THE BLOOD SMEAR TECHNIQUE

1. **The Blood Drawing**

It is made by the lateral finger part puncture with a sterilized needle. The skin is disinfected and degreased with an alcohol compress. We hold the patient finger with the hand and with a rapid movement, we prick the right hands needle. The first drop of blood is wiped a compress because if can have tissue liquids or skin impurities.

2. **The Smear Realisation**

It consists in a drop of blood expanding – in a thin and uniform stratum. The second drop of blood is moved with the little extremity of the ground lamina and is applied at one extremity of the horizontal port-object lamina.

We move laterally the lamina with the drop of port-object lamina to obtain a smear.

The smear thick depends on:

- The drop of blood volume (small drop – thin smear)
- The angle formed by the polished lamina and the port-object one, what is about 30-35 degrees (thin smear)
- The smear making speed (great speed-thick smear)

A correct smear have to be thin, to cover all the lamina, to have two limits and to end in fringes.

1. **The Smear Fixing**

The smear fixing is made by:

a) The drying or the dessication is made at the room temperature by moving the lamina (dry smear); the stain have to be made quickly to prevent the cellular alterations modify the tinctorial affinity.
b) The fixing solutions (wet smear); absolute ethylic alcohol, for 15 minutes, absolute ethylic and ether in equal parts, for 5-15 minutes, methyl alcohol for 2 minutes or by OsO_4 vapours (2% solution). After that, on make the specimen staining.

THE BLOOD SMEAR DYEING TECHNIQUE

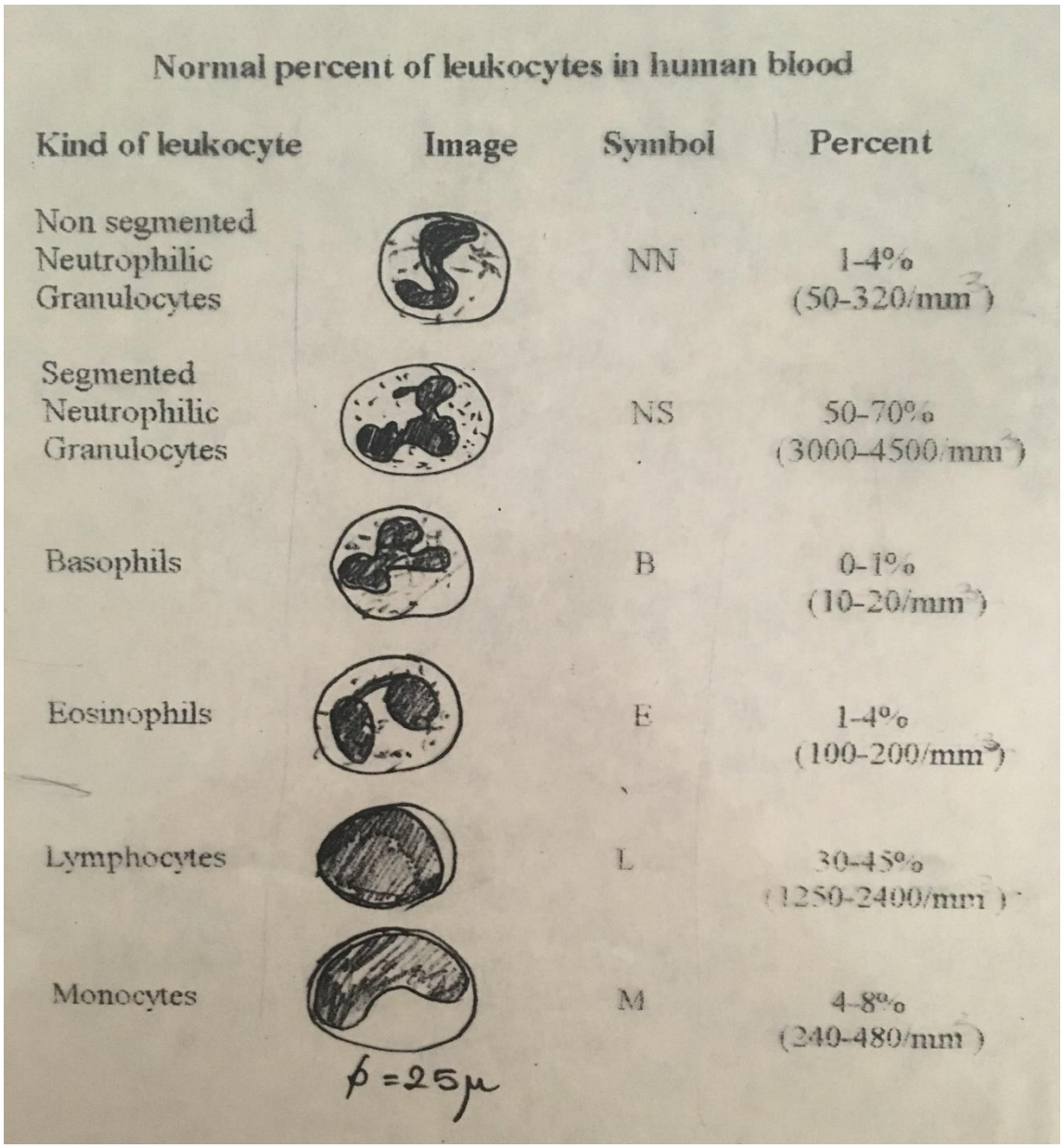
We use the May-Grunwald- Giemsa dyeing method or the Pappenheim staining. The colouring matter is formed by two solution: May-Grunwald- Giemsa solution an Giemsa solution.

The work technique is the following:

- The lamina with the dry smear up, is put on two parallel glass bars disposed in Petri boxes
- We cover the smear with 5-10 drops of May-Grunwald solution
- After 2-3 minutes, when the methyl alcohol from the solution fixes the cellular elements of the smear, over the May-Grunwald solution statum, we put the same number of drops of distilled neutral water; the mixture is homogenized with a Pasteur pipette and we keep it 2-3 minutes more, when the May-Grunwald solution acts like a colouring matter.
- We move off the former solution and, without washing, the smear is covered with a Giemsa solution diluted 1/1 with distilled neutral water, 20-30 minutes
- The smear wash with water
- The differentiation in distilled neutral water 1 minute
- We put the lamina on a stand and on wait to dry
- We realize the examination with the immersion objective

To prepare the leukocyte formula, we examine the blood smear on the broder, the glass lamina is displaced in only one direction, noting every type of leucocyte from 100 numbered leucocytes. In the following tables we present the normal data of the human being values.

Normal percent of leukocytes in human blood



Blood is a tissue which consists of a variety of cells suspended in a fluid medium called *plasma*. Blood functions principally as a vehicle for the transport of gases, nutrients, metabolic vaste products, cells and hormones throughout the body.

Physiological values of peripheral blood cells

Erythrocytes:

- Male 4.500.000 – 5.000.000/mm3
- Female 4.000.000 – 5.000.000/mm3
- New born 4.000.000 – 7.000.000/mm3
- Adult (until 12 years old) 4.000.000 – 5.000.000/mm3

Leukocytes:

- New born 15.000 – 25.000/mm3
- Adult 4.000 – 9.000/mm3

Platelets:

- 130.000 – 300.000/mm3

The cells of blood are of three major functional classes: red blood cells (erythrocytes), white blood cells (leukocytes) and platelets (thrombocytes).

Erythrocytes are primarily involved in oxygen and carbon dioxide transport, the leucocytes constitute an important part of the defense and immune systems of the body, and platelets are a vital component of the blood clotting mechanism. All these cell types are formed in the bone marrow by a process called hematopoiesis. Erythrocytes and platelets function entirely within blood vessels whereas leucocytes act mainly outside blood vessels in the tissues. Thus the leucocytes found in circulating blood are merely in transit between their various sites of activity.

Erythrocytes

In a stained smear of peripheral blood, the cells are stained pink due to their high content of hemoglobin. The pale staining of the central region of the erythrocyte is a result of its unusual biconcave disc shape. The unusual shape provides a large surface area relative to cell volume, which greatly enhances gaseous exchange. The fluidity of the plasma membrane, combined with its biconcave shape allows the erythrocyte to deform readly, thus erythrocyte (average diameter 6-8 μm) are able to pass through the smallest capillaries (3-4 μm in diameter).

White cell series

There are five cell types in the white blood cell series and these are subdivided into two main classes, granulocytes and agranulocytes, according to the granularity of their cytoplasm and general nuclear characteristics:

1. **Granulocytes:** the granulocytes are characterized by prominent cytoplasmic granules and a single, multilobate nucleus, which may give the erroneous impression that granulocytes are multinucleate cells. The highly variable shape of granulocytes nuclei has give rise to the common name of polymorphonuclear leucocytes or polymorphs.

There are three different types of granulocytes, neutophyls, eosinophils and basophils named according to the staining characteristics of their specific granules.

The specific granules of neutrophils have little affinity for either acidic or basic dyes whereas those of eosinophils are stained strongly by acidic dyes such as eosin, and those of basophils are stained intensely by basic dyes such as haematoxylin or methylene blue.

2. **Agranulocytes:** the agranulocytes, which comprise the lymphocytes and monocytes, are so named since they do not microscopy. In contrast to the granulocytes, the nuclei of the agranulocytes are not lobed although they may be deeply indented. This nuclear feature led to the application of the misleading term mononuclear leucocytes in reference to the agranulocytes.

All leucocytes exhibit amoeboid movement, which provides the means for migration in and out of the circulatory system and through the tissues.

Neutrophils

a) Distribution

Neutrophils, or polymorphonuclear leukocytes (PMNs), are the most common leukocytes in normal human peripheral blood. A cubic millimeter of blood contains about 4500 neutrophils.

b) Structure

Neutrophils are 12-15 μm in diameter. The nucleus has three to five lobes, is largely heterochromatin, and contains no nucleolus. The cytoplasm is moderately acidophilic and contains two types of granules:

- Azurophilic granules (primary, or type A) stain with azure dye and are diagnostic for neutrophils. These granules (0,4 μm) comprise about 20% of the granule population and are visible in the light microscope.
- Specific granules (secondary or type B) are smaller (0,2 μm) and may contain crystalloids. They comprise 80% of the granule population and are not visible in the light microscope.

c) Function

- The azurophilic primary granules, the lysosomes, contain a variety of hydrolytic enzymes plus potent antibacterial enzymes such as lysozyme, myeloperoxidase and D-amino-oxidase, which destroy bacterial cell walls. The principal function of neutrophils is to engulf invading microorganisms, particularly bacteria; neutrophils

are the main white cell type involved in acute inflammatory responses. Neutrophils first adhere to bacteria and then engulf them in a membrane-bound *phagosome.* Phagosomes fuse with secondary granules and then primary granules to from *phagolysosomes.*

- The specific granules of neutrophils contain a group of proteins with antibacterial action called *phagocytins* and the enzyme *alkaline phosphatase*. Specific granules contain the protein *lactoferrin,* with binds the ferric ions required for bacterial multiplication.

Eosinophils

a) Distribution

Eosinophils comprise about 1% of all leukocytes in blood; a cubic millimeter contains about 200.

b) Structure

Eosinophils are motile phagocytic cells, which have diameters similar to neutrophils yet appear quite distinct in the light microscope. The nucleus has two or three lobes, which contain a striking array of large (0,6 µm) red or orange *eosinophilic granules*. Eosinophilic granules have prominent crystalloids.

c) Function

Eosinophils kill parasitic larvae as they enter peripheral blood or the lamina propria of the gut. Eosinophilic granules contain lysosomal enzymes that destroy dead parasites.

Basophils

a) Distribution

Basophils are the rarest leucocytes. A cubic millimeter of blood contains about 5 basophils.

b) Structure

Basophils are about the size of neutrophils and eosinophils, and contain a nucleus with two or three lobes. Basophil granules are membrane-bound and contain crystalline regions, which suggests that they are modified lysosomes. Basophile granules contain *histamine* (a potent acute vasodilator), *heparin* (a glycosaminoglycan anticoagulant) and *slow reacting substance* (a slow-acting vasodilator).

c) Function

Basophils mediate the inflammatory response and secrete *eosinophil chemotactic factor*. In response to certain antigens, basophils stimulate the formation of *immunoglobulin* E (IgE) – a calss of antibodies.

Lymphocytes

a) Distribution

Lymphocytes are the most common agranulocytes. S\A cubic millimeter of blood contains about 2500. Lymphocytes are not confined to peripheral blood. They also exist in connective tissue lamina propria, lymph nodes, the spleen and tonsils, and bone marrow.

b) Structure

Lymphocytes diameter varies from 5-8 µm in small lymphocytes to 15 µm in large lymphocytes. Lymphocytes have a round, densely stained nucleus, which occupies most of the cell volume, and a thin shell of cytoplasma around the nucleus. As lymphocytes diameter increases, the cell's cytoplasma volume increases faster than the nuclear volume. Lymphocytes do not contain specific granules.

c) Function

Lymphocytes are key cells in the immune system.

Monocytes

a) Distribution

A cubic millimeter of blood contains about 300 monocytes.

b) Structure

Monocytes, the larges leukocytes, have diameters of 12-18 µm. Monocytes have an arganular cytoplasma and a rounded nucleus that has indentation on one side. They do chromatin, monocytes chromatin stains uniformly, revealing a delicate network.

c) Function

Monocytes are direct precursors to macrophages.

Plateles

a) Structure and distribution

Platelets are 2-4 µm in diameter. A cubic millimeter of blood contains 200.00-300.000

Platelets have central granulomere, which stains purple in blood smears, and a peripheral hyalomere, which stains faintly.

b) Function

Platelets are important for blood clotting.