Red Blood Cells (Erythrocytes) count
In healthy men, the average number of RBCs per cubic millimeter is 5,200,000 (±300,000);
In women, it is 4,700,000 (±300,000).

Hematocrit or Packed cell volume (PCV)
Normal adult female Range: 36 - 46 percent.
Normal adult male Range: 41 - 53 percent
Normal Newborn Range: 49 - 61 percent
It is normally higher in male than female due to higher RBC count in male than female.

Estimation of blood hemoglobin
Normal hemoglobin levels are:
Men: 13.8 to 18.0 g/dL
Women: 12.1 to 15.1 g/dL
Children: 11 to 16 g/dL

HEMATOLOGICAL INDICES:
These are useful for determining the type of anemia.
1. Mean Corpuscular Volume (MCV):
   This index estimates mean volume of erythrocytes in femptoliters and is calculated as follows:
   MCV (femp-to-liters) = [Hematocrit % x 10] / [RBC count (in millions/μL)]
   Normal Range: 87 ± 5 fempto-liters; where 1 fempto-liters = 1 X 10^-15 litter
2. Mean Corpuscular Hemoglobin (MCH):
   This is the mean quantity of hemoglobin (pico-grams) found in each red blood cell.
   The index is calculated as follows:
   MCH (pico-grams) = [Hemoglobin (in gm/dL) x 10] / [RBC count (in millions/μL)]
   Normal Range: 29 ± 2 pico-grams; where 1 picogram = 1.0 × 10^-12 grams
3. Mean Corpuscular Hemoglobin Concentration (MCHC)
   This index is an estimation of the concentration of hemoglobin in an average RBC and is expressed as gm/dL.
   The index is calculated as follows:
   MCHC (%) = [Hemoglobin (in gm/dL) x 100] / [Hematocrit (in %)]
   Normal Range: 34 ± 2 gm/dL
4. The red blood cell distribution width (RDW or RCDW):
   The "width" in RDW-CV is sometimes thought to be "misleading," since it in fact is a measure of deviation of the volume of RBCs, and not directly the diameter. However, "width" refers to the width of the volume curve (distribution width, here presented as the Coefficient of Variation, or CV), not the width of the cells. Thus, it is a reasonably accurate term
   Normal reference range in human red blood cells is 11 - 15%.
   RDW = (Standard deviation of MCV ÷ mean MCV) × 100.
   Iron Deficiency Anemia: usually presents with high RDW with low MCV
   Folate and vitamin B12 deficiency anemia: usually presents with high RDW and high MCV
   Mixed Deficiency (Iron + B12 or folate) anemia: usually presents with high RDW with MCV being high, low or often normal range
   Recent Hemorrhage: typical presentation is high RDW with normal MCV
Anemias are defined based on cell size (MCV) and amount of hemoglobin (MCH).

Anisocytosis: means variation in size
- MCV less than lower limit of normal: micro-cytic anemia
- MCV within normal range: normo-cytic anemia
- MCV greater than upper limit of normal: macro-cytic anemia

MCH less than lower limit of normal: hypo-chromic anemia
- MCH within normal range: normo-chromic anemia
- MCH greater than upper limit of normal: hyper-chromic anemia

Shape of RBCs may be determined by visual inspection of a stained blood smear through the microscope. Poikilocytes: means variation in shaped, and usually a further descriptive term which defines the shape is added, i.e., sickle cells, elliptical cells, club-shaped cells, spherical cells, etc.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (Hct) (%)</td>
<td>47</td>
<td>42</td>
</tr>
<tr>
<td>Red blood cells (RBC)</td>
<td>5.4</td>
<td>4.8</td>
</tr>
<tr>
<td>(10^6/μL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (Hb) (g/dL)</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>Mean corpuscular volume</td>
<td>87</td>
<td>87</td>
</tr>
<tr>
<td>(MCV) (fl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean corpuscular</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>hemoglobin (MCH) (pg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean corpuscular</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>hemoglobin concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(MCHC) (g/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean cell diameter</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>(MCD) (μm)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Cells with MCVs > 95 fl are called macrocytes; cells with MCVs < 80 fl are called microcytes; cells with MCHs < 25 g/dL are called hypochromic.*
Stained smears also contribute information concerning size (megalocyte, macrocyte, normocyte, and microcyte) and Hemoglobin content (hypochromia, normochromia, and hyperchromia) to the trained observer.

**What are the components of the complete blood count (CBC)?**
The complete blood count, or CBC, lists a number of many important values. Typically, it includes the following:

1. **White blood cell count (WBC or leucocyte count)**
2. **WBC differential count**
3. **Red blood cell count (RBC or erythrocyte count)**
4. **Hematocrit (Hct)**
5. **Hemoglobin (Hb)**
6. **Mean corpuscular volume (MCV)**
7. **Mean corpuscular hemoglobin (MCH)**
8. **Mean corpuscular hemoglobin concentration (MCHC)**
9. **Red cell distribution width (RDW)**
10. **Platelet count & Mean Platelet Volume (MPV)**
Erythrocyte sedimentation Rate (ESR)

**Mechanism:**
ESR is determined by the interaction between factors that promote (fibrinogen) and resist (negative charge of RBCs - that repel each other) sedimentation. Normal RBCs settle slowly as they do not form rouleaux or aggregate together. Instead, they gently repel each other due to the negative charge on their surfaces.

Increased rouleaux formation contributes to high ESR. Rouleaux are stacks of many RBCs that become heavier and sediment faster. Plasma proteins, especially fibrinogen, adhere to the red cell membranes and neutralize the surface negative charges, promoting cell adherence and rouleaux formation.

The aggregated RBCs in the rouleaux formation have a higher ratio of 'mass to surface area' as compared to single RBCs and hence sink faster in plasma.

**Important Note:**
- Erythrocyte sedimentation rate is a non-specific test and is not diagnostic of any particular disease.
- Erythrocyte sedimentation rate has a high sensitivity but low specificity.

\[
\text{sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}
\]

\[
\text{specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}
\]

- Never base a diagnosis solely on an ESR value, either normal or high.
- Interpretation of the result should always be along with the patient's clinical history, examination findings and results of other tests done.
- If high ESR is encountered without any obvious reasons, patient should be reassured and the test repeated after a reasonable amount of time (a couple of months). There is no need to extensively search for an occult disease without repeating it again.

**Normal ESR Values:**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>20</th>
<th>55</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (mm/hr)</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Women (mm/hr)</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>
Newborn: 0 to 2 mm/hr
Neonatal to puberty: 3 to 13 mm/hr, but other laboratories place an upper limit of 20.

Using the ESR to Make a Diagnosis
1. The ESR remains an important diagnostic criterion for only two diseases:
   A. polymyalgia rheumatica: is characterized by severe aching and stiffness in the neck, shoulder girdle
      or pelvic girdle areas
   B. temporal arteritis: is usually characterized by ① headaches, ② visual disturbances such as blindness, ③ a
      tender, reddened or nodular temporal artery, ④ facial pain and ⑤ jaw claudication.
2. Rheumatoid arthritis:
   ESR traditionally has been a diagnostic parameter for rheumatoid arthritis, but it is used as a means of
   staging the disease rather than as one of the major diagnostic criteria

Monitoring Disease Activity or Response to Therapy
In the past, the ESR was commonly used as an index of disease activity in patients who had certain
 disorders.
With the development of more specific methods of evaluation, the ESR has remained an appropriate
 measure of disease activity or response to therapy for only a few diseases: temporal arteritis, polymyalgia
 rheumatica, rheumatoid arthritis and, possibly, Hodgkin's disease.

Oncologic Diseases
In oncology, a high ESR has been found to correlate with overall poor prognosis for various types of cancer, including Hodgkin's disease, gastric carcinoma, renal cell carcinoma, chronic lymphocytic leukemia, breast cancer, colorectal cancer and prostate cancer.
In patients with solid tumors, a sedimentation rate greater than 100 mm per hour usually indicates metastatic disease.
In Hodgkin's disease elevated ESR may still be an excellent predictor of early relapse, especially if the value remains elevated after chemotherapy.

Discriminating Iron Deficiency from Anemia of Chronic Disease
The ESR may be useful in differentiating iron deficiency from anemia of chronic disease in patients with
 a background chronic inflammatory condition such as rheumatoid arthritis.

Screening for Systemic Disease or Neoplasia
Unfortunately, the ESR is neither sensitive nor specific when used as a general screening test. For
 instance, the ESR may be elevated in the presence of ① infectious disease, ② inflammatory or
destructive processes, ③ collagen vascular disease ④ malignancy
ESR may not be increased in a number of ① infectious diseases (e.g., typhoid fever, malaria, and
mononucleosis), ② allergic processes, ③ angina (which is opposed to myocardial infarction) ④ peptic ulcer
disease (as opposed to active inflammatory bowel disease).

Screening for Infection in Specific Clinical Settings
Recent studies have evaluated the ESR as a screening test for infection in specific clinical instances such as infection associated with orthopedic prostheses, pediatric bacterial infection and gynecologic inflammatory disease.

Blood Typing
TRANSFUSION REACTIONS
Dangerous hemolytic transfusion reactions occur when blood is transfused into an individual with an incompatible blood type; that is, an individual who has agglutinins against the red cells in the transfusion. The plasma in the transfusion is usually so diluted in the recipient that it rarely causes agglutination even when the titer of agglutinins against the recipient’s cells is high. However, when the recipient’s plasma has agglutinins against the donors red cells, the cells agglutinate and hemolyze. Free hemoglobin is liberated into the plasma.

The severity of the resulting transfusion reaction may vary from an asymptomatic minor rise in the plasma bilirubin level to severe jaundice and renal tubular damage leading to anuria and death. All transfusion reactions eventually cause either immediate hemolysis resulting from hemolysins or later hemolysis resulting from phagocytosis of agglutinated cells. The hemoglobin released from the RBCs is then converted by the phagocytes into bilirubin and later excreted in the bile by the liver. The concentration of bilirubin in the body fluids often rises high enough to cause jaundice—that is, the person’s internal tissues and skin become colored with yellow bile pigment. However, if liver function is normal, the bile pigment will be excreted into the intestines by way of the liver bile, so jaundice usually does not appear in an adult person unless more than 400 milliliters of blood are hemolyzed in less than a day.

One of the most lethal effects of transfusion reactions is kidney failure, which can begin within a few minutes to a few hours and continue until the person dies of acute renal failure. The kidney shutdown seems to result from three causes:

**First**, the antigen-antibody reaction of the transfusion reaction releases toxic substances from the hemolyzing blood that cause powerful renal vasoconstriction.

**Second**, loss of circulating RBCs in the recipient, along with production of toxic substances from the hemolyzed cells and from the immune reaction, often cause circulatory shock. The arterial blood pressure falls very low, and renal blood flow and urine output decrease.

**Third**, if the total amount of free hemoglobin released into the circulating blood is greater than the quantity that can bind with “haptoglobin” (a plasma protein that binds small amounts of hemoglobin), much of the excess leaks through the glomerular membranes into the kidney tubules. If this amount is still slight, it can be reabsorbed through the tubular epithelium into the blood and will cause no harm; if it is great, then only a small percentage is reabsorbed. Yet water continues to be reabsorbed, causing the tubular hemoglobin concentration to rise so high that the hemoglobin precipitates and blocks many of the kidney tubules. Thus, renal vasoconstriction, circulatory shock, and renal tubular blockage together cause acute renal shutdown. If the shutdown is complete and fails to resolve, the patient dies within a week to 12 days unless he or she is treated with an artificial kidney.
Persons with type AB blood are “universal recipients” because they have no circulating agglutinins and can be given blood of any type without developing a transfusion reaction due to ABO incompatibility. Type O individuals are “universal donors” because they lack A and B antigens, and type O blood can be given to anyone without producing a transfusion reaction due to ABO incompatibility. This does not mean, however, that blood should ever be transfused without being cross-matched except in the most extreme emergencies, since the possibility of reactions or sensitization due to incompatibilities in systems other than ABO systems always exists. In cross-matching, donor red cells are mixed with recipient plasma on a slide and checked for agglutination. It is advisable to check the action of the donor’s plasma on the recipient cells in addition, even though, as noted above, this is rarely a source of trouble.

A procedure that has recently become popular is to withdraw the patient’s own blood in advance of elective surgery and then infuse this blood back (autologous transfusion) if a transfusion is needed during the surgery. With iron treatment, 1000 to 1500 mL can be withdrawn over a 3-wk period. The popularity of banking one’s own blood is due primarily to fear of transmission of infectious diseases by heterologous transfusions, but of course another advantage is elimination of the risk of transfusion reactions.

**SIGNIFICANCE OF BLOOD GROUPS**

1. For transfusions
2. For solving paternity disputes
3. Disease linked with ABO groups (e.g.: peptic ulcer believed to occur commonly in O-group people and gastric malignancy in A-group).
4. Anthropological analyses
5. Establishing identity of subjects, e.g. tracing missing babies in maternity ward.

**HEMOLYTIC DISEASE OF THE NEWBORN**

Another complication due to Rh incompatibility arises when an Rh-negative mother carries an Rh-positive fetus. Small amounts of fetal blood leak into the maternal circulation at the time of delivery, and some mothers develop significant titers of anti-Rh agglutinins during the postpartum period. During the next pregnancy, the mother’s agglutinins cross the placenta to the fetus. In addition, there are some cases of fetal–maternal hemorrhage during pregnancy, and sensitization can occur during pregnancy. In any case, when anti-Rh agglutinins cross the placenta to an Rh-positive fetus, they can cause hemolysis and various forms of hemolytic disease of the newborn (erythroblastosis fetalis).
If hemolysis in the fetus is severe, the infant may die in utero or may develop anemia, severe jaundice, and edema (hydrops fetalis). Kernicterus, a neurologic syndrome in which unconjugated bilirubin is deposited in the basal ganglia, may also develop, especially if birth is complicated by a period of hypoxia. Bilirubin rarely penetrates the brain in adults, but it does in infants with erythroblastosis, possibly in part because the blood–brain barrier is more permeable in infancy. However, the main reasons that the concentration of unconjugated bilirubin is very high in this condition are that production is increased and the bilirubin-conjugating system is not yet mature.

About 50% of Rh-negative individuals are sensitized (develop an anti-Rh titer) by transfusion of Rh-positive blood.
Because sensitization of Rh-negative mothers by carrying an Rh-positive fetus generally occurs at birth, the first child is usually normal. However, hemolytic disease occurs in about 17% of the Rh-positive fetuses born to Rh-negative mothers who have previously been pregnant one or more times with Rh-positive fetuses. Fortunately, it is usually possible to prevent sensitization from occurring the first time by administering a single dose of anti-Rh antibodies in the form of Rh immune globulin during the postpartum period. Such passive immunization does not harm the mother and has been demonstrated to prevent active antibody formation by the mother. In obstetric clinics, the institution of such treatment on a routine basis to un-sensitized Rh-negative women who have delivered an Rh positive baby has reduced the overall incidence of hemolytic disease by more than 90%. In addition, fetal Rh typing with material obtained by amniocentesis or chorionic villus sampling is now possible, and treatment with a small dose of Rh immune serum will prevent sensitization during pregnancy.

**Rh₀(D) immune globulin or Rh₀(D) immune globulin** (letter o and digit zero are both widely attested; more at Rh blood group system - Rh nomenclature) is a medicine given by intramuscular injection that is used to prevent the immunological condition known as Rh disease (or hemolytic disease of newborn), an anti-D antibody that is administered to the expectant mother starting at 28 to 30 weeks of gestation. The anti-D antibody is also administered to Rh-negative women who deliver Rh-positive babies to prevent sensitization of the mothers to the D antigen. This step greatly reduces the risk of developing large amounts of D antibodies during the second pregnancy. The mechanism by which Rh immunoglobulin globin prevents sensitization of the D antigen is not completely understood, but one effect of the anti-D antibody is to inhibit antigen-induced B lymphocyte antibody production in the expectant mother. The administered anti-D antibody also attaches to D-antigen sites on Rh-positive fetal RBCs that may cross the placenta and enter the circulation of the expectant mother, thereby interfering with the immune response to the D antigen.

**Cross-matching blood**

Cross-matching blood, in transfusion medicine, refers to the complex testing that is performed prior to a blood transfusion, to determine if the donor's blood is compatible with the blood of an intended recipient, or to identify matches for organ transplants. Cross-matching is usually performed only after other, less complex tests have not excluded compatibility. Blood compatibility has many aspects, and is determined not only by the blood types (O, A, B, AB), but also by blood factors, (Rh, Kell, etc.). Cross matching is done by mixing a small sample of recipient blood with a small sample of the donor blood. The mixed blood is looked under a microscope to see if there is any clumping of blood. If there is no clumping it indicates that it is safe to transfuse the donor blood. People must receive blood of the same blood type else serious, sometimes fatal, transfusion reaction can occur. Cross matching is essential for organ transplantation since ABO antigens are also found on most body organs.

**Serological cross-matching**

In serological cross-matching, red blood cells from the donor unit are tested against the serum of the patient in need of the blood transfusion. If the patient’s serum contains antibodies against the antigens present on the donor red blood cells, agglutination will occur. Agglutination is considered a positive reaction indicating that the donor unit is incompatible for that specific patient. If no agglutination occurs the unit is deemed compatible and is safe to transfuse.
Cross-matching falls into two categories:

**Major Cross-match:** Recipient serum is tested against donor packed cells to determine if the recipient has performed antibodies against any antigens on the donor’s cells. This is the required cross-match prior to release of a unit of packed cells.

**Minor Cross-match:** Recipient red cells are tested against donor serum to detect donor antibodies directed against a patient’s antigens. This is no longer required. It is assumed that the small amount of donor serum and antibodies left in a unit of packed cells will be diluted in a recipient.

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**Leukocytes (White Blood Cells) count**

Normal WBC count values
At birth: 10,000-25,000 cell/cu mm (μl)
1 to 3 years: 6,000-18,000 cell/cu mm (μl)
4 to 7 years: 6,000-15,000 cell/cu mm (μl)
8 to 12 years: 4,500-13,500 cell/cu mm (μl)
Adult: 4,000-10,000 cell/cu mm (μl)
Pregnancy: 12,000 -15,000 cell/cu mm (μl)

The peripheral leukocyte count is determined by several mechanisms, including:

(1) The size of precursor and storage pool of myeloid and lymphoid cells,
(2) The rate of release of the cells from the storage pool in the bone marrow,
(3) The rate of marginating cells out of blood vessels into the tissues
(4) The rate of consumption of the cells in the tissues (i.e. cell loss).

An increase in the number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leukopenia.

Leukopenia (or leukocytopenia or pancytopenia)

A clinical condition known as leukopenia, in which the bone marrow produces very few WBCs, occasionally occurs. This condition leaves the body unprotected against many bacteria and other agents that might invade the tissues.

Normally, the human body lives in symbiosis with many bacteria because all the mucous membranes of the body are constantly exposed to large numbers of bacteria. The mouth almost always contains various spirochetal, pneumococcal, and streptococcal bacteria, and these same bacteria are present to a lesser extent in the entire respiratory tract. The distal gastrointestinal tract is especially loaded with colon bacilli. Furthermore, one can always find bacteria on the surfaces of the eyes, urethra, and vagina. Any decrease in the number of WBCs immediately allows invasion of adjacent tissues by bacteria that are already present.

Within 2 days after the bone marrow stops producing WBCs, ulcers may appear in the mouth and colon, or some form of severe respiratory infection might develop. Bacteria from the ulcers rapidly invade surrounding tissues and the blood. Without treatment, death often ensues in less than a week after acute total leukopenia begins.

Causes:
1. Aplastic anemia:
Leucopenia could be a part of aplastic anemia where the bone marrow fails to produce RBC, WBC and platelets.
Irradiation of the body by x-rays or gamma rays, or exposure to drugs and chemicals that contain benzene or anthracene nuclei, is likely to cause aplasia of the bone marrow.
2. Some common drugs such as chloramphenicol (an antibiotic), thiouracil (used to treat thyrotoxicosis), and even various barbiturate hypnotics on rare occasions cause leukopenia, thus setting off the entire infectious sequence of this malady.

Leukocytosis

The percentage of neutrophils is high for the first few weeks after birth, but then lymphocyte predominance is seen. Until about 8 years of age, lymphocytes are more predominant than neutrophils.
Physiological causes of leukocytosis:
(1) Age:
(2) Pregnancy: primarily due to an increase in neutrophils with a slight increase in lymphocytes.
(3) Severe pain
(4) Muscular exercise
Leukocytosis can occur due to an increase in any of the five types of white blood cells:
- Neutrophilic leukocytosis = neutrophilia
- Lymphocytic leukocytosis = lymphocytosis
- Eosinophilic leukocytosis = eosinophilia
- Monocytic leukocytosis = monocytosis
- Basophilic leukocytosis = basophilia

Pathological causes of leukocytosis:
A. Normally cellular responding bone marrow
   1. Infection
   2. Inflammation: tissue necrosis, infarction, burns, arthritis
   3. Stress: overexertion, seizures, anxiety, anesthesia and epinephrine administration
   4. Drugs: corticosteroids, lithium, beta agonists
   5. Trauma: splenectomy
   6. Hemolytic anemia
B. Abnormal cellular responding bone marrow:
   1. Acute leukemia
   2. Chronic leukemia

Leukocytes (WBC) differential count
Types & percentages:
A. Granulocytes (or poly-morpho-nuclears: poly: many, morph: morphology, nuclears: nucleus)
   1. Neutrophils (or segmented): 50 - 70% relative value (2500-7000 absolute values)
   2. Eosinophils: 1 - 3% relative value (100-300 absolute values)
   3. Basophils: 0.4% - 1% relative value (40-100 absolute values)
B. Agranulocytes (or mononuclears)
   1. Lymphocytes: 25 - 35% relative value (1700-3500 absolute values)
   2. Monocytes: 4 - 6% relative value (200-600 absolute values)

Neutropenia:
Neutropenia is a condition in which the number of neutrophils in the bloodstream is decreased.
Neutropenia may arise as a result of numerous medical conditions:
1. Infections (more commonly viral infections,
2. Medications that may damage the bone marrow or neutrophils, including cancer chemotherapy;
3. Vitamin deficiencies (megaloablastic anemia due to vitamin B12 and/or folate deficiency);
4. Diseases of the bone marrow such as aplastic anemia, Radiation therapy;
5. Hypersplenism, which refers to the increased sequestration and/or destruction of blood cells by the spleen

**Neutrophilia:**
It is a condition in which the number of neutrophils in the bloodstream is increased. It may arise as a result of numerous medical conditions:

**A. Physiological:**
Cold, heat, exercise, seizures, pain, labor, surgery, panic, rage, fear, excitement, and vigorous exercise cause epinephrine release, resulting in a transient (1 hour) mature neutrophilia.

**Pathological:**
1. Infections (i.e. bacterial or fungal infections or tuberculosis),
2. Inflammatory diseases such as rheumatoid arthritis, heart attack, other infarct or burns
3. Corticosteroids, whether from stress or when using drugs like prednisone, cause increased bone marrow release of mature neutrophils,

### Division of neutrophiles due to nuclear segments

A "left shift" refers to the presence of increased proportions of younger, less well differentiated neutrophils and neutrophil-precursor cells in the blood. This generally reflects early or premature release of myeloid cells from the bone marrow, the site where neutrophils are generated. A severe neutrophilia with left shift is referred to as a leukemoid reaction. The leukocyte alkaline phosphatase (LAP) score, which refers to the amount of alkaline phosphatase per neutrophil, will increase. Normal mature neutrophils contain small amounts of leukocyte alkaline phosphatase (LAP) in primary granules. The peripheral blood LAP is elevated during leukocytosis because of the "left shift".

**Shift to left mean increase band (younger neutrophils)**
Shift to right mean increase hyper-segmented (old neutrophils)

**Eosinophilia**

Eosinophils are weak phagocytes, and they exhibit chemotaxis, but in comparison with the neutrophils, it is doubtful that the eosinophils are significant in protecting against the usual types of infection.

**Causes:**

A. **Primary eosinophilia:**

Hyper-eosinophilic syndrome: is a disease characterized by a persistently elevated eosinophil count in the blood for at least six months without any recognizable cause, with involvement of either the heart, nervous system, or bone marrow

B. **Secondary eosinophilia:**

1. **Parasitic infection:** example schistosomiasis, trichinosis (“pork worm”). Although most parasites are too large to be phagocytized by eosinophils or any other phagocytic cells, eosinophils attach themselves to the parasites by way of special surface molecules and release substances (hydrolytic enzymes from their granules or highly reactive forms of oxygen) that kill many of the parasites.

2. **Allergic disorders:** example Asthma, Hay fever, Allergic skin diseases and Drug allergies

   The mast cells and basophils release an eosinophil chemotactic factor that causes eosinophils to migrate toward the inflamed allergic tissue so, the Eosinophils collect in tissues in which allergic reactions occur, such as in the peri-bronchial tissues of the lungs in people with asthma and in the skin after allergic skin reactions.

   The eosinophils are believed to detoxify some of the inflammation-inducing substances released by the mast cells and basophils and probably also to phagocytize and destroy allergen-antibody complexes, thus preventing excess spread of the local inflammatory process.

**Estimation of clotting mechanism**

**Prothrombin Time (PT)**

**Principle**

The prothrombin time (PT) is used to screen for inherited and acquired abnormalities defects in the extrinsic (factor VII) and common (factors V, X, protrombin and fibrinogen) pathways.

Normal range (depend on reagent used)

- 10-12 seconds
- 12-14 seconds

A PT greater than 20 seconds is indicative of coagulation deficit.

**Prothrombin Time.**

**Prothrombin time and international normalized ratio**

Prothrombin time gives an indication of the concentration of prothrombin in the blood.
The shortness of the prothrombin time is determined mainly by prothrombin concentration. The normal prothrombin time is about 12 seconds. In each laboratory, a curve relating prothrombin concentration to prothrombin time, is drawn for the method used so that the prothrombin in the blood can be quantified. The results obtained for prothrombin time may vary considerably even in the same individual if there are differences in activity of the tissue factor and the analytical system used to perform the test. Tissue factor is isolated from human tissues, such as placental tissue, and different batches may have different activity.

The international normalized ratio (INR) was devised as a way to standardize measurements of prothrombin time. The result (in seconds) for a prothrombin time performed on a normal individual will vary according to the type of analytical system employed. This is due to the variations between different batches of manufacturer’s tissue factor used in the reagent to perform the test. The INR was devised to standardize the results.

The ISI value (International Sensitivity Index) usually varies between 1.0 and 2.0. The INR is the ratio of the person’s prothrombin time (PT) to a normal control sample raised to the power of the ISI:

\[
\text{INR} = \left( \frac{\text{PT}_{\text{test}}}{\text{PT}_{\text{normal}}} \right)^{\text{ISI}}
\]

The normal range for INR in a healthy person is 0.9 to 1.3. A high INR level (e.g., 4 or 5) indicates a high risk of bleeding. A low INR (e.g., 0.5) suggests that there is a chance of having a clot. Patients undergoing warfarin therapy usually have an INR of 2.0 to 3.0.

Interpretation:

The common causes of prolonged PT are:
1. The administration of oral anticoagulant drugs (Vitamin K antagonist/ Warfarin)
2. Liver disease
3. Vitamin K deficiency
4. Disseminated intravascular coagulation
5. Deficiency of extrinsic coagulation factors Inhibitor to Factor VII
A prolonged prothrombin time indicates a deficiency in any of factors VII, X, V, prothrombin, or fibrinogen.

**Activated Partial Thromboplastin Time (APTT or PTT)**

**Principle**
The APTT assay is used to detect inherited and acquired coagulation factor deficiency and quality of the intrinsic pathway, to screen for lupus anticoagulant and to monitor heparin therapy. The assay will evaluate the function of fibrinogen, prothrombin, and factors V, VIII, IX, X, XI, and XII. The APTT is an assay of the intrinsic and common pathway. The APTT assay measures all factors except factors VII and XIII. Normal range: depending on the reagent used
25-45 seconds
23-35 seconds