The Complete Blood Count and Associated Tests

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Purpose

The purpose of this course is to explain the normal blood values for the complete blood count and associated tests as well as implications of increased and decreased values.

Course objectives

Upon completion of this course, the healthcare provider should be able to:

- Discuss the composition of blood.
- List 4 types of progenitor cells that produce blood cells.
- List 8 tests included as part of the complete blood count.
- Discuss normal values, causes and implications of increase and decrease for the following:
  - Red blood cell count.
  - Hemoglobin.
  - Hemoglobin A1C.
  - Hematocrit.
  - Erythrocyte/Red blood cell indices.

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    - Erythrocyte sedimentation rate (ESR).
    - Erythropoietin (EPO).
    - Leukocyte/White blood cell count and differential.
    - Neutrophils.
    - Eosinophils.
    - Basophils.
    - Lymphocytes.
    - Monocytes.
    - Platelets.
- Explain a “shift to the left.”
- Explain flow cytometry.

Introduction

Blood is an essential living tissue that circulates throughout the body—about 5 liters in the adult. Blood comprises:

- Liquid plasma (78%): 90% water with albumin and blood clotting factors, such as fibrinogen and globulin.
- Cells (22%): platelets, red blood cells, and white blood cells.

Blood cells are formed in the cancellous bone of the bone marrow in the shafts of the arms, legs, ribs, sternum, and vertebrae in adults. Bone marrow is yellow in areas with many lipid cells but red in areas where formation of blood (hematopoiesis) occurs. Almost the entire marrow area is red in infants, but red marrow recedes as people mature and is replaced with yellow marrow.

Blood cells are produced by stem cells, which comprise 3-5% of all marrow cells. The type of blood cells formed by progenitor stem cells is controlled by cytokines (proteins secreted by cells to signal other cells) and hormones (poietins):

- Interleukin-7: B and T cell lymphocytes.
- Erythropoietin: Erythrocytes.
- Thrombopoietin (with Interleukin-7): Megakaryocytes (which fragment into platelets).
- Granulocyte-monocyte-colony-stimulating factor (with Interleukin-3 and Interleukin-5): Granulocytes and monocytes.

The immature cells that are formed are called blast cells. These blast cells continue to differentiate and develop into different types of mature cells.

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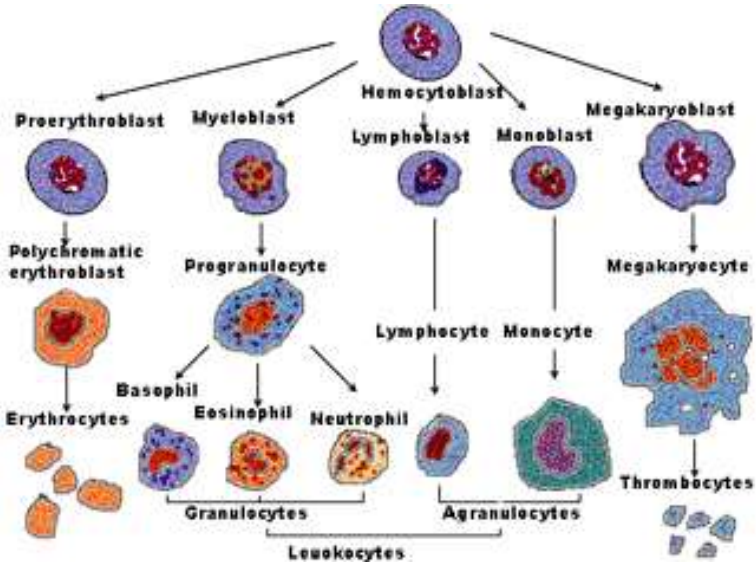

US National Cancer Institute

Red blood cells mature in the bone marrow before they are released into the blood, but some lymphocytes (a type of white blood cell) are immature when they leave the bone marrow and enter the bloodstream. They travel to the thymus and other lymphoid tissue to mature. Each day the bone marrow produces huge numbers of cells (per kilogram of body weight):

- 2.5 billion erythrocytes
- 2.5 billion platelets
- 1 billion granulocytes

Abnormalities anywhere in this blood-producing system can effect the production of blood cells and the blood count.

CBC overview

The complete blood count (CBC) is one of the most frequently ordered screening laboratory tests. The CBC includes a number of different determinations, including the number, type, percentage, concentration, and quality of blood cells. In most cases, the CBC is done using an automated hematology analyzer, which can provide results in about a minute.

Tests usually part of a CBC include:

- Red blood cell (erythrocyte) count (RBC)
- Hemoglobin (Hb or Hgb)
- Hematocrit (Hct)
- Red blood cell indices:(mean corpuscular volume [MCV], mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration [MCHC]
- White blood cell (leukocyte) count (WBC)
- Differential white blood cell count or "diff"

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- Platelet (thrombocyte) count (estimated)
- Blood cell morphology

Norma values differ somewhat according to age and gender. The laboratory references provided in this course are meant as a guide and may vary somewhat from references used in different institutions.

Laboratory tests performed on plasma are done using blood samples taken by venipuncture, usually with vacuum tubes used to collect the specimen. Tubes come in various sizes, and using the proper size is important because the tubes contain various types of anticoagulants and the volume of the specimen must be correct for the type of anticoagulant. If the ratio is incorrect, it can interfere with test results. For most hematology studies, including cell counts, blood is collected in tubes with lavender stoppers. These tubes contain ethylene diamine tetraacetic acid (EDTA).

Nursing Alert:
Because the CBC count may fluctuate during the day, with regular CBC counts (such as daily or weekly), blood should be drawn at approximately the same time of day. Blood counts may be altered by hydration status. Overhydration (such as from IV fluids) increases the plasma component, and this dilutes percentages of blood elements. Dehydration, such as from inadequate fluids or NPO status, however, results in hemoconcentration, increasing the percentages of blood elements relative to plasma.

Red blood cell (RBC) count

Erythrocytes, commonly referred to as red blood cells (RBCs), have two primary functions:

- Carry oxygen from the lungs to body tissues
- Transfer carbon dioxide from the tissues to the lungs.
-

As RBCs mature, they become biconcave disks and produce hemoglobin, which makes up about 90% of the weight of the cell. Hemoglobin combines readily with oxygen (oxyhemoglobin) and carbon dioxide (carboxyhemoglobin). Oxyhemoglobin in arterial blood is bright red in color while the carboxyhemoglobin of venous blood appears dark red. The biconcave shape enables the maximum oxygen saturation of hemoglobin by providing more surface area for exposure of hemoglobin to

dissolved oxygen.

In response to hypoxia, the hormone erythropoietin, secreted primarily by the kidneys, stimulates the bone marrow to produce red blood cells. Red blood cells are able to change shape to permit passage through small capillaries that connect arteries with veins. Normal red blood cells survive about 120 days and are then ingested by phagocytic cells in the liver and kidneys.

RBCs comprise about 40% of total blood volume; the **RBC count** is the number of red blood cells per cubic millimeter of blood. Normal red blood cells values vary according to age and gender:

Age/gender	**RBC: Number per cubic millimeter**
Newborns	4.1 – 6.1 million
Children (1-6)	3.9– 5.3 million
Adult males:	4.5 – 6.0 million
Adult females	4.2 – 5.0 million (slightly↓with pregnancy)

Immature erythrocytes are called reticulocytes. They usually mature into red blood cells within 2 days after release into the blood stream. A **reticulocyte count** measures the percentage of reticulocytes in relation to the RBC count, and it is specifically used to monitor bone marrow function. Reticulocyte counts are normally very stable. When the RBC count increases, the reticulocyte count can help to determine the degree and rate of RBC overproduction. Normal ranges for reticulocytes include:

Age/gender	**Reticulocytes: %-age of RBCs**
Newborns	3.0% - 7%
Infants >12 wks	0.2% - 2.0%
Adult males:	0.5% - 1.5%
Adult females	0.5% - 2.5%

An increased reticulocyte count indicates that the bone marrow is responding to the need for increased red blood cell production, such as may occur with acute loss of blood, iron-deficiency anemia, hemolytic anemia, megaloblastic anemia, or treatment for anemia. Thus, the reticulocyte count is often used to monitor response to treatment for anemia. In conditions in which red cell production is stimulated, a concomitant increase in reticulocytes is usually present, such as at high altitudes. Pregnant women and newborns also tend to show increased reticulocyte counts.

A decreased reticulocyte count occurs with alcoholism, aplastic anemia, renal disease, folic acid deficiency, and bone marrow failure. Some drugs, such as azathioprine, dactinomycin, hydroxyurea, methotrexate, and zidovudine may

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decrease reticulocyte counts. The reticulocyte count may be falsely decreased after transfusion because of the dilutional effect.

Increased RBC count

Various disorders and treatments may involve an increase in RBC count (polycythemia), A very high RBC mass will slow blood velocity and increase the risk of intravascular clotting.

Primary polycythemia vera is abnormal increase in red blood cells with cause unknown and does not result from hypoxia or physiological need. Polycythemia vera may be treated by hydroxyurea to slow down bone marrow overproduction of red blood cells. Interferon is sometimes used to lower RBC count. Phlebotomy may be done weekly to decrease the concentration of red blood cells. Hydration is especially important as dehydration may increase viscosity of the blood.

Secondary polycythemia may be caused by a variety of factors:

- At high altitudes, less atmospheric weight pushes air into the lungs, causing a decrease in the partial pressure of oxygen and resultant hypoxia, so the RBC count increases at high altitude increases to compensate for lower atmospheric pressure and oxygen, making more hemoglobin available to transport oxygen.
- Erythropoietin-secreting tumors increase production of red blood cells.
- Renal disorders may increase red blood cell count.
- Increased exertion and muscle mass related to strenuous exercises increase RBC count in order to keep pace with demands for oxygen.
- Some drugs, such as gentamicin, corticosteroids, methyldopa, may cause an increased RBC count.
- Hypoxia associated with smoking may trigger increase in RBC production to compensate.
- Chronic lung disease in adults and children with congenital heart defects characterized by cyanosis cause polycythemia.
- Excessive exercise, anxiety, pain, and dehydration.

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Nursing Alert:

Maintaining adequate hydration is critical to preventing venous thrombosis in those with polycythemia vera, so long periods without fluids should be avoided. If patients with polycythemia require fluid restriction, such as those with heart failure or congenital heart defects, the physician should specify the amount of daily fluids and the patients should be carefully monitored.

RBC counts are affected by position, stasis, and hydration. Counts decrease slightly if a patient is recumbent and increase slightly when the patient is upright. Leaving the tourniquet on for more than 60

seconds when obtaining a blood sample may increase the RBC count. Dehydration increases RBC count and overhydration decreases it.

Decreased RBC count

A decreased RBC count (anemia) may result from a decrease in the number of red blood cells although it can also be caused by a decrease in hemoglobin content. A decreased RBC count is caused by:

- Hemolytic anemia.
- Chemotherapy.
- Hemorrhage resulting in loss of RBCs.
- Decreased plasma volume from severe burns, shock, severe vomiting, or intestinal obstruction.
- Pregnancy.
- Malignancies: Hodgkin's disease, multiple myeloma, leukemia.
- Addison's disease.
- Rheumatic fever.
- Nutritional deficit.
- Subacute endocarditis.
- Lack of substances necessary for the production of RBCs.
- Bone marrow suppression, often related to chemotherapy (amphotericin B, floxuridine, and phenylbutazone) or radiation.
- Hemodilution from excess IVs.

Hemoglobin

The hemoglobin molecule is comprised of four subunits (alphas and betas), with each containing an iron-containing pigment (heme) and a protein (globulin). One gram of hemoglobin can carry up to 1.34 mL of oxygen. While the amount of oxygen carried on each molecule of hemoglobin may vary, in general the ability of the blood to carry oxygen directly relates to the hemoglobin concentration. More hemoglobin, more oxygen, and vice versa.

Hemoglobin measures the amount of the oxygen-carrying protein (hemoglobin) in a volume of blood, providing an indication of the ability of the blood to oxygenate the tissue. Hemoglobin determination is used to screen for anemia and determine response to treatment. Hemoglobin is measured in grams per deciliter and closely corresponds to RBC numbers since 90% of red blood cells are composed of hemoglobin.

Age/gender	Hemoglobin: grams/deciliter
Newborns	14.5 – 24.5 g/dl
Children (1-6 years)	9.5 – 14. 1 g/dl
Adult males:	14.0 – 17.5 g/dl
Adult females	12.0 – 16.0 g/dl

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Hemoglobin: critical low and high values:

- A hemoglobin value under 6 g/dl may result in heart failure.
- A hemoglobin value over 18 g/dl may cause vascular occlusion because of hemoconcentration.

Increased hemoglobin

Increased hemoglobin occurs with the following:

- Burns
- Heart failure.
- COPD.
- Dehydration.
- Erythrocytosis.
- Hemoconcentration.
- High altitudes.
- Polycythemia vera.

Decreased hemoglobin

Because hemoglobin is carried on red blood cells, any decrease in circulating RBCs affects the hemoglobin level. Conditions with abnormal types of hemoglobin often result in lower total hemoglobin because the red blood cells with abnormal hemoglobin are readily damaged. Hemoglobin electrophoresis is used to distinguish among the different types of abnormal hemoglobin.
Specific disorders that result in decreased hemoglobin include:

- Thalassemia is an autosomal recessive hemoglobinopathy resulting in insufficient globulin in either the alpha or beta subunits.
- Sickle cell disease causes an abnormally-shaped hemoglobin known as sickle hemoglobin (hgbS). These misshapen RBCs tend to clump and form vessel occlusions.
- Iron deficiency anemia (hypochromic anemia) is a condition in which red blood cells have less hemoglobin than normal because of inadequate iron, so the cells are able to transport less oxygen. This results in a normal RBC count but low hemoglobin.
- Physiological anemia of pregnancy: During pregnancy, the plasma proportion of the blood expands, so the hemoglobin falls because of the increased volume.
- Serial blood draws: Neonates and premature infants are especially vulnerable to anemia from repeated blood draws.
- Hemolytic disorders.
- Malignancies, such as leukemia, carcinoma, lymphomas.
- Hodgkin's disease.
- Pregnancy.
- Nutritional deficit.
- Fluid retention, and IV overload.

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Hemoglobin A1C

Although not part of the standard complete blood count, an additional test associated with hemoglobin, hemoglobin A1C is used to determine an average plasma concentration of glucose over a 3-month period—compared to a standard fasting blood glucose that reflects blood glucose during a one-time fasting state. The fasting glucose level may fluctuate according to diet and other factors, so it is less reliable as an indicator of actual glucose levels than hemoglobin A1C.

Erythrocytes survive about 120 days, and during that time the erythrocytes are exposed to glucose circulating in the plasma. The glucose molecules join with the hemoglobin forming glycated (glycosylated) hemoglobin. The percentage of glucose in the cells indicates the average glucose level that the cell was exposed to. Different laboratory procedures may result in slightly different readings, but normal readings are usually in the 4-6% range, and diabetics are usually advised to maintain readings <7%. A1c readings correlate with blood glucose levels:

A1C level (%)	Blood glucose level (mg/dL)
6	135
7	170
8	205
9	240
10	275
11	310
12	345

Nursing Alert:
Hemoglobin A1C is used to assess diabetic control over a period of time. A value exceeding 7% indicates poor diabetic control. The patient may need further education about diet and diabetic control. Every 1% reduction in A1C reduces risks of microvascular complications by 10%.

Hematocrit

The **hematocrit**, or PVC (packed cell volume), measures the percentage of red blood cells in the total volume of blood. The term hematocrit refers to the separation of blood that occurs when a blood sample is placed in a centrifuge that separates components. The red blood cells sink to the bottom while the white blood cells and plates rise into a layer referred to as the “buffy coat.”

Age/gender	Hematocrit: % of blood volume
Newborns	44% - 64%
Children (1-6 years)	30% - 40%
Adult males:	45% - 52%

Adult females	36% - 48%

Hematocrit: critical low and high values:

- A hematocrit of less than 18% can result in cardiac failure.
- A hematocrit of over 54% may result in spontaneous blood clotting.

If the **RBC count** and the **hemoglobin** are both within normal limits, one can estimate the hematocrit as approximately three times the hemoglobin. For example, a person whose hemoglobin is 14 would have a hematocrit of about 42%. The heart rate usually increases with hematocrit <30% as a compensatory measure.

Increased hematocrit

Because the hematocrit measures the percentage of red blood cells in total blood volume any change in volume affects the hematocrit in relation to the total volume of blood. For example, a patient with extensive burns loses significant amounts of plasma, resulting in hemoconcentration and increased hematocrit. Increased hematocrit leads to polycythemia. Other causes of increase include:

- Heart failure.
- COPD.
- Erythrocytosis.
- High altitudes.
- Shock.
- Polycythemia.
- Dehydration/Hemoconcentration.

Hydration status should always be considered when interpreting the hematocrit. An early morning blood draw may show a higher hematocrit because of normal dehydration that occurs during the night.
When administering packed red blood cells, each unit should increase the hematocrit by about 3%.

Decreased hematocrit

The hematocrit is used done to evaluate the extent blood loss. Decreased hematocrit indicates anemia. If a person is hemorrhaging, initially plasma and blood cells are lost in equal proportions, so a hematocrit done immediately afterwards may not show a drop. However, the body tries to compensate for the loss of plasma by moving fluid from the interstitial spaces into the vascular system, diluting the blood. The red blood cells take much longer to produce, so a hematocrit taken several hours after hemorrhage will show a decrease.
Overhydration, such as with IV fluids may also dilute the blood, resulting in a decreased hematocrit.

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Other causes of decreased hematocrit include:

- Anemia.
- Bone marrow hyperplasia.
- Hemolytic disorders.
- Fluid retention.
- Pregnancy.
- Splenomegaly.
- Nutritional deficit.
- Medications may induce hemolysis in sensitive individuals.

Patients who tend to have chronically low hemoglobin, such as those receiving renal dialysis, may have few symptoms as their bodies have adjusted to the low level; however, those with a sudden drop, such as from hemorrhage, may develop indications of shock, with pallor, hypotension, and hypoxia.

Nursing Alert:
Activity level is a concern with low hemoglobin. If low hematocrit is caused by hemorrhage, the BP may stay stable initially as the plasma volume increases from interstitial fluid although the BP will fall with fluid deficit. The heart is often a better test to determine activity tolerance. **Tilt test:** Take blood pressure supine and after sitting. If the pulse rate increases from the act of sitting, tolerance for activity may be impaired. Patients may need slow, steady exercise or activities with rest periods. Low hematocrit requires increased production of red blood cells, so dietary modifications may include increased protein and iron Stasis from leaving the tourniquet in place during venipuncture for >60 seconds may increase Hct values by 2-3%. Values taken within a few hours of blood transfusion or acute blood loss may appear normal. Elevated glucose levels cause RBCs to swell and may cause a falsely elevated hematocrit.

Erythrocyte/Red blood cell indices

When hemoglobin levels are low, erythrocyte/red blood cell indices provide information about the size of red cells and the concentration of hemoglobin in order to diagnose the type of anemia

Mean corpuscular volume (MCV)

MCV measures the average size of red blood cells and classifies anemia. Calculation:

MCV = Hematocrit / total RBC count

The red blood cells are classified according to size:

- ***Microcytic:*** MCV is low because of small RBCs. Microcytic red blood cells are found in iron deficiency anemia, vitamin B12/folate anemia, lead poisoning and Thalassemias.
- ***Macrocytic:*** MCV is high because of large RBCs. Pernicious anemia and folic acid deficiencies are characterized by macrocytic RBCs. MCV is also increased in alcoholism, antimetabolite therapy, and hepatic disease.
- ***Normocytic:*** MCV is within the normal range and RBCs are normal in size. Anemia associated with acute hemorrhage is usually normocytic.
-

Age/gender	Mean Corpuscular volume: femtoliters
Infants (6-12 mo.)	73 – 87 fL
Children (1-6 years)	70 – 84 fL
Adult males:	84 – 96 fL
Adult females	76 – 96 fL

Some drugs may increase the MCV—colchicine, pentamidine, pyrimethamine, and triamterene—while nitrofurantoin may decrease MCV.

Mean corpuscular hemoglobin (MCH)

MCH measures the amount, or the mass, of hemoglobin present on average in one RBC, helping to determine if the cells are normochromic, hypochromic (pale), or hyperchromic (dark). Calculation:

MCH = Hgb / Total RBC count

Age/gender	Mean Corpuscular Hgb: picogram/cell
Infants (6-12 months)	24 – 30 pg
Children (1-6 years)	23 – 29 pg
Adult males:	27 – 32 pg
Adult females	27 – 32 pg

MCH is increased in macrocytic anemias and decreased in hypochromic and microcytic anemias.

Mean corpuscular hemoglobin concentration (MCHC)

MCHC measures the average concentration of hemoglobin in each cell. Calculation:

MCHC = Hgb / Hct

The results are reported in grams per deciliter reflecting the amount of

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hemoglobin in the RBC. In some cases, the results are reported in percentages. Calculation:

MCHC = (Hgb / Hct) x 100

Age/gender	MCHC: Gram/deciliter & percentage	
Infants (6-12 months)	32 – 36 g/dL	32-36%
Children (1-6 years)	31 – 35 g/dL	31-35%
Adult males:	30 – 35 g/dL	30-35%
Adult females	30 – 35 g/dL	30-35%

MCHC is also used to determine if red blood cells are normochromic, hypochromic, or hyperchromic. MCHC is increased in spherocytosis and thalassemia and decreased in iron-deficiency anemia.

Anemias can be classified using erythrocyte indices in the following way:

- **Decreased MCV, MCH, and MCHC**: Microcytic, hypochromic anemia, usually related to iron deficiency anemia.
- **Increased MCV, variable MCH and MCHC:** Macrocytic anemia, usually related to vitamin B12 deficiency or folic acid deficiency.

Red blood cell distribution width (RDW) RDW measures the variation in RBC size and is based on the mean corpuscular volume (MCV), which measures RBC size. Calculation:

RDW = (standard deviation or RBC width / mean RBC width) x 100

The results are expressed in percentages to show how many RBCs vary in size.

Age/gender	RDW: Percentage
Reference range	12.8 – 15.2%

Values may increase with various types of anemias (megaloblastic, hemolytic, sickle cell), liver disease, alcoholism, and folate and vitamin B12 deficiency.

Erythrocyte sedimentation rate (ESR)

The erythrocyte sedimentation rate (ESR) is not part of the complete blood count, but is an associated test or RBCs. The ESR, also called the “sed rate,” measures the rate at which RBCs in anticoagulated blood precipitate after an hour in an upright test tube (Westergren tube). The rate at which the RBCs fall is reported in mm/hr. This test is now more commonly done with automated analyzers.

The ESR is used as an indicator of inflammation for both acute and chronic inflammation associated with infections, cancers, and autoimmune diseases. Inflammation increases immune and clotting factors, such as globulins and fibrinogen, in the blood. Fibrinogen, a clotting factor, causes RBCs to clump into

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stacks called “rouleaux.” The rouleaux settle faster than separate RBCs, so an increased rate indicates inflammation. Since ESR elevations relate to increased fibrinogen and globulins, follow-up fibrinogen level and protein electrophoresis may be ordered to determine whether fibrinogen, globulins, or both are affecting the ESR.

ESR is a non-specific test as it does not indicate the type or cause of infection, but increases and decreases are associated with certain disorders. Acute infections are often better identified with the C-reactive protein test, which shows signs of infection earlier (within 6-8 hours) and is less sensitive to other variables. However, ESR is a simple test and is often done first when inflammation is suspected. It is commonly used to aid in diagnosis of pediatric rheumatoid arthritis and Kawasaki disease. The ESR may be used to monitor response to therapy for some conditions:

Age/gender	**Erythrocyte sedimentation rate: mm/hr.**
Neonates	0.0 – 2.0
Children (to puberty)	3.0 – 20.0
Adult males:	10.0 – 19.0 (increases with age)
Adult females	15.0 – 23 (increases with age & pregnancy

Increased ESR

Increased ESR is commonly found in severe infection and globulin-increasing tumors, such as multiple myeloma and Waldenstrom’s macroglobulinemia. Elevations in ESR associated with disease-specific symptoms are commonly used to diagnose and monitor two specific conditions: temporal arteritis and polymyalgia rheumatica. Some drugs may increase the sedimentation rate: methyldopa (Aldomet), oral contraceptives, penicillamine procainamide, theophylline, and vitamin A. A sustained elevation in ESR after chemotherapy for Hodgkin’s disease may be a predictor of early relapse.

The ESR combined with the hemoglobin has been shown to be a more effective screening tool for inflammatory bowel disease in children and adolescents than serology testing (and less expensive). IBD is identified by increased ESR (31 mm/hr.) and decreased hemoglobin (indicating anemia).

Decreased ESR

A decreased ESR is usually not clinically significant although it is associated with some disorders, such as polycythemia, severe leukocytosis, sickle cell anemia, and some protein abnormalities. Some drugs may decrease the sedimentation rate—aspirin, cortisone, and quinine—either because they affect inflammatory processes or clotting.

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Erythropoietin (EPO)

The erythropoietin (EPO) test is not part of the complete blood count but is often ordered when RBC levels are abnormal to help to identify the cause of the abnormality. Erythropoietin (EPO) is a hormone produced primarily by the kidney but some is produced in the liver as well. EPO is a glycoprotein that stimulates the production and development of RBCs in the bone marrow and promotes synthesis of hemoglobin in the RBCs and is, thus, the primary regulator of RBC production.

Tests are used to determine EPO level in order to evaluate bone marrow and kidney disorders as well as EPO abuse. EPO is sometimes taken as a performance-enhancing drug in athletes, such as cyclists, runners, speed skaters, and cross-country skiers. Testing is done after overnight fasting and after a 20-30 minute period of lying quietly. Lab values vary considerably from one laboratory to another, so conclusive reference ranges are not available. Each laboratory has reference values, so the following reference ranges are approximate.

Age/gender	EPO: mU/ml
Reference range	Authorities vary: 0.0 – 24.0 OR 11.0 – 48.0

Increased EPO

Elevated EPO is characteristic of secondary polycythemia as the EPO increases and stimulates the bone marrow to produce an excess of RBCs. If EPO levels are elevated but the hemoglobin is low (anemia), then this suggests that the disorder is in the bone marrow rather than the kidneys. Increased levels may also associated be with abuse of EPO (blood doping), but EPO has been banned by sports organizations, including the Tour de France and the Olympics. EPO is taken by athletes to increase RBC production, oxygen capacity, and endurance; however, the resultant polycythemia coupled with strenuous exercise and dehydration increases the risk of stroke and heart attacks.

Decreased EPO

A decreased EPO level is associated with some types of anemia, including anemia secondary to kidney failure, AZT treatment (for AIDS) and cancer. A low level is also found in polycythemia vera (as opposed to secondary polycythemia). EPO has been synthesized (recombinant erythropoietin) and is used as a treatment in some cases, especially cancer-related anemia, to increase the RBC count although RBC count must be monitored carefully because side effects include blood clots, heart attacks, strokes, increase in tumor size, and death. In chronic kidney disease, EPO tests may be done regularly to monitor kidney production of EPO.

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Leukocyte/White Blood Cell Count (WBC) and Differential

White blood cells, or **leukocytes**, are classified into two primary groups:

- **Granulocytes** (granules in cell cytoplasm): **Neutrophils, eosinophils**, and **basophils**. Because these cells have a multilobed nucleus, they are also called polymorphonuclear leukocytes or "polys." Neutrophils have segmented nuclei, so they are sometimes referred to segmented neutrophils or "segs."

- **Agranulocyte** (no granules and nonlobular nuclei): **Lymphocytes** and **monocytes** are sometimes referred to as mononuclear leukocytes.

White blood cells have a much shorter life span than red blood cells, approximately 13 to 20 days, after which they are destroyed by the lymphatic system and excreted in feces. These cells are produced in the bone marrow although lymphocytes can be produced elsewhere as well. When immature white cells are released from the bone marrow, they are referred to as “bands” or “stabs.”

WBCs usually make up a small percentage of the total blood (about 1%), but they serve a number of purposes for the immune system. Through phagocytosis, WBCs surround and destroy foreign organisms. WBCs also produce and transport antibodies in response to antigens.

The CBC includes two measurements of white blood cells:

- Total number of white blood cells in a microliter ($1x10^{-9}$ liters) of blood, providing an absolute number (in thousands) of WBCs.
- Differential: Percentage of each of the five types of white blood cells, totaling 100%. Calculation:

  Specific cell percentage = (Specific cell / Total WBC) X 100

  Absolute value = % / 100 X Total WBC

Age & gender	WBC ($x10^3$)	Bands %	Neut/segs %	Eos %	Baso %	Lymph %	Mono %
Newborn	9.0-30.0	10-18	36-62	0-2	0-1	26-36	0-6
1-6 yr	5.0-19.0	5-11	13-33	0-3	0-0	46-76	0-5
Adults	5.0-10.0	3-6	50-62	0-3	0-1	25-40	3-7

Leukocytes: critical low and high values

- WBC of less than 2500 increase risk of severe life-threatening infection.
- WBC over 30,000 indicates severe infection or a serious disease, such as leukemia.

When evaluating the WBC count and differential, one must consider both relative

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and absolute values. For example a relative value of 60% neutrophils may seem within normal limits; however, if the total WBC were 30,000, the absolute value (60% x 30,000) would be 18,000, an elevation.

Increased WBC

Leukocytosis, a WBC >10,000, is usually due to an increase in one of the five types of white blood cells rather than in increases in a number of cells. The condition is named depending on the cell that shows the most significant increase: Neutrophilia, lymphocytosis, eosinophilia, monocytosis, and basophilia.

White blood cells release colony stimulating factor to stimulate bone marrow to increase production of WBCs in response to trauma, acute infection, or inflammation. The WBC count can increase by thousands within a few hours. Some conditions, such as sepsis and measles, result in very high increases. With leukemia, the WBC count may also increase, but the increase is permanent and progressive.

Leukocytosis occurs in early infancy, as a response to stress, from cold exposure, after strenuous exercise, and with exposure to ultraviolet light. WBCs also increase with pregnancy and labor and menstruation. Pathologic conditions associated with leukocytosis include all kinds of bacterial infections, transfusion reactions, hemolysis, anemias, appendicitis, collagen disease, Cushing's disease, leukemias and other malignancies, parasitic infestations, and polycythemia vera.

Nursing Alert:
Severe stress that results in production of epinephrine can cause a rapid increase in WBC as the body prepares to "fight." Corticosteroids usually increase the WBC count in normal health individuals. However, in the presence of infection, the infection can spread without a rise in the WBC count, so severe infections can be masked in those receiving corticosteroids.

Decreased WBC

Leukopenia occurs when the WBC falls below 4,000. Viral infections, overwhelming bacterial infections, and bone marrow disorders can all cause leukopenia. Severe leukopenia puts patients at severe risk of opportunistic infections, so treatments that involving interrupting skin integrity, such as injections, may increase risk.

Leukopenia may occur with alcoholism, anemias, bone marrow suppression, malaria, malnutrition, radiation, viral infection, SLE and other autoimmune disorders, and chemotherapy. A number of drugs may decrease the white blood

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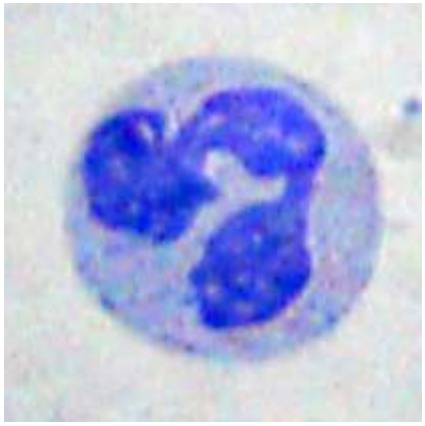
count: antibiotics, anticonvulsants, cardiovascular drugs, NSAIDS, and diuretics.

Differential

Neutrophils

Age & gender	Bands %	Neut/segs %
Newborn	10-18	36-62
1-6 yr	5-11	13-33
Adults	3-6	50-62



Neutrophils, also calls polymorphonuclear cells, usually comprise the largest percentage of white blood cells. They are the body's primary defense through phagocytosis and contain enzymes and pyogens to combat foreign organisms. Most circulating neutrophils are mature, and the nucleus is segmented.

Normally, most of the neutrophils circulating in the bloodstream are in a mature form, with the nucleus of the cell being divided or segmented. The nucleus of immature neutrophils is not segmented but has a banded or rod-like appearance.

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The nucleus of less mature neutrophils is not segmented, but has a band or rod-like shape. These immature cells are referred to as “bands” or “stabs.”

Increased neutrophil count

Both mature and immature neutrophils increase in response to an acute bacterial infection. When laboratory reports were written out by hand, by tradition, band and neutrophils were the first two cells on the left. Because neutrophils usually increase in response to inflammation, this increase is often referred to as a “shift to the left.” This term is still used to refer to an infective process that results in increased bands.

Neutrophilia also occurs with acute hemolysis, acute hemorrhage, temperature extremes, malignancies, metabolic disorders, myelocytic leukemia, physiological stress (surgery, allergies, childbirth, exercise), toxin/venom poisoning, and

inflammatory conditions, such as gout, rheumatoid arthritis, and vasculitis.

Decreased neutrophil count

Neutropenia, a decrease in neutrophils, may also occur with some types of bacterial infections, such as typhoid fever and brucellosis, and with many viral diseases. If infection overwhelms the ability of the bone marrow to produce neutrophils, neutropenia may also occur. Neutropenia is often a side effect of chemotherapeutic agents used to treat malignancies, such as leukemia, as well as lithium, phenothiazines, and tricyclic antidepressants.

Neutropenia also occurs with acromegaly, anaphylaxis, nutritional impairment (anorexia, starvation), bone marrow depression, SLE, thyrotoxicosis, and vitamin B12 or folate deficiency.

Absolute neutrophil count

Age & gender	Absolute neutrophil count
1 year	1500 to 8500
10 years	1800 to 8000
Adult	1800 to 7700

Absolute neutrophil count **(**ANC) is an important measure used to determine the degree of neutropenia. The total ANC should be about 1800 to 2000 mmm^3 or higher. Risk of infection is significant if the level falls to 1000 and severe at 500. ANC is calculated indirectly from the total white count and the percentages of neutrophils and bands:

- **ANC = Total WBC X (% neutrophils + % bands/100)**

Using this formula, if the white blood count is 3200 with 70% neutrophils and 3% bands, the calculation would indicate that despite the low WBC count, the patient does not have neutropenia:

- ANC = 3000 X 73/100 = 2190

If the white count is 5300 with 10% neutrophils and 1% bands, neutropenia is evident despite the normal WBC:

- ANC = 5300 X 11/100 = 583

Nursing Alert:
Patients with neutropenia are at increased risk of endogenous and exogenous infection, so they should avoid people with respiratory or other infections. They should be encouraged to use good hygiene and eliminate potential sources of infection, such as unpeeled fresh fruit or raw vegetables and fresh flowers.

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Patients with ANC <1000 should be placed in private rooms

Procedures that may interfere with the integrity of the skin or mucous membranes, such as injections, enemas, and suppositories, should be avoided.

Patients who develop a fever along with severe neutropenia usually have an infection and require immediate hospitalization for broad-spectrum antibiotics. Patients receiving antibiotics should be monitored for fungal super infections, such as moniliasis.

Eosinophils

Age & gender	Eos %
Newborn	0-2
1-6 yr	0-3
Adults	0-3

Eosinophils are associated phagocytosis of antigen-antibody complexes. Eosinophils become active in later stages of inflammation and response to allergens and parasitic infections. Eosinophil granules contain histamine, which helps to kill foreign cells, but is also implicated in lung pathology and asthma because it causes smooth muscle contraction in the bronchioles and upper respiratory tract, increased mucous production, and constriction of pulmonary vessels (asthma). Eosinophil granules also contain proteolytic enzymes that damage parasitic worms.

Increased eosinophil count:

Eosinophils increase with:

- Parasitic infections (may increase to >30%).
- Addison's disease
- Allergy, hay fever.
- Asthma.
- Drug reaction (allergic response).
- Hodgkin's disease.
- Pernicious anemia.

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- Rheumatoid arthritis.
- TB.
- Polycythemia vera.

Decreased eosinophil count:

Eosinophils decrease with:

- Aplastic anemia.
- Stress.
- Infections (associated with shift to the left).
- Eclampsia.
- Drugs: ASA, amphotericin B, corticotropin, desipramine, glucocorticoids, hydrocortisone, interferon, niacin, prednisone, procainamide.

Nursing Alert:
Eosinophil levels vary according to the time of day, lowest in the morning and rising until midnight, at which time the level begins to fall again. Serial tests should be done at the same time of day.

Basophils

Age & gender	Baso %
Newborn	0-1
1-6 yr	0-0
Adults	0-1

Basophils occur in very small number in the blood but are important as part of the immune system and have a phagocytic function. They are involved in allergic (hypersensitivity) reactions. The granules contain heparin, histamines, and serotonin. Basophils are similar to mast cells, which are found in the tissue.

Basophils are evaluated to assist in determining cause of increased WBC count, to detect hematologic diseases and monitor progression of other disorders, such as COPD, malabsorption syndromes, renal disease, and cancer. Basophils may be noted in leukemia, Hodgkin's disease, ulcerative colitis, polycythemia vera, and nephrosis.

Lymphocytes

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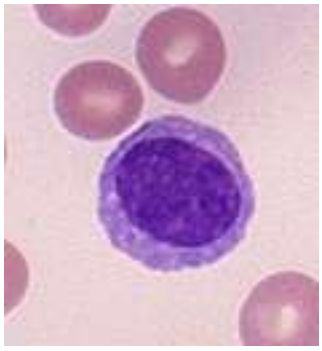
Age & gender	Lymph %
Newborn	26-36
1-6 yr	46-76
Adults	25-40



Lymphocytes are the second most common white blood cell in adults and first most common in young children and are an important part of the immune system. Lymphocytes are produced in the bone marrow but migrate through the lymphatic system to other parts of the body. B cells (for bone marrow) mature within the bone marrow, but T cells (for thymus) mature in the thymus.

There are both large and small lymphoctyes. Large lymphocytes include natural killer (NK) cells. NK cells, part of the innate immune response, help defend the body against tumors and viruses. NK cells are activated by interferons and then release cytokines that modulate T and B cell response and also produce cytotoxic granules that destroy cells. NK cells help to contain a viral infection while cytotoxic T cells clear the infection. NK cells destruction of organisms is not antigen specific.

Small lymphocytes include both T-cell and B-cell lymphocytes, which are part of the adaptive immune response with T cells active in cell-mediated immunity and B cells in humoral (antibody-related) immunity. Some cytotoxic T cells produce cytotoxic granules with enzymes that destroy pathogen-infected cells. Helper T cells (CD4) direct the immune response by activating killer cells and B cells. CD4 is a glycoprotein found on the surface of T helper cells and is an important measure of the progression of HIV. CD8 is found on cytotoxic T cells.

B cells recognize foreign antigens and produce antibodies to them. Additionally, they act as antigen presenting cells. That is, they attach to free antigens and present them to T cells for destruction. B cells form memory cells that remember particular antigens to increase speed of response with the next encounter.

Increased lymphocytes:

Lymphocytes increase in many viral infections as well as a number of other disorders:

- Lymphocytic leukemia.
- Addison's disease.
- Felty's syndrome.
- Thyrotoxicosis.

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- Ulcerative colitis.
- Lymphoma, lymphosarcoma, myeloma.
- Rickets.

Decreased lymphocytes:

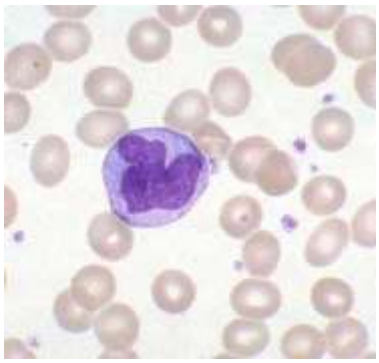
HIV results in a decrease in the total number of lymphocytes as well as changes in the ratios of the different types of T lymphocytes because CD4 (T4) cell counts decrease as HIV counts increase. Other causes of decreased counts include:

- Antineoplastic drugs.
- Aplastic anemia.
- Bone marrow failure.
- Burns.
- Hodgkin's disease.
- Immunodeficiency disorders.
- Malnutrition.
- Pernicious anemia.
- Radiation.
- Septicemia.
- Thrombocytopenic purpura.
- Transfusion reaction.

Nursing Alert:
Immunosuppressive drugs, such as corticosteroids, may decrease lymphoctyes, increasing risk of infection. As lymphocyte levels fall, the patient is especially at risk for developing viral infections.

Monocytes

Age & gender	Mono %
Newborn	0-6
1-6 yr	0-5
Adults	3-7



Monocytes are the largest cells normally found in blood and are produced in the bone marrow from the same progenitor cells as neutrophils. Their primary function is phagocytosis.

Monocytes stay in peripheral blood for approximately 70 hours and then migrate into tissues where they act as macrophages. As macrophages, the cells

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enlarge and increase the amount of digestive enzymes in their intracellular vesicles in order to more effectively carry out phagocytosis. Large numbers of macrophages are in the liver and spleen (known then as Kupffer cells) to clean bacteria from the blood.

Increased monocytes:
Monocytes increase with the following:

- Carcinomas.
- Cirrhosis.
- Collagen disease.
- Gaucher's disease.
- Hemolytic anemia.
- Infections.
- Lymphomas
- Monocytic leukemia.
- Polycythemia vera.
- Cirrhosis.
- Radiation.
- Sarcoidosis.
- Ulcerative colitis.
- SLE.
- Thrombocytopenic purpura.

Decreased monocytes:
A decrease in monocytes may indicate injury to the bone marrow or some types of leukemia.

Platelets

Age & Gender	Platelets
Newborn	140,000 – 300,000
1-6 yr	150,000 – 450,000
Adult males	150,000 – 400,000
Adult females	150,000 – 400,000

Platelets, or thrombocytes, are non-nucleated round or oval fragments of larger cells (megakaryocytes) found in the bone marrow and are very tiny—only about 20% the average diameter of RBCs. The primary functions of platelets are in coagulation, hemostasis, and thrombus formation. Platelets contain granules that contain substances that allow the platelets to stick to each other and to damage

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blood vessels.

Platelets break off the mother cells in response to release of the hormone thrombopoietin. Platelets enter the blood stream and circulate for about 10 days before they end life in the spleen. In a normal healthy patient, thrombopoietin is able to maintain an adequate production.

Decreased platelets

Thrombocytopenia is a platelet count <50,000. Thrombocytopenia markedly increases the patient's risk of bleeding with injury or complicating condition, such as liver disease or coagulopathy. As the count continues to fall to <10,000 the patient may experience spontaneous and uncontrolled internal bleeding that results in death.

Thrombocytopenia may result from increased platelet destruction or impaired platelet production associated with megakaryocytic hypoproliferation, impaired thrombopoiesis, bone marrow replacement, increased platelet loss or destruction, increased immune response destruction, increased destruction related to immune response to secondary infection.

- In thrombotic thrombocytopenic purpura and disseminated intravascular coagulation (DIC), platelets are rapidly destroyed, causing a marked decrease in the platelet count.
- Immune-related thrombocytopenic purpura, a condition that may occur early in HIV disease, causes antibodies to attach to platelets as though they are foreign organisms. When the damaged platelets circulate through the spleen, the attached antibody destroys them.
- Malignancies of the bone marrow, such as leukemia, cause proliferation of other cells so that platelet production is effectively curtailed.

Other causes include:

- Acute infections.
- Exercise (transient).
- Posthemorrhagic, hemolytic, aplastic, megaloblastic and iron-deficiency anemia.
- Chronic heart disease.
- Cirrhosis.
- Chronic pancreatitis.
- Rheumatoid arthritis.
- Splenectomy (2 months postoperative).
- Trauma.
- Polycythemia vera.
- TB.
- Alcohol toxicity.
- Drug toxicity.
- Prolonged hypoxia.
- Viral infection.
- Extensive transfusion.
- Severe hemorrhage.
- Uremia.
- Bacterial infection.
- Burns.
- Malaria.
- Rocky Mountain spotted fever.
- Histoplasmosis.
- Radiation.

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- Ulcerative colitis.
- Splenomegaly.

Signs of bleeding due to a low platelet count include:

- Ecchymosis, petechiae.
- Epistaxis.
- Hematuria.
- Tarry stools.
- Rectal bleeding.
- Hematemesis
- Symptoms consistent with intracranial hemorrhage.
- Gingival bleeding
- Heavy vaginal bleeding.

Treatment focuses on controlling the underlying disorder. In some cases, patients may require platelet transfusions.

Nursing Alert:
• Many drugs may decrease platelet count while corticosteroids may increase the count. • Traumatic venipuncture can result in activation of the coagulation sequence and lead to erroneous counts. • Patients with low counts must take precautions to avoid bleeding, including use of soft toothbrush, electric razor rather than sharp razor and avoiding constipation and intramuscular injections. • Patients must also avoid ASA and other anticoagulants.

Increased platelets

Thrombocytosis, an increased platelet counts, is usually not a problem until the count exceeds about 750,000. High counts are usually in response to an inflammatory process but may be related to myeloproliferative disease.

Thrombocytosis is classified as either primary (essential) or secondary (reactionary):

- Primary: Essential thrombocytosis, chronic myelogenous leukemia, polycythemia vera, myelofibrosis.
- Secondary: Post-surgical inflammation, iron deficiency, hemorrhage, hyposplenism (decreased breakdown of cells).

Thrombocytosis may increase the danger of thrombosis. Treatment depends upon identifying and treatment the underling cause. Usually increased levels of platelets do not require treatment although aspirin may be used to prevent clotting and with severely high levels (>1 million) hydroxyurea may be used.

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What is flow cytometry?

Flow cytometry is a sophisticated laboratory technique that is increasingly being used to sort cells and their components. It analyzes multiple parameters of individual cells in heterogeneous populations of cells, such as blood cells, and sorts by size and cell type. Flow cytometry may also be used to analyze bacteria, or sub-cellular elements (RNA, DNA, chromosomes, proteins, hormone receptors, and enzymes) and for phenotyping.

For laboratory testing, a sample of material, such as blood, tissue, or bone marrow is broken down into single cells in liquid (such as saline) in a test tube that is placed into the flow meter. The liquid is drawn into the flow chamber with cells flowing through the chamber in single file (500 cells/second). A small laser beam passes through the cells, causing light to bounce off of the cells in either a forward scatter or side scatter.

A light detector analyzes the scatter pattern (the magnitude of forward scatter indicates size and side scatter indicates granular cells) and sends this information to a computer. The scattered light is translated into a voltage pulse, and a histogram (a type of graph) of forward scatter shows the distribution by size in the population of cells while a combination scatter and side scatter shows the distribution of different cells within the population. Each cell has a unique pattern that identifies it.

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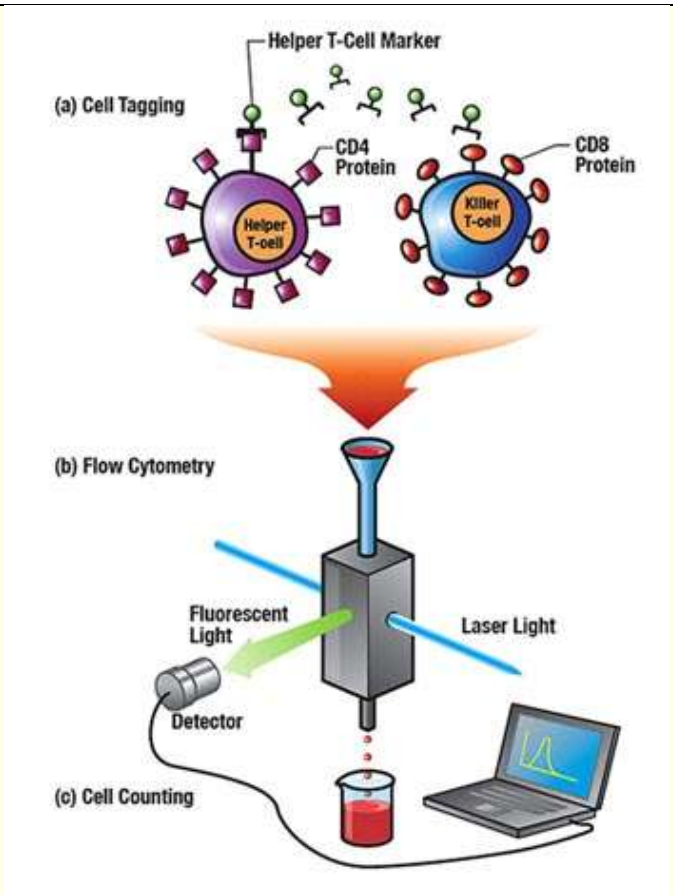

Los Alamos National Laboratory

Another way to study cells is to use fluorophore-labeled antibodies (fluorescent molecules), which are added to the cell sample (cell tagging). The antibodies bind to specific molecules on cell surfaces or inside the cell. When the laser light of the right wavelength strikes the flourophore, a fluorescent signal is produced. One, two, or more compatible fluorophores may be used.

Fluorophores attached to the cells emit lights of different colors (depending on which fluorophore is attached) and filters send these color signals to color detectors, which send the information to the computer. Different cells pick up fluorophores in different amounts, so graphs can be produced that show the proportion of different types of cells.

Information about both the scatter pattern and the color can be combined and plotted on a various histograms and graphs. Cellular characteristics, such as size, complexity, phenotype, and condition or health may be reported.

Flow cytometry is the future of laboratory testing. Some laboratories are now applying this technology for phenotyping leukocytes in leukemias, lymphomas, immunological disorders, transplant patients, and infectious diseases. There are many applications for flow cytometry: for example, flow cytometry is the most accurate method for reticulocyte count because thousands of cells can be

counted. Reference values are still being developed and may vary from one laboratory to another.

Conclusion

Blood is an essential living tissue that circulates about the body. Blood comprises 78% plasma and 22% cells, which includes red blood cells, white blood cells, and platelets. Blood cells are produced in the bone marrow. The complete blood count comprises the red blood cell (erythrocyte) count, hemoglobin, hematocrit, white blood cell (leukocyte) count and differential, and the platelet (thrombocyte) count. Immature erythrocytes are reticulocytes, and counts used to monitor bone marrow function.

An increased RBC count results in primary or secondary polycythemia and a decreased count results in anemia. Hemoglobin measures the amount of oxygen-carrying protein (hemoglobin) in a volume of blood. Hemoglobin A1C determines the average concentration of glucose in the plasma over a 3-month period. The hematocrit determines the percentage of red blood cells in a particular volume of blood. Erythrocyte indices provide useful information to determine which type of anemia a person has. The erythrocyte sedimentation rate (ESR) is used to evaluate inflammation, and the erythropoietin test to evaluate bone marrow and kidney disease.

The white blood cell count and differential provide important information about bone marrow production of cells and response to inflammation. Platelet counts determine the blood's clotting ability. Flow cytometry is a sophisticated laboratory technique that is increasingly being used to sort cells and their components.

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