**LAB 3**

**WHITE BLOOD CELL COUNT, RBC count and platelets counts**

**Hemacytometer Counting Areas**

❖ Hemacytometer has 2 identical ruled counting areas, four corner squares and the center square

❖ The central square is further subdivided into 25 smaller squares and each smaller square is further subdivided into 12 smaller squares

❖ Each corner square is further subdivided into 12 smaller squares

❖ Platelets and RBCs are counted in the entire large center squares

❖ WBC cells are counted in the four corner squares.





**Using The Hemacytometer**

Clean cover slip slide (especially made for the hemacytometer).

Position a clean, dust free, coverslip so it covers the ruled counting areas of a clean hemacytometer.

Fill the hemacytometer with the fluid containing

cells to be counted, by touching the tip of the capillary tube or micropipette tip to the point where the coverslip and raised platform meet on one side, the fluid will drawn under the coverslip and over the counting area by capillary action, this requires about 10 μl.

The chamber must not be overfilled or underfilled, if accurate results are needed!.

Place the hemacytometer on the microscope stage, so one of the ruled counting areas is aligned directly above the light source (condenser); rotate the low power objective (x10) into place and adjust your vision.

**The Counting Pattern**

Either left to right or right to left counting pattern can be used but with the insurance that each cell is counted only once, to accomplish this, cells that touch the right boundary lines or the bottom boundary lines are not counted, because they will be counted with the other squares.

 

**White blood cell (WBC) count**

It is the count of the actual number of white blood cells per volume of blood.

**Preparation solution (*Turkey's solution*):**

3% glacial acetic acid (Weak acids will lyse red blood cells, and will darken WBC‘s to facilitate counting by the hemacytometer.)

**Note:** *1 ml of Leishman stain can be added to the diluent to color the white blood cells, thus counting will be easier.*

**Dilution factor:**

1: 20 (i.e. 380 μl + 20 μl)

**Note:** *Use a piece of cotton to wipe the outside of the pipette before inserting in the tube*

**Method**

Mix the blood sample gently but thoroughly by inversion before preparation

Pipette (380 μl) of diluting fluid into a tube.

Pipette (20 μl) of well mixed blood and wipe the tip with cotton piece

Put blood into the tube containing diluting fluid and mix well with the tip.

Let the tube stand for 2-3 minutes to ensure complete RBC lyses, then mix well.

Prepare the clean hemacytometer and cover it with the designed cover slip.

Load one side of the hemacytometer with the aid of a micropipette

Allow the hemacytometer to sit for several minutes to allow the WBC‘s to settle in the counting chamber, to avoid drying effect, place the loaded hemacytometer in a covered Petri dish with a moist gauze, until counting.

Place the hemacytometer in the microscope stage.

The WBC‘s are counted in the four corner large squares

**Note:** *If the number of cells in a square varies from any other square by more than 9 cells, the count must be repeated, because this represents an uneven distribution of cells, which is may be caused by improper mixing of the dilution or improperly filled hemacytometer.*

**Sources of Error:**

❖ Contaminated diluting fluid

❖ Incorrect dilution

❖ Uneven distribution of WBC‘s

❖ Presence of clumped WBC‘s

❖ Unclean hemacytometer or cover slips

❖ Presence of air bubbles

❖ Incompletely filled hemacytometer

❖ Over flow

❖ Drying of the dilution in the hemacytometer

**Calculations**

Total WBC Count / cumm = no. of WBCs in 4 squares x 50

**Results:**

 High leucocytes count is known as ***leucocytosis***

Low leucocytes count is known as ***leucopenia***

**Red blood cell (RBC) count**

It is the count of the actual number of red blood cells per volume of blood.

**Preparation solution:**

Isotonic saline (0.85% sodium chloride (NaCl) in distilled water)

**Note:** *saline cause slight crenation of RBCs and allow rouleaux formation*

**Dilution factor:**

1:200

**Calculations**

Total RBCs Count / cmm = no. of RBCs in 5 squares x 10,000

**Results:**

High erythrocytes count (and hemoglobin) is known as ***polycythemia***

Low erythrocytes count (an d hemoglobin) is found in cases of ***anemia***

**Hemoglobin concentration**

It measures the amount of oxygen-carrying protein in the blood.

**Reagent**

***Drabkin’s solution contains the following:-***

*1. Potassium Ferricyanide*

*2. Potassium Cyanide.*

*3. Non- ionic Detergent* Triton X-100

*4. Dihydrogen Potassium Phosphate.*

***Working Drabkin’s Solution Preparation:***

Ready made commercial Drabkin‘s reagent is diluted with distilled water in the proportion 1:10.

**Principle**

Well mixed EDTA anticoagulated blood is diluted in Drabkin‘s solution; non-ionic detergent will lyse the red cells to liberate hemoglobin, and to decrease the turbidity caused by red cell membrane fragments by dissolving them.

Then, hemoglobin is oxidized and converted to methemoglobin (Hi) by potassium ferricyanide, this step is accelerated by the dihydrogen potassium phosphate, and requires approximately 3 minutes for total conversion. Potassium cyanide will provide cyanide ions to form cyanomethemoglobin (HiCN), which have a broad spectrum of absorption at 540 nm.

**Method**

5 ml Drabkin‘s solution + 20 ul whole blood

Mix well and incubate for 5 min at room temperature then read absorbance against reagent blank at **540 nm**

**Calculations**

Read hemoglobin concentration directly from the hemoglobin standard curve.

**The platelet count**

Platelets are the smallest formed elements in the blood, normally ranging in size from 2-4 microns. They are actually fragments of cytoplasm that have broken off platelet precursors. Platelets function in the coagulation of blood.

**Preparation solution:**

3.8 % ammonium oxalate

**Dilution factor:**

1:20

**Method**

Carefully charge hemacytometer with diluted blood by gently squeezing sides of reservoir until properly filled.

Place the hemacytometer in a moist chamber, e.g., covered Petri dish, bottom lined with moistened gauze squares, and allow to stand for 10 minutes for cells to settle.

Using bright light or phase microscope, place hemacytometer on microscope stage. With low power objective, bring ruled area into focus.

Switch to 40X objective, locate large center square, and bring into focus.

Count platelets in all 5 small squares within the large, center square.

**Platelets are difficult to count due to:**

small size - often hard to differentiate from bacteria and debris

adhesiveness - affinity for adhering to glass

aggregation - tendency to clump together

**Sources of Error:**

Platelets count should be carried out within 2h of blood collection , delay cause clumbing and disintegration of platelets

Specimens stored for more than 24 hours allow aggregation of platelets

Light adjustment is critical. If the condenser is not lowered it will fade out the platelets.

Bacteria and debris can be misinterpreted as platelets. This type of artifact is generally much more retractile than platelets.

**Calculations**

Total platelets Count / cumm = no. of platelets in 5 squares x 1,000

**Results:**

High platelets count is known as ***thrombocytosis***

Low platelets count is known as ***thrombocytopenia***