VOLUME - XVII
ISSUE - CII
NOV/DEC 2020

The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

CONTENTS

Editorial

Iron-deficiency anemia is anemia caused by a lack of iron. Anemia is defined as a decrease in the number of red blood cells or the amount of hemoglobin in the blood. When onset is slow, symptoms are often vague such as feeling tired, weak, short of breath, or having decreased ability to exercise. Anemia that comes on quickly often has more severe symptoms, including: confusion, feeling like one is going to pass out or increased thirst. Anemia is typically significant before a person becomes noticeably pale. Children with iron deficiency anemia may have problems with growth and development. There may be additional symptoms depending on the underlying cause.

Iron-deficiency anemia is caused by blood loss, insufficient dietary intake, or poor absorption of iron from food. Sources of blood loss can include heavy periods, childbirth, uterine fibroids, stomach ulcers, colon cancer, and urinary tract bleeding. Poor absorption of iron from food may occur as a result of an intestinal disorder such as inflammatory bowel disease or celiac disease, or surgery such as a gastric bypass. In the developing world, parasitic worms, malaria, and HIV/AIDS increase the risk of iron deficiency anemia. Diagnosis is confirmed by blood tests.

Iron deficiency anemia can be prevented by eating a diet containing sufficient amounts of iron or by iron supplementation. Foods high in iron include meat, nuts, spinach, and foods made with iron-fortified flour. Treatment may include dietary changes and dealing with underlying causes, for example medical treatment for parasites or surgery for ulcers. Iron supplements and vitamin C may be recommended. Severe cases may be treated with blood transfusions or iron injections.

Iron-deficiency anemia affected about 1.48 billion people in 2015. A lack of dietary iron is estimated to cause approximately half of all anemia cases globally. Women and young children are most commonly affected. In 2015, anemia due to iron deficiency resulted in about 54,000 deaths – down from 213,000 deaths in 1990.

The foregoing text is the "**DIASEASE DIAGNOSIS**" of this issue.

"**INTERPRETATION**" true to its name interprets FERRITIN for you.

Lastly "**TROUBLE SHOOTING** "explains the art and science of PHLEBOTOMY – both pictorially and textually.

The most colourful and interesting page aptly called "**BOUQUET**" has not gone missing!

DISEASE DIAGNOSIS

IRON DEFICIENCY ANEMIA

Background

Iron deficiency is defined as a decreased total iron body content. Iron deficiency anemia occurs when iron deficiency is severe enough to diminish erythropoiesis and cause the development of anemia. Iron deficiency is the most prevalent single deficiency state on a worldwide basis. It is important economically because it diminishes the capability of individuals who are affected to perform physical labor, and it diminishes both growth and learning in children. Posthemorrhagic anemia is discussed in this article because it is an important cause of iron deficiency. The acute and potentially catastrophic problems of hypoxia and shock that can occur from significant hemorrhage or severe iron deficiency are discussed elsewhere; however, daily blood losses can be small and may be overlooked.

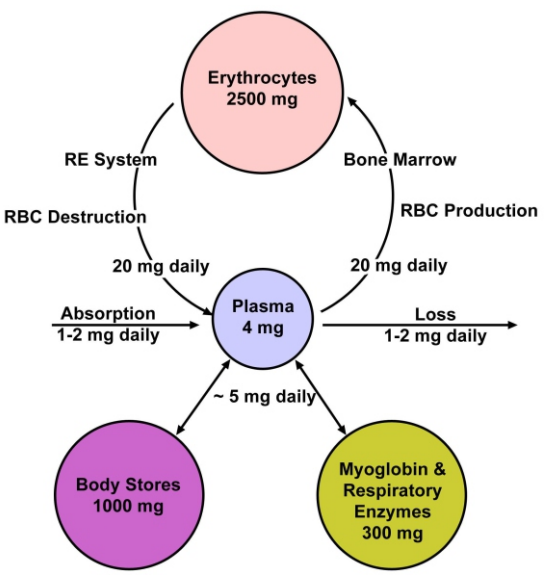
Other groups at elevated risk for iron deficiency anemia include the following:

- Adolescent girls with heavy menstrual bleeding
- Patients with congestive heart failure
- Renal transplant recipients
- Elite runners and triathletes
- Bariatric surgery patients

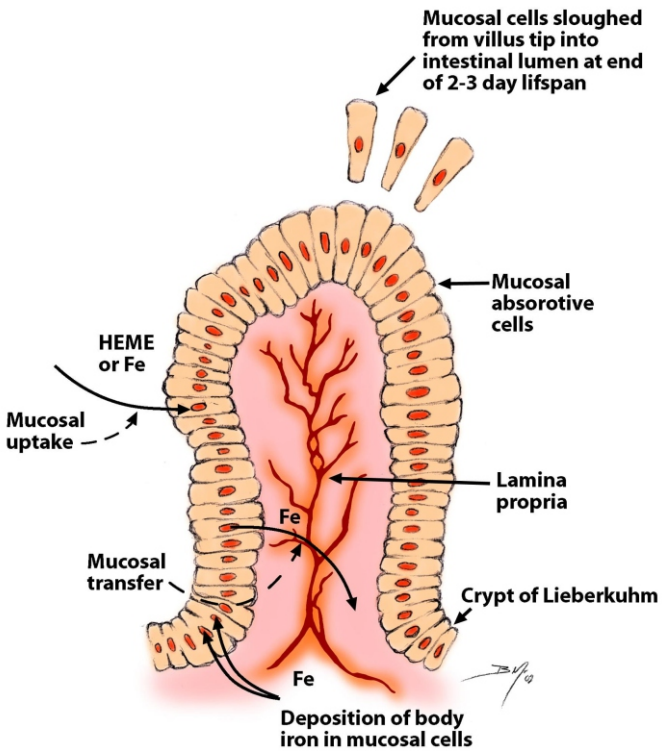
Occasionally, patients with severe iron deficiency anemia from slow but persistent gastrointestinal (GI) bleeding have repeatedly negative testing of stool for hemoglobin. Therefore, it is important for the clinician to be aware of characteristics of the anemia at all intervals after the onset of bleeding.

Pathophysiology

Iron is vital for all living organisms because it is essential for multiple metabolic processes, including oxygen transport, DNA synthesis, and electron transport. Iron equilibrium in the body is regulated carefully to ensure that sufficient iron is absorbed in order to compensate for body losses of iron (see the image below). Whereas body loss of iron quantitatively is as important as absorption in terms of maintaining iron equilibrium, it is a more passive process than absorption.



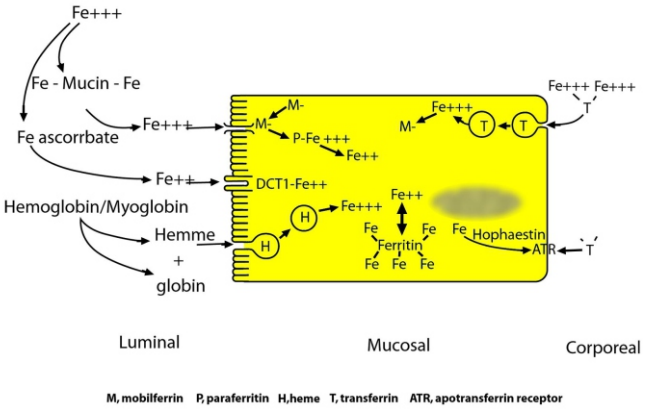
The total body iron in a 70-kg man is about 4 g. This is maintained by a balance between absorption and body losses. Although the body only absorbs 1 mg daily to maintain equilibrium, the internal requirement for iron is greater (20-25 mg). An erythrocyte has a lifespan of 120 days so that 0.8% of red blood cells are destroyed and replaced each day. A man with 5 L of blood volume has 2.5 g of iron incorporated into the hemoglobin, with a daily turnover of 20 mg for hemoglobin synthesis and degradation and another 5 mg for other requirements. Most of this iron passes through the plasma for reutilization. Iron in excess of these requirements is deposited in body stores as ferritin or hemosiderin. In healthy people, the body concentration of iron (approximately 60 parts per million [ppm]) is regulated carefully by absorptive cells in the proximal small intestine, which alter iron absorption to match body losses of iron (see the image below). Persistent errors in iron balance lead to either iron deficiency anemia or hemosiderosis. Both are disorders with potential adverse consequences.



Mucosal cells in the proximal small intestine mediate iron absorption. Intestinal cells are born in the crypts of Lieberkuhn and migrate to the tips of the villi. The cells are sloughed into the intestinal lumen at the end of their 2- to 3-day lifespan. Absorptive cells remain attuned to the body requirement for iron by incorporating proportionate quantities of body iron into the absorptive cells. This iron and recently absorbed iron decrease uptake of iron from the gut lumen by satiation of iron-binding proteins with iron, by stimulating an iron regulatory element, or both. The incorporation of iron into these cells in quantities proportional to body stores of iron also provides a limited method of increasing iron excretion in individuals replete in iron. Either diminished absorbable dietary iron or excessive loss of body iron can cause iron deficiency. Diminished absorption usually is due to an insufficient intake of dietary iron in an absorbable form. Hemorrhage is the most common cause of excessive loss of body iron, but it can occur with hemoglobinuria from intravascular hemolysis. Malabsorption of iron is relatively uncommon in the absence

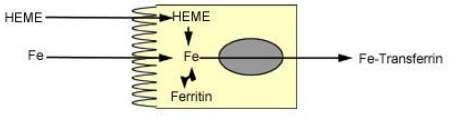
of small bowel disease (sprue, celiac disease, regional enteritis) or previous GI surgery. Iron uptake in the proximal small bowel occurs by 3 separate pathways (see the image below). These are the heme pathway and 2 distinct pathways for ferric and ferrous iron.

Postulated Mechanisms of Iron Absorption



M, mobilferrin P, paraferritin H,heme T, transferrin ATR, apotransferrin receptor

Three pathways exist in enterocytes for uptake of food iron. In the United States and Europe, most absorbed iron is derived from heme. Heme is digested enzymatically free of globin and enters the enterocyte as a metalloporphyrin. Within the cell iron is released from heme by heme oxygenase to pass into the body as inorganic iron. Most dietary inorganic iron is ferric iron. This can enter the absorptive cell via the integrin-mobilferrin pathway (IMP). Some dietary iron is reduced in the gut lumen and enters the absorptive cell via the divalent metal transporter-1 (DMT-1/DCT-1/Nramp-2). The proteins of both pathways interact within the enterocyte with paraferritin, a large protein complex capable of ferrireduction. Excess iron is stored as ferritin to protect the cell from oxidative damage. Iron leaves the cell to enter plasma facilitated by ferroportin and hephaestin, which associate with an apotransferrin receptor. The enterocyte is informed of body requirements for iron by transporting iron from plasma into the cell using a holotransferrin receptor. In the West, one third of dietary iron is heme iron, but two thirds of body iron is derived from dietary myoglobin and hemoglobin. Heme iron is not chelated and precipitated by numerous dietary constituent that render nonheme iron nonabsorbable (see the image below), such as phytates, phosphates, tannates, oxalates, and carbonates. Heme is maintained soluble and available for absorption by globin degradation products produced by pancreatic enzymes. Heme iron and nonheme iron are absorbed into the enterocyte noncompetitively.



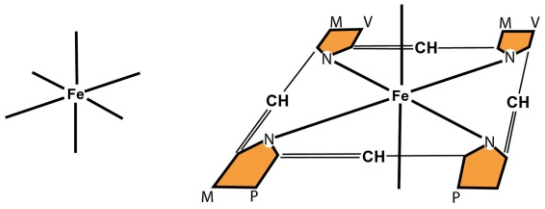
Intraluminal Factors	Mucosal Factors	Corpreal Factors
Dietary iron Quantity Chemical form pH Chelation & precipitation	Anatomic factors Diminished absorptive surface Defective epithelial cells Mucosal life span Intestinal motility Mucosal iron Quantity Chemical form Certain metals Drugs affecting protein synthesis	Body iron stores Iron Turnover Erythropoiesis Anabolism/catabolism Reticuloendothelial blockade Ineffective erythropoiesis Thalassemia, Sideroblastic anemia Cong. dyserythropoietic anemia Hypoxia Idiopathic hemochromatosis Neonatal hemochromatosis Atransferrinemia Ferrochaltase deficiency Porphyria cutanea tarda Ceruloplasmin deficiency Transferrin immune complex disease Nonalcoholic steatohepatitis Cirrhosis Portacaval shunt Familial hypochromic anemia with siderosis Sub-Saharan hemochromatosis (Bantu siderosis)

Dietary iron contains both heme and nonheme iron. Both chemical forms are absorbed noncompetitively into duodenal and jejunal mucosal cells. Many of the factors that alter the absorption of nonheme iron have little effect upon the absorption of heme iron because of the differences in their chemical structures. Iron is released from heme within the intestinal absorptive cell by heme oxygenase and then transferred into the body as nonheme iron. Factors affecting various stages of iron absorption are shown in this diagram. The simplest model of iron absorption must consider intraluminal, mucosal, and corporeal factors. Heme enters the cell as an intact metalloporphyrin, presumably by a vesicular mechanism. It is degraded within the enterocyte by heme oxygenase with release of iron so that it traverses the basolateral cell membrane in competition with nonheme iron to bind transferrin in the plasma. Ferric iron utilizes a different pathway to enter cells than ferrous iron. This was shown by competitive inhibition studies, the use of blocking antibodies against divalent metal transporter-1 (DMT-1) and beta3-integrin, and transfection experiments using DMT-1 DNA. This research indicated that ferric iron utilizes beta3-integrin and mobilferrin, while ferrous iron uses DMT-1 to enter cells. Which pathway transports most nonheme iron in humans is not known. Most nonheme dietary iron is ferric iron. Iron absorption in mice and rats may involve more ferrous iron because they excrete moderate quantities of ascorbate in intestinal secretions. Humans, however, are a scorbutic species and are unable to synthesize ascorbate to reduce ferric iron. Other proteins appear to be related to iron absorption. These are stimulators of iron transport (SFT), which are reported to increase the absorption of both ferric and ferrous iron, and hephaestin, which is postulated to be important in the transfer of iron from enterocytes into the plasma. The relationships and interactions among the newly described proteins are not known at this time and are being explored in a number of laboratories. The iron concentration within enterocytes varies directly with the body's requirement for iron. Absorptive cells of iron-deficient humans and animals contain little stainable iron, whereas those of subjects who are replete in iron contain significantly higher amounts. Untreated phenotypic hemochromatosis creates little stainable iron in the enterocyte, similar to iron deficiency. Iron within the enterocyte may operate by up-regulation of a receptor, saturation of an iron-binding protein, or both. In contrast to findings in iron deficiency, enhanced erythropoiesis, or hypoxia, endotoxin rapidly diminishes iron absorption without altering enterocyte iron concentration. This suggests that endotoxin and, perhaps, cytokines alter iron absorption by a different mechanism. This is the effect of hepcidin and the balance of hepcidin versus erythropoietin. Most iron delivered to nonintestinal cells is bound to transferrin. Transferrin iron is delivered into nonintestinal cells via 2 pathways: the classical transferrin receptor pathway (high affinity, low capacity) and the pathway independent of the transferrin receptor (low affinity, high capacity). Otherwise, the nonsaturability of transferrin binding to cells cannot be explained. In the classical transferrin pathway, the transferrin iron complex enters the cell within an endosome. Acidification of the endosome releases the iron from transferrin so that it can enter the cell. The apotransferrin is delivered by the endosome to the plasma for reutilization. The method by which the transferrin receptor–independent pathway delivers iron to the cell is not known. Nonintestinal cells also possess the mobilferrin integrin and DMT-1 pathways. Their function in the absence of an iron-saturated transferrin is uncertain; however, their presence in nonintestinal cells suggests that they may participate in intracellular functions in addition to their capability to facilitate cellular uptake of iron.

Etiology

Dietary factors

Meat provides a source of heme iron, which is less affected by the dietary constituents that markedly diminish bioavailability than nonheme iron is. The prevalence of iron deficiency anemia is lower in geographic areas where meat is an important constituent of the diet. In areas where meat is sparse, iron deficiency is commonplace. Substances that diminish the absorption of ferrous and ferric iron include phytates, oxalates, phosphates, carbonates, and tannates (see the image below). These substances have little effect upon the absorption of heme iron. Similarly, ascorbic acid increases the absorption of ferric and ferrous iron and has little effect upon the absorption of heme iron.

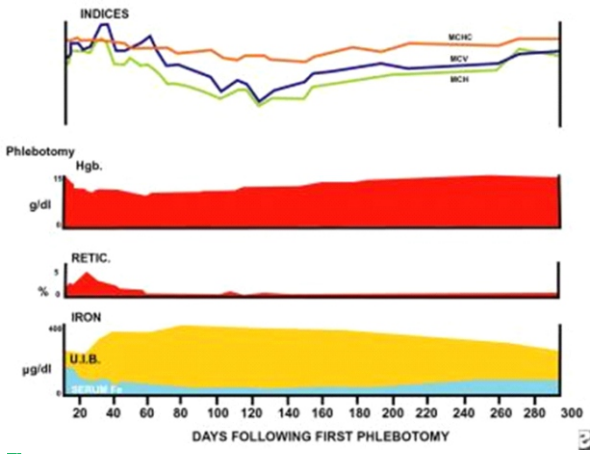


Inorganic Iron Heme

Both nonheme iron and heme iron have 6 coordinating bonds; however, 4 of the bonds in heme bind pyrroles, making them unavailable for chelation by other compounds. Therefore, ascorbic acid chelates nonheme iron to enhance absorption but has no effect upon heme iron. Many dietary components, such as phytates, phosphates, oxalates, and tannates, bind nonheme iron to decrease nonheme iron absorption. They do not affect heme. This explains why heme is so effectively absorbed with foods containing these chelators. Iron hemoglobin structure. Purified heme is absorbed poorly because heme polymerizes into macromolecules. Globin degradation products diminish heme polymerization, making it more available for absorption. They also increase the absorption of nonheme iron because the peptides from degraded globin bind the iron to prevent both precipitation and polymerization; thus, absorption of the iron in spinach is increased when the spinach eaten with meat. Heme and nonheme iron uptake by intestinal absorptive cells is noncompetitive.

Hemorrhage

Bleeding for any reason produces iron depletion. If sufficient blood loss occurs, iron deficiency anemia ensues (see the image below). A single sudden loss of blood produces a posthemorrhagic anemia that is normocytic. The bone marrow is stimulated to increase production of hemoglobin, thereby depleting iron in body stores. Once they are depleted, hemoglobin synthesis is impaired and microcytic hypochromic erythrocytes are produced.



Sequential changes in laboratory values following blood loss are depicted. A healthy human was bled 5 L in 500-mL increments over 45 days. A moderate anemia ensued, initially with normal cellular indices and serum iron. Subsequently, the mean corpuscular volume (MCV) increased as iron was mobilized from body stores and reticulocytosis occurred. The serum iron decreased, followed by an increase in the total iron-binding capacity. Gradual decreases in the red blood cell indices occurred, with maximal microcytosis and hypochromia present 120 days after bleeding. Values returned to normal approximately 250 days after blood loss. At the end of the experiment, iron was absent from body stores (marrow) because hemoglobin has a first priority for iron. Iron-59 absorption was increased after all values returned to normal in order to replenish the body store with iron. This suggests that the serum iron, total iron-binding capacity, hemoglobin concentration, and indices were not the primary regulators of iron absorption. Maximal changes in the red blood cell (RBC) cellular indices occur in approximately 120 days, at a time when all normal erythrocytes produced prior to the hemorrhage are replaced by microcytes. Before this time, the peripheral smear shows a dimorphic population of erythrocytes, normocytic cells produced before bleeding, and microcytic cells produced after bleeding. This is reflected in the red blood cell distribution width (RDW); thus, the earliest evidence of the development of an iron-deficient erythropoiesis is seen in the peripheral smear, in the form of increased RDW.

Hemosiderinuria, hemoglobinuria, and pulmonary hemosiderosis

Iron deficiency anemia can occur from loss of body iron in the urine. If a freshly obtained urine specimen appears bloody but contains no red blood cells, suspect hemoglobinuria. Obtain confirmation in the laboratory that the pigment is hemoglobin and not myoglobin. This can be accomplished easily because 60% ammonium sulfate precipitates hemoglobin but not myoglobin. Hemoglobinuria classically is ascribed to paroxysmal nocturnal hemoglobinuria, but it can occur with any brisk intravascular hemolytic anemia. In the early days of heart surgery with implantation of artificial valves, this mechanism of producing iron deficiency anemia was commonplace in large university hospitals. Today, with better prostheses, it has become a less frequent clinical problem. With less severe hemolytic disorders, there may be no significant hemoglobinuria. Investigate renal loss of iron by staining the urine sediment for iron. Hemosiderin is detected intracellularly. Most of these patients have a low or absent plasma haptoglobin. Similarly, pulmonary hemosiderosis can result in sufficient loss of iron as hemosiderin from the lungs.

Malabsorption of iron

Prolonged achlorhydria may produce iron deficiency because acidic conditions are required to release ferric iron from food. Then, it can be chelated with mucins and other substances (eg, amino acids, sugars, amino acids, or amides) to keep it soluble and available for absorption in the more alkaline duodenum. Starch and clay eating produce malabsorption of iron and iron deficiency anemia. Specific inquiry is required to elicit a history of either starch or clay eating because patients do not volunteer the information. Extensive surgical removal of the proximal small bowel or chronic diseases (eg, untreated sprue or celiac syndrome) can diminish iron absorption. In patients who have undergone bariatric surgery, postoperative gastric hypochlorhydria impairs iron absorption; in those who have undergone Roux-en-Y gastric bypass surgery, bypass of the duodenum impairs reduction of iron to the ferrous (absorbable) state. In addition, patients tend to eat less food after bariatric surgery, often less meat, which leads to decreased intake of heme iron. Rarely, patients with no history of malabsorption have iron

deficiency anemia and fail to respond to oral iron therapy. Most merely are noncompliant with therapy. Before placing these patients on parenteral therapy, document iron malabsorption either by measuring absorption of radioiron or by obtaining a baseline fasting serum-iron concentration and repeating the test 30 minutes and 1 hour after administration of a freshly prepared oral solution of ferrous sulfate (50-60 mg of iron) under observation. The serum iron should increase by 50% over the fasting specimen.

Iron-refractory iron deficiency

Iron-refractory iron deficiency anemia (IRIDA) is a hereditary disorder marked by with iron deficiency anemia that is typically unresponsive to oral iron supplementation and may be only partially responsive to parenteral iron therapy. IRIDA results from variants in the *TMPRSS6* gene that lead to uninhibited production of hepcidin. IRIDA is characterized by microcytic, hypochromic anemia and serum hepcidin values that are inappropriately high for body iron levels. Most patients with IRIDA are women. Age at presentation, disease severity, and response to iron supplementation are highly variable, even within families, with a few patients responding to oral iron but most requiring parenteral iron supplementation. An uncommon form of IRIDA occurs in postmenopausal women with androgen deficiency that leads to primary defective iron reutilization. This condition only responds to androgen replacement.

Epidemiology

International statistics

A study of national primary care database for Italy, Belgium, Germany, and Spain determined that annual incidence rates of iron deficiency anemial ranged from 7.2 to 13.96 per 1,000 person-years. Higher rates were found in females, younger and older persons, patients with gastrointestinal diseases, pregnant women and women with a history of menometrorrhagia, and users of aspirin and/or antacids. In countries where little meat is in the diet, iron deficiency anemia is 6-8 times more prevalent than in North America and Europe. This occurs despite consumption of a diet that contains an equivalent amount of total dietary iron; the reason is that heme iron is absorbed better from the diet than nonheme iron. In studies of children and adolescents from Sudan and Nepal, iron deficiency anemia was found in as many as two thirds of subjects. In certain geographic areas, intestinal parasites, particularly hookworm, worsen the iron deficiency because of blood loss from the GI tract. Anemia is more profound among children and premenopausal women in these environs.

Age-related demographics

Healthy newborn infants have a total body iron of 250 mg (80 ppm), which is obtained from maternal sources. This decreases to approximately 60 ppm in the first 6 months of life, while the baby consumes an iron-deficient milk diet. Infants consuming cow milk have a greater incidence of iron deficiency because bovine milk has a higher concentration of calcium, which competes with iron for absorption. Subsequently, growing children must obtain approximately 0.5 mg more iron daily than is lost in order to maintain a normal body concentration of 60 ppm. During adult life, equilibrium between body loss and gain is maintained. Children are more likely to develop iron deficiency anemia. In certain geographic areas, hookworm adds to the problem. Children are more likely to walk in soil without shoes and develop heavy infestations. During childbearing years, women have a high incidence of iron deficiency anemia because of iron losses sustained with pregnancies and menses. Gastrointestinal neoplasms become increasingly more prevalent with each decade of life. They frequently present with GI bleeding that may remain occult for long intervals before it is detected. Usually, bleeding from neoplasms in other organs is not occult, prompting the patient to seek medical attention before developing severe iron depletion. Investigate the etiology of the iron deficiency anemia to evaluate for a neoplasm.

Sex-related demographics

An adult male absorbs and loses about 1 mg of iron from a diet containing 10-20 mg daily. During childbearing years, an adult female loses an average of 2 mg of iron daily and must absorb a similar quantity of iron in order to maintain equilibrium. Because the average woman eats less than the average man does, she must be more than twice as efficient in absorbing dietary iron in order to maintain equilibrium and avoid developing iron deficiency anemia. Healthy males lose body iron in sloughed epithelium, in secretions from the skin and gut lining, and from small daily losses of blood from the GI tract (0.7 mL daily). Cumulatively, this amounts to 1 mg of iron. Males with severe siderosis from blood transfusions can lose a maximum of 4 mg daily via these routes without additional blood loss. A woman loses about 500 mg of iron with each pregnancy. Menstrual losses are highly variable, ranging from 10 to 250 mL (4-100 mg of iron) per period. These iron losses in women double their need to absorb iron in comparison to males. A special effort should be made to identify and treat iron deficiency during pregnancy and early childhood because of the effects of severe iron deficiency upon learning capability, growth, and development.

Race-related demographics

Race probably has no significant effect upon the occurrence of iron deficiency anemia; however, because diet and socioeconomic factors play a role in the prevalence of iron deficiency, it more frequently is observed in people of various racial backgrounds living in poorer areas of the world.

Prognosis

Iron deficiency anemia is an easily treated disorder with an excellent outcome; however, it may be caused by an underlying condition with a poor prognosis, such as neoplasia. Similarly, the prognosis may be altered by a comorbid condition such as coronary artery disease. Promptly and adequately treat a patient with iron deficiency anemia who is symptomatic with such comorbid conditions. Chronic iron deficiency anemia is seldom a direct cause of death; however, moderate or severe iron deficiency anemia can produce sufficient hypoxia to aggravate underlying pulmonary and cardiovascular disorders. Hypoxic deaths have been observed in patients who refuse blood transfusions for religious reasons. Obviously, with brisk hemorrhage, patients may die from hypoxia related to posthemorrhagic anemia. Whereas a number of symptoms, such as ice chewing and leg cramps, occur with iron deficiency, the major debility of moderately severe iron deficiency is fatigue and muscular dysfunction that impairs muscular work performance. In children, the growth rate may be slowed, and a decreased capability to learn is reported. In young children, severe iron deficiency anemia is associated with a lower intelligence quotient (IQ), a diminished capability to learn, and a suboptimal growth rate.

Clinical Presentation

History

Although iron deficiency anemia is a laboratory diagnosis, a carefully

obtained history can facilitate its recognition. The history can also be useful in establishing the etiology of the anemia and, perhaps, in estimating its duration. Iron deficiency anemia often develops gradually, with small amounts of blood loss. Such persons may remain asymptomatic until their iron stores become sufficiently depleted to compromise red cell production and other tissues, at which point fatigue and other symptoms arise. One half of patients with moderate iron deficiency anemia develop pagophagia. Usually, they crave ice to suck or chew. Occasionally, patients are seen who prefer cold celery or other cold vegetables in lieu of ice. Leg cramps, which occur on climbing stairs, also are common in patients deficient in iron. Often, patients can identify a distinct point in time when these symptoms first occurred, providing an estimate of the duration of the iron deficiency. Fatigue and diminished capability to perform hard labor are attributed to the lack of circulating hemoglobin; however, they occur out of proportion to the degree of anemia and probably are due to a depletion of proteins that require iron as a part of their structure. Increasing evidence suggests that deficiency or dysfunction of nonhemoglobin proteins has deleterious effects. These include muscle dysfunction, pagophagia, dysphagia with esophageal webbing, poor scholastic performance, altered resistance to infection, and altered behavior.

Dietary history

A dietary history is important. Vegetarians are more likely to develop iron deficiency, unless their diet is supplemented with iron. National programs of dietary iron supplementation are initiated in many portions of the world where meat is sparse in the diet and iron deficiency anemia is prevalent. Unfortunately, affluent nations also supplement iron in foodstuffs and vitamins without recognizing the potential contribution of iron to free radical formation and the prevalence of genetic iron overloading disorders. Elderly patients who are in poor economic circumstances and do not wish to seek aid may try to survive on a "tea and toast" diet. They may also be hesitant to share this dietary information. This group is far more likely to develop protein-calorie malnutrition before they develop iron deficiency anemia. A fundamental concept is that after age 1 year, dietary deficiency alone is not sufficient to cause clinically significant iron deficiency, so a source of blood loss should always be sought as part of the management of a patient with iron deficiency anemia. Infants and toddlers are the primary risk groups for dietary iron deficiency anemia. Neonates who double their birthweight are a special risk group. Also see Pediatric Acute Anemia and Pediatric Chronic Anemia. Pica is not a cause of iron deficiency anemia; pica is a symptom of iron deficiency anemia. It is the link between iron deficiency anemia and lead poisoning, which is why iron deficiency anemia should always be sought when a child is diagnosed with lead poisoning. Hippocrates recognized clay eating; however, modern physicians often do not recognize it unless the patient and family are specifically queried. Both substances decrease the absorption of dietary iron. Clay eating occurs worldwide in all races, though it is more common in Asia Minor. Starch eating is a habit in females of African heritage, and it often is started in pregnancy as a treatment for morning sickness.

History of hemorrhage

Two thirds of body iron is present in circulating red blood cells as hemoglobin. Each gram of hemoglobin contains 3.47 mg of iron; thus, each mL of blood lost from the body (hemoglobin 15 g/dL) results in a loss of 0.5 mg of iron. Bleeding is the most common cause of iron deficiency, either from parasitic infection (hookworm) or other causes of blood loss. With bleeding from most orifices (hematuria, hematemesis, hemoptysis), patients will present before they develop chronic iron deficiency anemia; however, gastrointestinal bleeding may go unrecognized. Patients often do not understand the significance of a melanotic stool. Excessive menstrual losses may be overlooked. Unless menstrual flow changes, patients typically do not seek medical attention for menorrhagia. If the clinician asks, these patients generally report that their menses are normal. Because of the marked differences among women with regard to menstrual blood loss (10-250 mL per menses), query the patient about a specific history of clots, cramps, and the use of multiple tampons and pads. For more information, also see Menorrhagia.

Physical Examination

Anemia produces nonspecific pallor of the mucous membranes. A number of abnormalities of epithelial tissues are described in association with iron deficiency anemia. These include esophageal webbing, koilonychia, glossitis, angular stomatitis, and gastric atrophy. The exact relationship of these epithelial abnormalities to iron deficiency is unclear and may involve other factors. For example, in publications from the United Kingdom, esophageal webbing and atrophic changes of the tongue and the corner of the mouth are reported in as many as 15% of patients with iron deficiency; however, they are much less common in the United States and other portions of the world. Splenomegaly may occur with severe, persistent, untreated iron deficiency anemia. This is uncommon in the United States and Europe.

Complications

Iron deficiency anemia diminishes work performance by forcing muscles to depend on anaerobic metabolism to a greater extent than they do in healthy individuals. This change is believed to be attributable to deficiency in iron-containing respiratory enzymes rather than to anemia. Severe anemia due to any cause may produce hypoxemia and enhance the occurrence of coronary insufficiency and myocardial ischemia. Likewise, it can worsen the pulmonary status of patients with chronic pulmonary disease. Defects in structure and function of epithelial tissues may be observed in iron deficiency. Fingernails may become brittle or longitudinally ridged, with the development of koilonychia (spoon-shaped nails). The tongue may show atrophy of the lingual papillae and develop a glossy appearance. Angular stomatitis may occur with fissures at the corners of the mouth. Dysphagia may occur with solid foods, with webbing of the mucosa at the junction of the hypopharynx and the esophagus (Plummer-Vinson syndrome); this has been associated with squamous cell carcinoma of the cricoid area. Atrophic gastritis occurs in iron deficiency with progressive loss of acid secretion, pepsin, and intrinsic factor and development of an antibody to gastric parietal cells. Small intestinal villi become blunted. Cold intolerance develops in one fifth of patients with chronic iron deficiency anemia and is manifested by vasomotor disturbances, neurologic pain, or numbness and tingling. Rarely, severe iron deficiency anemia is associated with papilledema, increased intracranial pressure, and the clinical picture of pseudotumor cerebri. These manifestations are corrected with iron therapy. Impaired immune function is reported in subjects who are iron deficient, and there are reports that these patients are prone to infection; however, because of the presence of other factors, the current evidence is insufficient to establish that this impairment is directly due to iron deficiency. Children deficient in iron may exhibit behavioral disturbances. Neurologic development is impaired in infants and scholastic performance is reduced in children of school age. The intelligence quotients (IQs) of schoolchildren deficient in iron are

reported to be significantly lower than those of their nonanemic peers. Behavioral disturbances may manifest as an attention deficit disorder. Growth is impaired in infants with iron deficiency. The neurologic damage to an iron-deficient fetus results in permanent neurologic injury and typically does not resolve on its own. Iron repletion stabilizes the patient so that his or her status does not further decline.

A case-control study of 2957 children and adolescents with iron deficiency anemia and 11,828 healthy controls from the Taiwan National Health Insurance Database found that iron deficiency anemia is associated with an increased risk for psychiatric disorders. After adjusting for demographic data and risk factors for iron deficiency anemia, children and adolescents with iron deficiency anemia were at higher risk for the following :

- Unipolar depressive disorder
- Bipolar disorder
- Anxiety disorder
- Autism spectrum disorder
- Attention-deficit/hyperactivity disorder
- Tic disorder
- Delayed development
- Mental retardation

Differential Diagnoses

Diagnostic Considerations

Other conditions to be considered include the following:

- Anemia of chronic disorders
- Hemoglobin CC disease
- Hemoglobin DD disease
- Lead poisoning
- Microcytic anemias
- Autoimmune hemolytic anemia
- Hemoglobin S-beta thalassemia

Go to Anemia, Sideroblastic Anemias, and Chronic Anemia for complete information on these topics. In a meta-analysis of indices for discriminating between iron deficiency anemia and thalassemia trait in subjects with microcytic red blood cells (RBCs), the ratio of microcytic to hypochromic RBCs (M/H ratio) showed the best performance. An M/H ratio >6.4 was strongly indicative of thalassemia. The authors concluded that the sensitivity and specificity of the M/H ratio are not high enough for making a definitive diagnosis, but the ratio can be valuable for identifying patients with microcytic RBC who should undergo diagnostic tests for confirming thalassemia.

Differential Diagnoses

- Alpha Thalassemia
- Beta Thalassemia
- Hereditary Spherocytosis
- Sideroblastic Anemias

Workup

Approach Considerations

Although the history and physical examination can lead to the recognition of the condition and help establish the etiology, iron deficiency anemia is primarily a laboratory diagnosis.

Useful tests include the following:

- Complete blood count (CBC)
- Peripheral smear
- Serum iron, total iron-binding capacity (TIBC), and serum ferritin
- Evaluation for hemosiderinuria, hemoglobinuria, and pulmonary hemosiderosis
- Hemoglobin electrophoresis and measurement of hemoglobin A_2 and fetal hemoglobin
- Reticulocyte hemoglobin content

Other laboratory tests (eg, stool testing, incubated osmotic fragility testing, measurement of lead in tissue, and bone marrow aspiration) are useful for establishing the etiology of iron deficiency anemia and for excluding or establishing a diagnosis of 1 of the other microcytic anemias.

Complete Blood Count

The CBC documents the severity of the anemia. In chronic iron deficiency anemia, the cellular indices show a microcytic and hypochromic erythropoiesis—that is, both the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) have values below the normal range for the laboratory performing the test. Reference range values for MCV and MCHC are 83-97 fL and 32-36 g/dL, respectively. Often, the platelet count is elevated (>450,000/µL); this elevation normalizes after iron therapy. The white blood cell (WBC) count is usually within reference ranges (4500-11,000/µL), but it may be elevated. If the CBC is obtained after blood loss, the cellular indices do not enter the abnormal range until most of the erythrocytes produced before the bleed are destroyed at the end of their normal lifespan (120 d).

Peripheral Smear

Examination of the peripheral smear is an important part of the workup of patients with anemia. Examination of the erythrocytes shows microcytic and hypochromic red blood cells in chronic iron deficiency anemia. The microcytosis is apparent in the smear long before the MCV is decreased after an event producing iron deficiency. Platelets usually are increased in this disorder. In iron deficiency anemia, unlike thalassemia, target cells usually are not present, and anisocytosis and poikilocytosis are not marked. This condition lacks the intraerythrocytic crystals seen in hemoglobin C disorders. Combined folate deficiency and iron deficiency are commonplace in areas of the world with little fresh produce and meat. The peripheral smear reveals a population of macrocytes mixed among the microcytic hypochromic cells. This combination can normalize the MCV.

Serum Iron, Total Iron-Binding Capacity, and Serum Ferritin

Low serum iron and ferritin levels with an elevated TIBC are diagnostic of iron deficiency. While a low serum ferritin is virtually diagnostic of iron deficiency, a normal serum ferritin can be seen in patients who are deficient in iron and have coexistent diseases (eg, hepatitis or anemia of chronic disorders). These test findings are useful in distinguishing iron deficiency anemia from other microcytic anemias. Serum ferritin and stainable iron in tissue stores are the most sensitive laboratory indicators of mild iron deficiency and are particularly useful in differentiating iron deficiency from the anemia of chronic disorders. The percentage saturation of transferrin with iron and free erythrocyte protoporphyrin values do not become abnormal until tissue stores are depleted of iron. Subsequently, a decrease in the hemoglobin concentration occurs because iron is unavailable for heme synthesis. Red blood cell indices do not become abnormal for several months after tissue stores are depleted of iron.

Evaluation for Hemosiderinuria, Hemoglobinuria, and Pulmonary Hemosiderosis

Iron deficiency anemia can occur from loss of body iron in the urine. If a

freshly obtained urine specimen appears bloody but contains no red blood cells, suspect hemoglobinuria. Obtain confirmation in the laboratory that the pigment is hemoglobin and not myoglobin. This can be accomplished easily because 60% ammonium sulfate precipitates hemoglobin but not myoglobin. Hemoglobinuria classically is ascribed to paroxysmal nocturnal hemoglobinuria, but it can occur with any brisk intravascular hemolytic anemia. In the early days of heart surgery with implantation of artificial valves, this mechanism of producing iron deficiency anemia was commonplace in large university hospitals. Today, with better prostheses, it has become a less frequent clinical problem. With less severe hemolytic disorders, there may be no significant hemoglobinuria. Investigate renal loss of iron by staining the urine sediment for iron. Hemosiderin is detected intracellularly. Most of these patients have a low or absent plasma haptoglobin. Similarly, pulmonary hemosiderosis can result in sufficient loss of iron as hemosiderin from the lungs.

Hemoglobin Studies

Hemoglobin electrophoresis and measurement of hemoglobin A
Hemoglobin electrophoresis and measurement of hemoglobin A_2 and fetal hemoglobin are useful in establishing either beta-thalassemia or hemoglobin C or D as the etiology of the microcytic anemia. Unfortunately, simple tests do not exist for alpha-thalassemia in most laboratories, and it is a diagnosis of exclusion.

Reticulocyte hemoglobin content

Mateos Gonzales et al assessed the diagnostic efficiency of commonly used hematologic and biochemical markers, as well as the reticulocyte hemoglobin content (CHr) in the diagnosis of iron deficiency in children, with or without anemia. The investigators identified CHr and iron serum as the only parameters that were independently associated with iron deficiency ($P< .05$), and CHr was the strongest predictor of iron deficiency and iron deficiency anemia. Mateos Gonzalez et al concluded that measurement of CHr may be a reliable method to assess deficiencies in tissue iron supply and, in combination with a CBC, may be an alternative to the traditional biochemical panel for the diagnosis of iron deficiency in children.

Other Laboratory Tests

Stool testing

Testing stool for the presence of hemoglobin is useful in establishing gastrointestinal (GI) bleeding as the etiology of iron deficiency anemia. Usually, chemical testing that detects more than 20 mL of blood loss daily from the upper GI tract is employed. More sensitive tests are available; however, they produce a high incidence of false-positive results in people who eat meat. Severe iron deficiency anemia can occur in patients with a persistent loss of less than 20 mL/d. To detect blood loss, the patient can be placed on a strict vegetarian diet for 3-5 days and the stool can be tested for hemoglobin with a benzidine method, or red blood cells (RBCs) can be radiolabeled with radiochromium and retransfused. Stools are collected, and the radioactivity is quantified in a gamma-detector and compared to the radioactivity in a measured quantity of the patient's blood. An immunologic method of detecting human species-specific hemoglobin in stool is under development and could increase specificity and sensitivity.

Incubated osmotic fragility

Incubated osmotic fragility is useful. Microspherocytosis may produce a low-normal or slightly abnormal MCV; however, the MCHC usually is elevated rather than decreased, and the peripheral smear shows a lack of central pallor rather than hypochromia. Spherocytosis can normally be separated from iron deficiency anemia by peripheral blood smear.

Tissue lead concentrations

Measure tissue lead concentrations. Chronic lead poisoning may produce a mild microcytosis. The anemia probably is related to the anemia of chronic disorders. The incidence of lead poisoning is greater in individuals who are iron deficient than in healthy subjects because increased absorption of lead occurs in individuals who are iron deficient. Paint in old houses has been a source of lead poisoning in children and painters.

Bone marrow aspiration

A bone marrow aspirate can be diagnostic of iron deficiency. The absence of stainable iron in a bone marrow aspirate that contains spicules and a simultaneous control specimen containing stainable iron permit establishment of a diagnosis of iron deficiency without other laboratory tests. A bone marrow aspirate stained for iron (Perls stain) can be diagnostic of iron deficiency, provided that spicules are present in the smear and that a control specimen containing iron is performed at the same time. Although this test has largely been displaced in the diagnosis of iron deficiency by serum iron, TIBC, and serum ferritin testing, the absence of stainable iron in a bone marrow aspirate is the criterion standard for the diagnosis of iron deficiency. This test is diagnostic in identifying the sideroblastic anemias by showing ringed sideroblasts in the aspirate stained with Perls stain. Occasionally, it is useful in separating patients with the anemia of chronic disorders or alpha-thalassemia from patients with iron deficiency, and it is useful in identifying patients with both iron deficiency and the anemia of chronic disorders.

Histologic Findings

The absence of stainable iron in body tissues, including the bone marrow and liver, is the most useful histologic finding in individuals who are iron deficient. Nonspecific abnormalities of epithelial tissues are reported in iron deficiency. These include gastric atrophy and clubbing of the small intestinal villi. While they suggest that iron deficiency is a pantropic disorder, they have little clinical diagnostic value.

Treatment & Management

Management

Treatment of iron deficiency anemia consists of correcting the underlying etiology and replenishing iron stores. Iron therapy is as follows:

- Oral ferrous iron salts are the most economical and effective form
- Ferrous sulfate is the most commonly used iron salt
- Better absorption and lower morbidity have been claimed for other iron salts
- Toxicity is generally proportional to the amount of iron available for absorption
- Reserve parenteral iron for patients who are either unable to absorb oral iron or who have increasing anemia despite adequate doses of oral iron
- Reserve transfusion of packed RBCs for patients who are experiencing significant acute bleeding or are in danger of hypoxia and/or coronary insufficiency.

INTERPRETATION

Ferritin

Reference Range

Ferritin is the cellular storage protein for iron. It is present in small concentrations in blood, and the serum ferritin concentration normally correlates well with total-body iron stores, making its measurement important in the diagnosis of disorders of iron metabolism.

Reference ranges

Male : 12-300 ng/mL
Female : 10-150 ng/mL {ref}
Children :
- Newborn: 25-200 ng/mL
- ≤1 month: 200-600 ng/mL
- 2-5 months: 50-200 ng/mL
- 6 months-15 years: 7-142 ng/mL

*** PLEASE REFER TO NORMAL BIOLOGICAL REFERENCE RANGES AS GIVEN IN PRODUCT INSERTS OF INDIVIDUAL MANUFACTURERS.**

Interpretation

Ferritin levels are increased in the following:
- Acute and chronic liver disease
- Infection
- Inflammation
- Alcoholism
- Malignancies
- Hyperthyroidism
- Gaucher disease
- Myocardial infection
- Iron overload (hemochromatosis)
- End-stage renal disease
- Renal cell cancer
- Anemia other than iron deficiency

Ferritin levels are decreased in the following:
- Iron deficiency
- Hemodialysis

Collection and Panels

Collection: Tiger top or red tube
Panel: Iron panel usually includes ferritin

Background

Description

Ferritin is the cellular storage protein for iron. Its molecular weight is about 440 kilodalton. It is a 24-subunit protein that consists of light and heavy chains and can store up to 4500 atoms of iron. Ferritin is an acute-phase reactant that coordinates cellular defense against oxidative stress and inflammation along with transferrin and its receptor. Usually, human plasma contains little ferritin; however, plasma ferritin levels are markedly increased in persons with excess iron.

Serum ferritin levels can be measured with a sensitive and specific immunoassay, serving as an index of body iron stores.

Iron is stored either as hemosiderin or ferritin. Ferritin is water-soluble, while hemosiderin is water-insoluble. Ferritin is found in virtually all cells of the body and in tissue fluids. Ferritin in lysosomes is converted into hemosiderin upon partial degradation of its protein shell by lysosomal enzymes. By contrast, ferritin that is degraded within the cytosol results in complete release of the iron.

Ferritin is present in small concentrations in blood. The serum ferritin concentration normally correlates well with total-body iron stores, making its measurement important in the diagnosis of disorders of iron metabolism.

Indications/Applications

Ferritin testing is indicated for the following:
- To predict and monitor iron deficiency
- To monitor response to therapy or compliance with treatment
- To differentiate iron deficiency from chronic disease as a cause of anemia
- To monitor iron status in patients with renal diseases with or without dialysis
- To conduct population studies of iron level and response to supplements
- To detect and monitor response to iron depletion therapy in iron overload states.

Considerations

In hepatic, inflammatory, and malignant conditions, the ferritin level may be normal. In these scenarios, bone marrow stain of iron may be necessary to exclude iron deficiency.

Transferrin saturation is more sensitive in early hemochromatosis; serum ferritin is used to confirm the diagnosis of iron excess.

A high serum ferritin level is also an indication for liver biopsy.

Ferritin level increases with age, and the level is higher in men than women. It is particularly high in women taking contraceptives and in persons who eat red meat (in contrast to vegetarians).

TROUBLESHOOTING

BLOOD COLLECTION

ROUTINE VENIPUNCTURE AND SPECIMEN HANDLING

VENIPUNCTURE PROCEDURE

The venipuncture procedure is complex, requiring both knowledge and skill to perform. Each phlebotomist generally establishes a routine that is comfortable for her or him. Phlebotomists are considered to have occupational exposure to blood borne pathogens. The performance of routine vascular access procedures by skilled phlebotomists requires, at a minimum, the use of gloves to prevent contact with blood. Laboratory coats or work smocks are not typically needed as personal protective equipment during routine venipuncture, but an employer must assess the workplace to determine whether certain tasks, workplace situations, or employee skill levels may result in an employee's need for laboratory coats or other personal protective equipment to prevent contact with blood. It is an employer's responsibility to provide, clean, repair, replace, and/or dispose of personal protective equipment/clothing. As part of presenting a professional appearance, an institutional dress code may include wearing of a laboratory coat or smock.

Several essential steps are required for every successful collection procedure:

1. Patient comfort. Is the seating comfortable and has the patient been seated for at least 5 minutes to avoid being rushed or confused?
2. Carry out hand hygiene before and after each patient procedure, before putting on and after removing gloves.
3. Identify the patient using two different identifiers, asking open ended questions such as, "What is your name?" and "What is your date of birth?"
4. Assess the patient's physical disposition (i.e. diet, exercise, stress, basal state).
5. Check the requisition form for requested tests, patient information, and any special requirements.
6. Label the collection tubes at the bedside or drawing area.
7. Select a suitable site for venipuncture.
8. Prepare the equipment, the patient and the puncture site.
9. Perform the venipuncture, collecting the sample(s) in the appropriate container(s).
10. Recognize complications associated with the phlebotomy procedure.
11. Assess the need for sample recollection and/or rejection.
12. Promptly send the specimens with the requisition to the laboratory.

ORDER FORM / REQUISITION

A requisition form must accompany each sample submitted to the laboratory. This requisition form must contain the proper information in order to process the specimen. The essential elements of the requisition form are:

- Patient's surname, first name, and middle initial.
- Patient's ID number.
- Patient's date of birth and sex.
- Requesting physician's complete name.
- Source of specimen. This information must be given when requesting microbiology, cytology, fluid analysis, or other testing where analysis and reporting is site specific.
- Date and time of collection.
- Initials of phlebotomist.
- Indicating the test(s) requested.

An example of a simple requisition form with the essential elements is shown below:

LABORATORY SERVICE - UNIVERSITY OF UTAH HOSPITAL

Patient Name: _____

Patient ID: _____

Patient Birthdate: _____ Sex: _____

Source of Specimen: _____

Date Collected: _____ Time: _____ Phleb: _____

Physician: _____ Location: _____

Diagnosis: _____

Tests Requested:

_____ Electrolyte Panel _____ Complete Blood Count

_____ Hepatic Panel _____ Protime / PTT

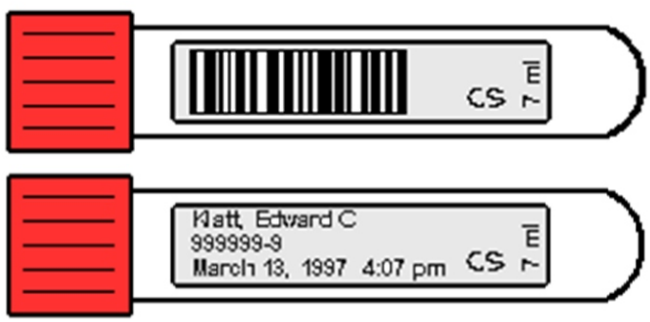
LABELING THE SAMPLE

A properly labeled sample is essential so that the results of the test match the patient. The key elements in labeling are:

- Patient's surname, first and middle.
- Patient's ID number.
- **NOTE:** Both of the above MUST match the same on the requisition form.
- Date, time and initials of the phlebotomist must be on the label of EACH tube.

Automated systems may include labels with bar codes.

Examples of labeled collection tubes are shown below:



EQUIPMENT:

THE FOLLOWING ARE NEEDED FOR ROUTINE VENIPUNCTURE:

- Evacuated Collection Tubes - The tubes are designed to fill with a predetermined volume of blood by vacuum. The rubber stoppers are color coded according to the additive that the tube contains. Various sizes are available. Blood should **NEVER** be poured from one tube to another since the tubes can have different additives or coatings (see illustrations at end).
- Needles - The gauge number indicates the bore size: the larger the gauge number, the smaller the needle bore. Needles are available for evacuated systems and for use with a syringe, single draw or butterfly system.

- Holder/Adapter - use with the evacuated collection system.
- Tourniquet - Wipe off with alcohol and replace frequently.
- Alcohol Wipes - 70% isopropyl alcohol.
- Povidone-iodine wipes/swabs - Used if blood culture is to be drawn.
- Gauze sponges - for application on the site from which the needle is withdrawn.
- Adhesive bandages / tape - protects the venipuncture site after collection.
- Needle disposal unit - needles should NEVER be broken, bent, or recapped. Needles should be placed in a proper disposal unit IMMEDIATELY after their use.
- Gloves - can be made of latex, rubber, vinyl, etc.; worn to protect the patient and the phlebotomist.
- Syringes - may be used in place of the evacuated collection tube for special circumstances.

ORDER OF DRAW

Blood collection tubes must be drawn in a specific order to avoid cross-contamination of additives between tubes. The recommended order of draw for plastic collection tubes is:

1. First - blood culture bottle or tube (yellow or yellow-black top)
2. Second - coagulation tube (light blue top). If just a routine coagulation assay is the only test ordered, then a single light blue top tube may be drawn. If there is a concern regarding contamination by tissue fluids or thromboplastins, then one may draw a non-additive tube first, and then the light blue top tube.
3. Third - non-additive tube (red top)
4. Last draw - additive tubes in this order:
    1. SST (red-gray or gold top). Contains a gel separator and clot activator.
    2. Sodium heparin (dark green top)
    3. PST (light green top). Contains lithium heparin anticoagulant and a gel separator.
    4. EDTA (lavender top)
    5. ACDA or ACDB (pale yellow top). Contains acid citrate dextrose.
    6. Oxalate/fluoride (light gray top)

    NOTE: Tubes with additives must be thoroughly mixed. Erroneous test results may be obtained when the blood is not thoroughly mixed with the additive.

PROCEDURAL ISSUES

PATIENT RELATIONS AND IDENTIFICATION:

The phlebotomist's role requires a professional, courteous, and understanding manner in all contacts with the patient. Greet the patient and identify yourself and indicate the procedure that will take place. Effective communication - both verbal and nonverbal - is essential. Proper patient identification MANDATORY. If an inpatient is able to respond, ask for a full name and always check the armband or bracelet for confirmation. **DO NOT DRAW BLOOD IF THE ARMBAND OR BRACELET IS MISSING.** For an inpatient the nursing staff can be contacted to aid in identification prior to proceeding. An outpatient must provide identification other than the verbal statement of a name. Using the requisition for reference, ask a patient to provide additional information such as a surname or birthdate. A government issued photo identification card such as a driver's license can aid in resolving identification issues. If possible, speak with the patient during the process. The patient who is at ease will be less focused on the procedure. Always thank the patient and excuse yourself courteously when finished.

PATIENT'S BILL OF RIGHTS:

The Patient's Bill of Rights has been adopted by many hospitals as declared by the Joint Commission on Accreditation of Healthcare Organizations (JCAHO). The basic patient rights endorsed by the JCAHO follow in condensed form are given below.

The patient has the right to:

- Impartial access to treatment or accommodations that are available or medically indicated, regardless of race, creed, sex, national origin, or sources of payment for care.
- Considerate, respectful care.
- Confidentiality of all communications and other records pertaining to the patient's care.
- Expect that any discussion or consultation involving the patient's case will be conducted discretely and that individuals not directly involved in the case will not be present without patient permission.
- Expect reasonable safety congruent with the hospital practices and environment.
- Know the identity and professional status of individuals providing service and to know which physician or other practitioner is primarily responsible for his or her care.
- Obtain from the practitioner complete and current information about diagnosis, treatment, and any known prognosis, in terms the patient can reasonably be expected to understand.
- Reasonable informed participation in decisions involving the patient's health care. The patient shall be informed if the hospital proposes to engage in or perform human experimentation or other research/educational profits affecting his or her care or treatment. The patient has the right to refuse participation in such activity.
- Consult a specialist at the patient's own request and expense.
- Refuse treatment to the extent permitted by law.
- Regardless of the source of payment, request and receive an itemized and detailed explanation of the total bill for services rendered in the hospital.
- Be informed of the hospital rules and regulations regarding patient conduct.

VENIPUNCTURE SITE SELECTION:

Although the larger and fuller median cubital and cephalic veins of the arm are used most frequently, the basilic vein on the dorsum of the arm or dorsal hand veins are also acceptable for venipuncture. Foot veins are a last resort because of the higher probability of complications.

Certain areas are to be avoided when choosing a site:

- Extensive scars from burns and surgery - it is difficult to puncture the scar tissue and obtain a specimen.
- The upper extremity on the side of a previous mastectomy - test results may be affected because of lymphedema.
- Hematoma - may cause erroneous test results. If another site is not available, collect the specimen distal to the hematoma.
- Intravenous therapy (IV) / blood transfusions - fluid may dilute the specimen, so collect from the opposite arm if possible. Otherwise, satisfactory samples may be drawn below the IV by following these procedures:
    - Turn off the IV for at least 2 minutes before venipuncture.
    - Apply the tourniquet below the IV site. Select a vein other than the one with the IV.
    - Perform the venipuncture. Draw 5 ml of blood and discard before drawing the specimen tubes for testing.
- Lines - Drawing from an intravenous line may avoid a difficult venipuncture, but introduces problems. The line must be flushed first. When using a syringe inserted into the line, blood must be withdrawn slowly to avoid hemolysis.

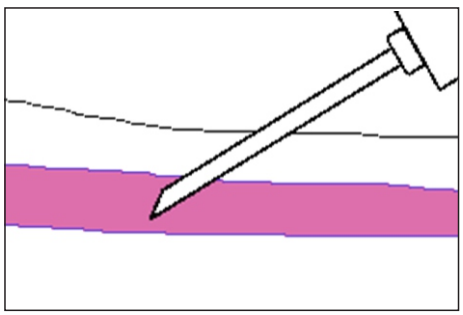
- Cannula/fistula/heparin lock - hospitals have special policies regarding these devices. In general, blood should not be drawn from an arm with a fistula or cannula without consulting the attending physician.
- Edematous extremities - tissue fluid accumulation alters test results.

PROCEDURE FOR VEIN SELECTION:

- Palpate and trace the path of veins with the index finger. Arteries pulsate, are most elastic, and have a thick wall. Thrombosed veins lack resilience, feel cord-like, and roll easily.
- If superficial veins are not readily apparent, you can force blood into the vein by massaging the arm from wrist to elbow, tap the site with index and second finger, apply a warm, damp washcloth to the site for 5 minutes, or lower the extremity over the bedside to allow the veins to fill.

PERFORMANCE OF A VENIPUNCTURE:

- Approach the patient in a friendly, calm manner. Provide for their comfort as much as possible, and gain the patient's cooperation.
- Identify the patient correctly.
- Properly fill out appropriate requisition forms, indicating the test(s) ordered.
- Verify the patient's condition. Fasting, dietary restrictions, medications, timing, and medical treatment are all of concern and should be noted on the lab requisition.
- Check for any allergies to antiseptics, adhesives, or latex by observing for armbands and/or by asking the patient.
- Position the patient. The patient should either sit in a chair, lie down or sit up in bed. Hyperextend the patient's arm.
- Apply the tourniquet 3-4 inches above the selected puncture site. Do not place too tightly or leave on more than 2 minutes (and no more than a minute to avoid increasing risk for hemoconcentration). Wait 2 minutes before reapplying the tourniquet.
- The patient should make a fist without pumping the hand.
- Select the venipuncture site.
- Prepare the patient's arm using an alcohol prep. Cleanse in a circular fashion, beginning at the site and working outward. Allow to air dry.
- Grasp the patient's arm firmly using your thumb to draw the skin taut and anchor the vein. The needle should form a 15 to 30 degree angle with the surface of the arm. Swiftly insert the needle through the skin and into the lumen of the vein. Avoid trauma and excessive probing.



- When the last tube to be drawn is filling, remove the tourniquet.
- Remove the needle from the patient's arm using a swift backward motion.
- Press down on the gauze once the needle is out of the arm, applying adequate pressure to avoid formation of a hematoma.
- Dispose of contaminated materials/supplies in designated containers.
- Mix and label all appropriate tubes at the patient bedside.
- Deliver specimens promptly to the laboratory.

PHLEBOTOMY PROCEDURE ILLUSTRATED:

- **Patient identification**
- **Filling out the requisition**
- **Equipment**
- **Apply tourniquet and palpate for vein**
- **Sterilize the site**
- **Insert needle**
- **Drawing the specimen**
- **Drawing the specimen**
- **Releasing the tourniquet**
- **Applying pressure over the vein**
- **Applying bandage**
- **Disposing needle into sharps**
- **Labeling the specimens**

PERFORMANCE OF A FINGERSTICK:

- Follow the procedure as outlined above for greeting and identifying the patient. As always, properly fill out appropriate requisition forms, indicating the test(s) ordered.
- Verify the patient's condition. Fasting, dietary restrictions, medications, timing, and medical treatment are all of concern and should be noted on the lab requisition.
- Position the patient. The patient should either sit in a chair, lie down or sit up in bed. Hyperextend the patient's arm.
- The best locations for fingersticks are the 3rd (middle) and 4th (ring) fingers of the non-dominant hand. Do not use the tip of the finger or the center of the finger. Avoid the side of the finger where there is less soft tissue, where vessels and nerves are located, and where the bone is closer to the surface. The 2nd (index) finger tends to have thicker, callused skin. The fifth finger tends to have less soft tissue overlying the bone. Avoid puncturing a finger that is cold or cyanotic, swollen, scarred, or covered with a rash.
- Using a sterile lancet, make a skin puncture just off the center of the finger pad. The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges.
- Wipe away the first drop of blood, which tends to contain excess tissue fluid.
- Collect drops of blood into the collection device by gently massaging the finger. Avoid excessive pressure that may squeeze tissue fluid into the drop of blood.
- Cap, rotate and invert the collection device to mix the blood collected.
- Have the patient hold a small gauze pad over the puncture site for a couple of minutes to stop the bleeding.
- Dispose of contaminated materials/supplies in designated containers.
- Label all appropriate tubes at the patient bedside.
- Deliver specimens promptly to the laboratory.

FINGERSTICK PROCEDURE ILLUSTRATED:

- **Equipment**
- **Proper location on finger**
- **Puncture with lancet**
- **Drop of blood**
- **Wipe first drop**
- **Collecting the specimen**
- **Specimen container**

ADDITIONAL CONSIDERATIONS:

To prevent a hematoma:

- Puncture only the uppermost wall of the vein
- Remove the tourniquet before removing the needle
- Use the major superficial veins
- Make sure the needle fully penetrates the upper most wall of the vein. (Partial penetration may allow blood to leak into the soft tissue surrounding the vein by way of the needle bevel)
- Apply pressure to the venipuncture site

To prevent hemolysis (which can interfere with many tests):

- Mix tubes with anticoagulant additives gently 5-10 times
- Avoid drawing blood from a hematoma
- Avoid drawing the plunger back too forcefully, if using a needle and syringe, or too small a needle, and avoid frothing of the sample
- Make sure the venipuncture site is dry
- Avoid a probing, traumatic venipuncture
- Avoid prolonged tourniquet application or fist clenching.

Indwelling Lines or Catheters:

- Potential source of test error
- Most lines are flushed with a solution of heparin to reduce the risk of thrombosis
- Discard a sample at least three times the volume of the line before a specimen is obtained for analysis

Hemoconcentration: An increased concentration of larger molecules and formed elements in the blood may be due to several factors:

- Prolonged tourniquet application (no more than 1 minute)
- Massaging, squeezing, or probing a site
- Long-term IV therapy
- Sclerosed or occluded veins

Prolonged Tourniquet Application:

- Primary effect is hemoconcentration of non-filterable elements (i.e. proteins). The hydrostatic pressure causes some water and filterable elements to leave the extracellular space.
- Significant increases can be found in total protein, aspartate aminotransferase (AST), total lipids, cholesterol, iron
- Affects packed cell volume and other cellular elements
- Hemolysis may occur, with pseudohyperkalemia.

Patient Preparation Factors:

- Therapeutic Drug Monitoring: different pharmacologic agents have patterns of administration, body distribution, metabolism, and elimination that affect the drug concentration as measured in the blood. Many drugs will have "peak" and "trough" levels that vary according to dosage levels and intervals. Check for timing instructions for drawing the appropriate samples.
- Effects of Exercise: Muscular activity has both transient and longer lasting effects. The creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and platelet count may increase.
- Stress: May cause transient elevation in white blood cells (WBC's) and elevated adrenal hormone values (cortisol and catecholamines). Anxiety that results in hyperventilation may cause acid-base imbalances, and increased lactate.
- Diurnal Rhythms: Diurnal rhythms are body fluid and analyte fluctuations during the day. For example, serum cortisol levels are highest in early morning but are decreased in the afternoon. Serum iron levels tend to drop during the day. You must check the timing of these variations for the desired collection point.
- Posture: Postural changes (supine to sitting etc.) are known to vary lab results of some analytes. Certain larger molecules are not filterable into the tissue, therefore they are more concentrated in the blood. Enzymes, proteins, lipids, iron, and calcium are significantly increased with changes in position.
- Other Factors: Age, gender, and pregnancy have an influence on laboratory testing. Normal reference ranges are often noted according to age.

REASONS FOR CANCELING A LABORATORY TEST

A test that has been ordered may be cancelled due to problems unrelated to drawing the specimen, and these are the most common causes for cancellations:

- Duplicate test request
- Incorrect test ordered
- Test no longer needed

A test may be cancelled due to a technical problem in the specimen collection process:

- Hemolysis of the specimen
- Clotted specimen
- Quantity of specimen not sufficient
- Collection of specimen in incorrect tube
- Contaminated specimen
- Identification of the specimen is suspect
- Delay in transport - specimen too old

SAFETY AND INFECTION CONTROL

Because of contacts with sick patients and their specimens, it is important to follow safety and infection control procedures.

PROTECT YOURSELF

- Practice universal precautions:
  - Wear gloves and a lab coat or gown when handling blood/body fluids.
  - Change gloves after each patient or when contaminated.
  - Wash hands frequently.
  - Dispose of items in appropriate containers.
- Dispose of needles immediately upon removal from the patient's vein. Do not bend, break, recap, or resheath needles to avoid accidental needle puncture or splashing of contents.
- Clean up any blood spills with a disinfectant such as freshly made 10% bleach.
- If you stick yourself with a contaminated needle:
  - Remove your gloves and dispose of them properly.
  - Squeeze puncture site to promote bleeding.
  - Wash the area well with soap and water.
  - Record the patient's name and ID number.
  - Follow institution's guidelines regarding treatment and follow-up.
  - NOTE: The use of prophylactic zidovudine following blood exposure to HIV has shown effectiveness (about 79%) in preventing seroconversion

PROTECT THE PATIENT

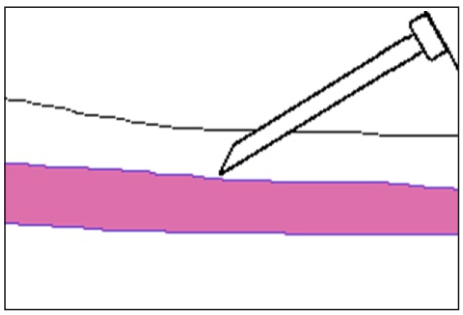
- Place blood collection equipment away from patients, especially children and psychiatric patients.
- Practice hygiene for the patient's protection. When wearing gloves,

change them between each patient and wash your hands frequently. Always wear a clean lab coat or gown.

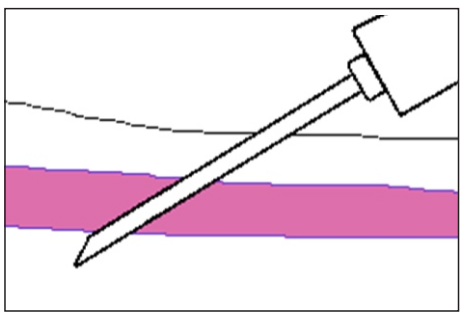
TROUBLESHOOTING GUIDELINES:

IF AN INCOMPLETE COLLECTION OR NO BLOOD IS OBTAINED:

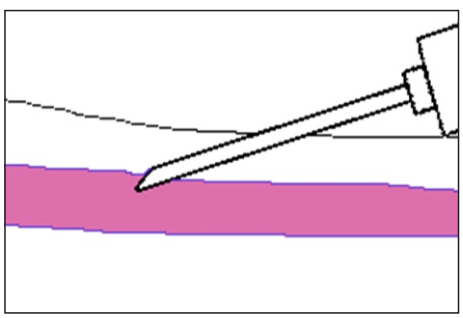
- Change the position of the needle. Move it forward (it may not be in the lumen)



- or move it backward (it may have penetrated too far).



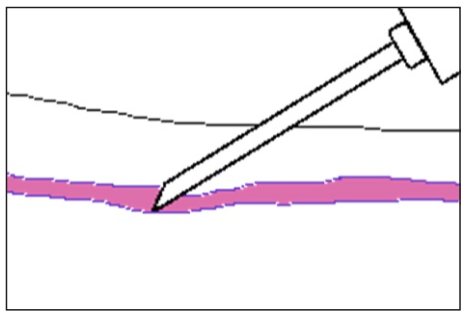
- Adjust the angle (the bevel may be against the vein wall).



- Loosen the tourniquet. It may be obstructing blood flow.
- Try another tube. Use a smaller tube with less vacuum. There may be no vacuum in the tube being used.
- Re-anchor the vein. Veins sometimes roll away from the point of the needle and puncture site.
- Have the patient make a fist and flex the arm, which helps engorge muscles to fill veins.
- Pre-warm the region of the vein to reduce vasoconstriction and increase blood flow.
- Have the patient drink fluids if dehydrated.

IF BLOOD STOPS FLOWING INTO THE TUBE:

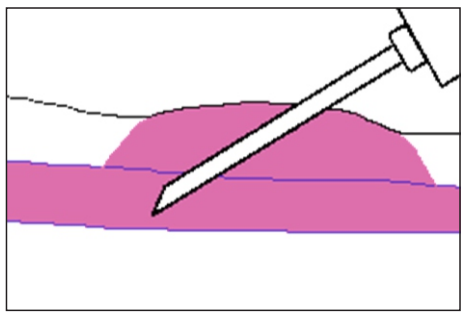
- The vein may have collapsed; resecure the tourniquet to increase venous filling. If this is not successful, remove the needle, take care of the puncture site, and redraw.



- The needle may have pulled out of the vein when switching tubes. Hold equipment firmly and place fingers against patient's arm, using the flange for leverage when withdrawing and inserting tubes.

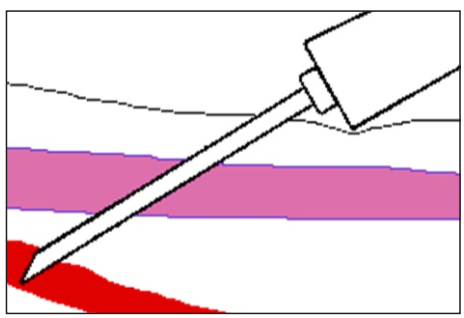
PROBLEMS OTHER THAN AN INCOMPLETE COLLECTION:

- A hematoma forms under the skin adjacent to the puncture site - release the tourniquet immediately and withdraw the needle. Apply firm pressure.



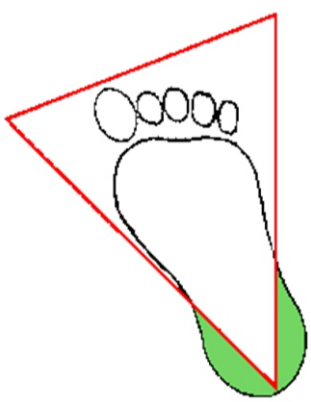
Hematoma formation is a problem in older patients.

- The blood is bright red (arterial) rather than venous. Apply firm pressure for more than 5 minutes.



BLOOD COLLECTION ON BABIES:

- The recommended location for blood collection on a newborn baby or infant is the heel. The diagram below indicates in green the proper area to use for heel punctures for blood collection:



- Prewarming the infant's heel (42 C for 3 to 5 minutes) is important to obtain capillary blood gas samples and warming also greatly increases the flow of blood for collection of other specimens. However, do not use too high a temperature warmer, because baby's skin is thin and susceptible to thermal injury.
- Clean the site to be punctured with an alcohol sponge. Dry the cleaned area with a dry cotton sponge. Hold the baby's foot firmly to avoid sudden movement.
- Using a sterile blood lancet, puncture the side of the heel in the appropriate regions shown above in green. Do not use the central portion of the heel because you might injure the underlying bone, which is close to the skin surface. Do not use a previous puncture site. Make the cut across the heelprint lines so that a drop of blood can well up and not run down along the lines.
- Wipe away the first drop of blood with a piece of clean, dry cotton. Since newborns do not often bleed immediately, use gentle pressure to produce a rounded drop of blood. Do not use excessive pressure or heavy massaging because the blood may become diluted with tissue fluid.
- Fill the capillary tube(s) or micro collection device(s) as needed.
- When finished, elevate the heel, place a piece of clean, dry cotton on the puncture site, and hold it in place until the bleeding has stopped.
- Be sure to dispose of the lancet in the appropriate sharps container. Dispose of contaminated materials in appropriate waste receptacles. Remove your gloves and wash your hands.

HEELSTICK PROCEDURE ILLUSTRATED:

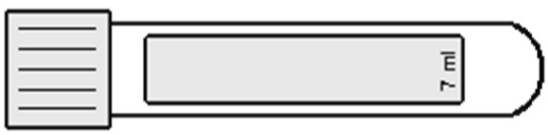
- **Heelstick on baby**

PEDIATRIC PHLEBOTOMY:

- Children, particularly under the age of 10, may experience pain and anxiety during the phlebotomy procedure.
- A variety of techniques can be employed to reduce pain and anxiety. Effective methods use distraction. These may include listening to music or a story, watching a video, playing with a toy, having a parent provide distraction with talk or touch, using flash cards, and squeezing a rubber ball. (Uman et al, 2013)

COLLECTION TUBES FOR PHLEBOTOMY

- Collection tubes can vary in size for volume of blood drawn, appropriate to the tests ordered with sample size required, and vary in the kind of additive for anticoagulation, separation of plasma, or preservation of analyte. Larger tube sizes typically provide for collection of samples from 6 to 10 mL.



- Smaller collection tubes for sample sizes of 2 mL or less may be appropriate in situations where a smaller amount blood should be drawn, as in pediatric patients, or to minimize hemolysis during collection, or to avoid insufficient sample volume in the collection tube.

Red Top

ADDITIVE	None
MODE OF ACTION	Blood clots, and the serum is separated by centrifugation
USES	Chemistries, Immunology and Serology, Blood Bank (Crossmatch)

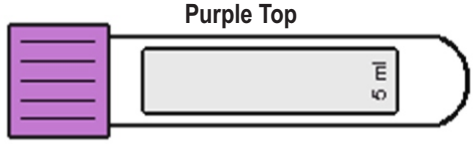
Gold Top

ADDITIVE	None
MODE OF ACTION	Serum separator tube (SST) contains a gel at the bottom to separate blood from serum on centrifugation
USES	Chemistries, Immunology and Serology

Light Green Top

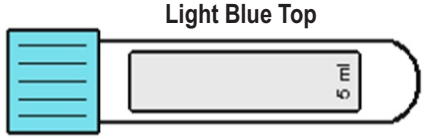
ADDITIVE	Plasma Separating Tube (PST) with Lithium heparin
MODE OF ACTION	Anticoagulates with lithium heparin; Plasma is separated with PST gel at the bottom of the tube
USES	Chemistries

Purple Top



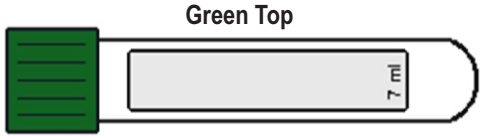
ADDITIVE	EDTA
MODE OF ACTION	Forms calcium salts to remove calcium
USES	Hematology (CBC) and Blood Bank (Crossmatch); requires **full draw** - invert 8 times to prevent clotting and platelet clumping

Light Blue Top



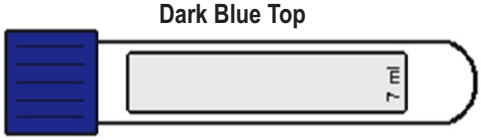
ADDITIVE	Sodium citrate
MODE OF ACTION	Forms calcium salts to remove calcium
USES	Coagulation tests (protime and prothrombin time), **full draw** required

Green Top



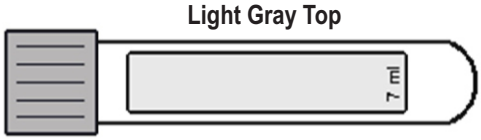
ADDITIVE	Sodium heparin or lithium heparin
MODE OF ACTION	Inactivates thrombin and thromboplastin
USES	For lithium level, use sodium heparin For ammonia level, use sodium or lithium heparin

Dark Blue Top



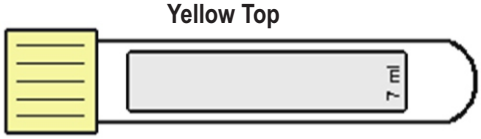
ADDITIVE	EDTA-
MODE OF ACTION	Tube is designed to contain no contaminating metals
USES	Trace element testing (zinc, copper, lead, mercury) and toxicology

Light Gray Top



ADDITIVE	Sodium fluoride and potassium oxalate
MODE OF ACTION	Antiglycolytic agent preserves glucose up to 5 days
USES	Glucoses, requires **full draw** (may cause hemolysis if short draw)

Yellow Top

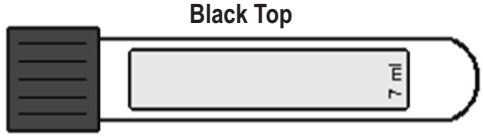


ADDITIVE	ACD (acid-citrate-dextrose)
MODE OF ACTION	Complement inactivation
USES	HLA tissue typing, paternity testing, DNA studies

Yellow - Black Top

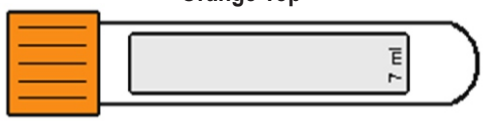
ADDITIVE	Broth mixture
MODE OF ACTION	Preserves viability of microorganisms
USES	Microbiology - aerobes, anaerobes, fungi

Black Top



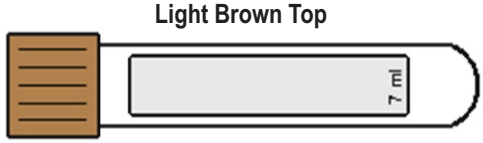
ADDITIVE	Sodium citrate (buffered)
MODE OF ACTION	Forms calcium salts to remove calcium
USES	Westergren Sedimentation Rate; requires **full draw**

Orange Top



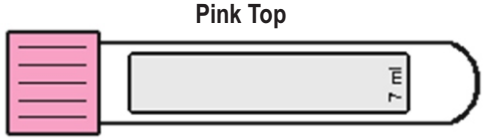
ADDITIVE	Thrombin
MODE OF ACTION	Quickly clots blood
USES	STAT serum chemistries

Light Brown Top



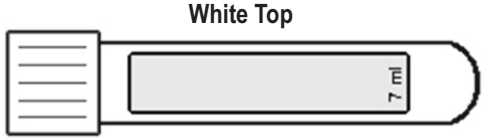
ADDITIVE	Sodium heparin
MODE OF ACTION	Inactivates thrombin and thromboplastin; contains virtually no lead
USES	Serum lead determination

Pink Top



ADDITIVE	Potassium EDTA
MODE OF ACTION	Forms calcium salts
USES	Immunohematology

White Top



ADDITIVE	Potassium EDTA
MODE OF ACTION	Forms calcium salts
USES	Molecular/PCR and bDNA testing

BOUQUET

In Lighter Vein

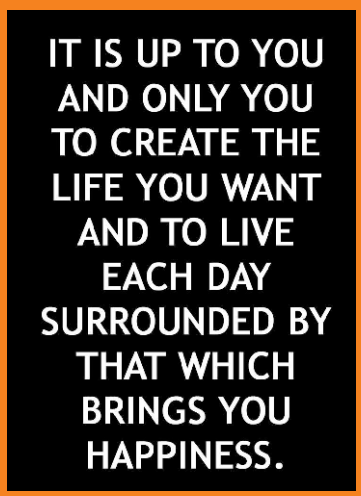
My wife told me she needs more space I said no problem and locked her out of the house

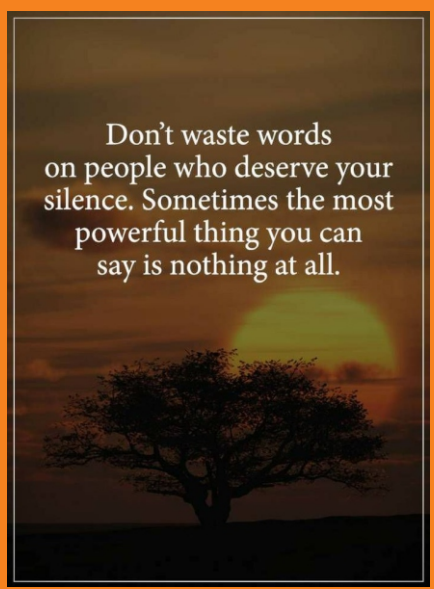
😁😁Got a call from an unknown number you got married I said no and you?

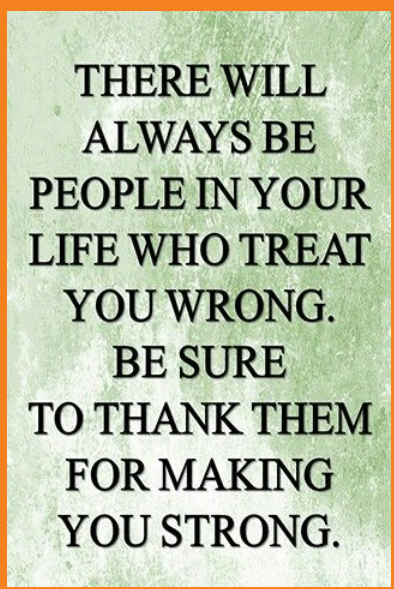
The answer came, come home and then I tell you.😁😁

Little Sam (On Phone): My Son Is Having High Fever And He Won't Be Able To Come To School Today.**Teacher:** Who Is This? **Little Sam:** This Is My Father Speaking!

Wisdom Whispers







Brain Teasers

1. **Reduction in the concentration of hemoglobin in the peripheral blood below normal for age and sex known as:**
   A. Anemia C. Polycythemia
   B. Hematuria D. Methemoglobinemia

2. **Hemoglobin from amount of oxygen it absorbs is estimated by:**
   A. Gasometric method C. Sahli's method
   B. Oxyhemoglobin method D. Cyanmethemoglobin.

3. **The method which does not estimate fetal hemoglobin is:**
   A. Gasometric method C. Sahli's method
   B. Akali hematin method D. Cyanmethemoglobin.

4. **The causes of anemia are:**
   A. Blood loss C. Increased destruction of RBC's
   B. Impaired red cell formation D. All.

ANSWER: 1.A, 2.A,3. B,4. D

Printed and published by D.G. Tripathi, Edited by Dr. Ramnik Sood, M.D. (Path.) for and on behalf of Tulip Diagnostics Private Ltd., Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex Post Office, Goa - 403 202, INDIA.
E-mail: sales@tulipgroup.com Website: www.tulipgroup.com