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

optimal shape of thin blood film

Tail

Body

head

optimal

Nb of cells
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 “methylene blue”)
  2. acidic part (eosin Y)

- methylene blue different than new methylene 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 nucleic acid basic part (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- 1 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

<table>
<thead>
<tr>
<th>Appearance</th>
<th>causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Too blue or pale staining</td>
<td>1-impure dyes</td>
</tr>
<tr>
<td></td>
<td>2-over used</td>
</tr>
<tr>
<td></td>
<td>3-incorrect preparation</td>
</tr>
<tr>
<td>Too pink</td>
<td>1-impure dyes</td>
</tr>
<tr>
<td></td>
<td>2-excessive washing in buffer</td>
</tr>
<tr>
<td>Stain deposit on film</td>
<td>1-stain solution in uncovered jar</td>
</tr>
<tr>
<td></td>
<td>2-stain solution is not filtered</td>
</tr>
<tr>
<td>Blue background</td>
<td>1-inadequate fixation</td>
</tr>
<tr>
<td></td>
<td>2-blood collected into heparin tube</td>
</tr>
</tbody>
</table>
- **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

**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:

<table>
<thead>
<tr>
<th>Normal</th>
<th>Macrocyte (Large)</th>
<th>Microcyte (small)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td><img src="image" alt="Normal Blood Cell" /></td>
<td><img src="image" alt="Macrocyte Blood Cell" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1. Liver disease</td>
<td>1. Iron deficiency anemia.</td>
</tr>
<tr>
<td></td>
<td>3. Megaloblastic anaemia</td>
<td></td>
</tr>
</tbody>
</table>
## 2-Variation in shape:

<table>
<thead>
<tr>
<th>Poikilocyte (tear shape)</th>
<th>1. Extra medullary haemopoiesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elliptocyte (rod like)</td>
<td>Hereditary elleptocytosis</td>
</tr>
<tr>
<td>Ovalocyte (oval)</td>
<td></td>
</tr>
</tbody>
</table>
| 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 |

- Poikilocyte: Tear shape
- Elliptocyte: Rod-like shape
- Ovalocyte: Oval shape
- Shistocyte: RBC fragments, pyknocyte, helmet cell, bite cell
- Sickled cell: Banana shape
<table>
<thead>
<tr>
<th>Burr cells (Spine projection)</th>
</tr>
</thead>
</table>
| 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:

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hypochromasia</td>
<td>(colour paler)</td>
<td>Than normal</td>
</tr>
<tr>
<td>2. Anisochromasias</td>
<td>(Dimorphic) picture</td>
<td></td>
</tr>
<tr>
<td>3. Target cells</td>
<td>iron deficiency anaemia, liver disease, thalassemia, post splenectomy</td>
<td></td>
</tr>
<tr>
<td>4. Leptocyte</td>
<td>Very thin cells with colour less central part. (ring shape)</td>
<td></td>
</tr>
<tr>
<td>5. Spherocyte</td>
<td>Sphere like with deep colour and no central pallor found in hereditary spherocytosis.</td>
<td></td>
</tr>
<tr>
<td>7. Stomatocyte</td>
<td><strong>Cause by:</strong> liver disease</td>
<td></td>
</tr>
</tbody>
</table>
4-Variation in content

Late normoblast (nucleated RBC)
- normally present In BM
- abnormal in peripheral blood
  - e.g: thalassemia

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 methylene 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