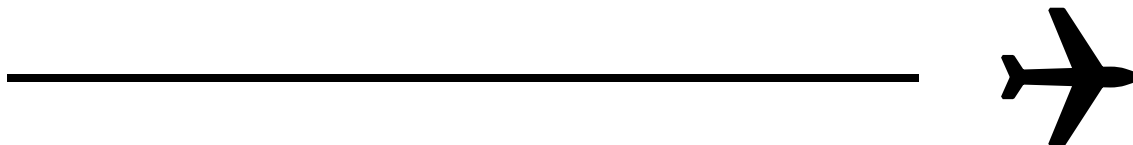


**CDC 4T051P**

**Medical Laboratory  
Journeyman: Hematology,  
Immunology, and Blood  
Banking**

**Volume 1. Hematology**



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THIS IS the final course for the Medical Laboratory Journeyman series. It contains three volumes—*Hematology*, *Immunology*, and *Blood Banking*. Unit 1 provides an introduction to hematology with the characteristics of blood and the hematology laboratory environment. Unit 2 is a study of erythrocyte, leukocyte, and thrombocyte maturation and characteristics. Unit 3 provides a brief review of the complete blood count and hematology procedures. Finally, unit 4 rounds out hematology with the hemostasis mechanism and evaluation of coagulation studies.

A glossary of terms, abbreviations and acronyms is included at the end of this volume.

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## Acknowledgments

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Date	TMHBC Plate Title	TMHBC Plate Number	CDC Figure Number
1985	ERYTHROCYTIC SYSTEM	PLATE 8	Figure 2-1
1985	MYELOCYTIC (GRANULOCYTIC) SYSTEM	PLATE 3	Figure 2-8
1985	MONOCYTES	PLATE 4	Figure 2-12
1985	LYMPHOCYTES	PLATE 5	Figure 2-13

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### NOTE:

In this volume, the subject matter is divided into self-contained units. A unit menu begins each unit, identifying the lesson headings and numbers. After reading the unit menu page and unit introduction, study the section, answer the self-test questions, and compare your answers with those given at the end of the unit. Then do the unit review exercises.

	<i>Page</i>
<b>Unit 1. Hematology Introduction.....</b>	<b>1-1</b>
1-1. The Characteristics of Blood.....	1-1
1-2. The Hematology Laboratory Environment .....	1-14
<b>Unit 2. Maturation and Characteristics of Blood Cells .....</b>	<b>2-1</b>
2-1. Erythrocyte Maturation and Characteristics.....	2-1
2-2. Leukocyte and Thrombocyte Maturation and Characteristics .....	2-24
<b>Unit 3. The Complete Blood Count and Related Studies .....</b>	<b>3-1</b>
3-1. Manual Cell Counts.....	3-1
3-2. Automated Cell Counts .....	3-16
3-3. Related Studies.....	3-34
<b>Unit 4. Coagulation Studies.....</b>	<b>4-1</b>
4-1. The Hemostasis Mechanism .....	4-1
4-2. Hemostasis Evaluation .....	4-12
 <i>Glossary.....</i>	 <i>G-1</i>
<i>Bibliography .....</i>	<i>B-1</i>



# Unit 1. Hematology Introduction

<b>1–1. The Characteristics of Blood .....</b>	<b>1–1</b>
001. The composition and functions of blood and basic cell structure.....	1–1
002. Origin of cellular elements .....	1–4
003. The circulatory system.....	1–8
<b>1–2. The Hematology Laboratory Environment .....</b>	<b>1–14</b>
004. Safety and specimen collection .....	1–14
005. Quality improvement, quality control, and hematology terminology.....	1–20

**F**OR CENTURIES humans have been aware of the importance of blood in maintaining life, but why is it so important? Antony Van Leeuwenhoek’s (1632–1723) use of the microscope not only affected the study of microorganisms, but also that of blood. It permitted blood to be studied microscopically. Thus, the science of studying blood—*hematology*—was born. The word hematology is derived from the Greek words *haima*, meaning blood, and *logos*, meaning study. This unit will introduce you to the world of hematology. Hematology is concerned with normal and abnormal development, physiology, function, and death or destruction of the formed components of blood.

## 1–1. The Characteristics of Blood

The average adult has a total blood volume of about six liters, which accounts for seven to eight percent of total body weight. Nearly 45 percent of the total blood volume is formed elements. The remaining 55 percent is a liquid medium made of approximately 90 percent water and a ten percent mixture of proteins (albumin, globulin, and fibrinogen), carbohydrates, vitamins, hormones, enzymes, lipids, and salts. This complex composition is necessary if blood is to maintain its many varied functions.

### 001. The composition and functions of blood and basic cell structure

What are the formed elements and what are their functions? Where and how are they formed? This lesson will answer these questions and perhaps many more!

#### Composition

Blood is a tissue that consists of microscopically visible formed elements suspended in a pale yellow liquid medium. The formed elements are red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes); the liquid medium is plasma. Each formed element has a unique function. We’ll discuss each element separately, even though it is unrealistic to completely separate these functions because they intertwine. The composition and function of plasma is discussed at length in the chemistry portion of CDC 4T051A.

#### Functions

The functions of blood are:

- Transporting oxygen from the lungs to the tissues.
- Transporting nutrients from the digestive system to the cells of the body.
- Preventing accumulation of waste by transporting metabolic waste from the cells to the excretory organs (skin, kidneys, and lungs).
- Transporting hormones from the endocrine glands and neurosecretory cells to the target tissues.
- Protecting the body from disease and foreign invaders.

- Aiding in hemostasis.
- Regulating fluid and salt balance, and body temperature.

### Basic cell structure

Remember that the cell is considered the basic unit of life and is the smallest part of the body that is capable of self-sufficiency. Before we look at the individual cellular elements of the blood, we'll review basic cellular structures of the animal cell (animal versus bacterial or plant). Knowledge of these structures will aid in identification and understanding of cell function. Take a look at the schematic diagram of a basic cell and its components in figure 1-1.

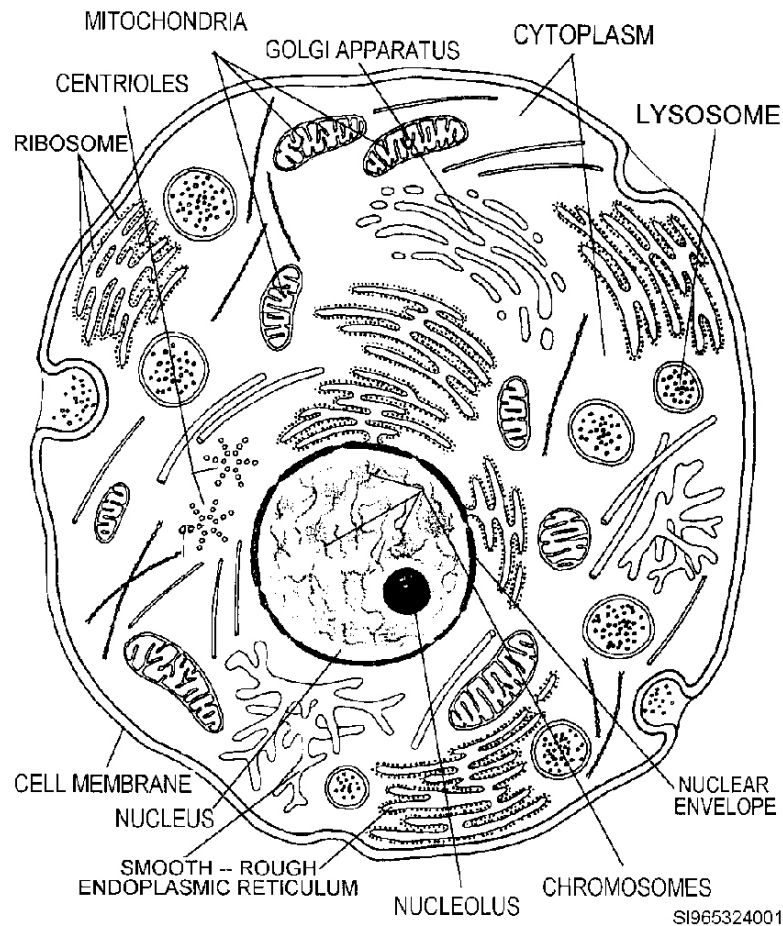


Figure 1-1. Schematic diagram of a basic cell.

### Cellular membrane

The cellular or plasma membrane surrounds the cell. It maintains the shape and integrity of the interior part of the cell. It's composed of three different layers:

1. An outer protein layer.
2. A lipid layer in the middle.
3. An inner protein layer.

Certain proteins on the outer layer are equipped for communication and permit other cells to recognize it. The membrane also has small pores through which substances may pass by osmosis, diffusion, and actual transport. Fortunately, the cellular membrane has selective permeability.



### ***Cytoplasm***

The cytoplasm is a jelly-like material inside the cellular membrane but outside the nucleus. It contains tiny structures called organelles. These components perform specific jobs within the cell. The cytoplasm is made of approximately 90 percent water containing amino acids, simple sugars, and other substances used to manufacture larger molecules. The other 10 percent is made of thousands of different kinds of enzymes and molecules used in cellular metabolism and ions to maintain a proper biochemical environment.

<b>Organelles</b>	<b>Explanation</b>
<i>Lysosomes</i>	Membranous sacs scattered throughout the cytoplasm containing enzymes that break down ingested materials, secretions, and wastes. The lysosomes are also responsible for a “self-destruction” system that release enzymes into the cytoplasm that breaks down the cell following death.
<i>Vacuoles</i>	Membranous sacs that transport and store ingested material, water, and waste.
<i>Ribosomes</i>	Granules made of ribonucleic acid (RNA) and protein. Their function is to synthesize polypeptides and manufacture proteins. They may attach to the endoplasmic reticulum or float freely in the cytoplasm.
<i>Endoplasmic reticulum (ER)</i>	ER can be compared to a complex underground drainage system that extends throughout a city. These “pipes” or “tubules” transport materials from one part of the cell to another. They also divide the cytoplasm into “blocks” that separate cell contents and activities. There are two types of ER—rough and smooth. The rough ER has a granular appearance that results from the attached ribosomes. Its purpose is to manufacture proteins for transportation to other parts of the cell or the proteins to be incorporated into the ER tubule membrane. The smooth ER is so called because it lacks ribosomes on its outer surface. It is the main site of fatty acid and phospholipid metabolism and is important in breaking down drugs and other chemicals such as carcinogens.
<i>Centrioles</i>	Two tiny centrioles that anchor and organize the microtubule formations are in each cell. The microtubules provide structural support, assist in cell and organelle movement, and play a role in cell division.
<i>Golgi apparatus</i>	The Golgi apparatus or complex is a group of flattened membrane sacs stacked together and dispersed throughout the cell. They act as a protein processing and packaging plant. They modify proteins and direct them to a particular location or organelle in the cell, depending on the proteins function. They could also be called a “traffic director” for the proteins.
<i>Mitochondria</i>	Mitochondria act as the cell's powerhouse where most of the reactions of cellular respiration take place. They transform energy originating from glucose and/or lipids into adenosine triphosphate (ATP). The mitochondria are sacs consisting of two membranes. The inner membrane is folded to form cristae, which increase the available membrane surface: similar to storage shelves.

### ***Nucleus***

The nucleus is the largest organelle in the cell. It is spherical in shape and is the “control center” of the cell. It is enclosed by a double membrane called the nuclear envelope or membrane. The envelope contains small pores that permit materials to pass in and out of the nucleus. It is also believed that these pores allow the RNA to carry messages from the deoxyribonucleic acid (DNA) to the cytoplasm. The fluid in the nucleus is thicker than the fluid of the cytoplasm and is called nucleoplasm or karyoplasm. In a cell that is not dividing, a loosely coiled fibrous material known as chromatin is present. When the cell starts to divide, the chromatin becomes more tightly coiled and condenses into discrete rod-shaped bodies termed chromosomes. Each chromosome contains several hundred genes made of DNA arranged in a specific linear order. The nucleolus is within the nucleus. It actively synthesizes protein and produces the RNA, which is a component of the ribosomes.

**Staining characteristics**

Some of the structures within a cell have their own staining properties. The table below is a review of the staining characteristics for the structures with Wright's stain. This will help you understand why cellular staining is not even or consistent throughout the cell.

Structure	Staining Characteristic
Mitochondria	Does not stain with Wright's stain
Golgi apparatus	Does not stain with Wright's stain
Ribosomes	Stains deep blue with Wright's stain
Nucleus	Stains dark purple with Wright's stain
Nucleoli	Stains light blue or bluish with Wright's stain

**002. Origin of cellular elements**

The production of formed or cellular elements of blood is called hematopoiesis. As we go through different stages to become a mature adult, our blood cells also go through different stages before becoming mature adult cells. All the cells within the blood are thought to start from an uncommitted pluripotent stem cell also called a colony forming unit-spleen (CFU-S). Because of a colony stimulating factor (CSF), these stem cells have the ability to reproduce themselves and to differentiate into committed lymphoid stem cells (CFU-L) or hematopoietic stem cells (CFU-C). The lymphoid stem cell gives rise to T- and B-cell lymphocytes. The myeloid or hematopoietic stem cell gives rise to erythrocytes, platelets, granulocytes (neutrophils and eosinophils), and monocytes. The differentiated cells produce blood cells through nuclear division called mitosis. Mitosis is nuclear division without reduction in the number of chromosomes. This type of cell division is unlike meiosis, which involves reduction in the number of chromosomes, as noted in sex cell production—that is, sperm and egg formation.

**Where does hematopoiesis start?**

Hematopoiesis for red blood cells begins at about two weeks of fetal development, when primitive blood cells begin to form blood islands in the yolk sac. At about the second month the liver and spleen phase of hematopoiesis begins. At this time, granulocytes and megakaryocytes start to appear. Around the fourth and fifth months lymphocytes and monocytes are being produced and the bone marrow assumes its primary role in hematopoiesis. At birth and through adulthood, the production of the major blood cells is accomplished in the bone marrow and is called medullary hematopoiesis. Unless the body is placed under some kind of stress, the stem cells go to a “standby” status. Extramedullary hematopoiesis may take place in the liver and spleen if the bone marrow is unable to produce sufficient numbers of hematopoietic cells in certain disease states. In an adult, this red marrow is located in the ribs, sternum, vertebrae, certain skull bones, pelvis, and epiphyses (ends) of the femur and humerus. The rest of the bones become filled with fat, and the tissue is known as yellow marrow. In times of crisis, the yellow marrow space is easily filled with red marrow to meet the increased need for hematopoiesis. Now let's briefly review each cellular element.

**Lymphopoiesis**

Lymphocytes are produced in the bone marrow. B-lymphocytes mature into B-cells in the bone marrow, whereas T-lymphocytes travel to the thymus where they will mature into T-cells. Once mature, the lymphocytes travel to the spleen and lymph nodes to support the immune system. They are leukocytes that lack specific granules in their cytoplasm. They also account for about 30 percent of all circulating leukocytes. The lymphocytes are primarily broken down into three groups based on their function:

Lymphocyte Type or Subpopulation	Maturation Location	Function	Other
B-cell lymphocytes	Bursa equivalent*	Humoral immunity	Produce 'daughter' or memory B cells. Memory B-cells are long-lived
-- Memory B-cells			Develop to respond to specific antigen(s), rapidly respond upon a second exposure
-- Plasma B-cells			Secretes antibodies that bind to and cause antigen destruction by making them easier targets for phagocytes
T-cell lymphocytes	Thymus	Cell mediated immunity	Includes the subpopulations of T-helper and T-suppressor cells.
-- T-helper cells			Initiates immune response through cytokines promoting B-cell proliferation & differentiation
-- T-suppressor cells			Initiates cessation of lymphocytic attack
<i>Null</i> cells			Lack surface/cell membrane markers for B- & T-cells
-- Cytotoxic Killer cells		Antibody dependent cell mediated lysis	Lyses target cell w/o complement via cell-to-cell contact
-- Natural killer cells		Direct cytotoxic activity	Mediate cytotoxic response w/o prior sensitization against the target

**\*Bursa equivalent:** The bursa is an organ unique to birds and is known as the bursa of Fabricius and is where B-cells mature. In all other vertebrates (except rabbits) the term bursa equivalent is used. Bursa equivalent is a theorized lymphatic tissue where B-cell maturation takes place. Further studies reveal that most B-cell maturation occurs in the bone marrow.

Approximately 60 to 80 percent of the lymphocytes are T lymphocytes. The immature T-cells migrate from the bone marrow to the thymus to mature. Once matured, they travel through the peripheral blood or lymphatic system where they interact with different antigens. They are responsible for delayed hypersensitivity reactions, suppression of tumors, graft rejection, and act against some intracellular organisms. In short, cellular immunity is the body's interaction of T-cells in the immune system. The B-cells account for ten to 20 percent of normal blood lymphocytes. They leave the bone marrow and travel through the lymphatic system (spleen), where they interact with antigens, then differentiate into plasma cells, which is the final stage of their maturation. The plasma cell secretes the immunoglobulins IgG, IgM, and IgA (used in defense against infections). The immune system's use of B-cells is called humoral immunity. The null lymphocyte (of which there are two distinct subpopulations – killer cells and natural killer cells, see table above) lacks the characteristics of the T- and B-cells and accounts for less than ten percent of mature lymphocytes. The majority of lymphocytes are long-lived with a life span of about 4 years. It has been noted that some lymphocytes may live as long as ten years providing long term immunity and protection. Others are short-lived, surviving only 3 to 4 days. They are transient and recirculate through the peripheral blood and lymphatic system. The discussion of T- and B-cells are continued in greater detail in volume 2—*Immunology*.

### Erythropoiesis

Erythrocytes are produced in the bone marrow. Stem cell production of erythrocytes is stimulated by a glycoprotein called erythropoietin, produced, for the most part, in the kidney. In the fetus it is produced by the liver. This hormone is produced in response to tissue oxygen tension, as in the case of hypoxia. Erythropoietin and interleukin-3 (produced by T-cells) are responsible for the formation of erythrocyte colony-forming units (CFU-E). Not only are the stem cells stimulated to produce more

cells, but also the sinuses of the bone are acted upon to release more cells. This then balances normal erythrocyte loss due to destruction and maintains the erythron, or total red cell mass, for proper homeostasis. Various materials are required for erythropoiesis and hemoglobin production: iron, folic acid, vitamin B<sub>12</sub>, manganese, cobalt, zinc, vitamins C, E, B<sub>6</sub>, thiamine, riboflavin, and pantothenic acid, and hormones (erythropoietin, thyroxine, and androgens). The erythrocytes' function is to manufacture hemoglobin, which transports oxygen to the tissues and carbon dioxide from the tissues to the lungs. The tagging of red blood cells (RBCs) with radioactive chromium has demonstrated that the erythrocyte has a half-life of 28 to 30 days (i.e., the time elapsed after tagging, when one-half of the tagged cells are still in circulation). In more common terms, tests have shown the RBC to live about 120 days  $\pm$  20 days after leaving the bone marrow. The "old" RBCs are broken down by the mononuclear phagocytic system (MPS), which has replaced the term reticuloendothelial system (RES). The MPS is composed of monocytes and differentiated macrophages [Kupffer cells (liver), microglial cells (nervous system), osteoclasts (bone), histiocytes (connective tissue), and pulmonary alveolar macrophages (lungs)]. This extravascular destruction mostly occurs in the spleen. Ninety percent of RBC destruction is extravascular. However, severely damaged RBCs are removed by the liver, while others are destroyed during circulation; this is termed intravascular destruction. The hemoglobin is broken down by the macrophages of the MPS by the enzyme heme oxygenase. The released heme or iron is recycled and used for production of new erythrocytes or stored by the MPS. The globin is broken down and added to the amino acid pool. The protoporphyrin ring is reduced in the MPS as bilirubin and eventually excreted.

### **Megakaryocytopoiesis**

As in the case of the erythropoiesis and lymphopoiesis, megakaryocytopoiesis takes place in the bone marrow. This form of a committed stem cell arises from CFU-C and develops into a megakaryocyte (CFU-Meg)—the largest cell found in normal bone marrow. Platelets are liberated in fragments from the cytoplasm of mature megakaryocytes. As the megakaryocyte matures its cytoplasm becomes more lobulated and granular, producing platelets at its cytoplasmic edge. The megakaryocyte cytoplasm produces 2,000 to 4,000 platelets that have a life span of nine to 12 days in the peripheral blood. The nucleus degenerates and is processed by the MPS. Only about two-thirds of the mature platelet population is found in the blood. The remaining third is sequestered as a reserve in the spleen. Platelets function in primary hemostasis. They also maintain capillary integrity. In unit 4 of this volume, we further examine the role of platelets in the clotting mechanism.

### **Granulopoiesis**

Granulopoiesis is the term used for the production of granulocytes and monocytes and is also accomplished in the bone marrow. The granules (mostly lysosomes) of these white blood cells (WBCs) contain potent enzymes that destroy ingested bacteria. They move quite freely into and out of the extravascular compartment. They use the circulating blood as transportation until a chemical stimulus (chemotactic response) attracts them to a site that requires leukocyte function. Here, in the tissues, they move by pseudopodal (false foot) movement to engulf and destroy such invading antigens as bacteria. This phagocytosis is a prime function of the granulocytes. There are three types of mature granulocytes based on their unique staining properties: neutrophils, eosinophils, and basophils.

### **Neutrophils**

Neutrophils have neutral staining properties (light lavender) and account for about 60 percent of all the WBCs. Because their nucleus takes on different shapes, they are often called polymorphonuclear leukocytes. The neutrophils only stay in the blood for about ten hours, after which they move through the capillary walls and enter the connective tissues. Neutrophils are excellent macrophages that phagocytize bacteria and other microscopic particles within the tissues. These cells engulf invaders

with pseudopodia and incorporate the material into a cell vacuole that protects the neutrophil from harm, although the cells eventually disintegrate, and in an inflammatory process they are replaced by monocytes. After a day or two they are swept away as waste through the digestive or urinary tract.

### ***Eosinophils***

The eosinophil was once considered to be a variety of a polymorphonuclear neutrophil. Their granules stain bright red with the acid dye eosin. It is now known that after leaving the bone marrow this cell lives only about eight hours in the circulating blood and then completes its several day life span in the tissues. For every one cell in the circulating blood, there are probably 300 to 500 eosinophils in the tissues. They are located primarily in the skin, nasal membranes, lungs, and gastrointestinal tract. This cell is actively phagocytic, but it is a less avid attacker of bacteria than the neutrophil. The eosinophil is thought to react to chemotactic agents produced when basophils, assisted to some extent by neutrophils and lymphocytes, produce histamines in response to invading antigens. The responding eosinophil then phagocytizes the resulting antigen-antibody complex. This reaction explains the dense population of eosinophils in the area of an allergic reaction, such as nasal swabs, revealing large numbers of this cell in allergic rhinitis. The mobilization of eosinophils is, in part, due to hormonal response. This is demonstrated by injecting the patient with adrenocorticotrophic hormone (ACTH) produced by the adrenal cortex. If the adrenal cortex is functioning properly, the number of circulating eosinophils and lymphocytes decrease, and the number of neutrophils increase. Although not yet proven, it is thought that the eosinophil may actually fight helminthic infections. Through production of peroxidases and other oxidative products, this cell may actually destroy the larvae of roundworm invaders. Studies using mice infected with *Trichinella spiralis* support this conclusion and partially explain the eosinophilia seen in certain round worm infections.

### ***Basophils***

When stained with a basic dye, the basophils' granules stain deep blue. The basophil is another tissue-dwelling granulocyte that exhibits both chemotaxis and phagocytosis, but accomplishes both poorly. They also circulate in the blood for about ten hours before entering the surrounding tissues where their life span is about four to five days. The primary function seems to be the basophil's ability to "dump" its histamine granules in the area of antigen-antibody binding in order to increase inflammation. It is thought that the eosinophil produces a *histaminase* as a reaction to the release of histamine, thereby balancing the reaction and inhibiting hypersensitivity reactions. The basophil granules are rich in heparin, which seems to aid in clot dissolution. In short, the pharmacological aspects of the granulocytes and their respective granules are undergoing a great deal of study. Once explained in the simplest of terms, these granules are now found to be extremely complex in function and worthy of our further investigation.

### ***Monocytes***

The monocyte arises from the same stem cell as the neutrophil; however, the monocyte is not granular. Monocytes are the largest WBCs in the peripheral blood and stain less intensely. The cells circulate in the blood for about 12 hours, and then become a fixed tissue macrophage or a wandering (unfixed) macrophage. When the monocyte transforms into an activated macrophage, the cell surface becomes sticky and numerous fan-like folds develop. Macrophages are scavenger cells with an enormous capacity for phagocytizing bacteria, dead cells, and other substances that litter tissue. They are part of the MPS. While circulating, the monocytes respond to inflammatory and cellular immune stimuli and are thought to be quite helpful in antigen processing and immunity. In this role, monocytes are often thought of as killer cells due to their search and destroy abilities. The monocyte is even capable of engulfing erythrocytes and protozoa. Bacteria that have a lipid capsule, such as *Mycobacterium tuberculosis*, fall victim to the monocyte with its high amount of lipase. Long known as perhaps the most efficient of the phagocytes, the monocyte has gained its most recent attention

since it was discovered that it is able to synthesize many important products such as interferon and certain growth factors. They also remove plasma proteins and lipids, and participate in iron metabolism.

### **003. The circulatory system**

Now that you know the composition of blood and its functions, how does it travel from its production site to the rest of the body in order to perform its life-saving functions? The answer is the circulatory system! The circulatory system is, in essence, a transportation system that links all the organ systems together. The circulatory system delivers nutrients, oxygen, and hormones to the cells and transports cellular waste to the excretory organs. It also plays a vital role in defending the body against disease and maintaining hemostasis. The cardiovascular system and lymphatic system are two subsystems of the circulatory system.

#### **Cardiovascular system**

The cardiovascular system is comprised of the heart, arteries, veins, and capillaries. It is responsible for transporting the nutrients, oxygen, hormones, and waste. The blood could be called “the vehicle of transportation” in this system. The heart is a double or two-sided pump that circulates the blood through the arteries, veins, and capillaries. The heart has four chambers:

1. Right atrium.
2. Right ventricle.
3. Left atrium.
4. Left ventricle.

The atria are receiving chambers and hold the blood between contractions of the heart. The ventricles pump the blood into the great arteries leaving the heart: the aorta and pulmonary artery.

#### ***Circulation***

The circulation of blood depends on several factors. The most important of these are the rhythmic beating of heart, the volume of blood circulated, the condition of the blood vessels, and a system of valves to control the direction of flow. Follow the course of blood, as shown in figure 1–2, during circulation through the heart. As the heart beats, muscles of the atria contract in a wave-like manner, sending blood into both ventricles and into one of two arteries. Depending on which artery the blood enters, the start of pulmonary circulation or systemic circulation has begun. The pulmonary artery marks the start of the pulmonary circulation, while the aorta is the first part of systemic circulation.

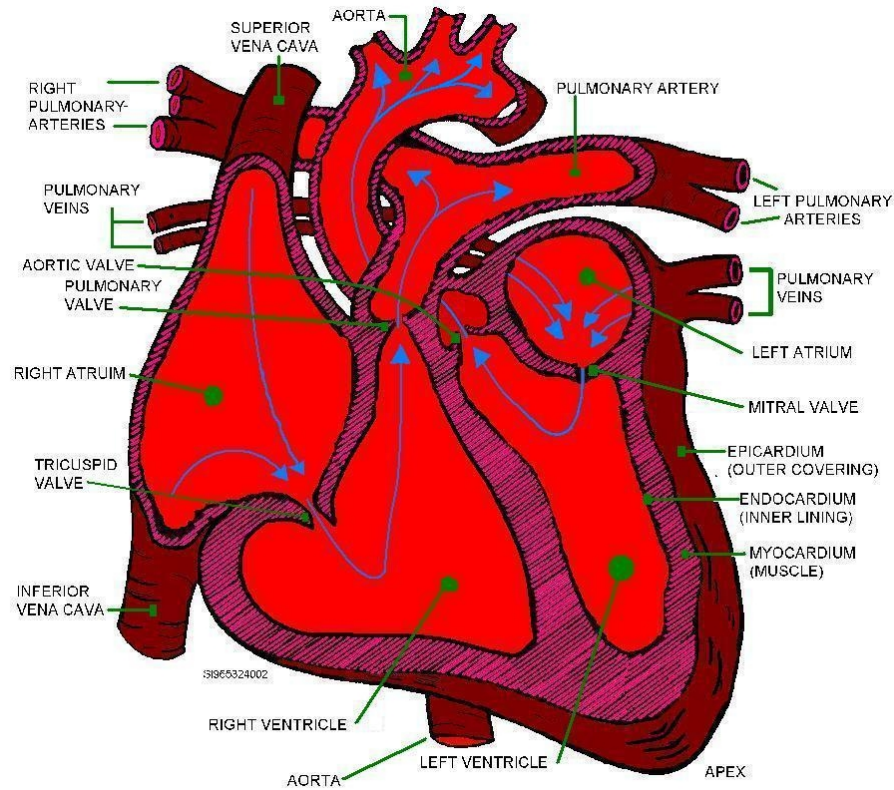


Figure 1–2. Blood circulation through the heart.

### *Pulmonary circulation*

Sometimes called the lesser circulation, this circulation takes blood through the lungs for the purpose of oxygenation. All venous blood returning from the body enters the right atrium through the superior vena cava or the inferior vena cava and enters the right ventricle through the atrioventricular (tricuspid) valve. As the right ventricle contracts, venous blood is forced through the pulmonary valve to the pulmonary artery, which carries the blood to the lungs. There the blood, through diffusion, exchanges waste carbon dioxide for oxygen. Oxygenated blood is then returned to the left atrium via the pulmonary veins, and passes through the mitral valve into the left ventricle.

### *Systemic circulation*

From the left ventricle, blood flows through the aorta en route to all parts of the body and follows various courses through arteries, arterioles, and capillaries until it returns by way of the veins, through the vena cava, to the right atrium. Systemic circulation includes coronary circulation, which feeds the heart itself, and portal circulation, which involves passage of venous blood from the gastrointestinal tract and spleen, through the liver, and out to the inferior vena cava through the hepatic veins.

### *Respiration*

Respiration is defined as the exchange of gases between the body and air. External respiration, then, is the exchange of gases between the atmosphere and the cells of the lungs, and internal respiration is the exchange of gases between the individual body or tissue cells and the circulating blood.

### *External respiration*

The exchange of oxygen between the alveoli of the lungs and the capillary is due to the great difference between the pressures. Since, according to the law of gaseous diffusion, gases flow from a high pressure area to one of lower pressure, oxygen diffuses through the walls of the alveoli and into the bloodstream. At the same time that oxygen is being exchanged, carbon dioxide—waste gas—is going through a similar transfer. The exchange proceeds from the capillary blood into the alveoli. Although the carbon dioxide pressure gradient is lower, a large quantity of gas flows across into the alveoli. This is explained by the fact that carbon dioxide has a greater coefficient of diffusion than does oxygen. As this exchange continues throughout the course of the capillary, the pressure of carbon dioxide in the blood falls until it reaches its normal value. As the two gases flow in and out of the alveoli, the blood is oxygenated. The oxygen content is high, and the carbon dioxide content is low. The blood then returns to the left side of the heart to be pumped throughout the body to all tissue cells. At this level, the second phase of respiration occurs—internal respiration.

### *Internal respiration*

As pure, highly oxygenated blood with low carbon dioxide leaves the left side of the heart, blood enters the capillaries and begins respiration between the tissue cells and the blood, this is known as internal respiration. In the cell, oxidation is occurring constantly and, consequently, the oxygen content is at a low level. It varies in value, depending on the activity of the cell at the time. During exercise, for example, a muscle cell has a lower oxygen pressure than it does during a period of rest. Since the oxygen pressure in the blood on the arterial side of the capillary is always higher than it is in the tissue cell, a large pressure gradient exists, and there is again a flow of oxygen to the lower pressure—in this case, from the blood into the cell. This transfer of oxygen is actually occurring through the length of the capillary until the pressure of oxygen in the blood gains equilibrium with the oxygen pressure in the cell. While the transfer of oxygen is occurring, there is also a transfer of carbon dioxide. The constant metabolic activity of the cell results in a high production of carbon dioxide. Even though a much smaller pressure gradient exists for this gas than for oxygen, a larger amount of carbon dioxide is forced from the cell into the blood due to the diffusion characteristics of carbon dioxide. Some of the carbon dioxide combines with hemoglobin and some is carried in the plasma. Thus, with the total exchange completed, as the arterial blood passes the cell and enters the venous system, the blood returns to the heart to be pumped to the lungs via the pulmonary artery for another cycle of gaseous exchange.

### *Blood pressure*

Blood pressure is that pressure exerted on any blood vessel; however, it is more often thought of as the pressure that exists in the large arteries at the height of the pulse or heart beat. This peak of the pulse wave results from contraction of the atria, called the atrial systole, causing blood to be forced through the atrioventricular valve into the ventricles. The peak pressure is the systolic blood pressure. When it is measured in the brachial artery of the arm, a normal adult should exhibit a systolic pressure of 90 to 140 mmHg. The atrial systole is followed immediately by an atrial diastole as blood flows into the empty atria. Normal adult diastolic blood pressure is considered to be 60 to 90 mmHg.

### **Lymphatic system**

The lymphatic system consists of: a clear, watery fluid called lymph, lymphatic vessels, diffuse lymphatic tissue, lymph nodules, lymph nodes, thymus, spleen, and tonsils. The principle functions of the lymphatic system are:

- Collecting excess interstitial fluid.
- Defending the body against disease.
- Absorbing lipids from the intestine and transporting them to the blood.



***Collecting excess interstitial fluid***

Because blood is under a high amount of pressure when entering the capillaries, some plasma is forced out through the pores of the capillary wall. The plasma that has left the blood is called interstitial fluid or tissue fluid. Interstitial fluid contains oxygen, glucose, amino acids, protein, other nutrients, and only a few white blood cells (no red blood cells or platelets). This fluid bathes the surrounding tissue's cells and supplies the needed nutrients to these cells. Most of the interstitial fluid reenters the blood capillaries at the venous end of the network. The rest of the fluid is absorbed by the lymph capillaries and is now called lymph. The lymph is carried through the capillaries to the lymphatics, to the lymph ducts, and then is returned to the blood as plasma. If not for the activity of the lymphatic system, the fluid balance of the body would be seriously disturbed within a few hours. Death would occur in about 24 hours.

**NOTE:** Edema is the excess accumulation of interstitial fluid which can be caused by an obstruction of the lymphatic vessels. The obstruction may be due to injury, inflammation, surgery, or parasitic infection.

***Defending the body against disease***

Lymph nodules are small masses of lymph tissue that produce lymphocytes. Tonsils are actually aggregates of lymph nodules, strategically placed, to defend against invading bacteria. Lymph nodes or lymph glands are small capsule-like masses that filter the lymph fluid and also produce lymphocytes. The spleen is the largest lymphatic organ and is similar to the lymph nodes. However, the spleen filters blood instead of lymph. Its main function is to bring the blood into contact with the lymphocytes. If there are any disease organisms or foreign invaders in the blood, they activate the lymphocytes, which in turn start the body's immune system. This system is discussed in more detail in 4T051C, volume 2—*Immunology*. The function of the thymus gland is still not completely understood, although we do know that it plays a vital role in immunity and production of T-lymphocytes.

***Absorbing lipids from the intestine and transporting them to the blood***

There are over 100 lymph nodes in the mesenteries of the intestine that filter the lymph returning from the intestinal walls. The lipids and nutrients absorbed from the filtered lymph are transported back to the blood through the lymphatic vessels.

As you can see, understanding the composition and functions of the blood can be very complicated. With this basic foundation of the blood we'll look at the hematology laboratory environment.

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**Self-Test Questions**

**After you complete these questions, you may check your answers at the end of the unit.**

**001. The composition and functions of blood and basic cell structure**

1. What is blood?
2. What are the three formed elements of blood?
3. What are the seven functions of blood?

4. Match each basic cell structure in column B with the statement in column A. Element in column B may be used once, more than once, or not at all.

____ (1)	Column A	Column B
____ (1)	Known as the powerhouse of the cell.	a. Cellular membrane.
____ (2)	Contains the tiny structures called organelles.	b. Cytoplasm.
____ (3)	Manufactures proteins.	c. Lysosomes.
____ (4)	They act as a protein processing and packaging plant.	d. Vacuoles.
____ (5)	Composed of three layers; outer protein, middle lipid, and inner protein.	e. Ribosomes.
____ (6)	Membranous sacs that contain enzymes that break down ingested materials.	f. Endoplasmic reticulum.
____ (7)	These pipes transport material from one part of the cell to another.	g. Centrioles.
____ (8)	Largest organelle in the cell.	h. Golgi apparatus.
____ (9)	Contains the gene chromosomes.	i. Mitochondria.
____ (10)	Two types; rough and smooth.	j. Nucleus.

### 002. Origin of cellular elements

1. When and where does hematopoiesis for red blood cells start?
2. Around what month(s) in fetal development does the bone marrow assume its primary role in hematopoiesis?
3. What is medullary hematopoiesis?
4. What is extramedullary hematopoiesis?
5. Where are the lymphocytes produced?
6. What are the three groups of lymphocytes and what is their percentage of total lymphocytes?
7. The body's interaction of T-cells in the immune system is called?
8. The immune system's use of B cells is called?

9. What glycoprotein stimulates erythropoiesis and where is it mostly produced?
10. What is the erythrocytes' function?
11. What system breaks down old RBCs?
12. What is the MPS composed of?
13. What is the largest cell found in normal bone marrow?
14. As the megakaryocyte matures, it liberates what type of cell and what is the platelet's function?
15. What are the three types of granulocytes?
16. What is the largest WBC in the peripheral blood?

**003. The circulatory system**

1. What comprises the cardiovascular system?
2. What is the function of the cardiovascular system?
3. What are the most important factors on which the circulation of blood depends?
4. What are the two types of circulation?
5. What circulation includes coronary circulation?

6. Name the two types of respiration and what they do.
7. What is blood pressure?
8. What makes up the lymphatic system?
9. What are the three principle functions of the lymphatic system?
10. What is the largest lymphatic organ?

## **1-2. The Hematology Laboratory Environment**

As in 4T051B, the content of this lesson, “hematology laboratory environment,” refers to the overall physical and functional design of a workplace dedicated to the specific task of diagnostic clinical hematology. Thus, it is clearly distinct from other types of work environments by virtue of the nature of the work performed. The environment must be conducive for specimen analyses, yet, also provide a safe working environment.

### **004. Safety and specimen collection**

This lesson is a general review of good laboratory safety practices and specimen collections unique to the hematology laboratory. In-depth studies of laboratory safety and specimen collection are presented in 4T051A, therefore, only a brief discussion follows.

#### **Laboratory safety**

Laboratory safety is an important and complex subject. The most important element of laboratory safety is achieved primarily through the education and training of laboratory personnel about safety procedures and equipment.

#### ***Fire safety***

Basic fire safety equipment includes the telephone and fire alarm system, fire retarding doors, Class A, B, and C fire extinguishers, fire hoses, and fire blankets. However, the most important element of a good fire safety system is knowledgeable and trained laboratory personnel. This should be reinforced by periodic fire drills and continuing training sessions.

#### ***Chemical safety***

Basic chemical safety equipment includes: acid and alkali spill stations where material is stored and used to neutralize and contain those types of chemical spills; protective clothing, monitoring devices, and absorbent material that can be used for containing and decontaminating radioactive spills; and drench showers and eye washes for removing and diluting toxic or caustic chemicals.

***Mechanical safety***

Mechanical or physical hazards include equipment, compressed gases, glassware, and sharp instruments. Inappropriate storage, usage, and disposal of the aforementioned can cause serious injuries. Carefully reading manufacturers instructions before operating any equipment ensures safe handling. Always follow guidelines for storage and handling of compressed gasses. Treat glassware carefully to avoid breakage. Dispose of glassware and sharp objects in puncture-proof containers in order to avoid injury or exposure to infectious agents. Handle sharp instruments (including needles) carefully to avoid punctures or cuts.

***Electrical safety***

Electrical hazards are caused by improper use or maintenance of electrical equipment. Inappropriate use can cause electrical shock, burns, fire, and/or explosions. We know water and electricity are a dangerous combination, but, nonetheless, certain laboratory instruments contain water or other liquids. Be careful when operating this type of equipment. Immediately wipe up all spills and don't operate instruments with wet hands. Inspect equipment for frayed wires and electrical connections. Ground all equipment by using a three-pronged plug.

***Biohazard safety***

The basic strategy of a biohazard safety program is to prevent exposure to infectious disease agents. The Center for Disease Control and Prevention (CDC) Standard Precautions covers exposure to blood and body fluids for protection against biohazards. These precautions have come about as a response to the problems associated with the spread of the human immunodeficiency virus (HIV). As a laboratory technician, you have no way of knowing whether your patients have HIV or any other blood borne pathogen; so you must conduct yourselves accordingly to prevent the possible spread of this or any other disease. Biohazards probably cause the greatest concern in hematology because hematology's primary function is the examination of whole blood and body fluids. Listed is a review of safety rules for the hematology laboratory.

- Always wear proper personal protective equipment (i.e., gloves and lab). If at risk of blood exposure from aerosols or splashes, use masks, face shields, and/or goggles.
- Practice safe personal habits such as no eating, drinking or smoking in the work area.
- Never pipette by mouth! Don't put pens, pencils, or any other objects in your mouth.
- Wash hands frequently and keep hands away from mouth, nose, eyes, and other mucous membranes.
- Keep work areas free of chemicals, spills, and dirty glassware. Disinfect biohazard spills immediately.
- Properly label all reagents and solutions. Post warning signs as needed.
- Use an absorbent material when removing stoppers from a blood specimen tube, either behind a safety shield or wearing a face shield to protect from splash.
- Always centrifuge tubes with stoppers in place to prevent infectious aerosols.
- Use a clear safety shield when manipulating blood samples.
- Frequently replace clay slabs for microhematocrit tube sealing. Never remold to recycle because there may be small shards or other sharp remnants embedded in the clay.
- Decontaminate sedimentation tube racks regularly.
- Properly dispose of contaminated laboratory supplies in biohazard waste containers.
- Do not operate new or unfamiliar equipment until trained.
- Read all labels and instructions thoroughly.

- Unfixed or unstained specimen slides should be considered infectious.
- Learn emergency procedures.

### **Specimen collection**

Collecting a proper specimen is the first step toward reporting accurate and reliable test results. The majority of hematology specimens are acquired through venipuncture, capillary puncture, and skin puncture. Proper specimen collection requires knowledge of the routine and special methods employed in hematology. Most hematology tests are performed on whole blood specimens, which are usually collected by laboratory personnel. However, the hematology laboratory is responsible for the examination of other body fluids that are collected by physicians or patients. Understanding their origins and collection procedures aids in the examination process.

### **Whole blood specimens**

Because nearly all hematology procedures require whole blood, and once blood leaves the body it begins to clot, it must be mixed with anticoagulants. In hematology, the most commonly used anticoagulants are ethylenediaminetetraacetic acid (EDTA), trisodium citrate, and heparin. The choice of anticoagulant depends on the test method. It is important to remember that an appropriate ratio of anticoagulant to blood is critical; significant errors may result with incorrect anticoagulant concentrations.

#### ***EDTA***

The disodium and tripotassium salts of EDTA are excellent anticoagulants that either chelate or bind calcium ions. EDTA is the anticoagulant of choice because it is in liquid form, and, therefore, goes into solution easier. At a concentration of 1.5 mg/mL blood, EDTA prevents artifacts and preserves cellular morphology when blood films are made within two hours. You can store blood for 24 hours at 4 to 6° C without changing hemoglobin, hematocrit, leukocyte count, or erythrocyte count. However, you may see artifactual changes in WBC morphology (i.e., reactive lymphocytes, degenerating neutrophils) and RBC morphology (i.e., pyknotic nucleus). EDTA is excellent in prevention of platelet clumping; however, EDTA blood cannot be used for platelet function studies. In concentrations above 2 mg/mL blood, erythrocytes shrink, causing sedimentation and hematocrit values to be falsely decreased.

#### ***Citrate***

The anticoagulant of choice for coagulation studies is trisodium citrate. Coagulation is prevented by binding calcium in a soluble complex, thereby protecting most labile clotting factors. Proper concentration is one part 0.129 M (3.8 percent) buffered sodium citrate to nine parts blood. Citrate tubes must be adjusted for hematocrit values of <20 percent and >55 percent. Also, the 9:1 ratio of blood to anticoagulant is critical. The Clinical and Laboratory Standards Institute [CLSI; formerly NCCLS] allows for ± 10 percent of blood to anticoagulant. The formula for adjusting citrate tubes for low or high hematocrit values is as follows. Examples are given in unit 4.

$$\text{Volume of anticoagulant} = 0.00185 \times \text{Volume of blood} \times (100 - \text{hematocrit percent})$$

#### ***Heparin***

This anticoagulant prevents clot formation by neutralizing thrombin when mixed at a concentration of 15 to 20 U/mL of blood. Heparin is the anticoagulant of choice for osmotic fragility tests. It is unsatisfactory for automated cell counters and making blood films (due to distortion of platelets and leukocytes). Also, it should never be used for coagulation studies because it inhibits thrombin but it may be used for STAT chemistries that require plasma.

**Bone marrow specimens**

Hematology personnel usually assist a pathologist or physician performing a bone marrow procedure. The laboratory is responsible for the sterile bone marrow collection trays used during the procedure. Disposable trays may be purchased from commercial vendors; however, additional supplies may be necessary. The laboratory designs the trays according to the pathologist and/or physician's requirements and recommendations. If supplies are to be reused, they must be cleaned and sterilized. Sterile central supply or central processing distribution (CPD) will sterilize the supplies and may set up the trays.

***Recommended equipment***

The recommended equipment includes an aspiration needle, a trephine biopsy needle, two or more 30 to 35 mL syringes (glass or plastic), one small syringe for the administration of a local anesthetic, sponges, forceps, drapes or towels, 4 x 4 sterile gauze, lancet, sterile gloves, and cleaning or disinfecting solutions.

***At the patients bedside***

If requested, finger stick blood films should be prepared before the bone marrow is obtained. The trephine or core biopsy is obtained before aspiration to avoid any disruption of marrow structure. The specimen is placed on sterile gauze and "touch preps" are made by carefully touching the slides to the specimen. The biopsy specimen is then placed in a fixative solution for histology. Immediately after the biopsy, the bone marrow is aspirated. Because megakaryocytes release tissue thromboplastin, which shortens the clotting time, speed is very important during the aspiration. Direct films must be made immediately. Some of the aspirate may be placed in EDTA (1 mg/mL of marrow) and mixed gently for making additional smears if needed.

**Body fluids specimens**

There are basically four types of body fluids: (1) cerebrospinal fluid (CSF), (2) synovial fluid, (3) serous fluids (i.e., peritoneum, pleura, and pericardium), and (4) seminal fluid. Seminal fluid is quite different from all the other body fluids mentioned in this lesson, so it will be discussed under its own heading. These fluids are known as ultrafiltrates. They are a product of ultrafiltration, which is filtration through filters with minute pores that separate extremely minute particles. Ultrafiltration occurs naturally within the body through the filtration of plasma at the capillary membrane in the various areas where these fluids are produced. Be aware that analysis of body fluids may be a "one shot deal" because of the scarcity of fluid in these areas so treat the fluid as a precious commodity. Therefore, immediate processing is important due to the deterioration of these fluids. Body fluid examinations are required to rule out or diagnosis infections, bleeding, presence of crystals, malignancies, and other disorders.

***CSF***

CSF fills the cavities within the brain and flows over the brain and the spinal cord. CSF is 99 percent water, but also contains glucose, proteins, urea, and salts. It is produced by the choroid plexuses (networks of capillaries) through ultrafiltration and active secretion. The capillary endothelium that contacts CSF makes up the anatomic structure of the blood-CSF barrier. The endothelium of these capillaries controls the passage of various substances into the CSF from the blood. The total volume of CSF for adults is 90 to 150 mL, and for neonates it is 10 to 60 mL. The CSF protects the brain from sudden changes in pressure and acts as a protective cushion for the underlying central nervous system (CNS) tissue. It lubricates the CNS and is a site for metabolic exchange of nutrients and waste. Lumbar punctures are performed because of four major reasons:

1. Meningeal infections (e.g. meningitis, encephalitis, syphilis).
2. Subarachnoid hemorrhage.

3. CNS malignancy (e.g., acute leukemia and lymphoma, and spinal cord or brain tumor with CSF involvement).
4. Demyelinating diseases (e.g., multiple sclerosis, Guillain Barré syndrome).

### ***Synovial***

Synovial fluid is also an ultrafiltrate of plasma and contains a significant amount of a mucopolysaccharide—hyaluronic acid. The synovial cells (that line the synovial membrane) phagocytize debris within the synovial fluid as it passes along the membrane. Synovial fluid is present in the joints and provides lubrication and nourishment for the cartilage. Concentrations of protein and immunoglobulins present in the synovial fluid are about one-fourth of those in plasma. However, concentrations of glucose, uric acid, and electrolytes are similar to those in blood. Bacteriologic, immunologic, mechanical, and chemical changes may alter the permeability of the membranes and capillaries, resulting in an inflammatory response.

### ***Serous***

Serous fluid is located in between layers of connective tissue which forms a sac. A sac surrounds the abdominal organs, lungs, and heart where it reduces friction and facilitates movement between the surfaces. The sac that encloses the abdominal organs is the peritoneum; for the lungs is the pleura; and the heart is the pericardium. The accumulation of fluid within these sacs creates a cavity. The fluid is referred to as an effusion. Two terms that describe the effusion are transudate and exudate. Transudate is a fluid that passes through the membranes by hydrodynamic forces and results in high fluidity with a low content of protein, cells, and/or solid material derived from cells. Exudate is a fluid that escapes from the blood vessels (usually due to inflammation) and results in a high content of protein, cells, and/or solid material from cells. Causes of transudate effusions are congestive heart failure, cirrhosis of the liver, hypoproteinemia with nephrotic syndrome, postoperative abdominal surgery, postpartum effusion, peritoneal dialysis, and superior vena cava obstruction. Causes of exudate effusions are infectious diseases (e.g., bacterial, viral, fungal, parasitic, and tuberculosis), neoplastic disease, pulmonary embolization or infarction, rheumatoid arthritis, systemic lupus erythematosus, gastrointestinal disease, postmyocardial infarction, and trauma.

### ***Peritoneum***

The peritoneum is a delicate, smooth serous membrane that covers the walls and viscera of the abdomen and pelvis, and it holds the abdominal organs in place. An inflammation of the peritoneum is called peritonitis. Peritonitis can be serious if the infection spreads to the adjoining organs. In certain disorders (e.g., alcoholic cirrhosis of liver) fluid may accumulate within the peritoneal cavity causing a condition known as ascites. In addition to ascites, a paracentesis is performed to alleviate discomfort from the build up of large amounts of fluid and for diagnosis of liver disease, bacterial infections, biliary tract disease, ruptured bowel, malignant disorders, pancreatitis, intestinal infarction, and results of trauma. The fluid submitted to the laboratory is interchangeably called paracentesis fluid, peritoneal fluid, or ascitic fluid.

### ***Pleura***

The pleura consists of a thin double-layered membrane that separates the lung from the chest wall, diaphragm, and mediastinum. The space between the two layers is called the pleural cavity. The pleural fluid acts as a lubricant and reduces the friction between the two pleural membranes during breathing. Pleurisy is an inflammation of the pleura. The aspiration of pleural fluid is called thoracentesis.



### *Pericardium*

Similar to the pleura, the pericardium is a double-walled sac that surrounds the heart and the origins of the great vessels entering and leaving the heart. The double walls are separated by a small amount of fluid called pericardial. This fluid allows the heart to move easily during contraction and relaxation. The space between the two layers is known as the pericardial cavity. The procedure for aspirating pericardial fluid is called pericardiocentesis. Inflammation of the pericardium is referred to as pericarditis.

### *Body fluid aspirations*

All body fluid aspirations are collected under sterile conditions by a physician. As much fluid as possible is collected and placed in three tubes for:

TUBE	SECTION
1	Chemistry. [Most contaminated from skin, cell and bacterial components. Not sterile.]
2	Microbiology. [Most likely to contain the bacteria causing an infection. Sterile.]
3	Cellular/crystal examinations. [Furthest specimen from the trauma of the puncture. Sterile.]

Remaining supernatant from the other procedures can be used for immunological and special studies. Body fluids for cellular or crystal examinations are placed in EDTA tubes and transported to the laboratory as soon as possible. Other studies may be collected in sterile, plain, red-top tubes or according to your laboratory's operating instructions.

### *Seminal fluid specimens*

Semen analysis is performed in the hematology section because it encompasses a cell count. Semen examinations are required for infertility investigations, post vasectomy checks, and possibly for forensic studies in alleged or suspected rape and sexual assault cases.

### *Composition of seminal fluid*

Seminal fluid is composed of spermatozoa and seminal plasma. The spermatozoa originate from the testis. They are the only cellular elements found in normal semen, and they constitute less than 5 percent of the seminal volume. Approximately 60 percent of the seminal volume comes from the seminal vesicles and is a viscid, neutral, or slightly alkaline, yellow pigmented fluid. The prostate is the source of a milky, slightly acidic fluid that contributes about 20 percent of the volume. Less than 10 to 15 percent of the total volume is from the epididymides, vasa deferentia, bulbourethral glands, and urethral glands. These different fractions are rapidly mixed together in the urethra during ejaculation.

### *Instructions to the patient for specimen collection*

Give your patient direct, fully explained, and professional instructions. Although it would seem that the attending physician should give these instructions, the task is almost universally conducted by the laboratory. Quite often, the patient's wife requests instructions from the laboratory. For this reason, and many others, it is wise to make a written set of instructions for semen analysis. Have these instructions available at your reception desk along with plastic, wide-mouth containers with screw-on lids. If polyethylene containers are used, the specimen must be examined immediately upon liquefaction or should be transferred to a glass container since polyethylene may lower sperm motility results. Instruct the patient or his representative that sexual abstinence (normally 3 days) is required before specimen collection. Hand the individual a small brown sack containing full printed instructions and a specimen container. After the individual has read the instructions, ask him or her whether there are any questions; this completes the job. Nobody is embarrassed, and this type of briefing can be handled discreetly by male or female technicians alike. Additionally, it may be

prudent to include the phone number for the laboratory as an added convenience. The specimen must be collected, by either masturbation or coitus interruptus, in a prewarmed, room temperature container. It is recommended that the specimen collection time and the percent of specimen collected be annotated either on the container or on the laboratory slip. It is very important to know if the entire specimen has been collected since the sperm count varies considerably in different portions of the ejaculate. Once collected by the patient, the specimen should be received by the laboratory within 1 hour. (During transportation, the specimen must be protected from extreme temperatures to prevent loss of motility; keep at body temperature. This can be accomplished by placing the container in the front pants pocket.) For these reasons, it is best to collect the specimen in a private area near the laboratory if at all possible.

### **005. Quality improvement, quality control, and hematology terminology**

Quality improvement in hematology (and throughout the laboratory) should be an established integral part of our daily operation. Earlier it was stated that proper specimen collection is the first step in providing accurate and reliable test results. Once the correct specimen is obtained, what are the other steps involved in providing correct results? We could probably make an extensive list of the different steps, but, I would venture to say that almost all, if not all, the steps would fall under the umbrella of quality improvement. Quality improvement (QI) is basically doing “the right thing better!”

#### **Quality improvement**

Quality improvement, also known as quality assurance, is a program intended to monitor all the steps or methods used to assure accurate and reliable results. This includes overall laboratory management and components of quality control. The Joint Commission on Accreditation of Healthcare Organizations (JCAHO) defines quality assurance (improvement) as a well-defined, organized program designed to enhance patient care through the ongoing objective assessment of important aspects of patient care and the correction of identified problems.

A meaningful quality improvement program must be able to monitor both quality control (QC) and other laboratory activities. The activities can be divided into two basic categories: (1) analytic QC and (2) nonanalytic control. The category QC is discussed shortly in this volume. Nonanalytic control encompasses all activities not directly associated with the performance of the clinical assay itself. This includes both preanalytical and postanalytical activities.

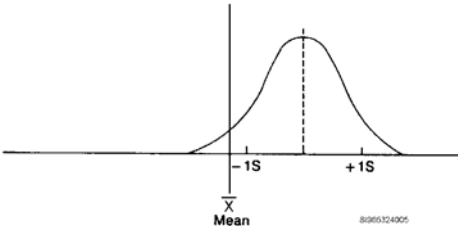
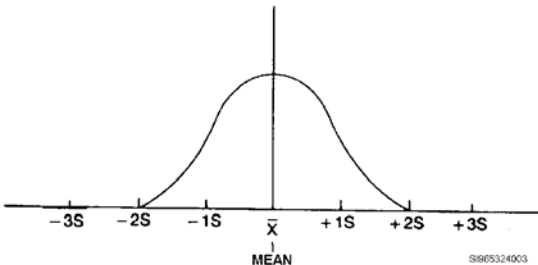
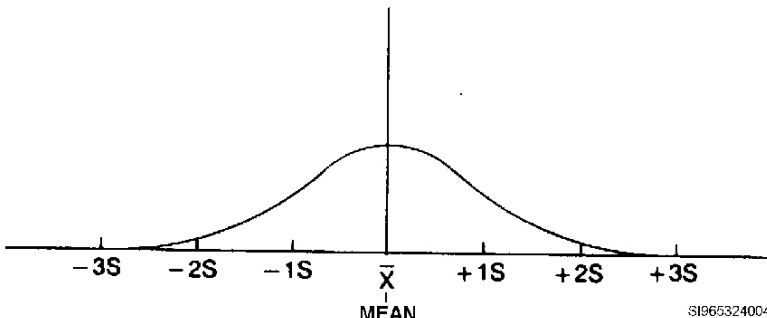
Activities	Explanation
Preanalytical	Ordering test; ensuring correct patient identification and preparation; specimen collection, transport, identification, and accessing; and specimen processing for analysis.
Postanalytical	Result validation, reporting results, workload recording or test charging, and specimen storage.

#### **Quality control**

Chemistry QC is relatively well established and direct. A pure chemical substance can be weighed and placed into a solution to create a standard of an exact known value. For example, a 75 mg/dL glucose solution can be made and used for calibration. However, hematology measures living cellular elements that lack stability. Therefore, it is difficult to produce stable standards and controls for hematology procedures. Quality control for hematology, to a degree, still lacks clear-cut standardized approaches. In volume 1, 4T051A, information on QC was presented in detail. A brief review of important terms and QC specific for hematology will be given in this area.

## Definitions

The following table gives you terms and definitions you need to know about quality programs.

Term	Definition
Systematic errors	Systematic errors may be due to deteriorating reagents or improper instrument calibration. They affect all results within the run or batch and can usually be detected by testing control specimens. Systematic errors introduce a bias (change of mean) into a procedure, as shown in figure 1-3, compared with a stable analytical procedure in figure 1-4.
<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  <p>Figure 1-3. Example of systemic error.</p> </div> <div style="text-align: center;">  <p>Figure 1-4. Stable procedure with a normal curve.</p> </div> </div>	
Term	Definition
Random errors	Are the result of chance, normally do not affect an entire run or batch of samples, and may not be detected by testing control specimens. Random errors introduce increased variability into a procedure as shown in figure 1-5.
<div style="text-align: center;">  <p>Figure 1-5. Example of increase in random error.</p> </div>	
Accuracy	Defined by the International Committee for Standardization in Hematology (ICSH) as agreement between the best estimate of a quantity and its true value.
Precision	The reproducibility or the variation of repeat measurements of the same analyte. In hematology an example of this is the consistency of a cell count between the first and second measurement or the measurement done on two different days
Primary standards	Are pure, stable, precise in concentration, and are used for calibration. There are no primary standards used in hematology.
Secondary standards	Whole blood samples that have been measured by a reference method and has a known value that is a reasonable estimate of the true value. Reference methods that are acceptable for instrument calibration are selected by the ICSH and Clinical and Laboratory Standards Institute (formerly NCCLS).
Controls	Defined by the ICSH as a substance used in routine practice for checking the concurrent performance of an analytical process or instrument. Controls must be similar in properties to, and be analyzed along with, patient specimens.

Term	Definition
<i>Mean</i>	<p>The average value of a group of measurements. See figure 1–6 for the equation.</p> $\bar{x} = \frac{\sum x_i}{n}$ <p> <math>\bar{x}</math> = mean  <math>x_i</math> = individual observation  <math>\sum</math> = sum of  <math>n</math> = number of observations </p> <p><b>MEAN EQUATION</b> SI965324007</p> <p><b>Figure 1–6. Mean equation.</b></p>
<i>Range</i>	The difference between the smallest and largest values in a data group.
<i>Variance</i>	<p>Standard deviation, as shown in figure 1–7.</p> $SD^2 = \frac{\sum (\bar{x} - x_i)^2}{n - 1}$ <p><math>SD^2</math> = variance</p> <p><b>VARIANCE EQUATION</b> SI965324010</p> <p><b>Figure 1–7. Variance equation.</b></p>
Term	Definition
<i>Standard Deviation (SD)</i>	<p>Sum of the squared deviation of each observation from the mean divided by the number of observations minus 1 as in figure 1–8.</p> $SD = \sqrt{\frac{\sum (\bar{x} - x_i)^2}{n - 1}} \text{ or } SD = \sqrt{\frac{\sum d^2}{n - 1}}$ <p><math>d</math> = difference of <math>\bar{x}</math> and <math>x_i</math></p> <p><b>STANDARD DEVIATION EQUATION</b> SI965324008</p> <p><b>Figure 1–8. Standard deviation equation.</b></p>
<i>Coefficient of variation (CV)</i>	<p>Is calculated as the SD divided by the mean and expressed as a percentage as in the equation shown in figure 1–9.</p> $\% CV = \frac{SD}{\bar{x}} \times 100 \text{ or } CV = \frac{SD}{\bar{x}} \times 100\%$ <p><b>COEFFICIENT OF VARIATION EQUATION</b> SI965324009</p> <p><b>Figure. 1–9. Coefficient of variation equation.</b></p>

***Quality control program***

A good quality control program includes procedures that ensure accurate and precise results through instrument calibration, controls, preventive maintenance, troubleshooting, documentation, and internal and external monitoring. The College of American Pathologists (CAP) publishes a *Laboratory Instrument Verification and Maintenance Manual*, which includes information on requirements, regulations, and standards of CAP, JCAHO, and CLSI. Sample forms for documentation, frequency, and type of maintenance for all laboratory equipment are presented in this publication.

***Standard deviation***

Each procedure you perform is subject to error due to mechanical, procedural, or technical difficulties. Operating a well defined and well disciplined quality control program can help overcome mechanical, procedural or technical difficulties that are sometimes encountered in each procedure. The use of controls greatly adds to your ability to detect these systematic errors. Standard deviation is an expression used to describe the permissible range into which a control must fall to be considered in control. See figure 1-10 for an example of calculating a monthly standard deviation for a hemoglobin control. If the control is outside these limits, the reason must be sought, explained, and annotated. You equate this acceptable range to be  $\pm 2$  SD from the mean value of the control. When the SD is used in conjunction with the mean, the shape of the normal distribution curve is completely defined. When plotting the observations, 68 percent of the values will fall within  $\pm 1$  SD, 95 percent of the values will fall within  $\pm 2$  SD, and 99.7 percent of the values will fall within  $\pm 3$  SD as shown in figure 1-11. Any value outside of  $\pm 3$  SD is statistically unacceptable. If the procedure is performed correctly and is in control, the values should be within  $\pm 3$  SD. The coefficient of variation, expressed as a percentage, gives a more understandable picture of the deviation regardless of the nature of the measurement. These methods or principles are embodied in a large portion of QC programs in the laboratory.

***Reference ranges***

Each laboratory should establish its own reference range (formerly termed normal range) for all tests performed. You do this when the tests are introduced or modified. A statistical study design is produced before specimens or data are collected. Specimens are drawn from a variety of demographic factors, such as, sex, age, and race. At least 40 subjects should be used, but only 5 to 10 samples are drawn and tested per day. The measurements or analyses are to be performed by different technicians to avoid an erroneously narrow reference range.

***Calibration***

It is no longer acceptable to use preserved cells used as controls to calibrate or adjust instrumentation. Perform calibration using fresh whole blood and reference methods. If reference method results on fresh blood are not available in your laboratory, commercial calibration material with assigned values for each parameter should be purchased.

***Method validation***

Linearity checks determine or verify the capabilities of an instrument at high and low ends. (Commercial kits may be available for these checks). After January 1996, all new procedures also require method validation to verify linearity. Furthermore, a method validation is performed with major reagent changes, major part replacements, and, if indicated by QC problems.

PROCEDURE: HEMOGLOBIN		DATE: 3 Apr 05	CONTROL POOL NO.: 4	TECHNICIAN(S): SSgt Earle
A. Test Results	D. Differences From Average	E. Squared Diff. From Average	F. Sum of Squared Differences From Average: <u>0.4270</u>	
1. 14.1	.13	.0169	G. Calculation of Standard Deviation (SD):  $SD = \sqrt{\frac{\text{Sum of Squared Differences From Average}}{\text{No. Tests} - 1}}$ $SD = \sqrt{\frac{0.4270}{29}}$ $SD = \sqrt{0.014727}$ $SD = 0.12$	
2. 14.3	.07	.0049		
3. 14.3	.07	.0049		
4. 14.4	.17	.0289		
5. 14.1	.13	.0169		
6. 14.0	.23	.0529		
7. 14.1	.13	.0169		
8. 14.3	.07	.0049		
9. 14.1	.13	.0169		
10. 14.4	.17	.0289		
11. 14.4	.17	.0289		
12. 14.2	.03	.0009		
13. 14.3	.07	.0049		
14. 14.1	.13	.0169		
15. 14.1	.13	.0169		
16. 14.2	.03	.0009		
17. 14.2	.03	.0009		
18. 14.4	.17	.0289		
19. 14.4	.17	.0289		
20. 14.1	.13	.0169		
B. Sum of Results: <u>427.0</u>			H. Calculation of Acceptable SD Range:  Acceptable limits = $\pm 2 \times SD$ or: $2 \times 0.12 = 0.24$	
C. Average Result: <u>14.23</u>				
			I. Test Control Limits  Average result: <u>14.2 g per dL</u> Upper limit: <u>14.4 g per dL</u> Lower limit: <u>14.0 g per dL</u>	
			* Consult a table of square roots	

Figure 1-10. Example of a monthly standard deviation calculation.

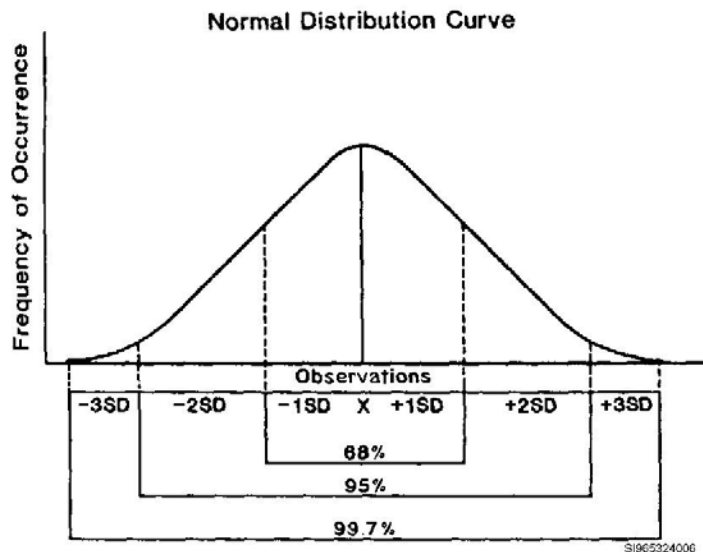


Figure 1-11. Gaussian distribution or normal bell-shaped curve with X as the mean.

### ***Controls***

Controls should be of the same matrix or material that is to be tested. For example, when the procedure requires whole blood then a whole blood control is used, when serum or plasma is required then the control should be serum or plasma. Controls for automated cell counters can be purchased commercially. These controls can provide day-to-day monitoring, but because of their short shelf life, they don't provide month-to-month monitoring. Three control levels, based on RBC parameters, are available: normal, low, and high. Lot-to-lot correlation of controls should be performed. This means that the new control lot is ran with the old control lot until the new lot's target value is established. The laboratory's target values do not have to match the manufacturer's mean values. However, if the values are outside the manufacturer's suggested ranges, the new lot has deteriorated, the control bottle is mislabeled, or the instrument is malfunctioning. Notify the manufacturer and do not use that lot number until the problem is resolved.

### ***Trends***

A trend is a series of control values that continue to move in the same direction (increase or decrease) over a period of time. They may be radical and easy to spot or develop over a period of days or weeks. The mean value will be seen to rise or fall continually, while the control values may still be within acceptable limits. Plotting control results makes the identification of trends much simpler. Trends are usually due to the deterioration of standards, controls, or instrumentation.

### ***Shifts***

This term implies that there has been a shift in the mean value for a given control. Whereas the previous control was giving the characteristic random distribution of values on both sides of the mean, all results now appear on one side or the other. The mean has actually moved or shifted, and now the values appear scattered accordingly around the new mean. Shifts demand immediate attention to control specimens, especially standards.

### ***Fresh whole patient blood controls***

In order to monitor instrument stability during the day or from day to day, the use of fresh, whole blood patient controls have been developed. A few patient blood samples are tested repeatedly throughout a 24-hour day to monitor the reproducibility of all values. Testing should be done at least every four hours and duplicate testing is encouraged to increase observations or measurements. Standard deviation and CV information is calculated and documented on these specimens (controls). Day-to-day monitoring consists of taking a specimen that is 24 hours old, warming it to room temperature, mixing, and testing again. This serves as a check for instrument reproducibility. Take deterioration of leukocyte and platelet counts into consideration before coming to an incorrect conclusion about the instrument. For procedures that do not have appropriate control material, (i.e., erythrocyte sedimentation rate, certain manual counts, and CSF counts), accuracy and reproducibility are maintained through strict attention to operating instructions (OIs), procedures, and proper training of personnel.

### ***X-bar-b analysis***

X-bar-b analysis is a method of quality control that frequently compares patient indices with known target values used to monitor automated hematology instruments. The Bull algorithm is a statistical system that incorporates patient data into a statistical formula and updates the data after every batch of 20 patient samples. Analysis of indices from various hospitals throughout the United States and the world revealed that the Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) are similar population groups. Indices are stable from day to day, week to week, and month to month. Once the mean value for each red cell index has been established for a given laboratory's patient population, the Bull algorithm is applied to evaluate the indices of consecutive groups of specimens as they are produced. The Bull algorithm

provides a sensitivity of one percent to changes in instrument calibration or patient population when the batch size is set at 20 samples. Laboratories that test more than 100 patient specimens per day should perform this analysis. Some hematology cell counters incorporate the Bull algorithm program. Others have computer programs in which you can load the data.

### **Documentation**

I'm sure you have heard the saying, "If it wasn't documented, it wasn't done." Documentation may be the single most important component of a good QC program. For that reason you must always document all QC and corrective actions. Without proper documentation your laboratory can be shut down, receive sanctions, as well as have criminal investigations in some cases.

### **Control charts**

Today, most multiparameter instruments include computer programs that automatically store and chart control results. However, if an instrument does not have its own QC program, you must manually chart the control data. Levey-Jennings Charts, Youden plots, and Cumulative sum charts are examples of ways to annotate control material (shown in figs. 1-12, 1-13, and 1-14, respectively).

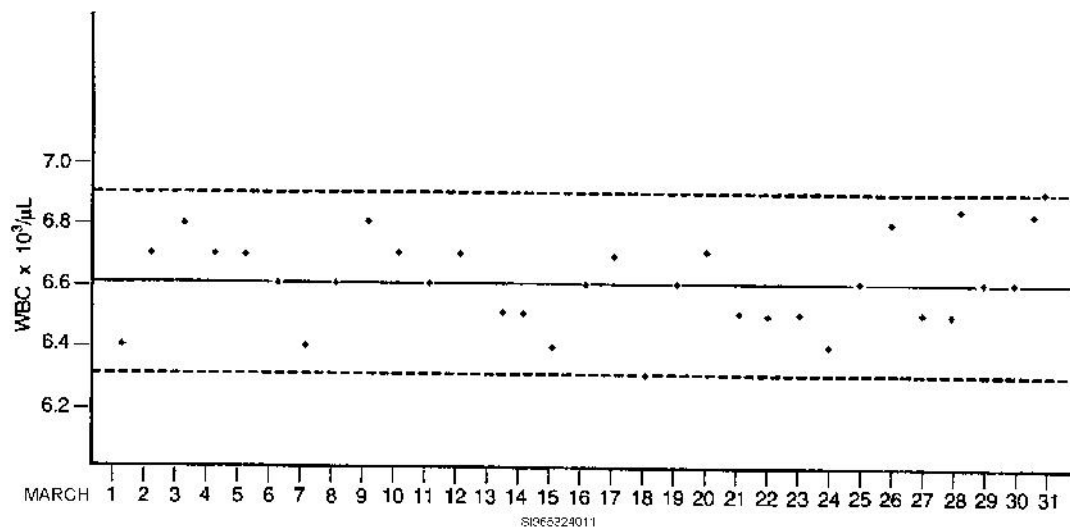


Figure 1-12. Example of Levey-Jennings chart.



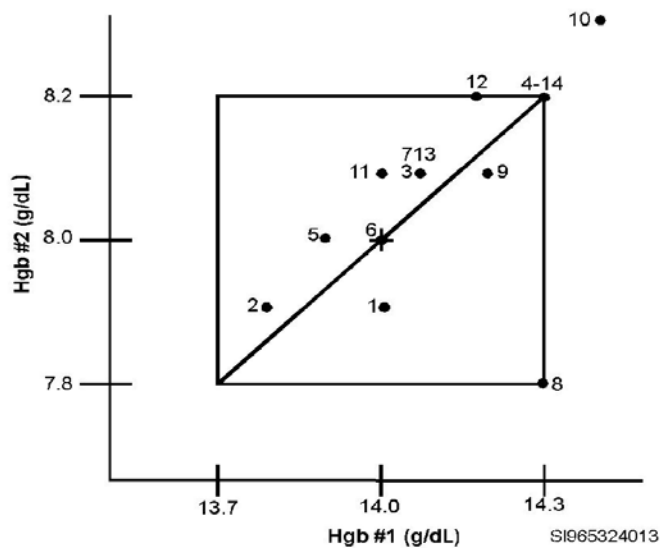


Figure 1-13. Example of a Youden twin-plot chart.

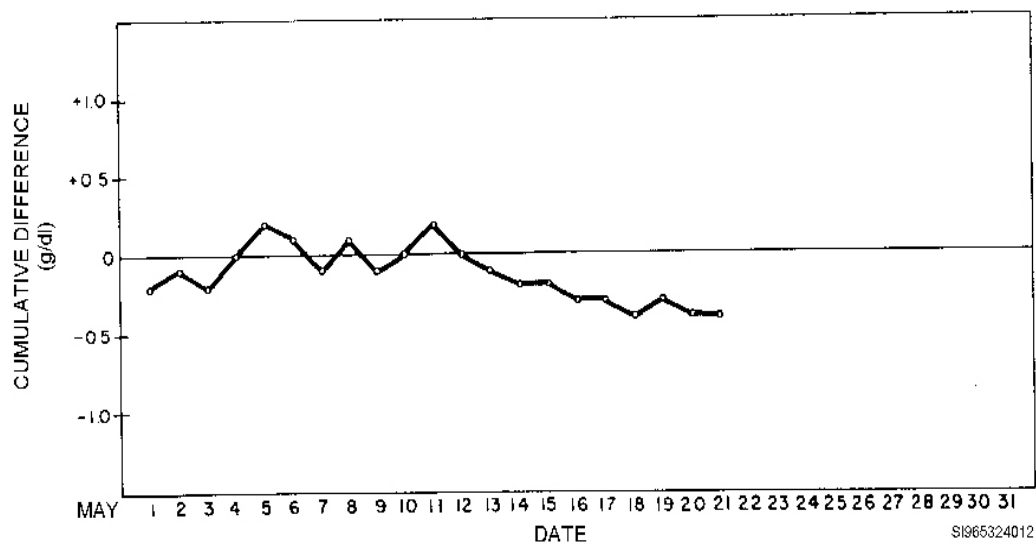


Figure 1-14. Example of a cumulative sum or difference chart.

#### *Instrument maintenance*

Routine instrument maintenance and troubleshooting procedures are also documented and maintained. The documentation should include the following information for troubleshooting instruments or equipment:

- Detailed information on the nature and frequency of the problem.
- The exact sequence of events.
- Identify instrumentation parameters, the cycle, or area involved.
- Record all corrective actions performed.

### ***Internal and external laboratory monitoring (proficiency testing)***

Internal monitoring can be accomplished by exchanging patient samples and blood films between laboratories within the same institution, exchange samples between instruments within the same lab, or between laboratories in the local area with similar instrumentation. Blood films can be exchanged on a weekly or bimonthly basis. Laboratories are required by federal agencies [(e.g., Clinical Laboratory Improvement Act (CLIA))] to monitor how patient specimen results statistically compare to the peer group. External laboratory monitoring can include regional, state, federal, and professional programs (i.e., College of American Pathologists (CAP) surveys). The data analyses from these programs can provide invaluable information on your QC program. For specific information on your laboratory's QC programs, consult your OIs, OIC, or technical supervisor.

### **Hematology terminology**

Hematology has a distinct terminology. A working understanding of the unique terminology will aid in learning and comprehending maturation and characteristics of blood cells and hematology procedures discussed in upcoming units. The table below presents the common prefixes and suffixes derived from Greek and Latin.

Prefix	Meaning	Suffix	Meaning
a-/an-	Without, lack, absent, decreased	-cyte	Cell
aniso-	Dissimilar, unequal	-emia	Blood
cyt-	Cell	-itis	Inflammation
dys-	Abnormal, difficult, bad	-lysis	Destruction of dissolving
erythro-	Red	-oma	Swelling, tumor
ferr-	Iron	-opathy	Disease
hypo-	Beneath, under, deficient, decreased	-osis	Abnormal increase or disease
hyper-	Above, beyond, extreme	-penia	Deficiency, decreased
iso-	Equal, alike, same	-phil(ic)	Attracted to, affinity for
leuk(o)-	White	-plasia (-plastic)	Cell production or repair
macro-	Large, long	-poiesis	Cell production, formation, and development
mega-	Large, giant	-poietin	Stimulates production
meta-	After, next, or change		
micro-	Small		
myel(o)-	From bone marrow or spinal cord		
pan-	All, overall, all-inclusive		
phleb-	Vein		
phago-	Eat, ingest		
poikilo-	Varied, irregular		
poly-	Many		
schis-	Split		
scler-	Hard		
splen-	Spleen		
thromb(o)-	Clot, thrombus		
xanth-	Yellow		

### Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

#### 004. Safety and specimen collection

1. What is the most important element of laboratory safety?
2. What are mechanical hazards?
3. What reaction can inappropriate use of electrical equipment cause?
4. What type of hazard probably causes the greatest concern in hematology?
5. Why is it considered unsafe to reshape or recycle clay for microhematocrit testing? What should be done instead?
6. What is the first step in reporting accurate and reliable test results?
7. What are the most commonly used anticoagulants in hematology?
8. At what concentration of EDTA to blood prevents artifacts and preserves cellular morphology when blood films are made within two hours?
9. Heparin is the anticoagulant of choice for what procedure?
10. What role do hematology personnel play during a bone marrow procedure?
11. When fingerstick blood films are requested in a bone marrow procedure, when are they prepared?
12. How is a bone marrow “touch prep” slide made?

13. What are the four types of body fluids?
14. Why is seminal fluid examined?
15. What is the only cellular element found in normal semen?
16. If polyethylene containers are used, the specimen must be examined immediately upon liquefaction or should be transferred to a glass container, why?

**005. Quality improvement, quality control, and hematology terminology**

1. What is quality improvement?
2. How does JCAHO define quality assurance (improvement)?
3. Match each quality improvement or quality control term in column B with the statement in column A. Each item in column B may be used once, more than once, or not at all.

<i>Column A</i>	<i>Column B</i>
____ (1) Pure, stable, precise in concentration, used for calibration.	a. Preanalytical.
____ (2) The difference between the smallest and largest values in a data group	b. Postanalytical.
____ (3) Includes ordering test, specimen collection, and processing.	c. Systematic error.
____ (4) The square root of the variance.	d. Random error.
____ (5) The result of chance, normally does not affect entire run.	e. Accuracy.
____ (6) Due to deteriorating reagents or improper instrument calibration.	f. Precision.
____ (7) Reproducibility or variation of repeat measurements.	g. Primary standard.
____ (8) Expressed as a percentage.	h. Secondary standard.
____ (9) Reporting results and specimen storage.	i. Control.
____ (10) Agreement between the best estimate of a quantity and its true value.	j. Mean.
____ (11) A substance used for checking the concurrent performance of an analytical process.	k. Range.
	l. Standard deviation.
	m. Coefficient of variation

4. Each procedure you perform is subject to what errors?
5. What is a standard deviation?
6. What percentage of values falls within  $\pm 1$  SD,  $\pm 2$  SD and  $\pm 3$  SD, respectively?
7. What does lot-to-lot correlation of controls mean?
8. What is a trend in control values?
9. What is a shift in control values?
10. What are fresh, whole patient blood controls used for and how often should they be run?
11. What may be the single most important component of a good QC program?
12. What are the four things you document when troubleshooting instruments or equipment?
13. How does a working understanding of hematology terminology help you?

---

## Answers to Self-Test Questions

### 001

1. Blood is a tissue that consists of microscopically visible formed elements suspended in a pale yellow liquid medium.
2. Red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes).
3.
  - (1) Transporting oxygen from the lungs to the tissues.
  - (2) Transporting nutrients from the digestive system to the cells of the body.
  - (3) Preventing accumulation of waste by transporting metabolic waste from the cells to the excretory organs (skin, kidneys, and lungs).
  - (4) Transporting hormones from the endocrine glands and neurosecretory cells to the target tissues.

- (5) Protecting the body from disease and foreign invaders.
  - (6) Aiding in hemostasis.
  - (7) Regulating fluid and salt balance, and body temperature.
4. (1) i.
- (2) b.
  - (3) e.
  - (4) h.
  - (5) a.
  - (6) c.
  - (7) f.
  - (8) j.
  - (9) j.
  - (10) f.

**002**

- 1. At about 2 weeks of fetal development, in the yolk sac.
- 2. Around the fourth and fifth months.
- 3. The production of the major blood cells is accomplished in the bone marrow, from birth and through adulthood.
- 4. The production of blood cells in the liver and spleen if the bone marrow is unable to produce sufficient numbers of hematopoietic cells. This may happen in certain disease states.
- 5. In the bone marrow.
- 6. T- (thymus)–60 to 80 percent, B- (bone)–10 to 20 percent, and null cells-less than ten percent.
- 7. Cellular immunity.
- 8. Humoral immunity.
- 9. Erythropoietin, produced, for the most part, in the kidney.
- 10. To manufacture hemoglobin.
- 11. Mononuclear phagocytic system (MPS)
- 12. Monocytes and differentiated macrophages [Kupffer cells (liver), microglial cells (nervous system), osteoclasts (bone), histiocytes (connective tissue), and pulmonary alveolar macrophages (lungs)].
- 13. Megakaryocyte.
- 14. Platelet, platelets function in primary hemostasis.
- 15. Neutrophils, eosinophils, and basophils.
- 16. Monocyte.

**003**

- 1. Heart, arteries, veins, and capillaries.
- 2. Transports the nutrients, oxygen, hormones, and waste throughout the body.
- 3. The rhythmic beating of heart, the volume of blood circulated, the condition of the blood vessels, and a system of valves to control the direction of flow.
- 4. Pulmonary circulation and systemic circulation.
- 5. Systemic.
- 6. External respiration is the exchange of gases between the atmosphere and the cells of the lungs. Internal respiration is the exchange of gases between the individual body cell and the circulating blood.
- 7. That pressure exerted on any blood vessel; however, it is more often thought of as the pressure that exists in the large arteries at the height of the pulse or heart beat.

8. Lymph, lymphatic vessels, diffuse lymphatic tissue, lymph nodules, lymph nodes, thymus, spleen and tonsils.
9. (1) Collecting excess interstitial fluid.  
(2) Defending the body against disease.  
(3) Absorbing lipids from the intestine and transporting them to the blood.
10. The spleen.

**004**

1. Education and training of laboratory personnel about safety procedures and equipment.
2. Equipment, compressed gases, glassware, and sharp instruments.
3. Electrical shock, burns, fire, and/or explosions.
4. Biohazard.
5. There may be small shards or other sharp remnants embedded in the clay. Frequently replace clay slabs for microhematocrit tube sealing.
6. Proper specimen collection.
7. EDTA, citrate, and heparin.
8. At a concentration of 1.5 mg/mL blood,
9. Osmotic fragility tests.
10. Assist pathologist or physician and are responsible for preparing the sterile bone marrow collection trays used during the procedure.
11. Before the bone marrow is obtained.
12. The specimen is placed on sterile gauze and “touch preps” are made by carefully touching the slides to the specimen.
13. Cerebrospinal (CSF), synovial, serous, and seminal.
14. Infertility investigations, post vasectomy checks, and possibly for forensic studies in alleged or suspected rape and sexual assault cases.
15. Spermatozoa.
16. The polyethylene container may lower sperm motility results.

**005**

1. Quality improvement (QI) is a program intended to monitor all the steps or methods used to assure accurate and reliable results.
2. A well-defined, organized program designed to enhance patient care through the ongoing objective assessment of important aspects of patient care and the correction of identified problems.
3. (1) g.  
(2) k.  
(3) a.  
(4) l.  
(5) d.  
(6) c.  
(7) f.  
(8) m.  
(9) b.  
(10) e.  
(11) i.
4. Mechanical, procedural, and technical difficulties.

5. An expression used to describe the permissible range into which a control must fall to be considered in control.
6. 68, 95, and 99.7 percent.
7. This means that the new control lot is ran with the old control lot until the new lot's target value is established.
8. A series of control values that continue to move in the same direction (increase or decrease) over a period of time.
9. Implies that there has been a shift in the mean value for a given control.
10. To monitor instrument stability during the day or from day to day and ran at least every four hours during a 24-hour period.
11. Documentation.
12.
  - (1) Detailed information on the nature and frequency of the problem.
  - (2) Always record the exact sequence of events.
  - (3) Identify instrumentation parameters, the cycle, or area involved.
  - (4) Record all corrective actions performed.
13. A working understanding of the unique terminology will aid in learning and comprehending maturation and characteristics of blood cells and hematology procedures.

**Do the unit review exercises before going to the next unit.**

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## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter. When you have completed all unit review exercises, transfer your answers to ECI (AFIADL) Form 34, Field Scoring Answer Sheet.

**Do not return your answer sheet to AFIADL.**

1. (001) Which one of the following is *not* a formed element found in peripheral blood?
  - a. Histocyte.
  - b. Leukocyte.
  - c. Erythrocyte.
  - d. Thrombocyte.
2. (001) Which of the following is *not* a function of blood?
  - a. Aiding in hemostasis.
  - b. Lubrication and nourishment for the organs.
  - c. Transporting hormones from the endocrine glands.
  - d. Regulating fluid and salt balance, and body temperature.
3. (001) What cell organelle is responsible for the self-destruct system that breaks down the cell after death?
  - a. Nucleus.
  - b. Lysosome.
  - c. Mitochondria.
  - d. Golgi apparatus.



4. (001) The control center and largest organelle in the cell is the
  - a. nucleus.
  - b. ribosomes.
  - c. mitochondria.
  - d. endoplasmic reticulum.
5. (001) What effect does Wright's stain have on the nucleus?
  - a. Turns it pink.
  - b. Turns it light blue or bluish.
  - c. Turns it dark purple.
  - d. Has no effect.
6. (001) What effect does Wright's stain have on the mitochondria?
  - a. Turns it pink.
  - b. Turns it light blue or bluish.
  - c. Turns it dark purple.
  - d. Has no effect.
7. (002) If bone marrow is unable to produce sufficient numbers of hematopoietic cells in certain disease states, what may occur?
  - a. Extramedullary hematopoiesis may take place in the liver and spleen.
  - b. Extramedullary hematopoiesis may take place in the lungs and kidneys.
  - c. Erythropoiesis and Megakaryocytopoiesis may take place in the liver and spleen.
  - d. Erythropoiesis and Megakaryocytopoiesis may take place in the lungs and kidneys.
8. (002) What group of lymphocytes is responsible for cellular immunity?
  - a. B-cells.
  - b. T-cells.
  - c. Null cells.
  - d. Plasma cells.
9. (002) What group of lymphocytes is responsible for humoral immunity?
  - a. B-cells.
  - b. T-cells.
  - c. Null cells.
  - d. Microglial cells.
10. (002) Stem cell production of erythrocytes is stimulated by the glycoprotein called
  - a. lipoprotein produced in the liver.
  - b. lipoprotein produced in the kidney.
  - c. erythropoietin produced in the liver.
  - d. erythropoietin produced in the kidney.
11. (002) The tagging of RBCs with radioactive chromium has demonstrated that erythrocytes have a half-life of
  - a. 16 to 20 days.
  - b. 22 to 26 days.
  - c. 28 to 30 days.
  - d. 32 to 36 days.

12. (002) Made up of such cells as histiocytes and Kupffer's cells, what body system breaks down old RBCs?
  - a. Alimentary.
  - b. Cardiovascular.
  - c. Proprioceptive nervous.
  - d. Mononuclear phagocytic.
13. (002) The mature megakaryocytes, whose cytoplasm becomes more lobulated and granular with age, fragments off, and liberates the cell, is called a
  - a. platelet.
  - b. basophil.
  - c. monocyte.
  - d. lymphocyte.
14. (002) For every one cell in the circulating blood, there are probably 300 to 500
  - a. basophils in the cerebrospinal fluid.
  - b. eosinophils in the cerebrospinal fluid.
  - c. basophils in the tissues.
  - d. eosinophils in the tissues.
15. (002) What leukocyte is *most* likely to destroy bacteria of *M. tuberculosis*?
  - a. Monocyte, because it produces lipase.
  - b. T lymphocyte, because it uses antibodies.
  - c. Eosinophil, because it produces peroxidases.
  - d. Basophil, because it contains histamine granules.
16. (002) What phagocyte is considered the *most* efficient?
  - a. Monocyte.
  - b. Granulocyte.
  - c. Lymphocyte.
  - d. Megakaryocyte.
17. (003) Which one of the following is *not* part of the cardiovascular system?
  - a. Heart.
  - b. Arteries.
  - c. Capillaries.
  - d. Lymph nodes.
18. (003) The exchange of oxygen between tissue cells and blood is called
  - a. internal respiration.
  - b. external respiration.
  - c. systemic circulation.
  - d. pulmonary circulation.
19. (003) The pressure exerted on the arteries at the height of the pulse beat is called
  - a. systolic.
  - b. brachial.
  - c. diastolic.
  - d. atrioventricular.

20. (003) *Normal* diastolic blood pressure, in terms of mmHg, is
- 30 to 60.
  - 60 to 90.
  - 90 to 120.
  - 120 to 150.
21. (003) One of the principle functions of the lymphatic system is
- collecting excess interstitial fluid.
  - exchanging waste carbon dioxide for oxygen.
  - exchanging gases between the atmosphere and the cells of the lungs.
  - transporting the nutrients, oxygen, hormones, and waste throughout the body.
22. (004) Which one of the following is *not* an anticoagulant *commonly* used in hematology?
- EDTA.
  - Heparin.
  - Sodium fluoride.
  - Trisodium citrate.
23. (004) What anticoagulant is excellent in prevention of platelet clumping; however, it *cannot* be used for platelet function studies?
- EDTA.
  - Heparin.
  - Sodium fluoride.
  - Trisodium citrate.
24. (004) Which one of the following is the anticoagulant of choice for osmotic fragility tests?
- EDTA.
  - Heparin.
  - Sodium fluoride.
  - Trisodium citrate.
25. (004) Seminal fluid should be delivered to the laboratory in
- $\frac{1}{2}$  to 1 hour and kept at body temperature.
  - 1 to 2 hours and kept at body temperature.
  - $\frac{1}{2}$  to 1 hour and kept at room temperature.
  - 1 to 2 hours and kept at room temperature.
26. (005) Preanalytical and postanalytical activities are a part of
- quality control.
  - documentation.
  - quality improvement.
  - laboratory monitoring.
27. (005) An agreement between the best estimate of a quantity and its true value is called
- mean.
  - range.
  - precision.
  - accuracy.
28. (005) When plotting observations, 95 percent of the values fall within
- $\pm 1$  SD.
  - $\pm 2$  SD.
  - $\pm 3$  SD.
  - $\pm 4$  SD.

29. (005) A series of control values that continue to move in the same direction (increase or decrease) over a period of time is called a
- a. shift.
  - b. trend.
  - c. mean variation.
  - d. linear regression.
30. (005) During a 24 hour day, fresh whole patient blood controls are ran every
- a. two hours.
  - b. four hours.
  - c. six hours.
  - d. eight hours.
31. (005) What may be the single *most* important component of a good QC program?
- a. Documentation.
  - b. Instrument calibration.
  - c. Internal laboratory monitoring.
  - d. Fresh whole blood patient controls.

**Please read the unit menu for unit 2 and continue ➡**

## Unit 2. Maturation and Characteristics of Blood Cells

	<i>Page</i>
<b>2–1. Erythrocyte Maturation and Characteristics .....</b>	<b>2–1</b>
006. General characteristics of cellular maturation .....	2–1
007. Normal erythrocyte morphology and maturation sequence .....	2–2
008. Abnormalities in erythrocyte morphology .....	2–6
009. Erythrocyte production and destruction abnormalities .....	2–10
<b>2–2. Leukocyte and Thrombocyte Maturation and Characteristics .....</b>	<b>2–24</b>
010. Normal leukocyte morphology and maturation sequence .....	2–24
011. Leukocyte abnormalities .....	2–34

**I**N UNIT 1 we discovered the functions of blood, basic cell structures, and the production of cellular elements or hematopoiesis. We know that the blood is responsible for transporting oxygen and wastes, protecting the body from disease, and aiding in hemostasis. We also know that the blood contains many components, but which component is responsible for which function or functions? As radio personality Paul Harvey would say, “Now for the rest of the story.” In this unit we’ll outline the maturation sequence of the erythrocytes, leukocytes, and thrombocytes. We’ll also look at their functions in greater detail. Once you have the normal cells firmly in your mind, you’ll progress to the study of abnormal cells and their associated disorders.

### 2–1. Erythrocyte Maturation and Characteristics

As red cells mature, they undergo physical changes and reduce in size. The evaluation of these developmental stages, correlated with hemoglobin studies and differential analysis, give physicians valuable information. This information assists them in evaluating their patients’ oxygen-carrying capacity and disease state.

#### 006. General characteristics of cellular maturation

As a human being, you start as a single living cell, develop into a fetus, become an infant at birth, grow into a toddler, and so on. You didn’t become an adult from an infant; you went through developmental stages along the way to adulthood. Although these stages are well defined, they may overlap and be different for each person. As stated in unit 1, all blood cells start from a pluripotent stem cell, but they don’t evolve from a stem cell to a mature cell. They also go through defined developmental stages known as maturation.

#### Normal cell maturation

During normal maturation, changes occur in the size of the cell, cytoplasm, and nucleus. These general changes are described below.

Change	Description
<b>Cell size</b>	As a whole, the cell decreases in size as it matures (which is opposite from our development). It goes from a larger cell to a smaller cell.
<b>Cytoplasm</b>	The cytoplasmic changes include loss of ribonucleic acid (RNA), which decreases the blue staining characteristics (high RNA content stains deep blue, low RNA content stains light blue). The immature cytoplasm stains a deep blue (high RNA), which may be termed basophilic. Also, cytoplasmic granules may appear and increase as the cell matures and the amount of cytoplasm in relationship to the rest of the cell usually increases.

Change	Description
<b>Nucleus</b>	The nucleus, on the other hand, usually decreases in size, becomes more coarse and clumped and may take on various shapes. It changes its staining properties from reddish-purple to a bluish-purple, and the nucleoli usually disappear as the cell matures. ( <b>NOTE:</b> As the cell matures, the nucleus decreases and the cytoplasm increases. This relationship is known as the nuclear to cytoplasmic ratio or N/C ratio.)

### Identification guidelines

If you can remember these basic changes, you can formulate questions to ask yourself while identifying cells.

Changes	Questions
<b>Cell size</b>	What is the size of the cell, small, medium or large?
<b>Cytoplasm</b>	What does the cytoplasm look like? (Note its characteristics.) <ul style="list-style-type: none"> <li>What is its staining properties or color?</li> <li>Does it contain granules and, if so, are they specific or nonspecific?</li> <li>What is its size in comparison the rest of the cell?</li> </ul>
<b>Nucleus</b>	What does the nucleus look like? (Note its characteristics.) <ul style="list-style-type: none"> <li>What is its size or N/C ratio?</li> <li>What is its shape?</li> <li>What is its color?</li> <li>Is the nuclear chromatin smooth, fine, or coarse, and is it clumped?</li> <li>Does it contain nucleoli and, if so, how many and what size?</li> </ul>

These questions can be asked for any cell type. Keep them in mind as we go through this unit and description of each cell during its maturation sequence.

### 007. Normal erythrocyte morphology and maturation sequence

When van Leeuwenhoek first examined blood under his microscope, he described the erythrocyte as a small round globule. During this period of time, erythrocytes were thought to be unimportant. However, today we know that they play a vital role in the life of humans, as well as all creatures. The erythrocyte may appear simple looking under a light microscope, but it is actually quite complicated. Slight modifications or changes in its features can be associated with numerous disorders. It is important that you learn the normal maturation sequence in order to identify abnormal cells; hence, you can report abnormal findings to the physician.

### Identification terms

There are three sets of terms used to identify the cells of the erythrocytic series. The Air Force has chosen to use those terms outlined by the American Society of Clinical Pathologists (ASCP). Paul Ehrlich (1854-1915), a German physician and biochemist, used the terms normoblasts to describe (normal precursors) and erythroblasts to describe (large abnormal precursors). You should be familiar with the other terms as they may be used in different text or references. The terms are given in the following table.

Rubri (ASCP)	Normoblast	Erythroblast
Rubriblast	Pronormoblast	Proerythroblast
Prorubricyte	Basophilic normoblast	Basophilic erythroblast
Rubricyte	Polychromatophilic normoblast	Polychromatic erythroblast

Rubri (ASCP)	Normoblast	Erythroblast
Metarubricyte	Orthochromatophilic normoblast	Orthochromatic erythroblast
Reticulocyte*	Reticulocyte*	Reticulocyte*
Erythrocyte	Erythrocyte or Normocyte	Erythrocyte
*Reticulocyte is also termed as diffusely basophilic erythrocyte and polychromatophilic erythrocyte		

### **Rubriblast (pronormoblast)**

This is the earliest recognizable cell in the erythrocytic series, measuring about 14 to 25  $\mu\text{m}$  in diameter. The rubriblast has only a small amount of nongranular cytoplasm, which appears as a narrow, dark blue band surrounding the nucleus. A lighter blue band may form a perinuclear halo around the nucleus. (This halo is the Golgi apparatus.) It has a round, slightly oval nucleus with a fine chromatin pattern that stains reddish-purple. The nucleus occupies about 80 percent of the cell and the N/C ratio is about 8:1. There may be one or two faint, pale blue nucleoli seen. If present, they differentiate this cell from the prorubricyte. The rubriblast gives rise to two prorubricytes. Refer to figure 2-1 for the normal maturation erythrocytic sequence. Figure 2-1, *Reproduction of Morphology of Human Blood Cells*, is used with the approval of Abbott Laboratories, all rights reserved by Abbott Laboratories.

### **Prorubricyte (basophilic normoblast)**

This cell is similar to the rubriblast, but slightly smaller (12 to 17  $\mu\text{m}$ ). The nongranular cytoplasm, usually a uniform blue with very few colorless areas, is more abundant than in the rubriblast. The color may vary from basophilic, with perinuclear areas of pink- or orange-staining hemoglobin, to diffusely lilac. When seen among other “blast” cells, the prorubricyte is rounder, with more abundant cytoplasm and a somewhat eccentric nucleus. The nucleus is round or slightly oval, contains clumped chromatin that may exhibit a so-called spoke wheel formation. It occupies about 75 percent of the cell and the N/C ratio is 6:1. The chromatin is very deep and dark, and parachromatin (light or unstained areas) is distinct. If nucleoli are present, they are usually not visible. The prorubricyte divides twice and gives rise to four rubricytes.

### **Rubricyte (polychromatophilic normoblast)**

Most recognizable of the rubricyte’s characteristics is the change from basophilic (blue) cytoplasm to acidophilic chromatin (blue-gray to pink-gray). This change is due to the rapid accumulation of hemoglobin. This first appearance of hemoglobin may vary from a perinuclear zone of pink to local areas of cytoplasm showing an acidophilic staining reaction. The cell measures from 10 to 15  $\mu\text{m}$  with a round nucleus that continues to decrease in size. It exhibits the spoke wheel chromatin pattern; however, the number of clumps has diminished. The nucleus to cytoplasm ratio is about 4:1.

### **Metarubricyte (orthochromatophilic normoblast)**

Full hemoglobinization has occurred in this cell. The cytoplasm now stains pinkish, as seen in the adult erythrocyte. The nucleus now appears as a small, shrunken, dense, dark-staining mass. It is eccentrically located and may be round, oval, or have various bizarre forms. The spoke wheel is no longer seen, and the nucleus now has a uniformly dark violet appearance. The cell usually measures about 7 to 12  $\mu\text{m}$  and the N/C ratio is 1:2. The metarubricyte becomes a reticulocyte.

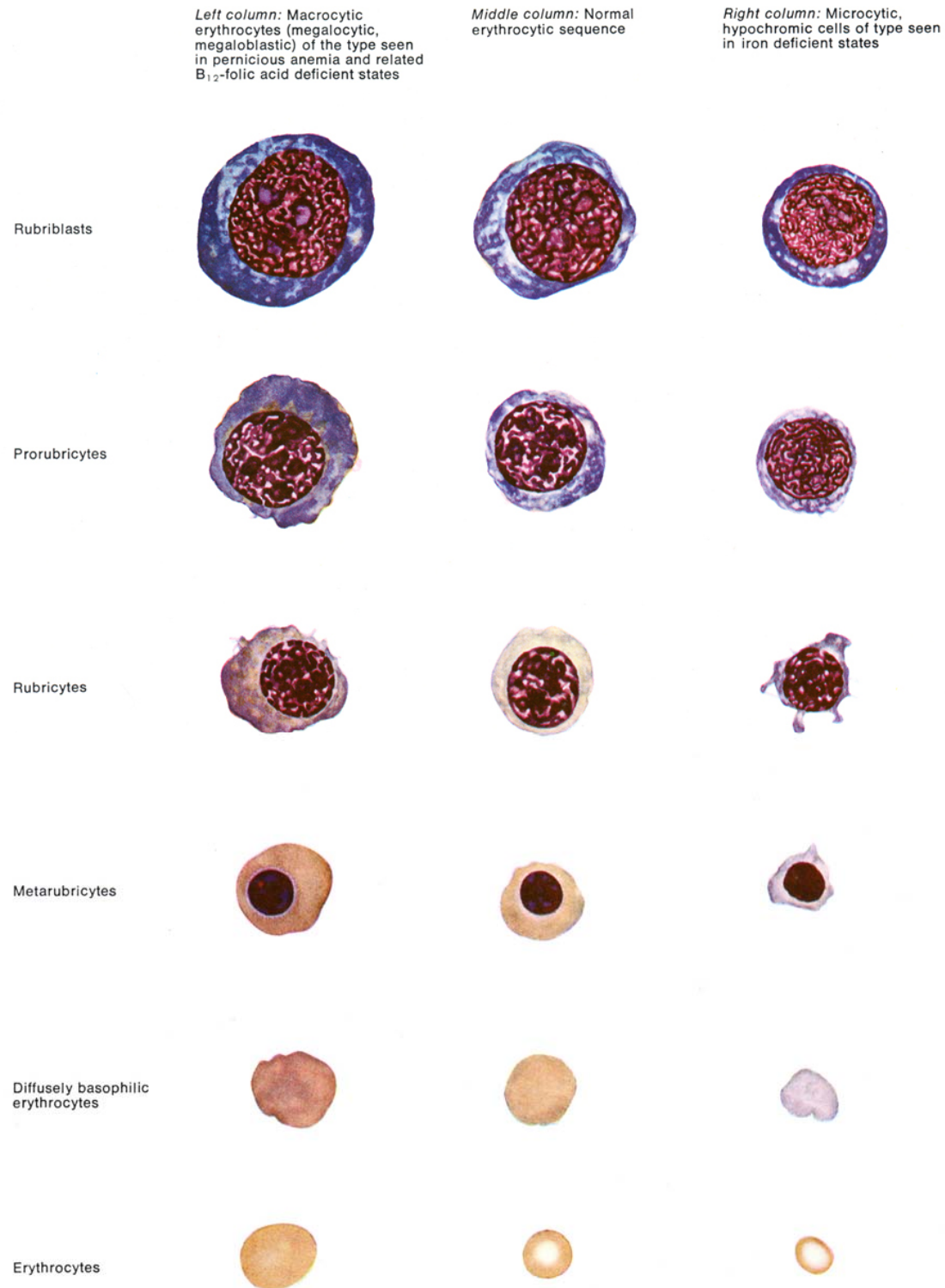


PLATE 8—ERYTHROCYTIC SYSTEM

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Figure 2-1. Morphology of human blood cells; erythrocytic system.



**Reticulocyte**

This immediate precursor of the adult erythrocyte or red blood cell (RBC) stains a uniform pink to a slight pinkish gray color. It is slightly larger than the erythrocyte, measuring about 7 to 9  $\mu\text{m}$ . A nucleus is not present. Reticulocytes usually account for about 2.5 to 6.5 percent of the total circulating RBCs in the newborn infant, but that percentage drops to the adult level of 0.5 to 1.5 percent, within two weeks of birth. Supravital staining with new methylene blue will distinguish these cells from basophilic stippling (punctate basophilia). Methylene blue stains the RNA remnants (the reticulum) in the cells. These reticula are not demonstrable in the Wright-stained smear. Increased numbers of these cells are indicative of red cell proliferation, such as an urgent need for circulating cells in the case of prolonged bleeding. Other factors, such as iron administration for anemia, will stimulate increased erythropoiesis and reticulocytes will be seen in larger numbers.

**Erythrocyte or mature red blood cell**

The mature RBC is one of the more highly specialized of all cells. It is devoid of cytoplasmic organelles and consists of little more than a membrane surrounding a solution of protein and electrolytes. The erythrocyte is a biconcave disk with a diameter of 7 to 8  $\mu\text{m}$  and a mean thickness of 2.14  $\mu\text{m}$ . Normally, the reticulocyte and erythrocyte are the only two cells in the maturation sequence to be seen in peripheral blood.

***Erythrocytic membrane***

The erythrocytic membrane is a dynamic component of the cell. It has selective permeability and facilitates changes in shape to move through the microvasculature. The chemical composition of the membrane is approximately 50 percent protein, 40 percent lipids, and 10 percent carbohydrate. The membrane is a lipid bilayer model, which means there is an orderly arrangement of phospholipids into a sheet two molecules thick. The molecules are oriented so that the nonpolar group of two layers is directed towards one another, forming lipid-to-lipid interactions. The polar group is directed outward and interacts with protein on both the extracellular and intracellular surfaces. Unesterified cholesterol is one of the lipids that maintain membrane fluidity and permeability for the exchange of electrolytes and nonelectrolytes.

***Protein classifications***

The two classifications of proteins are integral and peripheral. The integral proteins give the erythrocyte its negative charge. The negativity within the cells, known as zeta potential, causes the cells to repel one another. The integral membrane proteins, along with the glycolipid portions, carry some 500 identified red blood cell antigens. Most of the antigens are intrinsic components appearing on the membrane during early erythropoiesis. The peripheral proteins are responsible for the framework or cytoskeleton of the cell, and regulating membrane shape and deformability.

***Membrane function***

The membrane is responsible for three basic functions:

1. Allows nutrient and selective ion passage in and out of the cell.
2. Separates the intracellular fluid environment (cytoplasm) from the extracellular fluid environment (plasma).
3. Allows the cell to deform or change shape to travel through the small vessels.

***Erythrocyte function***

Although the mature erythrocyte doesn't contain a nucleus or other cellular organelles, all the components needed for survival and function are present. Its principle source of energy is glucose. Its primary function is to manufacture hemoglobin, which transports oxygen to the tissues and carbon dioxide ( $\text{CO}_2$ ) from the tissues to the lungs. Hemoglobin is about 90 percent of the cell's dry weight.

The normal hemoglobin molecule is composed of four subunits (consisting of heme and the protein globin). This molecule has an affinity for oxygen which allows for maximum transport. This attraction binds the oxygen molecule to the heme or iron molecules within the hemoglobin molecule.

### 008. Abnormalities in erythrocyte morphology

Erythropoiesis is regulated by the intake of different substances used in manufacturing cells and storage for later use. Recall from unit 1 that these substances include: iron; folic acid; vitamin B<sub>12</sub>; manganese; cobalt; zinc; vitamins C, E, B<sub>6</sub>, thiamine, riboflavin, and pantothenic acid; and hormones (erythropoietin, thyroxine, and androgens). In normal erythropoiesis the cytoplasm and nucleus of a cell grows at a synchronized rate. Individual differences in physiology and physical structure of the erythrocyte account for minor morphological changes. In certain diseases, these morphological changes can vary greatly. Abnormalities of erythrocytes fall into two primary categories—those that demonstrate variations in size and those that vary in shape.

#### Normal erythrocyte (RBC)

Before moving ahead with the abnormalities, let's review the morphology of a normal RBC. Normal RBCs are round, have a small area of central pallor (amount of hemoglobin), and show only a slight variation in size. The area of central pallor will increase or decrease according to the amount of hemoglobin present. When an RBC is within the mean corpuscular volume (MCV) range of 80 to 100 femtoliter (fL), it is considered normocytic. An RBC is termed normochromic when the mean corpuscular hemoglobin concentration (MCHC) is within 31 to 36 percent. The term hypochromic is used when the MCHC is below 31 percent and hyperchromic for an MCHC above 36 percent. The MCV and MCHC will be discussed in greater detail in the next unit.



#### Abnormalities in size

Variations in the size of RBC populations include those involving anisocytosis, microcytosis, and macrocytosis.

#### *Anisocytosis*

Although not a routine finding, the term *anisocytosis* means that the red cells are of unequal size. It is arbitrarily graded 1+ to 3+ or by using *slight*, *moderate*, or *marked* to describe the degree of variability. Significant anisocytosis is found in a variety of situations. Severe anemias are accompanied by the most severe anisocytosis, while sideroblastic anemias are generally accompanied by dual populations of erythrocytes.

**NOTE: sideroblastic anemia** are any of a heterogeneous group of acquired and hereditary anemias with diverse clinical manifestations; commonly characterized by large numbers of ringed sideroblasts in the bone marrow, ineffective erythropoiesis, variable proportions of hypochromic erythrocytes in the peripheral blood, and usually increased levels of tissue iron. The two most common kinds are hereditary sideroblastic anemia and refractory sideroblastic anemia.

#### *Microcytosis*

Microcytes are <6 µm in diameter and are commonly found in iron deficiency and thalassemia. In microcytosis, the MCV is <80 fl and the RBC is called microcytic.

#### *Macrocytosis*

Erythrocytes having a diameter >8.5 to 9.0 µm are said to be macrocytic. These large cells are commonly seen in vitamin B<sub>12</sub> and folic acid deficiency, alcoholism, liver disease, certain leukemia's, and megaloblastic anemias. In macrocytosis, the MCV is >100 fl, and the RBC is termed macrocytic.

### Abnormalities in shape

*Poikilocytosis* is a general term that indicates the presence of RBCs varying from the normal, biconcave shape. The grading is once again 1+ to 3+ on an arbitrary basis or by using *slight*, *moderate*, or *marked* to describe the degree of variability. The significance of poikilocytosis is limited because it is a generalized term that should be further defined by identifying the type of poikilocytosis present. Refer to figure 2-2 for an example of the following RBC shapes.

#### SHAPES OF RED BLOOD CELLS

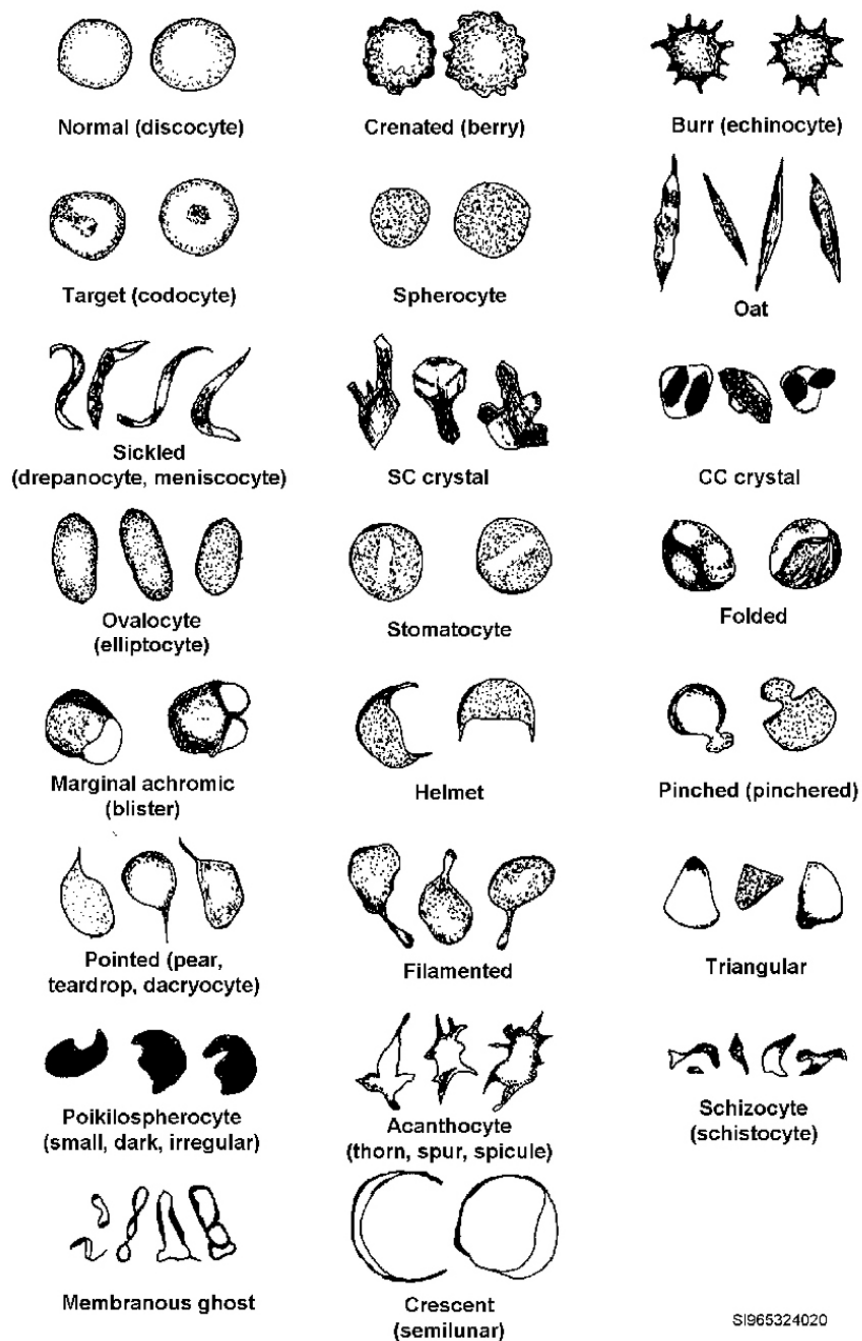


Figure 2-2. Shapes of red blood cells.

<b>RBC Shape</b>	<b>Explanation/Description</b>
<b>Normal</b> ( <i>discocyte</i> )	The normal biconcave disk-shaped erythrocyte is called a discocyte.
<b>Crenated</b> ( <i>berry</i> )	Crenated cells resemble the burr cells, but their spicules are more blunt. They can be seen as a common artifactual process due to faulty drying of the blood smear, or may result from hyperosmolarity. They may also be associated with altered membrane lipids.
<b>Burr cells</b> ( <i>echinocyte</i> )	Are red cells whose surface shows uniformly spaced pointed projections or spicules of the outer membrane. Uremia, bleeding peptic ulcers, carcinoma of the stomach, and pyruvate kinase deficiency show increased numbers of these cells.
<b>Target cell</b> ( <i>leptocytes, codocyte</i> )	This cell displays a characteristic "target shape" when viewed on a fixed smear. Seen in a wet state, the cells have a definite bell shape. Target cells appear dark in the center with a lighter ring between the center and periphery (hypochromic). They are seen in certain hemoglobinopathies: Hemoglobin CC, E, and SC diseases and sickle-cell anemia; liver disease which can be seen in most anemias; iron deficiency anemia; and thalassemia.
<b>Spherocytes</b>	These cells are erythrocytes that have a reduced or absent central zone of pallor and appear denser with a decreased diameter. The presence of spherocytes always indicates a hemolytic process. They are seen in hereditary spherocytosis, ABO hemolytic disease of the newborn, and immunohemolytic anemia secondary to autoimmune or isoimmune antibodies. Spherocytes have a shorter life span because they are sequestered by the spleen and then destroyed. They are more fragile; therefore, they have an increased osmotic fragility. Treatment for most patients with hereditary spherocytosis includes a splenectomy. However, if spherocytes are still present in these patients, the abnormality involves the red cell membrane itself, and not splenic damage to the cells. Blood units stored in the blood bank for a long time also develop spherocytes. In a patient that has been recently transfused, you may see both normal blood cells and spherocytes.
<b>Oat</b>	These cells are called oat cells due to the oat shape they display on fixed smears.
<b>Sickle cells</b> ( <i>drepanocytes, meniscocyte</i> )	These cells are called sickle cells due to the crescent-shaped sickles they display on fixed smears. Sickle cells are discocytes deformed by rod-like polymers of abnormal hemoglobins such as the polymers of hemoglobin S seen in sickle-cell disease. They show a decreased osmotic fragility. Mechanical fragility is increased, which is believed to be the cause of the hemolytic episodes of intravascular hemolysis seen in sickle-cell disease. These cells are also associated with hemoglobin SC disease and hemoglobin S $\beta$ thalassemia.
<b>Hemoglobin SC crystals</b>	Hemoglobin SC crystals are dark-hued that distort the red cell membrane. They are parallel, with one blunt end that looks like the Washington monument. There may be one or more crystals protruding from the cell membrane.
<b>Hemoglobin CC crystals</b>	Intracellular hemoglobin CC crystals appear hexagonal with blunt ends and stain very dark. These crystals form within the cell membrane when hemoglobin C crystallizes. At times the crystals appear free because the membrane is not visible. The crystals are more frequent after splenectomy.
<b>Ovalocytes</b> ( <i>elliptocytes</i> )	Ovalocytes are oval-shaped and elliptocytes have a more cigar shape. However, both cells are seen in smears from patients with hereditary elliptocytosis (from 25 to 90 percent of the cells may be affected). They may also be present in various anemias, including iron deficiency anemia, megaloblastic anemia, and myelophthisic anemia.
<b>Stomatocytes</b>	These cells, in a fixed smear, exhibit an elongated or a rectangle central zone of pallor that may resemble a mouth-like or slit-like appearance. The detection of sheets of stomatocytes on routine smears is indicative of hereditary stomatocytosis. These cells may also be noted in acute alcoholism, obstructive liver disease, Rh null disease, and electrolyte imbalance. Underlying change is membrane defect with abnormal cation permeability. Stomatocytes can also be an artifact of drying.

RBC Shape	Explanation/Description
<b>Marginal achromic</b> (blister)	Blister cells are red blood cells that appear to have large vacuoles with fuzzy margins due to the concentration of hemoglobin on one side of the cell.
<b>Keratocytes</b> (helmet cell)	These are RBCs with one or more notches in the edge of the erythrocyte. The border of the notches form projections that look like horns. They are damaged cells—deformed by incomplete cuts. These cells have also been called helmet cells.
<b>Pointed</b> (pear, teardrop dacryocytes)	The unusual teardrop shape can be partially explained by the pitting function of the spleen. Teardrop cells are seen in myelofibrosis, myeloid metaplasia, pernicious anemia, cancers metastasizing to the bone marrow, tuberculosis, $\beta$ -thalassemia, after Heinz body formation, and as an artifact. Artifact cells, caused by mechanical rather than physiological factors, point in the same direction. True teardrop cells will point in all directions.
<b>Acanthocytes</b> (thorn, spur, spicule)	RBCs exhibiting a striking degree of irregularly shaped spiny projections are called acanthocytes. They have sharp, pointed projections from the RBCs membrane. The acanthocyte is characteristic of abetalipoproteinemia. Acanthocytes can also be seen in certain liver and lipid metabolism disorders, post splenectomy, and alcoholic cirrhosis with hemolytic anemia.
<b>Fragmented Red Cell</b> (Schistocytes, helmet cells, triangular cells)	These are erythrocytic fragments caused by fibrin strands, altered blood vessels, or damaged heart valve prosthesis. They are not hereditary but are from localized erythrocytic membrane damage. They are irregular in shape and form, and include the helmet and triangular cells. They may be seen in microangiopathic hemolytic anemia, uremia, severe burns, and hemolytic anemia due to disseminated intravascular coagulation (DIC). When present in large numbers they may cause the mean corpuscular volume (MCV) to fall into the microcytic range.
<b>Teardrop cells</b> (dacryocytes, pear, pointed)	The unusual teardrop shape can be partially explained by the pitting function of the spleen. Teardrop cells are seen in myelofibrosis, myeloid metaplasia, pernicious anemia, cancers metastasizing to the bone marrow, tuberculosis, $\beta$ -thalassemia, after Heinz body formation, and as an artifact. Artifact cells, caused by mechanical rather than physiological factors, point in the same direction. True teardrop cells will point in all directions.

### Erythrocyte inclusions

Under this heading we include siderotic granules (pappenheimer bodies), malaria, cabot rings, basophilic stippling, and howell-jolly bodies as shown in figure 2–3.

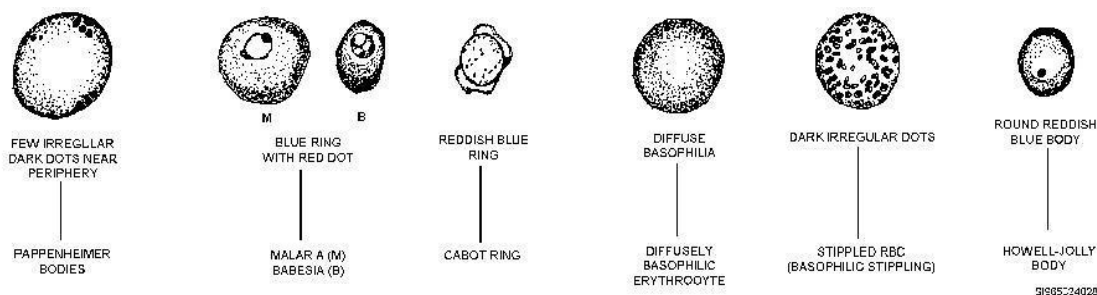


Figure 2–3. Wright's stain of red blood cell inclusions.

### Howell-Jolly bodies

These small, round bodies are remnants of DNA nuclear material after the normoblast nucleus is extruded and stain reddish-blue with Wright's stain. They can be distinguished from superimposed granules because they are not refractive. There is usually only one per cell, but occasionally two or more may be seen in severe anemias and alcoholism. Howell-Jolly bodies are common in hemolytic

anemias, but they are rarely seen in iron deficiency anemias. The spleen usually removes these inclusions without destroying the cell. This action is called pitting. Therefore, they are frequently seen in postsplenectomy.

### ***Basophilic stippling***

Here the stained cell contains many fine or coarse, irregular shaped, granules that stain deep blue to purple on a dried Wright-stained blood film. They are a result of ribosome aggregation and appear homogeneously throughout the cell. It has been shown that stipple formation is dependent on the desiccation of the cells. In lead poisoning, stippling may be striking, reflecting both an abnormality in ribosomal aggregates and iron-laden mitochondria. Basophilic stippling may also be seen in beta thalassemia, megaloblastic anemias, arsenic intoxication, and alcoholism.

### ***Siderotic granules (Pappenheimer bodies)***

These irregular-shaped granules are iron containing structures. The granules are usually multiple but may be single, positioned near the periphery of the cell, and stain very dark with Wright's or supravital stains. With Perls' Prussian blue stain, the cells stain positively, representing ferric iron aggregates. Siderotic granules are composed, in part, of iron-laden mitochondria. They are found in large numbers when hemoglobin synthesis is impaired as in siderotic anemias or in iron overload (hemosiderosis and hemochromatosis). In Wright-stained films, they can be confused with basophilic stippling; however, basophilic stippling does not stain with Prussian blue stain.

### ***Cabot rings***

Cabot rings are thought to represent nuclear remnants, but they may be merely artifacts. In Wright-stained smears, they are fine, ring-like or figure-eight-like, reddish-purple structures that are usually single, but at times double. They are distinguished from the ring forms of *Plasmodia* species by their larger size and the absence of a red chromatin mass. These rings are found in megaloblastic anemias, severe anemias, lead poisoning, and dyserythropoiesis (RBCs are destroyed before leaving the bone marrow).

## **009. Erythrocyte production and destruction abnormalities**

The red blood count, based on total erythrocyte volume, may be subject to a slight, even hourly, variation. In normal individuals, this range of variation remains essentially the same throughout adult life. The rate of destruction of aged cells is approximately the same as the rate of production. If the production-destruction ratio is not in equilibrium and more cells are produced than destroyed, the condition is known as *polycythemia* or *erythrocytosis*; if fewer cells are produced than destroyed, lowering the hemoglobin concentration, the condition is known as *anemia*.

### **Polycythemia or erythrocytosis**

Polycythemia and erythrocytosis are terms used to indicate an above normal hemoglobin, hematocrit, and red blood cell count. A hematocrit of >52 percent for males and >50 percent for females is often the diagnosis criterion. Polycythemia is classified as relative or absolute.

### ***Relative polycythemia***

This condition is normally transient and is due to a decrease in fluid (plasma). It is the result of fluid loss due to burns, excess sweating, dehydration, vomiting, diarrhea, smoking, use of diuretics, or severe anxiety and stress (spurious polycythemia or Gaisböck's syndrome). The red cells do not increase in actual numbers; the fluid that they bath in decreases, making it appear that there are more RBCs. White blood cell count is normal or slightly elevated.

***Absolute polycythemia***

Absolute polycythemia is subdivided into polycythemia vera and secondary polycythemia.

***Polycythemia vera***

In this condition, there is general hyperplasia of the bone marrow. It is a chronic myeloproliferative disorder of unknown origin. Erythrocytosis, leukocytosis, and thrombocytosis are all noted. The blood volume may be increased by two or three times normal, which causes the blood to become more viscous. High blood pressure, headaches, dizziness, vertigo, and formation of intravascular thrombi are common due to the increased viscosity. Immature cells are often seen in the peripheral blood. Strangely enough, there is a decrease of erythropoietin.

***Secondary polycythemia***

Elevated levels of erythropoietin can usually be detected in this condition. Increased erythropoietin production can be abnormal or a normal response to hypoxia. Erythrocyte numbers are increased. The arterial oxygen saturation of the blood is decreased, or there is a decrease in the capacity of the hemoglobin molecule to carry oxygen. The other factors seen in polycythemia vera are absent. The blood is normally fighting a general lack of oxygen (such as high altitude) by producing as many red cells as possible to carry the available oxygen to the tissues. Individuals transferred from Keesler AFB to the Air Force Academy would expect to show this type of polycythemia until their bodies adjusted to the lowered oxygen tension at the higher altitude. Other causes include chronic pulmonary disease, chronic congestive heart failure, heavy smoking, abnormal hemoglobins that have a high affinity for oxygen, and methemoglobinemia. The white blood cell count is normal in this condition.

**Anemia**

A rather oversimplified definition of this condition is the existence of a reduced red blood cell count, or a decreased concentration of blood hemoglobin. Anemia is not a diagnosis in itself, but merely an objective sign of the presence of a disease. The correct diagnostic terminology for a patient with anemia requires the inclusion of the causative physical condition. Some examples are:

- Pernicious anemia resulting from malabsorption of vitamin B<sub>12</sub>, which yields macrocytic RBCs.
- Iron deficiency anemia that may exhibit RBCs so hypochromic as to appear as only thin rims of hemoglobin.
- Hemolytic anemia with fragmentation due to sickle-cell hemoglobinopathy.

A general statement can be made that anemia is a decreased hemoglobin in the blood resulting in decreased oxygen reaching the body tissues and organs. There are two basic reasons for this condition.

1. Too many RBCs being destroyed.
2. Too few RBCs being produced.

**Classification of anemias**

Several different classifications of anemia have been invented, but none are completely satisfactory. However, they do help in learning the basics of anemia. Keep in mind, as you study this area, that an anemia is not a disease; it is a condition associated with a disease.

- Etiologic or cause classification of the anemia.
- Morphologic classification of anemia.
- Physiologic classification of anemia.

***Etiologic or cause classification***

This classification focuses on the underlying etiological mechanism or cause of the anemia. It is difficult to classify anemias by their cause due to the overlap of multiple factors or conditions and underlying mechanisms. The table below is an overview of the etiologic classification of anemia.

<b>Etiologic Classification of Anemia</b>	<b>Examples</b>
Relative anemia	Pregnancy Hyperproteinemia Intravenous fluids
Defective hemoglobin synthesis	Iron deficiency Primary sideroblastic anemia Secondary sideroblastic anemia Thalassemia Anemia of chronic disease
Vitamin B <sub>12</sub> or Folate deficiency	Vitamin B <sub>12</sub> deficiency Folate deficiency Pernicious anemia
Impaired bone marrow or stem cell function	Primary aplastic anemia Secondary aplastic anemia Myelodysplastic anemia Anemia of renal failure Anemia of endocrine disorders Anemia of chronic disease Paroxysmal nocturnal hemoglobinuria
Decrease red cell survival and increase red cell destruction	Membrane defect --Hereditary spherocytosis --Hereditary elliptocytosis --Hereditary stomatocytosis --Hereditary acanthocytosis --Lecithin-cholesterol acyltransferase deficiency Enzyme defect --G6PD deficiency --Pyruvate kinase deficiency Hemoglobinopathies --Sickle cell disease --Beta-thalassemia --Hemoglobin S/Beta-thalassemia Extrinsic red cell defect --Isoimmune hemolytic anemia --Autoimmune hemolytic anemia --Drug-induced immune hemolytic anemia --Microangiopathic hemolytic anemia --Hemolytic anemia associated with infection --Hemolytic anemia associated with toxic agents --Hypersplenism
Anemia secondary to blood loss	Acute Chronic



***Morphologic classification***

This classification uses the red blood cell indices and direct examination of red cell morphology for classifying anemias.

<b>Morphologic Classification of Anemia</b>	<b>Example</b>
Normocytic and normochromic red cells	<ul style="list-style-type: none"> <li>• Hypoproliferative anemia</li> <li>• Myelophthisic anemia</li> <li>• Refractory dysmyelopoietic anemia</li> <li>• Hemolytic anemia</li> <li>• Hemoglobinopathies</li> <li>• Blood loss anemia</li> <li>• Anemia of chronic disease</li> <li>• Acquired sideroblastic anemia</li> <li>• Early iron deficiency</li> <li>• Refractory anemia</li> </ul>
Macrocytic and normochromic red cells	<ul style="list-style-type: none"> <li>• Folic acid deficiency</li> <li>• Vitamin B<sub>12</sub> deficiency</li> <li>• Hypoproliferative anemia</li> <li>• Refractory anemia</li> <li>• Liver disease</li> <li>• Hemolytic anemia</li> <li>• Blood loss anemia</li> </ul>
Microcytic and hypochromic red cells	<ul style="list-style-type: none"> <li>• Iron deficiency</li> <li>• Thalassemias</li> <li>• Hereditary sideroblastic anemia</li> <li>• Anemia of chronic disease</li> <li>• Hemoglobinopathies</li> </ul>

***Physiologic classification***

Physiologic classification is based on the bone marrow's ability to respond to the anemia with increased erythropoiesis. If the bone marrow doesn't respond, it is termed ineffective erythropoiesis. If the anemia is due to other reasons than the bone marrow, it is termed effective erythropoiesis. This involves using the reticulocyte count for assessing erythrocyte production. The table on the next page is an overview of the physiologic classification of anemias.

<b>Physiologic Classification of Anemia</b>	<b>Example</b>
Ineffective erythropoiesis	<p>Hypoproliferative anemias</p> <ul style="list-style-type: none"> <li>--Bone marrow failure</li> <li>--Endocrine disease</li> <li>--Anemia of chronic disease</li> <li>--Renal disease</li> <li>--Refractory dysmyelopoietic</li> <li>--Myeloid metaplasia</li> <li>--Metastatic cancer</li> </ul> <p>Maturation disorders</p> <ul style="list-style-type: none"> <li>--Sideroblastic anemias</li> <li>--Thalassemias</li> <li>--Iron deficiency</li> <li>--Anemia of chronic disease</li> <li>--Liver disease</li> </ul>

Physiologic Classification of Anemia	Example
	--Hemolytic anemia --Folate deficiency --Vitamin B <sub>12</sub> deficiency
Effective erythropoiesis	Hemolytic anemia --Hereditary spherocytosis --Hereditary stomatocytosis --Hereditary acanthocytosis --Hemoglobinopathies, hemoglobin S, hemoglobin C, others --Autoimmune hemolytic anemia --Isoimmune hemolytic anemia --Drug-induced hemolytic anemia --Enzyme disorders --Paroxysmal nocturnal hemoglobinuria --Unstable hemoglobins --Hypersplenism --Microangiopathic hemolytic anemia ---Renal disease ---Malignancy ---Infections ---Pregnancy ---DIC --Cardiac hemolytic anemia --Infectious hemolytic anemia --Burns Blood loss anemia --Chronic

### General considerations

As you can see, classifying anemias is complicated at best. As a laboratory technician, it is not your job to diagnose, but a basic understanding of the disease processes and laboratory findings aids in providing quality laboratory results. A brief review of the more common causes of anemia follows.

### Hypoproliferative anemias

These disorders can present a normal to neoplastic (malignant) bone marrow picture with normocytic, normochromic red blood cell morphology. If the anemia is from bone marrow failure, there are three major causes of marrow damage that yield a hypoproliferative anemia:

1. Marrow displacement in leukemia or lymphoma, neoplasms, myelofibrosis, or osteosclerosis.
2. Marrow damage due mostly to Xrays, toxic drugs, glue sniffing, atomic radiation, benzene, or infections (aplastic anemias).
3. Intrinsic lesions—congenital, acquired, or thymus-related.

### Aplastic anemia

Aplastic anemia occurs when there is a complete failure of the bone marrow to produce red blood cells, white blood cells, and platelets. This results in a decreased number of all these cells in the peripheral blood. Another term used to describe this condition is *pancytopenia*. It is thought that the hematopoietic stem cells are damaged, and, therefore, can't proliferate or differentiate. In aplastic anemia, there are no immature cells seen on the blood film (as seen in leukemia patients). Aplastic anemia is more common in infants <1 year old and in adults >50 years old. It can be inherited or acquired. If acquired, it may be idiopathic (no known reason), or due to drugs, chemicals, radiation, or infections. Laboratory findings are normocytic, normochromic, and occasionally slight

macrocytosis red blood cell morphology. Reticulocyte count is also decreased. Bone marrow films show increased fat cells with a decrease in hematopoietic cells. Bone marrow transplant is usually the treatment of choice, rarely, does aplastic anemia go into a spontaneous remission.

### ***Other hypoproliferative anemias***

Myelophthisic anemia is classified as an anemia of chronic disease and is commonly due to metastatic carcinoma (replacement of normal bone marrow cells with abnormal cells). Hypoproliferative anemias can also be due to system failure, as seen in chronic renal disease and endocrine disorders. Renal disease or failure can result in the underproduction of erythropoietin, resulting in the marrow not being demanded to produce red cells. Endocrine deficiencies, such as decreases in thyroid, pituitary, or testosterone function, can decrease the amount of oxygen required by the body for physiologic maintenance.

### **Maturation disorders**

Maturation disorder anemias can be subdivided into two groups based on their MCV values. Macrocytic anemias that are considered true megaloblastic anemias have a MCV of  $>115$  fl and are associated with vitamin B<sub>12</sub> and folate deficiencies. Macrocytic cells with MCV values of  $<115$  fl are associated with the hemolytic anemias and liver disease. Microcytic anemias include iron deficiency anemias, sideroblastic anemias, and thalassemia.

### ***Megaloblastic anemias***

These anemias are characterized by increased MCV, macrocytic, normochromic morphology and may be a result of defective DNA synthesis. It is proposed that this biochemical abnormality leads to a state of unbalanced cell growth, in which RNA and protein synthesis continue while DNA synthesis is retarded. Therefore, hemoglobin is synthesized in excessive amounts during the delay between cell divisions. Most megaloblastic anemias are caused by vitamin B<sub>12</sub> and folate deficiencies. Laboratory data yields the following results:

- Hemoglobin about 7 to 8 g/dl.
- MCV consistent with severity of anemia.
- Red cell distribution width (RDW) markedly elevated.
- Reticulocyte count is very low.
- Increased anisocytosis and poikilocytosis, hypersegmentation of neutrophils, and bone marrow exhibits megaloblast erythrocyte precursors.

Erythrocyte inclusions are common—basophilic stippling, Howell-Jolly bodies, Cabot rings, and nucleated RBCs. WBC and platelet counts are usually within the reference range. See figure 2-4 for a schematic of a blood film with examples of maturation disorders.

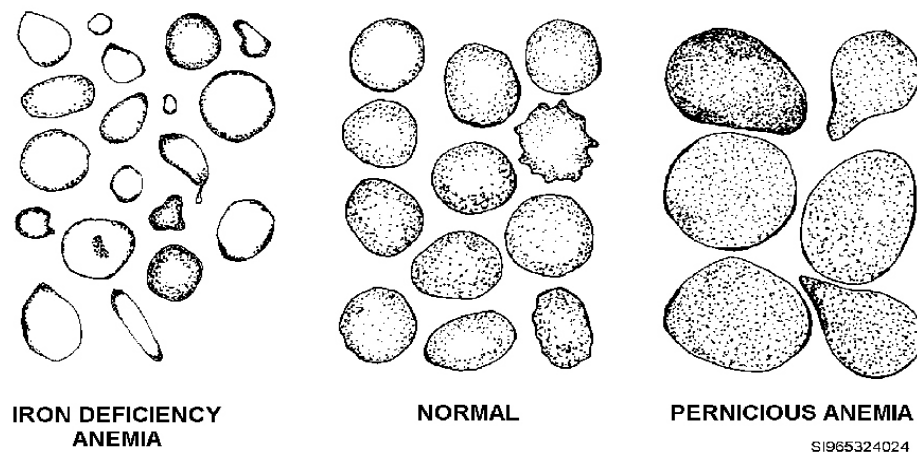


Figure 2-4. Examples of maturation disorders in RBCs.

### *Pernicious anemia*

Of the megaloblastic anemias, pernicious anemia (vitamin B<sub>12</sub> deficiency) is the most common. It is usually found in people over 60 years of age. The fundamental defect in this anemia is a qualitative or quantitative deficiency of the intrinsic factor that carries vitamin B<sub>12</sub> and folic acid (folate) across the distal ileum mucosal cell. The intrinsic factor is produced by the gastric parietal cells, which also contribute hydrochloric acid and pepsin. Vitamin B<sub>12</sub> is required for all normal cell replications to take place. This slowing of the replication of all cells results in depressed RBC, WBC, and platelet counts.

### *Other situations*

Other unusual situations that can result in megaloblastic anemia or combinations of folic acid and vitamin B<sub>12</sub> deficiencies are listed below.

- Infections of *Diphyllobothrium latum* (broad fish tape worm) that competes for absorption of vitamin B<sub>12</sub>.
- Imerslund's syndrome, a lack of receptors for vitamin B<sub>12</sub> on the ileum.
- Drug-induced vitamin B<sub>12</sub> malabsorption.
- Gastrectomy affecting absorption of vitamin B<sub>12</sub> and folate.
- Crohn's disease of the ileum and colon that affects vitamin B<sub>12</sub> and folate absorption.
- Folic acid deficiency is attributed to alcoholic cirrhosis, dietary deficiency, infant malnutrition, pregnancy, and folate antagonists.

### *Iron deficiency anemias*

Iron deficiency anemia is the most common form of a nutritional deficiency in both developing and developed countries. The daily requirement for iron is 10 to 30 mg per day. This is the most common form of anemia encountered in the practice of hematology. Iron deficiency comes about as either a late manifestation of prolonged negative iron balance or a failure to meet an increased physiologic need for iron in the production of hemoglobin. Negative iron balance can be due to inadequate dietary iron or impaired absorption of iron. Achlorhydria (reduced gastric acidity), gastric surgery, or severe iron deficiency in children are examples of this malabsorption. Iron loss can be due to gastrointestinal bleeding, resulting from a long list of causes or one or more of the following.

- Excessive menstrual flow.
- Regular blood donation.

- Hemoglobin passing in the urine (hemoglobinuria).
- Self-induced bleeding (primitive cultures).
- Or other more obscure reasons.

Iron deficiency can also result from increased requirements for iron, as in the case of pregnancy or lactation. At the onset, iron deficiency anemia is almost insidious and the progression of symptoms is gradual. Fatigue is the most common complaint. Laboratory findings yield lowered hemoglobin (about 8 g/dl), microcytic RBCs (about 74 fl), hypochromia, and moderate poikilocytosis (elongated elliptocytes and target cells), as shown in figure 2-4.

### ***Sideroblastic anemia***

Iron deficiency anemias previously discussed are microcytic in nature and associated with a maturational defect only in that there is a longer maturation time required for the cells to become fully hemoglobinated. The term sideroblastic refers to the formation of ringed sideroblasts that are iron-laden mitochondria granules, seen arranged around the nucleus of maturing erythrocyte precursors with Prussian blue stain. The sideroblastic anemias includes porphyrias, a group of disorders that demonstrate excess production and secretions of porphyrins and porphyrin precursors. They result from either a hereditary or acquired defect in the heme biosynthesis pathway. Etiological factors in acquired porphyrias include alcoholic cirrhosis, lupus erythematosus, hepatic adenoma, hemolytic anemia, and leukemia. Along with these causes are drugs and toxins.

### ***Abnormal globin development***

Abnormal globin development can be caused by genetic disorders characterized by reduction in globin chain synthesis for hemoglobin (thalassemia) or structural abnormalities of the globin polypeptide chains (hemoglobinopathies-sickle cell anemia).

### ***Hemoglobinopathies***

The hemoglobinopathies are discussed in the hemolytic anemia area.

### ***Thalassemia***

Thalassemias are associated with structural hemoglobin variants that are characterized by the presence of mutant genes that suppress the rate of synthesis of globin chains. They are probably the most common single-gene disorders found throughout the world. It is a hereditary disease found in people of the Mediterranean, Asian, and African ancestry. They are involved in the reduced production of the alpha ( $\alpha$ ), beta ( $\beta$ ), delta ( $\delta$ ), and gamma ( $\gamma$ ) globin chains. Homozygous beta thalassemia (Cooley's anemia or thalassemia major) is almost always a fatal disease. It is characterized by diminished or absent synthesis of the beta chains; however, the synthesis of alpha chains continues at a normal rate. This imbalance damages the red cell membrane resulting in hemolysis. Laboratory findings include:

- Decreased osmotic fragility.
- Decreased RBC count, hemoglobin around 2.5 to 6.5 g/dl.
- Hematocrit between 11 and 24 percent, MCV between 48 and 72 fl.
- Extreme anisocytosis, and poikilocytosis with numerous target cells and Howell-Jolly bodies.

Hypochromasia is extreme, and there are numerous nucleated RBCs. Thalassemia intermedia and thalassemia minor (Cooley's trait) are milder anemias where the patients general live a normal life span. Hemoglobin S  $\beta$  thalassemia shows moderately severe anemia, microcytosis, hypochromia, anisocytosis, and poikilocytosis as seen in figure 2-5. Tests for sickling are positive. Hemoglobin

electrophoresis shows hemoglobin S always in excess of hemoglobin A and increased hemoglobin A<sub>2</sub> and hemoglobin F. Mild cases have little or no anemia, but the smear appears thalassemic with numerous target cells.

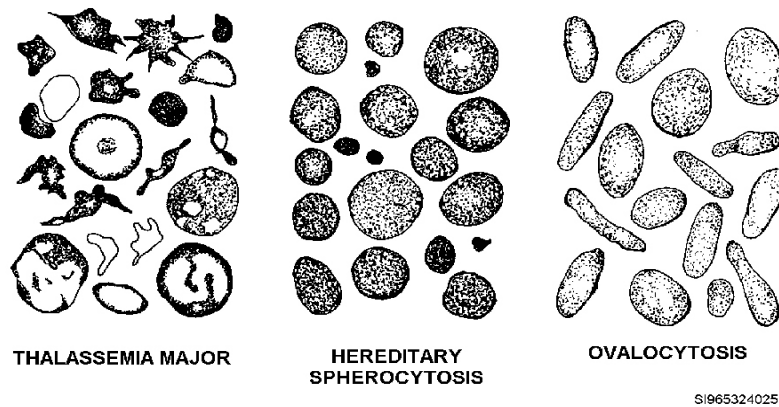


Figure 2-5. Examples of thalassemia and hemolytic anemias.

### Hemolytic anemia

The aforementioned anemias result from a combination of decreased production and increased destruction; this is not true for the hemolytic anemias. All hemolytic anemias are characterized by severe reduction in erythrocyte survival with a normal bone marrow that is unable to keep up with the destruction. Under maximal stimulation, the normal marrow is capable of undergoing hyperplasia until its production rate is increased six to eight fold. This situation can leave the condition undetectable; however, RBC life span can drop to as low as 15 to 20 days and leave the marrow unable to keep up with cell loss. The hemolytic anemias can be subdivided by the type of destruction or defect—intrinsic or extrinsic. Intrinsic defects come from the erythrocyte itself, where as the extrinsic defects result from environmental factors that damage normal erythrocytes. The intrinsic defects are usually inherited anemias, and the extrinsic defects are usually acquired anemias.

#### *Inherited hemolytic anemias*

These anemias involve red cell membrane and enzyme defects and plasma constituent abnormalities. Hemolysis results from premature destruction by the spleen and increased membrane rigidity in the red cell membrane anemias.

##### *Hereditary spherocytosis*

Hereditary spherocytosis is the most common disorder in the group of red cell membrane defect anemias. Hemoglobin levels are usually between 9 and 12 g/dl, but can be as low as 6 g/dl. MCV is normally between 77 and 87 fl. MCHC is high due to the increased capacity of the cell. RDW is normal. Polychromatophilia is high; RBC fragility is very increased; autohemolysis testing is positive; serum bilirubin is increased. There are also such states as hereditary elliptocytosis, acanthocytosis, and stomatocytosis as shown in figure 2-5. These, as spherocytosis, derive their names from the predominate RBC conformation.

##### *Glucose-6-phosphate dehydrogenase deficiency (G6PD)*

G6PD deficiency is an inherited sex-linked trait that generally expresses itself after the erythrocyte has been exposed to an increased oxygen load. G6PD catalyzes the initial step in the pathway for carbohydrate metabolism within the RBC for the production of an antioxidant that protects the cell against oxidant stress. This insufficiency results in hemoglobin denaturation and precipitation of the globin. As a result, these cells are then removed by the MPS, and the copious hemoglobin released is passed in the urine. There are episodes of a hemolytic disorder in these individuals after the

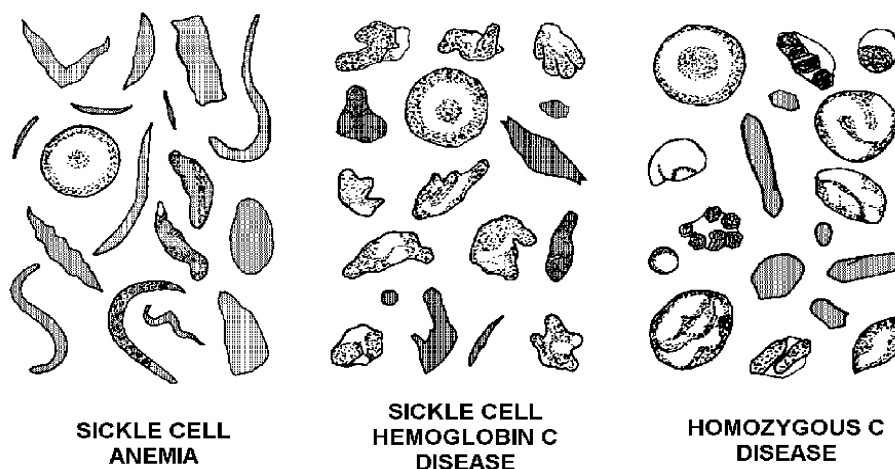
administration of various drugs. Some of these are sulfonamides, antipyretic drugs, and such anti-malarials as Primaquine. It is the latter that leads the Air Force to screen its total force for this deficiency.

### *Hemoglobinopathies*

Hemoglobinopathies involve abnormal structures of the polypeptide  $\alpha$ ,  $\beta$ ,  $\delta$ , and  $\gamma$  chains. The abnormalities include amino acid substitutions or deletions in the chains or elongation or fusion of different chains. They may or may not cause clinical disorders. Sickle-cell anemia is the best known hemoglobinopathy. It is a chronic hemolytic anemia resulting from the homozygous sickle-cell gene (Hb SS genotype). Normal hemoglobin is Hb AA—one gene type from each parent. In sickle-cell anemia the person is homozygous Hb SS; in sickle-cell trait, the person is heterozygous Hb AS; in hemoglobin C disease, persons may be homozygous Hb CC or heterozygous Hb AC; and in hemoglobin SC disease, the person is Hb SC. The major hemoglobins include, A, F, S, C, D, and E. As with ABO blood grouping, you can have a number of genotypes. The genotype decides the degree of clinical severity. Hb SS is severe and Hb SC is moderate to severe. Hemoglobin electrophoresis studies are needed to identify specific genotypes. Other hemoglobin variants include Hb O-Arab, Harlem, G-Philadelphia, Barts and H, I, Casper/Southampton, Genova, Torino, Gun Hill, Constant Spring, Bristol, and Kansas.

### *Sickle-cell anemia*

The biochemical defect of sickle-cell anemia is the result of an amino acid substitution in the  $\beta$  chains. There are several factors that affect the onset, severity and clinical results of RBC sickling as shown in figure 2-6. The disease is less severe with higher oxygen tension. Laboratory findings of the heterozygous (trait) form of the disease include normochromic, normocytic RBCs with occasional target cells. In sickle-cell disease, the anemia is normochromic with increased bilirubin. There is erythroid hyperplasia of the marrow and marked anisocytosis. Poikilocytosis is marked, demonstrating many target cells, sickled cells, and schizocytes. Nucleated RBCs are from 1 to 100 per 100 WBCs. Erythrocyte fragility studies are decreased. A simple screening is available for this hemoglobin prior to confirmation by hemoglobin electrophoresis.



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Figure 2-6. Examples of hemoglobinopathies.

### ***Acquired hemolytic anemias***

Acquired hemolytic anemias can be from immune (antibody) or nonimmune mechanisms.

#### ***Immune disorders***

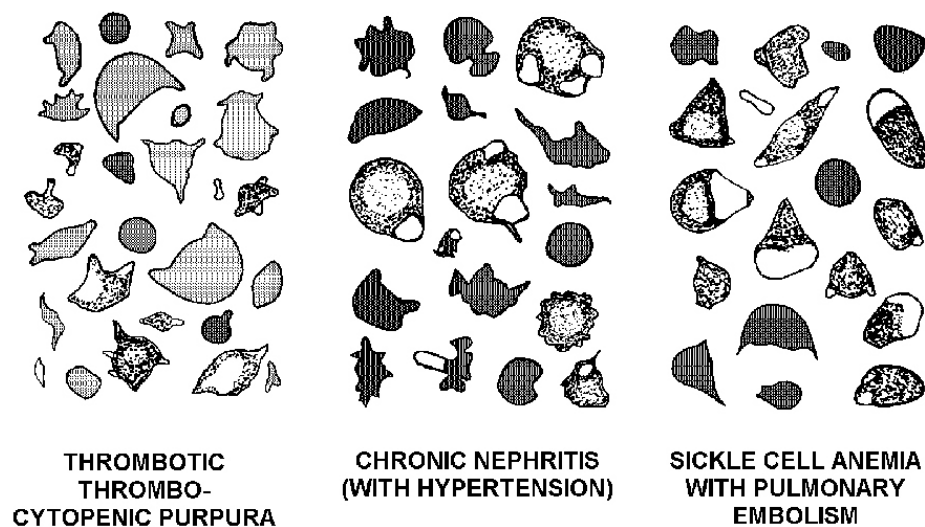
Immune hemolytic anemias include isoimmune antibodies (produced from antigens from another person; example, transfusion reactions), autoimmune antibodies (produced against “self” antigens; example warm- or cold-antibodies), or drug induced antibody production. These disorders are identified by a positive direct antiglobulin test (DAT) that detects the presence of anti-red cell antibodies present in the plasma. Hemolytic disease of the newborn (HDN) is caused by fetal cells crossing the placenta, where they stimulate production of maternal antibodies against the fetal cells. These antibodies can then cross the placental barrier and induce an anemia in the fetus. The most frequent cause of hemolytic fetal cells is by ABO or Rh incompatibility. ABO incompatibilities produce a large IgM antibody that does not cross the placental barrier, while Rh incompatibility produces IgG antibodies. These antibodies can cross the barrier, and the hemolytic anemia is much more severe. ABO incompatibilities have a negative or weakly reactive DAT, positive antibody screen, possibly lowered hemoglobin, and demonstrate nucleated RBCs and striking spherocytosis. Rh incompatibilities show positive DAT and normal hemoglobin at the time of birth that drops to about 3 g/dl, numerous macrocytes, marked polychromatophilia, and pronounced lymphocytosis. Other immunohemolytic anemias due to antibody production demonstrate similar symptoms.

#### ***Paroxysmal nocturnal hemoglobinuria (PNH)***

This disorder is characterized by an increased sensitivity of erythrocytes to complement. This increased sensitivity is seen in attacks of intravascular hemolysis and hemoglobinuria that occur at night. The patient’s RBCs have a tendency to lyse while the body is at rest due to a change pH, causing the individual to urinate large amounts of hemoglobin.

#### ***Nonimmune mechanisms***

These anemias can be caused by small or abnormal blood vessel structure; disseminated intravascular coagulation (DIC); thrombotic thrombocytopenic purpura (TTP); bacterial or parasitic infections, such as malaria; chemicals, toxins, or drugs, such as arsenic, lead, and sulfonamides; or from extensive burns. See figure 2-7 for examples.



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Figure 2-7. Examples of various anemias.



### Blood loss anemia

Blood loss anemias can be from acute or chronic blood loss. Acute blood loss is considered a sudden loss of 25 to 30 percent of total blood volume (1,000 to 1,500 ml), reaching as high as 50 percent of total blood volume, which can result in death. Acute blood loss is seen in car accidents, gun shots, stabbing, aneurysms, etc. The hemoglobin and hematocrit levels remain normal due to vasoconstriction for about 3 to 4 hours. After this time, the body's initial defense mechanism is to restore plasma volume. This dilutes the red blood cells, which decreases the hemoglobin and hematocrit values. The blood film reveals normocytic, normochromic anemia with slight anisocytosis, and poikilocytosis. Chronic anemia results from blood loss for example; excessive menstrual loss or gastrointestinal (GI) bleeding.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 006. General characteristics of cellular maturation

1. What are the general changes that occur in normal cell maturation?
2. How is N/C ratio defined?

### 007. Normal erythrocyte morphology and maturation sequence

1. Match the terms in column B with the statement in column A. Each term in column B may be used once, more than once, or not at all.

<i>Column A</i>	<i>Column B</i>
____ (1) When seen among other blasts, it is rounder with more abundant cytoplasm, and eccentric nucleus.	a. Rubriblast.
____ (2) Slightly larger than an erythrocyte, about 7 to 9 $\mu\text{m}$ .	b. Prorubricyte.
____ (3) The nucleus occupies about 80 percent of the cell.	c. Rubricyte.
____ (4) Shows a change from basophilic cytoplasm to acidophilic chromatin.	d. Metarubricyte.
____ (5) Contains clumped chromatin in a spoke wheel formation.	e. Reticulocyte.
____ (6) Contains cytoplasm devoid of organelles.	f. Erythrocyte.
____ (7) Contains reticula which do not stain with Wright's stain.	g. Erythrocytic membrane.
____ (8) Contains one or two faint, pale blue nucleoli.	h. Erythrocytic function.
____ (9) It has selective permeability and facilitates changes in shape to move through the microvasculature.	
____ (10) The first stage of development showing full hemoglobinization.	
____ (11) Demonstrates the first appearance of hemoglobin.	
____ (12) One of the more highly specialized cells in the body.	
____ (13) Is a lipid bilayer model.	
____ (14) Manufacturing hemoglobin.	
____ (15) It has selective permeability.	

**008. Abnormalities in erythrocyte morphology**

1. What are the three terms used to describe size variations in RBC populations?
2. What is the general term that describes different shapes of RBCs?
3. Match the terms in column B with the statement in column A. Each term in column B may be used once, more than once, or not at all.

*Column A*

- \_\_\_1. Exhibit an elongated or rectangle central zone of pallor that may resemble a mouth-like or slit-like appearance.
- \_\_\_2. Erythrocytic fragments caused by fibrin strands, altered blood vessels or damaged heart valve prosthesis.
- \_\_\_3. Remnants of nuclear material from the normoblast nucleus.
- \_\_\_4. Normal biconcave erythrocyte.
- \_\_\_5. Ring or figure-eight-like artifacts.
- \_\_\_6. Uniformly spaced pointed projections of the outer membrane.
- \_\_\_7. Crescent-shaped cells.
- \_\_\_8. Irregular shaped iron containing structures.
- \_\_\_9. Fine or coarse granules seen in lead poisoning
- \_\_\_10. Erythrocytes that have a reduced or absent central zone of pallor.
- \_\_\_11. Projections that look like horns.
- \_\_\_12. Sharp, pointed irregularly shaped projections from RBC membrane.
- \_\_\_13. Appear dark in the center with a lighter ring between the center and periphery.
- \_\_\_14. Seen in patients with hereditary elliptocytosis.

*Column B*

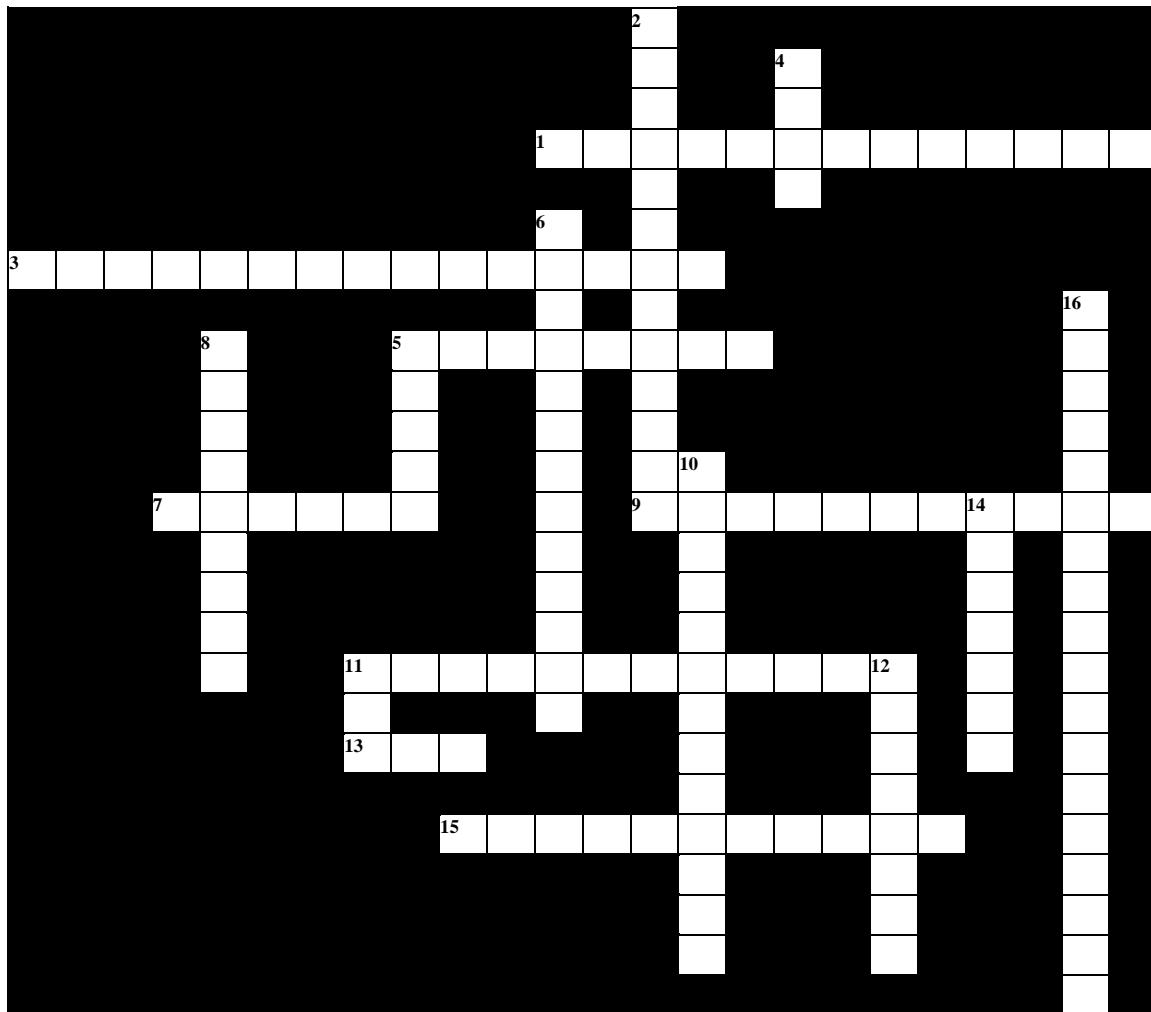
- a. Discocyte.
- b. Burr cells.
- c. Crenated.
- d. Stomatocytes.
- e. Acanthocytes.
- f. Target cells.
- g. Ovalocytes.
- h. Spherocytes.
- i. Schizocytes.
- j. Keratocytes.
- k. Teardrop cells.
- l. Semilunar bodies.
- m. Sick cells.
- n. Howell-Jolly bodies.
- o. Basophilic stippling.
- p. Siderotic granules.
- q. Cabot rings.

**009. Erythrocyte production and destruction abnormalities**

1. What is polycythemia?
2. What are the three types of polycythemia?
3. What is anemia?
4. What are the two basic reasons for anemia?
5. What are the different ways to classify anemias?

6. What is the physiologic classification based on?

7. Complete the following crossword puzzle using the different types of anemia.



ACROSS	DOWN
1. A hypoproliferative anemia due to metastatic carcinoma.	2. Most common hereditary disorder in the group of red cell membrane defect anemias.
3. The most common form of a nutritional deficiency anemia in developing countries.	4. A deficiency that is an inherited sex-linked trait expressed when exposed to increased oxygen.
5. Occurs when there is a complete failure of the bone marrow to produce all cell lines.	5. A sudden loss of 25 to 30 percent of total blood volume, seen in car accidents, etc.
7. Hemolytic anemia that includes antibody production.	6. These anemias are characterized by increased MCV, macrocytic, normochromic morphology.
9. Best known hemoglobinopathy.	8. Anemia caused by DIC, TTP, infections, etc.
11. Most common megaloblastic anemia.	10. Iron-laden mitochondria granules seen with Prussian blue stain, includes porphyrias group.
13. A cause of acquired hemolytic anemia of newborns.	11. A disorder characterized by an increased sensitivity of RBCs to complement.
15. Associated with structural hemoglobin variants by the presence of mutant genes.	12. Hemolytic anemia from immune or nonimmune mechanisms.
	14. Anemia associated with excessive menstrual loss or gastrointestinal bleeding.
	16. Involved in abnormal structures of the polypeptide $\alpha$ , $\beta$ , $\delta$ , and $\gamma$ globin chains.

## 2-2. Leukocyte and Thrombocyte Maturation and Characteristics

A study of leukocyte and thrombocyte maturation necessarily involves studying the various stages that the cells pass through prior to assuming the characteristics of mature cells. The five types of leukocytes that originate from the pluripotent stem cell have similar morphological characteristics. They begin as primitive cells, evolve into blasts, and eventually attain the nuclear and cytoplasmic characteristics you are familiar with. Their changes are distinctive enough to differentiate them into developmental stages and cells. In a healthy person only the mature cells are found in the peripheral blood. However, in many diseases, immature and abnormal forms of cells may be present. For this reason, it is important for you to be able to identify abnormal cell forms for both erythrocytes and leukocytes. Their presence and number in the peripheral blood provides the clinicians with valuable insights into the patients' condition and prognoses.

### 010. Normal leukocyte morphology and maturation sequence



As you know, developing blood cells follow a set series of events as the cell approaches maturity. Cells in the granulocytic, agranulocytic, and megakaryocytic series all demonstrate the aforementioned (006.) characteristics. These and other special differences form the basis for our discussion on maturation. The five types of leukocytes or white blood cells (WBC), like erythrocytes, are identified by various terms. *Granulocytes* or *polymorphonuclear* are used to identify the neutrophils, eosinophils, and basophils. *Agranulocytes*, *nongranulocytes*, and *mononuclear* are used to describe the monocytes and lymphocytes. These terms may not be satisfactory because there are exceptions. Other terms, based on function, are *phagocytes* and *immunocytes*.

#### Function

Leukocytes are found in bone marrow, blood, and tissue. Most of their function is carried out in the tissues; thus, the blood is just the transport system. The primary function of the leukocyte is to protect the body from foreign invaders. The phagocytes seek, engulf, and destroy these invaders, while the immunocytes are concerned with antibody production and other immune response activities. Both functions depend on each other and are interrelated for maximum efficiency. The phagocytes are neutrophils, eosinophils, basophils, and monocytes. The immunocytes are the lymphocytes. Possibly, the terms, based on their function, would be more descriptive and should be used. Nonetheless, this text will use the more familiar, conventional terms: granulocytic, agranulocytic, and megakaryocytic series.

#### Granulocytic series

Specific granules develop in cells of the granulocytic series as the cells mature. Azurophilic (nonspecific) granules initially develop after the blast stage. Consequently, they are found in early stages of the granulocytic series. As the granulocyte matures, azurophilic cytoplasmic granules give way to more specific granules. The granulocytes are named according to their cytoplasmic granule staining properties. Eosinophil granules stain bright red-orange because of their affinity to eosin, the acidic dye in Wright's stain. Basophil granules stain dark blue-purple because of their affinity to methylene blue, which is the basic dye in Wright's stain. Neutrophil granules appear pinkish-tan because they don't have an affinity for either dye. Normally, only band and segmented granulocytes are observed in the peripheral blood. The other cells are normally seen *only* in bone marrow preparations. Refer to figure 2-8 for a color chart of the granulocytic system. Figure 2-8, *Reproduction of Morphology of Human Blood Cells*, is used with the approval of Abbott Laboratories, all rights reserved by Abbott Laboratories.

The stages of normal granulocyte maturation are listed below.

- Myeloblast.
- Promyelocyte.
- Myelocyte (neutrophilic, eosinophilic, and basophilic).
- Metamyelocyte (neutrophilic, eosinophilic, and basophilic).
- Band cell (neutrophilic, eosinophilic, and basophilic).
- Segmented cell (neutrophilic, eosinophilic, and basophilic).

### ***Myeloblast***

Let's consider size, cytoplasm, and nucleus.

Characteristics	Explanation/Description
<i>Size</i>	Myeloblasts range from 15 to 20 $\mu\text{m}$ in diameter.
<i>Cytoplasm</i>	The amount of cytoplasm is small in relationship to the rest of the cell. The cytoplasm is very smooth in texture, is usually nongranular, moderate blue in color, and forms a thin rim around the nucleus.
<i>Nucleus</i>	The nucleus is round or oval. It occupies about four-fifths of the cell and has a delicate, interlaced chromatin pattern. The N/C ratio is 4:1. The nucleus stains reddish-purple and contains 2 to 5 nucleoli.

### ***Promyelocyte***

Let's consider size, cytoplasm, and nucleus.

Characteristics	Explanation/Description
<i>Size</i>	Promyelocytes range in size from 15 to 21 $\mu\text{m}$ and are just slightly larger than the myeloblast.
<i>Cytoplasm</i>	Cytoplasm stains pale blue. A few large nonspecific azurophilic granules are present. More mature promyelocytes may show a few specific granules (neutrophilic, eosinophilic, or basophilic).
<i>Nucleus</i>	The nucleus of promyelocytes is oval or round and occupies one-half or more of the cell. It contains two to three nucleoli, which are not as distinct as in the myeloblast. The chromatin is slightly clumped, appearing more coarse and less evenly stained. The N/C ratio is 3:1 to 2:1. A cell ceases to be a promyelocyte and becomes a myelocyte when specific, definitive granules are present and the nucleus becomes slightly indented.

### ***Myelocyte***

Let's consider size, cytoplasm, and nucleus.

Characteristics	Explanation/Description
<i>Size</i>	Myelocytes range in size from 12 to 18 $\mu\text{m}$ in diameter.
<i>Cytoplasm</i>	The cytoplasm contains a few-to-moderate number of nonspecific granules and a few patches of blue. The first sign of neutrophilic differentiation is a small, relatively light area of ill-defined, pink granules that develop in the cytoplasm among the nonspecific azurophilic granules. The azurophilic granules become less prominent and disappear as the myelocyte ages. The chromatin appears more clumped in the myelocyte than in the promyelocyte. Neutrophilic myelocytes are usually smaller than promyelocytes and have a relatively larger cytoplasm. Specific granules begin to appear at this stage and are easily distinguished as being neutrophilic, eosinophilic, or basophilic.
<i>Nucleus</i>	The nuclei of the myelocytes are oval or round. The chromatin pattern becomes coarser, and nucleoli are either not visible or absent. Nuclei can be centrally located or eccentric. The N/C ratio 1:1. This is the last stage capable of cell division.

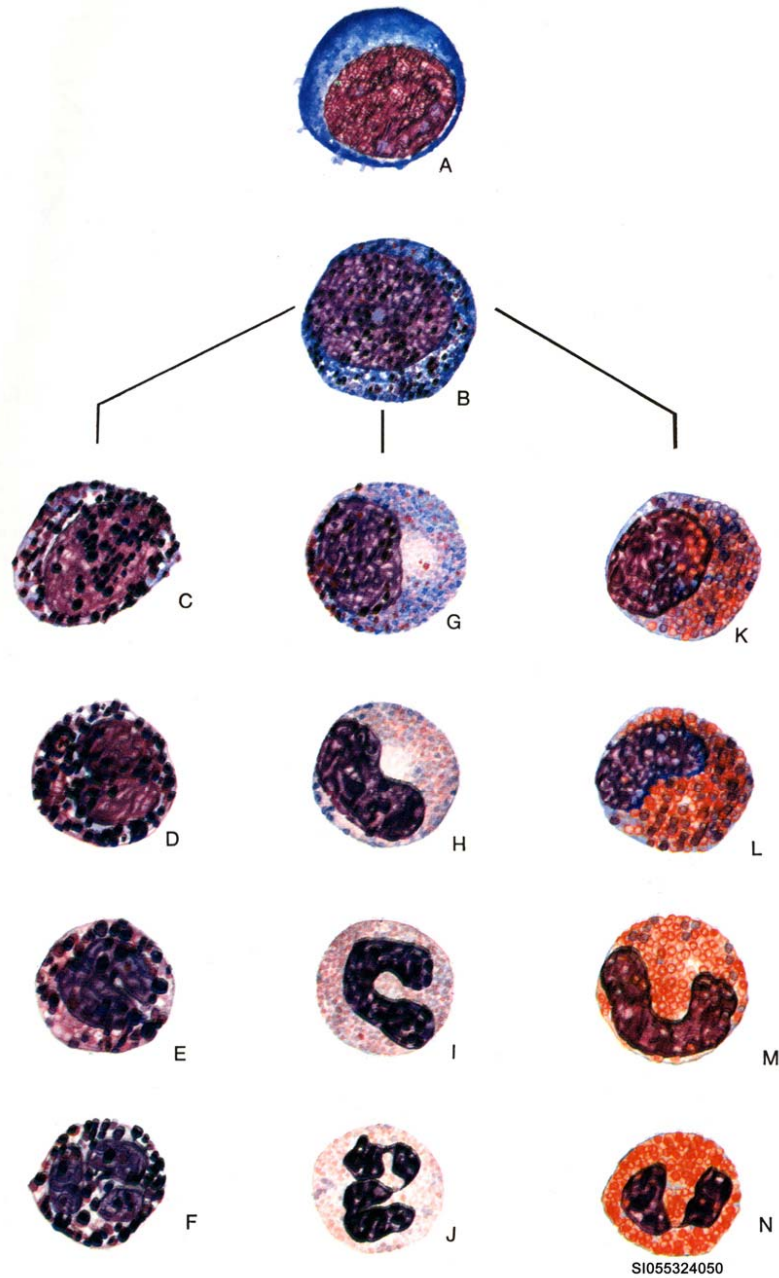


PLATE 3—MYELOCYTIC (GRANULOCYTIC) SYSTEM

A Myeloblast  
B Promyelocyte (progranulocyte)

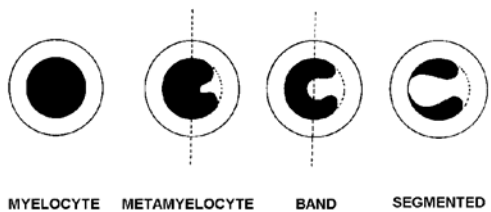
C Basophilic myelocyte	G Neutrophilic myelocyte	K Eosinophilic myelocyte
D Basophilic metamyelocyte	H Neutrophilic metamyelocyte	L Eosinophilic metamyelocyte
E Basophilic band	I Neutrophilic band	M Eosinophilic band
F Basophilic segmented	J Neutrophilic segmented	N Eosinophilic segmented

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Figure 2-8. Morphology of human blood cells; myelocytic (granulocytic) system.

### Metamyelocyte

Neutrophilic metamyelocytes are slightly smaller than myelocytes and have a relatively smaller nucleus and less defined chromatin net. Neutrophilic metamyelocytes are not seen in peripheral blood of normal people, but are often found in acute conditions in which there is a marked increase in myelocytic proliferation. Let's consider size, cytoplasm, and nucleus.

Characteristics	Explanation/Description
Size	Metamyelocytes range in size from 10 to 15 $\mu\text{m}$ in diameter.
Cytoplasm	<p>Their amount of cytoplasm is moderate to abundant. Nonspecific granules are not seen, but there is a full amount of specific granules. The N/C ratio is about 1.5:1.</p> <p>Neutrophilic metamyelocyte—granules are pinker and numerous.</p> <p>Eosinophilic metamyelocyte—granules are brighter red-orange and more numerous.</p> <p>Basophilic metamyelocyte—dark purple to black granules are numerous.</p>
Characteristics	Explanation/Description
Nucleus	<p>The nucleus of the metamyelocyte is kidney-shaped or indented, with a heavy nuclear membrane as shown in figure 2-9. The chromatin pattern is coarser and has no visible nucleoli. The chromatin stains deep purple, much darker than that of younger cells.</p> <div style="text-align: center;">  <p>MYELOCYTE   METAMYELOCYTE   BAND   SEGMENTED</p> <p>SI965324021</p> </div> <p><b>Figure 2-9. Example of maturation changes of a nucleus.</b></p>

### Band granulocyte

In band form, the nuclear indentations are more indented than the kidney-shaped nucleus of the metamyelocytes, but it does not have filaments typical of the segmented neutrophil. Neutrophilic bands are slightly smaller than metamyelocytes. The specific cytoplasmic granules of band neutrophils are small and evenly distributed and stain various shades of lilac to pink with Wright's stain. Let's consider size, cytoplasm, and nucleus.

Characteristics	Explanation/Description
Size	These cells range from 9 to 15 $\mu\text{m}$ in diameter.
Cytoplasm	Their cytoplasm is similar to that of the myelocyte.
Nucleus	The nucleus is sausage or band-shaped. The chromatin pattern is coarse. It can be constricted at one or more points, with a sufficient amount of chromatin visible in the constriction.

### Segmented granulocyte

Mature neutrophils are approximately twice the size of erythrocytes. These cells differ from neutrophilic bands in that the nucleus has two or more definite lobes separated by very thin filaments rather than an indentation. See figure 2-10 for examples of the band and segmented neutrophil. The cytoplasm in an ideal Wright-stained preparation is buff or pink, and the small, numerous, and evenly distributed neutrophilic granules have a lilac color. The transition among the various stages of neutrophilic cells is gradual. Differentiation of cells is made almost exclusively from the nuclear configuration. Borderline cells are difficult to distinguish. Cells should be placed in the more mature category when there is doubt; however, in the distinction between bands and segmented cells, the

assumption that a filament must be present, even though it cannot be seen, should not be made. Such cells should classify as bands. If a question arises as to whether a cell is a metamyelocyte or a band, it should be counted as a band. Let's consider size, cytoplasm, and nucleus.

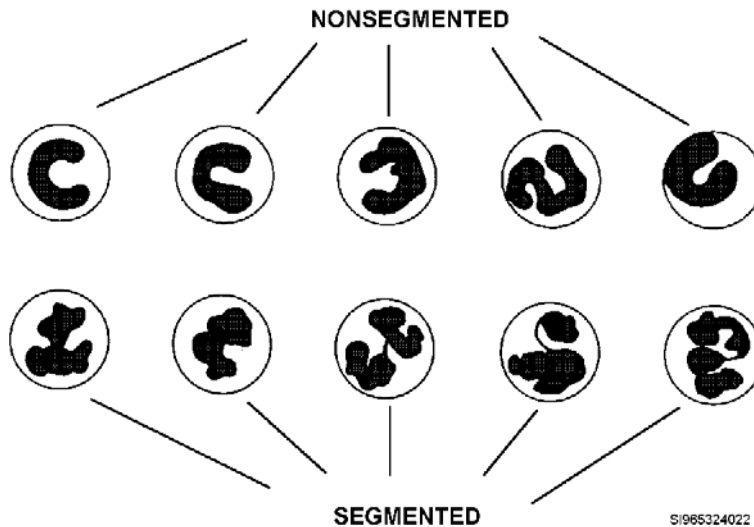


Figure 2-10. Comparing unsegmented (band) and segmented neutrophils.

Characteristics	Explanation/Description
<i>Size</i>	Its sizes range from 9 to 15 $\mu\text{m}$ in diameter.
<i>Cytoplasm</i>	The N/C ratio is 1:3, color is light pink or blue. Specific granules are: Neutrophilic—fine granules, pink or lilac in color. Eosinophilic—large, brick-red granules. Basophilic—large, blue-black granules.
<i>Nucleus</i>	The nucleus of the segmented granulocyte appears clumped with a coarse chromatin pattern. It stains deep purple-blue with scant parachromatin. Two or more lobes of nuclear chromatin are connected by thin filaments.

### Agranulocytic series

The agranulocytic series is composed of leukocytes without specific granulation. This series includes monocytes and lymphocytes.

#### Monocyte

Monoblasts are derived from the CFU-GM (G for neutrophils and M for macrophage), which is derived from the hematopoietic stem cell. The monoblasts mature to form promonocytes and then monocytes.

#### Monoblast

This cell is extremely difficult to differentiate from the myeloblast. It is 12 to 20  $\mu\text{m}$  in diameter. There is a moderate amount of basophilic or gray-tinged cytoplasm. There are no granules in the cytoplasm. The nucleus is round or oval and appears more lightly stained than in the myeloblast. The nuclear chromatin is fine and delicate with abundant, sharply defined, pale pink or blue parachromatin. There are one or two nucleoli present. The N/C ratio is 4:1 to 3:1.



### *Promonocyte*

Some authorities do not differentiate the promonocyte from the monoblast. It is 14 to 18  $\mu\text{m}$  in size. The cytoplasm is blue-gray, ground glass in appearance with granules so small that they are called *azurophilic dust*. The nucleus is oval, single folded, with one to five nucleoli and a fine chromatin pattern. N/C ratio is 3:1 to 2:1.

### *Monocyte*

Mature monocytes are usually larger than other leukocytes in peripheral blood (14 to 20  $\mu\text{m}$ ). Unstained monocytes exhibit slow, amoeboid movement and can be seen on the wet mount with single or multiple pseudopodia. The cytoplasm of a monocyte is opaque, blue-gray without the clear perinuclear zone described for most lymphocytes. It has been described as foamy or having a ground glass appearance and may occasionally contain vacuoles. There is a large amount of cytoplasm in relation to the nucleus, which is also in contrast to the lymphocyte. The nonspecific, fine, azurophilic granules can be seen in overstained smears. The nucleus of the monocyte is usually folded, but it can be round, kidney-shaped, or deeply indented as shown in figure 2-11. One of the more distinctive features of the monocyte is the fine, diffuse chromatin strands with abundant parachromatin in the nucleus.

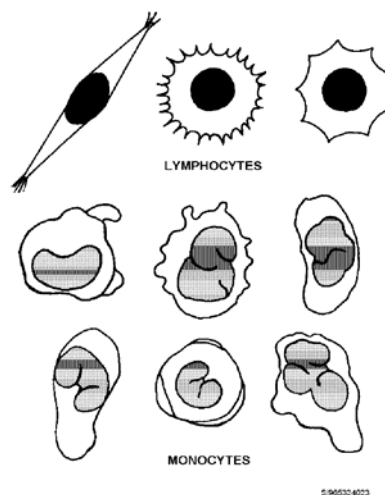
This diffuse, very light-staining nucleus differentiates the monocyte from the lymphocyte and the metamyelocyte. This delicate chromatin pattern is in contrast to the lymphocyte and metamyelocyte chromatin, which is clumped. Nucleoli are absent. If overstained, the monocyte can be confused with metamyelocytes. Monocytes can be difficult to differentiate from other cells, particularly if the stain is not good. In poorly stained smears, the delicate nuclear morphology appears less distinct, and the coarse granules confuse the picture. When other differential features are absent, the brain-like convolutions of the nucleus and the dull blue-gray color of the cytoplasm are usually sufficient to classify the cell as a monocyte. Refer to figure 2-12 for color comparison of the different monocytes. Figure 2-12, *Reproduction of Morphology of Human Blood Cells*, is used with the approval of Abbott Laboratories, all rights reserved by Abbott Laboratories.

### *Lymphocyte*

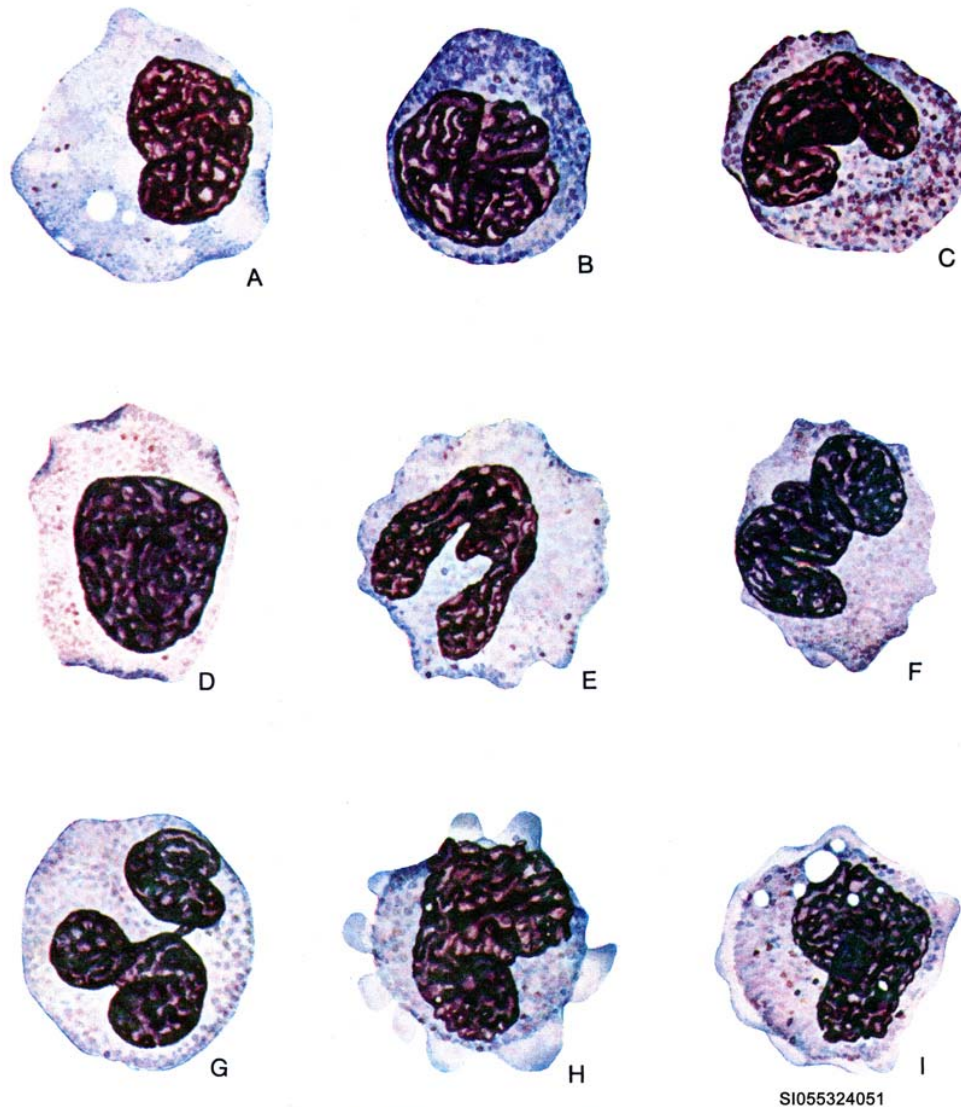
Lymphoblasts originate from the CFU-BL (B-lymphocyte) and CFU-BT (T-lymphocyte), which are derived from the lymphoid stem cell. The lymphoblast mature to form the prolymphocyte which forms small, medium, or large lymphocytes.

### *Lymphoblast*

These cells are similar to other blast cells. They are 10 to 18  $\mu\text{m}$  in diameter. The cytoplasm is moderately basophilic and nongranular, forming a lighter, thin rim around the nucleus. The nucleus is round or oval, staining light red-purple with Wright's stain. The nuclear chromatin is finer than that of the lymphocyte, but not as delicate as in the myeloblast. There is a moderate amount of light-blue parachromatin. There are one to two nucleoli present and the N/C ratio is 4:1.



**Figure 2-11. Examination of different monocyte and lymphocyte shapes.**



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## PLATE 4—MONOCYTES

- |                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>A Monocyte with "ground-glass" appearance, evenly distributed fine granules, occasional azurophilic granules, and vacuoles in cytoplasm</p> <p>B Monocyte with opaque cytoplasm and granules and with lobulation of nucleus and linear chromatin</p> <p>C Monocyte with prominent granules and deeply indented nucleus</p> <p>D Monocyte without nuclear indentations</p> | <p>E Monocyte with gray-blue color, band type of nucleus, linear chromatin, blunt pseudopods, and granules</p> <p>F Monocyte with gray-blue color, irregular shape, and multilobulated nucleus</p> <p>G Monocyte with segmented nucleus</p> <p>H Monocyte with multiple blunt nongranular pseudopods, nuclear indentations, and folds</p> <p>I Monocyte with vacuoles and with nongranular ectoplasm and granular endoplasm</p> |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

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Figure 2-12. Morphology of human blood cells; monocytic series.

### *Prolymphocyte*

Some authors do not differentiate this cell from the lymphoblast. When these cells are differentiated, it is more by comparison with lymphoblasts and lymphocytes than by any unique, morphological characteristics. There is more abundant, light-blue to moderately basophilic, cytoplasm. In addition, there may be a few azurophilic granules. The nuclear chromatin is described as more coarse than in the lymphoblast, being slightly clumped. One nucleus is usually present with one to two nucleoli. Generally, the prolymphocyte is smaller than the lymphoblast.

### *Lymphocyte*

The lymphocyte is more distinctive than its precursor. The cell is described as approximately 8 to 16  $\mu\text{m}$  in diameter or from about the size of a normal RBC to more than twice the size of an RBC. They can be divided into three categories: *small* (8 to 10  $\mu\text{m}$ ), *medium* (10 to 12  $\mu\text{m}$ ), and *large* (12 to 16  $\mu\text{m}$ ) for descriptions. The cytoplasm may vary in quantity from scant to moderate, depending on the thickness of the smear and the size of the cell. The cytoplasm is normally clear and homogeneous and described as light blue, sky blue, lightly basophilic, or moderately basophilic. The cytoplasm normally contains a few nonspecific azurophilic granules that are peroxidase negative. A clear perinuclear zone is often observed in the cytoplasm. The nucleus of a normal lymphocyte can be round, oval, or slightly indented. It contains clumped chromatin, which appears in Wright-stained preparations as very dark staining bluish-purple aggregates in the nucleus separated by lighter staining, indistinct areas of parachromatin. This description of the normal lymphocyte indicates the wide biological variations that must be thoroughly appreciated before attempting to differentiate the normal from the atypical or variant lymphocytes see figure 2-11. Also, refer to figure 2-13 for a color comparison of the different lymphocytes. Figure 2-13, *Reproduction of Morphology of Human Blood Cells*, is used with the approval of Abbott Laboratories, all rights reserved by Abbott Laboratories.

### **Thrombocyte maturation sequence and abnormalities**

The cells of the megakaryocytic series arise from the committed stem cell, the megakaryoblast (CFU-Meg), which is produced by the hematopoietic stem cell (CFU-C). Megakaryocyte colony stimulating factor (CSF-Meg) is responsible for the proliferation of the committed stem cells into megakaryoblasts. In addition, thrombopoietin stimulates differentiation and maturation of the megakaryocytes. This cell line differs from the others in that during thrombocyte maturation the cells increase in size instead of decrease, except for the platelet.

### ***Megakaryoblast (stage I)***

These cells range from 20 to 50  $\mu\text{m}$  in diameter and are irregular in shape with blunt protrusions. The cytoplasm is scant, irregularly basophilic, and usually nongranular. The nucleus is unique in that it is capable of undergoing multiple mitotic divisions without cytoplasmic division termed endomitosis. This creates giant multinucleated or polyploid cells. There may be up to 32 nuclei within one megakaryoblast. Multiple nucleoli are found in the nuclei. The nuclei may be round, oval, or kidney shaped with a fine chromatin pattern. The N/C ratio is approximately 10:1.

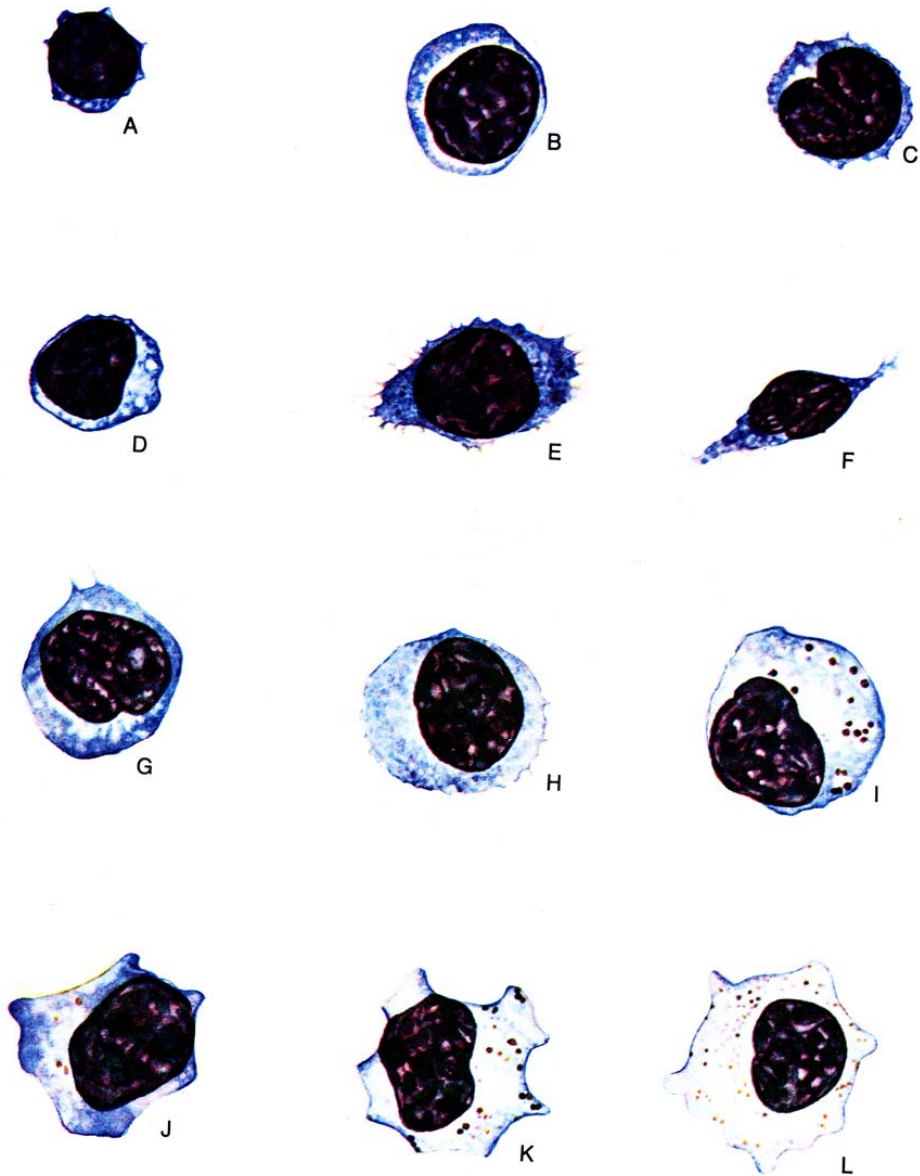


PLATE 5—LYMPHOCYTES

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- |                                                                                       |                                                                                                                      |
|---------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| A Small mature lymphocyte                                                             | H Large lymphocyte                                                                                                   |
| B Lymphocyte of intermediate size                                                     | I Large lymphocyte with purplish-red (azurophilic) granules                                                          |
| C Lymphocyte with indented nucleus                                                    | J Large lymphocyte with irregular cytoplasmic contours                                                               |
| D Lymphocyte of intermediate size                                                     | K Large lymphocyte with purplish-red (azurophilic) granules and with indentations caused by pressure of erythrocytes |
| E Lymphocyte with pointed cytoplasmic projections (frayed cytoplasm); typical nucleus | L Large lymphocyte with purplish-red (azurophilic) granules                                                          |
| F Spindle-shaped lymphocyte with indented nucleus                                     |                                                                                                                      |
| G Large lymphocyte with indented nucleus and pointed cytoplasmic projections          |                                                                                                                      |

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Figure 2-13. Morphology of human blood cells; lymphocytic series.

***Promegakaryocyte (stage II)***

These cells are generally larger than the preceding cells, ranging from 20 to 70  $\mu\text{m}$  in diameter. The cell membrane is still irregular with blunt protrusions. The cytoplasm is less basophilic than in the blast cell with granules beginning to appear. The nuclei are also irregular in shape with multiple nucleoli and a coarse nuclear chromatin. The N/C ratio is between 4:1 and 7:1, depending on the ploidy.

***Megakaryocyte (stage III)***

The megakaryocyte is about 30 to 90  $\mu\text{m}$  in diameter. It may also be called a granular megakaryocyte. The abundant cytoplasm has numerous, rather uniformly dispersed, fine pinkish-blue granules. The multiple nuclei are small with a coarse chromatin. N/C ratio is 2:1 to 1:1.

***Metamegakaryocyte (stage IV)***

These cells are the largest of the blood cells, ranging from 40 to 120  $\mu\text{m}$  in diameter.

Metamegakaryocytes may also be known as mature megakaryocytes. The cytoplasm contains coarse clumps of granules aggregating into little “packets.” These packets bud off from the outer edge of the cell to become platelets. The nucleus or nuclei are present with an N/C ratio of 1:1.

Metamegakaryocytes usually do not appear in the peripheral blood, although its fragments may be seen. In bone marrow preparations, there should be little difficulty in recognizing cells of this series due to their enormous size.

***Thrombocyte (platelet)***

The thrombocyte cytoplasm stains light blue to purple and is very granular. There is no nucleus. The platelet is only 1 to 4  $\mu\text{m}$  in diameter; yet, it has a very complex structure. The platelet is composed of approximately 60 percent protein, 30 percent lipid, and 8 percent carbohydrate, various minerals, water, and nucleotides. It also contains over 90 different enzymes, glycogen, and coagulation factors. It has four specific areas.

Area	Explanation
Peripheral zone	Consists of the outer membrane, which is responsible for platelet adhesion and aggregation.
Submembrane area	Is a microtubular system that connects the inner part of the cell to the outer membrane.
Sol-gel zone	Is the cytoskeleton part of the cell that maintains platelet shape.
Organelle zone	Is composed of the mitochondria, dense bodies, lysosomes, and alpha granules.

***Platelet function***

The primary function of the platelet is to stop bleeding and maintain capillary integrity.

***Thrombocyte abnormalities***

The general accepted platelet reference range is  $140$  to  $440 \times 10^9/\text{L}$ . Platelet abnormalities include quantitative (thrombocytopenia and thrombocytosis or thrombocythemia) and qualitative (inherited and acquired defects) disorders. A review of these abnormalities is given in the table below.

Abnormality	Classification or Cause
<b><i>Thrombocytopenia</i></b>	Decreased platelet production Increased platelet loss or destruction Increased platelet sequestration by spleen Dilution of platelets by multiple transfusions
<b><i>Thrombocytosis</i></b>	Reactive thrombocytosis is a moderate increase in response to anemia, surgery, infections, or neoplasm's Primary or autonomous thrombocytosis is an increase due to myeloproliferative disorders
<b><i>Inherited defects</i></b>	Platelet adhesion disorders

Abnormality	Classification or Cause
	Platelet aggregation disorders Platelet secretion disorders
<b>Acquired defects</b>	Drugs (i.e., aspirin, penicillin's, and alcohol) Diet (i.e., vitamin deficiencies or overdoses) Diseases (i.e., uremia, DIC, AML, Multiple myeloma, and myeloproliferative disorders)

More information will be given in unit 4 and the role of platelets in hemostasis and how these abnormalities affect hemostasis.

## 011. Leukocyte abnormalities

Leukocyte abnormalities can be caused by mechanical means, stimulation, or disease states. These abnormalities can be divided into two categories: malignant or nonmalignant. Malignant is defined as becoming progressively worse, resulting in death. In this lesson we'll use these divisions in order to study leukocyte abnormalities.

### Leukocyte terminology

Before moving further, we must review the following terms associated with leukocyte abnormalities.

Term	Definition
<b>Leukopenia</b>	Leukopenia is present if the WBC result is $<3.0 \times 10^9/\text{Liter}$ of blood or below the reference range for the age and sex of that person.
<b>Leukocytosis</b>	Leukocytosis refers to a WBC result $>11.0 \times 10^9/\text{Liter}$ of blood or above the reference range for the age and sex of that person.
<b>Leukemia</b>	<i>Leukemia</i> is a progressive, malignant disease of the blood-forming organs.
<b>Polycythemia vera</b>	In this disease, leukocytes, as well as thrombocytes and erythrocytes, show increased counts. Histochemical studies of the leukocytes produced reveal normal cells; however, it has been reported that granulocytic leukemia has developed from this disease.
<b>Acute, chronic, and subacute</b>	These terms are associated with the course or the disease and with certain disorders.
	<i>Acute</i> Means a disorder has a short and relatively severe course.
	<i>Chronic</i> Means that a disease persist over a long period of time.
	<i>Subacute</i> Is between acute and chronic.
<b>Malignant and nonmalignant</b>	As stated above, the tendency for a disorder to become progressively worse and result in death is termed <i>malignant</i> , hence, <i>nonmalignant</i> would not result in death.
<b>Atypical or variant lymphocytes</b>	Certain disease states can change or transform lymphocytes into an immature cell with the appearance of a blast cell. These cells are termed <i>atypical</i> . Other terms used to describe these atypical lymphocytes include virocytes; variant, leukocytoid, reticular, reactive, and transformed lymphocytes; plasmacytoid lymphocyte; lymphocytoid plasma cell; or Turk cells. The Clinical and Laboratory Standards Institute has proposed the term <i>variant lymphocyte</i> be used in place of <i>atypical lymphocyte</i> .
<b>Reference ranges</b>	The International Committee for Standardization in Hematology has recommended the liter (l) as the unit of volume for use in hematology, however the United States uses (L). The traditional unit cubic millimeters (cu mm, $\text{mm}^3$ ) is no longer used. See the example below.  $1 \text{ cu mm} = 1.00003 \mu\text{L}$ (or $1 \mu\text{L} = 10^{-6} \text{ liters}$ or $1 \times 10^6 \mu\text{L} = 1 \text{ liter}$ , therefore; $5,500/\text{cu mm} = 5.5 \times 10^3/\text{cu mm}$ $5,500/\mu\text{L} = 5.5 \times 10^3/\mu\text{L}$ $5,500 \times 10^6/\text{Liter} = 5.5 \times 10^9/\text{L}$ These are all the same WBC count.

Reference ranges are age dependent so they would be different for infants, children, and adults. In order to compare abnormal values, you must know the normal or expected reference range. The below table uses the adult reference ranges.

Cell Type	Relative Cell Count	Absolute Cell Count
Neutrophils-Band	0.0 to 0.6%	0.0 to $0.70 \times 10^9/L$
Neutrophils-Segmented	47.0 to 78.9%	$1.8$ to $7.00 \times 10^9/L$
Eosinophils	0.0 to 7.5%	0.0 to $0.45 \times 10^9/L$
Basophils	0.0 to 2.0%	0.0 to $0.20 \times 10^9/L$
Monocytes	2.0 to 11.0%	0.0 to $0.80 \times 10^9/L$
Lymphocytes	12.5 to 40.0%	$1.0$ to $4.80 \times 10^9/L$
<b>Total leukocytes</b>	NA	<b><math>4.5</math> to <math>11.0 \times 10^9/L</math></b>

### Nonmalignant leukocyte abnormalities

We know that the primary function of the leukocytes is to protect the body against foreign invaders. As these “stand-by” cells become “active warriors” they must transform in order to prepare for battle. The transformation includes:

- Increase in cell size.
- Cytoplasm becomes basophilic and increases.
- Toxic granules and vacuoles may appear.
- Nucleus changes shape, becomes less dense with nucleoli present, and may be hypersegmented.
- Alterations are made in total number of leukocytes present in the blood.

Morphological changes that occur in neutrophils in response to stress, infection, or inflammation are called *toxic changes*. Nonmalignant leukocyte changes or abnormalities may be caused by a number of various responses or reasons. They are summarized in the table below. Keep in mind that malignant leukocyte changes can overlap with nonmalignant changes.

Change or Disorder	Cause	Laboratory Findings
Neutrophilia—Acute or Chronic	Infections, inflammation, and malignancy	Neutrophil count $>8.0 \times 10^9/L$ with increased numbers of immature forms
Neutropenia	Inherited disorders, bone marrow damage, destruction due to infections, viral infections, neonatal isoimmune reaction, drug hypersensitivity, and sequestration of neutrophils	Neutrophil count $<1.8 \times 10^9/L$ , <i>agranulocytosis</i> is used when the count is $<0.5 \times 10^9/L$
Eosinophilia	Inherited, malignant, parasitic invasion, and hypersensitivity disorders	Eosinophil count $>0.7 \times 10^9/L$
Eosinopenia	Decreased bone marrow production, acute bacterial infections, and administration of ACTH (adrenocorticotrophic hormone)	Hemocytometer chamber counts of $<0.05 \times 10^9/L$
Basophilia	Immediate hypersensitivity reactions, myeloproliferative disorders, long-term antigen stimulation, hypothyroidism, ulcerative colitis, and estrogen therapy	Basophil count $>0.3 \times 10^9/L$
Basopenia	Acute infections, stress, hyperthyroidism, and increase in glucocorticoids levels	Basopenia has been described but it is difficult to measure in the clinical laboratory

Change or Disorder	Cause	Laboratory Findings
Monocytosis	Bacterial, tuberculosis, and syphilis infections, inflammation, neutropenia recovery, and myeloproliferative disorders	Monocyte count $>0.9 \times 10^9/L$
Monocytopenia	Rare inherited disorders, glucocorticoids administration, and acute bacterial and viral infections	Monocyte count $<0.03 \times 10^9/L$
Lymphocytosis-Absolute with variant morphology	Infectious mononucleosis, acute viral hepatitis, and cytomegalovirus infections	Lymphocyte count $>4.8 \times 10^9/L$
Lymphocytosis-Absolute with normal morphology	Acute infectious lymphocytosis and <i>Bordetella pertussis</i> infection	Lymphocyte count $>4.8 \times 10^9/L$
Lymphocytosis-Relative with variant morphology	Viral infections, immune disorders, drug reactions, serum sickness, tuberculosis, syphilis, malaria, typhus, brucellosis, rickettsia, and diphtheria	Increase in lymphocyte percentage, not increase in cell count
Lymphocytosis-Relative with normal morphology	Neutropenia	Increase in lymphocyte percentage, not increase in cell count

### ***Leukemoid reactions***

In leukemoid reactions, the blood picture can be that of a true leukemia. A large increase in leukocyte counts without extreme immaturity suggests a leukemoid reaction. However, a shift to the left, so extreme as to show promyelocytes, and a rare blast form may occur in leukemoid reactions. When the disease is accompanied by anemia, thrombocytopenia, splenomegaly, or lymph node enlargement, it may be difficult to distinguish the leukemoid reaction from leukemia. A bone marrow aspiration may be necessary to differentiate this condition from true leukemia, even though bone marrow studies are not always diagnostic. Leukemoid reactions most commonly occur in whooping cough, chicken pox, tuberculosis, infectious mononucleosis, and when bone marrow is replaced by metastatic tumors.

### ***Leukoerythroblastic reaction***

Leukoerythroblastic reactions reveal both nucleated red blood cells and immature neutrophils. This reaction is nonspecific, but it does provide evidence of underlying disease or stress to the bone marrow. It may be mild to severe. Mild leukoerythroblastic reactions may be associated with hemolytic anemia, severe infections, cardiac failure, uremia, and megaloblastic anemia. Severe leukoerythroblastic reactions may be associated with metastatic tumors, lymphoma, leukemia, and fibrosis.

### ***Variant lymphocyte morphology***

Variant lymphocytes are normal cells that have gone through changes because of their response to stimuli. It is important to remember that they are benign. You may have heard of the *Downey* cell morphology of infectious mononucleosis; it is so named because in 1923 Hal Downey (1877-1959) and Dr. C.A. McKinlay provided a description of reactive lymphocytes. They described three distinct types of variant lymphocytes. Their morphology is summarized in the table below.

<b>VARIANT LYMPHOCYTES</b>			
Parameter	Type I	Type II	Type III
Cell size	9 to 20 $\mu m$	15 to 25 $\mu m$	12 to 35 $\mu m$
Shape	Round to oval	Scalloped or Irregular	Round to Irregular
Cytoplasm	Moderately basophilic, foamy appearance, vacuolated, may contain azurophilic granules,	Pale with basophilia at the periphery, abundant and indented, few	Abundant basophilia with vacuoles and a clear perinuclear area



VARIANT LYMPHOCYTES			
Parameter	Type I	Type II	Type III
	darker areas of basophilia at periphery	vacuoles	
Nucleus	Indented or oval, distinct nuclear membrane, heavy strands, dense irregularly clumped	Round or oval rarely lobulated, coarse chromatin strands but not as condensed as type I, nucleoli are usually not visible	Nucleoli visible and elongated or irregular, finely dispersed chromatin strands with loose clumping
Association	Immunocompetent B cell, also called plasmacytoid lymphocyte	Predominant type in infectious mononucleosis	Transformation cell, also called transformed or reticular lymphocyte

### ***Infectious mononucleosis***

IM (infectious mononucleosis) is a self-limiting disease caused by the Epstein-Barr virus that usually affects teenagers or young adults. The incubation period is 10 to 14 days. The onset is characterized by fever, sore throat, and enlarged cervical lymph nodes. There may be involvement of the whole body, especially the lymphatic tissues, with symptoms and signs varying with the organs and tissues involved. The diagnosis of IM infection is made by microscopic blood examination and heterophil antibody-positive serological test. IM will be discussed in greater detail in volume 2—*Immunology*. There is an increase of lymphocytes ( $>5.0 \times 10^9/L$ ) with more than 20 percent variant lymphocytes seen on the differential. The classic Downey cells (type II) of infectious mononucleosis are probably the best examples of cells reported as atypical lymphocytes. This scrutiny of lymphocytes is often helpful in diagnosis; however, positive serological procedures are still the definitive answer to the physician. Cytomegalovirus infections (CMV) closely resemble IM, but the patient doesn't have a sore throat or enlarged lymph nodes. Variant lymphocytes will also be seen in CMV, but the heterophil-antibody test will be negative.

### ***Infectious lymphocytosis***

This is a contagious, benign, and self-limited disease of young children. Clinical manifestations are mild or may even be absent. The leukocytosis may be as high as  $50 \times 10^9/L$ , and sometimes as high as  $100 \times 10^9/L$ . There is both an absolute and a relative increase of normal, small, mature lymphocytes. The lymphocytosis may last three to five weeks or slightly longer. The bone marrow is not remarkable in the disease. Although, there is a lymphocytosis, there may also be a granulocytopenia. Heterophil-antibody tests are also negative.

### ***Leukocyte hereditary disorders***

There are different nonmalignant hereditary disorders that cause morphological and functional abnormalities in leukocytes. The following is a brief list of these disorders and their associations with disease states:

Disorders	Associated Diseases
<i>Granulocyte Morphology Abnormalities</i>	Nuclear abnormalities Cytoplasmic abnormalities
<i>Granulocyte Function Disorders</i>	Job's Syndrome Lazy Leukocyte Syndrome
<i>Defective Killing of Microorganisms Syndrome</i>	Chediak-Higashi Syndrome Congenital C3 Deficiency Chronic Granulomatous Disease G6PD Deficiency Myeloperoxidase Deficiency

Disorders	Associated Diseases
<i>Monocyte Macrophage System Disorders</i>	Mucopolysaccharidoses Lipidoses
<i>Immune Leukocytes Disorders</i>	B-cell Deficiencies T-cell Deficiencies Combined Deficiencies

### Malignant leukocyte abnormalities

Malignant cells are cells that are “out of control.” They don’t respond to the body’s natural checks and balances. Environmental agents, chemicals, drugs, viruses, and genetic disorders can trigger normal stem cells or mature cells to transform into malignant cells. If these malignant cells are found in the peripheral blood, the leukocyte count can be normal or increased (leukocytosis). Leukopenia is noted if the abnormal cells are confined to the bone marrow. The term *leukemia* is used when malignant cells are present in both the peripheral blood and bone marrow, and *aleukemic leukemia* for those present only in the bone marrow. If malignant cells are present in the lymphatic system and they produce a solid growth or tumor, it is known as *lymphoma*. The spread of the tumor to the bone marrow or peripheral blood is called *leukemic lymphoma*.

### Classification of leukemias

Classifying leukemias can be done by several methods. They can be grouped by the stem cell line involved—myeloid (hematopoietic) or lymphoid, or by acute or chronic forms. The myeloid classification includes the granulocytes, monocytes, megakaryocytes, as well as the erythrocytes. The lymphoid classification involves T-cells, B-cells, and lymphomas. Acute and chronic (defined earlier) can describe myeloid and lymphoid forms of leukemia. Acute leukemias are subdivided by morphological characteristics. These divisions are known as FAB classifications; a product of a conference consisting of individuals from France, America, and Britain. In this course, myeloid and lymphoid classifications will be used. I’m sure you are aware that this could be a very exhaustive study; therefore, use the table below to briefly review these disorders. To help with the table, review the prefixes and suffixes from unit 1.

Disorder	Comments	Laboratory Findings
<b>Chronic Myeloproliferative Disorders</b>	Acquired, malignant disorder developed from proliferation of abnormal pluripotent stem cells that coexist with normal stem cells, can transform into an acute leukemia	One or more of the stem cell lines may be effected, chromosome abnormalities may also be found
<i>Chronic Myelogenous Leukemia (CML)</i>	Accounts for 20% of all leukemias, disease of adults 30 to 50 year olds, chronic phase 2- to 5-year life span, blastic phase 3-month life span	WBC $50.0-300.0 \times 10^9/L$ , All cell lines counts increased with immature granulocytes and large platelet forms
<i>Polycythemia Vera</i>	See lesson 009	Erythrocytosis
<i>Myelofibrosis</i>	Affects middle age or elderly, 1- to 5-year life span, bone marrow becomes fibrotic, defect is in a single pluripotent stem cell	Granulocytic hyperplasia with teardrop RBCs
<i>Thrombocythemia</i>	Closely related to polycythemia vera with no apparent cause, characterized by thrombocytosis with spontaneous aggregation	Platelet count $>1000.0 \times 10^9/L$
<b>Dysmyelopoietic Disorders or Myelodysplastic</b>	Affects persons over 50 years old, generally long life span, associated with exposure to carcinogens (benzene and toluene) or	Macrocytic anemia, neutropenia, thrombocytopenia, and/or monocytosis, with nucleated red blood cells, basophilic stippling, and

Disorder	Comments	Laboratory Findings
<b>Syndromes</b> <i>Refractory Anemias</i>	chemotherapeutic agents, abnormalities in hematopoietic stem cells	Howell-Jolly bodies present
<b>Acute Nonlymphocytic Leukemias (ANLL)</b> <b>FAB classifications M1 thru M7</b>	Affects all age groups but mostly adults, involves myelocytic, monocytic, erythrocytic, and megakaryocytic cell lines, associated with radiation, chemicals, genetics, and viruses, if left untreated it is rapidly fatal <b>Treatments</b> are chemotherapy, radiation, immunotherapy, and bone marrow transplantation	All cell line counts decreased, blasts seen on blood films, anemia present: • M1 and M2 - Acute Myeloblastic Leukemia - M3 - Acute Promyelocytic Leukemia - M4 - Acute Myelomonocytic Leukemia - M5 - Acute Monocytic Leukemia - M6 - Di Guglielmo's Syndrome - M7 - Acute Megakaryocytic Leukemia
<b>Acute Lymphoblastic Leukemias (ALL)</b> <b>FAB classifications</b>	T-cells account for 10 to 20% of the cases and B-cells 60 to 70% of the affected cell lines. <b>Treatments</b> are chemotherapy and bone marrow transplantation	In approximately: - 60% of the patients, WBC is $>10.0 \times 10^9/L$ . - 15% WBC is $>100.0 \times 10^9/L$ . - 25% WBC is $<5.0 \times 10^9/L$ .
L1	Children age 15 years and younger constitutes 74% of the infections, best prognosis of the ALLs	Lymphoblasts are small.
L2	Affects children (14% of total cases) and young adults (66%)	Lymphoblasts are large.
L3	Also known as Burkitt type, it's rare and makes up 3 to 5% of the cases, very poor prognosis	Lymphoblasts are normal to large.
<b>Chronic Lymphoproliferative Leukemic Disorders</b>	B-cell line affected with cells accumulating in the bone marrow, peripheral blood, lymph nodes, and other organs	
<i>Chronic Lymphocytic Leukemia (CLL)</i>	Most common type of leukemia in Western hemisphere, twice as many men than women affected, usually over 50 years of age, Rai classification stages 0 thru 5, stage 0 prognosis 10 or more years, stages 1 thru 5 prognosis is $<2$ years <b>Treatment</b> alkylating agents, corticosteroids, and radiation.	WBC is usually $20.0$ to $200.0 \times 10^9/L$ , blood film shows 60 to 95% small mature lymphocytes, lymphoblast are not present, prolymphocytes are rare
<i>Prolymphocytic Leukemia (PLL)</i>	80% of the cases involve B cells and 20% T-cells, males over 70 years of age are mostly effected, prognosis is poor, survival is about 2 years <b>Treatment</b> includes chemotherapy and splenectomy	WBC is $>100.0 \times 10^9/L$ , prolymphocyte is predominant cell in bone marrow and peripheral blood
<i>Hairy Cell Leukemia (HCL)</i>	So named because of the hair-like projections that surround the outer border of the cell, occurs in males between 40 and 60 years of age, average survival rate is between 5 and 6 years <b>Treatment</b> is splenectomy	WBC may be increased but pancytopenia or depression of all cell lines is usually seen

Disorder	Comments	Laboratory Findings
<b>Lymphomas</b>	These disorders result in abnormal lymph node enlargement	Ordinarily they don't effect the peripheral blood until late in the disease
<b>Hodgkin's Disease (HD)</b> <i>Rye classifications</i>  Lymphocytic predominant form  Lymphocyte depleted form  Mixed cell types  Nodular sclerosis	Cause is unknown, cells in the lymph nodes are variable, high percentage have normal appearance while only a small population with malignant features, twice as many men affected than women, a bimodal incidence occurs with the first age group of 15 to 35 years and the second over 50 years affected, stages based on location and extent of involved tissue, stage I and II- 85% 10 year survival rate, stage III- 70%, and stage IV-50% if found at a younger age <b>Treatment</b> includes radiotherapy and chemotherapy	Lymph node biopsy to identify Reed-Sternberg cells is used for definitive diagnosis , WBC ranges from 12.0 to $25.0 \times 10^9/L$ , neutropenia is seen when the bone marrow is involved
<b>Non-Hodgkin's Lymphoma (NHL)</b> <i>Rappaport classifications</i> Multiple myeloma  Well-differentiated lymphocytic lymphoma  Poorly-differentiated lymphocytic lymphoma  Histiocytic lymphoma  Mixed histiocytic lymphoma	Exact cause unknown, but in 60% of the patients evidence shows chromosomal damage, 95% of the patients exhibit B cell malignancies with most cells in the lymph nodes affected, more common in men than women (2:1), three times as common as HD, predominate in 40 years or older age group, earlier stages have an average 5 year survival rate, late stages have a 40 to 50% chance of survival after 2 years <b>Treatments</b> are radiotherapy and chemotherapy	Definitive diagnosis made by lymph node biopsy, WBC is usually normal but the blood film may show abnormal lymphocytes

### Causes of death

There are many complications that arise from the malignant, as well as nonmalignant, disorders. It is usually these complications that bring about the death of the patient. Severe leukocytopenia and thrombocytopenia, due to the disorder or from therapy, is very dangerous. Without the leukocytes to protect from foreign invaders, the patient may succumb to bacterial, viral, and/or fungal infections. Thrombocytopenia can lead to spontaneous bleeding which may be uncontrollable. Thrombocytopenia will be discussed further in the next lesson and in unit 4.

### Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

#### 010. Normal leukocyte morphology and maturation sequence

1. What are the five types of leukocytes?
2. What is the primary function of the leukocytes and where is it carried out?
3. How are the granulocytes named?
4. What are the staining properties of the three granulocytes?
5. Match the terms in column B with the statement in column A. Each term in column B may be used once, more than once, or not at all.

##### Column A

- \_\_\_ 1. First stage in the lymphocytic series.
- \_\_\_ 2. 12 to 18  $\mu\text{m}$  in diameter and first cell to reveal specific granules.
- \_\_\_ 3. Ground glass-looking cytoplasm with granules called azurophilic dust.
- \_\_\_ 4. 14 to 20  $\mu\text{m}$  in diameter, features include brain-like convolutions of the nucleus.
- \_\_\_ 5. Nucleus has two or more lobes connected by thin filaments.
- \_\_\_ 6. N/C ratio is 4:1, nucleus stains reddish-purple and contains two to five nucleoli.
- \_\_\_ 7. Extremely difficult to differentiate from the myeloblast.
- \_\_\_ 8. Kidney-shaped or indented nucleus with a heavy nuclear membrane and full amount of specific granules.
- \_\_\_ 9. Divided into three categories—small, medium, and large.
- \_\_\_ 10. Exhibits slow, amoeboid movement in wet mount with single or multiple pseudopodia.
- \_\_\_ 11. Some authors do not differentiate this cell from the lymphoblast.
- \_\_\_ 12. Sausage-shaped nucleus.
- \_\_\_ 13. 15 to 21  $\mu\text{m}$  in diameter, N/C ratio 3:1 to 2:1, contains two to three nucleoli.
- \_\_\_ 14. 8 to 16  $\mu\text{m}$  in diameter with a clear perinuclear zone in the cytoplasm.

##### Column B

- a. Myeloblast.
- b. Promyelocyte.
- c. Myelocyte.
- d. Metamyelocyte.
- e. Band granulocyte.
- f. Segmented granulocyte.
- g. Monoblast.
- h. Promonocyte.
- i. Monocyte.
- j. Lymphoblast.
- k. Prolymphocyte.
- l. Lymphocyte.

6. What are the five stages of megakaryocytic maturation?
7. What is the diameter and the N/C ratio of the megakaryoblast?
8. How many nuclei can the megakaryoblast have and what are they called?
9. Which is larger, the promegakaryocyte or the megakaryoblast?
10. What is the largest blood cell?
11. Initial platelet formation is seen in what stage of development?
12. What is the composition of the platelet?
13. What are the four specific areas of the platelet?
14. What is the primary platelet function?
15. What are the two basic types of platelet abnormalities?

#### **011. Leukocyte abnormalities**

1. What is leukopenia?
2. What is leukocytosis?
3. What is leukemia?

4. What is the difference between acute and chronic?
5. What term does the Clinical and Laboratory Standards Institute (formerly NCCLS) propose to use in place of atypical lymphocyte?
6. What are some of the changes (transformations) you'll see in the leukocytes when they become active?
7. Match the disorder in column B with the cause in column A. Items in column B may be used once, more than once, or not at all.

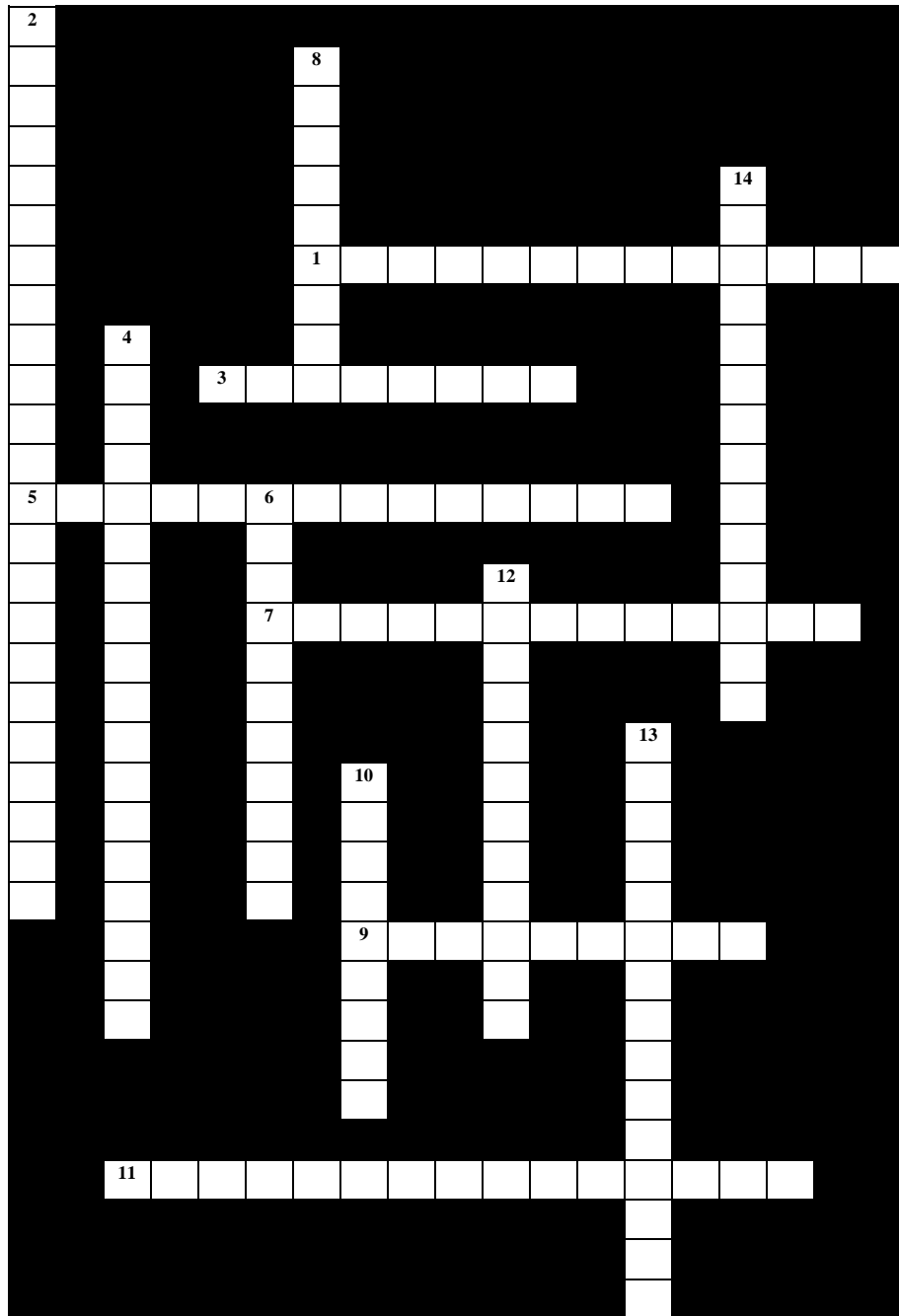
*Column A*

- \_\_\_1. Infectious mononucleosis, *B. pertussis* infection and infectious lymphocytosis.
- \_\_\_2. Bacterial, tuberculosis, and syphilis infections.
- \_\_\_3. Infections, inflammation, and malignancy.
- \_\_\_4. Administration of ACTH.
- \_\_\_5. Viral infections, immune disorders, drug reactions, and neutropenia.
- \_\_\_6. Immediate hypersensitivity reactions.
- \_\_\_7. Stress and hyperthyroidism.
- \_\_\_8. Inherited disorders and bone marrow damage.
- \_\_\_9. Rare inherited disorders and glucocorticoids administration.
- \_\_\_10. Parasitic invasion and hypersensitivity disorders.

*Column B*

- a. Neutrophilia.
- b. Neutropenia.
- c. Eosinophilia.
- d. Eosinopenia.
- e. Basophilia.
- f. Basopenia.
- g. Monocytosis.
- h. Monocytopenia.
- i. Lymphocytosis-absolute.
- j. Lymphocytosis-relative.

8. Complete the following crossword puzzle using the different types of nonmalignant and malignant leukocyte abnormalities.



ACROSS	DOWN
1. Affects middle age or elderly, bone marrow becomes fibrotic.	2. Type II variant lymphocytes predominant, self-limiting disease.
3. Lymph node biopsy used to identify Reed-Sternberg cells.	4. Most common type of leukemia in the Western hemisphere.
5. FAB classifications M1 to M7, affects all age groups.	6. Accounts for 20% of all leukemias, disease of adults 30 to 50 year olds.



ACROSS	DOWN
7. FAB classifications L1 to L3, 10 to 20% T-cells and 60 to 70% B-cells affected.	8. Common reaction that occurs in chicken pox, tuberculosis, IM, and whooping cough.
9. Hair-like projections surround outer border of cell, occurs in males.	10. Disorders that result in abnormal lymph node enlargement.
11. Characterized by thrombocytosis with spontaneous aggregation.	12. Cause unknown but 60% of patients show chromosomal damage.
	13. Associated with exposure to carcinogens, generally long life span.
	14. Seen in males over 70 years, prognosis is poor.

### Answers to Self-Test Questions

#### 006

- As a whole, the cell decreases in size as it matures, the cytoplasm changes size and color and granules may appear, and the nucleus changes its staining properties from reddish-purple to a bluish-purple and the nucleoli usually disappear as the cell matures.
- As the cell matures the nucleus decrease and the cytoplasm increases. This relationship is known as the nuclear to cytoplasmic ratio or N/C ratio.

#### 007

- b.
  - e.
  - a
  - c.
  - b
  - f.
  - e.
  - a.
  - f.
  - d.
  - c.
  - f.
  - g.
  - h.
  - g.

#### 008

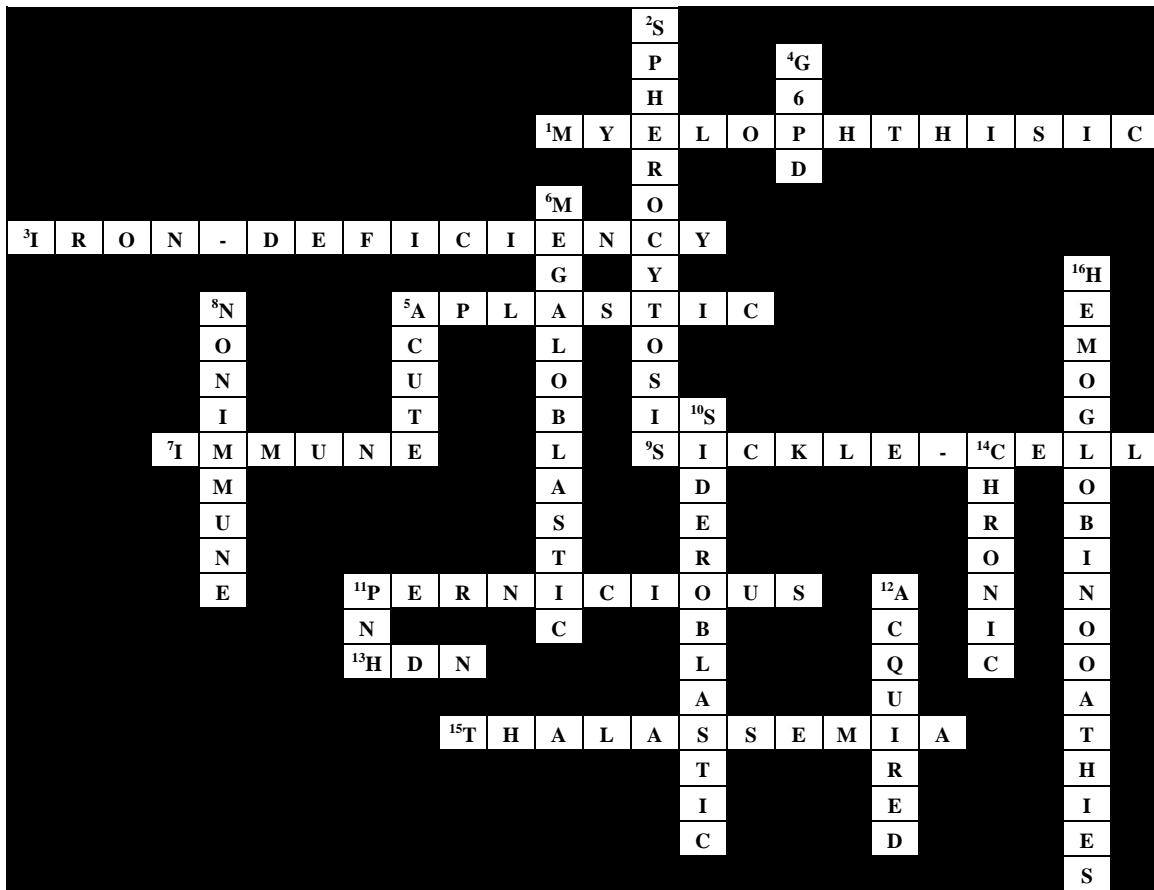
- Anisocytosis refers to a variation in size outside normal limits.
  - Microcytosis refers to RBCs <6 µm in diameter.
  - Macrocytosis refer to RBCs with a diameter >8.5 to 9.0 µm.
- Poikilocytosis.
- d.
  - i.
  - n.
  - a.

- (5) q.
- (6) b.
- (7) m.
- (8) p.
- (9) o.
- (10) h.
- (11) j.
- (12) e.
- (13) f.
- (14) g.

**009**

- 1. A term used to indicate an above normal hemoglobin, hematocrit, and red blood cell count.
- 2.
  - (1) Relative polycythemia is a condition that is normally transient and is due to a decrease in fluid (plasma).
  - (2) Polycythemia vera a condition where there is general hyperplasia of the bone marrow.
  - (3) Secondary polycythemia is increased erythropoietin production can be abnormal or a normal response to hypoxia.
- 3. The existence of a reduced red blood cell count, or a decreased concentration of blood hemoglobin.
- 4. Too many RBCs being destroyed or too few RBCs being produced.
- 5. Etiologic or cause, morphologic, or physiologic.
- 6. The bone marrow's ability to respond to the anemia with increased erythropoiesis or not being able to respond called ineffective erythropoiesis. If the bone marrow fails to respond it is ineffective erythropoiesis. If the anemia is caused by reasons outside of the bone marrow it is termed effective erythropoiesis.

7.



010

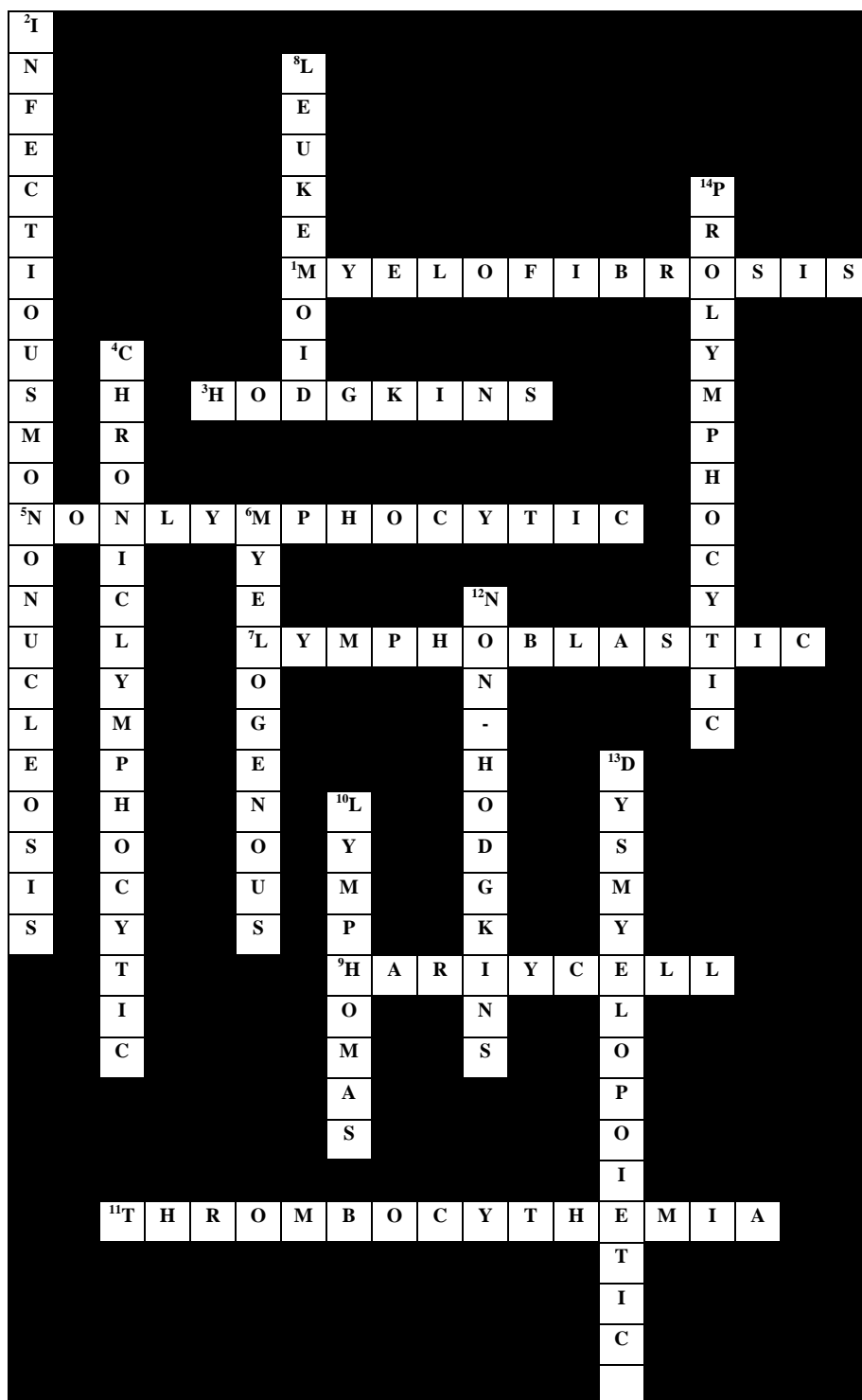
1. Neutrophils, eosinophils, basophils, monocytes, and lymphocytes.
2. To protect the body from foreign invaders and most of their function is carried out in the tissues.
3. According to their cytoplasmic granule staining properties.
4. (1) Eosinophil granules stain bright red-orange because of their affinity to eosin, the acidic dye in Wright's stain.  
 (2) Basophil granules stain dark blue-purple because of their affinity to methylene blue, which is the basic dye in Wright's stain.  
 (3) Neutrophil granules appear pinkish-tan because they don't have an affinity for either dye.
5. (1) j.  
 (2) c.  
 (3) h.  
 (4) i.  
 (5) f.  
 (6) a.  
 (7) g.  
 (8) d.  
 (9) l.  
 (10) i.  
 (11) k.

- (12) e.
- (13) b.
- (14) l.
- 6. (1) Megakaryoblast (stage I), (2) promegakaryocyte (stage II), (3) megakaryocyte (stage III), (4) metamegakaryocyte (stage IV), and (5) thrombocyte.
- 7. 20 to 50  $\mu\text{m}$ ; 10:1.
- 8. Up to 32; polyploid cells.
- 9. Promegakaryocyte.
- 10. Metamegakaryocyte.
- 11. Metamegakaryocyte (stage IV).
- 12. The platelet is composed of approximately 60 percent protein, 30 percent lipid, and 8 percent carbohydrate, various minerals, water, and nucleotides. It also contains over 90 different enzymes, glycogen, and coagulation factors.
- 13. Peripheral zone, submembrane area, sol-gel zone, and organelle zone.
- 14. To stop bleeding and maintain capillary integrity.
- 15. Platelet abnormalities include quantitative (thrombocytopenia and thrombocytosis or thrombocythemia) and qualitative (inherited and acquired defects) disorders.

**011**

- 1. Leukopenia is present if the WBC result is  $<3.0 \times 10^9/\text{Liter}$  of blood or below the reference range for the age and sex of that person.
- 2. Leukocytosis refers to a WBC result  $>11.0 \times 10^9/\text{Liter}$  of blood or above the reference range for the age and sex of that person.
- 3. Leukemia is a progressive, malignant disease of the blood-forming organs.
- 4. Acute means a disorder has a short and relatively severe course. Chronic means that a disease persists over a long period of time.
- 5. Variant lymphocyte.
- 6. The transformation includes (1) increase in cell size; (2) cytoplasm becomes basophilic and increases; (3) toxic granules and vacuoles may appear, (4) nucleus changes shape, becomes less dense with nucleoli present may be hypersegmented; and (5) alterations are made in total number of leukocytes present in the blood.
- 7. (1) i.
  - (2) g.
  - (3) a.
  - (4) d.
  - (5) j.
  - (6) e.
  - (7) f.
  - (8) b.
  - (9) h.
  - (10) c.

8.



## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter.

32. (006) When identifying cells during maturation, formulating a question about specific or nonspecific granules applies to the
  - a. nucleus.
  - b. cell size.
  - c. cytoplasm.
  - d. deoxyribonucleic acid (DNA).
33. (007) Which one of the following is the *earliest* recognizable cell in the erythrocytic series?
  - a. Rubriblast.
  - b. Erythrocyte.
  - c. Prorubricyte.
  - d. Metarubricyte.
34. (007) In what cell has *full* hemoglobinization occurred?
  - a. Rubriblast.
  - b. Reticulocyte.
  - c. Prorubricyte.
  - d. Metarubricyte.
35. (007) What cell is the immediate precursor of the adult erythrocyte and may be found in a healthy adult?
  - a. Rubriblast.
  - b. Reticulocyte.
  - c. Prorubricyte.
  - d. Metarubricyte.
36. (007) Which one of the following is *not* one of the three basic functions for which the erythrocytic membrane is responsible?
  - a. It allows nutrient and selective ion passage in and out of the cell.
  - b. It allows the cell to deform or change shape to travel through the small vessels.
  - c. It recognizes and protects the body from foreign invaders, for example bacteria.
  - d. It separates the intracellular fluid environment (cytoplasm) from the extracellular fluid environment (plasma).
37. (008) What term is used to describe erythrocytes that are *less* than 6  $\mu\text{m}$ ?
  - a. Microcytic.
  - b. Macrocytic.
  - c. Anisocytosis.
  - d. Poikilocytosis.
38. (008) Cells that appear dark in the center with a lighter ring between the center and periphery and are seen in certain hemoglobinopathies (hemoglobin CC, E, and SC diseases) are called
  - a. ovalocytes.
  - b. target cells.
  - c. schizocytes.
  - d. stomatocytes.

39. (008) Cabot rings are distinguished from the rings of the *Plasmodia* species because
- they are not refractile on Perls' Prussian blue-stained films.
  - they are larger and they don't have of a red chromatin mass.
  - Plasmodia* rings stain positive for ferric iron aggregates with supravital stains.
  - Plasmodia* rings are larger in size and they don't have of a red chromatin mass.
40. (009) If a patient has an increased erythrocyte count due to a decrease in fluid as a result of burns, excess sweating, or dehydration, the condition is known as
- polycythemia vera.
  - relative polycythemia.
  - absolute polycythemia.
  - secondary polycythemia.
41. (009) If the body destroys too many or produces too few RBCs, the condition is called
- uremia.
  - anemia.
  - leukemia.
  - polycythemia.
42. (009) Anemia can be classified on the basis of all the following *except*
- physiologic.
  - morphologic.
  - psychological.
  - etiological or cause.
43. (009) What anemia is due to a complete failure of the bone marrow to produce RBCs, WBCs, and platelets?
- Aplastic.
  - Sickle-cell.
  - Megaloblastic.
  - Iron deficiency.
44. (009) Which one of the following is the *best* known hemoglobinopathy?
- Thalassemia.
  - Hemoglobin Bart.
  - Sickle-cell anemia.
  - Hemoglobin SC disease.
45. (010) Which one of the following is *not* considered a phagocyte?
- Monocyte.
  - Neutrophil.
  - Eosinophil.
  - Lymphocyte.
46. (010) In the granulocytic series, specific granules begin to appear at what stage and are easily distinguished as being neutrophilic, eosinophilic, or basophilic?
- Myelocyte.
  - Myeloblast.
  - Promyelocyte.
  - Metamyelocyte.

47. (010) A cell, in which the nucleus has two or more definite lobes separated by very thin filaments rather than an indentation, is called a
- myelocyte.
  - metamyelocyte.
  - band granulocyte.
  - segmented granulocyte.
48. (010) In the agranulocytic series, what cell exhibits an opaque, blue-gray cytoplasm with a foamy or ground glass appearance?
- Monocyte.
  - Monoblast.
  - Lymphocyte.
  - Lymphoblast.
49. (010) What cell is described as about 8 to 16  $\mu\text{m}$  in diameter or from about the size of a normal RBC to more than twice the size of an RBC?
- Monocyte.
  - Monoblast.
  - Lymphocyte.
  - Lymphoblast.
50. (010) The *largest* blood cell, ranging from 40 to 120  $\mu\text{m}$  in diameter, is the
- megakaryocyte.
  - megakaryoblast.
  - promegakaryocyte.
  - metamegarkaryocyte.
51. (010) What specific area of the platelet is responsible for adhesion and aggregation?
- Sol-gel zone.
  - Organelle zone.
  - Peripheral zone.
  - Submembrane area.
52. (010) Which of the following are quantitative platelet abnormalities?
- Inherited and acquired defects.
  - Thrombocytosis and inherited defects.
  - Thrombocytopenia and thrombocytosis.
  - Thrombocytopenia and acquired defects.
53. (011) What is a contagious, benign and self-limited disease of young children where the leukocyte count may be as high as  $50 \times 10^9/\text{L}$ , and sometimes as high as  $100 \times 10^9/\text{L}$ ?
- Leukemoid reaction.
  - Infectious lymphocytosis.
  - Infectious mononucleosis.
  - Leukoerythroblastic reaction.
54. (011) If a solid growth tumor spreads to the bone marrow or peripheral blood, it is called
- leukemia.
  - lymphoma.
  - aleukemic leukemia.
  - leukemic lymphoma.



55. (011) What leukemia is characterized by a WBC count of  $50.0 - 300.0 \times 10^9/L$ , all cell lines counts increased with immature granulocytes and large platelet forms, and accounts for 20 percent of all leukemias?
- a. Prolymphocytic.
  - b. Acute lymphoblastic.
  - c. Chronic lymphocytic.
  - d. Chronic myelogenous.
56. (011) The *most* common type of leukemia in the Western hemisphere, where the WBC count is usually  $20.0$  to  $200.0 \times 10^9/L$  and the blood film shows 60 to 95 percent small mature lymphocytes, is
- a. prolymphocytic.
  - b. acute lymphoblastic.
  - c. chronic lymphocytic.
  - d. acute nonlymphocytic.

**Please read the unit menu for unit 3 and continue ➡**

## **Student Notes**

## Unit 3. The Complete Blood Count and Related Studies

<b>3–1. Manual Cell Counts.....</b>	<b>3–1</b>
012. Manual procedures .....	3–1
013. Body fluid procedures.....	3–8
<b>3–2. Automated Cell Counts.....</b>	<b>3–16</b>
014. Hemoglobin, hematocrit, erythrocyte indices, and miscellaneous measurements .....	3–16
015. Instrument principles, histograms, and scattergrams .....	3–20
<b>3–3. Related Studies .....</b>	<b>3–34</b>
016. Routine and special studies.....	3–34

**T**HE COMPLETE BLOOD count or CBC is one of the most frequently performed tests in the clinical laboratory. It is a very important screening procedure and its value should not be underestimated. It is useful in diagnosing infections, anemia's, leukemias, and hemoglobin abnormalities. It is also useful in monitoring disease states and their therapy. The different related studies complement the CBC and provide additional information about patients' health or disorder.

### 3–1. Manual Cell Counts

Long gone are the days when the complete blood count (CBC) consisted of a manual white blood cell (WBC) count, colorimetric hemoglobin, spun hematocrit, and differential leukocyte count. Today, even the smallest laboratories have automated hematology analyzers that furnish a CBC that includes RBC, WBC, and platelet counts; hemoglobin; hematocrit; red blood cell indices; red cell distribution width (RDW); mean platelet volume (MPV); and platelet distribution width (PDW). Most even furnish a preliminary or estimated leukocyte differential.

#### 012. Manual procedures

Manual cell counting will never be entirely replaced. Many lucky technicians haven't performed a manual cell count since their initial technical training days. Some have lived through the panic that usually accompanies a major equipment failure in which backup procedures include manual cell counts. Others may perform manual cell count procedures only on body fluids. (**Note:** Your laboratory may employ a semi-automated method as a backup procedure. However, we will not discuss these procedures in this text. This text will contain basic subject knowledge of manual procedures.) Be aware that there are numerous ways to perform these procedures and your lab may perform them differently. Also, please keep in mind that the purpose of these CDCs is to provide subject knowledge. They do not replace your laboratory's operating instructions (OIs), but are meant to increase your theory or knowledge about a task. Nonetheless, a study of hematology would not be complete without a review of equipment and procedures used in hematology. This is also a good time to brush up on **scientific notation** which is used extensively in hematology calculations. The following chart should help in that regard and will serve as a handy reference for the remainder of the volume.

EXPONENT	EXPANSION	NAME & [REPRESENTATION]	METRIC PREFIX	SYMBOL
$10^{-15}$	0.000 000 000 000 001	<i>One quadrillionth</i> [1/1,000,000,000,000,000]	Femto-	f
$10^{-12}$	0.000 000 000 001	<i>One trillionth</i> [1/1,000,000,000,000]	Pico-	p
$10^{-9}$	0.000 000 001	<i>One billionth</i> [1/1,000,000,000]	Nano-	n

EXPONENT	EXPANSION	NAME & [REPRESENTATION]	METRIC PREFIX	SYMBOL
$10^{-6}$	0.000 001	One millionth [1/1,000,000]	Micro-	$\mu$
$10^{-3}$	0.001	One thousandth [ 1/1,000]	Milli-	m
$10^{-2}$	0.01	One hundredth [1/100]	Centi-	c
$10^{-1}$	0.1	One tenth [1/10]	Deci-	d
$10^0$	1	One	_____	_____
$10^1$	10	Ten	Deca-	D
$10^2$	100	One Hundred	Hecto-	H
$10^3$	1,000	One Thousand	Kilo-	K
$10^4$	10,000	Ten thousand	_____	10k
$10^5$	100,000	One hundred thousand	_____	100k
$10^6$	1,000,000	One Million	Mega-	M
$10^7$	10,000,000	Ten million	_____	_____
$10^8$	100,000,000	One hundred million	_____	_____
$10^9$	1,000,000,000	One billion	Giga-	G
$10^{10}$	10,000,000,000	Ten billion	_____	_____
$10^{11}$	100,000,000,000	One hundred billion	_____	_____
$10^{12}$	1,000,000,000,000	One trillion	Tera-	T
$10^{15}$	1,000,000,000,000,000	One quadrillion	Peta	P

### Red blood cell counts

Manual RBC counts are rarely (*if at all*) performed in today's laboratory because they are inaccurate and unreliable. Regardless, it will be briefly reviewed in this unit in order to compare it with the WBC and other counts.

### Principle

The principle of the manual RBC count is to aid counting and prohibit lysis of the red blood cells. It is used as a backup for equipment failure, for specimens that are out of the instruments' linearity range, and for medical contingency operations.

### Specimen

Capillary and venous whole blood with EDTA or heparin anticoagulants may be used.

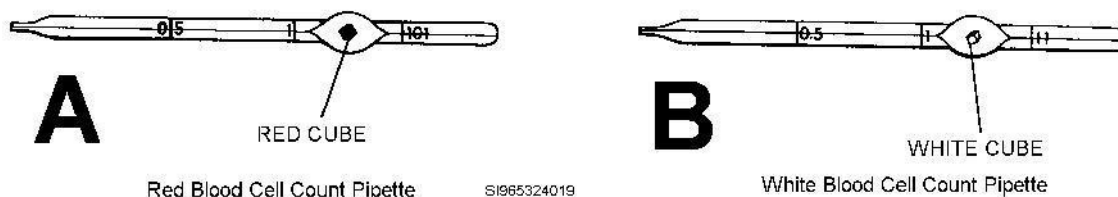


Figure 3-1. Pipettes used for manual cell counts.

### Equipment and reagents

To help you recognize the Thoma diluting pipettes refer to figure 3-1. Diluting pipettes such as this are rarely if ever used due to the need for aspirating tubes and suction (often performed with mouth tubes). Thoma red cell diluting pipette, with a red cube, as shown in figure 3-1, RBC diluting fluid (trisodium citrate with or without formalin), microscope, gauze, moisture chamber, and an improved Neubauer hemocytometer with cover glass, as shown in figure 3-2, are used for manual RBC counts. Haymen's diluting fluid is usually not recommended because of rouleaux and clumping of RBCs due to hyperglobulinemia. All hemocytometers must meet the specifications of the National Bureau of Standards (NBS) and are identified with those initials. If using the Unopette system, you don't need a red cell count pipette or RBC diluting fluid.

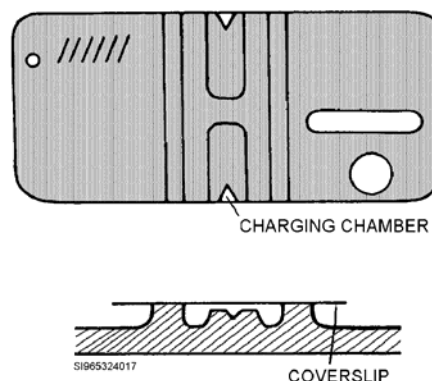


Figure 3-2. Drawing of a Neubauer hemocytometer.

### Using the hemacytometer

When cleaning the hemocytometer, use a lint free cloth and, if needed, 95 percent ethanol. A glass cover is placed on top of the ruled area of the counting chamber. After allowing the dilution to mix, the first four to five drops of the mixture is discarded from the pipette and then the chamber is charged. Be careful not to over- or under fill the counting chamber. Charge the other chamber of the hemocytometer with the second dilution. Carefully place the hemocytometer in a moist chamber. The easiest moist chamber to construct is the time-honored Petri dish with a layer of moistened gauze. It must rest for three to five minutes to allow the red blood cells to settle before counting. After three to five minutes, gently place the hemocytometer on the microscope platform. Care must be taken not disrupt or move the cover glass, if this happens, recharge a new hemocytometer. A 10 X objective (low power) is used to examine the hemacytometer for even cell distribution. A 40 X objective (high dry) is used to count the red cells in each of the five squares indicated by an R in figure 3-3. Because the hemacytometer is much thicker than a routine slide, care must be exercised so the objectives are not damaged by moving them into the hemacytometer.

**CAUTION:** When performing manual procedures always wear gloves and practice safety procedures outlined in unit 1.

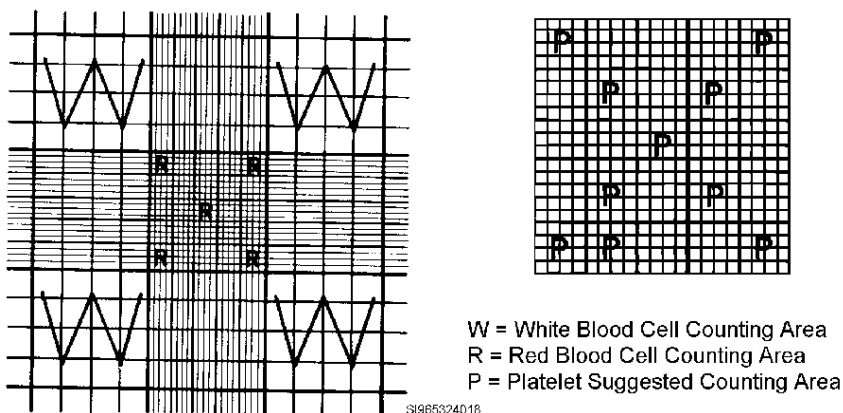


Figure 3-3. Microscopic view of hemocytometer counting areas.

### Unopette

The advent of pre-measured dilutions is a welcome change from the Thoma red cell pipette. Almost every laboratory uses the Unopette system for manual red blood counts instead of the Thoma pipette. The Unopette (Becton Dickinson Vacutainer Systems, Rutherford, New Jersey) and other generic products have made the drudgery of manual cell counting almost bearable. The reservoirs contain a measured amount of diluting fluid appropriate for the cellular element to be counted. The pipettes are

available in different sizes (i.e., 3  $\mu\text{L}$ , 3.3  $\mu\text{L}$ , 10  $\mu\text{L}$ , 20  $\mu\text{L}$ , 25  $\mu\text{L}$ , and 44.7  $\mu\text{L}$ ), depending on the procedure to be performed. For the RBC Unopette, the enclosed capillary pipette delivers 10  $\mu\text{L}$  of blood, giving the normal 1:200 dilution for RBC counts.

### Unopette procedure

The shield from the capillary pipette should be removed with a twisting motion. The reservoir is placed on a flat surface and held firmly with one hand as shown in figure 3-4. The other hand punctures it, using the pipette shield. Once open a dilution can be made. Hold the pipette, as indicated, for either finger-stick use or for filling from an EDTA tube. The specimen draw stops automatically when blood reaches the end of the capillary pipette. Wipe the outside of the pipette carefully, making sure that you do not remove or wick away any specimen from the end of the pipette. If this occurs you will affect the overall dilution and eventual results and you set up another dilution. Squeeze the reservoir gently to remove some of the air and insert the capillary pipette into the punctured top of the reservoir. Seat the pipette firmly into the reservoir and release pressure. The specimen is drawn into the diluent. Next, squeeze the diluent chamber gently several times, allowing diluent to completely wash the pipette, and bring the entire specimen into solution.

**NOTE:** Do not lose any fluid or the dilution will be incorrect.

Finally, remove the pipette, squeeze the chamber, and reseal the pipette. When pressure is released, the entire specimen will be in solution and well mixed. Allow the dilution to rest for ten minutes. Prior to charging the counting chamber, hold your gloved finger over the pipette opening and invert the chamber several times to ascertain proper mixture of the specimen. Remove the pipette from the container and reseal it firmly in the reverse position to form the dropper assembly. Discard the first four to five drops; then charge both sides of the hemocytometer and allow it to rest in a moist chamber (Petri plate) for three to five minutes.

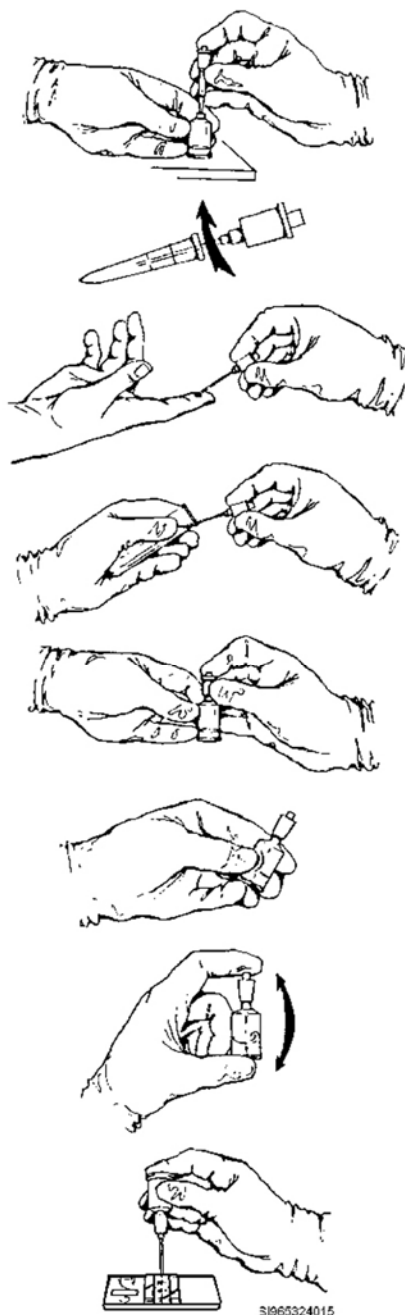


Figure 3-4. Using the Unopette system.

### Calculation

Count the RBCs in five of the 25 smaller squares that comprise the larger center square. Count both sides of the counting chamber; then average the counts. Both counts should be within ten percent of each other; if not, repeat the procedure. The average figure is used to calculate the red blood cell count per liter of blood. Remember, the dilution is 1:200. The entire ruled area on one side of the hemocytometer is  $0.9\ \mu\text{L}$  (width  $\times$  length  $\times$  depth or  $3\ \text{mm} \times 3\ \text{mm} \times 0.1\ \text{mm}$ ). Hence, each large square on the hemocytometer contains  $0.1\ \mu\text{L}$  ( $0.9\ \mu\text{L} \div 9 = 0.1\ \mu\text{L}$ ). Accordingly,  $0.1\ \mu\text{L}$  is divided by 25 (small squares), which equals  $0.004\ \mu\text{L}$ . However, we counted five small squares, therefore, it is  $5 \times 0.004\ \mu\text{L} = 0.02\ \mu\text{L}$ . To find out how many red blood cells are in  $1.0\ \mu\text{L}$  of blood, we must multiply  $0.02\ \mu\text{L}$  by 50 ( $1.0\ \mu\text{L} \div 0.02 = 50$ ). Recall from unit 2 that  $1.0\ \mu\text{L} = 10^{-6}$ , thus, to convert to liters we must multiply by  $10^6$ . To calculate the erythrocyte count, use the following formula and review the examples.

# of Cells in five squares		Correction for dilution		Correction for volume		Conversion to liter		
RBCs Counted	$\times$	200	$\times$	$1.0 \div 0.02$	$\times$	$10^6$	=	RBCs/L
<i>Examples:</i>								
300	$\times$	200	$\times$	50	$\times$	$10^6$	=	$3.0 \times 10^{12}/\text{L}$
750	$\times$	200	$\times$	50	$\times$	$10^6$	=	$7.5 \times 10^{12}/\text{L}$

### Reporting results

In unit 1 it was stated that each laboratory should establish its own reference range for each age group, therefore, the reference range below is considered general guidelines.

Age Group	Reference Range
Adult Females	$3.6$ to $5.6 \times 10^{12}/\text{L}$
Adult Males	$4.2$ to $6.0 \times 10^{12}/\text{L}$
After 50 years of age	Slightly lower than the adult range.
Newborn	$5.0$ to $6.5 \times 10^{12}/\text{L}$
At 1 year of age	$3.5$ to $5.1 \times 10^{12}/\text{L}$
Childhood and Adolescence	Slightly lower than the adult range.

### Procedural notes or hints

Mix venous blood for RBC counts thoroughly, but gently, prior to diluting it. It is also advisable to ring the tube of blood with a wooden applicator stick to check for clots. If very small clots are present, they should show up on the wooden surface of the sticks. The presence of clots *invalidates* the specimen for cell counts. When performing RBC counts on capillary blood, act rapidly and visually check for minute clots. Perform diluting fluid background counts periodically.

### Limitations of the procedure

Make sure all reagents are not contaminated and pipettes are free from blood and debris. All dilutions must be performed accurately. Once the hemocytometer is charged, don't let it set too long, since the fluid may evaporate causing inaccurate counts. When using a Unopette-like system, use the diluting equipment only once so that contamination of fluids is not a concern. To avoid counting artifacts, make certain that the counting chamber and cover glass are clean and dry. The specimen of choice is venous, EDTA blood, which must be pipetted accurately. When performing the count on capillary blood, it is wise to fill the capillary pipette from a hemolet or other such microcollector—one that yields a small anticoagulated specimen. If filling the pipette from a capillary puncture site, the blood must be free flowing. Always wipe away the first drop and don't squeeze the site. Tissue fluid expressed from the site will alter results drastically.

### White blood cell count

The WBC count is performed as a normal part of the routine CBC. As with the RBC count, the use of a reagent system, like the Unopette, makes the WBC count much simpler and more reliable.

#### Principle

The principle of the manual WBC count is to aid counting and hemolyze red blood cells by using a weak acid solution as the diluent. It may be used as a backup for equipment failure, specimens that are out of the instruments' linearity range, problematic specimens (i.e., platelet clumps), or medical contingency operations.

#### Specimen

Capillary or venous whole blood with EDTA as the anticoagulant may be used.

#### Equipment and reagents

The same equipment used for RBC counts is also used for WBC counts. The different diluting fluids that may be used are acetic acid in distilled water, hydrochloric acid in distilled water, and Turk's diluting fluid (glacial acetic acid with aqueous gentian violet in distilled water).

#### Procedure

The technique for use of the Thoma white count pipette and the Unopette system is identical to that of the RBC procedure. The only difference is in the dilution and the resting time. When using the Thoma white count pipette, blood is drawn to the 0.5 mark and the diluent to the 11.0 mark. This makes a final dilution of 1:20. The Unopette system also provides a 1:20 dilution. After performing the dilutions, allow them to stand for 10 minutes so that red cells can lyse. Charge both sides of the hemocytometer counting chamber, and allow the cells to settle for three to five minutes in a moist chamber (Petri plate).

#### Calculation

Count the four large corner squares of the chamber indicated by a W in figure 3-3 under the 10 X objective. Count the four corner squares on the other chamber and average these two counts. The two counts should be within ten percent of each other. This average figure is used to calculate the white blood cell count per liter of blood. Remember the dilution is 1:20. The entire ruled area on one side of the hemocytometer is 0.9  $\mu\text{L}$  (width  $\times$  length  $\times$  depth or 3 mm  $\times$  3 mm  $\times$  0.1 mm). Hence, each large square on the hemocytometer contains 0.1  $\mu\text{L}$  ( $0.9 \mu\text{L} \div 9 = 0.1 \mu\text{L}$ ). Therefore, if four large squares are counted, the total volume is 0.4  $\mu\text{L}$  ( $4 \times 0.1 \mu\text{L} = 0.4$ ). In order to find out how many WBCs are in 1.0  $\mu\text{L}$  of blood, 0.4  $\mu\text{L}$  is multiplied by 2.5 ( $1.0 \mu\text{L} \div 0.4 = 2.5$ ). Recall from unit 2 that 1.0  $\mu\text{L} = 10^{-6}$ , thus, to convert to liters we must multiply by  $10^6$ . To calculate the leukocyte count, use the following formula and review the examples.

# of Cells in four squares		Correction for dilution		Correction for volume		Conversion to liter	
WBCs Counted	$\times$	20	$\times$	$1.0 \div 0.4$	$\times$	$10^6$	= WBCs/L
Examples:							
135	$\times$	20	$\times$	2.5	$\times$	$10^6$	= $6.75 \times 10^9/\text{L}$
485	$\times$	20	$\times$	2.5	$\times$	$10^6$	= $24.25 \times 10^9/\text{L}$



### Reporting results

A general reference range is given here:

Age Group	Reference Range
Adults	4.0 to $11.0 \times 10^9/\text{L}$
Newborn	10.0 to $30.0 \times 10^9/\text{L}$
At 1 year of age	6.0 to $17.0 \times 10^9/\text{L}$
After 1 year the WBC count drops slowly to normal levels by age 21	

### Procedural notes or hints

Review procedural notes or hints for RBC counts, they also apply. If the WBC count is above  $30.0 \times 10^9/\text{L}$ , a larger dilution should be used. One method involves using a Thoma *red cell* pipette, draw the blood to the 1.0 mark and dilute to the 101 mark with WBC diluting fluid. This will make a dilution of 1:100. If the WBC count is  $100.0$  to  $300.0 \times 10^9/\text{L}$ , as seen in certain leukemias, use the RBC diluting procedure for a 1:200 dilution. If the WBC count is less than  $3.0 \times 10^9/\text{L}$ , use a smaller dilution. Using a Thoma white cell pipette, draw the blood to the 1.0 mark and dilute to the 11 mark for a dilution of 1:10.

### Limitations of the procedure

Review limitations of the procedure for RBC counts, they also apply to the WBC count. The diluting fluids used for WBC counts hemolyze all non-nucleated RBCs, consequently nucleated RBCs may be present and appear as WBCs. If there are more than five nucleated red blood cells (NRBCs) per 100 WBCs reported from a differential, the WBC count must be corrected. Use the formula below for a corrected WBC count.

$$\text{Corrected WBC} = \frac{\text{Uncorrected WBC}}{100 + \# \text{ of NRBC per } 100 \text{ WBC}} \times 100$$

### Platelet and eosinophil counts

Platelets and eosinophils can be counted separately from the other blood cells. A Unopette system is available for both. The platelet Unopette system contains ammonium oxalate, which hemolyzes the RBCs. See figure 3-3 for the suggested squares to be used for the platelet count. The reference range for platelets is 140,000 to 440,000  $\mu\text{L}$  or  $140$  to  $440 \times 10^9/\text{L}$ . The eosinophil Unopette system employs a 25  $\mu\text{L}$  pipette and a reservoir containing phyloxine B as the diluting fluid. Phyloxine B contains a dye that stains only the eosinophils red. The resulting 1:32 dilution is used to charge both sides of the counting chamber. Count all nine squares on each side of the chamber and *add* them together for a total of eosinophils. Plug result into appropriate formula. The reference range for eosinophils is  $50$  to  $350 \times 10^6/\text{L}$ .

### Alternate methods

There are two general methods for determining the absolute eosinophil count; the direct method, just described, and the indirect method. The indirect method is a calculation using the WBC and differential counts. The WBC count is multiplied by the percentage of eosinophils in the differential. You can also use this method as a QC check for manual (direct) eosinophil counts. For example:

$$\text{WBC} = 8.3 \times 10^3/\mu\text{L} \text{ or } 8.3 \times 10^9/\text{L}.$$

$$\text{Differential eosinophil percentage} = 7.0 \text{ percent}$$

$$\begin{aligned} \text{Absolute eosinophil count} &= 8.3 \times 0.07 = 0.58 \times 10^3/\mu\text{L} \\ &\text{or } 580 \text{ eosinophils}/\text{mm}^3 \end{aligned}$$

**REMINDER:** Always follow the manufacturer's instructions; check the OIs in your laboratory for the exact procedures and formulas used for calculations.

### 013. Body fluid procedures

Routine analysis of body fluids include gross examination, total and differential cell counts, inspection for abnormal cells and crystals, chemical analysis, microbiology procedures, and cytologic examinations. For the purpose of this lesson, we will briefly review gross examination, total and differential cell counts, and inspection for abnormal cells and crystals. The most common body fluids received in hematology are cerebrospinal, synovial, serous, and seminal fluids.

#### Cerebrospinal fluid (CSF)

The examination of cerebrospinal fluid (CSF) requires strict adherence to biohazard safety procedures because of the highly infectious pathogens that may be present in the CSF. As stated in 4T051B, volume 1, unit 2, tube #3 is usually used for cellular analysis because it is most likely to be representative of the *in vivo* specimen and void of cells picked up during the tap.

#### Gross examination

Normally, CSF is a sterile, crystal clear, water-like substance that bathes the brain and central nervous system. If a disease is present, the CSF may appear cloudy, turbid, bloody, viscous, or clotted. Its color may be pink-red, green-tinged, or xanthochromic, which refers to pink, orange, or yellow after centrifugation.

Appearance or Color	Cause	Indication
Cloudy or Turbid	The presence of WBCs, microorganisms, or protein.	Meningitis, disorders that affect the blood-brain barrier, or an abnormal production of IgG within the CSF.
Bloody	The presence of RBCs.	Traumatic tap, subarachnoid hemorrhage, intracerebral hemorrhage, or infarct (an area of tissue necrosis).
Viscous	The presence of mucus, capsular polysaccharide, liquid nucleus pulposus.	Metastatic mucin-producing carcinomas, cryptococcosis, or from a needle injury during the tap.
Clotted	The presence of blood, microorganisms, or elevated protein levels.	Traumatic tap, suppurative or tuberculous meningitis or seen with Froin's syndrome.
Pink-red	Presence of blood.	See bloody appearance.
Green-tinged	Presence of myeloperoxidase which is an enzyme found in azurophilic granules of neutrophils and mononuclear phagocytes.	Purulent (pus) fluid.
Xanthochromic	Presence of hemoglobin, bilirubin, melanin, or protein.	Old hemorrhage or lysed cells from traumatic tap, RBC breakdown or elevated serum bilirubin (jaundice), meningeal malignant melanoma, or see protein above.

It is difficult to differentiate between bleeding from a traumatic tap and bleeding from a pathological reason. The table below gives general guidelines in distinguishing between the two.

Traumatic Tap	Pathologic Bleeding
A traumatic tap displays a large amount of blood in the first tube, with a progressive decrease in the subsequent tubes.	In a subarachnoid hemorrhage the blood is evenly mixed in all the tubes.
After centrifugation the supernatant is usually clear.	After centrifugation the supernatant is xanthochromic.
A clot may be present.	Usually no clot formation is seen.
<b>*These guidelines remain true only if the specimen is processed within 1 hour or less after the spinal tap.</b>	

### *Total and differential cell counts*

CSF cell counts are performed using a hemocytometer because normal CSF counts are well below the allowable background limits of electronic cell counters. A differential can be performed directly from the hemocytometer or by concentrating the CSF, making a slide out of the sediment and staining.

**NOTE:** Urgency of testing is important for not only clinical information, but, because of specimen deterioration over time, i.e., lysed RBCs.

### *Total cell count*

To perform the cell count, using a 100  $\mu\text{L}$  pipette, charge an improved Neubauer hemocytometer with well-mixed, undiluted specimen. Allow three to five minutes for cells to settle in a moist chamber. Count the entire ruled area on both sides of the hemocytometer and obtain an average. This technique is used to count WBCs and RBCs. Results can be recorded as  $\#/\mu\text{L}$  or  $\text{WBCs} \times 10^6/\text{L}$  or  $\text{RBCs} \times 10^6/\text{L}$ . Be sure to note the condition of the RBCs—fresh or crenated. This may give the physician insight as to the patient's condition. If the cells are too numerous to count, a 1:1, 1:10, or 1:100 dilution may be required. There are various ways to make the dilutions. You can use a 75-mm test tube, the Unopette system, or Thoma RBC and WBC pipettes. Be sure to use the appropriate formula with corrections for dilutions and volumes. The lymphocyte is the predominant cell in most viral infections, syphilis, and tuberculosis meningitis. Pyogenic infections due to meningococci and pneumococci usually result in a predominance of the neutrophil. Cerebral and extradural abscesses, as well as subdural hemorrhages, produce a neutrophilic response, although no bacteria can be demonstrated. See the table here for the reference range of WBCs and RBCs in CSF.

Age Group	Reference Range for CSF WBCs
Adults	0.0 to $5.0 \times 10^6/\text{L}$ or 0.0 to 5.0/ $\mu\text{L}$
5 years to puberty	0.0 to $10.0 \times 10^6/\text{L}$ or 0.0 to 10.0/ $\mu\text{L}$
1 to 4 years	0.0 to $20.0 \times 10^6/\text{L}$ or 0.0 to 20.0/ $\mu\text{L}$
Less than 1 year	0.0 to $30.0 \times 10^6/\text{L}$ or 0.0 to 30.0/ $\mu\text{L}$
Age Group	Reference Range for CSF RBCs
All ages	$0.0 \times 10^6/\text{L}$ or 0.0/ $\mu\text{L}$

You should realize that, to reflect the true condition of the CSF and the patient's disorder, hematology, microbiology, immunology, and cytology procedures along with chemical and immunocytochemistry examinations are all necessary.

### *Differential count*

While on the chamber, WBCs can be differentiated into polymorphonuclear and mononuclear. This procedure is not as accurate as using a stained smear for the differential. Perform the differential on a concentrated specimen. The CSF can be concentrated by different methods, including sedimentation, filtration centrifugation, or cytocentrifuge (the method of choice for most laboratories). It is sometimes difficult to make CSF adhere to the slide for a differential. If you experience this situation, add a drop of 22 percent albumin to the sediment before spreading the cells on a slide (the same technique as used for peripheral blood films), then air dry.

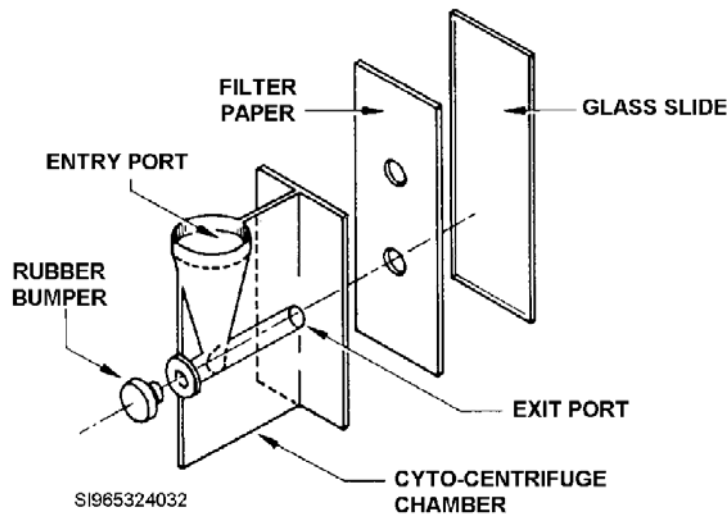


Figure 3-5. Cytocentrifuge technique for cellular concentration.

### *Cytocentrifuge method*

In the cytocentrifuge method, a predetermined amount of CSF is centrifuged at a low speed. The fluid passes through a tube, is absorbed by filter, and the cells are deposited directly on a glass slide as shown in figure 3-5. The advantages are that it is rapid, simple, and the slides are air dried so they can be stained with Wright-Giemsa. Some disadvantages are:

- It requires special equipment.
- Occasionally there is a reduced cell yield.
- There is less-than-optimal preservation of cellular integrity.

These can be alleviated if appropriate calibrations of speed and time are performed. Also, quality control for cytocentrifuge cell recovery should be done and compared with the manual WBC count.

### *Stained slide*

Once the slide is stained, perform a WBC differential. Note *all* the various cell types and if any microorganisms are seen. The CSF normally contains a small number of lymphocytes and monocytes. The other types of cells that may be seen include neutrophils, eosinophils, macrophages, plasma cells, malignant lymphoid cells, blasts, ependymal/choroid plexus cells, cartilage cells, bone marrow cells, and erythrocytes.

### **Other body fluids**

Other body fluids include synovial (joint) and serous (serum-containing) fluids. The serous fluids are found in the pleural, peritoneal, and pericardial cavities. All body fluid routine examinations should include gross descriptions (color, etc.), cell counts, and chemical analysis (i.e., glucose, protein). Enzyme, lipid, pH, microbiology, immunological, immunocytochemistry, and biochemical malignancy marker studies may also be ordered.

#### ***Synovial fluid***

Normal synovial fluid is a clear, pale yellow, viscous liquid that does not clot. Turbidity is indicative of the presence of WBCs, crystals, or cartilage debris. Cell counts can be done on an undiluted specimen using a hemocytometer. If the counts are too high, the specimen can be diluted with normal saline. The differential can be accomplished on a phase-contrast microscopy or by Wright-stained smears from a concentrated or diluted specimen. Normal synovial fluid contains less than 25 percent neutrophils and 75 percent normal lymphocytes and monocytes *without* RBCs, cartilage cells, cells with inclusions, or crystals. The types of cells seen in abnormal synovial fluid include neutrophils, lymphocytes, plasma cells, monocytes, eosinophils, macrophages, histiocytes, synovial lining cells, and lupus erythematosus cells. The crystals that are associated with clinical disorders and may be present are monosodium urate monohydrate, calcium pyrophosphate dihydrate, basic calcium phosphates, calcium oxalate, and cholesterol esters.

#### ***Serous fluid***

Normal serous fluid is clear and pale yellow. Bloody fluids are due to traumatic puncture or disease. Milky white fluids are due to the presence of lipids, cholesterol crystals, or cellular debris. Cell counts are performed the same as for synovial fluid. Differentials should be performed on concentrated specimens. The types of cells that may be seen in these types of fluids include lymphocytes, plasma cells, monocytes, macrophages, neutrophils, eosinophils, RBCs, and mesothelial cells. Malignant cells are commonly found in these fluids and are indicative of a neoplastic disease. All cells types are reported.

### **Semen analysis**

The main indications for semen analysis include infertility workup and post vasectomy check.

#### ***Gross examination***

Upon ejaculation, semen is liquid, but it gels almost immediately; then it liquefies again in 10 to 20 minutes due to the action of fibrinolysin. Spermatozoa exhibit maximum motility once this liquefaction takes place. While it is in this liquefied state, laboratory analysis can take place. On receipt, note the time of specimen collection and begin the preliminary workup. Measure the total volume. The average is 1.0 to 5.0 mL. Volumes of less than 1.0 mL are associated with male infertility or gross anatomy problems. Some physicians request color (white, yellow, gray, etc.) and turbidity (clear, opalescent, opaque, etc.), although these factors have no relationship to sperm counts in humans. Record these parameters if they are requested. Viscosity can be assessed by pouring the liquefied specimen from the collection container into the graduate cylinder (for measurement) and checking to see if it can be poured drop by drop. This represents normal viscosity. Last, test the pH of the specimen with a reagent strip or litmus paper. The pH should be 7.5 to 8.0.

#### ***Examination for motility***

Mix the specimen, place a drop on a slide, and cover slip. Read the slide under high dry and record the percent of spermatozoa that exhibit directional motility. At least 40 percent of the sperm should show progressive motility. Some laboratories report out the percentage in three categories—progressive motility, regressive motility, and non-motile. A viability should be determined and correlated with the motility results. The viability can be determined by staining the spermatozoa and counting the number of dead and alive sperm. Normally, 50 percent of the spermatozoa should be alive.

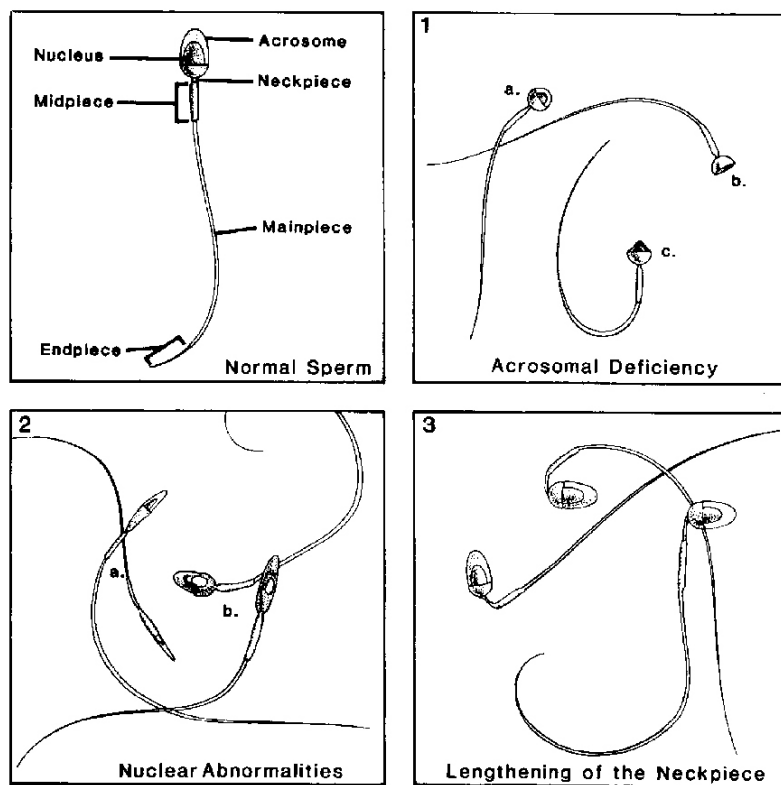
### ***Sperm count***

To count spermatozoa accurately, dilute an aliquot of the specimen 1:20 in a mixture of sodium bicarbonate, formalin (neutral), and distilled water. Use a Thoma WBC pipette or add 1.9 mL of this solution to 0.1 mL of semen and mix well. Charge an improved Neubauer hemocytometer and allow three to five minutes for spermatozoa to settle. Count number of spermatozoa in two large squares and multiply by 100,000. This gives you the number of spermatozoa per milliliter (mL). The reference range is 10 to  $20 \times 10^6/\text{mL}$ . A sperm count of  $<10$  million is considered abnormal. Repeat the procedure and average the value. If the specimen is highly viscous, dilute semen 1:1 with a mucolytic agent and multiply the number by two.

### ***Morphological examination***

The accurate reporting of sperm morphology is critical to the reliability of semen analysis results. Unfortunately, until recently little data has been available to the technician to guide the assessments of normal versus abnormal. Refer to figure 3-6. You might be surprised to learn that double heads and double tails are not among the more commonly encountered spermatozoa abnormalities. Begin the sperm morphology by placing a liquefied drop of semen on the end of a plain glass slide. Place a second slide on top of this slide and slip the slides apart. This will make a thin, full-slide smear. While the specimen is still wet, spray the slide with a cytological spray or fix with 95 percent ethanol, and allow drying. Stain the slide with Papanicolaou stain. If your hospital has a cytology department, you can ask them to stain your semen slides along with their Pap smears.

**NOTE:** George Nicholas Papanicolaou, 1883–1962, is given credit for the development of the Pap test.



The three most common Sperm abnormalities

SI965324035

Figure 3-6. Abnormalities of human spermatozoa.

### *Head*

The head of the sperm cell, which carries the paternal hereditary material, is composed of two regions—the acrosome and the nucleus. The acrosome forms the cap-like structure of the head and contains numerous enzymes that aid in penetration of the outer layer of the ovum. The nucleus is located in the posterior of the sperm head and covered by the postnuclear cap that overlaps with the acrosome. It is this structure, composed of conjugated protein that carries the genetic material and represents 65 percent of the sperm head.

### *Tail*

Through electron microscopy, you see the tail to be a very complex structure composed of four parts—the neck, midpiece, main piece, and end piece. The sperm head is connected to the midpiece by a barely discernible neck. The midpiece, composed of inner and outer fibrils, is wrapped by a mitochondrial sheath. Metabolic enzymes, which give motility to the sperm cell, are found inside this sheath. The main piece and end piece comprise the rest of the tail and are used for locomotion. Surrounding the entire spermatozoon is a cellular membrane called the plasmalemma.

### *Abnormalities*

Count at least 200 spermatozoa under oil immersion. Report the percentage of abnormal forms and the presence of RBCs, WBCs, and epithelial cells. Abnormal sperm are seen in the semen of practically all normal males; however, it is when abnormalities are in excess of 75 percent that infertility is suspected. It has been documented that 40 to 50 percent of all infertility cases indicate that it is the male partner who has the infertility. Three abnormalities are commonly seen in sperm cells—Acrosomal deficiency, nuclear abnormalities, and lengthening of the neckpiece. Acrosomes may appear to be entirely deficient, small and acorn-like, giant, or only as tiny peaks on top of the nucleus. Sperm exhibiting acrosomal absence often appear in pairs. When this phenomenon is present, both acrosomal absence and pairing should be noted. Nuclear abnormalities often include bizarre shapes such as dumbbells or bullets. Varicoceles often produce elongated nuclei with tapering heads. Another nuclear abnormality seen on the stained smear is vacuolization, which is common and appears to be of no consequence. Lengthening of the neck piece results in the sperm cell appearing to be dragging the head at a 90 degree angle while propelling forward. This condition is often accompanied by cytoplasmic droplets being seen clinging to the neck piece. One cause can be an improperly functioning epididymis. It may also be necessary to differentiate WBCs from immature sperm. These germ cells (spermatids, spermatocytes, and spermatogonia) vary in size and normally have three or more residual bodies that are not connected. It is easy to note the WBCs because of their uniform size, lack of nuclei, and lobes that are connected.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 012. Manual procedures

1. What is the principle of the manual red blood cell count?
2. Haymen's diluting fluid usually not recommend for RBC counts - why?
3. What welcome changes are provided by the Unopette systems?
4. When manually calculating RBCs, within what percentage should the RBC counts of both chambers be?
5. What is the formula for counting RBCs?
6. What is the principle of the WBC count?
7. What is the formula used for counting WBCs?
8. If the WBC count is over  $100.0 \times 10^9/L$ , what manual procedure can be used?
9. Using a Thoma white cell count pipette, what mark is the blood and diluting fluid drawn to and what is the final dilution?

### 013. Body fluid procedures

1. What collection tube is used for CSF cellular studies?
2. What does normal CSF look like?
3. What does CSF look like from disease states?



4. Match the CSF related terms in column B with the statement in column. Each item in column B may be used once, more than once, or not at all.

*Column A*

- \_\_\_1. After centrifugation the supernatant is xanthochromic.
- \_\_\_2. Presence of myeloperoxidase.
- \_\_\_3. Presence of hemoglobin, bilirubin, melanin, or protein.
- \_\_\_4. Meningitis, disorders that affect the blood-brain barrier or an abnormal production of IgG within the CSF.
- \_\_\_5. Metastatic mucin-producing carcinomas, cryptococcosis, or from needle injury during the tap.
- \_\_\_6. Purulent (pus) fluid.
- \_\_\_7. Lots of blood in the first tube, with progressive decrease in the subsequent tubes.
- \_\_\_8. Traumatic tap, subarachnoid hemorrhage, intracerebral hemorrhage, or infarct.
- \_\_\_9. The presence of blood, microorganisms, or elevated protein levels.

*Column B*

- a. Cloudy or turbid.
- b. Bloody.
- c. Viscous.
- d. Clotted.
- e. Pink-red.
- f. Green-tinged.
- g. Xanthochromic.
- h. Traumatic tap.
- i. Pathologic bleeding.

- 5. What is the predominant cell in most viral infections, syphilis, and tuberculosis meningitis?
- 6. What is the predominant cell in pyogenic infections?
- 7. What are the advantages of the cytocentrifuge method for performing CSF differentials?
- 8. What are the disadvantages?
- 9. What are the various cell types seen in CSF?
- 10. What does normal synovial fluid look like and what normal cells can be seen?
- 11. What are some of the abnormal cells seen in synovial fluid?
- 12. What is indicated by the presence of malignant cells in serous fluid?
- 13. What are the main indications for semen analysis?

14. When do spermatozoa exhibit maximum motility?
15. What are the three sperm motility categories?
16. What are the three common spermatozoa abnormalities?

## 3-2. Automated Cell Counts

How many cell counts can you perform in one duty day? In 1955, an average technician could perform about 30 manual cell counts in a day. Today, with proper equipment, 300, 500, or even 1,000 cell counts can be accomplished in a 24-hour period. The advent of automated hematology analyzers has not only enhanced our workload capability, but it has also vastly improved precision and reproducibility. Before we discuss these amazing instruments, we should review foundational procedures.

### 014. Hemoglobin, hematocrit, erythrocyte indices, and miscellaneous measurements

The H&H, hemoglobin and hematocrit tests, is one of the most common screening procedures. Boy Scouts going to summer camp, expectant mothers, the seriously ill, prospective insurance customers, accident victims, high school athletes, housewives, paupers to presidents rely on these two simple tests to monitor their general health and oxygen carrying capacity. Erythrocyte indices and platelet counts have been added to the basic CBC with the advent of advanced instrumentation.

#### Hemoglobin

The measurement of hemoglobin is a simple way to assess anemia, polycythemia, and follow the treatment of both.

#### *Measurement by the cyanmethemoglobin method*

Cyanmethemoglobin is a very stable pigment that does not deteriorate for several years if properly refrigerated.

#### *Principle*

Whole blood is added to a cyanmethemoglobin reagent (potassium cyanide and potassium ferricyanide). The hemoglobin iron is converted from the ferrous state ( $\text{Fe}^{++}$ ) to the ferric state ( $\text{Fe}^{+++}$ ) by the ferricyanide to form methemoglobin, which then combines with potassium cyanide to form the stable pigment, cyanmethemoglobin ( $\text{HiCN}$ ).

#### *Specimen*

Venous blood anticoagulated with EDTA and capillary blood is acceptable.

### Procedure

The resulting color of the HiCN is measured using a spectrophotometer at 540 nm. The optical density is directly proportional to the hemoglobin concentration. The solution must be clear. All forms of hemoglobin are measured by this technique, except sulfhemoglobin. Its values are expressed in grams per deciliter (g/dL) and vary greatly with age and sex, as shown in the following table.

Age Group	Reference Range
Adult Females	12 to 16 g/dL
Adult Males	13 to 18 g/dL
After 50 years of age	Slightly lower than the adult range.
Newborn	15 to 20 g/dL
Around 2 months	9 to 14 g/dL
Childhood and Adolescence	12 to 15 g/dL

### Abnormal hemoglobin pigments

Hemoglobin that has been converted to an abnormal pigment cannot transport oxygen. This “inactive” condition can lead to cyanosis and hypoxia if concentrations are adequate. The following are the three most significant abnormal hemoglobin pigments:

Pigment	Explanation
Carboxyhemoglobin	This reversible hemoglobin formation is due to hemoglobin's higher affinity (>200x) for carbon monoxide than for oxygen. Carbon monoxide readily binds at all sites on the molecule intended for oxygen transportation. This hemoglobin is found in smokers at concentrations of 1 to 10 percent.
Methemoglobin	In this type of hemoglobin, the ferrous ion has been oxidized to the ferric state and is incapable of combining with or transporting the oxygen molecule. Methemoglobinemia can be inherited or acquired. This formation is reversible and normally present in blood at concentrations of 1 to 2 percent.
Sulfhemoglobin	This hemoglobin is not normally found in blood. If present, its formation is irreversible and it will remain unchanged for the life of the carrier cell. It is incapable of transporting oxygen, and its exact nature is unknown. It is thought to be produced by the action of certain drugs and chemicals such as sulfonamides and aromatic amines. It can also combine with carbon monoxide to form carboxysulfhemoglobin.

### Hematocrit

The percentage of centrifuged whole blood that is composed of red blood cells is the hematocrit, or packed cell volume (PCV). This procedure is simple, reproducible, and one of the most accurate hematology procedures. Its values closely parallel red blood cell counts and hemoglobin values. It can be reported out as a percentage or a decimal fraction (i.e., 43 percent or 0.43).

### Principle

A small amount of blood is centrifuged for maximum red blood cell packing and is expressed as a percentage of the whole blood volume.

### Specimens

Venous blood anticoagulated with dipotassium EDTA or heparin, or capillary blood.

### Procedure

Two capillary tubes are filled two-thirds full with capillary blood or well-mixed anticoagulated blood. If anticoagulated blood is used, the capillary tubes should not contain anticoagulant. For capillary blood, the tubes used should contain an appropriate amount of heparin. One end of the tubes are sealed with clay. They are placed into the radial grooves of the centrifuge head, across from each

other, clay end to the outside. To achieve maximum packing of cells, the tubes must be centrifuged between relative centrifugal force (**RCF**) of 10,000 to 15,000  $\times g$  or 10,000 to 12,000 RPM for five minutes. The tubes are then removed from the centrifuge as soon as it stops spinning and read both tubes on a microhematocrit reader. If a variation of more than two percent exists between the two tests, repeat the procedure. The general reference range is given in this table:

Age Group	Reference Range
Adult Females	36 to 48%
Adult Males	40 to 55%
After 50 years of age	Slightly lower than the adult range.
Newborn	45 to 60%
By 1 year of age	27 to 44%
Childhood and Adolescence	Increases slightly from 1 year old to adult range.

### ***Other considerations***

The electronically calculated hematocrit does not normally read the same as a spun hematocrit or PCV, as it is correctly called. This discrepancy is due to plasma trapped between the cells of the PCV and normally account for the PCV being 1.3 to 3.0 percent higher. This inborn error in the procedure can vary as much as five to six percent in a patient with sickle cell anemia. The following is a list of other sources of error that may be encountered in performing this procedure:

- Severe dehydration can drastically raise hematocrit values.
- Inadequate speed or time of centrifugation can falsely raise values.
- Specimen leaking around the clay invalidates the test.
- Excessive EDTA will cause cell shrinkage and decreased PCV.
- Variation of reading techniques, from technician to technician, can account for up to a two percent variation in readings.
- Over anticoagulation, EDTA blood placed into a heparinized tube, can alter results.

### **Erythrocyte indices**

Three red cell, or erythrocyte, indices are used in the classification and study of anemias—the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC). These indices are based on three determinations—the RBC count, hemoglobin, and hematocrit values. The indices are used to define the hemoglobin content and the size of the RBC.

#### ***Mean corpuscular volume (MCV)***

This is a calculation of the mean volume of the average erythrocyte in the population of the specimen, expressed in femtoliters (fL). Since the value is an average, it is possible to have a normal MCV, yet have macrocytes and microcytes present. For this reason, MCV is confirmed by microscopic evaluation. Review the following formula:

Volume of RBC in femtoliters of blood

or

$$\text{MCV} = \frac{\text{Hct} \times 10}{\text{RBC (in millions)}}$$

See the table below for an example of the formula for a hematocrit value of 43 percent and a RBC count of  $5.0 \times 10^{12}/\text{L}$ , if  $1 \mu\text{L} = 10^9 \text{ fL}$  and  $1 \text{ L (liter)} = 10^{15} \text{ fL}$ .

$$\begin{aligned}\text{MCV} &= \frac{0.43 \times 10^{15} \text{ fL/L}}{5.0 \times 10^{12} / \text{L}} \\ &\text{or} \\ &= 0.086 \times 10^3 / \text{L} \\ &= 86 \text{ fL}\end{aligned}$$

The MCV normal range is 80 to 100 fL. If the MCV is below 80 fL, the specimen is predominantly microcytic, while an MCV of over 100 fL would indicate macrocytic. Be aware that cold agglutinins may also increase the MCV.

#### ***Mean corpuscular hemoglobin***

This parameter of the indices, the MCH, is representative of the average weight of the hemoglobin in the red blood cell, expressed in picograms (pg). The MCH is directly proportional to the concentration of hemoglobin and the size of the RBC. It should also correlate with the MCV and MCHC. See the formula below.  $\text{RBC} / \text{L}$

$$\text{MCH} = \frac{\text{Hgb} \times 10}{\text{RBC count}}$$

See the formula below for a hemoglobin (Hgb) value of 12 g/dL and a RBC count of  $4.0 \times 10^{12}/\text{L}$ , if  $1 \text{ g} = 10^{12} \text{ pg}$  and  $1 \text{ L (liter)} = 10 \text{ dL}$ .

$$\begin{aligned}\text{MCH} &= \frac{\text{Hgb} \times 10 \times 10^{12} \text{ pg/L}}{\text{RBC} / \text{L}} \\ \text{MCH} &= \frac{12 \times 10^{13} \text{ pg/L}}{4.0 \times 10^{12} / \text{L}} \\ &= \frac{12 \times 10 \text{ pg/L}}{4.0 / \text{L}} \\ &= 30 \text{ pg}\end{aligned}$$

The normal range for the MCH is 27 - 31 pg. The MCH is elevated in macrocytic anemia's and in some cases of spherocytosis in which hyperchromia is indicated. If the MCH is lower than 27 pg, it is indicative of microcytic anemia.

#### ***Mean corpuscular hemoglobin concentration (MCHC)***

The MCHC is an expression of the average concentration of hemoglobin in the RBCs. It denotes the ratio of hemoglobin to the volume of the red blood cells and is expressed in grams per deciliter (g/dL) or a percentage.

$$\text{MCHC} = \frac{\text{Hgb in g/dL}}{\text{Hct in g/dL}} \times \text{to convert to a percentage}$$

or

$$\text{MCHC} = \frac{\text{Hgb} \times 100\%}{\text{Hct}}$$

See the formula for a Hgb value of 13 g/dL and a hematocrit of 43 percent.

$$\begin{aligned}\text{MCHC} &= \frac{13.0 \text{ g/dL}}{43 \text{ g/dL}} \times 100\% \\ &= 0.302 \times 100\% \\ &= 30.2\% \\ &\text{or} \\ \text{MCHC} &= 30.2 \text{ g/dL or } 302 \text{ g/L}\end{aligned}$$

The MCHC normal range is 31 to 36 percent. It indicates whether the RBCs will be normochromic, hyperchromic, or hypochromic. An MCHC within the normal range is normochromic, above 36 percent but below 38 percent (because an RBC is limited and can only contain a maximum concentration amount) is hyperchromic, and below 31 percent is hypochromic. Newborns have a higher than normal MCV and MCH. An MCH up to 40 pg has been seen in newborns. Elevated MCH and MCHC may indicate problems with falsely elevated hemoglobins (i.e., lipemic, icteric, and hemolysis). Anytime you have an increased MCH and MCHC, you must consider a problematic specimen. These formulas need to be learned because, although your analyzers calculate indices, your understanding of the concept will be broadened by your increased knowledge.

#### **Miscellaneous measurements**

Most of the new multiparameter analyzers provide one or more of the following: RDW, MPV, PDW, and PLCR. The RDW or red cell distribution width is an indication of the degree of dispersion of RBC size (anisocytosis) compared with normal size distribution. The RDW is useful because it is increased in iron deficiency anemias, is normal in the microcytic thalassemias, and it may provide valuable clues for classifying other anemia's. The MPV (mean platelet volume), PDW (platelet distribution width), and PLCR (platelet large cell ratio indicates platelets above 12  $\mu\text{m}$ ) are helpful in evaluating abnormal platelet populations and the disorders they are associated with (i.e., thrombocytopenia, thrombocytosis, hyperthyroidism, and myeloproliferative disorders).

#### **015. Instrument principles, histograms, and scattergrams**

In 1958, Joseph and Wallace Coulter presented their ideas for the Model A Coulter<sup>®</sup> counter to the National Electronics Conference. This instrument was hand-wired and contained 30 vacuum tubes. Although primitive by today's standards, it employed the same basic principles of cell counting by impedance used in today's sophisticated analyzers. The other principle utilized today is the physical scattering of light. Automated hematology instruments currently manufactured have evolved from single-parameter, particle-counting instruments to multiparameter state-of-the-art instruments with onboard computers.

### Cell counts by electronic impedance

Instruments using the impedance principle utilize a regulated constant current that is passed between two electrodes immersed in an electrically conductive diluent. The electrodes are insulated from each other by an insulator containing a small aperture (60 to 100  $\mu\text{m}$ ). The cells to be counted are, first, diluted and suspended evenly in a diluent fluid. As the suspension is drawn through the aperture, each cell displaces its own volume of the conducting electrolyte solution. The cells are poorer conductors of electricity than is the diluent; therefore, they produce a measurable change in impedance or voltage. This change in impedance is proportional to their individual volumes. The resulting electrical voltage pulses are amplified, discriminated, and counted during a given period of time for a known volume of suspension to be drawn through the aperture. The cell count is determined by the number of pulses generated in the timeframe. There are separate channels for RBCs and WBCs. In addition to the particle count per unit of volume, a number of other values can be computed electronically from the same measuring process or actually measured depending on the instrument. These are directly measured by the instrument—the mean corpuscular volume (MCV), red cell distribution width (RDW), and mean platelet volume (MPV). The instruments using this technique usually measure hemoglobin by the cyanmethemoglobin procedure. From the MCV hemoglobin concentration and the red blood cell count, hematocrit, the indices, such as the MCH and MCHC, can be determined. The companies that use this principle are Coulter Electronics Inc. (S-Plus series, STKR), Toa Medical Electronics Co. (E-5000, Sysmex CC-800), DuPont de Nemours and Co. (Cell-Dyn 2000), Instrumentation Laboratory (Collect 8E), and Baker Instruments Corp. (System 9000). Some of these instruments may not be on the market at the time you read this or the companies may have been purchased by other companies, and changed the names.

#### *Model S Plus Coulter® counter*

Although not the only impedance counters on the market, the Coulter® instruments are still the analyzers you will most likely use in Air Force laboratories. The S-Plus is Coulter's improvement of the old model S. The S-Plus has a smaller, less complicated pneumatic system and offers two more parameters—whole blood platelet counts and RDW—the last of which tests the degree of anisocytosis on patient samples. The model S-Plus Coulter® counter is composed of five interconnected units:

Unit	Explanation
Power supply	Provides regulated voltage to operate the electronics, vacuum and diluter units, and reagent system.
Diluter unit	Actually moves the sample through the unit. It aspirates, pipettes, dilutes, mixes, and lyses red blood cells.
Analyzer unit	Controls the diluter through a series of electronic cards. The diluter sends messages to the analyzer, which counts, sizes, measures and computes the data received. This computed data is then sent to the X-Y recorder.
Printer unit	Prints hard copy results obtained from the instrument.
X-Y recorder	Produces a graph that shows platelet size distributions and simulated platelet distributions.

#### *Coulter® Models S-Plus II, III, IV, V, and VI*

Coulter® has modified and improved its basic Plus system. Here is a brief look at several of the improvements and a comparison of them to the systems of the basic Plus.

- A modification of the reagent system resulted in the S-Plus II reporting a lymphocyte count and percent of total leukocyte count. This change also resulted in the RDW being reported as a true C.V. (coefficient of variation) of red cell size. The S-Plus II can be fitted with a data terminal that can plot the number of lymphocytes per microliter of blood and per cent of lymphocytes present, perform many quality control features, flag abnormal results, and print

RBC, WBC, and platelet histograms. For these functions, the normal X-Y recorder is replaced by a matrix printer.

- The Coulter® counter Model S-Plus III has included an automatic sample handler. This instrument can handle up to 115 specimens per hour. The specimens are loaded onto the instrument in trays, and the analyzer obtains the specimen from the sealed container.
- Sample size and reagent usage have both been reduced in the S-Plus IV. The sample has been changed from 1 to 100 mL, and the reagent usage is lowered by 25 percent.
- The S-Plus V incorporates a needle assembly that punctures the specimen's rubber cap for specimen sampling.
- The S-Plus VI uses an additional unit, the auto sampler. This unit contains two rotating wheel-shaped sample trays that each hold 32 patient samples. They also rotate to premix the specimens before testing.

#### ***Coulter® Model S-Plus STKR (“stacker”) and STKS (“stack-S”)***

The STKR, the stacker, the last model in the S-Plus series, is capable of accepting and mixing up to 144 patient samples at one time. Bar code labeled specimens are placed in a 12-sample cassette. The cassettes are transported from the right side of the instrument to the center of the diluter unit. At this point the sample is identified from the bar code label and the specimen is aspirated by a cap piercing aspirator. (There is a secondary aspirator for hand-held, uncapped specimens.) It also incorporates a three-part leukocyte differential displayed as a histogram. The STKS is the new generation fully automated hematology analyzer with a leukocyte differential counter (VCS). VCS was a stand-alone differential counter until it was combined with the STKR to become the STKS. The V stands for volume (sizing and counting cells using direct current). The C is for conductivity that uses high frequency electromagnetic probe to compile information about the cell nuclear density and size. The S is for scatter of laser light. This new technology differentiates the leukocytes into five subpopulations. Components of the STKS include a power supply, diluter unit, analyzer, data management system, graphic printer (optional), and autoreporter ticket printer (optional).

#### **Cell counting by light scattering**

The principle of light scattering is based on cells that pass through a flow cell on which a beam of light is focused. Individual or single cells pass through a sensing zone where they interrupt a beam of light. This disruption causes the light to scatter in all directions. However, only scattered light rays at specific angles are collected and sensed by the photodetectors.

#### ***Detected and analyzed***

The detected scattered light is analyzed and converted into digital forms, which provides cell counts and size information. A modified cyanmethemoglobin colorimetric procedure is used to determine hemoglobin values. RBC and platelet counts are performed on the same diluted sample and WBCs are counted from a different dilution in which the RBCs have been lysed. Indices and RBC size variation are usually calculated parameters.

#### ***Light scattering combined with absorbance***

In this system, a narrow stream of cells, uniformly suspended in a diluent, pass the focal point in single file, in a darkfield illumination system. Each cell causes a forward scattering of light on a photomultiplier. This creates an electrical pulse that is amplified and counted. The pulses are then correlated to the volume of fluid analyzed in a given time period. In the absorbance channel, when either unstained cells or no cells pass through, the photodiode receives maximum light. As stained cells pass through the same area, the cell absorbs a portion of the light. The decrease of light detected by the photodiode is proportional to the amount of light absorbed by the cell, which is proportional to the amount of staining. Cells are differentiated on this basis. This type of system can be seen in Technicon (Technicon Instrument Corp.) hematology instruments.



### Cell counting with lasers

The word *laser* is actually an acronym for light amplification by stimulated emission of radiation. Laser light has different characteristics and effectiveness from a beam or ordinary light. Laser light is:

- monochromatic-light of a single wavelength
- coherent-travels great distance with very little spread
- in phase-its parallel waves travel in one direction
- very bright

In this technique, a low-powered helium/neon laser is focused to a point measuring about 20  $\mu\text{m}$  on a fast moving column of liquid, the center of which is the specimen diluted in an isotonic fluid. Refer to figure 3-7. As RBCs, WBCs, and platelets pass by the laser, they are counted, and the RBC-size is determined by the degree to which the laser is occluded. From this data, the hematocrit is calculated. Hemoglobin is measured as cyanmethemoglobin by an LED colorimeter at 539 nm. A photomultiplier is used as the detector. MCV, MCH, and MCHC are also calculated values. The Ortho (Ortho Diagnostics Systems, Inc.) "Hemac" was the original instrument using this principle. It was replaced by the ELT series. These instruments are no longer manufactured, but some are still in service. The Cell-Dyn 3500/4000 SL automated hematology analyzers, manufactured by Abbott Diagnostics, use electrical impedance to count RBCs and platelets, a combination of light scattering and laser light to count and differentiate WBCs. The MCV is measured and the hematocrit, MCH, and MCHC are calculated values. Hemoglobin is measured by the cyanmethemoglobin method.

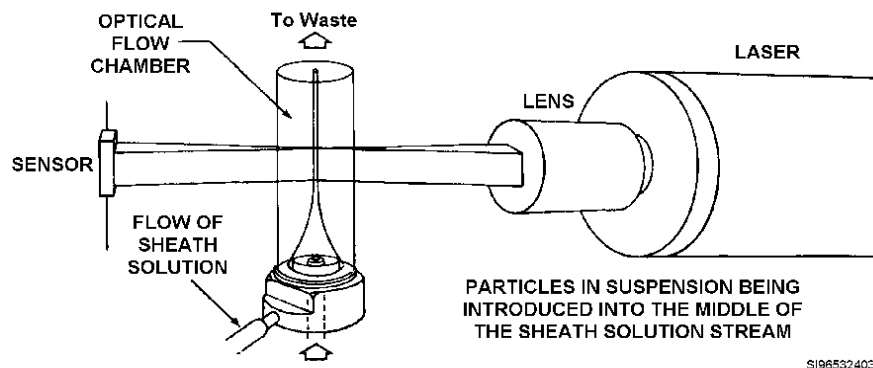


Figure 3-7. Cell counting using laser technology.

### Errors to be anticipated when performing electronic cell counting

There are many sources of error inherent to automated cell counting. Let's look at a partial list of the major problems you can encounter.

#### *Diluent errors*

One of the less than obvious errors may simply be the use of incorrect diluent or lysis reagent. As you troubleshoot your analyzer, check all fluids to ascertain proper levels and types. Your fluids, especially diluents, must be particle-free to avoid falsely elevated cell counts, especially platelets. Most systems check these fluids by background checks at each start-up.

#### *Abnormal cells*

Cells that result from disease or mechanical deformities can alter the cell count. In chronic myelocytic leukemia, megakaryocytic nuclei may be present in the peripheral blood and falsely elevate the leukocyte parameter. To the contrary, in chronic lymphocytic leukemia, the lymphocytes may be extremely susceptible to mechanical lysis and, therefore, the count will be falsely lowered. Giant platelets may be counted as larger cells, causing the platelet count to be lowered. The platelet count may be artificially elevated if your analyzer counts microcytic RBCs or RBC fragments. If platelets

clump, the result will likely be a lowered count or a wide variance of counts on successive specimens. Platelet satellitosis, the adherence of platelets to neutrophils, may cause an apparent thrombocytopenia. Another source of falsely increased platelet counts is found in automated optical instruments that lyse RBCs prior to counting platelets. In this situation, fragmented neutrophils, Howell Jolly bodies, Heinz bodies, Pappenheimer bodies, or even malarial parasites can be counted as platelets. Hemolytic anemia can also cause falsely elevated platelet results. Nucleated RBCs will likely be counted as WBCs and elevate the leukocyte count. In analyzers that use a spun hematocrit, the usual three percent trapped plasma between RBCs will artificially raise hematocrit values. RBCs containing sulfhemoglobin result in lowered hemoglobin results since this hemoglobin does not convert to cyanmethemoglobin. In the case of hemoglobin S or C, the WBC count may be elevated due to the high resistance of the RBCs to lysis. Falsely lowered MCV will be seen in the case of hypernatremia due to RBC shrinkage.

### ***Abnormal proteins and pigments***

Autoagglutinins, such as cold agglutinins, can result in decreased RBC counts and increased WBC counts. *In vivo* RBC lysis will show spurious increased hemoglobin levels. Severe plasma lipemia will yield elevated hemoglobin values, but can be corrected in some instruments by using a hemoglobin blank. Monoclonal proteins in high concentration can sometimes precipitate with various lysing agents. The resulting turbidity may elevate the WBC count and hemoglobin value. MCH and MCHC >40 can help you recognize these abnormalities.

### ***Mechanical problems***

It is impossible to list every mechanical problem found in each type of instrument. Most systems have checks to look for possible problems. The following is a very abbreviated list of malfunctions you may encounter on your hematology analyzer.

- Partial obstruction of the aperture will result in fewer cells counted and a falsely lowered count.
- Two or more cells passing the orifice at one time and being counted as one cell—coincidence loss—will give a falsely lowered count.
- Carryover from one count to another such as one specimen having a falsely elevated count when run immediately after a specimen with a severely high cell count.
- Spurious signals from the sensing region of the counter is a mechanical problem.
- Spurious signals from electrical interference is an example of an electromechanical problem.
- Look for instability or intermittent failure of electronic components.
- The blood clot—one of the great enemies of the cell counter—may present every aberration from lowered RBC values to blocked apertures that necessitate instrument shutdown.

### **Histograms and scattergrams**

Histograms and/or scattergrams are produced by most multiparameter hematology analyzers, and they provide valuable information that is probably underutilized. As stated earlier, because a large majority of the instruments used in Air Force laboratories are manufactured by Beckman Coulter®, most of this text pertains to these analyzers, although the basic principles are the same for the other instruments.

#### ***Histograms***

Histograms are distributional plots for RBCs, WBCs, and platelets. The Y axis is the concentration or relative number of cells and the X axis is the measurement of cell size in femtoliters (fL). Particles measuring between two and 20 fL are considered platelets and those greater than 36 fL are categorized as RBCs. Remember that RBCs and platelets are measured by one channel and the WBCs in a different channel. WBCs measuring between 35 and 90 fL are normal lymphocytes, between 90 and 160 fL are considered mononuclear cells, and between 160 and 450 fL are granulocytes.

### *RBC histograms*

The RBC count and MCV is used to create the RBC histogram. A normal RBC population produces a histogram with Gaussian distribution (bell-shaped curve). The curve will widen when there is more variation in red cell size. The curve will move to the left as the MCV decreases and to the right as the MCV increases.

### *Platelet histograms*

A normal platelet population produces a log-normal distribution. The platelet histogram usually has two curves. The first curve or graph is a plot of the platelets between 2 and 20 fL. However, we know that there are smaller platelets below 2 fL and larger platelets above 20 fL; therefore, a fitted curve is graphed from 0 to 70 fL over the original curve. All the platelets contained within the second curve are counted and reported as the platelet count. The second curve is only graphed when the platelets show a log-normal distribution. If the graphs don't fit, only the first curve will be shown and a symbol signifying a "no-fit" platelet count will appear on the printout.

### *WBC histograms*

Newer analyzers use scatter plots for WBC distribution. However, if your system still reports a histogram, a normal WBC histogram shows three distinct populations of WBCs and is known as trimodal distribution. If your instrument has a WBC histogram, be aware that if nucleated RBCs are present there will not be the characteristic valley at 35 fL seen in normal populations. Also, the lymphocyte category will contain mature and some atypical lymphocytes; the mononuclear category includes monocytes, promyelocytes, myelocytes, and blasts; and the granulocyte category is comprised of neutrophils, eosinophils, basophils, bands, and metamyelocytes.

### *Scattergrams*

Scattergrams, also called cytograms and dot plots, are two-dimensional displays of cell analysis data. Each axis represents a measurement of cell characteristics and cells with similar characteristics form clusters. An increase in cell concentration produces a denser or thicker cluster. All instruments that perform a five-part screening differential evaluate cell size and at least one other cellular characteristic. The Technicon analyzers use light scatter analysis and cytochemical staining to separate WBCs into subpopulations. The other manufacturers (Beckman Coulter® and TOA) use electrical impedance and/or light scatter without the use of stains to differentiate WBCs.

### *Coulter®*

The DF1 or discriminant function 1 scattergram displays the cell volume on the Y axis and light scatter analysis characteristics on the X axis. The DF1 scattergram is divided into six sections as shown in figure 3-8. The table below identifies the sections and the suspected cell populations to be seen in that section.

Section	Suspected Cell Populations
Long bottom	Giant platelets, Nucleated RBCs
Middle left	Lymphocytes, Variant lymphocytes, Suspect blast forms outside main population concentration
Middle right	Aged or damaged neutrophils
Top left	Monocytes, Suspect blast forms outside main population concentration
Top middle	Neutrophils, Suspect immature granulocytes and blast forms toward the top outside main population concentration
Top right	Eosinophils
*Basophils will not be seen on the DF1 scattergram because they are located behind the lymphocytes in the middle left area.	

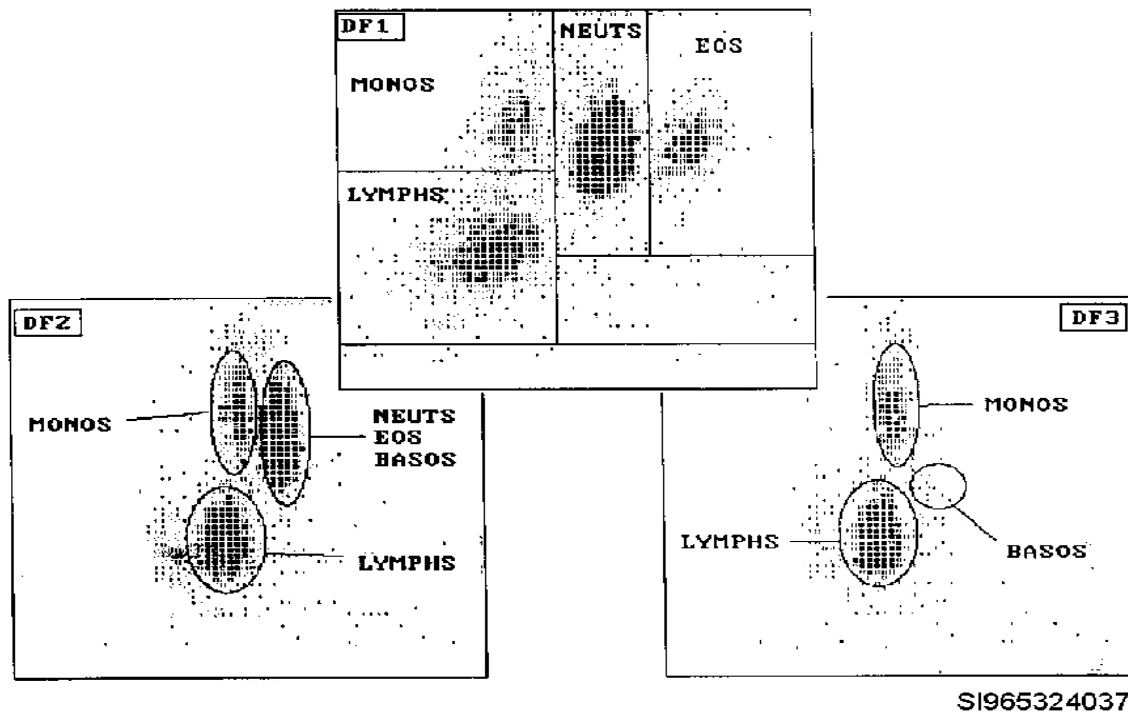


Figure 3-8. Schematic of DF1, DF2, and DF3.

#### *DF2 and DF3*

DF2, or discriminant function 2, plots data from cell volume and conductivity analysis for monocytes, lymphocytes, and granulocytes. DF3, or discriminant function 3, also plots data from cell volume and conductivity properties, but it reveals differentiation of the basophil population by gating out the neutrophils and eosinophils.

#### *Examples of histograms and scattergrams*

Examples are courtesy of Major Ron Hickman from Wilford Hall Medical Center, Lackland AFB, Texas, using the Coulter® STKS. Unfortunately, when reproducing scattergrams, the lightness versus the thickness of the plots are difficult to control. Remember, the thicker the plots the higher the count when scattergrams are produced from the instrument.

#### *Normal patient*

Figure 3-9 shows a scattergram and histograms of a normal patient. Note the cellular counts are reported out as  $\# \times 10^3/\mu\text{L}$  for WBCs and platelets and  $\# \times 10^6/\mu\text{L}$  for RBCs, this is the same as value  $\# \times 10^9/\text{L}$  and value  $\# \times 10^{12}/\text{L}$ , respectively.

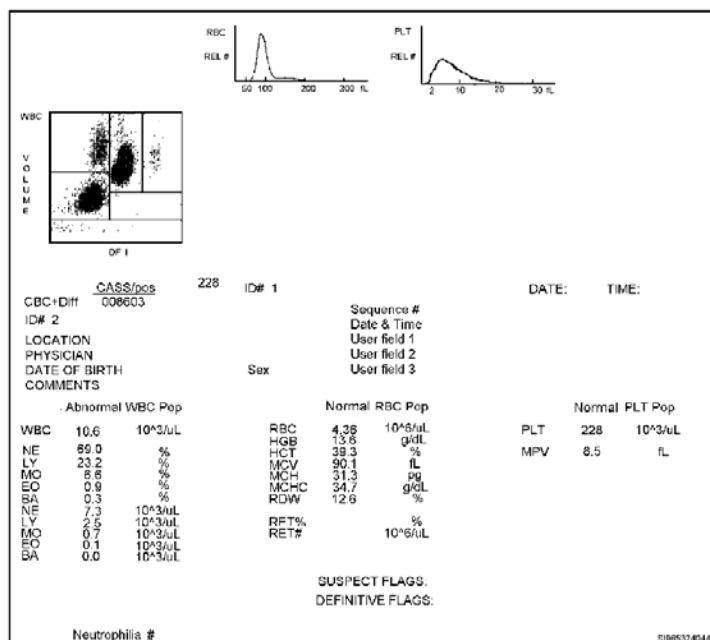


Figure 3-9. Scattergram of a normal patient.

### Nucleated RBCs

Figure 3-10 is from a patient with 29 nucleated red blood cells per 100 WBCs. Notice the two separate concentrations in the middle left area and an overlap in the long bottom area. Also, the RBC histogram distributional curve is quite different from the curve of the normal patients as shown in figure 3-9.

### Dimorphic RBC

The scattergram in figure 3-11 is from a patient who received blood. Did you notice the double peak on the RBC histogram and the plots in the long bottom area of DF1?

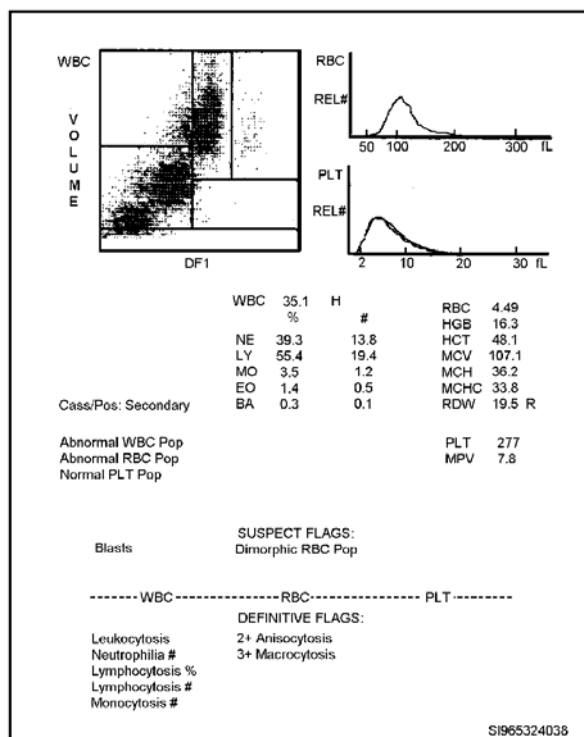


Figure 3-10. Scattergram of a patient with nucleated RBCs present.

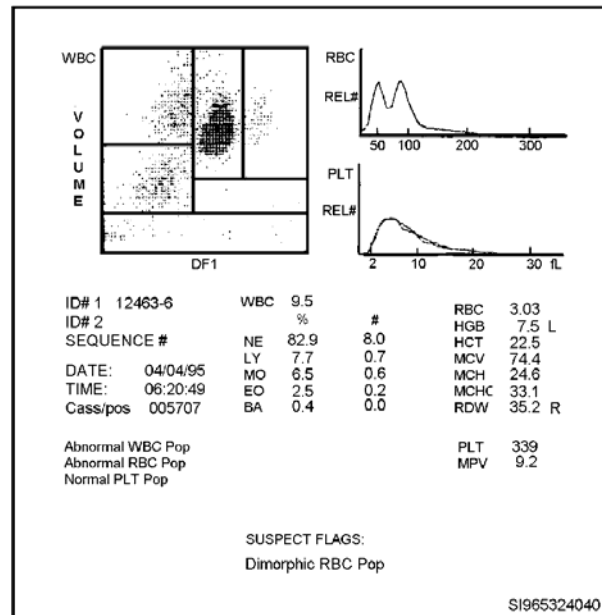


Figure 3-11. Scattergram of a patient with a dimorphic RBC population.

### Malaria

The RBC histogram is identical to the normal patients histogram in figure 3-12 and the scattergram is almost the same except for the concentration of plots in the long bottom area.

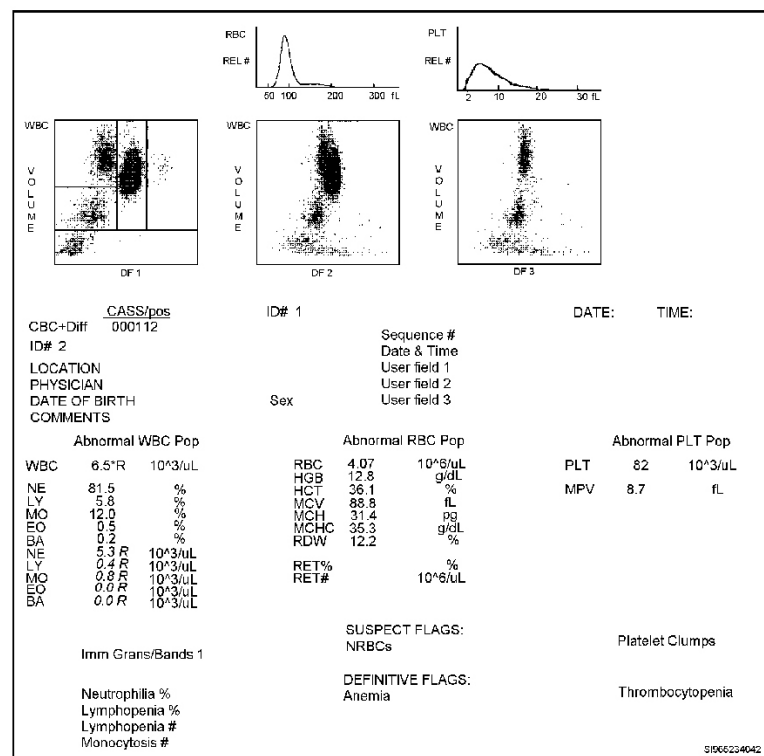


Figure 3-12. Scattergram of a patient with malaria.

### Eosinophilia

Figure 3–13 is a scattergram that is representative of a patient with eosinophilia as shown in the concentration of plots in the top right area.

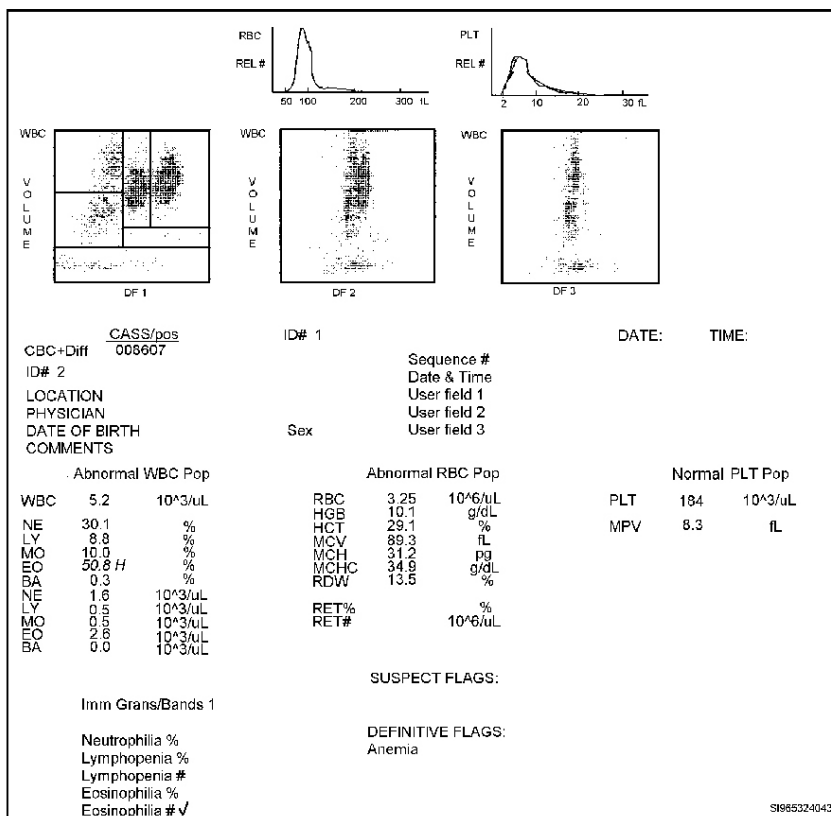


Figure 3–13. Scattergram of a patient with eosinophilia.

### Leukemoid reaction

As you can see, it can be very difficult to differentiate a leukemoid reaction, as shown in figure 3–14, from a patient with a true leukemia. Remember to suspect immature cells from plots in the very top middle area. Manual differential results were sent along with this scattergram and the results are shown here:

White blood cell	Number/100 WBC
Blasts	2
Myelocyte	4
Metamyelocyte	3
Band Neutrophil	59
Segmented Neutrophil	18
Lymphocytes	5
Basophil	1

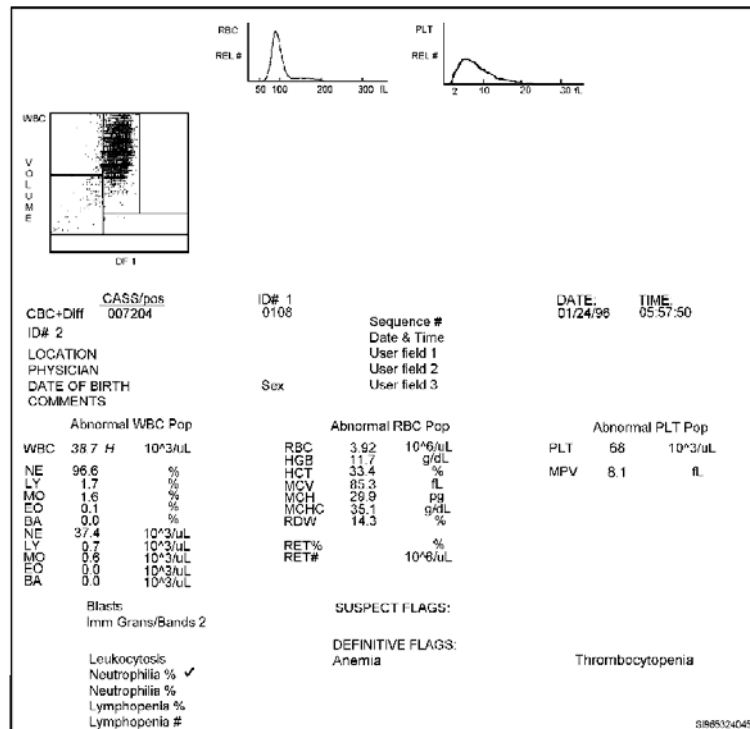


Figure 3-14. Scattergram of a patient with a leukemoid reaction.

### Lymphocytosis and thrombocytopenia

In figure 3-15 you see a patient with lymphocytosis and thrombocytopenia demonstrating an abnormal platelet histogram and RBC histogram with a shift to the right. Also, the scattergram is noticeably different with a concentration of plots only in the middle left area.

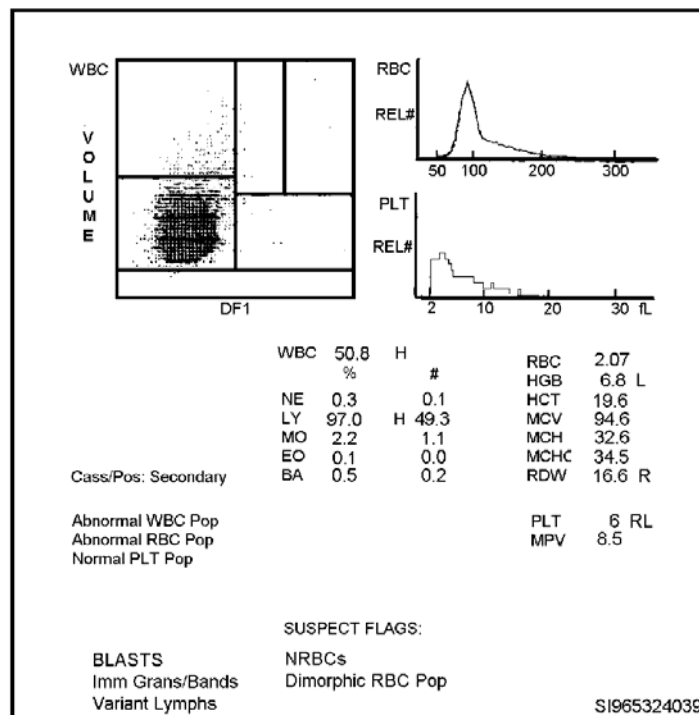
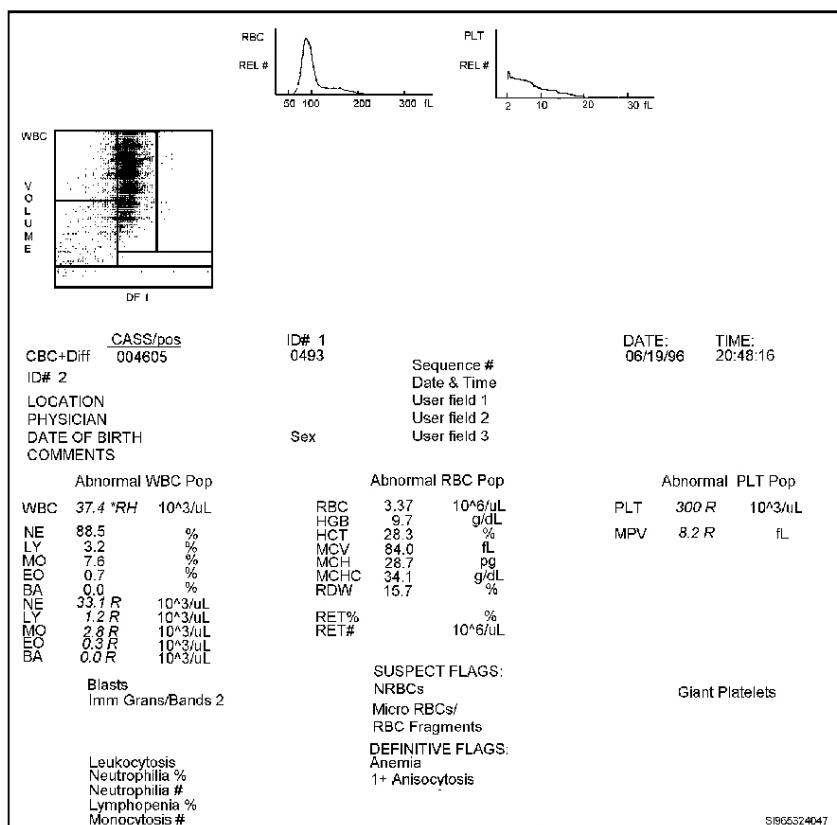


Figure 3-15. Scattergram of a patient with lymphocytosis and thrombocytopenia.



### Accelerated phase of CML

In this scattergram, as shown in figure 3–16, notice the concentration of plots in the upper area of the top middle section and the abnormal platelet histogram. This patient is in the accelerated phase of chronic myelogenous leukemia with approximately 30 percent blast found on the manual differential.



**Figure 3–16. Scattergram of a patient with accelerated phase of CML.**

### CLL

The patient's scattergram in figure 3–17 indicates an abnormal population of lymphocytes and monocytes seen in the middle left area and top left area, respectively. The WBC count of  $572 \times 10^3/\mu\text{L}$  is associated with chronic lymphocytic leukemia.

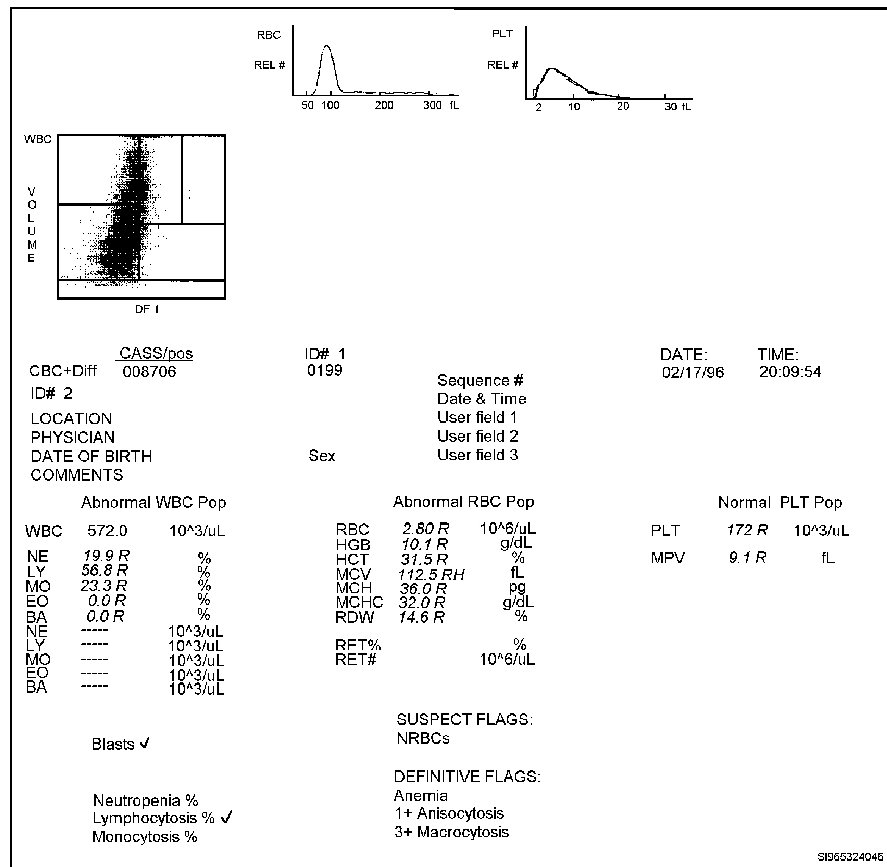


Figure 3-17. Scattergram of a patient with CLL.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 014. Hemoglobin, hematocrit, erythrocyte indices, and miscellaneous measurements

1. The measurement of hemoglobin is a simple way to assess and monitor what?
2. What is the principle of the cyanmethemoglobin procedure?
3. What form of hemoglobin is not measured using a spectrophotometer?
4. What are the three most significant abnormal hemoglobin pigments?
5. What hemoglobin pigment is found in smokers?

6. What is the principle of hematocrit testing?
7. What are the three red cell indices used in classifying and studying anemias?
8. What determinations are the erythrocyte indices based on and what do they define?
9. What is the MCV and how is it expressed?
10. What is the MCV normal range and what do the abnormal values indicate?
11. What is the MCH and how is it expressed?
12. What is the MCH normal range and what do the abnormal values indicate?
13. What is the MCHC and how is it expressed?
14. What is the MCHC normal range and what do the abnormal values indicate?
15. What is the RDW and what is it used for?

**015. Instrument principles, histograms, and scattergrams**

1. What is the impedance principle?
2. What does VCS used in automated analyzers stand for?
3. What is the principle of light scattering?
4. What does the acronym “laser” stand for?

5. What are some of the errors associated with automated cell counting?
6. What are histograms?
7. What type of distribution is seen in RBC, platelet, and WBC histograms, respectively?
8. What is a scattergram?

### 3-3. Related Studies

Although the CBC is a wonderful diagnostic tool, it may not always be able to reveal a total blood picture. The CBC can point the physician in many different directions. It might point to chemistry, special chemistry, immunology, microbiology, or other hematology procedures. This lesson will explore routine and special hematology procedures that still involve the cellular elements of blood.

#### 016. Routine and special studies

Routine studies include differentials, erythrocyte sedimentation rate (ESR), reticulocyte count, and sickle cell test. Even the smallest Air Force laboratories are familiar with these procedures. Consequently, an abbreviated study will follow. Special studies include G6PD, osmotic fragility tests, and bone marrow processing.

#### The differential

In a laboratory world that is becoming increasingly automated, one of the last areas requiring human expertise and judgment is differential microscopy. Although analyzers may read normal differentials, it is still the skilled technician that must be prepared to intervene with knowledge and experience before the final report is issued. There are those who say the most revealing, medically important procedure offered by the clinical laboratory is the differential. In this procedure, you report the relative number of each type of leukocyte and the condition and quality of the erythrocytes and platelets. As an experienced technician, you know that a high WBC count with a predominance of neutrophils may indicate appendicitis. This is just one example of how increases in leukocyte cell lines may help the physician diagnose many diseases. The following lists the “normal” differential and several increases that may be associated with diseases or conditions.

Cell Type	Relative Cell Count	Absolute Cell Count
Neutrophils-Band	0.0 to 0.6%	0.0 to $0.70 \times 10^9/L$
Neutrophils-Segmented	47.0 to 78.9%	1.8 to $7.00 \times 10^9/L$
Eosinophils	0.0 to 7.5%	0.0 to $0.45 \times 10^9/L$
Basophils	0.0 to 2.0%	0.0 to $0.20 \times 10^9/L$
Monocytes	2.0 to 11.0%	0.0 to $0.80 \times 10^9/L$
Lymphocytes	12.5 to 40.0%	1.0 to $4.80 \times 10^9/L$
<b>Total leukocytes</b>	NA	<b>4.5 to <math>11.0 \times 10^9/L</math></b>

<b>Neutrophilia may indicate:</b> Appendicitis. Pneumonia (bacterial). Myelogenous leukemia. Bacterial infection.	<b>Lymphocytosis may indicate:</b> Viral infections. Whooping cough. Infectious mononucleosis. Lymphocytic leukemia.
<b>Eosinophilia may indicate:</b> Allergies and allergenic reactions. Eosinophilic leukemia. Asthma. Scarlet fever. Parasitic infection.	<b>Basophilia may indicate:</b> Chronic granulocytic leukemia. Hemolytic anemia. Removal of the spleen. Irradiation (x-ray). Polycythemia vera.
<b>Monocytosis may indicate:</b> Tuberculosis. Brucellosis. Monocytic leukemia. SBE (subacute bacterial endocarditis). Typhoid Rickettsial infections. Collagen disease. Hodgkin's disease. Gaucher's disease.	

### *Preparing and processing blood films*

There are four ways to prepare a manual blood film:

1. Coverslip (used in bone marrow preps).
2. Automated centrifuge.
3. Automated spreader.
4. The most common—wedge or push method.

Let's discuss the wedge method. Glass slides to be used for differentials must be clean and oil-free. Occasionally, you may have to clean commercial slides even though they are pre-cleaned. Put a drop of blood to one end of the slide and immediately spread with another slide or hemocytometer coverslip (the best). A properly made blood smear covers more than half but less than three-quarters of the slide. Show a gradual transition from thick to thin and have a straight feathered end. Label the slide and air dry. Blood films made from EDTA should be made within two hours of collection to prevent changes in RBC and WBC morphology. You should know that platelet autoagglutinins cause platelet clumping in the presence of EDTA. Also, they have a tendency to adhere to neutrophils, a phenomenon known as satellitism.

### *Buffy coat preparations*

The severely leukopenic patient presents the laboratory with a unique challenge. These patients can have an almost unreadable differential slide, devoid of leukocytes. When this problem presents itself, use of a buffy coat prep can make life much easier. Simply fill a Wintrobe sedimentation tube with the patient's sample and centrifuge for five to ten minutes at  $1,000 \times g$ . Using a pipette, remove most of the plasma layer and discard. With the same pipette, carefully remove the rest of the plasma, the buffy coat layer, and a few red cells. Prepare a wedge smear and air dry. This slide can then be stained and read as usual.

### *Thick blood films*

If you remember, thick films are used for blood parasite identification. These films are usually prepared in hematology along with a regular or thin blood film. The thick film is prepared in the same manner as the wedge smear, but with a larger amount of blood. Newspaper print should be just visible through the blood.

### *Nasal eosinophil smears*

A nasal eosinophil count is used as a screening procedure for allergic rhinitis. At this time, there is a debate on whether or not it is truly diagnostic due to the high number of false positives/negatives and it doesn't correlate with blood eosinophil. An absolute eosinophil count may be more appropriate. Nonetheless, the procedure for a nasal eosinophil smear will be briefly discussed. The specimen may be collected by the clinician or patient. Using a nasopharyngeal swab, the clinician collects nasal mucus and submits the specimen as a smear on a labeled slide. If collected by the patient, the laboratory instructs the patient to blow into a waxed paper and, using a swab, thinly smear the mucus on a labeled slide. The latter procedure reveals more inflammatory cells and fewer contaminating epithelial cells that are obtained from the method when the mucosa is scraped by the nasopharyngeal swab. The slide is fixed with methanol and allowed to air dry. Then, it is stained, either manually or using an automated stainer, with Wright's stain. The stained smear is examined under low power for the presence of cellular elements. Move to high dry or oil immersion for identifying the presence of bacteria and the differentiation of the cells present. If there is a sufficient number of WBCs, perform a differential and report the number of eosinophils counted as a percentage of total WBCs. If there is not a sufficient amount of WBCs, estimate the number of eosinophils present in relation to the number of white cells present and report as *few*, *moderate*, or *many* eosinophils seen. Report any bacteria present as *few*, *moderate*, or *many* bacteria seen. If there is an increased number of neutrophils present, note on the report or report as a percentage of WBCs. The absence of eosinophils doesn't rule out an allergic condition because neutrophils also migrate into the nasal passages in allergic rhinitis. Also, if there is a secondary sinus infection, there will be a greater percentage of neutrophils in the specimen from an allergic subject. Nasal smears may be most helpful in confirming the diagnosis of nonallergic rhinitis with eosinophilia and differentiating acute viral rhinitis from allergic rhinitis. When a large percentage of the WBCs are eosinophils (usually greater than 50 percent) and all the skin tests to common aeroallergens are negative, a presumptive diagnosis of nonallergic rhinitis with eosinophilia can be made.

### *Staining methods*

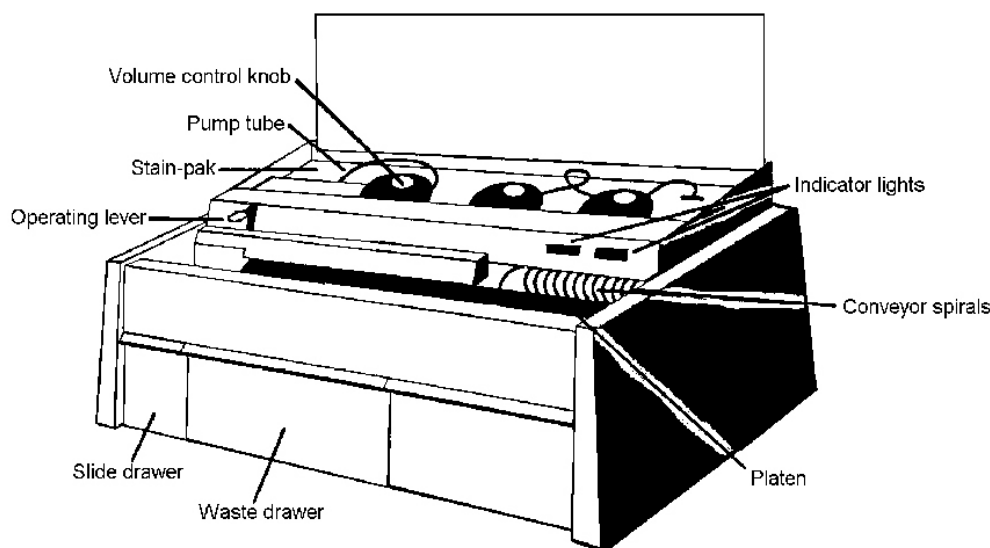
In Air Force laboratories, slides are stained with an automated slide stainer. When the stainer breaks down, a manual, single-reagent, dip stain is used. First let's go over the primary stains used in hematology.

#### *Stains*

Wright's, Giemsa, and Wright-Giemsa stains are an outgrowth of the Romanowsky class of stains, composed primarily of eosin, methylene blue, and oxidized methylene blue called azure. The oxidation of methylene blue is accomplished by molds, heating with potassium carbonate, adding borax, or heating to 100° F for one hour. This oxidation reaction is very important since it is this action that gives the stains their polychromatic staining ability. That is, it stains most normal and abnormal structures in the blood differently. Wright-Giemsa stain is used primarily for its ability to stain the malarial parasite. Fixing of the specimen is accomplished by the methyl alcohol contained in the stain or fixing the slides in chemical pure acetone-free methanol.

#### *Manual staining*

Currently, two stains are available for quick, manual staining. One involves fixation with methanol, a timed dipping of the slide into a stain, then a few minutes in water to remove extra stain. Simply, just air dry and read. The other method involves separate buffer and stain steps but is essentially the same. There are, of course, advocates of each procedure. Both of the procedures are best set up in staining jars so that they are ready in case they are needed. Coplin jars are excellent for this purpose. Make sure the jars are completely dry to avoid water contamination. The occasional filtering of the stain is recommended by both manufacturers.



**Figure 3-18. Example of the Hema-Tek automated slide stainer.**

#### *Automated staining*

The introduction of the Hema-Tek stainer, as shown in figure 3-18, put smiles on the faces of hematology workers—no more purple fingers, no more staining racks, and no more timers. Just drop in a stain pack, set the stain, load it, and let it go! This instrument provides a completely automated method for Wright's staining blood smears. The unit holds 25 slides in a spiral, gear-driven conveyor that moves them down a staining platform (platen). The slides are stained, rinsed, blown dry, then stacked in a slide drawer at a rate of one per minute. Slides are first loaded between two spiral gears. Care must be taken to properly align the slides between the gears. Improper alignment will result in broken slides as your stainer "eats" your blood films. As the slides are carried down the platen, a microswitch is triggered, and stain is applied to the slide. The next microswitch releases buffer solution, at which time most of the actual staining takes place. The last microswitch releases water to wash the stained smear and is followed by a fan that dries the slide before it is dropped into a holding drawer. Microscopically, properly stained red blood cells are buff pink. If RBCs are blue, the stain is too alkaline. With an alkaline stain, the WBCs stain dark and have only fair distinguishing characteristics. Abnormalities of the RBCs are also masked by this type of staining. Bright red RBCs result from a staining reaction that is too acid. In this situation, the WBCs (except for eosinophil granules) stain very poorly. The tendency toward acid staining is often caused by incomplete drying before staining. These staining reactions can be corrected by altering the amount of stain and buffer added to the slide. Each fluid release can be controlled by individual volume control knobs. If the Hema-Tek stainer is to operate properly, it must be cleaned properly. Periodically, clean the platen with methanol and a soft tissue. Clean from the loading area to the stacking area. This is necessary to preclude damage to the wires that operate the microswitches that control the staining process. The switches swing from right to left, but bend if pushed in the other direction. On a regular basis, empty the waste chamber located in the bottom of the unit. Another automated stainer is the dip-type manufactured by EM Diagnostic Systems, Inc., shown in figure 3-19.

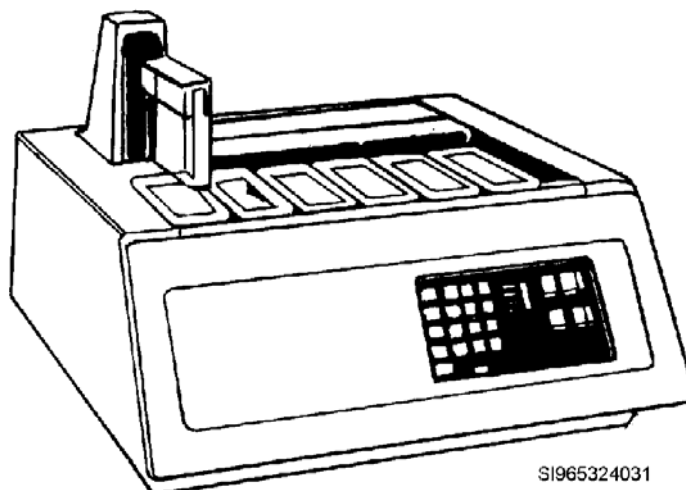


Figure 3-19. An automated dip-type stainer

### *Examining a stained blood film*

A differential blood film should be examined, under a 10 X objective (low power). The WBCs should be evenly distributed and not clumped. Large blast cells, platelet clumps, fibrin threads in platelets, monocytes, and neutrophils have a tendency to congregate at the edge of the slide, leaving the lymphocytes in the center of the slide. Do not read the cells at the extreme edge of the slide, as they are likely distorted and difficult to differentiate, but it is a good idea to scan the edge before going to high power. The RBCs in the thin, reading area of the slide should exhibit very little overlapping.

### *Counting the cells*

In routine laboratory work, counting 100 consecutive leukocytes on a stained blood smear is usually sufficient for a clinical workup on a patient. The reporting of differentials over 100 cells can be done as a percentage (as a 100-cell differential) or in absolute numbers of cells counted. This means that, if 300 cells are counted and 129 neutrophils are counted, it can be reported as 129 or 43 percent. The gradual transition from the metamyelocyte to the band and then to the segmented neutrophil makes precise classification difficult. This differentiation has been the source of many arguments. One rule of thumb is to identify the cell in the more mature form. So, what's a filament? Your hematology section should have a reference set of slides that graphically outlines the differentiation between band and segmented neutrophils. New personnel should study the reference set and consult senior technicians until they are properly trained.

### *Estimating platelet and leukocyte counts*

It is often useful to be able to get a quick estimate and verify these counts, if for no other reason than to ascertain the probability of an abnormally high or low count. Platelets can be quickly estimated by counting the average number seen in an oil immersion field and multiplying by 20,000. Estimate WBC counts by counting the number seen under the low power objective and multiplying by 200.

### **Reticulocyte count**

As studied in unit 2 of this volume, the reticulocyte is the precursor to the mature erythrocyte. It has lost its nucleus but retains aggregates of ribonucleic acid (RNA). We also know that it cannot be seen on a blood film stained by Wright's or Giemsa stains. The reticulocyte is found in small numbers in the peripheral blood of healthy people. However, the reticulocyte count is a very important diagnostic tool for determining bone marrow activity and is used in classifying anemias. It is also important for monitoring therapy. Many new analyzers perform automated reticulocyte counts.



### Principle

To detect the RNA, the reticulocyte must be stained during its living state, hence the need for the use of a supravital stain. New methylene blue and brilliant cresol blue are common supravital stains, but CLSI recommends the use of new methylene blue for the reticulocyte or retic stain. When mixed with a supravital stain, the reticulocyte will demonstrate two or more particles of blue-stained, granulofilamentous material as shown in figure 3-20.

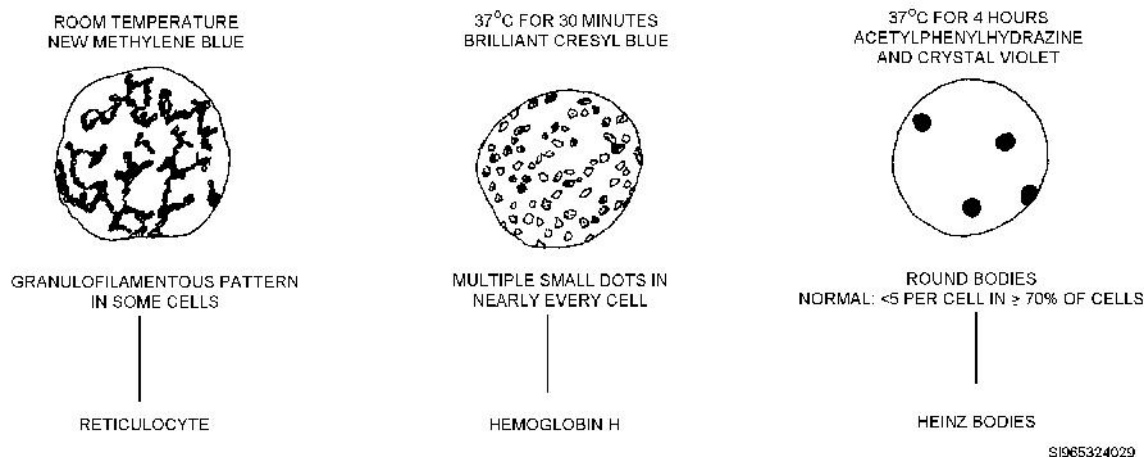


Figure 3-20. An example of a reticulocyte stained with new methylene blue supravital stain.

### Specimen

EDTA anticoagulated whole blood is recommended, but other common anticoagulants may be used. Capillary blood may also be used.

### Procedure

An equal amount of whole blood and stain is mixed in a small tube and incubated at room temperature for five to ten minutes. After the incubation, mix well and prepare two to three wedge method blood films. Allow to air dry and read under oil immersion (100 X objective). Count the number of reticulocytes observed in 1000 erythrocytes.

### Calculation

Use the formula below to calculate reticulocytes and note the example if 24 retics were counted in 1000 erythrocytes.

Retic (%) =

$$\frac{\text{\# of Retics}}{1000 \text{ RBCs observed}} \times 100$$

or

$$\frac{\text{\# of retics observed per 1000 RBCs}}{10}$$

**Reporting results**

Reticulocytes are reported out in a percentage as shown in the calculation or as and absolute number. The normal range for adults is 0.5 to 2.0 percent and in newborns 2.0 to 6.0 percent, and in absolute numbers the range is approximately  $50 \times 10^9/L$ .

**Corrected reticulocyte count and reticulocyte production index**

The corrected reticulocyte count (CRC) is sometimes referred to as a reticulocyte index or RI. The CRC is correlated with the hematocrit value. The reticulocyte production index (RPI) is a general indicator of the rate of erythrocyte production increase above normal in anemia. Different calculations are used to achieve the RPI. An RPI result over three generally represents an adequate bone marrow response to anemia, and RPI of less than two indicates an inadequate response. (Review unit 2 and the anemias classified by bone marrow response.) An elevated RPI is associated with chronic hemolysis, recent hemorrhage, and a response to therapy. A decreased RPI is seen in bone marrow failure (aplastic anemia) and ineffective erythropoiesis (vitamin B<sub>12</sub> and folate deficiency) causing megaloblastic anemia.

**Formula**

CRC and RPI formula for a patient with a 30 percent Hct and 2.4 percent retic.

$$\text{CRC} = \text{Retic \%} \times \frac{\text{Hct (liter / liter)}}{\text{Normal Hct (0.45 liter / liter)}}$$

$$\text{CRC} = 2.4 \times \frac{0.30}{0.45} = 1.6\%$$

$$\text{RPI} = \frac{\text{CRC}}{\text{Maturation time in peripheral blood (from a chart)}}$$

$$\text{RPI} = \frac{1.6\%}{1.5 \text{ days}} = 1.1 \text{ RPI}$$

**Sickle cell testing**

The sickle cell test is used to screen patients for sickle cell anemia and sickle cell trait. There are various methods for sickle cell testing; we'll look at the sodium dithionite, which is a solubility test.

**Principle**

When red blood cells are mixed with dithionite reagent, the red cells will immediately lyse. Once the sickling hemoglobins (hemoglobin S) are released from the red cells, they form liquid crystals to yield a turbid solution.

**Specimen**

EDTA, heparin, or sodium citrate anticoagulated blood may be used for up to three weeks.

**Procedure**

A 20  $\mu\text{m}$  sample of a patient's blood is added to 2 mL of dithionite reagent (commercially available) and mixed in a 12 x 75 mm glass test tube. After five minutes, the tube is examined against a lined reader for turbidity, as shown in figure 3-21. Hemoglobin S, if present, forms crystals in the reagent, and the resulting turbidity prevents viewing of the lines. If there is no HbS or non-S sickling hemoglobin present, the solution will be clear and the lines readily seen through the test tube. Should the screening procedure prove positive, hemoglobin electrophoresis is indicated. Run a positive and negative control with each set of tests.

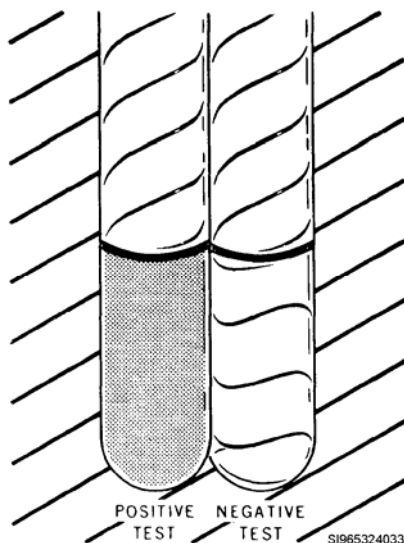


Figure 3-21. Dithionite tube test interpretation.

### **Discussion**

In patients with a hemoglobin of less than 7 g/dL, false negatives can occur. To preclude this error, double the concentration of blood (add 40  $\mu$ m to the reagent). False negatives can also occur in infants less than six months old due to hemoglobin F that is still predominant. Therefore, they should not be tested using this method. Positive dithionite tests are also seen in hemoglobin Bart, hemoglobin C-Harlem, and in the presence of certain abnormal proteins. At one time, the Air Force only tested African Americans and individuals of Mediterranean extraction for Hgb S. This policy has been changed to read that all Air Force personnel will be screened for HbS.

### **Erythrocyte sedimentation rate or ESR**

The ESR is a nonspecific assessment used to identify and monitor an inflammatory response to tissue injury. Our discussion will cover erythrocyte mass, plasma composition, and mechanical factors involved, as well as basic procedures.

#### ***Erythrocyte mass***

Macrocytes show an increased ESR, while microcytes have a decreased rate, both due to their respective mass. Zeta potential is the force that keeps these cells apart. It is a combination of the negative surface charges of the erythrocytes that repel each other, and pH and ionic strength of the plasma. Various proteins decrease the zeta potential. Those that affect it most are asymmetric molecules and molecules with high weight. Two examples of the proteins are fibrinogens and immunoglobulins. If the zeta potential decreases, erythrocytes form characteristic coin-like stacks called rouleaux. The sedimentation rate is increased due to increase in mass. Cells showing altered shapes, such as sickle cells or spherocytes, are unable to form rouleaux or agglutinate and, therefore, have decreased sedimentation rates. In anemia, the sedimentation rate is markedly increased due to the lowered concentration of cells in the plasma.

#### ***Plasma composition***

The plasma viscosity is related to the components of the blood. The higher the viscosity, the lower the sedimentation rate. Rouleaux and red cell aggregation are affected mainly by the levels of fibrinogen, alpha-1 globulin, and alpha-2 globulin. Rouleaux and aggregation increase in direct proportion to the plasma levels of the constituents.

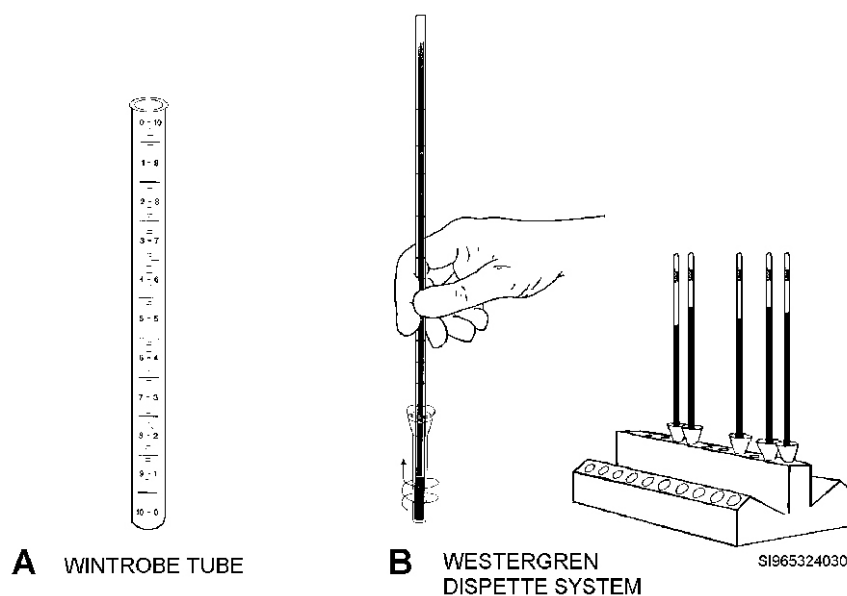
### ***Mechanical factors***

The sedimentation rate varies with the length and bore size of the sedimentation tube. Care must be taken to avoid the use of off-size tubes. This is easily avoided by ordering the standard item tube. The following factors must be carefully controlled:

- Tubes must be exactly perpendicular. Tubes allowed to slant from perfectly vertical increase sedimentation rate (a 3° slant equates to a 30 percent increase).
- Set up blood within 2 hours of drawing.
- Hemolized or clotted blood is totally unacceptable.
- Heparin and EDTA are anticoagulants of choice; double oxalates can increase values as much as five percent.

### ***The procedures***

For years, when a physician ordered an ESR, a Wintrobe sedimentation rate was performed. Today, there are two procedures to choose from—the Wintrobe and modified Westergren. New automated methods are being developed and possibly may be used at the time you read this text. There are no standard quality control specimens for the ESR test.



**Figure 3—22.**Wintrobe and Westergren erythrocyte sedimentation rate systems.

### ***Sedimentation rate by Wintrobe and Landsberg method***

The Wintrobe procedure is actually a three-stage reaction:

1. Cells rouleaux and drop slowly.
2. Cells drop rapidly.
3. Sedimentation slows due to buildup of cells at the bottom of the tube.

Using well-mixed, whole blood, fill a Wintrobe tube to the 0 mark as shown in figure 3-22, view A. Place the tube perfectly vertical for 60 minutes. When 60 minutes have elapsed, report the level the red cells have dropped to and record in millimeters per hour. The normal rates are 0 to 20 mm/hr for women and 0 to 9 mm/hr for men.

### *Modified Westergren method*

This technique is recommended by the Clinical and Laboratory Standards Institute (CLSI). A 2 mL aliquot of whole blood is mixed for two minutes with 0.5 mL of 0.85 percent saline or 0.5 mL of sodium citrate. Place blood container in the rack directly under each hole and insert a Westergren pipette through the whole into the blood mixture as shown in figure 3-22, view B. Fill the Westergren pipette to the 0 mark. Be certain that no air bubbles are permitted. After 60 minutes read the top of the erythrocytes and record results in Westergren ESR in millimeters/hour. The pipette can be made of glass or plastic but must have the following dimensions:

- Length 300 mm  $\pm$  5 mm.
- External diameter 5.5 mm  $\pm$  0.5 mm.
- Tube or internal bore 2.65 mm  $\pm$  0.15 mm.
- Bore uniformity  $\pm$  0.05.
- Graduated scale on pipette of 200 mm  $\pm$  0.35 mm.

ESR Normal Values	
Age Group	Reference Range
Men <50	0 to 15 mm/hr
Men >50	0 to 20 mm/hr
Women <50	0 to 20 mm/hr
Women >50	0 to 30 mm/hr
Children	0 to 10 mm/hr

### **Glucose-6-Phosphate Dehydrogenase (G6PD)**

G6PD deficiency is the most common red cell enzyme disorder associated with hemolysis. The disorder is a result of an abnormal gene that codes for G6PD and is an X-linked inherited disease. Therefore, men are homozygous and women are heterozygous with two populations of RBCs. One population has normal enzyme activity and the other is G6PD deficient. It is most common in West Africa, Mediterranean area, Middle East, and Southeast Asia. The disease can range from mild episodic hemolysis induced by drugs to severe chronic nonspherocytic anemia. The most useful laboratory screening test is the G6PD fluorescent spot test. A whole blood specimen is added to the reagent, incubated, and the mixture is then placed on filter paper and observed for fluorescence. Little or no fluorescence indicates a G6PD deficiency, moderate fluorescence indicates a mild deficiency, and bright fluorescence shows a normal G6PD. Use a normal patient specimen as the control to be used for patient comparison.

### **Osmotic fragility test**

The osmotic fragility test is a measure of the ability of the red blood cells to take up fluid without lysing. It is designed to evaluate the altered physical properties of erythrocytes in the diagnosis of anemias. Red blood cells are excellent indicators of osmotic pressure. The degree of distortion or eventual lysis that occurs to an RBC in a fluid is directly related to the osmotic pressure between the cell and the surrounding fluid. An erythrocyte goes through several stages in the process of lysis. In the first stage, the normal biconcave disc crenates. Then, the cell becomes spheroid along with crenation. Finally, the crenated spherocyte loses its crenation and hemolyzes, and a ghost cell (representing stroma without hemoglobin) remains. Since a spherocyte has progressed to a late stage of this system, very slight osmotic changes cause it to hemolyze. You'll recall that, in congenital spherocytosis anemia, the osmotic fragility test is increased (decreased resistance to lysis). It is also increased in hemolytic disease of the newborn, chemical poisoning, and burn cases. In the sickle-cell anemia and several other conditions, the osmotic fragility test is decreased (increased resistance to lysis).

***Principle***

The basis of the test is that, in isotonic saline (0.85 percent), the osmotic pressures are balanced between the red blood cell and saline so that there is no cellular distortion or hemolysis. In distilled water, the osmotic pressures are so imbalanced between the two that hemolysis of the cells occurs immediately upon exposure.

***Specimen***

The specimen of choice is heparinized venous blood and is set up within two hours of collection or within six hours if blood is refrigerated. A normal patient control should be drawn at the same time and treated the same as the patient sample.

***Procedure***

The test consists of a series of progressively lower salt concentrations from 0.85 to 0 percent solution—distilled water. When erythrocytes are placed into the solutions, the RBCs lyse at a certain concentration below 0.85 percent (initial hemolysis) and then lyse completely (complete hemolysis) between the initial hemolysis concentration and distilled water. In one modification of the fragility test, the amount of hemolysis is determined by measuring the amount of hemoglobin released. The percentage of hemoglobin in each tube is reported, equating the complete hemolysis tube to 100 percent released hemoglobin. Another modification that greatly increases the sensitivity of the test involves incubating the blood specimen at 37° C for 24 hours before running the test. For this modification, the blood must be collected aseptically in a sterile container with glass beads. The specimen is rotated before testing so that the blood is defibrinated by the glass beads. Becton-Dickinson also manufactures an Unopette test kit for determining osmotic fragility of red blood cells.

***Reporting results***

The salt concentration must be very exact for clear-cut reactions. The test report should state the concentration at which both initial and complete hemolysis occurred. Normally, initial hemolysis occurs at a saline concentration of 0.42 to 0.46 percent, and the hemolysis is complete between 0.30 and 0.34 percent saline.

***Processing bone marrow smears***

Bone marrow smears are critical for diagnosing many hematological disorders, including leukemia, megaloblastic anemia, and tumor cells metastasizing from other organs to name a few. Prepare wedge or coverslip films from the bone marrow specimen at the patient's bed side or from an EDTA tube with extra marrow. Particle smears are also prepared; crushed particle smears may also be required. Peripheral blood films collected from the patient on the same day of the bone marrow aspiration should be done. All of the above smears should be stained with Wright's stain along with special stains accomplished in the histology department. The special stains include Periodic Acid-Schiff, Acid phosphatase, Peroxidase, Leukocyte alkaline phosphatase, Sudan Black B, Chloroacetate esterase, and Nonspecific esterase. Your pathologist will decide on the staining protocol to be used in your military treatment facility. You may be required to screen (read) the Wright-stained films or just prepare them for the histology department.

## Self-Test Questions

After you complete these questions, you may check your answers at the end of the unit.

### 016. Routine and special studies

1. What do you report in the differential procedure?
2. What may neutrophilia indicate?
3. What may lymphocytosis indicate?
4. What may eosinophilia indicate?
5. What may basophilia indicate?
6. What may monocytosis indicate?
7. What are the four ways of preparing a manual blood film?
8. What preparation can you use to perform differentials on severely leukopenic patients?
9. How do you report eosinophils from a nasal smear?
10. What are the primary stains used in hematology and what are they composed of?
11. What areas of a differential slide should be read?
12. How can platelet counts be estimated from a differential slide?
13. The reticulocyte count is very important diagnostic tool for what disorder?

14. What type of stain is used in the reticulocyte count and give some examples?
15. What is the RPI and what does indicate?
16. What is the principle of the sodium dithionite test for sickle cell testing?
17. What is the ESR and what is it used for?
18. What is zeta potential?
19. Rouleaux and red cell aggregation are affected mainly by what constituents?
20. What are the three stages in the Wintrobe procedure?
21. What ESR technique is recommended by CLSI?
22. What is G6PD associated with?
23. What does the osmotic fragility test measure?
24. What disorders are increased osmotic fragility tests seen and what does it mean?
25. What disorders are decreased osmotic fragility tests seen and what does it mean?



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## Answers to Self-Test Questions

### 012

1. To aid counting and prohibit lysis of the red blood cells.
2. Rouleaux and clumping of RBCs due to hyperglobulinemia.
3. Premeasured dilutions that contain a measured amount of diluting fluid appropriate for the cellular element to be counted with pipettes in different sizes depending on the procedure to be performed.
4. 10 percent.
5.  $\text{RBCs Counted} \times 200 \times 1.0 \div 0.02 \times 10^6 = \text{RBCs/L}$ .
6. The principle of the manual WBC count is to aid counting and hemolyze red blood cells by using a weak acid solution as the diluent.
7.  $\text{WBCs Counted} \times 20 \times 1.0 \div 0.4 \times 10^6 = \text{WBCs/L}$ .
8. If the WBC count is  $100.0$  to  $300.0 \times 10^9/\text{L}$ , as seen in certain leukemias, use the RBC diluting procedure for a 1:200 dilution.
9. Using the Thoma white count pipette, draw the blood 0.5 mark and the diluent to the 11.0 mark for a 1:20 dilution.

### 013

1. Tube #3.
2. Crystal clear, water-like substance.
3. Cloudy, turbid, bloody, viscous, or clotted and its color may be pink-red, green-tinged, or xanthochromic.
4. (1) i.  
(2) f.  
(3) g.  
(4) a.  
(5) c.  
(6) f.  
(7) h.  
(8) b.  
(9) d.
5. Lymphocyte.
6. Neutrophil.
7. It is rapid, simple, and the slides are air dried so they can be stained with Wright-Giemsa.
8. Requires special equipment, occasionally there is a reduced cell yield, and there is less-than-optimal preservation of cellular integrity.
9. Lymphocytes, monocytes, neutrophils, eosinophils, macrophages, plasma cells, malignant lymphoid cells, blasts, ependymal/choroid plexus cells, cartilage cells, bone marrow cells and erythrocytes.
10. Clear, pale yellow, viscous liquid; neutrophils, lymphocytes and monocytes.
11. Neutrophils, lymphocytes, plasma cells, monocytes, eosinophils, macrophages, histiocytes, synovial lining cell, and lupus erythematosus cells.
12. Neoplastic disease.
13. Infertility workup and postvasectomy check.
14. Once liquefaction takes place.
15. Progressive motility, regressive motility, and nonmotile.
16. Acrosomal deficiency, nuclear abnormalities, and lengthening of the neck piece.

**014**

1. Anemia, polycythemia, and the treatment of both.
2. Whole blood is added to a cyanmethemoglobin reagent (potassium cyanide and potassium ferricyanide). The hemoglobin iron is converted from the ferrous state ( $\text{Fe}^{++}$ ) to the ferric state ( $\text{Fe}^{+++}$ ) by the ferricyanide to form methemoglobin, which then combines with potassium cyanide to form the stable pigment, cyanmethemoglobin ( $\text{HiCN}$ ).
3. Sulfhemoglobin.
4. Carboxyhemoglobin, methemoglobin, and sulfhemoglobin.
5. Carboxyhemoglobin.
6. A small amount of blood is centrifuged for maximum red blood cell packing and is expressed as a percentage of the whole blood volume.
7. (1) The mean corpuscular volume (MCV).  
(2) The mean corpuscular hemoglobin (MCH).  
(3) The mean corpuscular hemoglobin concentration (MCHC).
8. The RBC count, hemoglobin, and hematocrit values. They are used to define the hemoglobin content and the size of the RBC.
9. It is the mean volume of the average erythrocyte in the population of the specimen, expressed in femtoliters (fL).
10. The MCV normal range is 80 to 100 fL. If the MCV is below 80 fL, the specimen is predominantly microcytic, while an MCV of over 100 fL would indicate macrocytic.
11. The MCH is representative of the average weight of the hemoglobin in the red blood cell, expressed in picograms (pg).
12. The normal range for the MCH is 27 to 31 pg. The MCH is elevated in macrocytic anemia's and in some cases of spherocytosis in which hyperchromia is indicated. If the MCH is lower than 27 pg it is indicative of microcytic anemia.
13. It denotes the ratio of hemoglobin to the volume of the red blood cells and is expressed in grams per deciliter (g/dL) or a percentage.
14. 31 to 36 percent; hyperchromic or hypochromic.
15. Red cell distribution width; indication of the degree of anisocytosis

**015**

1. Regulated constant current is passed between two electrodes immersed in an electrically conductive diluent. The cells are poorer conductors of electricity than is the diluent; therefore, they produce a measurable change in impedance or voltage. This change in impedance is proportional to their individual volumes.
2. Volume, conductivity, and scatter.
3. Cells pass through a flow cell on which a beam of light is focused. Individual or single cells pass through a sensing zone where they interrupt a beam of light. This disruption cause the light to scatter in all directions. However, only scattered light rays at specific angles are collected and sensed by the photodetectors. The detected scattered light is analyzed and converted into digital forms, which provides cell counts and size information.
4. *Light amplification by stimulated emission of radiation.*
5. (1) Use of incorrect diluent or lysis reagent, (2) cell counts altered by abnormal cells, (3) presence of abnormal proteins and pigments alter results, and (4) various mechanical problems.
6. Distributional plots for RBCs, WBCs, and platelets.
7. A normal RBC population produces a histogram with Gaussian distribution (bell-shaped curve), normal platelet population produces a log-normal distribution, and normal WBC histogram shows three distinct populations of WBCs and is known as trimodal distribution.
8. Scattergrams, also called cytograms and dot plots, are two-dimensional displays of cell analysis data. Each axis represents a measurement of cell characteristics and cells with similar characteristics form clusters.

## 016

1. In this procedure, you report the relative number of each type of leukocyte and the condition and quality of the erythrocytes and platelets.
2. Appendicitis, pneumonia (bacterial), myelogenous leukemia, and bacterial infection.
3. Viral infections, whooping cough, infectious mononucleosis, and lymphocytic leukemia.
4. Allergies and allergenic reactions, eosinophilic leukemia, asthma, scarlet fever, and parasitic infection.
5. Chronic granulocytic leukemia, hemolytic anemia, removal of the spleen, irradiation (X ray) and polycythemia vera.
6. Tuberculosis, brucellosis, monocytic leukemia, SBE (subacute bacterial endocarditis), typhoid, rickettsial infections, collagen disease, Hodgkin's disease, and Gaucherie's disease.
7. (1) Coverslip (used in bone marrow preps).  
(2) Automated centrifuge.  
(3) automated spreader.  
(4) the most common-wedge or push method.
8. Buffy coat differential.
9. If there is a sufficient number of WBCs, perform a differential and report the number of eosinophils counted as a percentage of total WBCs. If there is not a sufficient amount of WBCs, estimate the number of eosinophils present in relation to the number of white cells present and report as few, moderate, or many eosinophils seen.
10. Wright's, Giemsa, and Wright-Giemsa stains are an outgrowth of the Romanowsky class of stains, composed primarily of eosin, methylene blue, and oxidized methylene blue called azure.
11. In the areas where the WBCs are evenly distributed and not clumped and where the RBCs exhibit very little overlapping.
12. Platelets can be quickly estimated by counting the average number seen in an oil immersion field and multiplying by 20,000.
13. Anemia.
14. Supravital; new methylene blue and brilliant cresol blue.
15. Reticulocyte production index and it is a general indicator of the rate of erythrocyte production increase above normal in anemia's.
16. When red blood cells are mixed with dithionite reagent, the red cells will immediately lyse, and once the sickling hemoglobins (hemoglobin S) are released from the red cells, they form liquid crystals to yield a turbid solution.
17. Erythrocyte sedimentation rate, and it is a nonspecific assessment used to identify and monitor an inflammatory response to tissue injury.
18. Zeta potential is the force that keeps RBCs apart and it is a combination of the negative surface charges of the erythrocytes that repel each other, and pH and ionic strength of the plasma.
19. Rouleaux and red cell aggregation are affected mainly by the levels of fibrinogen, alpha-1 globulin, and alpha-2 globulin.
20. (1) cells rouleaux and drop slowly.  
(2) cells drop rapidly.  
(3) sedimentation slows due to buildup of cells at the bottom of the tube.
21. Modified Westergren method.
22. Hemolysis.
23. The osmotic fragility test is a measure of the ability of the red cells to take up fluid without lysing.
24. Congenital spherocytosis anemia, hemolytic disease of the newborn, chemical poisoning, and burn cases; a decreased resistance to lysis.
25. In the sickle-cell anemia and several other conditions; an increased resistance to lysis.

**Do the unit review exercises before going to the next unit.**

## Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter.

57. (012) The *normal* dilution used for a manual RBC count is
- 1:10.
  - 1:50.
  - 1:100.
  - 1:200.
58. (012) When performing a manual RBC procedure, what squares on a Neubauer hemocytometer do you count?
- 4 of the 9 large squares.
  - 5 of the 25 smaller squares.
  - 9 of the 9 large squares.
  - 9 of the 25 smaller squares.
59. (012) The *normal* dilution used for a manual WBC count is
- 1:10.
  - 1:20.
  - 1:50.
  - 1:100.
60. (012) When performing a manual WBC procedure, what squares on a Neubauer hemocytometer are counted?
- 4 of the 9 large squares.
  - 5 of the 25 smaller squares.
  - 9 of the 9 large squares.
  - 9 of the 25 smaller squares.
61. (013) In a subarachnoid hemorrhage the blood in the CSF tube is
- undetectable.
  - evenly mixed in all tubes.
  - light in the first tube and progressively increases.
  - heavy in the first tube and progressively decreases.
62. (013) An advantage of the cytocentrifuge method for processing CSFs is
- it requires special equipment.
  - occasionally there is a reduced cell yield.
  - less-than-optimal preservation of cellular integrity.
  - the slides are air dried so they can be stained with Wright-Giemsa.
63. (013) Semen liquefies about 20 minutes after emission due to the action of
- bacteria.
  - fibrinolysin.
  - motile sperm cells.
  - seminal liquefaciens.
64. (013) Normal sperm specimens show progressive motility of at *least*
- 20 percent.
  - 30 percent.
  - 40 percent.
  - 50 percent.

- 
- 
65. (013) To count spermatozoa accurately, dilute an aliquot of the specimen at
- 1:5.
  - 1:10.
  - 1:20.
  - 1:30.
66. (013) The cellular membrane that surrounds the entire sperm cell is called the
- main piece.
  - plasmalemma.
  - postnuclear cap.
  - mitochondrial sheath.
67. (014) What hemoglobin is a reversible type found in concentrations of 1 to 10 percent in smokers?
- Sulfhemoglobin.
  - Methemoglobin.
  - Carboxyhemoglobin.
  - Carboxysulfemoglobin.
68. (014) An MCHC value below 31 percent indicates
- polychromic RBCs.
  - hypochromic RBCs.
  - hyperchromic RBCs.
  - normochromic RBCs.
69. (015) With an electronic cell counter, nucleated RBCs are likely counted as
- RBCs.
  - WBCs.
  - platelets.
  - malarial parasites.
70. (015) Which one of the following is a distributional plot of RBCs, WBCs, and platelets?
- Dot plot.
  - Cytogram.
  - Histogram.
  - Scattergram.
71. (015) Neutrophils are seen in what area of a DF1 Coulter® scattergram?
- Top right.
  - Top middle.
  - Left middle.
  - Right middle.
72. (016) A nasal eosinophil count is used as a screening procedure for
- viral rhinitis.
  - allergic rhinitis.
  - viral pneumonia.
  - bacterial pneumonia.
73. (016) Which one of the following is the *most* common stain used in hematology and is composed of eosin, methylene blue, and oxidized methylene blue?
- Wright-Giemsa.
  - Periodic acid-Schiff.
  - Chloroacetate esterase.
  - Leukocyte alkaline phosphatase.

74. (016) To estimate the platelet count of the differential slide, the average number of platelets seen under an oil immersion field is multiplied by
- a. 10,000.
  - b. 15,000.
  - c. 20,000.
  - d. 25,000.
75. (016) Which of the following is *not* a factor in the erythrocyte sedimentation rate?
- a. Air circulation.
  - b. Tube slant angle.
  - c. Erythrocyte mass.
  - d. Plasma composition.
76. (016) What test measures the ability of red blood cells to take up fluid without lysis?
- a. Pap smear.
  - b. Osmotic fragility.
  - c. Complete blood count.
  - d. G6PD fluorescent spot.

**Please read the unit menu for unit 4 and continue ➡**

## Unit 4. Coagulation Studies

<b>4-1. The Hemostasis Mechanism .....</b>	<b>4-1</b>
017. Hemostasis mechanisms and process .....	4-1
018. Coagulation factors, platelets, and fibrinolysis components.....	4-5
<b>4-2. Hemostasis Evaluation .....</b>	<b>4-12</b>
019. Specimen collection and coagulation instruments and principles.....	4-13
020. Coagulation procedures .....	4-15
021. Coagulation abnormalities .....	4-18

**I**N UNIT 1 we discussed the importance of blood in maintaining life, its characteristics, and its functions. One of its numerous functions is aiding hemostasis. Hemostasis is derived from the Greek words *haima* meaning blood and *stasis* meaning halt. It has been reported that the study of hemostasis dates to the time of Aristotle and Plato. The hemostatic mechanism has experienced renewed interest in the last few decades. This has been largely due to advances made in medical research laboratories, resulting in more highly purified coagulation proteins. Also, as reagent quality and equipment precision and accuracy have increased, so has the clinical application of coagulation studies. In this unit you'll discover the hemostatic mechanism, process, and its evaluation.

### 4-1. The Hemostasis Mechanism

Bleeding disorders can attribute to death in many conditions, but in order to understand and evaluate these disorders, you must know the basics of hemostasis. Hemostasis is defined as (1) the stoppage of bleeding through physiological properties (vasoconstriction and coagulation) or by surgical procedures, and (2) the interruption of normal blood flow throughout the body. Hemostasis is a very complex mechanism with many reactions occurring at the same time. Therefore, it is difficult to present text in a chronological order. Hopefully the text will be presented in an easy to understand manner.

#### 017. Hemostasis mechanisms and process

There are times when it is critical to stop the flow of blood, but on the other hand, it is dangerous to interrupt normal blood flow. When we speak of hemostasis, we are referring to the maintenance of vascular integrity and control of hemorrhage without compromising the free flow of blood. This requires a fine balance among blood vessel integrity, platelet function, interaction of circulating coagulation factors, and activation of fibrinolysis.

#### Elements of hemostasis

The three elements of hemostasis are extravascular, vascular, and intravascular. Think of each element as a leg on a three-legged stool, if one leg is missing or abnormal, the stool is unbalanced and falls down. The same is true for hemostasis; if one of the three elements is abnormal or altered in any way, the normal hemostatic balance is upset.

#### *Extravascular*

The extravascular element of hemostasis involves the tissues that surround the blood vessels. If a blood vessel is injured, the tissue provides back pressure (through swelling) to stop the bleeding. It also traps or holds the escaping blood. The tissue's ability to help in hemostasis is dependent on the amount, type, and tone of the tissue itself. The surrounding tissue also contains the tissue factor required to start the extrinsic pathway of coagulation.

#### *Vascular*

The vascular element of hemostasis involves the various blood vessels.

### *Arteries*

Blood vessels act as conduits for blood and nutrients throughout the body. Arteries are constructed in three layers. The tunica adventitia is composed of collagen fibers and connective-tissue fibroblast. It is the outer layer of the artery and covers the tunica media. The tunica media is the middle muscular layer of the artery wall, which provides strength to withstand hydrostatic pressures. It is made up of smooth muscle, collagen fibers, and rare fibroblasts. The inner layer, the tunica intima, forms the endothelium that lines the blood vessels and provides an inert, smooth surface over which blood flows without initiating coagulation. It consists of a basement membrane, elastin, collagen fibers, and epithelial cells that deposit von Willebrand's factor (used in normal platelet function) in the subendothelium matrix. If the arteries are injured or altered, substances or constituents are released that can initiate coagulation and fibrinolysis.

### *Veins*

The muscular, elastic tissue that comprises the veins is quite similar to that of the arteries, but it is not quite as developed. The veins' role in hemostasis is largely due to their contractile abilities (vasoconstriction). Vasoconstriction occurs immediately upon vessel injury under the control of the autonomic nervous system and is done by smooth muscle cells in the vessel wall. The benefit of venous constriction is a reduction in blood flow. This, coupled with the fact that platelets quickly occlude injuries, allows veins to adequately handle 70 percent of the body's blood supply.

### *Arterioles and venules*

These smaller vessels have roughly the same makeup and capabilities as the larger organ they modify. Arterioles are much more resistant to injury than venules due to the tunica media in their makeup. The venules have contractile capabilities and become occluded quickly with platelets at the time of injury.

### *Capillaries*

These are the smallest blood vessels that allow only a single erythrocyte or leukocyte to pass through. Capillaries consist of a basement membrane, endothelial cells, and junctions for the passage of oxygen, nutrients, and waste. Due to the minute size of the capillary lumen, injury is normally sealed immediately with little or no dependence on the hemostatic mechanism. Pericytes are cells located beneath the endothelium of the capillaries, veins, and arteries, and may differentiate into vessel-related cells if needed.

### ***Intravascular***

Platelets and biochemicals (coagulation factors, etc.) found in plasma are the elements of intravascular hemostasis. They are involved in coagulation and fibrinolysis and will be discussed in greater detail in the next lesson.

### **Primary hemostasis**

Primary hemostasis involves vascular and platelet responses to the damaged vessel. The injured vessel constricts to decrease the flow of blood; this is known as vasoconstriction and is a result of a nervous system response. Also, the platelets clump and adhere to the vessel to form a platelet plug in order to stop bleeding. This activation of the platelets makes the platelet surface instrumental for coagulation factor activation.

### **Secondary hemostasis**

During secondary hemostasis, coagulation factors and tissue factor interact to form a fibrin clot. The fibrin clot and platelet plug work together to more effectively stop bleeding. At the same time, naturally occurring inhibitors block the activated coagulation factors so that widespread coagulation doesn't occur throughout the body. Only the site of injury is involved in coagulation.



## The hemostasis process

When a blood vessel is injured, tissue factor, plasma proteins or coagulation factors, and calcium interact on the surface of the platelets to form a stable platelet-fibrin clot or thrombi. This process is known as coagulation. As the vessel heals, fibrinolysis occurs. This is the body's way of methodically and gradually dissolving the clot in order to restore normal blood flow.

## Coagulation

It was Paul O. Morawitz (1879–1936) who, in 1905, theorized the clotting cascade that still stands as a model today. The original theory states that prothrombin, in the presence of calcium and thromboplastin, converts to thrombin. Fibrinogen, in the presence of thrombin, converts to a fibrin clot. Of course, this is a skeleton diagram of a rather complex system of reactions that results in hemostasis. We'll now break apart Morawitz's simple statement and scrutinize the actual reaction that takes place. Coagulation is divided into three interrelated systems or pathways: (1) extrinsic, (2) intrinsic, and (3) common. Refer to the illustration below while reading the following information.

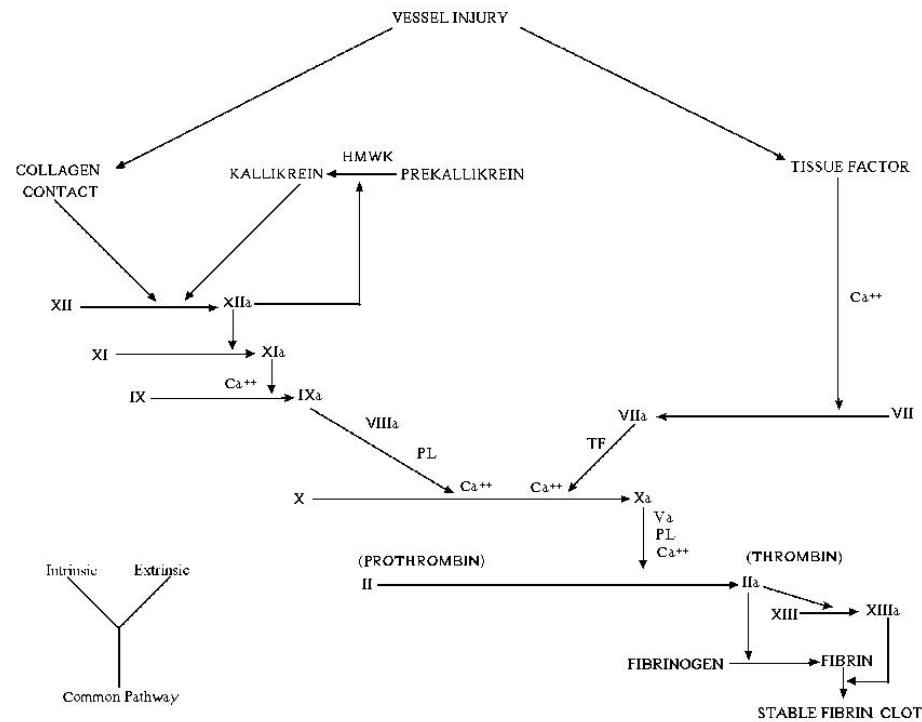


Figure 4-1. Schematic of the coagulation pathways.

### Extrinsic

The extrinsic pathway requires tissue factor (tissue thromboplastin) for activation. Tissue thromboplastin enters the blood from the damaged tissue that surrounds the vessel and activates the other factors present in the plasma. Tissue factor (factor III) activates proconvertin (factor VII) to the serine protease factor VII<sub>a</sub>. The subscript "a" indicates the serine protease or activated state of the factors. Together, factor VII<sub>a</sub>, calcium, and platelet phospholipid (PL) activate Stuart-Prower factor (factor X<sub>a</sub>).

### Intrinsic

The intrinsic pathway is activated when the vessel is injured and the collagen and subendothelial basement membrane are exposed. Both promote coagulation along with the platelets, coagulation

factors, and calcium. The intrinsic pathway begins when the subendothelial basement comes in contact with Hageman factor (factor XII), plasma thromboplastin antecedent (factor XI), high-molecular-weight kininogen (HMWK), and prekallikrein. It should be mentioned that absence of prekallikrein results in a much slower generation of XII<sub>a</sub> and, subsequently, activated factors. Factors XII and XI are activated and XI<sub>a</sub>, with calcium, activates plasma thromboplastin component (factor IX). Factor IX<sub>a</sub>, PL, calcium, and a cofactor (factor VIII<sub>a</sub>) work together to convert factor X to the serine protease factor X<sub>a</sub>.

#### Common

With the activation of Stuart-Prower factor or factor X<sub>a</sub>, the common pathway begins. As shown in the diagram, it can start from the extrinsic or intrinsic pathway. Factor X<sub>a</sub>, calcium, and a cofactor (proaccelerin or factor V<sub>a</sub>) converts prothrombin (factor II) to thrombin (factor II<sub>a</sub>), which converts fibrinogen (factor I) to fibrin—used to form the fibrin clot. Thrombin formation is the critical event in the hemostatic process. It must be present for the ensuing actions to take place. Thrombin splits off two small peptides on each side of the fibrinogen molecule. This produces fibrin monomers and the subsequent assembly of a highly ordered polymeric fibrin clot. The fibrin clot is a cross-weave of fibrin strands formed from fibrin polymers. Thrombin is also a potent stimulus of platelet activation. It activates VIII to VIII<sub>a</sub>, V to V<sub>a</sub>, and amplifies the activation of factors X and II. Fibrin stabilizing factor (factor XIII) stabilizes the fibrin clot.

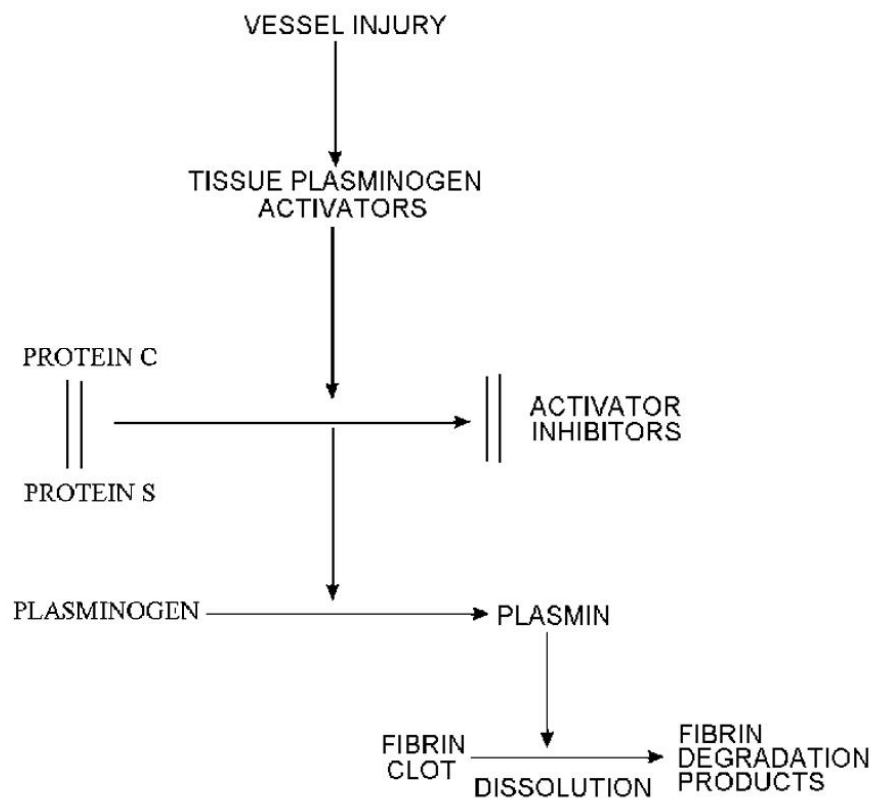


Figure 4-2. Schematic of the fibrinolytic pathway.

#### Fibrinolysis

The fibrinolysis mechanism is also referred to as the fibrinolytic system or pathway. The primary purpose of this pathway is to dissolve fibrin clots as they form in order to keep the circulatory system free of deposited fibrin or fibrin clots. Fibrinolysis begins when plasminogen is converted into

plasmin, which dissolves fibrin or fibrinogen into smaller fragments called fibrinogen degradation products as shown in figure 4-2. These components will also be discussed in greater detail in the next lesson.

### 018. Coagulation factors, platelets, and fibrinolysis components

We'll now take a closer look at the coagulation factors, platelets, and fibrinolysis components of the hemostasis process. First, look at the outline or review of the physiological properties of hemostasis (fig.4-3) seen here:

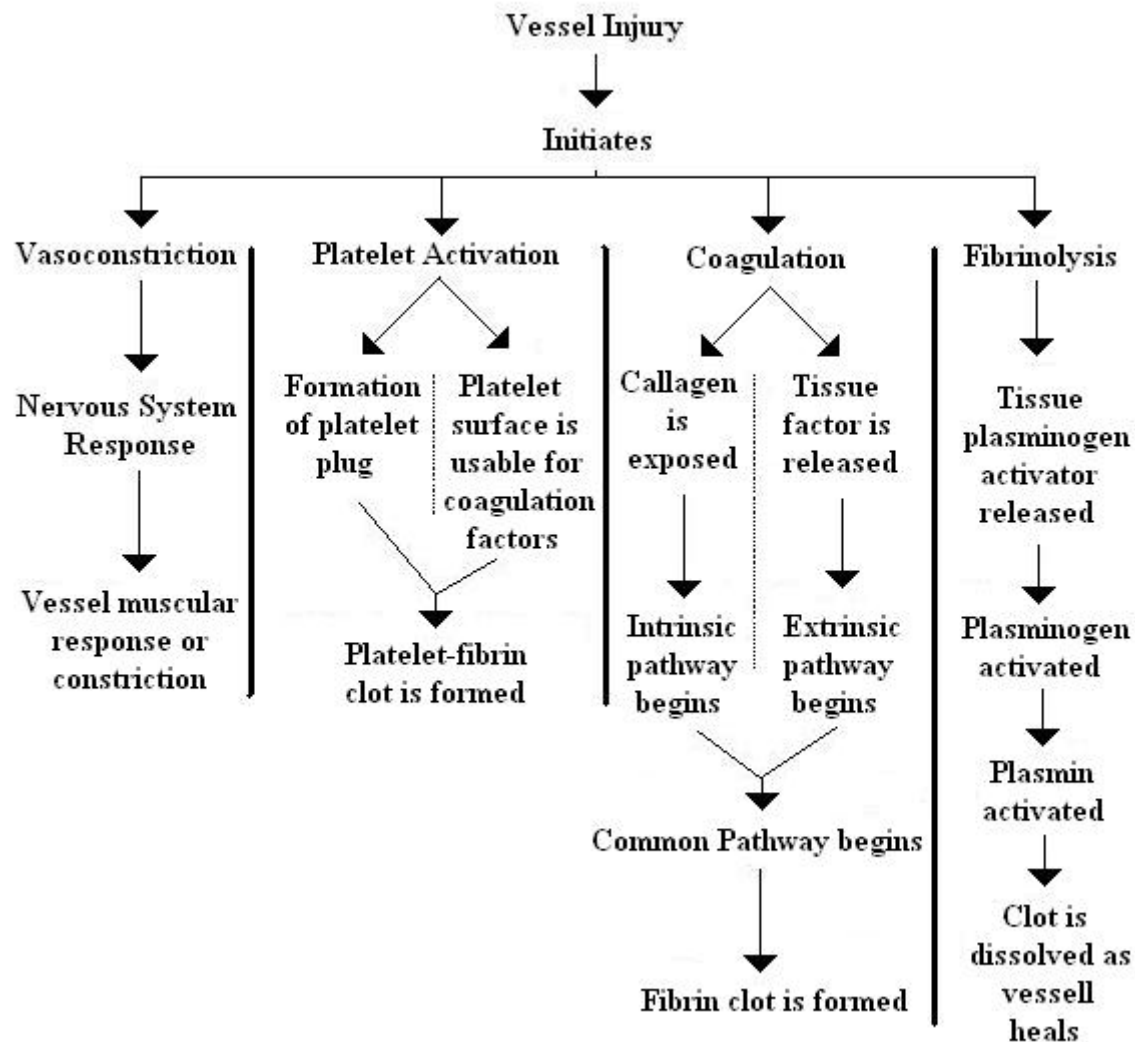


Figure 4-3. Properties of hemostasis.

### Coagulation factors

Coagulation factors can be categorized as substrates, cofactors, or enzymes. A substrate is that in which an enzyme acts upon. Cofactors are proteins that accelerate enzyme activity. An enzyme is a catalyst in a reaction, and the two groups are the serine proteases and transaminases (factor XIII). Coagulation factors are referred to by their Roman numeral and name assigned by the International

Committee on Nomenclature of Blood Coagulation Factors. The following headings will give the factor Roman numeral, the preferred name, and if applicable, synonyms in parentheses.

***Factor I: Fibrinogen***

Fibrinogen is made of three pair of peptide chains ( $\alpha$ -alpha,  $\beta$ -beta, and  $\gamma$ -gamma) and is produced by the liver. It is the main substrate in the blood coagulation system. Fibrinogen has an important role with platelets because they are able to compensate one another. This coagulation protein is *not* vitamin K dependent for production and precipitates from plasma if heated to 56° C. Severe liver disease may moderately lower plasma fibrinogen levels; however, this rarely causes hemorrhage to occur. Fibrinogen is clotted by thrombin and participates in the common pathway of both the extrinsic and intrinsic clotting pathways. Upon the action of proteolytic enzymes, thrombin leaves fibrinogen and yields fibrinogen degradation products. The normal plasma concentration is 200 to 400 mg/dl. A minimum level of 50 to 100 mg/dl is required for normal coagulation.

***Factor II: Prothrombin (prethrombin)***

Prothrombin is the precursor to thrombin and is a  $\alpha$ -2 globulin. It is synthesized in the liver and is vitamin K dependent. It participates in the common pathway of both the extrinsic and intrinsic clotting pathways and the normal plasma concentration is 10 to 15 mg/dl. Prothrombin is so heavily utilized in the clotting mechanisms that very little remains in the serum.

***Factor III: Tissue Factor (tissue thromboplastin)***

This tissue coagulant is a cofactor and is the initial activator of the extrinsic system. Its composition is 50 percent protein and 50 percent phospholipid (lipoprotein). Highest concentrations of tissue thromboplastin are found in the brain, lungs, and placenta.

***Factor IV: Calcium***

This circulating body electrolyte is necessary in numerous conversion steps of the coagulation cascade. It, in its ionized state, is essential for coagulation. For this reason, it is possible to produce anticoagulants that bind calcium and inhibit coagulation.

***Factor V: Proaccelerin (labile factor or accelerator globulin-aCg)***

Factor V increases the action of factor X<sub>a</sub> threefold. It is considered a cofactor that is *not* vitamin K dependent, and participates in the common pathway. It is synthesized in the liver and is extremely thermolabile (deteriorates rapidly at room temperature). Normal plasma concentration of proaccelerin is 0.5 to 1.0 mg/dl.

***Factor VI: Not assigned***

This factor was once thought to be the completion of factor V, but at this time it doesn't exist.

***Factor VII: Proconvertin (stable factor or serum prothrombin conversion accelerator-SPCA)***

Factor VII functions as a serine protease in its active form. It is a very stable compound and remains stable and undiminished in serum stored up to four days at 25 to 37° C. Its normal plasma concentration is 0.2 mg/dl. Proconvertin is synthesized in the liver and is vitamin K dependent. It is involved in the extrinsic pathway.

***Factor VIII:C Antihemophilic Factor-AHF (antihemophilic globulin-AHG or antihemophilic factor A)***

This glycoprotein is the low molecular weight subunit of the factor VIII molecule. The high molecular subunit of the molecule is called von Willebrand's factor. The von Willebrand's factor is synthesized in the endothelium and also acts as a cofactor for platelets. Measurements of this cofactor are obtained by antigenic determinations such as platelet aggregations, immunoelectrophoresis, or chromogenic determinations. Factor VIII has been subdivided into VIII/vWF (which is the entire molecule), VIII:vWF, VIII:C, VIIC:Ag, VIIR:Ag, and VIIR:RCo. These divisions are based on a portion of the molecule, antigenic property, or related antigens that they represent. Factor VIII:C is

*not* vitamin K dependent, is a cofactor, and is thermolabile. Its origin is uncertain at this time. It participates in the intrinsic pathway and plasma concentrations are 1 to 2 mg/dl.

***Factor IX: Plasma Thromboplastin Component-PTC (Christmas factor, antihemophilic factor B, or platelet cofactor 2)***

Factor IX is synthesized in the liver and participates in the intrinsic pathway. This very stable factor is vitamin K dependent and functions as serine protease in active form. Normal plasma concentration of PTC is 0.3 to 0.4 mg/dl.

***Factor X: Stuart-Prower Factor (Stuart factor, Prower factor, or Autoprothrombin III)***

Factor X is synthesized in the liver and is vitamin K dependent. It is a very stable factor and it is also found in serum, which acts as serine protease. Stuart-Prower factor is activated in both the intrinsic and extrinsic pathways and begins the common pathway. Its normal plasma concentration is 0.6 to 0.8 mg/dl.

***Factor XI: Plasma Thromboplastin Antecedent (antihemophilic factor C)***

This is one of the contact factors (i.e., it becomes activated by contact with a glass test tube). Specimens for factor XI activity must be drawn in plastic tubes or siliconized glass. It is produced in the liver and functions as a serine protease. It participates in the intrinsic pathway and is *not* vitamin K dependent. Normal plasma concentration of plasma thromboplastin is 0.4 mg/dl.

***Factor XII: Hageman Factor (glass factor or contact factor)***

Factor XII is a contact factor, and specimens must be handled accordingly. It functions as serine protease and is produced in the liver. It is *not* vitamin K dependent and its normal plasma concentration is 2.9 mg/dl. Factor XII is activated *in vivo* by collagen and other subendothelial tissue, as well as glass, kaolin, celite, and ellagic acid *in vitro*. It is present in both serum and plasma.

***Factor XIII: Fibrin Stabilizing Factor (Laki-Lorand factor, fibrinase, plasmin transglutaminase, or fibrinoligase)***

This factor is produced in the liver; however 50 percent is associated with megakaryocytes. It is not present in serum and is activated by thrombin. It participates in the common pathway. Factor XIII functions as a transglutaminase to stabilize the fibrin clot by forming intermolecular linkages between residues of adjacent fibrin monomers. It is *not* vitamin K dependent and plasma concentration is 2.5 mg/dl.

***Prekallikrein Factor (Fletcher factor)***

This factor functions as serine protease and is produced in the liver. It is present in serum, it is *not* vitamin K dependent, and it participates in the intrinsic pathway. It is also one of the contact factors. Normal plasma concentration of prekallikrein is 5.0 mg/dl. When small amounts of XII<sub>a</sub> become available, prekallikrein is converted to kallikrein.

***High-Molecular-Weight Kininogen-HMWK (Fitzgerald factor, contact activation cofactor, Williams factor, or Flaujeac factor)***

HMWK is produced in the liver, is *not* vitamin K dependent, it functions as a serine protease, and is also a contact factor. HMWK and kallikrein work together to activate more factor XII. It is involved in the intrinsic pathway and is present in the serum. HMWK normal plasma concentration is 4.7 to 12.2 mg/dl.

**Platelets**

We will now take a thorough look at the platelet and view it as much more than a small purple granule on a differential slide. Platelets are critical to proper hemostatic maintenance; they:

- Adhere to damaged vessels.

- Aggregate at the site of injury.
- Promote coagulation on their phospholipid surface.
- Release biochemicals important to hemostasis.
- Induce clot retraction.

### ***Platelet function***

Platelets accomplish their many roles in hemostasis through several functions—adhesion, secretion, and aggregation.

#### ***Adhesion***

When blood vessel injury occurs, the platelets are exposed to endothelium cells, subendothelium membrane collagen fibers, and the basement membrane. They become stimulated and adhere to the exposed collagen fibers and basement membrane of the vessel wall. Platelet adhesion to the collagen fibers requires the plasma protein von Willebrand's factor (VIII:vWF). VIII:vWF act as a link between the platelet receptor and connective tissue of the collagen fibers. Other plasma proteins may also stimulate platelet adhesion. For example, the plasma proteins albumin and fibrinogen can influence platelets to adhere to the artificial surfaces of prosthetic valves or vessel wall grafts.

#### ***Secretion***

Secretion occurs as a reaction to the activation of the platelet. The platelet secretes or releases the contents of its alpha and dense granules. The biological materials collagen, thrombin, epinephrine, thromboxane A<sub>2</sub>, and other arachidonic acid metabolites cause the platelet to release the granular contents. Alpha granules contain coagulation factors, a permeability factor, platelet-specific proteins (platelet factor 4 and  $\beta$ -thromboglobulin), and a cationic protein called platelet-derived growth factor (PDGF). PDGF stimulates smooth-muscle cell growth and proliferation while the platelet is attached to the vessel wall. This aids the healing process. It should be noted that platelets may not be the only source of PDGF. Dense granules contain adenosine, guanosine, diphosphates and triphosphates, calcium, magnesium, and serotonin. The most important substance that the dense granules secrete is nucleotide adenosine diphosphate (ADP). Once released from the platelets, ADP binds to specific receptors and initiates platelet aggregation.

#### ***Aggregation***

Aggregation takes place due to the stimulation of collagen, ADP, epinephrine thrombin, and thromboxane A<sub>2</sub>. Fibrinogen is also needed and is a cofactor in aggregation. Platelet secretion and aggregation happen simultaneously. These biochemicals cause the activated platelets to become “sticky” and adhere to one another.

**NOTE:** Adhesion is platelets sticking to the vessel wall and aggregation is platelets sticking to each other.

### **Fibrinolysis components**

As stated earlier, fibrinolysis is the body's way of dissolving a clot or thrombin.

#### ***Plasminogen***

Plasminogen is a single chain glycoprotein found in plasma at a concentration of 20 to 40 mg/dl. It is produced by the liver, but it is stored and transported by eosinophils. Small amounts of plasminogen are absorbed into the fibrin clot during coagulation. Plasminogen is converted to plasmin by the action of specific enzymes called plasminogen activators. Intrinsic activators include factor XII<sub>a</sub>, kallikrein, and HMWK, all of which are present in the blood. Extrinsic activators are a tissue-type plasminogen activator and a urokinase plasminogen activator, which is produced by the kidney. Urokinase and streptokinase can be administered as therapeutic agents for the activation of plasminogen to plasmin.

### ***Plasmin***

Plasmin is responsible for the slow digestion of fibrin or fibrinogen. It is formed inside the clot and gradually dissolves the clot. It is important that the clot dissolves gradually so that bleeding doesn't reoccur and that there is time for vessel repair. Plasmin is not *normally* found in circulating blood since it is actually activated plasminogen, as discussed previously. Plasmin can't distinguish between fibrinogen and fibrin. Therefore, it sequentially cleaves bonds of both fibrinogen and fibrin, thereby releasing fibrin(*ogen*) degradation products (FDP) or fibrin(*ogen*) split products (FSP). The four principle product fragments are X, Y, D (D-dimer), and E. X and Y are early degradation products and D and E are late degradation products.

### **Coagulation and fibrinolysis controls**

Once a clot is formed and the trauma site is repaired, blood flow must be restored to the area. This system of clotting, healing, and clearing forms one of the body's more important checks and balance systems. However, coagulation and fibrinolysis can be double-edge swords. If the body's coagulation system is "inactive," spontaneous bleeding can occur. If it is "overactive," dangerous spontaneous clots can appear. If the body's fibrinolytic system is "inactive," clots are not dissolved. If it is "overactive," it can destroy available coagulation factors and spontaneous bleeding can occur. Naturally occurring controls or inhibitors are present to keep both systems in check.

### ***Coagulation controls***

Clotting mechanism inhibitors shut down plasma mechanisms to prevent the overproduction of thrombin. Of the greatest importance in this area is antithrombin III.

### ***Antithrombin III***

Antithrombin III is produced in the liver. This is a naturally occurring inhibitor that works on the principle of physiologic inhibition of thrombin and factor X<sub>a</sub>. Furthermore, antithrombin III inhibits activity against factors XII<sub>a</sub>, XI<sub>a</sub>, and IX<sub>a</sub>. It also inhibits kallikrein and plasmin. Heparin enhances the activity of antithrombin III, thus constituting the primary mechanism by which heparin acts as an anticoagulant. Approximately 75 percent of the thrombin formed in the circulation is inhibited by antithrombin III.

### ***Complement component C1***

C1, the first component of complement, has been shown to exhibit inhibitory activity against factors XI<sub>a</sub> and XII<sub>a</sub>, and kallikrein.

### ***Alpha-2 Macroglobulin and Alpha-1 Antitrypsin***

Alpha-2 macroglobulin is a large plasma glycoprotein that inhibits thrombin, but it is slow acting compared to antithrombin III. It forms a complex with thrombin, kallikrein, and plasmin, thus inhibiting their activities. Alpha-1 antitrypsin is an alpha globulin that has demonstrated potent inhibition against factors XI<sub>a</sub>. It may also inactivate plasmin.

### ***Proteins C and S***

Protein C is a vitamin K dependent glycoprotein produced in the liver. Free protein S (also produced in the liver) is a cofactor for protein C. Protein C is a major inhibitor of blood coagulation. It degrades factors V<sub>a</sub> and VIII<sub>a</sub> and stimulates fibrinolysis by inactivating plasminogen activator inhibitors.

### ***In vivo anticoagulants***

Occasionally it may be necessary to intervene with artificial anticoagulants to control overactive coagulation and prevent the formation of clots. There are two therapeutic anticoagulants—heparin and coumarin drugs.

### *Heparin*

Heparin is an intravenous anticoagulant. Upon injection, heparin gives an immediate anticoagulant effect by attacking all activated (serine proteases) coagulation factors. It is a mucopolysaccharide that acts in conjunction with antithrombin III. Therapy with this anticoagulant results in prolonged prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin time. The APTT is used as a monitoring test for therapy. Heparin has a rather short half-life of about four hours. Reptilase counters the effects for testing and establishes a clotting time for such tests as fibrinogen. Protamine sulfate or polybrene normalizes test values.

### *Coumarin drugs*

Coumarin drugs are oral anticoagulants that interfere with the carboxylation of the vitamin K-dependent plasma factors in the liver by interrupting the enzymatic phase of this reaction. Warfarin is the most frequently used coumarin drug. Response is slower than for heparin, responding in about 16 to 24 hours after first administration. Factors II, VII, IX, and X show decreased production. The prothrombin (PT) test is used to monitor this anticoagulant therapy. The international normalized ratio (INR) is used for monitoring patients who are receiving oral anticoagulants. Because PT test results will vary depending on the reagent and method used, the INR was developed to decrease their effects.

### *Lupus-like anticoagulants*

These acquired coagulation inhibitors are seen to occur in five to ten percent of patients with systemic lupus erythematosus, but they can also occur as the result of drug therapy or lymphoproliferative disorders. The lupus-like inhibitors are directed at phospholipid or phosphoprotein components of the coagulant factors. They are not associated with a clinical bleeding tendency unless additional hemostatic abnormalities are present. Prolonged APTTs are to be expected. Prolonged PTs and kaolin clotting times may also be encountered.

### *Fibrinolysis controls*

The fibrinolytic system must be regulated so that unneeded clots are dissolved. Yet, free plasmin must not be able to reach the bloodstream where it can destroy other coagulation factors. If free plasmin is released into the blood from the dissolving clot, antiplasmins within the plasma immediately destroy the plasmin. Naturally occurring antiplasmins are alpha-2 antitrypsin, alpha-2 macroglobulin, alpha-1 antitrypsin, and, to some extent, antithrombin III. Alpha-2 antitrypsin is the prime inactivator of plasmin and is not found in clots.

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## **Self-Test Questions**

**After you complete these questions, you may check your answers at the end of the unit.**

### **017. Hemostasis mechanisms and process**

1. What is hemostasis?
2. What do we mean by balanced hemostasis?
3. What are the three elements of hemostasis?



4. What does the extravascular element of hemostasis involve?
5. What does the vascular element of hemostasis involve?
6. What do the intravascular elements of hemostasis involve?
7. What does primary hemostasis involve?
8. What does secondary hemostasis involve?
9. What does the process of coagulation entail?
10. There are three interrelated systems or pathways in coagulation, what are they?
11. When does the common pathway begin?
12. What is the primary purpose of fibrinolysis mechanism?

**018. Coagulation factors, platelets, and fibrinolysis components**

1. Match the element in column B with the statement in column A. Each element in column B may be used once, more than once, or not at all.

<i>Column A</i>	<i>Column B</i>
___ (1) A contact factor, activated by glass, kaolin, celite, and ellagic acid.	a. Factor I.
___ (2) It is responsible for the slow digestion of fibrin or fibrinogen.	b. Factor II.
___ (3) This circulating body electrolyte is necessary for coagulation.	c. Factor III.
___ (4) This cofactor is the initial activator of the extrinsic system.	d. Factor IV.
___ (5) This factor begins the common pathway.	e. Factor V.
___ (6) A transglutaminase that stabilizes the fibrin clot.	f. Factor VII.
___ (7) Adhesion, secretion, and aggregation are its functions.	g. Factor VIII:C.
___ (8) Main substrate in the blood coagulation system.	h. Factor VIII:vWF.
___ (9) Extremely thermolabile and is considered a cofactor.	i. Factor IX.
___ (10) Very stable and is a serine protease.	j. Factor X.
___ (11) Precursor to thrombin.	k. Factor XI.
___ (12) It is converted to plasmin.	l. Factor XII.
___ (13) A serine protease and a contact factor.	m. Factor XIII.
___ (14) Contact factor and also called antihemophilic factor C.	n. Prekallikrein factor.
___ (15) Antihemophilic factor B.	o. HMWK.
___ (16) Antihemophilic factor A.	p. Platelet.
	q. Plasminogen.
	r. Plasmin.

2. What are the coagulation controls or inhibitors?

3. What are the two therapeutic anticoagulants?

4. What are the naturally occurring antiplasmins?

**4-2. Hemostasis Evaluation**

As you can see from the first section, maintaining a proper hemostatic balance is crucial. Hemostasis evaluation procedures provide meaningful information to the physician. If testing for hemostatic maintenance is to be successful, specimens must be collected and handled properly. This includes not only proper use of anticoagulants and blood drawing techniques, but also the centrifugation of the specimen. David McGlasson, one of the country's leading researchers in coagulation, has been quoted to say, "... of all the telephone calls I receive on difficulties associated with coagulation testing, inadequate centrifugation seems to be one of the most recurring problems." The most dedicated technician cannot produce reliable results from improperly processed specimens.

## 019. Specimen collection and coagulation instruments and principles

Special care must be taken in the collection of blood specimens for coagulation studies. This lesson gives you a basic step-by-step breakdown for the drawing, processing, and storage of these delicate specimens. Check your laboratory's operating instructions for exact procedures.

### Specimen collection

The CLSI preferred anticoagulant for coagulation is 0.109 M (3.2 percent) buffered sodium citrate. Buffered sodium citrate helps maintain the proper pH. Sodium citrate is preferred due to its superior ability to preserve factors V and VIII. The proper ratio for anticoagulation is nine parts blood to one part (9:1) sodium citrate. Insufficient anticoagulant causes clotting, while too much causes prolonged testing results. If the specimen has a hematocrit of greater than 55 percent or less than 20 percent, the amount of sodium citrate must be altered using the following formula. Specimen should be collected in a plastic syringe (glass tubes can activate the contact factors) and then added to the correct amount of sodium citrate.

Volume of anticoagulant =  $0.00185 \times \text{Volume of blood} \times 100 \text{ percent hematocrit}$

For example:

#### **Hct = 66% with a 5 mL draw**

$$0.00185 \times 5 \text{ mL} \times (100 - 66)$$

$$0.00185 \times 5 \text{ mL} \times (34)$$

0.3 of anticoagulant and 4.7 mL of whole blood should be used.

#### **Hct = 16% with a 3 mL draw**

$$0.00185 \times 3 \text{ mL} \times (100 - 16)$$

$$0.00185 \times 3 \text{ mL} \times (84)$$

0.5 of anticoagulant and 2.5 mL of whole blood should be used.

### *Venipuncture technique*

The area should be cleansed with 70 percent isopropanol and dried. To prevent the accumulation of tissue factor, the stick must be atraumatic. Sodium citrate (blue-top) tubes used for coagulation studies should be plastic. However, if glass is used, it must have a siliconized surface. Most vacutainer tubes have a siliconized surface. A two-syringe technique or a vacutainer system can be used. When using the latter, draw the coagulation tube second or after red-top tubes and before any other anticoagulated tubes. If a coagulation test is the only test ordered, first draw a few milliliters in a red-top tube and discard tube in the laboratory. When using a two-syringe technique, part of the blood from the second syringe is used for the blue-top tube. If only one syringe is used, the first milliliter of blood obtained must be discarded. For special studies, especially platelet studies, use the vacutainer system. Also, when performing the venipuncture, release the tourniquet soon after the blood enters the first tube.

### *Specimen processing*

Once collected, the blood specimen must be centrifuged and separated as soon as possible. Ideally, separation should be accomplished within 30 minutes. Check for clots either before or after centrifugation. (If conducted after, remove plasma and check red cell layer.) Most coagulation tests require platelet poor plasma. This can usually be obtained by centrifuging the specimen at  $2,500 \times g$  for at least 15 minutes. These requirements offer sufficient force and time to drop the plasma platelet count below  $15,000/\mu\text{L}$ . If the specimen is for circulating anticoagulants, such as lupus anticoagulant, the centrifugation must be for at least 30 minutes to obtain a platelet count below  $5,000/\mu\text{L}$ . Once

separated, the specimen should be tested within two hours if stored at room temperature, or four hours if stored at 4° C. Maintain specimens for prothrombin time, factor VII assay, and platelet function studies at room temperature. Ideally, plasma for factor assays can be stored for several weeks at -20° C or below. However, freezing must be accomplished rapidly. Specimen thawing should also be done rapidly using a 37° C water bath. It must be mentioned that probably the single most important factor in specimen handling and testing is standardization. Procedures must be adequately researched and tested to assure validity, but when the operating instructions are finalized—*don't vary from the standard procedure*. Adherence to proper procedures and your expertise as a technician will yield a product you can be proud of and a test result that the physician can rely on.

### **Coagulation instruments and principles**

For years it wasn't a matter of which instrument to use, just a decision of how to use the Fibrometer®. Although this laboratory "workhorse" is now considered technologically inferior, when automated instruments break down, it is usually the backup instrument and is still used to confirm lipemic specimens measured by optical analyzers. The two most common principles of coagulation instruments are reviewed below.

#### ***Fibrometer®***

This instrument is semiautomated and requires constant attention. It works on the principle that fibrin strands cause a break in the electric circuit in the sensor probe. Reagents are manually pipetted. Since the instrument doesn't rely on an optical system, hemolysis and lipemia cause no difficulties. The Fibrometer® uses two different probes, which must be changed to fit the analysis: 0.1 mL and 0.2 mL. All in all, the Fibrometer® will probably never be entirely replaced. The simple mechanical timer, replaceable probes, and ease of maintenance still make it the backup instrument preferred by many technologists.

#### ***Photo-optic instruments***

Fibrometers® detect the presence of a fibrin clot by the breakage of an electric current, but, the photo-optic instruments detect clot formation by a photocell sensing circuit that reads change in optical density when clot formation occurs. Light passes through the reaction cup and is measured by a photo detector. When the opaque fibrin clot develops, the light path is broken and the electric timer stops. One obvious drawback to this technology is that lipemia and hemolysis greatly interfere with the testing—a clear specimen is required. However, most analyzers have a floating baseline based on a "specimen blank" from initial readings of lipemic samples.

#### ***Other instruments***

As with any analyzer in the clinical laboratory, there are many manufacturers and even more options. Your laboratory may have a manual, semiautomated, or automated system. Automated systems can be multichannel discrete analyzers with random access that sample closed coagulation tubes. They may also be capable of performing different procedures at the same time. Nonetheless, the ever-growing list of automated instruments contains several common characteristics:

- They require their own specialized reaction cups.
- They usually automatically pipette and dispense reagents (some may automatically pipette specimens).
- They automatically time incubations, and measure/record clotting times through various methods.

These methods include electric circuit sensors, nephelometry for chromogenic procedures, photometry for absorbance or optical density procedures, and immunoassay techniques. Most are computer operated. Your analyzer may perform only a few tests or many tests at one time. The tests that may be included (singly or in combination) are PT, APTT, thrombin time, fibrinogen, factor

assays, reptilase time, antithrombin III, plasminogen, p-nitrophenylphosphate (PNP) heparin dilution curve, heparin, protein C, alpha-2 antiplasmin, and D-dimer.

### ***Platelet instruments***

Various instruments may be used to measure platelet function. Only two are mentioned in the following text.

#### ***Platelet aggregometry***

A spectrophotometer is employed to measure the change in optical density of platelet rich plasma. As platelet rich plasma is stirred at 37° C, aggregating reagents are added. There are many reagents that can be used—ADP, epinephrine, collagen, thrombin, ristocetin, serotonin, or arachidonic acid. Results are gained from curve formation based on the increase of density of the solution as platelets aggregate and the type of reagent used.

#### ***Lumi-aggregometer***

This technique is used for ATP measurement and uses two luminescent reagents—firefly luminescent substrata and luciferin enzyme (luciferase). As platelets aggregate, the increased luminescence, due to tagged platelets, is measured.

## **020. Coagulation procedures**

In 1913 the Lee-White whole blood coagulation time was the first laboratory test used to evaluate the bloods' clotting mechanism. This lesson will familiarize you with current technologies on coagulation testing, but is not in any way a complete synopsis of methodologies. Also, to enumerate every abnormality of vascular clotting would be both lengthy and of little real use. Therefore, we'll look briefly at the more common abnormalities. For a complete list of procedures or abnormalities, see the bibliography or check other civilian references on hemostasis.

### **Coagulation procedures**

The normal values used in this lesson are for reference only. Each individual laboratory must assign its own normal or reference range for each instrument and/or procedure. Just a brief procedure principle is discussed; see your laboratory's operating instructions for specific information.

#### ***One-stage prothrombin time (PT) test***

In this procedure, tissue thromboplastin and calcium are added to patient plasma to initiate clot formation by activating factor VII, which in turn proceeds through the common pathway. This test is a screening procedure for the extrinsic and common pathways and for coumarin drug therapies. The primary factors measured are I, II, V, VII, and X. After mixing, the normal plasma clotting time is 10 to 12 seconds for photo-optical and 12 to 14 for manual (clot sensing) methods.

#### ***International normalized ratio (INR)***

The INR is used for monitoring patients who are receiving oral anticoagulants. Because PT results will vary depending on the reagent and method used, the INR was developed to decrease their effects. The INR is a ratio of the patient's prothrombin time to that of the normal control. An international reference thromboplastin reagent has been developed for use in standardizing the prothrombin time ratio.

#### ***World Health Organization (WHO)***

Manufacturers of thromboplastin calibrate their reagent against the standard thromboplastin reagent produced by the WHO. The results of the calibration are used to develop the International Sensitivity Index (ISI) for each lot number of reagent. The ISI is dependent on the instrument; therefore, each laboratory needs to be sure to identify its instrument when requesting the ISI from the thromboplastin manufacturer. The ISI is published by the manufacturer in the product insert. In order to determine the INR, the PT ratio and the ISI are needed. The PT ratio is obtained by dividing the patient's PT

value by the mean of the in-house determined normal PT range. The patient ratio is to the power of the ISI value. This formula or a precalculated table can be used for obtaining results—

$$\text{Formula: INR} = (\text{Patient PT/Normal PT})^{\text{ISI}}$$

#### ***Activated partial thromboplastin time (APTT) test***

The APTT is a screening test for all factors in the intrinsic pathway (except XIII and platelets) and for monitoring heparin therapy. This test involves the recalcification of plasma in the presence of a standardized amount of a platelet-like reagent and plasma activator. Factors XI and XII are fully activated by the addition of kaolin, ellagic acid, celite, and ground glass. Cephalin is then added to permit optimal phospholipid concentration. Calcium chloride is added last to allow the formation of a clot. Normal clotting time is 25 to 35 seconds. This procedure is performed in conjunction with the PT test.

#### ***Thrombin time test***

The principle of this test measures the rate of transformation of plasma fibrinogen to fibrin when a standard thrombin solution is allowed to clot plasma. The procedure involves the addition of excess thrombin to a standardized solution and, at the same time, to patient plasma. The clotting times are recorded and compared. Patient clotting time should be around 10 to 14 seconds. This is an excellent screen for heparin contaminated specimens. Results outside these parameters can be the result of hypofibrinogenemia (90 mg/dl or less), heparin or heparin-like anticoagulants, elevated FDP, or the presence of an abnormal fibrinogen monomer.

#### ***Fibrinogen assay***

Fibrinogen in plasma is the precursor to fibrin, which is converted by the addition of thrombin. This assay can be performed by clotting, colorimetric, or radial diffusion technique. The normal fibrinogen level is 200 to 400 mg/dl.

##### ***Clotting method***

A standard amount of thrombin is added to known amounts of fibrinogen dilution to prepare a standard curve. Dilutions of patient plasma are then run, and their clotting times are recorded. The results are calculated by comparing patient plasma to the standard curve.

##### ***Colorimetric method***

The biuret method of measuring fibrinogen is another technique. Protein is precipitated out by ammonium sulfate, and the biuret reagent reacts colorimetrically, allowing the test to be read, employing Beer's law, "an empirical relationship that relates the absorption of light to the properties of the material through which the light is traveling", against a known standard.

##### ***Radial immunodiffusion***

Plasma is added to a gel (agarose) impregnated with a fibrinogen standard antisera. After incubation, the resulting zone of diffusion is measured against a standard.

#### **Tests for fibrinogen degradation products**

When fibrinolysis is present, an increase in breakdown products of fibrinogen and fibrin are seen. These are just a few of the available tests for FDPs.

#### ***Staphylococcal clumping test***

A coagulase negative strain of *S. aureus* is used in this procedure. These cells are coated with bound coagulase, causing the visible agglutination when mixed with plasma containing FDPs.

#### ***Latex agglutination***

This is a quick, easy procedure using latex particles coated with D and E fragments of FDPs. When these coated particles are mixed with plasma containing FDPs, visible agglutination is noted.

***Protamine sulfate (by paracoagulation)***

When protamine sulfate is added to plasma, soluble complexes of fibrin monomers, or fragment X attached to FDPs, are split. The fibrin monomers or fragment X then form a gelatin suspension of fibrin strands.

***D-dimer***

During coagulation, thrombin converts fibrinogen to fibrin and activates factor XIII. Thrombin also activates the fibrinolysis system. Plasminogen activates plasmin and plasmin dissolves the clot. Factor XIII<sub>a</sub> is a crosslinked fibrin. D-dimer is the fibrin degradation product of this factor. As stated earlier, plasmin doesn't distinguish between fibrin and fibrinogen. However, fibrinogen doesn't produce the crosslinked D-dimer portion. The presence of D-dimer suggests that a stable fibrin clot has been lysed. D-dimer will be found in pulmonary embolism, deep vein thrombosis, DIC with secondary fibrinolysis, arterial thromboembolism, and sickle cell anemia. D-dimer can be performed on automated instruments or by procedures using latex coated particles with monoclonal antibodies to D-dimer.

**Factor assays**

This lesson makes no attempt to teach you every procedure for performing factor assay. However, it does familiarize you with those techniques and covers the generalities of the methods.

***Factor assay: II, V, VII, and X***

The principle of these assays is that factor activity can be measured by determining the degree of correction obtained when test plasma is mixed with the appropriate factor deficient plasma. Platelet-poor patient plasma is serially diluted, using a veronal buffer. The appropriate factor deficient plasma is added to the dilutions, and PT tests are performed on the resulting specimens. A curve is plotted based on a 1:10 dilution of standardized plasma being regarded as 100 percent. The percentage of the factor is obtained by locating the point on the straight line graph where the 1:10 dilution intercepts the normal curve. Results are reported in percent of plasma concentration.

***Factor assay: VIII, IX, XI, XII, Fletcher, or Fitzgerald***

The principal and dilutions are the same as previously mentioned. For this assay, APTT tests are performed instead of PT tests. Be certain to use plastic or nonwetable equipment if assaying for the contact factors, XI and XII. Results are the same as for the previous factors.

**Coagulation and fibrinolysis inhibitors**

Coagulation testing abnormalities may be caused by either a factor deficiency or a problem with coagulation or fibrinolysis inhibitors, regulatory proteins, or lysis products. These tests are performed on fully automated analyzers, as manual procedures, or shipped out to a civilian laboratory.

**Testing platelet function**

There are many tests for evaluating platelet function. We'll cover a few of the ones that are currently in use.

***Ivy bleeding time***

The Ivy bleeding time is a screening test for abnormalities of the primary hemostatic mechanism, particularly disorders of platelet function and may be used for correlation with thrombocytopenia. This test detects vascular defects of the blood, von Willebrand's disease, and drug-induced defects of the clotting mechanism. The Ivy method involves placing a blood pressure cuff on the patient's arm and inflating the cuff to 40 mmHg. With this pressure sustained, cleanse the forearm with 70 percent isopropanol, make a uniform cut 2 to 4 mm in depth, and touch the drop of blood every 30 seconds with a piece of filter paper. Be certain to gently cleanse the area to avoid trauma and misleading aggregation of platelets. The cut must be made the same size every time the procedure is performed.

Never touch the wound with the filter paper—only the drop of blood. Normal time for cessation of bleeding is two to nine minutes.

***Platelet adhesion (glass bead column)***

This test is difficult to standardize and, therefore, not commonly used; however, it is an easily performed procedure and bears mention. A platelet count is performed on whole blood; then the blood is run through a glass column containing glass beads. A platelet count is performed on the resulting blood. Aspirin damaged platelets or von Willebrand's disease fail to adhere to the glass beads.

***Tourniquet test or capillary fragility test***

This test is a rudimentary screen for capillary fragility. It is mentioned in the text at this point because it can also be useful in assaying platelet defects, vascular purpura, vitamin C deficiency (Scurvy), and decreased fibrinogen levels. A blood pressure cuff is placed on the patient's arm and inflated to a point between the systolic and diastolic pulse points. This allows for arterial pressure to enter the arm without venous release of the pressure. The cuff is left in place for five minutes. After removing the cuff, wait one to two minutes and count the petechiae present on the patient's arm. Normals are rather vague and are usually left up to the discretion of the attending physician.

***Platelet count***

This lesson cannot be considered complete without addressing the platelet count. The platelet count plays a major role in the hemostasis mechanism. Platelet counts ranging from  $20$  to  $60 \times 10^9/L$  are associated with minor bruising, excessive menstrual bleeding, and postoperative or post-traumatic bleeding. Platelet counts below  $20 \times 10^9/L$  are associated with spontaneous bleeding into skin and mucous membranes. See unit 3 for platelet count procedures and reference ranges.

**021. Coagulation abnormalities**

This clotting scheme points out the fact that bleeding can be produced by weak links in the hemostatic mechanism. The following table shows a general list of defects that can contribute to a defect in the clotting of whole blood:

Defect	Result
Abnormal plasma proteins	These proteins are seen in the hemophilias, von Willebrand's disease, or systemic lupus erythematosus.
Qualitative or quantitative platelet disorders	Such disorders are associated with von Willebrand's disease, Bernard Soulier, idiopathic thrombocytopenia (ITP), Glanzmann's thrombasthenia, or one of several defects in the granules of the platelets..
Vascular defects and connective tissue disorders	Disorders of this kind are exemplified by the May-Hegglin anomaly or the Ehlers-Ehlers-Danlos syndrome. They may be hereditary or acquired.



## Terms

The following are terms and their definitions associated with coagulation abnormalities:

Term	Definition
<b><i>Hypocoagulation</i></b>	Hypocoagulation or abnormal bleeding is associated with several conditions that can be inherited or acquired. Inherited conditions are hemophilia, von Willebrand's, and factor deficiencies. Acquired conditions include liver and kidney diseases, vitamin K deficiency, massive transfusions, and DIC.
<b><i>Hypercoagulation</i></b>	Hypercoagulation or thrombosis is associated with the production of thrombi that block the normal flow of blood. It is usually a defect in the fibrinolysis system. The thrombus is a mixture of WBCs, RBCs, and platelets held together by fibrin. They are painful and can cause an assortment of symptoms. If they block the blood supply to vital organs, they can be life-threatening. Hypercoagulation can be stimulated by malignancies or surgical procedures.
<b><i>Purpura</i></b>	Purpura are areas of discoloration produced by hemorrhage of blood into small areas of the skin, mucous membranes, and other tissues. The areas are red at first, then turn purple, and finally brownish yellow. <i>Ecchymosis</i> is a form of purpura that affects the larger areas of the skin and mucous membranes. These areas are black at first and then turn blue, and finally greenish brown or yellow.
<b><i>Petechiae</i></b>	Petechiae are pinpoint, purplish red spots in the skin caused by intradermal hemorrhage. The hemorrhage is due to poor capillary integrity and the inability of the capillaries to withstand normal blood pressure & trauma.

## Most common abnormalities

Let's look briefly at the most common coagulation abnormalities.

### *Hemophilia*

Hemophilia was the first recognized bleeding disorder and was described in the 12<sup>th</sup> century following the death of two male children due to excessive bleeding after circumcision. At that time it was recommended that if a child had died because of excessive bleeding after circumcision that subsequent male children born to the same mother should not be circumcised.

#### *Hemophilia A*

Hemophilia A is an inherited, sex-linked disorder carried on the X chromosome, transmitted by the female, and manifested by the male offspring. Approximately one-third of all new cases reported have no previous family history of the disease. This is attributable to either generations of silent carriers or recent mutations of the genes. Test results will show greatly decreased factor VIII, normal PT and Ivy bleeding time, prolonged APTT, and normal platelet function.

#### *Hemophilia B*

Hemophilia B, or Christmas disease, resembles hemophilia A in every way, except that the deficiency is in factor IX. This is the rarer form of the disease whose inhibitors are immediate acting as opposed to the time-dependent ones of hemophilia A. IX-deficiencies are seen in hemophilia B, also called Christmas disease (named after the family in which it was first discovered), vitamin K deficiencies, and liver disease.

### *von Willebrand's disease*

This disease is autosomal dominant and not sex-linked. Type I, Classic von Willebrand's, demonstrates prolonged bleeding times, decreased factor VIII, and decreased ristocetin cofactor assay. These patients are treated with factor rich cryoprecipitate or fresh frozen plasma.

***Glanzmann's thrombasthenia***

This autosomal recessive disease is due to intermarriage and, therefore, offspring are affected. Bleeding time is prolonged, platelet aggregation is abnormal, PT and APTT are normal, factor assays are normal, and platelets are normal to slightly decreased.

***Bernard-Soulier***

The clinical effects are prolonged bleeding times, decreased ristocetin aggregation, and ristocetin cofactor that does not correct with administration of cryoprecipitate. Thrombin aggregation may be affected, and thrombocytopenia will be evident. One outstanding characteristic of this condition is the presence of giant platelets, 18 to 20  $\mu\text{m}$  in diameter. These cells are nonfunctional; therefore, the patient exhibits bleeding associated with low platelet counts. Easy bruising, bleeding from teeth and gums, and prolonged menstrual periods are a few of the clinical symptoms of this condition.

***Disseminated intravascular coagulation (DIC)***

DIC is a complication of other primary disorders where coagulation and fibrinolysis occur at the same time. However, one process is usually dominant at a given time. If the coagulation process is dominant and secondary fibrinolysis is minimal, then DIC may be seen as diffuse thromboses. If the coagulation process is minimal and the secondary fibrinolysis is dominant, then the clinical picture is hemorrhage. DIC is clinically different from person-to-person, but what is common is the release of thromboplastic substances that accelerate clot formation. Once the coagulation process is activated, so is the fibrinolysis process. In an earlier lesson, you learned that vessel injury initiates these processes. Vessel and tissue (cellular) injury can be caused by bacterial toxins, shock, acidosis, antigen-antibody reactions, pregnancy complications and deliveries, overwhelming infections, and malignancies. Nonetheless, massive tissue trauma (burns, heat stroke, surgery, or crush injuries) is the most common cause of DIC. Also, thromboplastic substances can be injected directly in to the bloodstream through insect or snake bites. Acute DIC develops within a few hours with hemorrhage as the most common occurrence resulting in an estimated 60 percent mortality. Chronic DIC is usually seen in patients who have already exhibited thrombosis disorders. Hemorrhage is less frequent in chronic DIC. Bleeding and clotting times are increased, platelet counts are lowered, and clotting studies in general are all abnormal. The following tests are used for detection: fibrinogen, thrombin time, FDP, D-dimer, PT and APTT, platelet count, plasminogen, alpha-2 antitrypsin, and euglobulin lysis time.

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**Self-Test Questions**

After you complete these questions, you may check your answers at the end of the unit.

**019. Specimen collection and coagulation instruments and principles**

1. What is the proper ratio of blood to anticoagulant for coagulation studies?
2. Why should the venipuncture be atraumatic?
3. Ideally, how soon after collection should a blood specimen be centrifuged and separated?
4. Once separated, how soon should testing be done?

5. What principle does the Fibrometer® utilize?
6. What is the photo-optic principle used by some coagulation instruments?

**020. Coagulation procedures**

1. What are the primary factors measured by the PT test and what therapy is monitored by this procedure?
2. What is the INR?
3. Why was the international reference thromboplastin reagent developed?
4. The APTT test screens what factors and anticoagulant therapy?
5. What is the principle of the thrombin time test?
6. What tests are used to measure FDPs?
7. What test is specific for crosslinked portions of fibrin?
8. What conditions will the D-dimer test be positive for?
9. What are a few of the tests used to evaluate platelet function?

**021. Coagulation abnormalities**

1. What is hypocoagulation and what conditions is it associated with?
2. What is hypercoagulation and what can it be stimulated by?

3. What are petechiae?
4. What is hemophilia A?
5. What is DIC?
6. In relation to DIC, what happens if the coagulation process is dominant and the secondary process is minimal?
7. In relation to DIC, What happens if the secondary fibrinolysis is dominant?
8. What can cause vessel or tissue damage and cause DIC?
9. What tests are used for the detection of DIC?

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### **Answers to Self-Test Questions**

#### **017**

1. The maintenance of vascular integrity and control of hemorrhage without compromising the free flow of blood.
2. The balance among blood vessel integrity, platelet function, interaction of circulating coagulation factors, and activation of fibrinolysis.
3. Extravascular, vascular, and intravascular.
4. The tissues that surround the blood vessels.
5. The various blood vessels.
6. Platelets and biochemicals (coagulation factors, etc.) found in plasma.
7. Vascular and platelet responses to a damaged vessel.
8. Coagulation factors and tissue factor interact to form a fibrin clot.
9. When a blood vessel is injured, tissue factor, plasma proteins or coagulation factors, and calcium interact on the surface of the platelets to form a stable platelet-fibrin clot or thrombi.
10. Extrinsic, intrinsic, and common pathways.
11. With the activation of Stuart-Prower factor or factor X<sub>a</sub>.
12. Dissolve fibrin clots as they form in order to keep the circulatory system free of deposited fibrin or fibrin clots.

**018**

1. (1) l.  
(2) s.  
(3) d.  
(4) c.  
(5) j.  
(6) m.  
(7) q.  
(8) a.  
(9) e.  
(10) f.  
(11) b.  
(12) r.  
(13) p.  
(14) k.  
(15) i.  
(16) g.
2. Antithrombin III, C1 (the first component of complement), alpha-2 macroglobulin, alpha-1 antitrypsin, and protein C and S.
3. Heparin and coumarin drugs.
4. Alpha-2 antitrypsin, alpha-2 macroglobulin, alpha-1 antitrypsin, and, to some extent, antithrombin III.

**019**

1. The proper ratio for anticoagulation is 9 parts blood to 1 part sodium citrate.
2. To prevent the accumulation of tissue factor.
3. Within 30 minutes.
4. Within 2 hours if stored at room temperature or four hours if stored at 4° C.
5. It works on the principle that fibrin strands cause a break in the electric circuit in the sensor probe.
6. The photo-optic instruments detect clot formation by a photocell sensing circuit that reads change in optical density when clot formation occurs.

**020**

1. The primary factors measured are I, II, V, VII, and X; coumarin drugs.
2. The INR is a ratio of the patients prothrombin time to that of the normal control.
3. Used in standardizing the prothrombin time ratio.
4. Screening test for all factors in the intrinsic pathway (except XIII and platelets) and for monitoring heparin therapy.
5. The principle of this test measures the rate of transformation of plasma fibrinogen to fibrin when a standard thrombin solution is allowed to clot plasma.
6. Staphylococcal clumping test, latex agglutination, protamine sulfate (by paracoagulation), and D-dimer.
7. D-dimer.
8. D-dimer will be found in pulmonary embolism, deep vein thrombosis, DIC with secondary fibrinolysis, arterial thromboembolism, and sickle cell anemia.
9. Ivy bleeding time, platelet adhesion (glass bead column), and tourniquet test or capillary fragility test.

**021**

1. Abnormal bleeding; inherited conditions are hemophilia, von Willebrand's, and factor deficiencies; acquired conditions include liver and kidney diseases, vitamin K deficiency, massive transfusions, and DIC.
2. Thrombosis; malignancies or surgical procedures.

3. Pinpoint, purplish red spots in the skin caused by intradermal hemorrhage.
4. It is an inherited, sex-linked disorder carried on the X chromosome, transmitted by the female, and manifested by the male offspring.
5. DIC is a complication of other primary disorders where coagulation and fibrinolysis occur at the same time.
6. The DIC may be seen as diffuse thromboses.
7. The clinical picture is hemorrhage.
8. Vessel and tissue (cellular) injury can be caused by bacterial toxins, shock, acidosis, antigen-antibody reactions, pregnancy complications and deliveries, overwhelming infections, and malignancies. Nonetheless, massive tissue trauma (burns, heat stroke, surgery, or crush injuries) is the most common cause of DIC. Also, thromboplastic substances can be injected directly in to the blood stream through insect or snake bites.
9. Fibrinogen, thrombin time, FDP, PT and APTT, platelet count, plasminogen, alpha-2 antitrypsin, and euglobulin lysis time.

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### Unit Review Exercises

**Note to Student:** Consider all choices carefully, select the *best* answer to each question, and *circle* the corresponding letter. When you have completed all unit review exercises, transfer your answers to ECI Form 34, Field Scoring Answer Sheet.

**Do not return your answer sheet to ECI.**

77. (017) What system or pathway begins with the activation of factor X?
  - a. Intrinsic.
  - b. Extrinsic.
  - c. Common.
  - d. Fibrinolysis.
78. (017) What is the critical event in the hemostatic process?
  - a. Vasoconstriction.
  - b. Platelet response.
  - c. Thrombin formation.
  - d. Thromboplastin activation.
79. (018) What factor is the *main* substrate in the blood coagulation system?
  - a. I.
  - b. II.
  - c. III.
  - d. IV.
80. (018) Platelets, when exposed to collagen fibers, stick to the blood vessel. This process is called
  - a. release.
  - b. adhesion.
  - c. secretion.
  - d. aggregation.
81. (018) What component is responsible for the slow digestion of fibrin and fibrinogen?
  - a. Plasmin.
  - b. Plasminogen.
  - c. Prekallikrein.
  - d. Antithrombin III.

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82. (019) The Fibrometer® utilizes what principle?
- a. Immunoassay.
  - b. Photocell sensing circuit.
  - c. Chromogenic nephelometry.
  - d. Electric circuit in a sensor probe.
83. (020) What screening procedure is used for the extrinsic and common pathways and for coumarin drug therapies?
- a. PT test.
  - b. APTT test.
  - c. Factor assays.
  - d. Thrombin time.
84. (020) Which of the following was developed to monitor PTs on patients who are receiving oral anticoagulants?
- a. RDW.
  - b. PDW.
  - c. INR.
  - d. RPI.
85. (020) The APTT is a screening test for *all* factors in the intrinsic pathway *except*
- a. IX and X.
  - b. X and XIII.
  - c. IX and platelets.
  - d. XIII and platelets.
86. (020) What procedure for fibrinogen degradation products uses particles coated with D and E fragments of FDPs?
- a. Protamine sulfate.
  - b. Latex agglutination.
  - c. Radial immunodiffusion.
  - d. Staphylococcal clumping test.
87. (020) What platelet function test involves a uniform 2 to 4 mm cut on the patient's forearm?
- a. Ivy bleeding.
  - b. Duke's bleeding.
  - c. Forman's clotting.
  - d. Lee-White clotting.
88. (021) What term is associated with abnormal bleeding?
- a. Purpura.
  - b. Petechiae.
  - c. Hypocoagulation.
  - d. Hypercoagulation.
89. (021) What term is associated with pinpoint, purplish red spots in the skin caused by intradermal hemorrhage?
- a. Purpura.
  - b. Petechiae.
  - c. Hypocoagulation.
  - d. Hypercoagulation.

90. (021) A sex-linked coagulation disorder, carried on the X chromosome, transmitted by the female, and manifested by the male offspring is called
- a. Hemophilia A.
  - b. Bernard-Soulier.
  - c. von Willebrand's disease.
  - d. Glanzmann's thromboasthenia.

When you complete this course, please complete the student survey on the Internet at this URL:  
<http://www.maxwell.af.mil/au/afiadl/>. Click on Student Info and choose 9502 Survey.



## Glossary

### Terms

**acanthocyte**—An abnormal RBC having spiny projections, associated with low-density beta lipoproteins.

**acrosome**—The anterior end of the sperm head.

**aggregometer**—An instrument used to measure the rate of platelet aggregation.

**anisocytosis**—Variance in erythrocytic size.

**aplastic**—A deficient development or failure to develop.

**chemotaxis**—The attraction or repulsion of living protoplasm, as in leukocytes attraction to a site of infection.

**coagulant**—A substance that causes a fluid to clot or coagulate.

**coitus interruptus**—The removal of the penis from the vagina prior to the emission of semen.

**cristae**—An inward projection or fold of the inner membrane of a mitochondrion

**cytology**—The science that deals with the function and structure of body cells.

**dacryocyte**—A teardrop shaped erythrocyte.

**defibrinate**—To remove fibrin from plasma.

**discocyte**—A normally shaped erythrocyte.

**drepanocyte**—A sickle-shaped erythrocyte.

**hyperplasia**—Excessive formation of tissue.

**idiopathic**—Without known cause.

**impedance**—Resistance due to self induction; that resistance met by electricity in an alternating current.

**infarct**—Tissue that has died due to loss of blood flow.

**lymphoma**—Tumor of the lymphoid tissue.

**metaplasia**—The production of one type of tissue from another.

**monoclonal**—Cells derived from a single cell or clone.

**mucosa**—A mucous membrane.

**neoplasm**—A new tissue growth that is out of control.

**petechiae**—Small, pinpoint hemorrhages.

**prosthesis**—The substitution of a body part by an artificial replacement.

**purpura**—A group of disorders characterized by skin discoloration due to hemorrhages under the skin.

**pyogenic**—Pus producing.

**pyretic**—Having to do with fever.

**reptilase**—An enzyme produced from Russell's viper venom used in determining blood clotting time.

**serine**—A naturally occurring amino acid.

**serotonin**—A vasoconstrictor found in numerous animals.

**thromboxane**—An intermediate in the metabolic pathway to arachidonic acid.

**vasectomy**—The surgical removal of the ductus (vas) deferens.

## Abbreviations and Acronyms

<b>ACTH</b>	adrenocorticotrophic hormone
<b>ADP</b>	adenosine diphosphate
<b>ALL</b>	acute lymphoblastic leukemias
<b>ANLL</b>	acute nonlymphocytic leukemias
<b>APTT</b>	activated partial thromboplastin time
<b>ASCP</b>	American Society of Clinical Pathologists
<b>ATP</b>	adenosine triphosphate
<b>CAP</b>	College of American Pathologists
<b>CBC</b>	complete blood count
<b>CDC</b>	career development course; Center for Disease Control and Prevention
<b>CFU-BL</b>	colony-forming unit-B lymphocyte
<b>CFU-BT</b>	colony-forming unit-T lymphocyte
<b>CFU-C</b>	Colony-forming unit—culture
<b>CFU-E</b>	colony-forming unit—erythrocyte
<b>CFU-GM</b>	colony-forming unit—granulocyte-macrophage
<b>CFU-L</b>	colony-forming unit—leukaemic
<b>CFU-Meg</b>	colony-forming unit—megakaryocyte
<b>CFU-S</b>	colony-forming unit—spleen
<b>CLIA</b>	Clinical Laboratory Improvement Act
<b>CLL</b>	chronic lymphocytic leukemia
<b>CLSI</b>	Clinical and Laboratory Standards Institute (formerly NCCLS)
<b>CML</b>	chronic myelogenous leukemia
<b>CMV</b>	cytomegalovirus infections

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<b>CNS</b>	central nervous system
<b>CO<sub>2</sub></b>	carbon dioxide
<b>CPD</b>	central processing distribution
<b>CRC</b>	corrected reticulocyte count
<b>CSF</b>	cerebrospinal fluid; colony stimulating factor
<b>CSF-Meg</b>	colony stimulating factor-megakaryocyte
<b>CV</b>	coefficient of variation
<b>DAT</b>	direct antiglobulin test
<b>DIC</b>	disseminated intravascular coagulation
<b>DNA</b>	deoxyribonucleic acid
<b>EDTA</b>	ethylenediaminetetraacetic acid
<b>ER</b>	endoplasmic reticulum
<b>ESR</b>	erythrocyte sedimentation rate
<b>FAB</b>	France, America and Britain
<b>FDP</b>	fibrin( <i>ogen</i> ) degradation products
<b>FSP</b>	fibrin( <i>ogen</i> ) split products
<b>G6PD</b>	glucose-6-phosphate dehydrogenase deficiency
<b>GI</b>	gastrointestinal
<b>HCL</b>	hairy cell leukemia
<b>HD</b>	hodgkin's disease
<b>HDN</b>	hemolytic disease of the newborn
<b>Hg</b>	mercury
<b>HiCN</b>	cyanmethemoglobin
<b>HIV</b>	human immunodeficiency virus
<b>HMWK</b>	high-molecular-weight kininogen
<b>ICSH</b>	International Committee for Standardization in Hematology
<b>IM</b>	infectious mononucleosis

<b>INR</b>	international normalized ratio
<b>ISI</b>	International Sensitivity Index
<b>JCAHO</b>	Joint Commission on Accreditation of Healthcare Organizations
<b>LED</b>	light-emitting diode
<b>MCH</b>	mean corpuscular hemoglobin
<b>MCHC</b>	mean corpuscular hemoglobin concentration
<b>MCV</b>	mean corpuscular volume
<b>MPS</b>	mononuclear phagocytic system [ <b>NOTE:</b> this term replaced reticuloendothelial system (RES)]
<b>MPV</b>	mean platelet volume
<b>NBS</b>	National Bureau of Standards
<b>NHL</b>	non-hodgkin's lymphoma
<b>N/C</b>	nuclear to cytoplasmic
<b>OI</b>	operating instructions
<b>PCV</b>	packed cell volume
<b>PDGF</b>	platelet-derived growth factor
<b>PDW</b>	platelet distribution width
<b>PK</b>	pyruvate kinase
<b>PL</b>	platelet phospholipid
<b>PLCR</b>	platelet large cell ratio
<b>PLL</b>	prolymphocytic leukemia
<b>PNH</b>	paroxysmal nocturnal hemoglobinuria
<b>PNP</b>	p-nitrophenylphosphate
<b>PT</b>	prothrombin time
<b>PTC</b>	plasma thromboplastin component
<b>OIC</b>	officer in charge
<b>QC</b>	quality control

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<b>QI</b>	quality improvement
<b>RBC</b>	red blood cell
<b>RCF</b>	relative centrifugal force
<b>RDW</b>	red cell distribution width
<b>RI</b>	reticulocyte index
<b>RNA</b>	ribonucleic acid
<b>RPI</b>	reticulocyte production index
<b>RPM</b>	rotations per minute
<b>SD</b>	standard deviation
<b>SPCA</b>	serum prothrombin conversion accelerator
<b>STAT</b>	Statim (Latin: Immediately [medical])
<b>TTP</b>	thrombotic thrombocytopenic purpura
<b>VCS</b>	leukocyte differential counter (volume, conductivity, scatter of laser light)
<b>WBC</b>	white blood cell
<b>WHO</b>	World Health Organization

**Common Symbols**

<b>Unit</b>	<b>Symbol</b>	<b>Unit</b>	<b>Symbol</b>
acidity or basicity	pH	meter	m
alpha	$\alpha$	milligram	mg
beta	$\beta$	milligrams per deciliter	mg/dL
centimeter	cm	milligrams per milliliter	mg/mL
decimeter	dm	milliliter	mL
degrees celcius	°C	millimeter	mm
degrees ferhenheit	°F	millimeter of mercury	mmHg
delta	$\delta$	microgram	$\mu\text{g}$
femtoliter	fL	micrometer or micron	$\mu\text{m}$
gamma	$\gamma$	Moles	M
gram	g	nanometer	nm
grams per deciliter	g/dL	picograms	pg
kilogram	kg	plus or minus	$\pm$
kilometer	km	units per milliliter	U/mL
liter	L		

## Bibliography

### Books

- Brown, Barbara, *Hematology: Principles and Procedures*, Malvern, Pennsylvania, 6<sup>th</sup> ed., Lea & Febiger, 1993.
- Diggs, L.W., Sturm, Dorothy, and Bell, Ann, *The Morphology of Human Blood Cells*, Abbott Park, Illinois, 5<sup>th</sup> ed., Abbott Laboratories, 1985.
- Henry, John B., and et al (ed), *Clinical Diagnosis and Management by Laboratory Methods*, Philadelphia, Pennsylvania, 18<sup>th</sup> ed., W. B. Saunders Company, 1991.
- Kjeldsberg, C. R., and J.A. Knight, *Body Fluids: Laboratory Examination of Cerebrospinal, Seminal, Serous, & Synovial Fluids*, Chicago, Illinois, 3<sup>rd</sup> ed., American Society of Clinical Pathologists, 1993.
- Lotspeich-Steininger, Cheryl A., Stiene-Martin, E. Anne, and Koepke, John A., *Clinical Hematology Principles, Procedures, Correlations*, Philadelphia, Pennsylvania, J. B. Lippincott Company, 1992.
- Taylor, Elizabeth J., et al (ed), *Dorland's Illustrated Medical Dictionary*, Philadelphia, Pennsylvania, 27th ed., W. B. Saunders Co., 1988.

## **Student Notes**



**CAREER DEVELOPMENT COURSE (CDC)  
4T051C, Medical Laboratory Journeyman, Volume 1**

**ASSESSMENT SURVEY**

**STUDENT NAME:**  
**(Optional)**

**DATE:**

**PURPOSE:** This survey is designed to obtain definitive and measurable feedback on the CDC volume you have just completed. The feedback you provide will give me your assessment of the quality of the training provided, identify areas where I need to improve, and with consolidation of the data, provide an assessment of how well I am meeting your needs.

**INSTRUCTIONS:** Please respond to the following statements. Circle the appropriate response according to the following scale:

	1 Strongly Disagree	2 Disagree	3 Somewhat Disagree	4 Not Applicable	5 Somewhat Agree	6 Agree	7 Strongly Agree
1. The information presented in the CDC provided me with knowledge I need to perform on the job.	1	2	3	4	5	6	7
2. The information was presented in a logical sequence and was easy to read.	1	2	3	4	5	6	7
3. The CDC volume							
a. was well written,	1	2	3	4	5	6	7
b. provided sufficient details and examples,	1	2	3	4	5	6	7
c. emphasized key information, and,	1	2	3	4	5	6	7
d. the information was easily understood.	1	2	3	4	5	6	7
4. The provided figures consistently supported the text illustrating and/or clarifying principles, techniques, and procedures.	1	2	3	4	5	6	7
5. The format of the volume made information easy to read, reference, and locate.	1	2	3	4	5	6	7
6. Self-test Questions and Unit Review Exercises							
a. were relevant to tasks performed in the workplace and knowledge required to perform on the job.	1	2	3	4	5	6	7
b. and responses could be referenced to the text.	1	2	3	4	5	6	7

Additional write-in comments/recommendations:

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**Please complete this survey, remove the page, and return it to me at the address on the back of this page. If you need more room for your comments, add a page.**

**If you would like a response, include your name, address, and DSN. (E-mail address, if you have one.)**

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## **Student Notes**

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