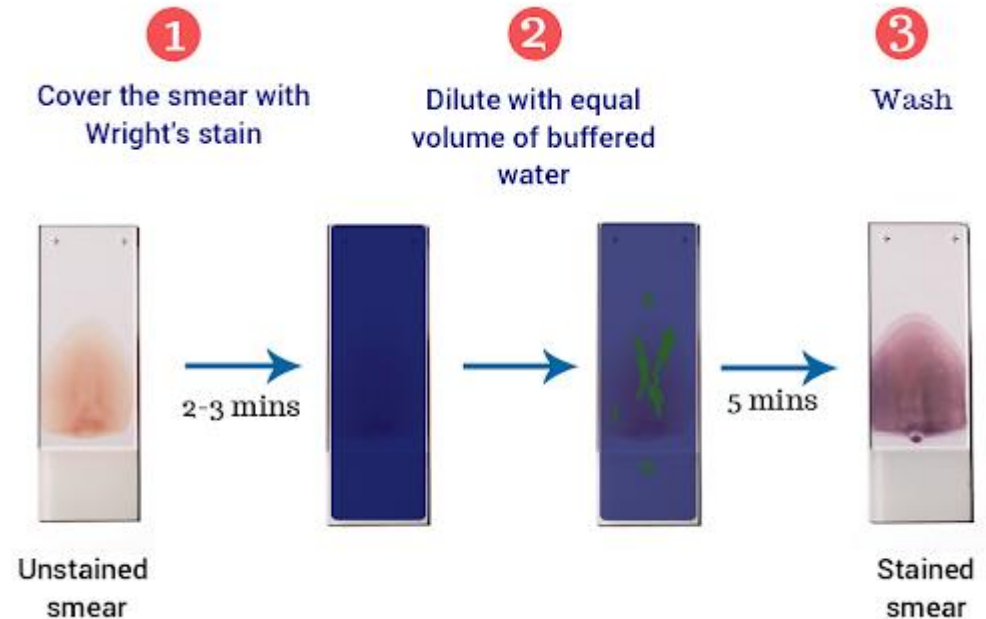
Staining Steps

1. **Fixation** of blood cells to **protect them from hemolysis due to washing**. Well-fixed cells resist the action of water. Fixation is done by:

- **Methanol** (1.5 min)
- B-Undiluted stain (neat stain) as in **leishman's** & wright stain

2. **Staining**

3. **Washing**



Manual Staining Method

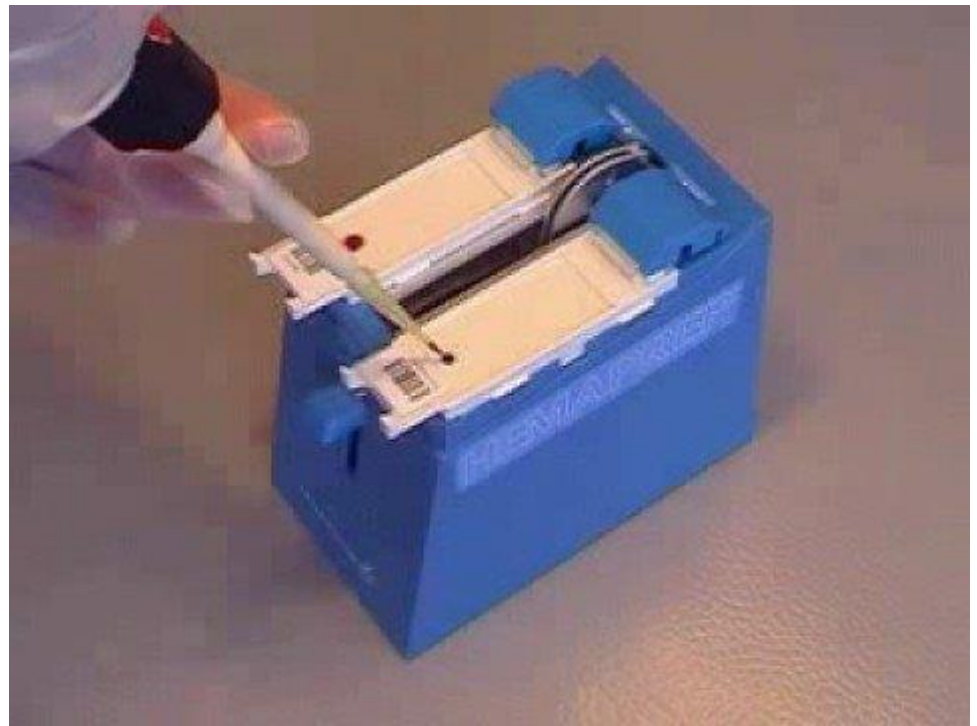
Leishman's and Write Method:

1. Place **dried film** on the **staining rack** with film facing upwards.
2. **Flood** slide with neat stain (1 volume of Pasteur pipette).
3. Allow to stain for **5 min.**
4. Add double volume (2 volumes of Pasteur pipette) of the buffer pH 6.8 on the stain. Do not wash.
5. Allow to stain for 10-15 min.
6. Wash gently with distilled water.
7. Clean underneath the slide and leave to dry.



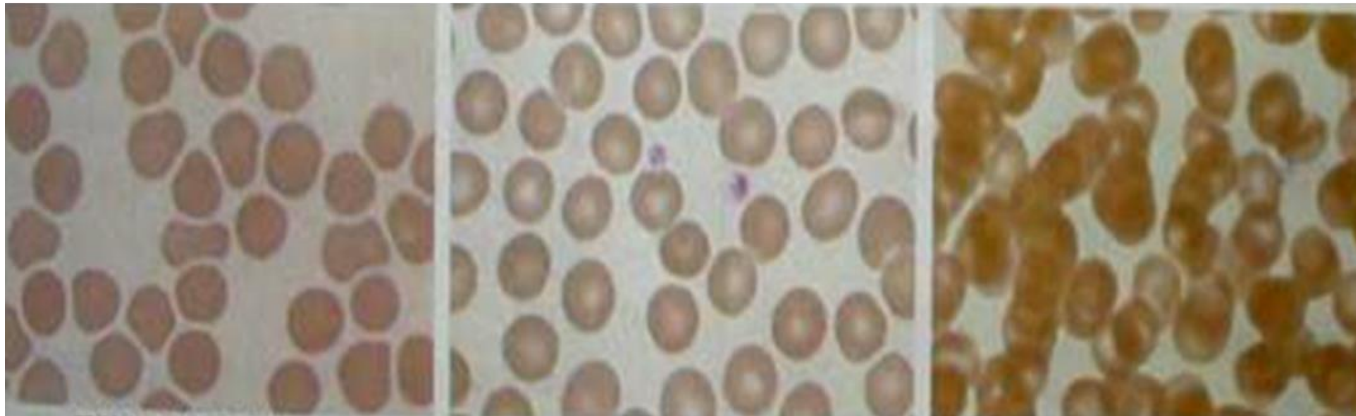
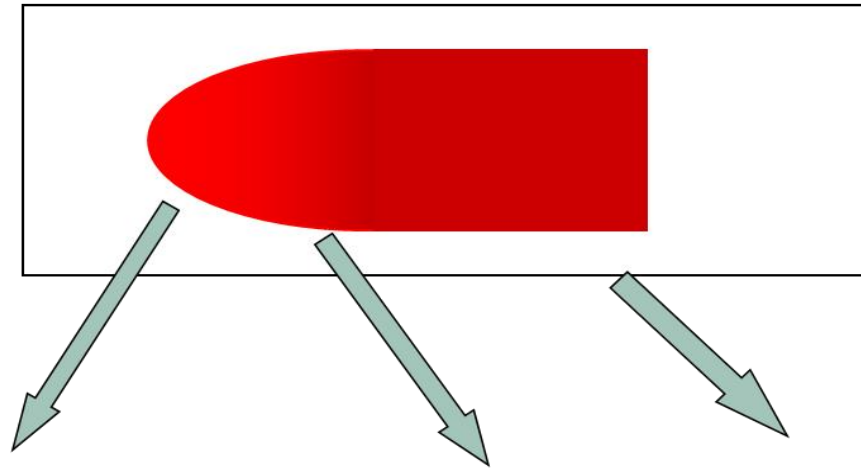
Automated Blood Film Makers

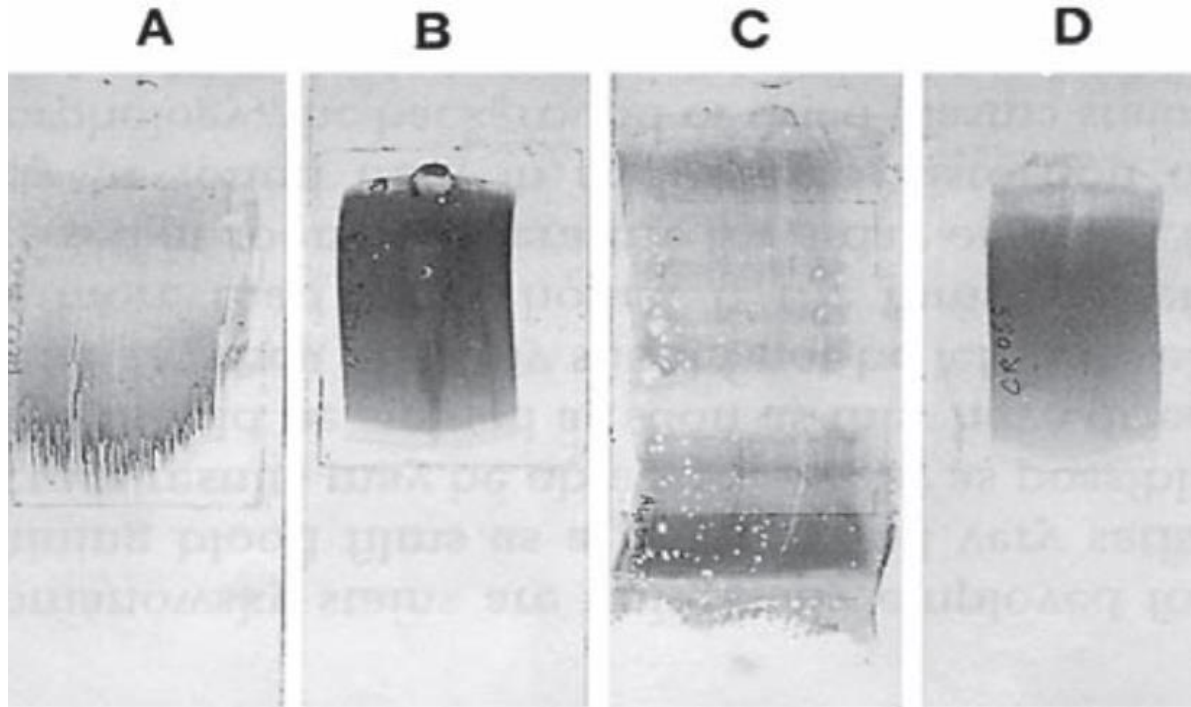
- In most hematology laboratories today, **automated blood film makers** and strainers are used.
- Studies are still being conducted to compare the slides made by automated slide makers to those that are made manually. The manufacturer's instructions should be followed unless local experience has demonstrated that variation of the recommended technique achieves better results.



The shape of blood film

tail body head





A: Blood film with jagged tail made from a spreader with a chipped end.

B: Film which is too thick

C: Film which is too long, too wide, uneven thickness and made on a greasy slide.

D: A well-made blood film

Examples of unacceptable smears

