CLS 291 Clinical Hematology 1

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Lecture 5

Blood Film Preparation, Staining, and RBCs Manual Counting

Outlines

I. Making blood film
II. Staining Blood Films
III. Red blood cell manual counting

I. Blood Film - Wedge Smear Preparation

Types of blood film

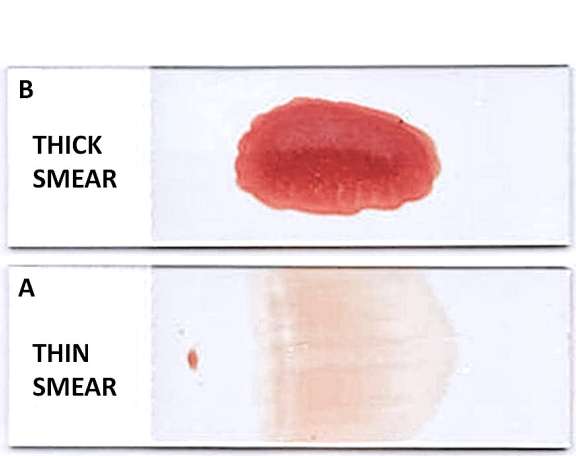
- **The aim of blood film** allows the evaluation of white blood cells (WBCs, leucocytes), red blood cells (RBCs, erythrocytes), and platelets (thrombocytes) size and morphology.

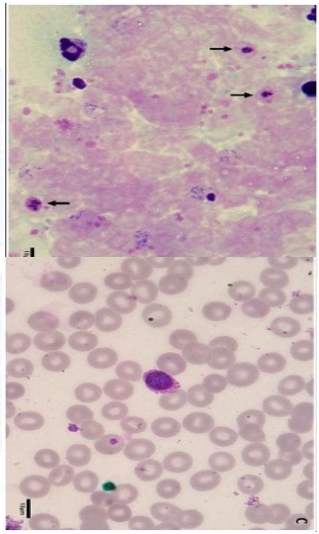
- **Staining** of blood film helps in differentiating cells and detecting the presence of abnormality in microscopic examinations of blood and bone marrow samples.

- There are two types of blood films:

  - Thick blood film
    - Allow a more efficient detection of parasites. (increased sensitivity 11 times than thin smear).
    - No Fixation is used.

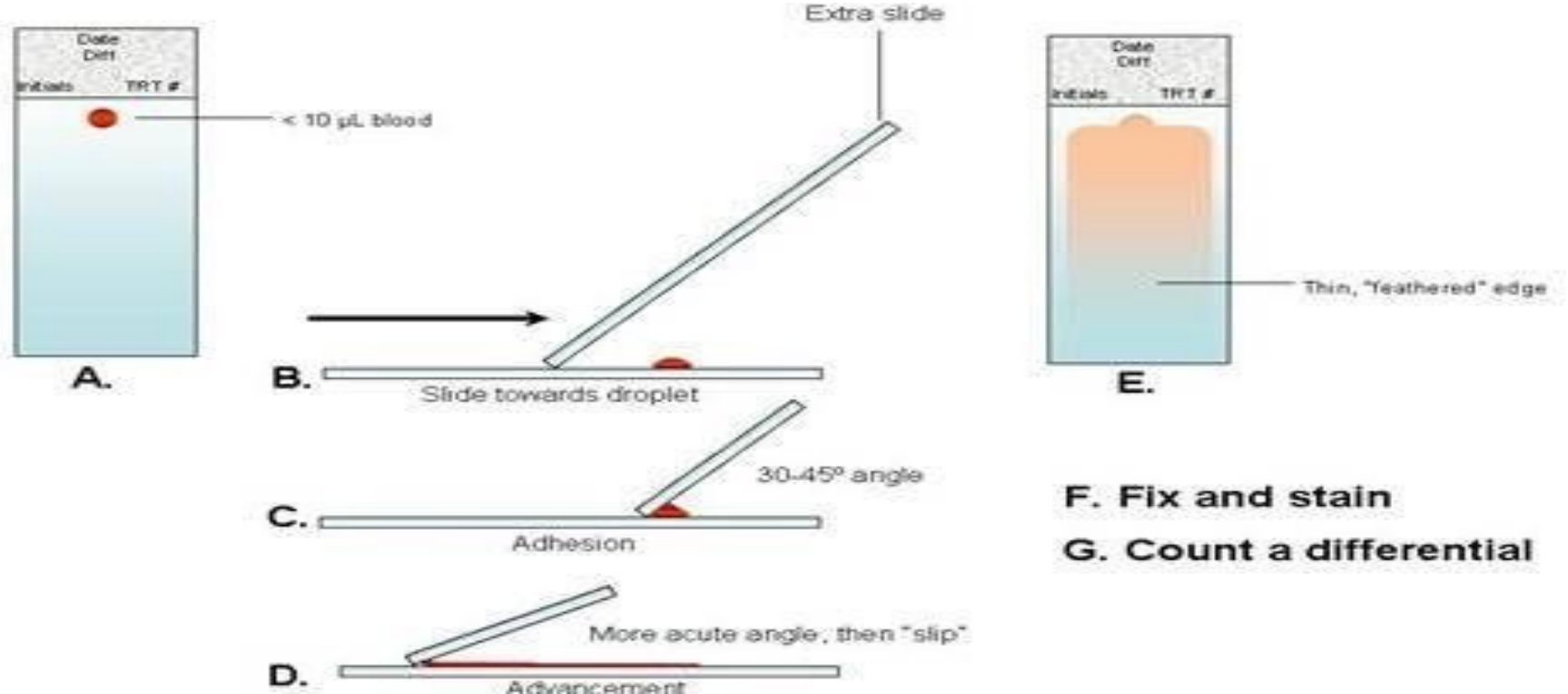
  - Thin blood film
    - Used for examining Blood Cell morphology.
    - Fixed smear.





Wedge Smear Preparation

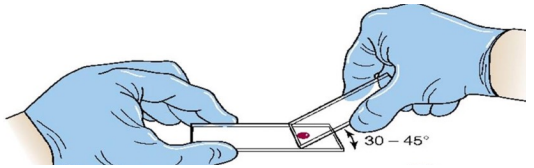
- Prepare the blood film as follows:



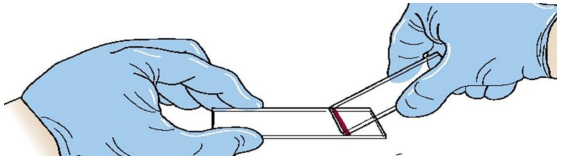
Wedge Smear Procedure

Prepare the blood film as follows:

1. Use 2 clean glass slides. (one for spreading and one for the blood film).
2. Label the slide with the patient's MRN# and your initial.
3. Add a small drop of EDTA blood sample about 3 mm in diameter placed at one end of the slide.
4. Holds the pusher slide securely in front of the drop of blood at a 30- to 45-degree angle to the smear slide (A).
5. The pusher slide is pulled back into the drop of blood and held in that position until the blood spreads across the width of the slide (B).
6. Then quickly and smoothly pushed forward to the end of the smear slide, creating a wedge smear (C).



(A)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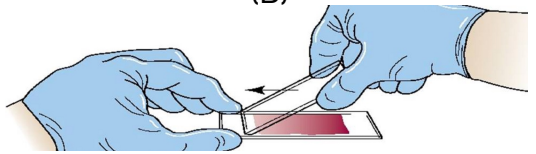
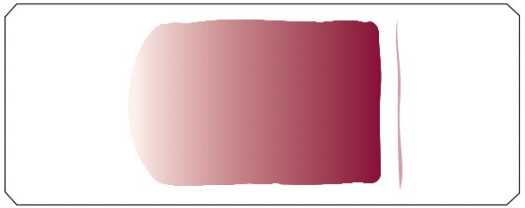

(C)


Well-made blood smear

https://www.youtube.com/watch?v=rqXy45sRJkw
https://www.youtube.com/watch?v=nbRUiWl2Qrs

Unacceptable Blood Smear

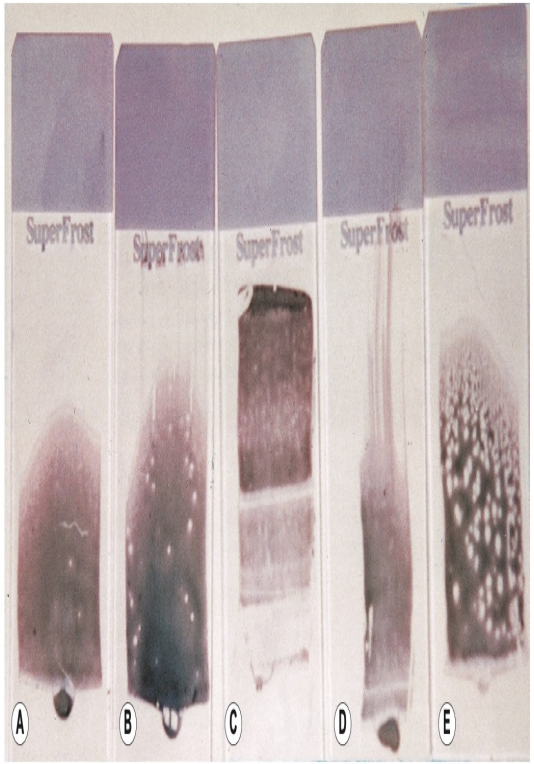


FIGURE 4-1 Blood films made on slides. (A) A well-made film. (B) An irregular patchy film on a dusty slide. (C) A film that is too thick. (D) A film that has been spread with inconsistent pressure and using an irregularly edged spreader, resulting in long tails. (E) A film made on a very greasy slide.

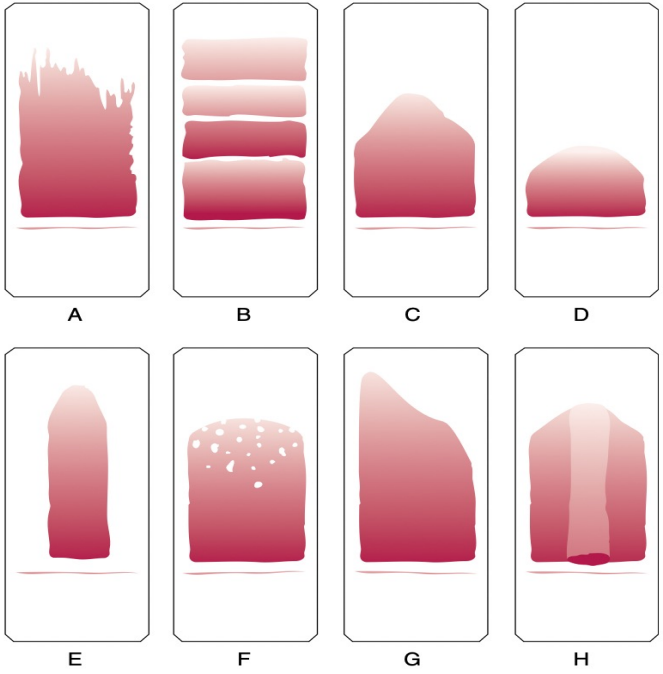
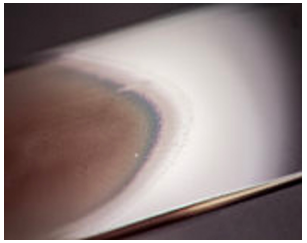


FIGURE 1–3 Unacceptable peripheral blood films. Slide appearances associated with the most common errors are shown, but note that a combination of causes may be responsible for unacceptable films. **A,** Chipped or rough edge on spreader slide. **B,** Hesitation in forward motion of spreader slide. **C,** Spreader slide pushed too quickly. **D,** Drop of blood too small. **E,** Drop of blood not allowed to spread across the width of the slide. **F,** Dirt or grease on the slide; may also be caused by elevated lipids in the blood specimen. **G,** Uneven pressure on the spreader slide. **H,** Time delay; drop of blood began to dry. (From Rodak BF, Fritsma GA, Keohane EM: *Hematology: clinical principles and applications*, ed 4, St. Louis, 2012, Saunders.)



In a **well-made** when the slide is held up to the light, the **feather edge** of the smear should have a **"rainbow"** appearance.

II. Staining Blood Films

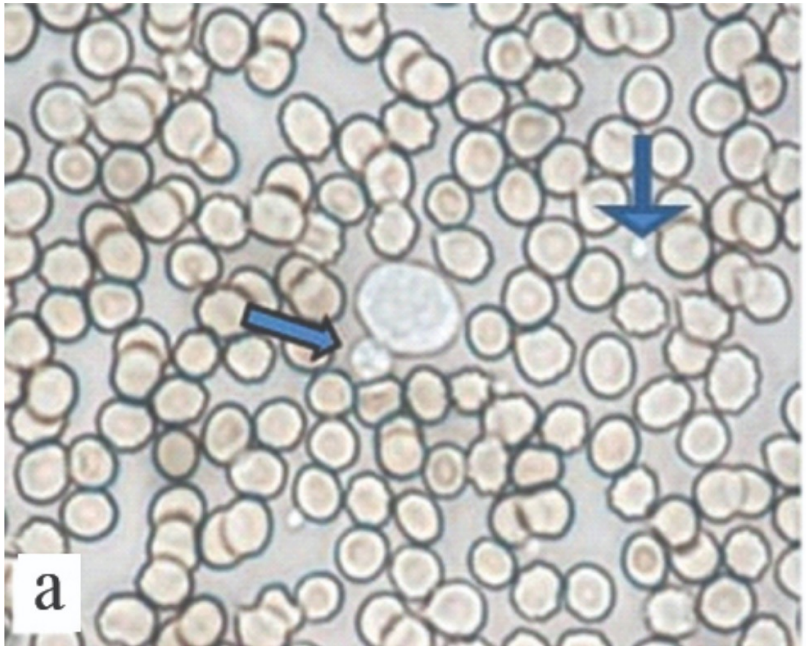
Staining of blood film

- **Types of staining:** Romanowsky stains (the routine stain in hematology).

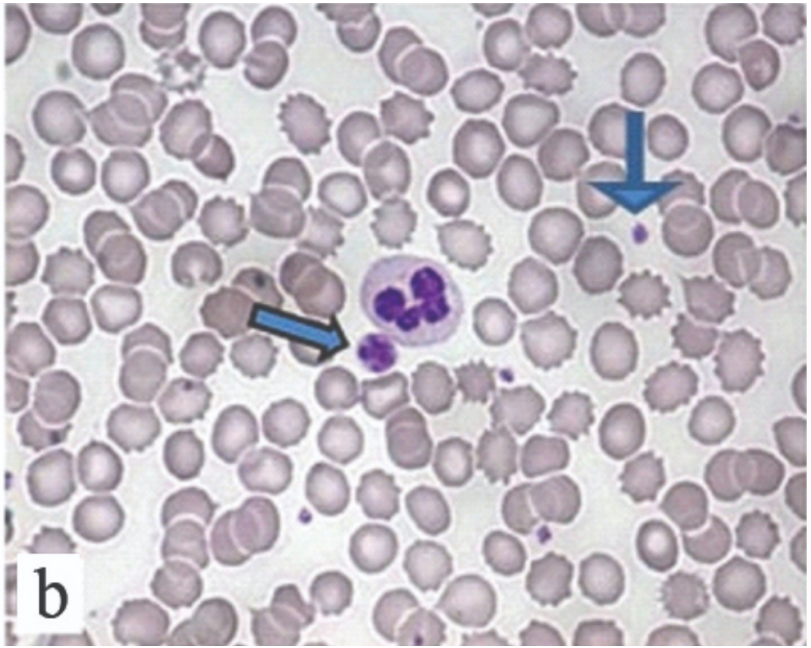
- Romanowsky stains include a number of stains, such as:

  1. Jenner
  2. Giemsa
  3. Leishman's stain
  4. Wright's stain

Unstained vs Stained Blood Film

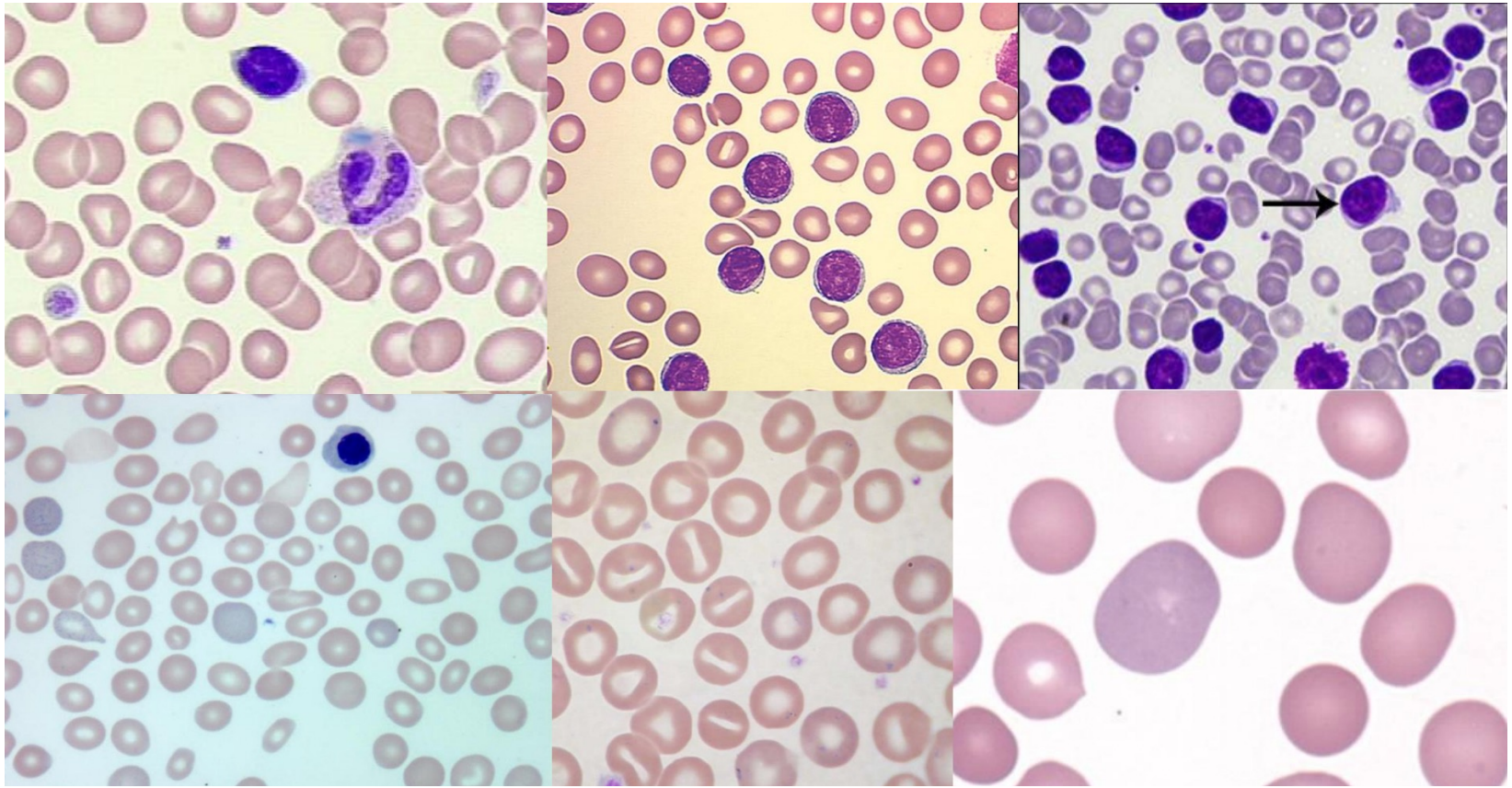


Unstained Blood Film



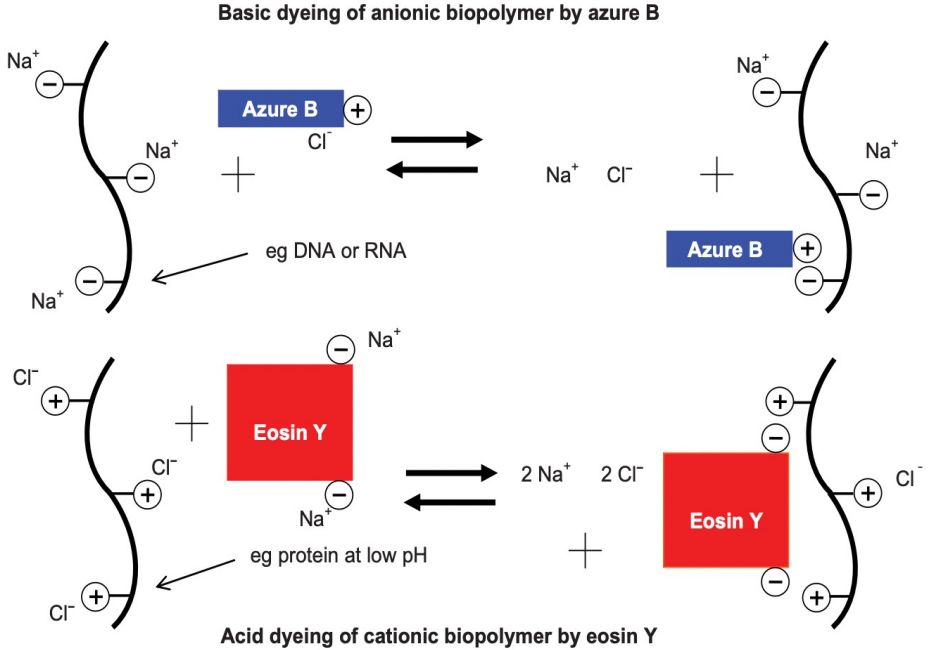
Stained Blood Film

What colors do you see?



Acidic and basic staining mechanisms

- It depends on two components:
  - Acidic dye (eosin Y):
    - Binds to basic parts of the cell as cytoplasm and Hb and gives them red color.
  - Basic dye (azure B):
    - Binds to acidic parts of the cell as the nucleus and gives them blue color.

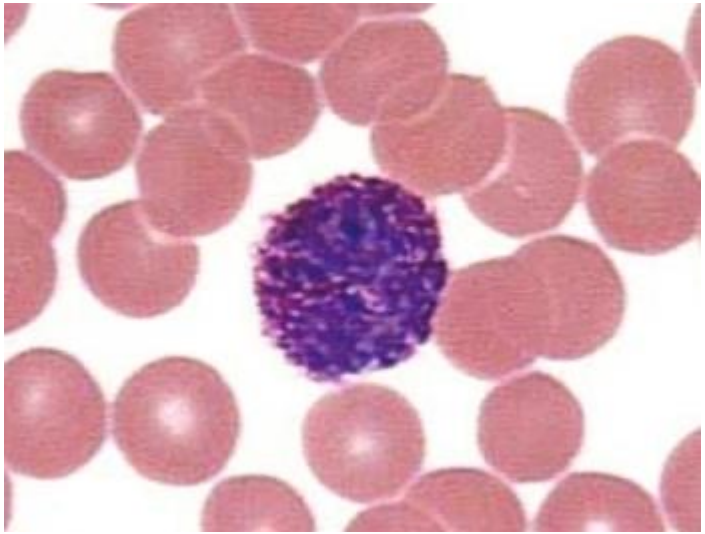


Acid and basic dyeing are seen as ion exchange processes. Mobile counterions, shown as Na+ and Cl^-, in actuality, depend on the species present in the staining solution.

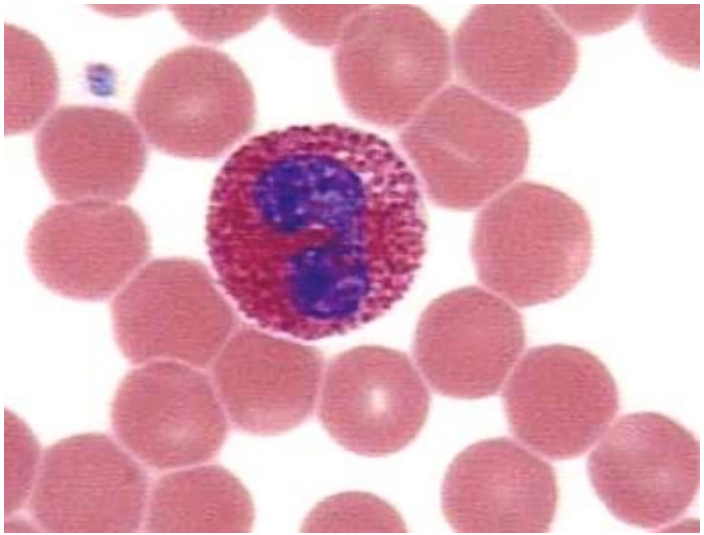
Acidic and basic staining mechanisms

pH of Cell Component	Color after staining with Romanowsky stains
Hb (basic-cationic)	Red by Eosin Y (acid-anionic)
Nucleus (acid-anionic) Due to the negative charge of phosphate groups of nucleic acids.	Blue–purple by azure B (basic-cationic)
Basophilic granules (acid-anionic)	Dark blue by azure B (basic-cationic)
Eosinophilic granules (basic-cationic)	Red by Eosin Y (acid-anionic)
Nutrophil granules	Purple mixed of both Eosin Y and azure B

Blood cell appearance after staining



Basophil has heparin in its granules which is acidic. Thus, the azure B dye (basic dye) of the stain will bind to the granules of basophil and stain it blue.

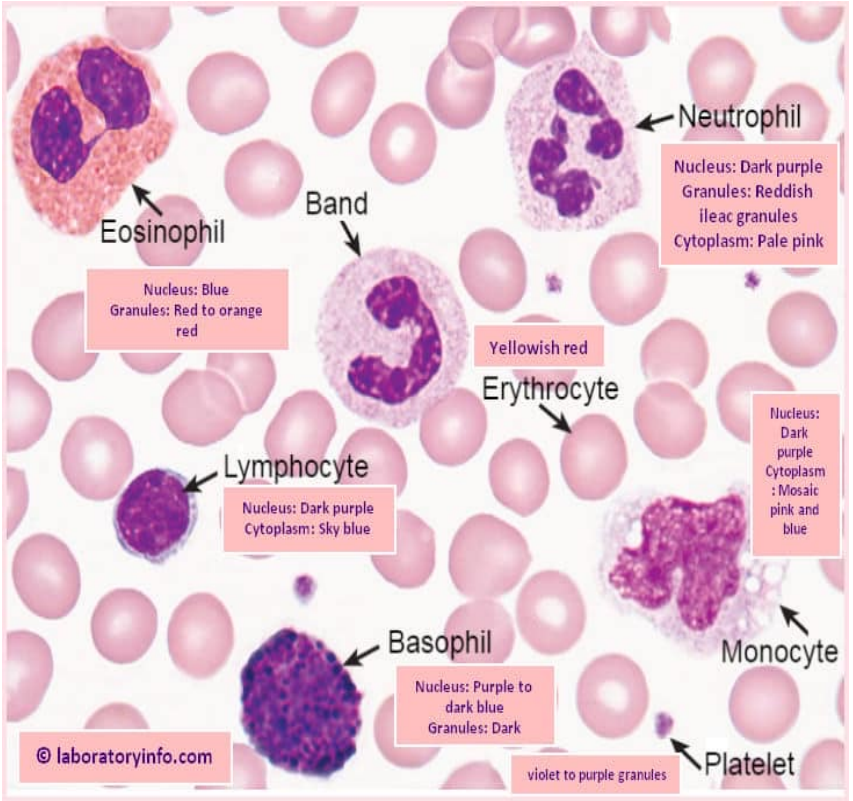


Eosinophil granules are basic. Thus, eosin Y (acidic dye) of the stain will bind to basic granules and gives them red color.

Blood cell appearance after staining

A well-made stained peripheral blood smear has the following characteristics:

1. The **red blood cells (RBCs)** should be pink to salmon.
2. **Nuclei** are dark blue to purple.
3. Cytoplasmic **granules** of **neutrophils** are lavender to lilac.
4. Cytoplasmic **granules** of **basophils** are dark blue to black.
5. Cytoplasmic **granules** of **eosinophils** are red to orange.
6. The area between the cells **(background)** should be colorless, clean, and free of precipitated stains.



Blood Film Staining Procedure

- Procedure steps

I. **Blood Smear Preparation:** Wedge Smear.

II. **Fixation: [**(physical fixation) by Air drying the smear]. Don't forget to label the slide.

III. **Staining:** [(chemical fixation) by Methanol, staining, and washing].

IV. **Microscopy:** Examine your slide under the microscope.

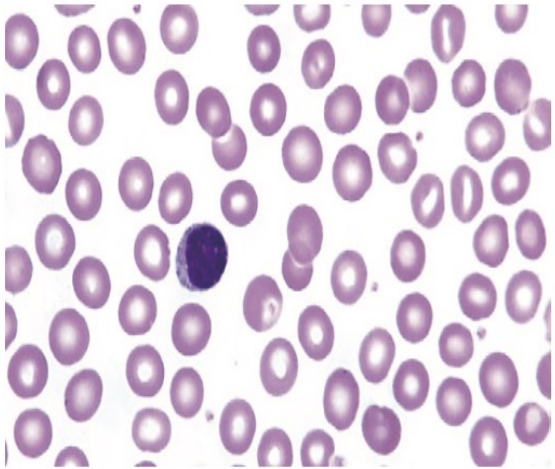
Blood Film Staining Procedure

III. Staining procedure: (Leishman's stain):

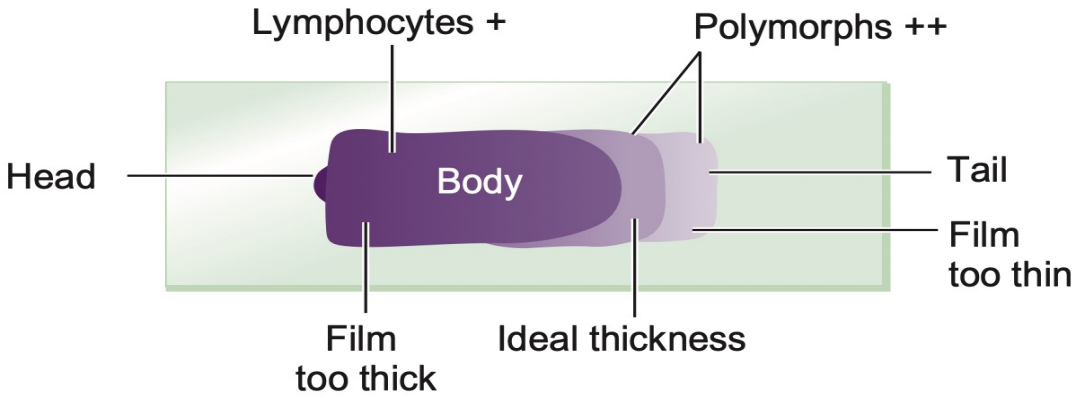
1. Immerse the slide for 2 minutes in 100% Methanol (fixation step).
2. Immerse the slide for 4 minutes in a solution containing: 100% Leishman's stain.
3. Immerse the slide in the first 100% Buffer (washing step) by immersing the slide for 1 minute.
4. Wash the slide in the second 100% Buffer (washing step) by immersing the slide 5 times.
5. Wash with water (carefully).
6. Air dry, then examine under the microscope.

Microscopic Examination

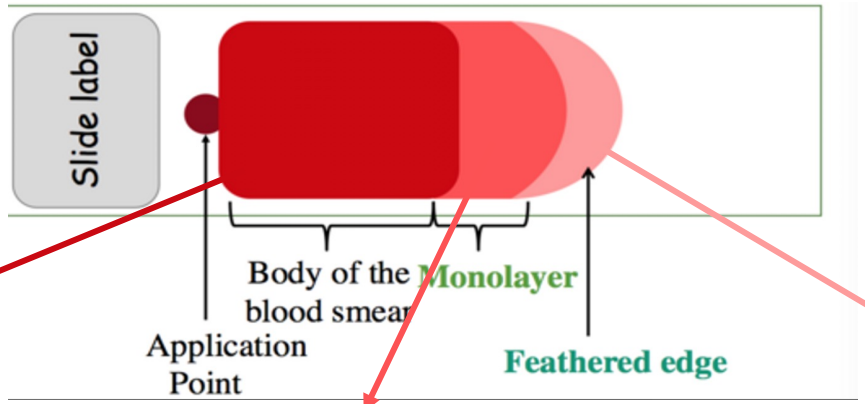
1. Examine under the microscope.
2. Start with low power (X10) to find the suitable area of ideal thickness where the cells are appropriately separated.
3. Change to (X40) to see the morphology. <<< Most of your time, you will use this magnification.
4. Change to (X100) to see inclusions (if present) – **(use immersion oil only with 100x)**

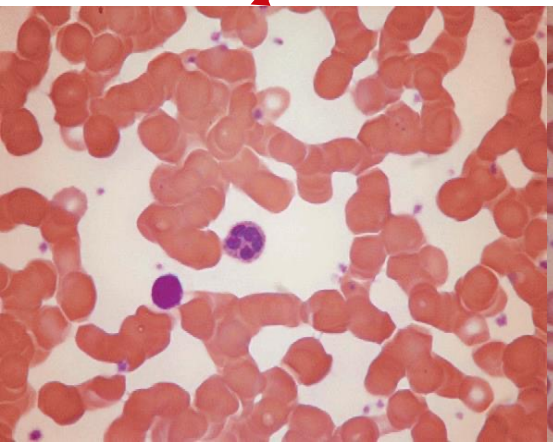


(X100)

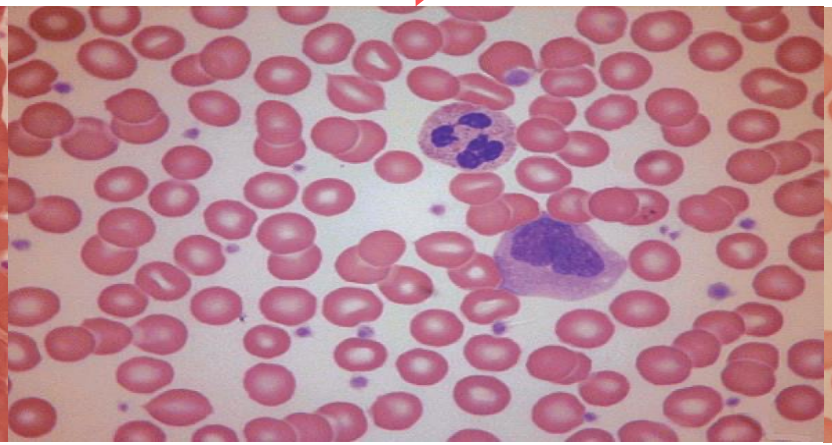


Different areas of thickness found in a smear

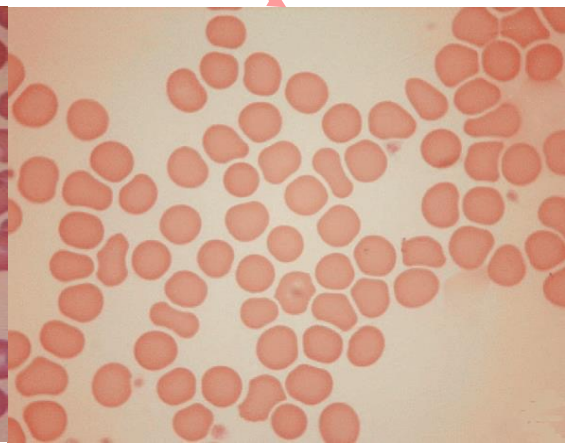




Body of smear – have too much Rouleaux

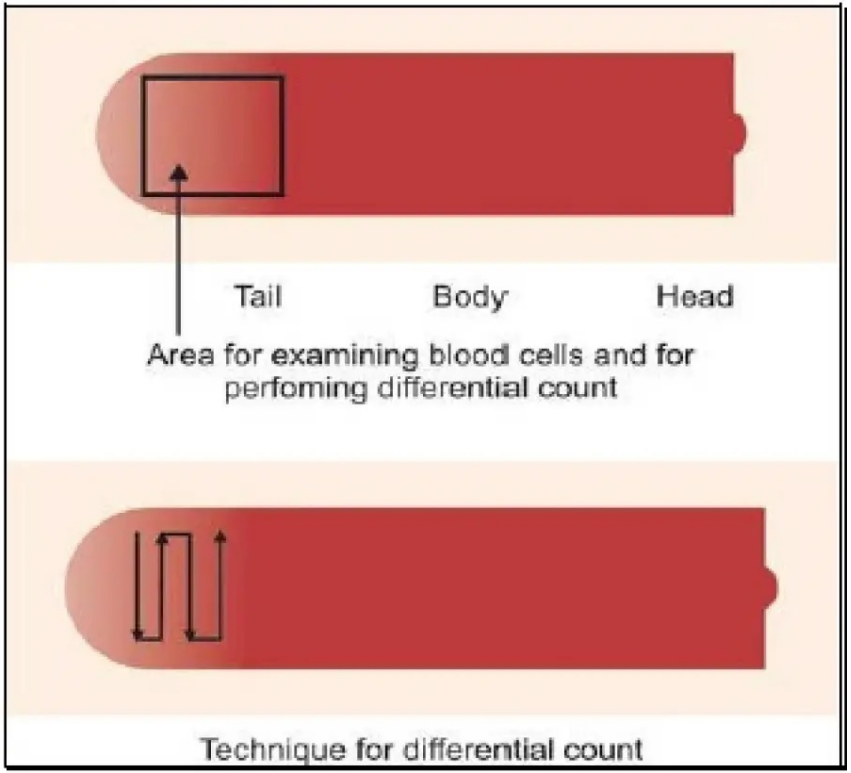


Monolayer - ideal area for investigation



Edge of smear – less cells

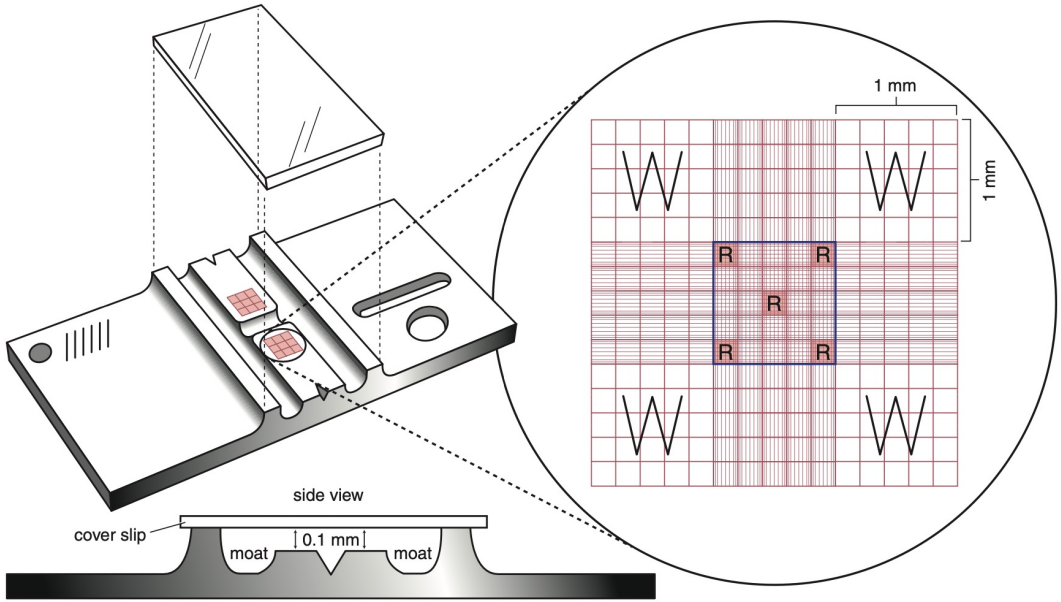
Microscopic Examination

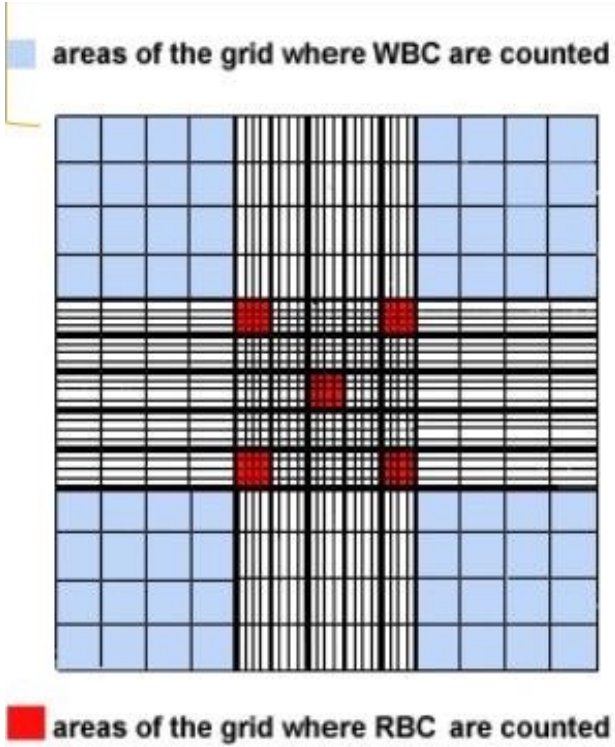


Use the (zig-zag motion) scanning technique for examining your blood smear on the 40X objective lens.

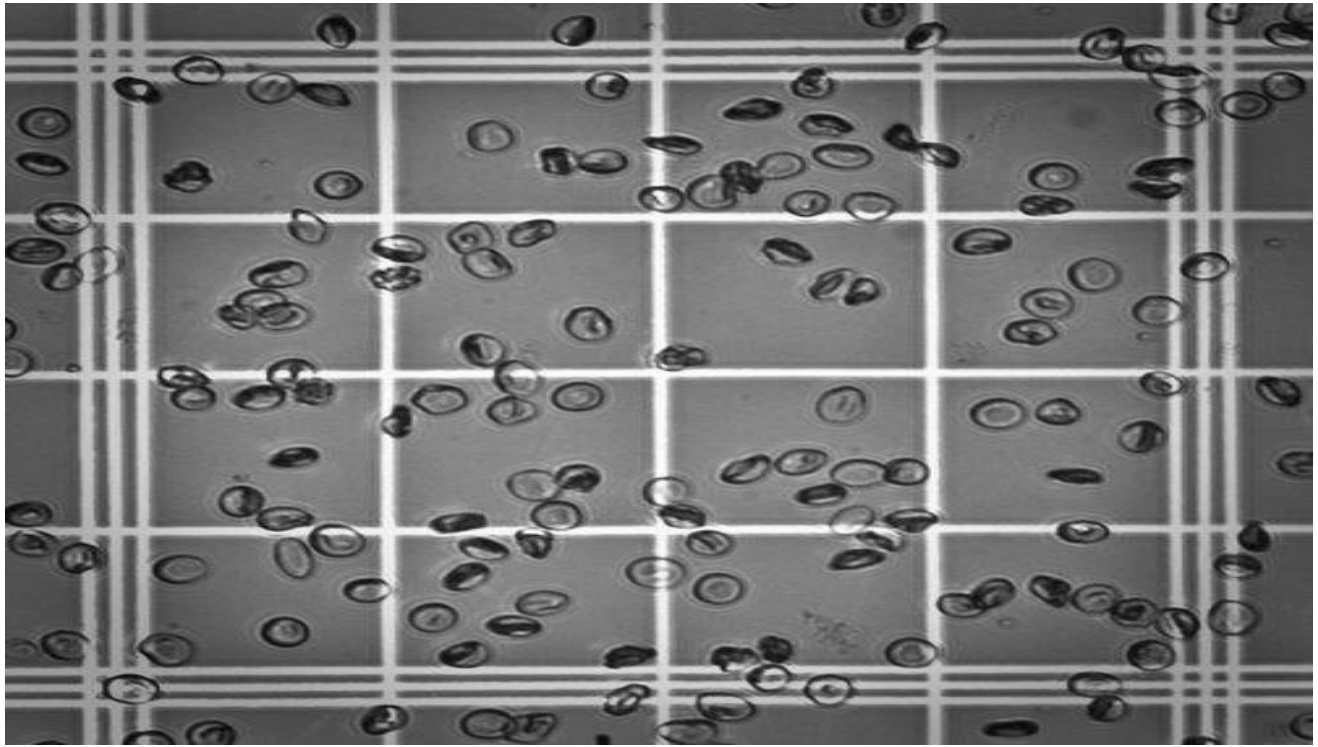
II. Red Blood Cells Counting

Manual counting of RBCs

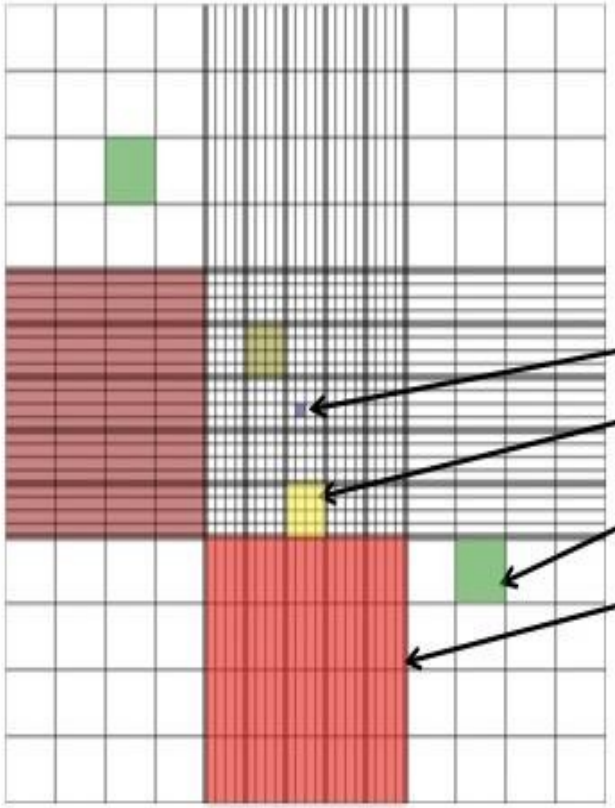




Manual counting of RBCs



Manual counting of RBCs



	Size	Volume
Blue	0.0025 mm^2	0.25 nl
Yellow	0.04 mm^2	4 nl
Green	0.0625 mm^2	6.25 nl
Red	1 mm^2	100 nl

Manual counting of RBCs

- In manual RBC counting, we are counting the number of RBCs in 1 µl.

Normal range of red blood cell count:

- Men 5.0 +/- 0.5 **x 10^12/L**
- Women 4.3 +/- 0.5 **x 10^12/L**

[x 10^12/L] is a unit. Don't use it in any of your calculations!