Preparation of Staining & examination of blood film

- we can see cells in :

1-venous blood

2-BM

- **blood film should made on clean slides and label the blood film by pencil.**

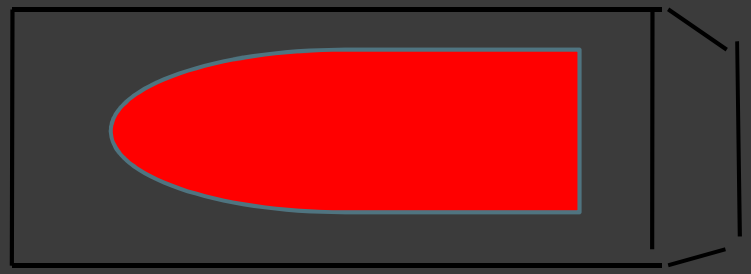
Blood film is important in:

1-haematological diagnosis as in anemia and leukemia to see the morphology of the cells.

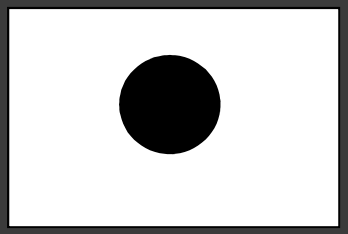
2-WBC differential count to see the account of each type of WBC.

3- estimate the nb of platelets

- Films may be spread by hand or by automated slide spreader.
- Blood film prepared from fresh blood ,use anticoagulant (EDTA) by using capillary tubes.
- No depressing from sample i.e clot
- not short, not long.



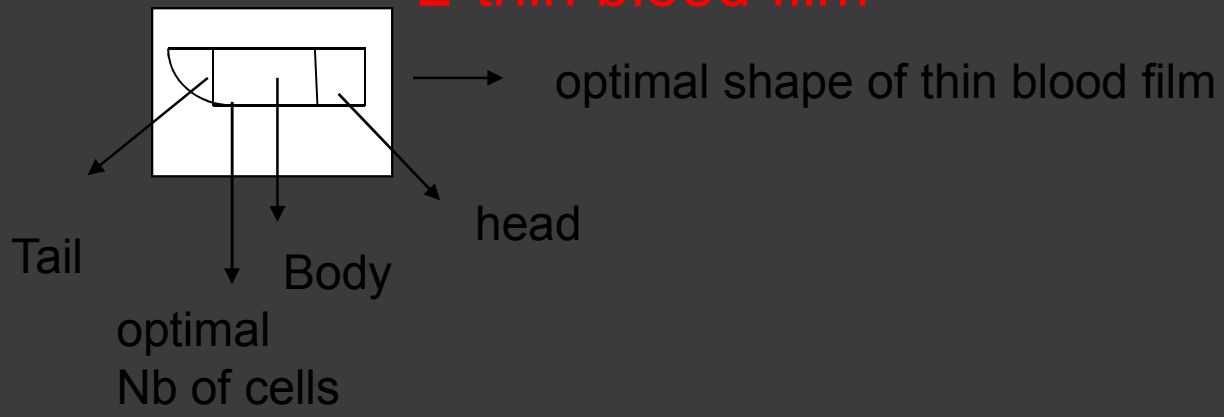
2 types of blood film:



1- Thick blood film → big drop

Used for parasite examination. e.g:Malaria

2-thin blood film



Staining of blood film :

- **Romanowsky stains** are universally employed for routine staining of blood film.

- **It depends on 2 components :**

1-basic part (azure B include “methelene blue”)

2-acidic part (eosin Y)

- methelene blue different than new methelene blue ← used in rectic count

Mechanism by which certain component of cell stain with particular dyes and other component can not stained

1-Azure B (Basic part of stain) bound to acidic part of the cell as nucleus and gives it the blue colour.

2-EosinY(Acidic part of stain) bound to basic part of the cell as protein, cytoplasm and Hb and give them the red colour

Blue ← nuclei has neucleic acid ← basicpart (azure B)

Red ← basic part ← acidic part (eosin y)

Cytoplasm and Hb

Red orange ← Eosinophilic granules (alkaline) ← Acidic part (eosin Y)
granules

Violet ← Basophil has heparin which is acidic ← basic part(azure B)

Types of Romanosky stain:

- **Leishman stains** widely used & simple
- **Wright stain** widely used & simple
- **May grunwald stain** rarely used
- **Jenners stain** rarely used(simplest)
- **Giemsa stain** most complex

staining steps:

1-**fixation** of blood cells to protect the cells from haemolysis due to washing.

If the cells are well fixed the cells resist the action of water

Done by:

Methanol (4 min)

2-**staining**

3-**washing**

Method of leishman&wright stain:

1-dry the film

2- 1 volume of Pasteur Pipette methanol (fixation step) for 4 min

2-1 volume of Pasteur Pipette of the stain (staining step) (neat stain)for 6 min

3- gradually 2 volume of Pasteur Pipette of the diluted stain (stain+buffer PH=6.8)for 6 min

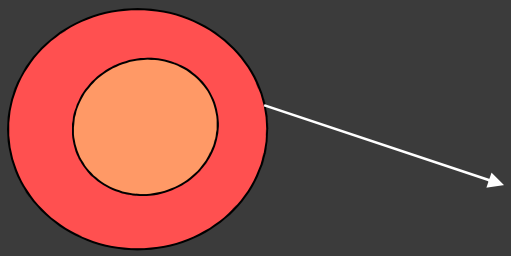
4- I volume of Pasteur Pipette of buffer for 1 min (washing step)

5- washing with water by using pasteur pipette for 1 min

Factors giving rise to faulty staining

Appearance	causes
Too blue or pale staining	1-impure dyes 2-0ver used 3-incorrect preparation
Too pink	1-impure dyes 2-excessive washing in buffer
Stain deposit on film	1-stain solution in uncovered jar 2-stain solution is not filtered
Blue background	1-inadequate fixation 2-blood collected into heparin tube

- **Red cell morphology:**
- In healthy person, the red cells in well spread ,well stained film, they appear as reddish brown round smooth contours with a pale center .
- Its size same the nucleus of lymphocyte and has diameters of 6 to 8.5 µ.m



RBC in Healthy person

Normocytic (size is normal)

Normochromic(Hb content i.e colour is normal)

(The red cells are stained with eosin Y component of rowmanowsky dyes)

Variation in RBCs morphology due to:

1-Abnormal erythropoiesis :production of RBC only

2- increased erythropoiesis

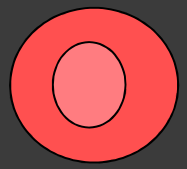
3-decreased Hb formation

4-RBC damage

These causes result in the following variation:

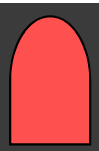
- **Anisocytosis** → variation in size (micro or macro)
- **Poikilocytosis** → variation in shape
- Variation in colour
- Variation in content

1. Variation in Size:

Normal 	
Macrocyte (Large) 	1. Liver disease 2. Alcoholism 3. Megaloblastic anaemia
Microcyte (small) 	1. Iron deficiency anemia. 2. Haemoglobinopathy.

2-Variation in shape:

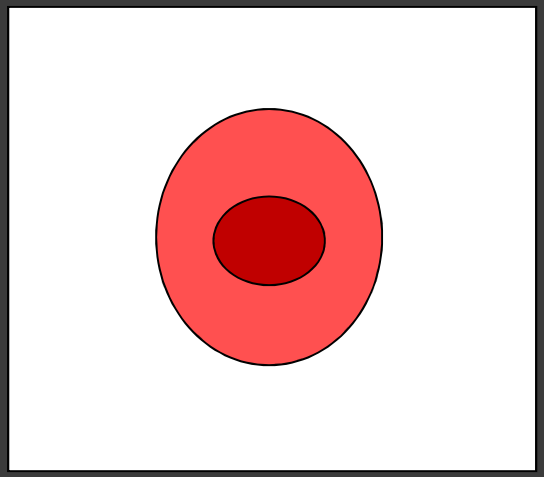
Poikilocyte (tear shape) 	1. Extra medullary haemopoiesis
Elliptocyte (rod like) 	Hereditary elleptocytosis
Ovalocyte (oval) 	
Shistocyte (RBC fragments, (pyknocyte, helmet cell ,bite cell) 	1. DIC → Disseminated intravascular coagulation. 2. Burns 3. Cardiac valves disease
Sickled cell (Banana shape) 	Sickle cell anaemia

Burr cells (Spine projection) 	
Crenation ((acanthocyte)) or spur cells 	1. liver disease 2. renal failure
Ecchinocyte 	1. liver disease 2. renal failure
Pencil cell 	- Iron deficiency anaemia
Basket cell 	G6pD deficiency anaemia
Blister cell 	G6PD deficiency anaemia.

3- Variation in colour:

1. Hypochromasia 	(colour paler) Than normal
2. Anisochromasia	(Dimorphic) picture
3. Target cells 	iron deficiency anaemia liver disease thalassemia post splenectomy
4. Leptocyte 	Very thin cells with colour less central part.(ring shape)
5. Spherocyte 	Sphere like with deep colour and no central pallor found in hereditary spherocytosis.
6. Polychromasia 	Pale greenish – blue color.
7. Stomatocyte 	**Cause by:** liver disease

4-Variation in content

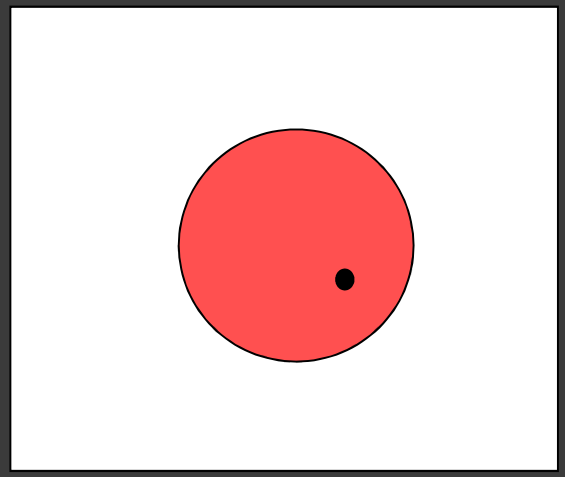


Late normoblast (nucleated RBC)

normally present In BM

abnormal in peripheral blood

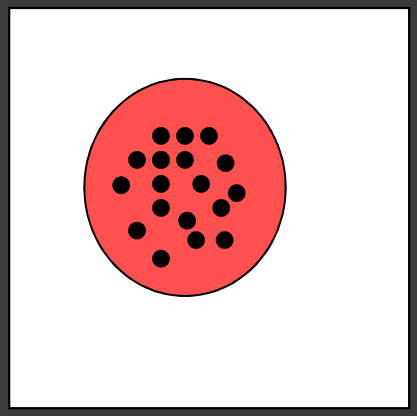
e.g: thalasemia

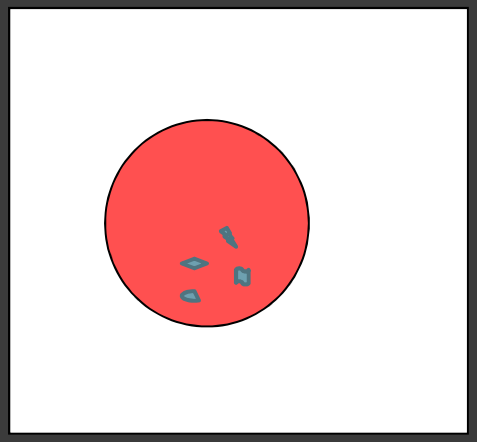


Howell-Jolly body:

DNA remnant

e.g:underdeveloped spleen





Basophilic stippling

denatured

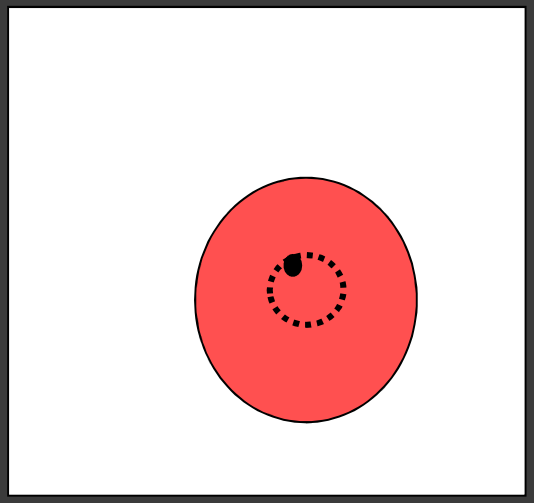
RNA

e.g:megaloblastic anaemia

Siderotic granules (Pappenheimer bodies)

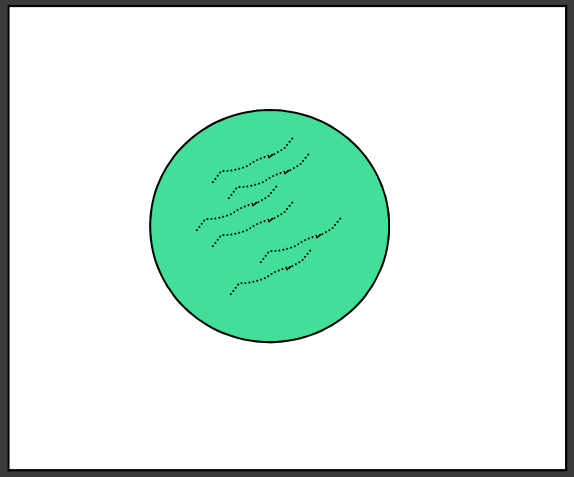
contain iron due to Hb oxidization

they are purple in conventional staining but blue with perl's stain



Malarial parasite

inside RBC



Reticulocyte (RNA)

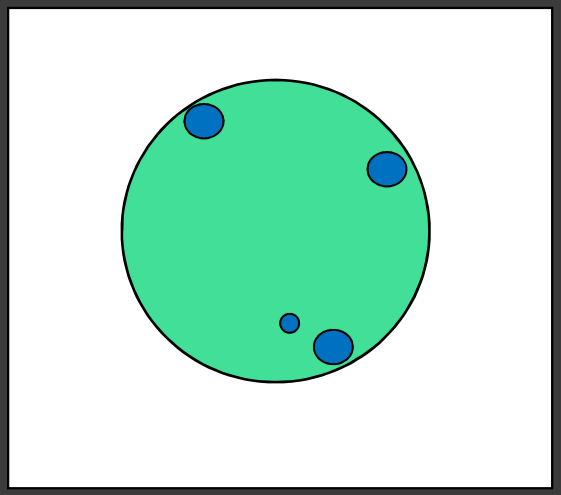
young RBC contain ribosome and RNA

present in BM and blood

Stain by supravital staining

e.g:new methelene blue

Brilliant cresyl blue



Heinz bodies

Oxidized denatured Hb

Found in G6PD defficiency

Stain by supravital staining

e.g:new methelene blue

Brilliant cresyl blue