Microscopic view of stained and non-stained blood

It is often profitable to observe blood in its living state, free from the artifacts resulting from drying, fixation, and staining. White cell motility and phagocytosis is best observed in wet preparations. Inclusions are easily seen with polarised light or phasemicroscopy, and abnormal poikilocytic forms (elliptocytosis, stomatocytosis, macrocytosis, microcytosis, etc.) can be evaluated. A drop of blood placed between a glass microscope slide and a coverglass permits observation of single cells, with or without staining. Dilution of the blood with physiological saline facilitates dispersion of the cells for observation or photography. In addition, smears can be made by a variety of staining methods. The essential step involves formation of the single layer of cells with a minimum of distortion or artifact introduction.

- **Coverglass preparation.** The blood can be spread between two coverglasses. When the glasses are pulled apart, a thin layer of blood is left on each. This way only a small droplet is required for two coverglasses.

- **Wedge smears.** A drop of blood placed on a glass slide can be spread by using a second slide held at an acute angle. The drop is placed at one end of the slide and a second “spreader” slide is brought into contact with the drop and moved along the surface of the first slide in a smooth motion. The goal is to achieve a wedge-shaped smear with a thin, feathery edge. Observations of cell morphology are optimal adjacent to the edge.

Procedure of panoptic Pappenheim staining (with May-Grunwald and Giemsa stain)

Wedge-shaped thin blood smears can be stained on horizontal racks. Smears are slowly air dried or dipped in methyl alcohol. 20-30 drops of May-Grunwald stain (containing acidic eosin, methylene blue, methyl alcohol and glycerol) are added to the slide covering the surface. In alkaline pH, methylene blue has an affinity for the acidic components of the nucleus and cytoplasm (RNA, DNA). Acidic derivatives of eosin combine with basic elements of the cell such as haemoglobin or eosinophilic granules. The dye also contains neutral components that stain other cell structure. Methyl alcohol serves as a fixative for the cell blood. The staining time is about 3 minutes. After 3 minutes equal volume of distilled water is added the stain and left for another 2 minutes. All the fluid is removed from the glass and Giemsa stain (containing azure II, eosin, methyl alcohol and glycerol) diluted in equal volume of distilled water should be added and left on the slide for 20 minutes. After 20 minutes, stain has to be washed off the slide by a gentle rinse with distilled water. The back of the slide is wiped with paper towel to remove excess stain before the slide dries. After drying, the slide can be examined directly under or without the coverglass with mounting medium (cedar wood oil) in high power microscopic field.

Microscopically, a properly stained smear appears pinkish-grey. Red blood cells stain pink to orange, eosinophil granules stain a bright orange, neutrophil cytoplasm stains a light pink, and lymphocyte cytoplasm a light to medium blue. The nuclei of granulocytes and lymphocytes stain dark blue to purple. Improperly stained slides usually result from buffers that are overly acidic (too pink) or overly basic (too blue).
Peripheral blood differential count (Schilling or Romanovsky stain)

Leukocytes are identified and enumerated from their morphological appearance on stained blood smears or by automated instruments that use a combination of optical characteristics and histo-chemical reactions. Blood smears are most often stained with a modification of one of methylene-based polychrome dyes (Wright's stain, Giemsa stain, Pappenheim stain, etc.).

The slide is examined with the high dry objective (40-45X) to observe cell distribution, red cell morphology, and to screen for infrequent, abnormal cells that might be missed during the differential count. A drop of oil is added to the slide and the oil immersion lens (95-100X) carefully lowered into focus. A consistent search pattern (meander pattern) should be used that permits examination of a representative portion of the optical area of the smear, but without counting the same area twice. Leukocyte counts are reported as a percentage or/and as an absolute cell count:

<table>
<thead>
<tr>
<th>Leukocytes</th>
<th>Table for differential count</th>
<th>Per 100 cells</th>
<th>Average values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Basophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myelocyte</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metamyelocyte</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Band</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Segmented</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Absolute cell count/µL = total WBC/µL x % of the cell on the differential

Search pattern and area for differential counts on a blood smears.
Evaluation of WBC Count with Bürker hemacytometer (chamber).

**Elements:**

1. Bürker (or Thoma) hemacytometer contains a net, which forms squares (dimension of the side $1/5 \text{ mm}$ and the surface of the square is $1/25 \text{mm}^2$). The height of the chamber after covering with a cover glass is $0.1 \text{ mm}$. It means that the volume of the single cuboid is $1/5 \text{mm} \times 1/5 \text{mm} \times 1/10 \text{ mm} = 1/250 \text{ mm}^3$. Cover glass should be stuck to the haemacytometer to form colour Newtonian rings.

2. Türk solution (3.0 mL of acetic acid, 1mL of gentian violet and distilled water up to 100mL). The solution should be used to dilute blood (ratio 1vol. of blood/10 or 20 vol. of Türk solution). Acetic acid haemolyses erythrocytes. Violet dye stains leukocytes.

**Procedure:**

1. Dilute; stain; haemolyse blood with Türk solution.
2. Put a cover glass on the hemacytometer to form a chamber which volume is $1/250 \text{ mm}^3$.
3. Inject some prepared blood into the chamber.
4. Count WBC in 100 to 200 cuboids. Do not count cells that “touch” the lower and left side of the cuboid.
5. Count the average value of leukocytes (AV.) per one cuboid.
6. Estimate WBC count by multiplication of average value of leukocytes per cuboid, rate of dilution in Türk solution and inverse of the cuboid volume to obtain the number of leukocytes per volume of $1 \text{ mm}^3 (\mu\text{L})$.

\[
\text{WBC count (per 1\muL)} = \text{Number per cuboid} \times \text{rate of dilution} \times 250
\]

Reference values of WBC 4 – 10 thousand/$\mu\text{L}$. 