



Al-Rasheed University College Pharmacy Department

2nd Stage / 2nd Semester

2021-2022



Differential WBC Counting

Physiology lab #4

Done by:

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Introduction

- White blood cells (WBCs) are also called leukocytes
- They are the first line of defense against invading pathogens and diseases
- Leukocytes are originated in bone marrow from stem cells (hemocytoblasts) in response to certain hormones.
- Once WBCs are fully mature, they enter the blood stream and then transported to the site of injury.

Types of WBC

- **Two main types of WBCs are identified:**
- **Granulated WBCs** (because of the presence of granules in cytoplasm)

Neutrophils

Eosinophils

Basophils

- **Agranulated WBCs** (because they lack granules in cytoplasm)

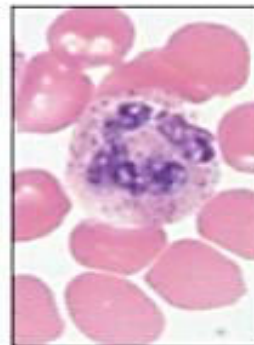
Lymphocytes (T cell & B cells)

Monocytes

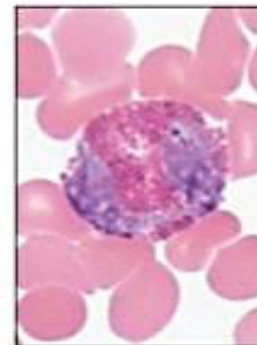
Characteristic differences

WBCs differ one from the other in:

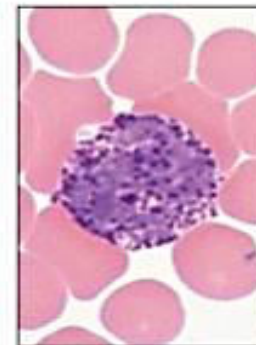
1. Cell size
2. Composition of the cytoplasm
3. Shape of the nucleus
4. Staining characteristics



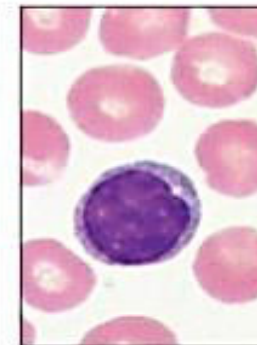
(a) Neutrophil;
multilobed
nucleus



(b) Eosinophil;
bilobed nucleus,
red cytoplasmic
granules



(c) Basophil;
bilobed nucleus,
purplish-black
cytoplasmic
granules



**(d) Small
lymphocyte;**
large spherical
nucleus



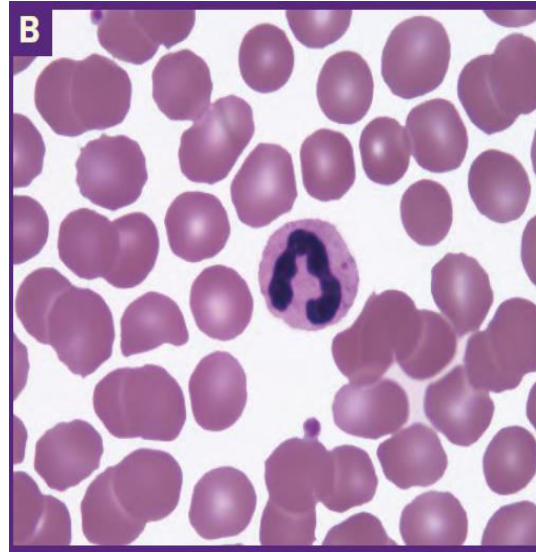
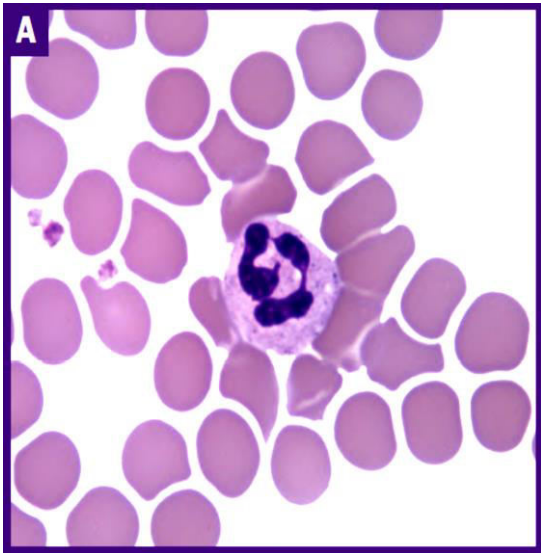
(e) Monocyte;
kidney-shaped
nucleus

Granulated WBC

1-Neutrophils

2-Eosinophils

3-Basophils



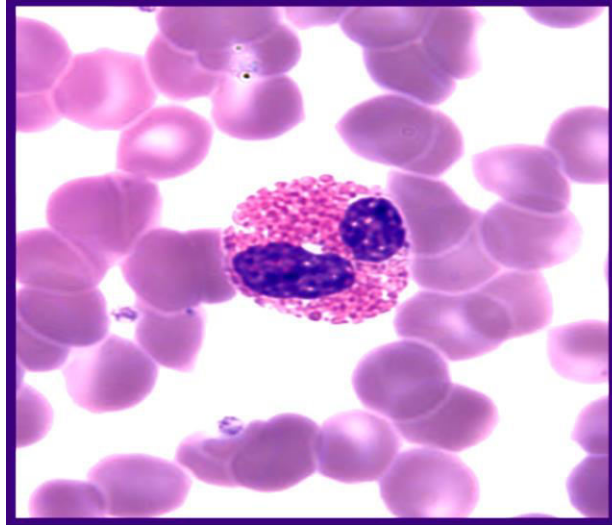
- Have fine granulated cytoplasm
- Mostly lobulated nucleus (2 – 5 lobes)
- Immature neutrophils, **nucleus not lobulated**
- Mature neutrophils, nucleus is lobulated (in this case the cells are called polymorphonuclear neutrophils = PMNs)
- Granules stains purple in color with acid-base stain
- **Most abundant type of WBCs (accounts for 55% - 65%)**
- Mature and specialized neutrophils are called microphages capable of phagocytosis
- ***First line of defense in acute bacterial infection or inflammation***
- Increased number of neutrophils called (neutrophilia) as in:
 - Acute inflammation (gouty arthritis)
 - Acute bacterial infection
- Decreased number of neutrophils called (neutropenia) as in:
 - Viral infection
 - Parasitic infection (malaria)

Granulated WBC:

1-Neutrophils

2-Eosinophils

3-Basophils



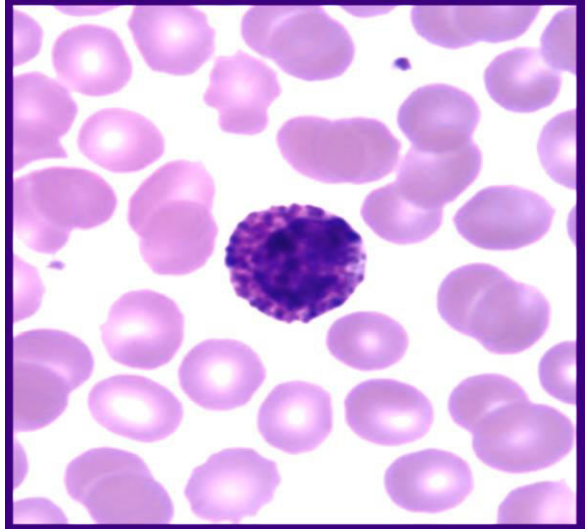
- Course granulated cytoplasm
- Lobulated nucleus (2 lobes)
- Granules stains bright red
- Accounts for about (1% - 3%) of WBCs
- Release the histaminase enzyme that combat the effect of histamine and other inflammatory mediators
- Active during moderate allergic reaction and parasitic worm infection. Capable of phagocytosis
- Increased number of eosinophils is called (eosinophilia)
 - Parasitic infection
 - Allergic reaction (hay fever)
- Decreased number of eosinophils is called (eosinopenia)
 - Cushing syndrome
 - Acromegaly

Granulated WBC

Neutrophils

Eosinophils

Basophils

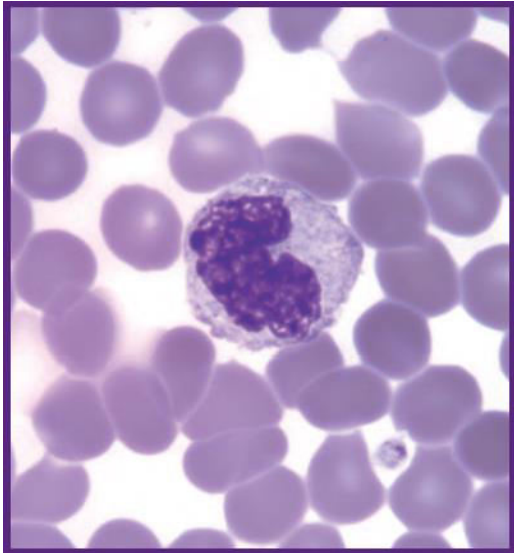


- Very coarse granulated cytoplasm
- Lobulated nucleus (2 lobes), usually obscured by the large granules
- Granules stains deep blue
- Least abundant type of WBCs, accounts for (1% or less)
- They release **histamine** to promote inflammation and **heparin** to prevent blood clotting and intracellular coagulation
- Increased number of basophils is called (basophilia)
 - TB infection
 - Hypothyroidism
- Decreased number of basophils is called (basopenia)
 - Hyperthyroidism
 - Cushing syndrome

Agranulated WBC

Monocytes

Lymphocytes

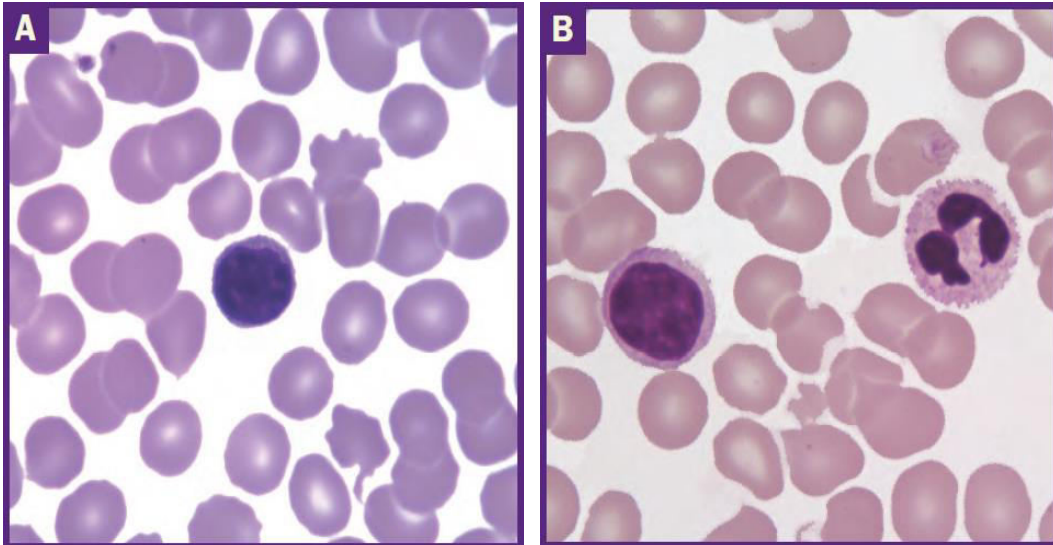


- Cytoplasm lacks granules, stains light blue
- Single nucleus, not lobulated (kidney-shaped or horse shoe-shaped), nucleus stains purple
- The largest cells of WBCs
- Accounts for 3% - 7% of all WBCs
- **Specialized monocytes** leave the blood stream and localize in different organs, then they are **called macrophages**
- They are the **first line of defense** in **chronic infection**, and they are capable of phagocytosis.
- They secrete anti-viral substance called **interferon** and inflammatory substance called **interleukin 1**
- Increased in number of monocytes is called **(monocytosis)**
 - Ulcerative colitis
 - Tuberculosis (TB)
- Decreased number of monocytes is called **(monocytopenia)**
 - Hairy cell leukemia
 - Steroid treatment

Agranulated WBC

Monocytes

Lymphocytes



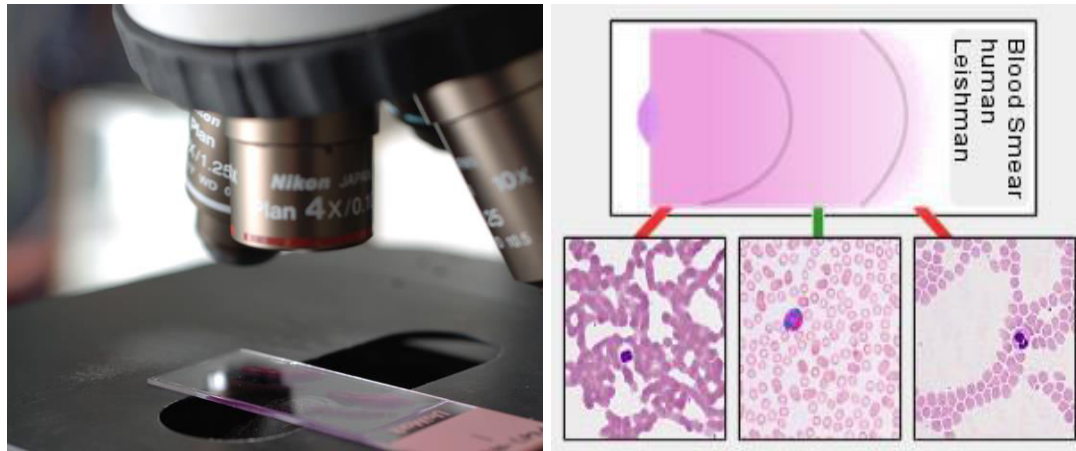
- Cytoplasm lacks granules, stains light blue
- Single nucleus, not lobulated. The nucleus is round and takes up most of the cell leaving a small amount of cytoplasm surrounding the nucleus, stains purple
- Smallest cells among WBCs
- Accounts for 25% - 33% of all WBCs
- **There are 2 types of lymphocytes:**
 - **T lymphocytes:** originate from bone marrow and mature in the thymus and important for cellular immunity
 - **B lymphocytes:** originates from bone marrow and mature in bone marrow and they secrete antibodies
- **Important in cellular immunity and antibodies production**
- Increased number of lymphocytes is called **(lymphocytosis)**
 - Viral infection
 - Chron disease
- Decreased number of lymphocytes is called **(lymphocytopenia)**
 - Malignancies
 - Aplastic anemia

Objective (aim) of the experiment

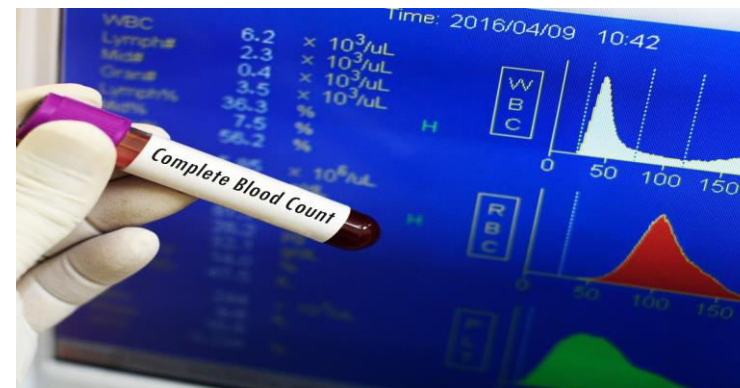
- A differential blood cell count is done to determine approximate numbers of the various leukocytes in blood.
- Excess or deficiency of all or a specific group is indicative of certain disease states

Methods of measurements

1- MANUAL (OLD FASHION)



2- AUTOMATED



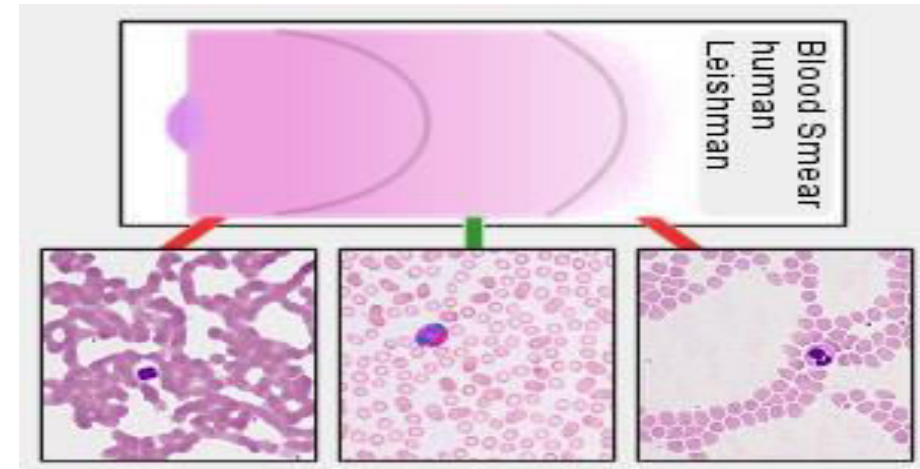
Manual method

- **Objective of the experiment:**

To learn how to prepare a proper, uniform and thin blood film.

Use the correct type of stain and how to make the staining of the blood film.

To be able to examine the stained blood film under the microscope in order to view the different types of WBCs and count them.



Basic Required Equipment:

1-70% alcohol and Cotton for sterilization.

2-Sterile blood lancet.

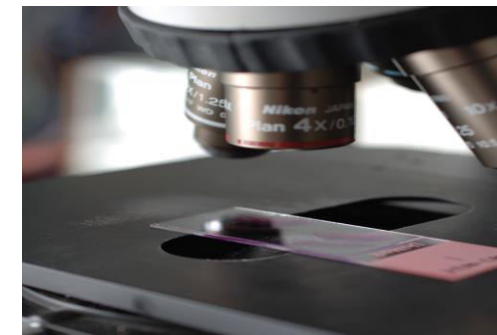
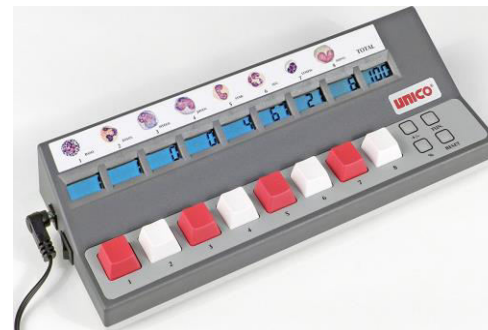
3-Two clean slides.

4-Heparinized capillary tube (red) or anticoagulant test tubes.

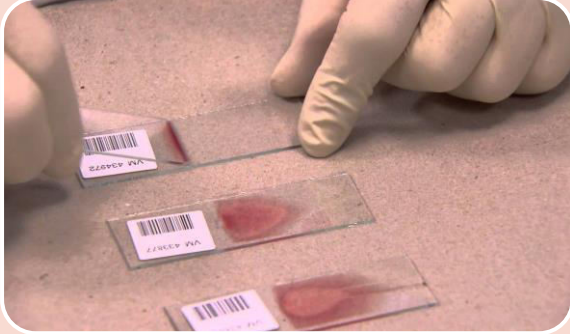
5-Staining solution (Leishman or Giemsa or Wright stain).

6-Light microscope.

7-Manual WBC counter



Procedure



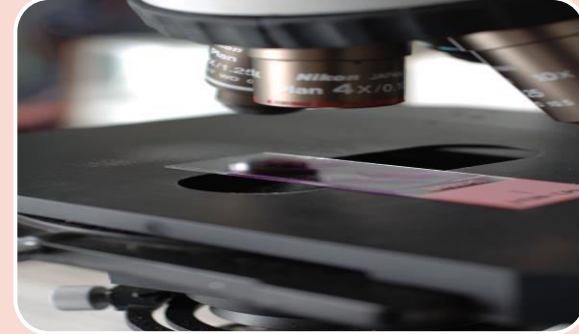
Making a
blood film
(smear)

(1)



Staining
the blood
smear

(2)

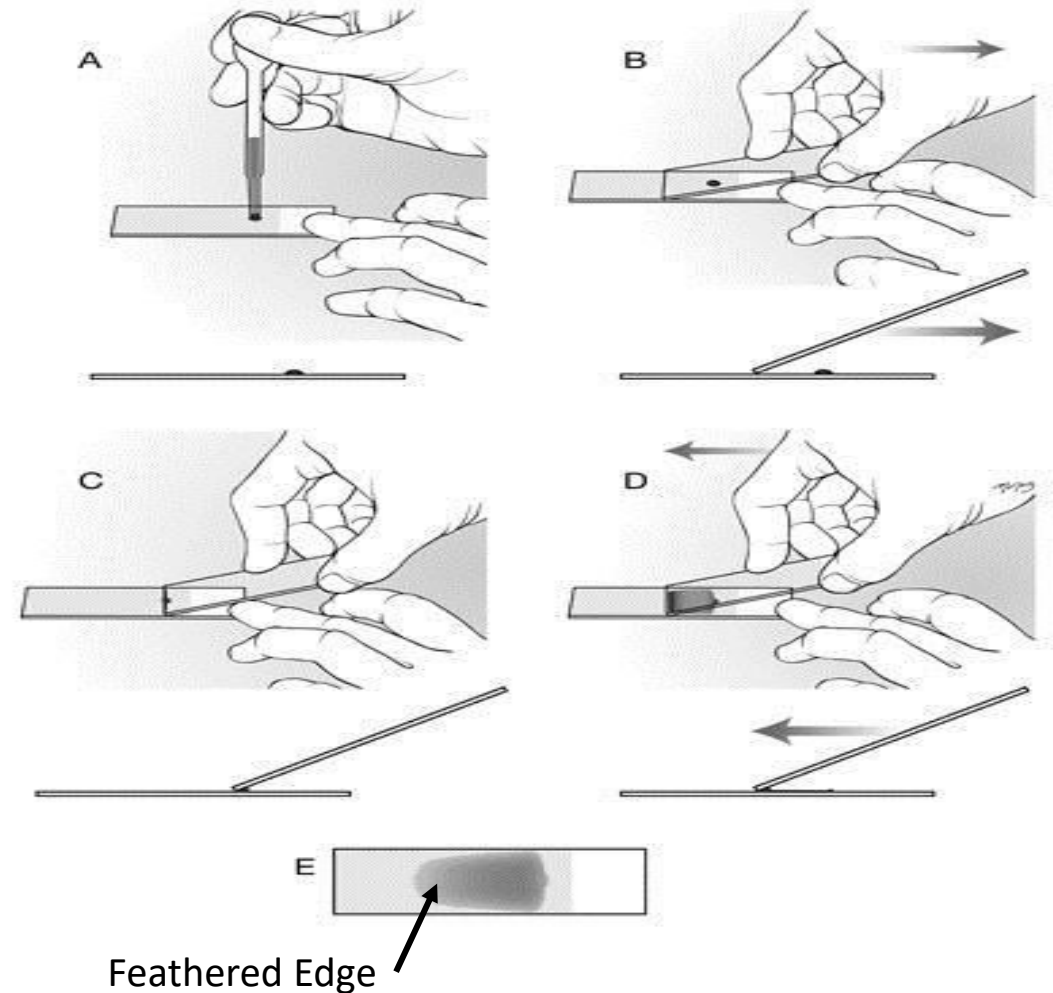


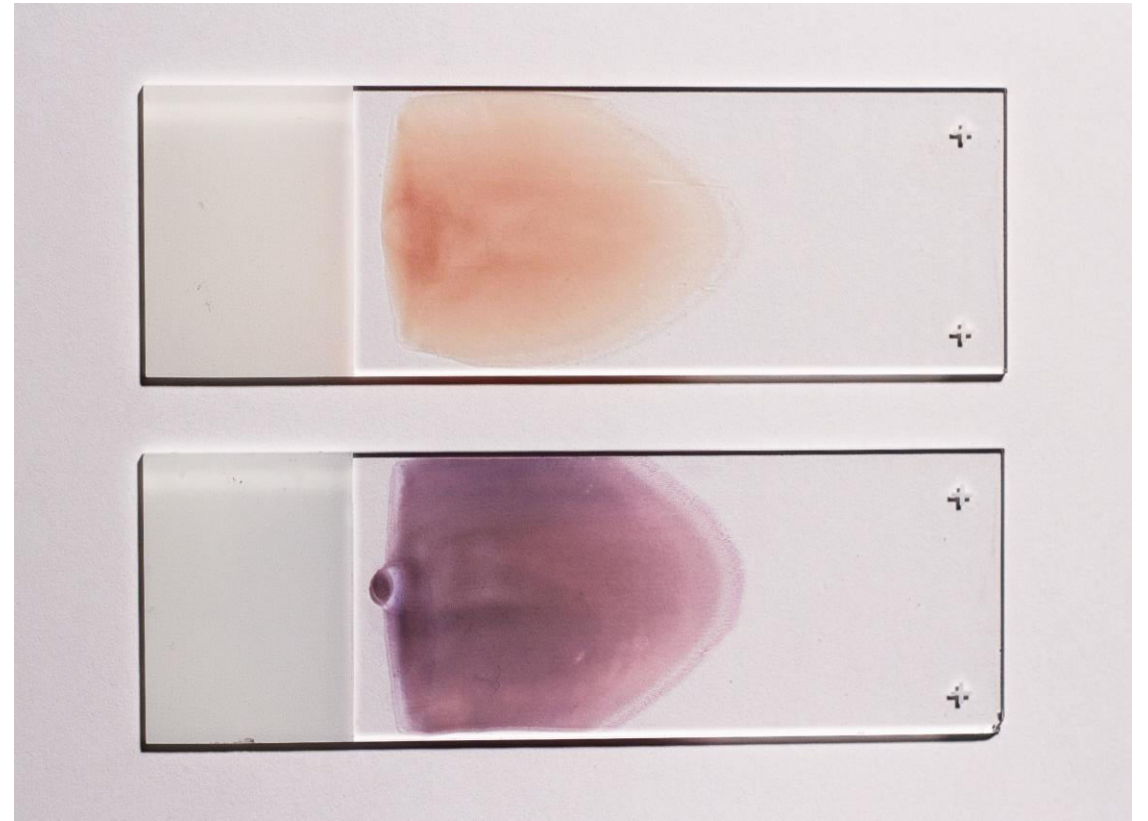
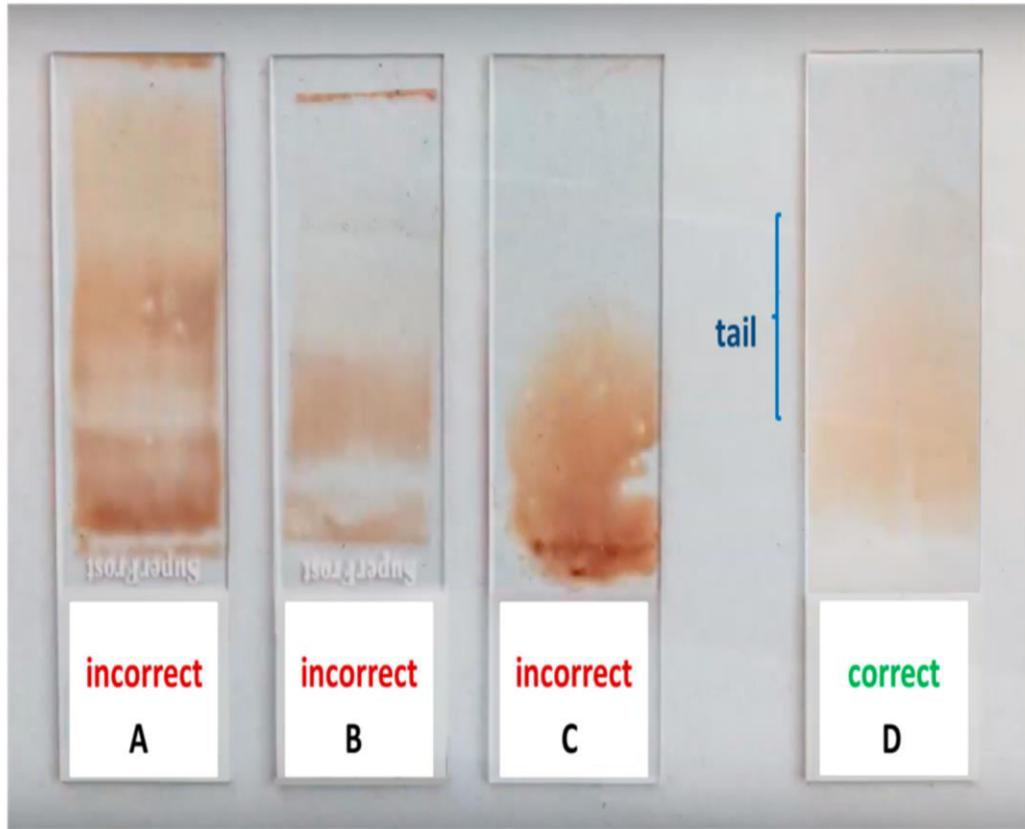
Counting
WBCs under
a
microscope

(3)

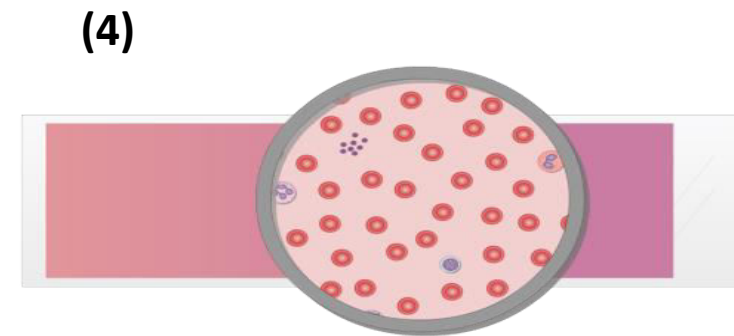
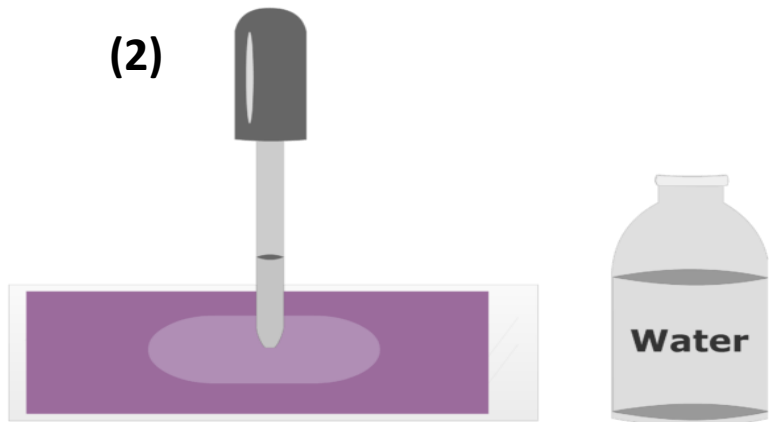
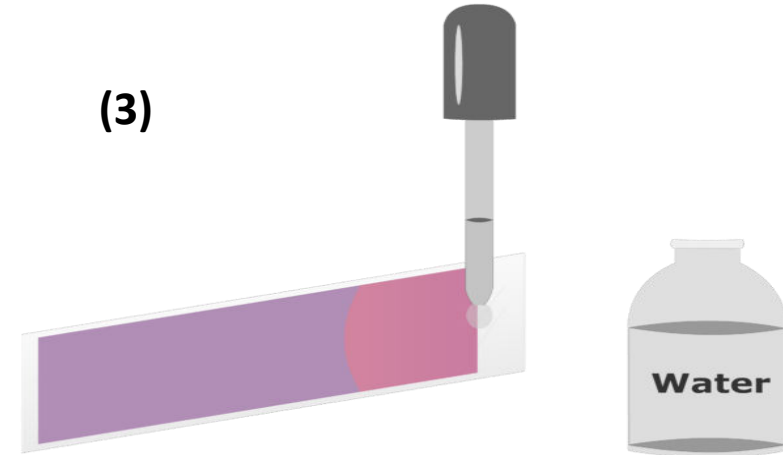
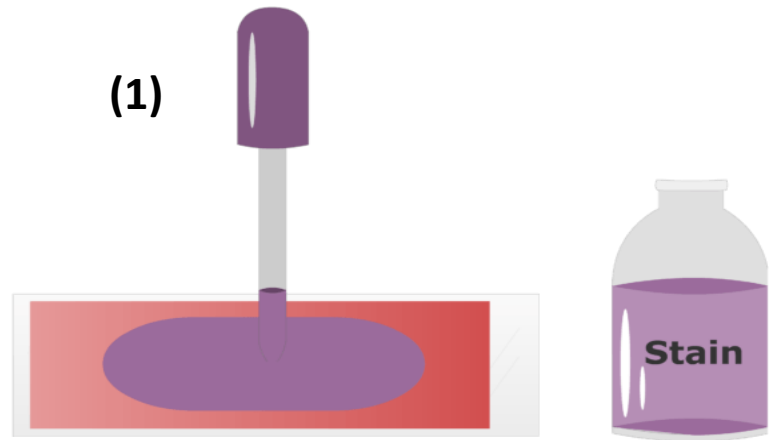
First step: Making blood film or smear

1. Collect finger puncture blood by capillary tube (red) or collect venous blood using anticoagulant tubes
2. Place a small drop of blood close to one end of the slide. hold the end of a second slide (the "spreader") against the surface of the first slide at an angle of 30 degrees. Slowly back the spreader into the blood drop
3. allowing the blood to pool at the angle between the two slides.
4. Gently push the spreader slide at a moderate speed forward, spreading the drop of blood out into a thin film.





Step Two: Staining the blood film



Characteristic of stains

- **Hematology stains are:**

1. Giemsa stain
2. Wright stain
3. Leishman stain

- All of these stain are a mixture of **basic stain** (methylene blue or azure 2) and **acidic stain** (eosin)

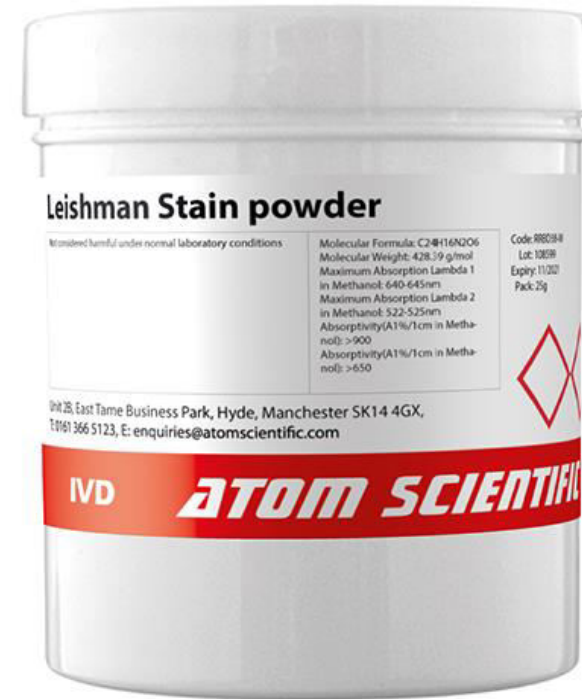
- **Methylene Blue or Azure 2:**

- Basic stain
- Blue – purple color
- Stains nucleus, ribosome and endoplasmic reticulum

- **Eosin:**

- Acidic stain
- Pink – red color
- Stains nucleus and most cytoplasmic granules and proteins

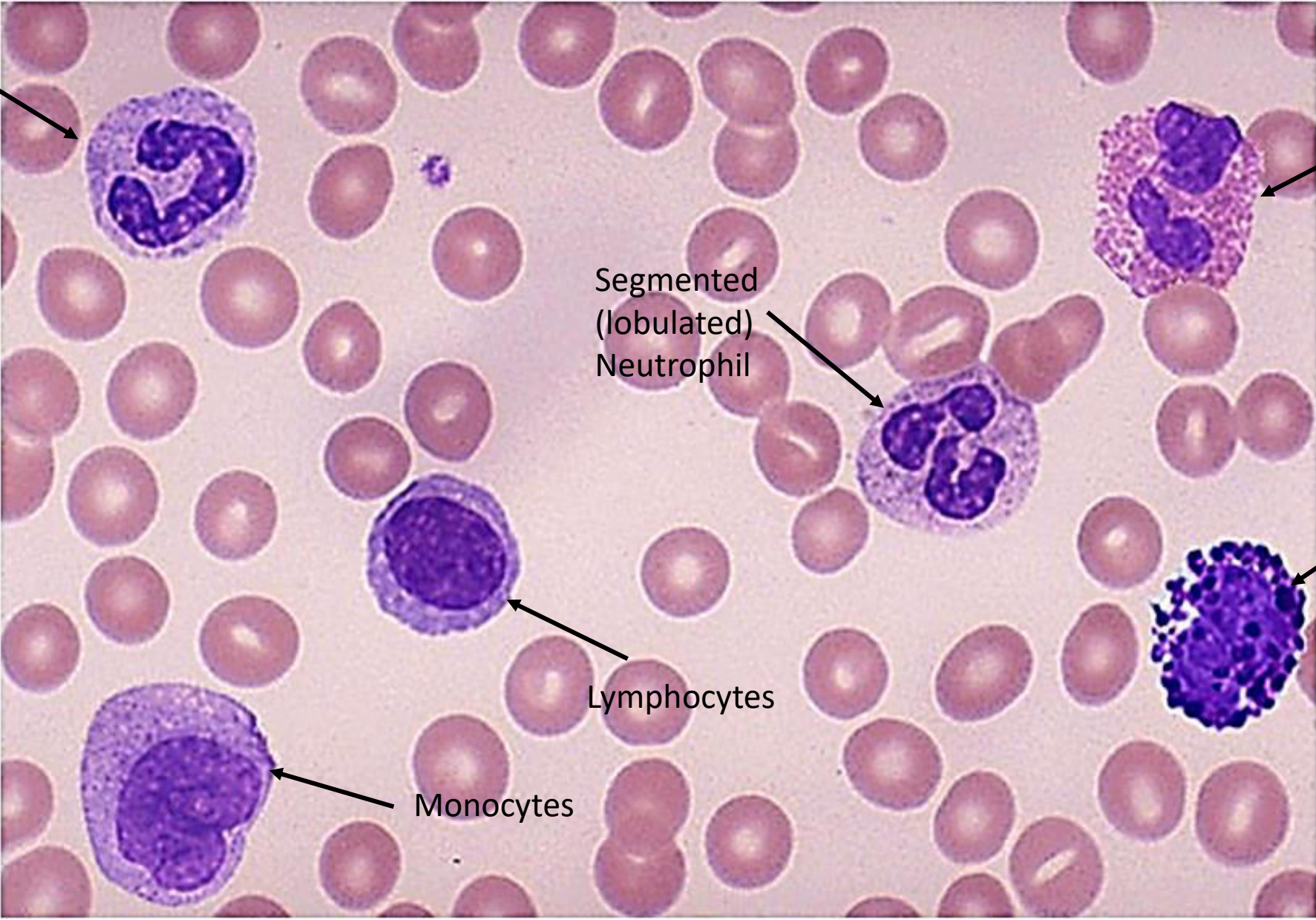




Solution of the stain consists of the following:

- Leishman stain powder for staining the blood film
- Methanol as a solvent for the stain powder and a fixative agent for the cells
- Glycerin to facilitate the entrance of the stain inside the cells

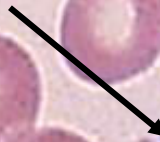
Non segmented Neutrophil



Eosinophil



Segmented (lobulated) Neutrophil



Basophil

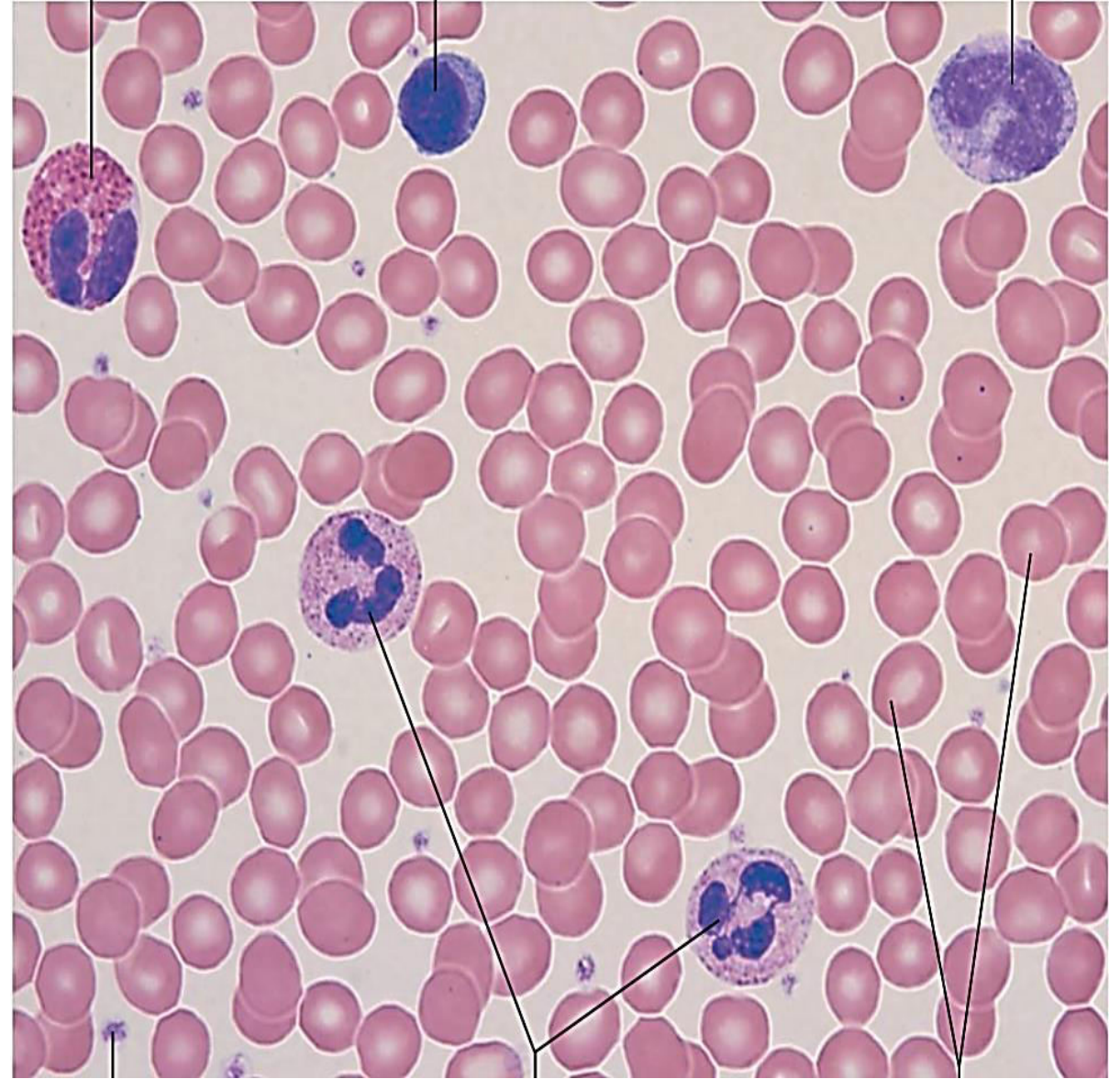
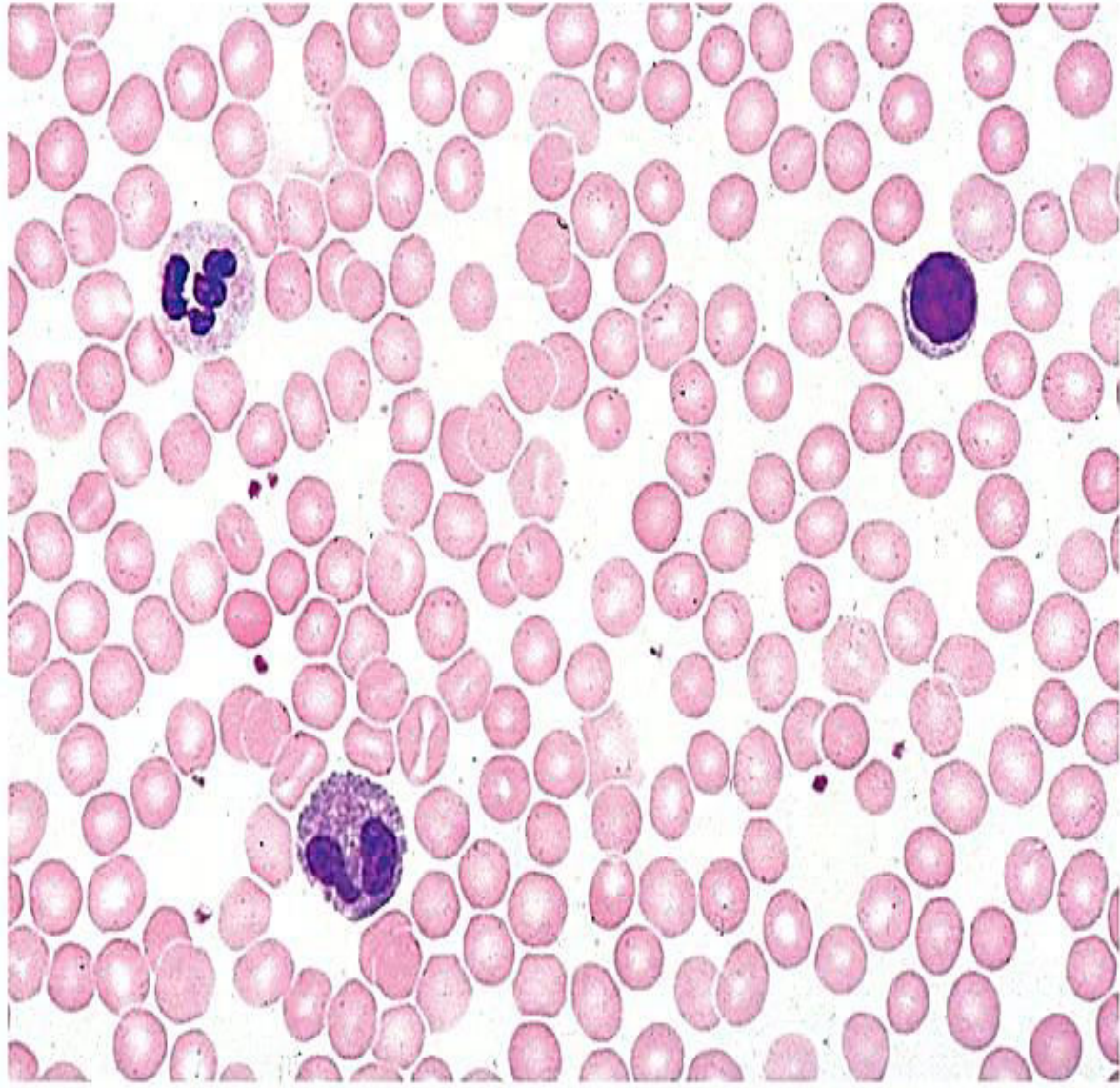


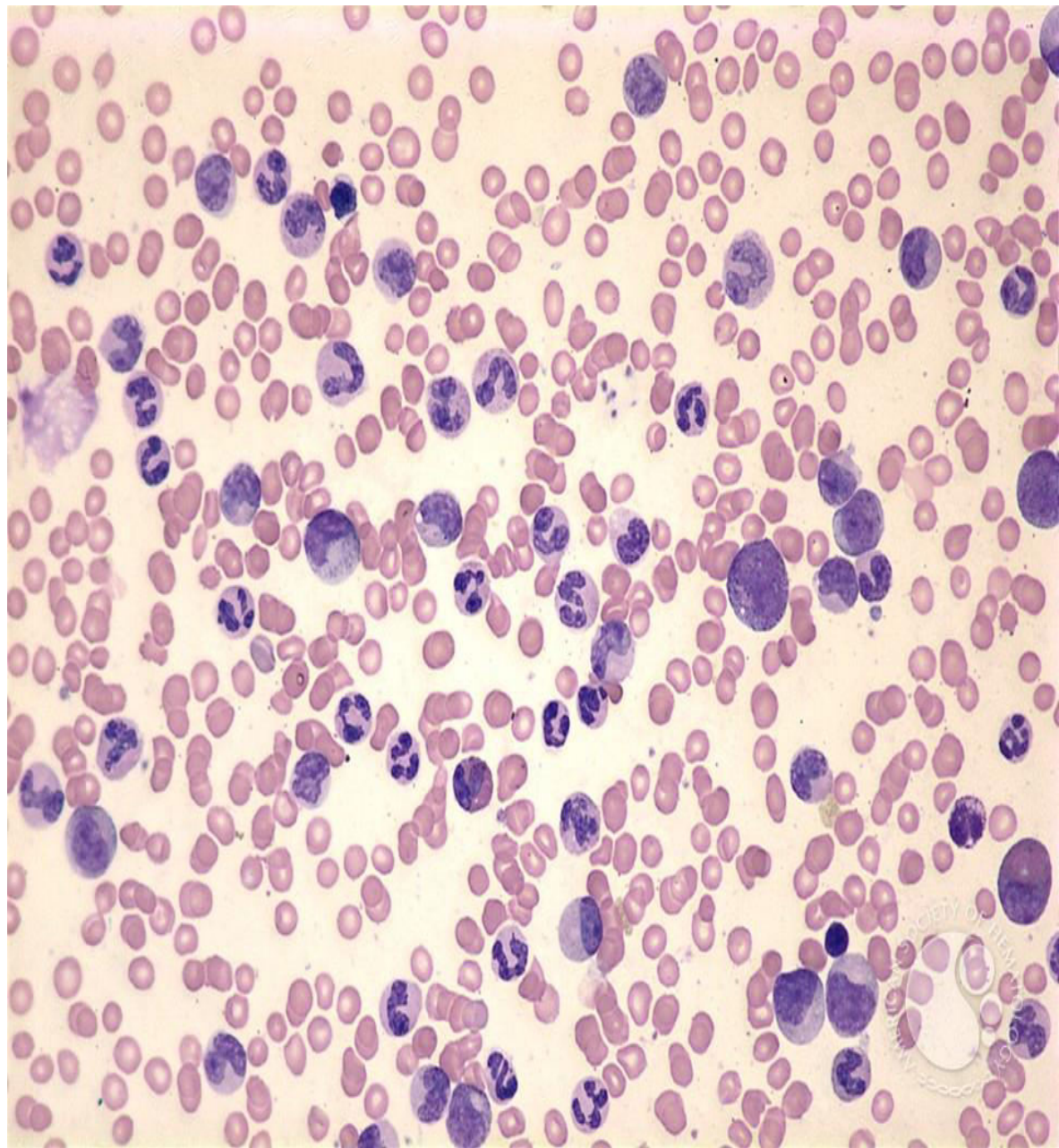
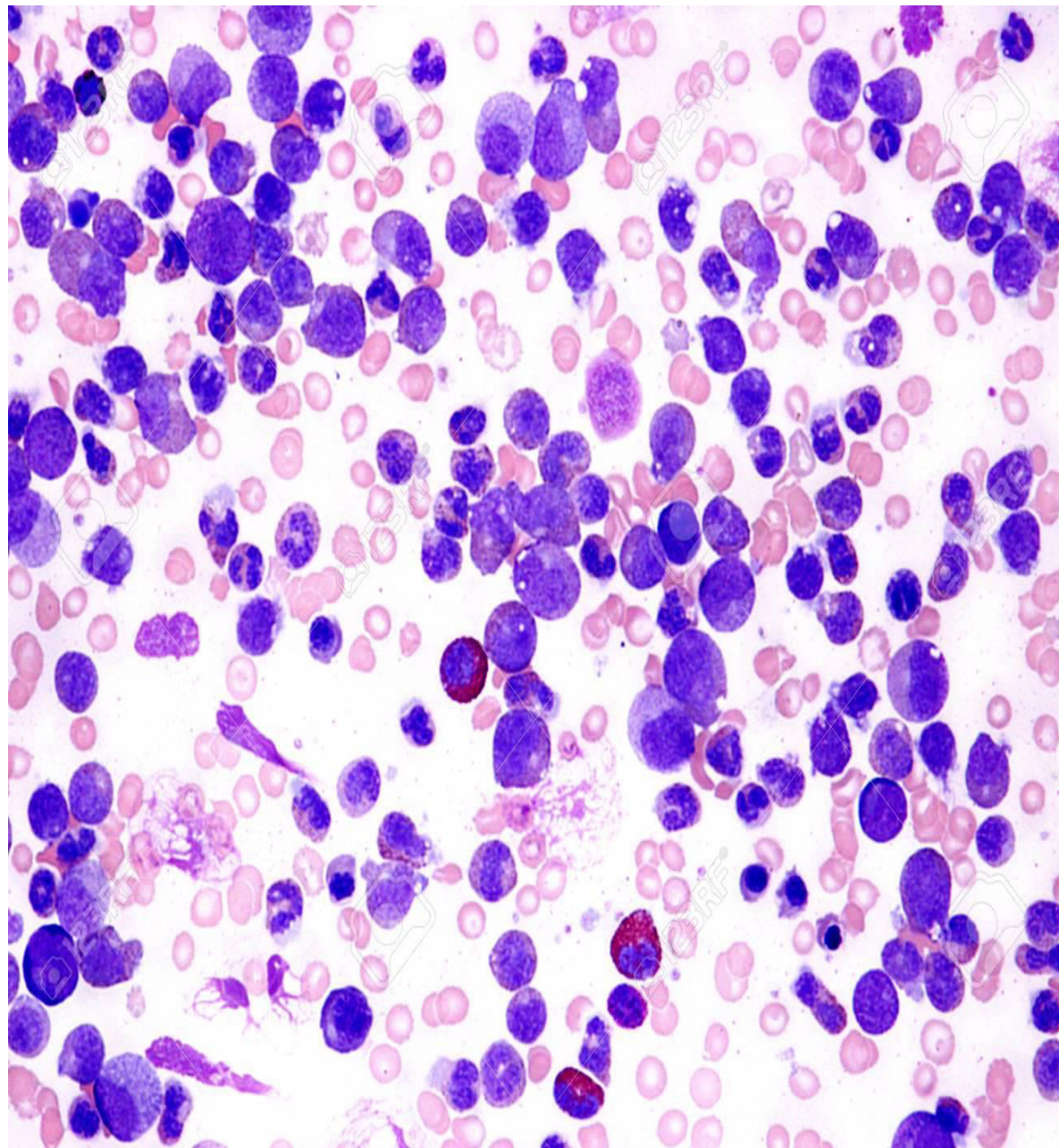
Lymphocytes



Monocytes







Automated Method



- You should know, this method is used to determine **CBC (complete blood count)** tests
- What are CBC tests?
 - Total WBCs count
 - Differential WBCs count
 - Total RBCs count
 - PCV value or Hematocrit (HCT)
 - Hemoglobin concentration (Hb)
 - Total platelets count

Hematology Analyzer

Automated method

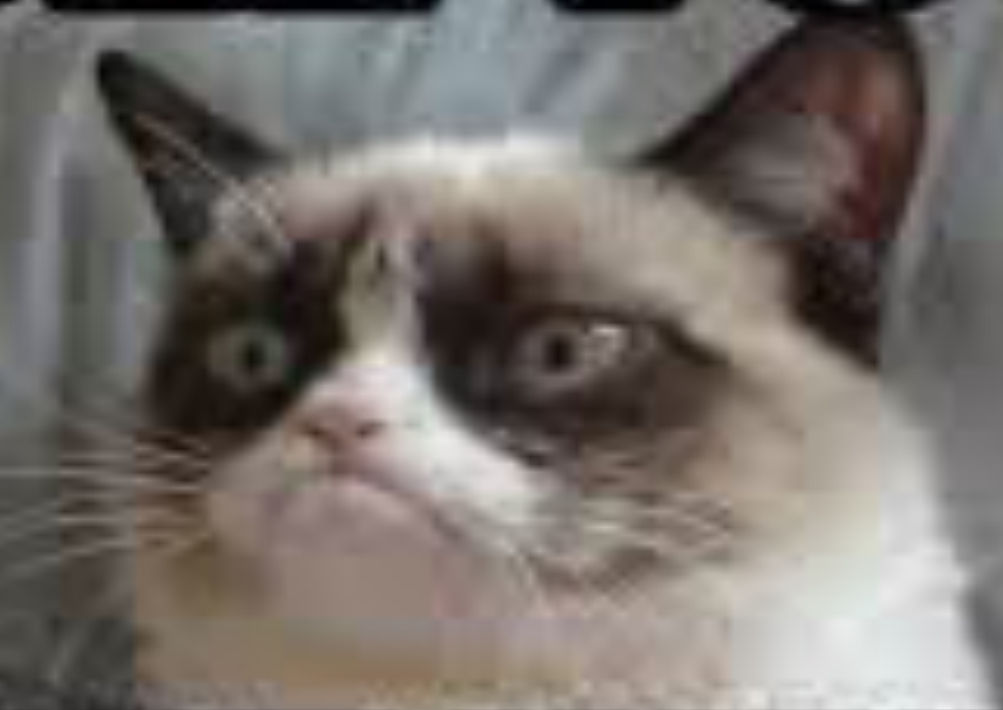
ADVANTAGES:

- Speed with efficient handling large number of samples
- Accurate and precise in quantitative blood tests
- Ability to perform multiple test on a single platform
- Reduction of labor requirement
- Process human and animals blood samples

DISADVANTAGES:

- Flagging of the laboratory test results demands intensive manual examination of the blood smear
- Red blood cells morphology cannot be determined
- Platelet clumps are counted as single (false low results)
- False increased or decreased results due to interfering factors
- Expensive

SEE YOU



NEXT WEEK!