Determination of the number of leukocytes in peripheral blood

The purpose of this exercise is to determine the number of leukocytes in liter of patient's peripheral blood. Leukocytes are a heterogeneous group of cells involved in immune processes. Determination of the total number of leukocytes is an essential diagnostic test that provides important information about the state of the immune system. Physiological values of leukocytes are approximately 5-10 x 10^9/L. Variations in the total number of white blood cells are called: leukopenia (reduction in the number of leukocytes), and leukocytosis (increased white blood cell count) . Deviations from the normal number may be due to an increase or decrease in their production, or increased destruction.

Although today the determination of the number of leukocytes is done by flow cytometry, still, in small and / or underprivileged laboratories this test is done "manually ". This is performed by using Türk solution: a dyed hypotonic solution, in which blood cells swell and burst , but leukocyte nuclei, which are more osmotic resistant - remain intact . Nuclei are stained with Gentian violet dye (from Türk solution), and are easier to perceive during microscopy .

In parallel with the determination of the total number of leukocytes , it is important to determine the prevalence of specific types of leukocytes in peripheral blood , which is done by test called ***Differential blood count***.

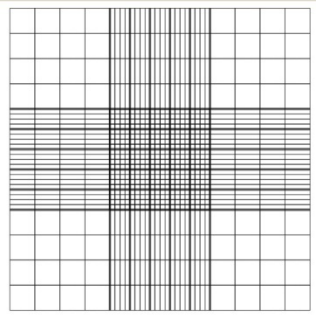
Procedure:

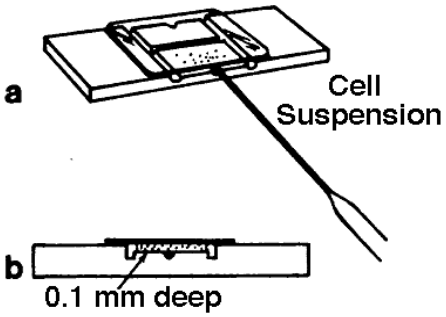
Before you start working with patient (subject) , it is necessary to prepare all the materials that will be used during this exercise :

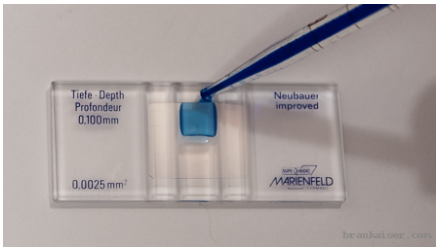
a) put on gloves
b) pipette 90 µl of Türk solution in marked tube
c) take a piece of parafilm , pipettes , pipette tips , rack for tubes
d) take cotton balls , ethanol and lancets
e) prepare hemocytometer chamber

Hemocytometer chamber by Bürker - Türk is a thick glass with precisely carved grid lines at the center, which form a square (or a net), with exact dimensions. Square surface measures 9mm^2 . The central part of the chamber, with gridlines, is separated from lateral columns by grooves. The central part is indented in comparison to columns, for 1/10 mm. Chamber is prepared by attaching a coverslip to columns. Chamber and coverslip should be clean and dry . Slightly moist the edges of the cover slip with water and position it on the columns of the chamber. Slide the cover slip up and down, while slightly pressing it with two fingers, until it is firmly in place (Newton circles are a sign of good attachment) .

3 mm x 3 mm

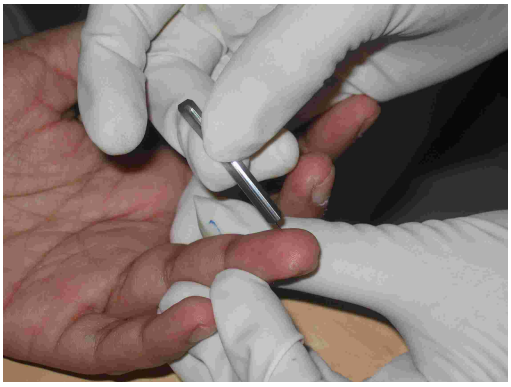






Working with the patient:

While working with biologically hazardous materials , such as blood , we must take care to protect the health of patients, as well as the medical staff . The person who prepares such samples must wear protective medical gloves, and nose/mouth mask and goggles, if needed . Part of the skin used for taking a blood sample should be properly disinfected , and only sterile, disposable materials should be used for this procedure . All waste /used materials must be disposed in properly labeled buckets for hazardous materials.



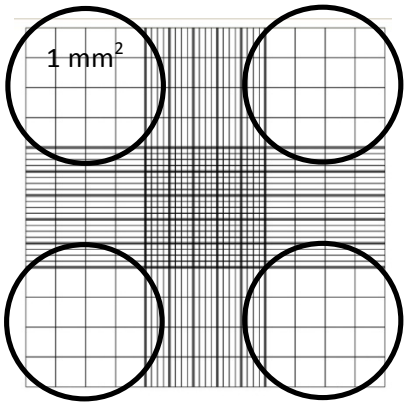
Before taking a blood sample from a subjects fingertip, disinfected the skin by wiping it with cotton ball, soaked in 70 % ethanol . Wait for the ethanol to evaporate . Use sterile lancet to make a tiny laceration on the skin; make sure to make a laceration on the soft part of the fingertip, not the bone . The motion should be precise and strong, so the depth of a wound will be approximately 2-3 mm . By pressing the finger tip form a drop of blood . Using a pipette with a sterile pipette tip, transfer the blood from the fingertip to a piece of Parafilm (a plastic foil) . When you have collected a sufficient amount of blood (approximately 30 µl) , take a new piece of cotton soaked in ethanol and wipe off the finger tip. With this, blood sampling is completed . Beware that the blood is not left for more than 1-2 minutes on parafilm , otherwise it will dry/coagulate and become useless for the test .

Previously, we measured 90 µl of Türk solution into a marked tube. Now take 10 µl of blood from parafilm and transferred it into solution in a tube. By pipetting up and down, rinse the remainings of blood from a pipette tip into Türk solution . Close the tube well and tap the bottom of the tube to mix the content for 1 minute . Then leave the tube aside , so the lyses of blood cells could proceed unhindered .

After 10 minutes , fill a hemocytometer chamber with prepared mixture of blood and Türk solution and count leukocyte nuclei by microscoping angular squares of the chamber net

(magnification: 10 x 10 = 100)

Positions for leukocyte counting in the chamber :



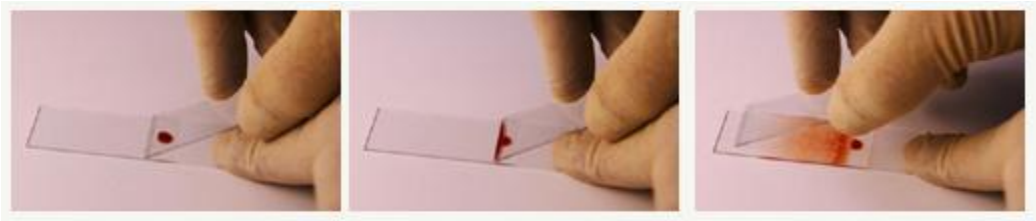
The Differential blood count

A test used for determining a proportion of different types of leukocytes in peripheral blood is called Differential blood count . This test is always done in parallel to determination of a total number of leukocytes , because only this way it will provide relevant information about the state of the immune system of an individual .

Test is based on microscopy of a thin, dyed smears of peripheral blood prepared on a glass slide, and distinguishing types of leukocytes on the basis of their morphological characteristics. When a total of 100 leukocyte cell is differentiated and counted, a numeric share of certain types of leukocytes is calculated: neutrophil granulocytes , lymphocytes , monocytes , eosinophil and basophil granulocytes .

Material preparation:

To prepare a thin smear of peripheral blood on a slide, we need approximately 10-15 µl of blood . Blood should be placed on a slide with pipette, near one of the ends of the slides. With another glass slide , spread the blood with continuous, rapid movement , while firming the slide in place with the other hand (see picture) .



Leave the smear to air-dry for at least 1 hour. Dried smear should be marked (the name of the patient) with graphite pencil and dyed using method by Papenheim (May Grunwald - Giemsa; MGG). After completion of staining, blood smear is again air dried, after which it is ready for microscopy.