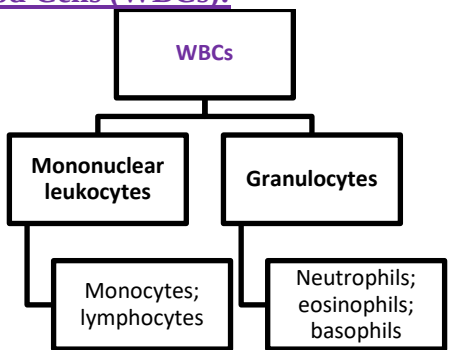
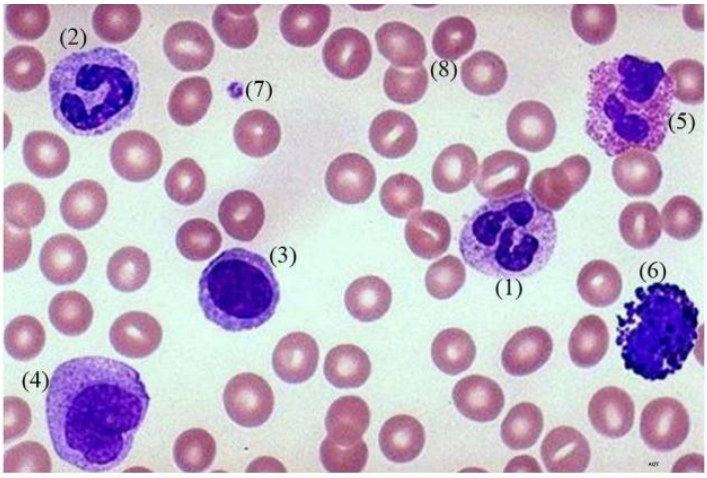
Unit I – Problem 6,7 – Microbiology Lab: Defenders

- **Identification of White Blood Cells (WBCs):**





(1) Neutrophil - (2) Neutrophil (rarely seen band cell)- (3) Lymphocyte – (4) Monocyte - (5) Eosinophil – (6) Basophil – (7) platele – (8) Red blood cells (RBC)

- **Normal values:**

Average number of leukocytes in blood	5000-9000 cells/mm^3
Leukocytosis	> 12,000 cells/mm^3
Leucopenia	< 5000 cells/mm^3

- Leukocytes are always found in sites of "potential infection" in tissues and organs where they are needed to fight against different organisms which can enter our body and threaten us.
- **Neutrophils and monocytes are also known as phagocytes**. They ingest foreign particles, bacteria and degenerating cells. Notice that when monocytes reach the infected tissue they are converted to macrophages (monocytes are only present in the blood stream).
- **Lymphocytes aid in the formation of antibodies** (especially B-lymphocytes which are converted to plasma cells that will start producing immunoglobulins).

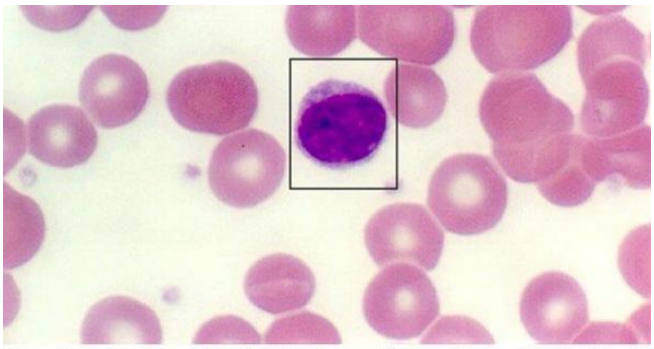
Cell type	Size (µm)	Number (mm^3)	Function
Neutrophil	12-15	300-700	Phagocytosis of cellular debris, bacteria, fungi, viruses… etc
Eosinophils	12-15	120-400	Phagocytosis (antigen-antibody complexes), anti-parasite agents
Basophils	12-15	30-100	Immediate hypersensitivity reaction

- **How to identify WBCs?**
  - ✓ Thin blood smear is prepared → air-dry → fixed with methanol → stained with Romanovsky stain (notice that Giemsa stain is also used with sampe characteristics appearing on cells):

- ❖ Fixative solution (thyazine dye in methanol) for 30 minutes.
- ❖ Transfer without drying to Eosin-Y in phosphate buffer for 15-30 seconds by immersing and withdrawing the slide in solution several times.
- ❖ Transfer without drying to Methylene blue in phosphate buffer for 15-30 seconds by immersing and withdrawing the slide in solution several times.
- ❖ Rinse slide briefly in water and allow to dry.
- ❖ Examine under the microscope.

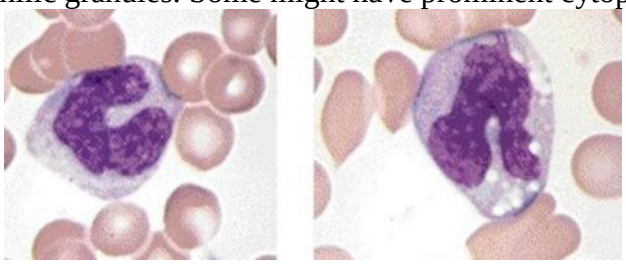
- **Mononuclear leukocytes:**
  - **Lymphocytes:**

Small lymphocytes (most common)	Large, dense, round nucleus and thin basophilic cytoplasm and are capable of ameboid movement and production of antibodies.
Large lymphocytes	Indented nucleus, abundant cytoplasm and azurophilic granules might be detected.

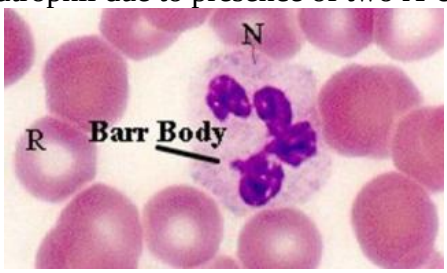


Lymphocytes are only slightly larger than red blood cells (small lymphocytes) and they have a relatively large nucleus:cytoplasm ratio. Note that the lymphocyte in the above photo has only a thin rim of light purple cytoplasm around the dense nucleus.

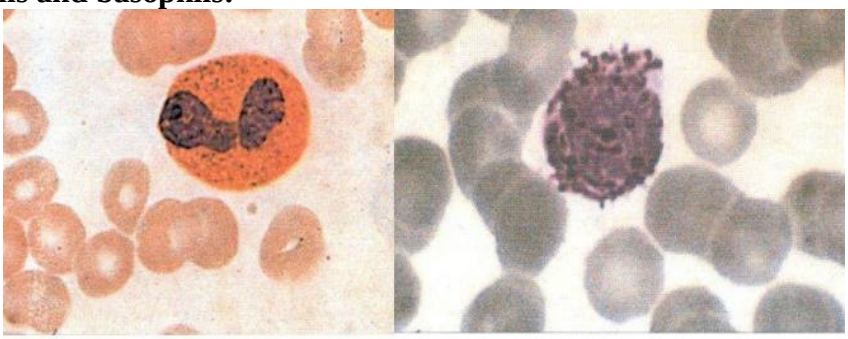
  - **Monocytes:**
    - ✓ Largest white cells found in normal blood. Nucleus is folded with moderately fine chromatin pattern. Cytoplasm has grey "ground glass' appearance with fine azurophilic granules. Some might have prominent cytoplasmic vacuoles.



- **Granulocytes:**
  - **Neutrophils:**
    - ✓ Typical nuclear lobe separation by fine filaments (up to 4 lobes). Bar body is typical of a female neutrophil due to presence of two X-chromosomes.



- **Eosinophils and basophils:**



Morphology of the Eosinophil
The bilobed nucleus and eosinophilic (red-orange) granules in the cytoplasm

Morphology of the Basophil
This blood smear shows a typical basophil with its deep violet-blue granules

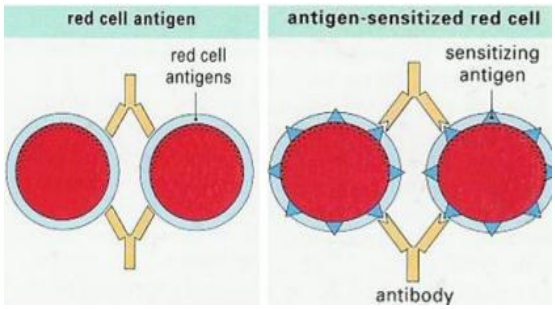
  - ✓ A basophil is characterized by lobed nucleus and it is filled with large blue-black granules that sometimes cover the nucleus.

- **Laboratory methods for the detection of antigens/antibodies:**

- **Agglutination:**
  - ✓ A reaction of cellular antigen with an antibody which results in clumping of antigen particles.
  - ✓ Examples: clumping of Red Blood Cells (RBCs) in presence of antibody. The antibody binds multiple particles and joins them creating a large complex.

The blood group is A+. Photo of bedside blood grouping card showing agglutination of blood with anti-A and anti-Rh(D), but not with anti-B.

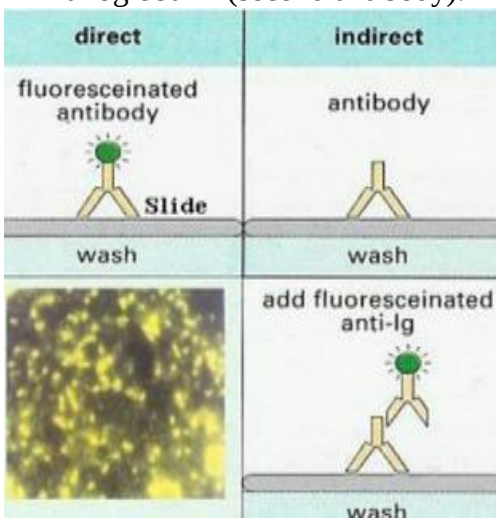
  - ✓ Direct agglutination: a cell or insoluble particulate antigen is agglutinated directly by antibody. Example: agglutination of group (A) RBC by anti-A sera.



  - ✓ Indirect agglutination: agglutination of antigen coated to inert particles which are passive carriers of otherwise soluble antigens. There are two types:
    - ❖ *Agglutination of antigen-coated erythrocytes. It is called hemagglutination test.*
    - ❖ *Agglutination of antigen-coated latex beads. It is called indirect agglutination test.*

- **Immunofluorescence:**
  - ✓ Detection of antigens by using fluorescent-labelled antibodies followed by observation of the specimen under fluorescent (UV) microscope.
  - ✓ This method is generally qualitative.
  - ✓ Direct immunofluorescence: a drop of known fluorescenitaed antibody is applied to the smear, incubated and washed-off. Any bound antibody is then revealed under the microscope.

- ✓ Indirect immunofluorescence: patient's serum (unknown) is applied to a smear containing relevant antigen and visualized following the addition of fluoresceinated anti-immunoglobulin (second antibody).



- **Enzyme-Linked Immunosorbent Assay (ELISA):**
  - ✓ Highly sensitive, quantitative method for the detection of both soluble antigen and antibody.
  - ✓ The method relies on the use of antibodies to which an enzyme has been attached (as a method to detect the antibody).