# Immunology

**Practical Immunology** 

Lecture 2
Microscopic examination of the blood smear and identification of immune cells

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# Major components of the blood

-Red blood cells (Erythrocytes): Carry the oxygen from the lung to various body organs while also transporting carbon dioxide from the tissue to the lung

-White blood cells (leukocytes): Fight the infection which consist of basophils, neutrophils, eosinophils, lymphocytes and monocytes

-Blood platelets (Thrombocytes):help clotting of the blood and homeostasis

## Complete blood counts (CBC)

Complete blood counts is a blood test which used to evaluate the overall health and giving an indication of such a disorders, anemia and leukemia.

\* All blood cells originate from pluripotent stem cells in the bone marrow.

## Microscopic blood examination procedure

- Blood smear is prepared by using a drop from blood.
- The drop of blood going through the following procedure:

A- Dryness

**B- Fixation** 

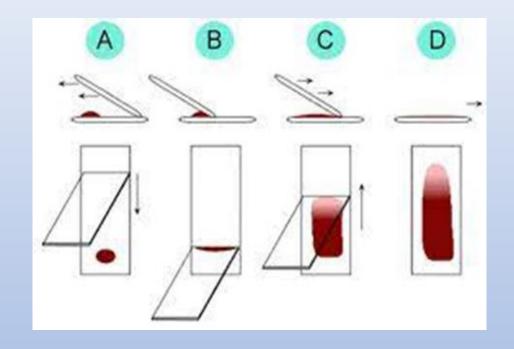
**C-Staining** 

D-Examination under microscope at a magnification of 1:40-1:1000.



# Preparation of the smear

- 1-Place a drop of the blood about 2-3 mm in diameter on one end of the slide.
- 2- Place the second slide (spreader) on the specimen slide just Infront of the blood drop.
- 3- Hold the spreader slide at 30-45 angle and draw it back against the drop of blood
- 4- Allow the blood to spread almost the edges of the slide
- 5- Push the spread forward with one light and smooth moderate speed
- 6- Label one edge with the patient's information or animal information.



### What is Romanowsky stain?

Romanowsky stains are group of stains which applied for differentiation of the cells, pathological examinations of samples such as blood and bone marrow samples .this groups include :

A- Giemsa stain

B- Jenner stain

C- Wright stain

D- May- Grunwald stain

E- Leishman stain

#### Giemsa stain

- -Giemsa stain is a gold standard technique that is used for blood films and blood parasites.
- -Giemsa stain composed of both anionic acidic(Eosin) and cationic basic dyes(Azure and methylene blue).
- -These dyes have different charges, so they have an affinity toward different cellular components such as Basic dye binds to the acid components and specifically to the phosphate groups of the rich part of AT (Adenine Thymine) of nucleic acid producing deep purple color.
- -Eosin is an acidic dye that is attached to the cytoplasm and cytoplasmic granules which are alkaline-producing pink color.

#### Major ingredients of Giemsa stain solution

- 1-Giemsa powder
- 2- Glycerol
- 3- Methanol

Note: The ingredients are the same for all diagnosis, However the concentrations may vary depends on their use.

#### **Staining Procedure**

- 1- Make a thin film of blood and leave it 1-2 minutes to dry.
- 2- Dip the smear few times inside the methanol for fixation and then leave it on air to dry for 30 seconds.
- 3- Flood the slide with Giemsa stain for 20-30 minutes.

Flush with tap water and leave it to dry

Note: Methanol as a fixative does not allow any further changes in the cells and makes them adhere to the glass slide.

# Application of Giemsa stain in the field of Immunology

Immune Hematology

Example: The blood smear is the target spot in order to obtain the hematological purposes each type of immune cells stain differentially and has their own morphology.

Eosin.

Neutrophils: Purple-red nucleus and pale pink cytoplasm

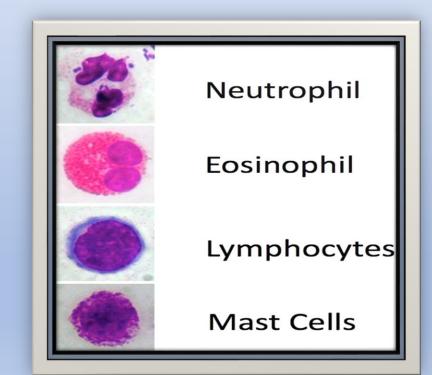
Basophils: Purple nucleus and bluish granules

Eosinophils / Mast cells : Purple blue nucleus

Lymphocytes: Navy blue nucleus and fainty blue cytoplasm

Monocytes: Purple

Platelets: small dark blue without much evident cytoplasm



#### Application of Giemsa stain in other fields

-Bacteriology

Example: staining of Helicobacter pylori infection in stomach.

-Virology: detection of intracellular viral inclusions such as Herpes simplex virus.

-Parasitology

Example: intraerythrocytic parasites such as plasmodia babesiae extraerythrocytic parasites such as Trypanosomes and Microfilaria.

-Mycology

Example: Detect the intracellular yeast forms of Histoplasma capsulatum

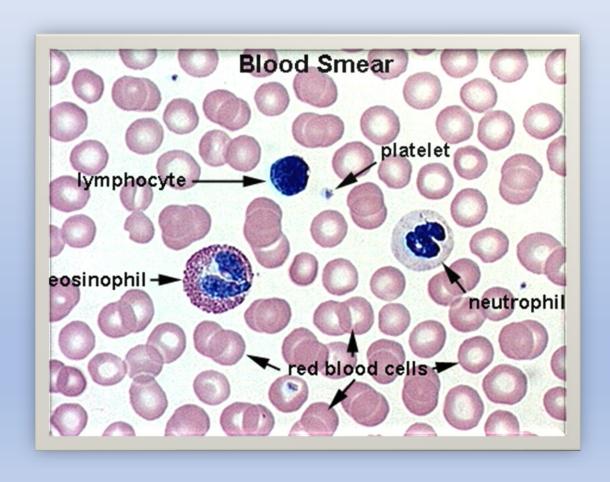
-Histology/Cytology

Example: Giemsa stain can be used as an alternative for Hematoxylin/Eosin.

-Histopathology

Example: Tumors shows a high number of immune cells.

Picture of Blood smear as it appear under the microscope after fixation and staining with Giemsa stain.



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