RBC Indices

MCV Mean Corpuscular Volume	MCH Mean Corpuscular Hemoglobin	MCHC Mean Corpuscular Hemoglobin Concentration
The volume of one Erythrocyte	Amount of hemoglobin in one Erythrocyte	How much of the volume in one erythrocyte is occupied by hemoglobin.
Normally 80-100 femtoliterfl	Normally 27-32 Picogram PG	Normally 30-38%
= PCV/RBC X 10	= Hb/RBC X 10	=Hb/PCV X 100
Terminology: MCV 80-100: -Normocyte -Normocytic Anemia, like active blood loss MCV > 100 -Macrocyte -Macrocytic Anemia, like in B12 deficiency. MCV < 80 -Microcyte. -Microcytic Anemia, like Iron deficiency anemia.	**Terminology:** MCH 27-30 -Normochromic -Normochromic Anemia, like active blood loss. MCH>32 -Hyperchromic - No true hyperchromic seen in B12 deficiency. MCH < 27 -Hypochromic -Hypochromic Anemia, like Iron deficiency anemia.	**Terminology:** MCHC 30-38% -Normochromic -Normochromic Anemia, like active blood loss. MCHC >38% -Hyperchromic - No true hyperchromic seen in B12 deficiency. MCHC < 30% -Hypochromic -Hypochromic Anemia, like Iron deficiency anemia.

Example: Blood sample, PCV = 45%, RBC Count: 5000000, Hb:15 gm.

Calculate MCV, MCH, MCHC.

Answer:

1- MCV = (PCV/RBC) X 10 = 45/5 X 10 = 90 FL

2- MCH = (Hb/RBC) X 10 = 15/5 X 10 =30 PG

3- MCHC = (Hb/PCV) X 100 = 15/45 X 100 = 30%

Differential White Blood Cells Count

- **Differential white cell count:** is a test that consists of an examination of stained blood film under microscope to determine the percentage and the number of different types of white blood cells.

- There are 5 main types of white blood cells, subdivided into two main groups:

1. **Polymorphonucleurleukocyte (granulocyte):**

- **The granulocytes** = 70% of all WBCs

They are characterized by:

- Irregular segmented nucleus.
- Specific granules (specific size, staining affinity).
- The granules are subdivided into 3 types: Neutrophils, Eosinophils, Basophils.

- **2.Mono-nucleur leukocyte (Agranulocyte) :**

They are characterized by:

- Regular nuclei (round or kidney-shaped)
- Nonspecific granules.
- The Agranulocytes are subdivided into 2 types: Monocyte and Lymphocyte.

Principles of this procedure:

A drop of blood is spread over a glass slide, then we let it dry, and stained with special quick stain (Wright-Giemsa stain), and viewed under microscope.

Differential Count Procedure

We do it in 4 steps:
1. Making blood smear.
2. Shaking the blood smear
3.Counting the cells
4.Reporting the count "cell identification"

Making Blood Smear

-Blood Smear: is spreading of the blood on a glass slide to be thin layer of blood.

The perfect slide consists of a smear that is exactly "one cell thick in the feathered edge" when viewed microscopically.

To make a good blood smear, follow these steps:

1. Select two glass slides that are clean and free of clipped edge.
2. Place a drop of blood 1-2mm in diameter on one of the slides, the drop should be in the center line, approximately ¼ inch from the frosted edge of the slide.
3. Make the smear immediately after you have applied the drop of blood.
4. Rest the left end of the spreader slide at a 45 degree angle, just in front of the blood drop.
5. Keep the spreader slide at 45 degree angle. Push the spreader slide rapidly across the stationary slide with one even stroke and pressure.

Notes:

1. Any pressure exerted on the spreader should be directed across the slide in the direction that the film is made rather than down on the stationary slide.
2. The faster the spreader slide is moved, the longer and thinner the film will be. The slower the spreader slide is moved, the shorter and thicker the film will be.
3. The Angle will also vary the result,
   - If angle > 45 degree, the smear will be thicker.
   - If angle < 45 degree, the smear will be thinner.

So, SPEED, ANGLE, and DROP SIZE can be varied slightly to produce good smear.

Finally, allow the slide to air dry and Fix the slide with Methanol.

Staining of the Cells:

- Staining the blood smear highlights the differences among the different types of leukocytes, for easier recognition during the counting process.
- The most popular stain used >>**Wright's Stain**.
- The Wright's Stain is a methyl alcohol "Methanol Solution" of:
  Acid dye: Known as **Eosin - Red color.**
  Basic dye: known as **Methylene Blue - Blue color.**

Generally, White blood cells are identified by their affinity to the dye.

To stain a blood smear with Wright's Stain, follow the steps below:

1. Prepare two staining containers by filling one with the stain solution and the other with deionized water.
2. Immerse the slide in the stain for (15-30) seconds.
3. Remove the slide and allow excess stain to drain from the edge of the slide.

4. Immerse the slide in the deionized or distilled water for (5-15) seconds. "Change water when it becomes dark blue"
5. Drain excess water and wipe the back of the slides to reduce background color.
6. Place slide in horizontal position on table and allow to air dry.
7. When slide is dry, proceed counting the cells.

Counting the cells:

1. Once the blood smear has been stained, place slide under the microscope and focus the thin area under a low power lens.
2. Place a large drop of immersion oil on the thin area of blood smear.
3. Switch the oil immersion objective 100X into position above thin area covered with oil drop, then see the cell and count 100 consecutive white cell, pressing the correct key on the cell counter for each type of white cell identified.

Relative count of certain cell% = number of cell counted/100 X 100%

Cell Identification

1. **Erythrocyte**
   - Biconcave discs without nucleus.
   - They are 5,000,000 RBC/cubic mm
   - About one third of the volume of each cell's hemoglobin.
2. **Platelets**
   - Small fragments of cell.
   - They are 200,000-400,000 platelets/cubic mm.
3. **Neutrophils**
   - The largest one of WBC's in number "40-60%"
   - Larger than RBCs, and their nuclei has 3-5 lobes
   - They are very active phagocytotically.
   - Their cytoplasmic granules stain with neutral dye.
   - Increase in their number means **bacterial infection.**

4. **Eosinophils:**
   - Their cytoplasmic granules stain with acidic dye, Red-Orange in color.
   - Their granules are larger than Neutrophils granules.
   - They represent 5% of WBCs.
   - They are same size of Neutrophils but have 2-3 lobes.
   - Increase in Eisonophills number means **1-Allergy2-Parasitic Infections.**

5. **Basophils:**
   - It is the least numerous in number, about 1% of WBCs.
   - Their granules stain with basic dye, black to blue in color.
   - The nuclei have 1-2 lobes.
   - Their granules contain:
     1.Histamine >> Vasodilator.
     2.Heparin>> Anticoagulant.

6. **Lymphocytes:**
   - Most abundant of the Agranulocytes, 20-40% of WBCs, 2500 cell/cubic mm.
   - Round, Densely stained Nucleus, which occupies most of the volume of cell.
   - They are the key cells of the immune system.
   - They are subdivided into B and T lymphocytes.
   - They are the smallest WBCs in size.

7. **Monocytes:**
   - Represent about 7% of WBCs, 300 cell/cubic mm
   - They are the largest WBC in size.
   - Kidney-Bean shaped Nucleus.
   - Monocytes move to tissue become macrophages and histiocytes.
   - Phagocytic activity.

Significance of the test

This procedure is useful in:

1. General Health Examination.
2. Help investigate a variety of illnesses including infections, allergies...etc.
3. Treatment monitoring

Leukocytosis is increase number of WBCs > it May result from bacterial or viral infection, Metabolic disorder, Chemical and drug poisoning and acute hemorrhage.

Leukopenia is decrease number of WBCs > it may result from typhoid infection, measles, infectious hepatitis, tuberculosis, cirrhosis of the liver.

Hematocrit

(Packed Cell Volume of Whole Blood)

Hematocrit:

Hematocrit is defined as percentage of erythrocytes to the whole volume of blood, and is usually expressed as a percentage of the volume of the whole blood sample (expressed as % (vol/vol)).

The Hematocrit may also be referred to as Packed Cell Volume (PVC).

Principle of the Test:

- The Hematocrit (PVC) is usually determined by spinning a blood-filled capillary tube in a Hematocrit centrifuge.

- Hematocrit is a common laboratory test can tell a physician a great deal about the **volume** of red cells in a blood sample.

- The volume of RBC's refers to the amount of space that the RBC's occupy within the blood. If whole blood is placed in a special **hematocrit** tube (a small test tube) and then spun very rapidly in a centrifuge, the heavier components will quickly settle to the bottom of the tube. When the centrifuge spins, the RBC's are forced to the bottom of the tube because they are the heaviest element in the blood. The WBC's and platelets are lighter so, they come to rest on top of the heavier RBC's in a layer called the **buffy coat**. Above the buffy coat rests the plasma.

Specimen:

- Venous blood anticoagulated with EDTA or capillary blood collected directly into haparinized capillary tubes can be used.

- Specimens should be centrifuged within 6 hours of collection.

Hemolyzed samples cannot be used for testing.

Procedure:

1- Draw well-mixed anticoagulated blood into two microhematocrit tubes by capillary action avoiding air bubbles. The tubes should be filled about 3/4 full.

2- Wipe off excess blood with a Kimwipe or gauze.

3- Seal one end of each tube with a small amount of clay material at 90 angles. Be sure the seal has a perfectly flat bottom.

4-Place the filled and sealed capillary tubes into the centrifuge. The sealed ends should point toward the outside of the centrifuge. The duplicate samples should be placed opposite each other in order to balance the centrifuge. Record the position number of each specimen.

5- Securely fasten the flat lid on top of the capillary tubes.

6- Centrifuge for 5 minutes at a set speed 11000 rpm. This separates the RBC's from plasma and leaves a band of buffy coat consisting of WBCs and platelets.

7- Allow the centrifuge to stop on its own. Do not use the hand brake.

8- After the centrifuge has stopped, open the top and remove the cover plate.

9- Promptly read the hematocrit on the hematocrit reader. (Instructor will review directions on using the hematocrit reader.) Do not include the buffy coat layer. (See illustration). If the buffy coat exceeds 2%, it should be recorded and noted as volume of backed WBC/plt.

Clinical Significance of the Test:

1- The PCV is an easily measure for detecting anemia or Polycethemia and can be useful in estimating changes in hemodilution or hemoconcentration.

2- The PCV is used together with the RBC's count, in calculating the mean cell volume (MCV), and together with hemoglobin content, in calculating the mean corpuscular hemoglobin concentration (MCHC).

- PCV is increased in:

- **Polycethemia**: increased RBC's count.

A. Pathological: bone marrow malignancy.

B. Physiological:

Age: (PCV is higher in infants).

Altitudes: (PCV is higher)

- PCV is decreased in:

- **Anemia:** RBC's number is decreased.

Reporting Results:

Normal values:

Newborn 53-65%

Adult male 42-52%

Adult female 37-47%

Blood Typing

- **Group A**individuals had an antigen, called A, on their red blood cells andantibodies to another antigen, called B, in their serum.

- **Group B** individuals had antigen B on their blood cells and antibodies to antigen A in their serum.

- Group O, had neither A or B on their red blood cells but had both anti-A and anti-B in their serum.

- Some time later, individual were described who had both A and B antigens on their red blood cells but no antibodies to A or B in their serum. This group was called AB.

Principle:

The test procedure based upon the agglutination (clumping) of red blood cell which carries a specific antigen in the presence of a corresponding specific antibody.

TO WHO CAN I DONATE BLOOD, AND EROM WHOM CAN I RECEIVE BLOOD?

	If you are:	You can donate to:	You can receive from:
Group	O	A, B, AB, O	O
	A	A, AB	O, A
	B	B, AB	O, B
	AB	AB	A, B, O, AB
Rh status	Rh +	Rh +	Rh +, Rh -
	Rh -	Rh +, Rh -	Rh -

Method:

1- Obtain a drop of blood from a finger prick in each of the circles of the disposable blood group slide.

2- Add one drop of anti A (blue) and a drop of anti B (yellow) and a drop of anti D sera in the proper circles on the disposable blood group slide.

3- Using clean (uncontaminated) glass rod to mix the blood with antiserum.

4- Tilt the slide from side occasionally, and after 2 minute read macroscopically for agglutination.

5-Any apparently negative tests should be read microscopically after 5 minute.

Report the result as follow:

- Agglutination (clumping) take place in the circle which has anti A, but not with that of anti B then Blood Group for this sample is A.

- Agglutination (clumping) takes place in the circle which has anti B, but not with that of anti A then Blood Group for this sample is B.

- Agglutination (clumping) takes place in the circle which has anti A and anti B then the Blood Group for this sample is AB.

- No agglutination takes place in both anti A and Anti B circles the Blood Group for this sample is O.

- For Rh if Agglutination (clumping) takes place in the anti D circle then this mean Rh positive while if not it mean Rh negative.

Hemoglobin Determination

- Hemoglobin, composed of:

1- Heme part, iron containing pigment, bind $4O_2$.

2- Protein part (Globulin), consist of four subunit (2α, 2β).

- Function of hemoglobin:

1- Main function is to carry O_2 from lungs to tissues, and CO_2 from tissue to lung.

2- Also serves as pH puffer in ECF.

- The formation of hemoglobin takes place in the developing red cells located in bone marrow.

- Hemoglobin in circulating blood is mixture of hemoglobin, Oxyhemoglobin, carboxyhemoglobin and other forms.

- 1 g of Hemoglobin---- carries 1.34 ml of CO_2.

- O_2 combining ability of blood is in direct proportion with Hemoglobin concentration, not the RBCs.

- **Hemoglobin determination is used to:**

1- Screen for anemia

2- Identify severity of anemia

3- Assist in evaluating the patient's response to anemia therapy.

- **Principles of hemoglobin determination:**

1- We used reagent solution called (*Drabkin's solution*) to determine Hemoglobin concentration.

2- This solution contain, *Potassium ferricyanid*, which convert the Ferrous ion (Fe2+) of hemoglobin to the ferric (Fe3+) by oxidation,--- to form *Methemoglobin*.

3- Methemoglobin (which is unstable) reacts with *cyanide ions* provided by*potassium cyanide* to form *Cyanmethemoglobin (HiCN)*.

4- The amount of Cyanmethemoglobin can be measured Spectrophotometrically at wavelength of 540 nm on spectrophotometer and compared to known hemoglobin standards in order to determine the Hemoglobin conc. of unknown sample.

- **Note:** All forms of circulating hemoglobin are readily converted to Cyanmethemoglobin, except for sulfhemoglobin, which is rarely present in significant amount.

- **Specimen:**

1- It can be obtained using capillary blood

2- EDTA- anticoagulated whole blood obtained by vein puncture is recommended.

3- Best result is obtained with blood drawn on the same day as testing.

- **Procedure:**

1- Add 5 ml of Drabkin's reagent to a test tube.

2- Add 20 blood to the test tube using Hb pipette or micropipette

3- Mix blood with Drabkin's and wait for 5 to 10 minute for the reaction to take place

4- Measure the absorbance of the sample against blank (Drabkin's reagent) at 540 nm by spectrophotometer.

- **Normal range of hemoglobin:**

1- Adult (Male) ---- (14-18) gm

2- Adult (Female) ---- (12-16) gm

3- Newborn ---- (17-19) gm

- **Physiological variation of Hemoglobin**, due to:

Age, Gender, Pregnancy and Altitude.

1- Pregnancy: gains fluid ---- diluting RBCs---- and thus decrease Hemoglobin.

2- Altitude:
   a- Higher altitude ---- increase Hb
   b- Lower altitude ---- decrease Hb

- Pathological variation of Hemoglobin:

1- Decrease Hemoglobin level, due to:

a- Conditions cause low RBCs, will decrease Hemoglobin

Ex. blood loss, bone marrow suppression

b- Patient with abnormal type of hemoglobin or hemogloinopatheis

c- Patients with normal RBCs, but low hemoglobin, example: Iron deficiency anemia.

2- Increase Hemoglobin level, due to:
In any condition that in which RBCs increase, Hb level will increase, for example in Polycethemia Vera.

1. **Drabkin's solution contains:**
   a. Magnesium sulfate
   b. Calcium chloride
   c. Sulfuric acid
   d. Copper sulfate
   e. Cyanide

2. **In the cyano Methemoglobin method of determining hemoglobin the diluent used is:**
   a. Distilled water
   b. Hayem's solution
   c. Oxidic acid
   d. Physiological saline solution
   e. Drabkin's solution