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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

Which disorder/ disease state is commonly found in infants, is usually found in pregnant women and is sometimes found in the elderly? It also has a higher incidence in the developing nations. Microcytosis and hypochromia are its hallmark features. Yes you have got it right. It is indeed, iron deficiency anaemia. At the insistence of several readers, we are now presenting to you iron deficiency anemia. This is the DISEASE DIAGNOSIS feature of this issue. It is the commonest form of anemia. Patients initially may be asymptomatic. Don't be surprised to see iron deficient individuals eating chalk or even clay! If the causative disorder can be taken care of and no complications have supervened, it is one of the simplest disorders to treat.

You will get a double dose of iron deficiency anemia in this issue as the INTERPRETATION discusses iron indices and ferritin for you. All ailments where testing for serum iron/ TIBC and ferritin can come to the aid of a clinician/ diagnostician are enumerated and considered threadbare for your assimilation. Age-wise reference values are provided. Disorders that lead to raised or lowered values is are listed for reference. Peep inside to get a detailed view.

TROUBLE SHOOTING segment of this issue is carrying the spillover from the previous one. First thing first, we must know how to prepare a sample aptly. Hopefully, by the end of this issue, we would have harvested the proper techniques of sample preparation in a diagnostic laboratory or even before that at the sample procurement site, the hospital. All shipping/ proper transportation details are also provided. At no stage, should a sample get spoilt because of lack of knowledge regarding its appropriate processing procedures. This, precisely, is the aim of the article.

BOUQUET is happily lurking somewhere within the covers. DISCOVER!

The Crux has come of age. It is no longer a toddler but at four years of age, it is constantly looking up and we are sure that with your active participation it can only rise vertically. We invest in this venture because you prompt us to do so. This eight-page communiqué has been appreciated considerably in the subcontinent as also in all the other habitable continents. The Crux Family now encompasses over twenty thousand members and is growing by every issue that is created.

The Crux team places its thanks on record to you the reader! A Laboratarian par excellence!

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DISEASE DIAGNOSIS

IRON DEFICIENCY ANEMIA

Description

Anemia due to decreased iron stores caused by inadequate iron intake, poor absorption, or blood loss. **Most** often seen in women with heavy menses and during pregnancy; cause in men is usually gastrointestinal bleeding. **Skin** and conjunctival pallor may be present, but otherwise physical examination may be normal. **Rarely**, glossitis, koilonychia, angular stomatitis are present. **Treatment** includes increasing dietary iron and oral or intravenous iron replacement.

Synonyms

Anemia of chronic blood loss /Hypochromic, microcytic anemia/ Chlorosis.

Immediate action

If patient is severely anemic with emergent symptoms such as hypoxemia, admit to hospital for possible blood transfusion

Urgent action

Red blood cell (RBC) transfusion is indicated if anemia is life-threatening, depending on patient's hematocrit, rate of blood loss, and comorbid conditions such as angina or other cardiac decompensation. Ferritin, serum iron, and total iron-binding capacity should be drawn prior to transfusion to help clarify diagnosis once patient is stable.

Key Features

Anemia due to reduced iron stores (decreased intake relative to loss). **Diagnosis** is by complete blood count, peripheral blood smears, and serum iron indices. **Hypochromic** microcytic anemia classically.**Treatment** by iron supplementation, manage clinically.

Background

Cardinal features: Most common form of anemia in primary care and clinical hematology. **Patients** may be asymptomatic with a normal physical examination. **Fatigue**, dizziness, exertional dyspnea may be reported by patient. **Conjunctival** and skin pallor may be present. **Koilonychia** - spoon-shaped fingernails that are flat and thin. **Triad** of dysphagia (web of mucosa between hypopharynx and esophagus), angular stomatitis, and lingual abnormalities including glossitis if the cause is malabsorption. **Pagophagia** - ingestion of abnormal amounts of ice. **Pica** - ingestion of dirt, clay, as well as 'food pica': ingestion of abnormal amounts of a specific food, usually crunchy like potato chips, carrots, celery, raw potatoes. **Low** hemoglobin - often< 8g/dL. **Reduced** mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). **Exaggeration** of central pallor of RBCs on peripheral blood smear. **Reduced** serum iron concentration, transferrin saturation, and serum ferritin. **Increased** total iron-binding capacity.

Causes

Common causes: In adults: Diet - rare causes of iron deficiency; more common in developing countries where poverty, war, agricultural practices, or religious and social customs favor diet low in heme iron. **Gastrointestinal** blood loss (common cause) - may be occult with steady loss of blood or frank and can be caused by colon or gastric cancer, diverticulosis, angiodysplasia, gastritis, peptic ulcer disease, inflammatory bowel disease, nonsteroidal anti-inflammatory drugs (NSAIDs), *Helicobacter pylori* and *H. pylori*-related gastritis, and parasitic infections, including hookworm infestation (*Necator americanus* or *Ancylostoma duodenale*). **Menstrual** bleeding (most common cause in women). **Regular** blood donation. **Alveolar** hemorrhage - hemoptysis may not be evident; patients may swallow sputum-tinged blood, leading to occult blood in stools and diagnostic confusion. **Long**-distance runners - mild hemolysis, hematuria of unclear origin, and gastrointestinal bleeding caused by stress, jarring motion, or ischemia **Nosocomial** blood loss - phlebotomy for diagnostic tests in hospitalized patients. **Chronic** renal failure treated with hemodialysis - due to dialysis process and laboratory evaluation. **Pregnancy** and lactation, causing increased demand for iron. **Impaired** absorption - achlorhydria with impaired absorption of inorganic iron. Whether histamine H_2 inhibitors or proton pump inhibitors increase this risk remains controversial. Although some iron-deficient animal models have demonstrated a decrease in iron absorption rates, it is unknown if this holds true for conditions in humans with higher iron absorption requirements such as iron-deficiency anemia. Some consider it more important to follow vitamin B12 than iron levels in patients taking chronic antacid therapy. In general, it is recommended that patients take iron supplements at least 2h before or 4h after antacids. **Pica** - ingestion of dirt, clay, laundry starch, ice, or other unusual food substances; may be cultural. **After** gastric or small bowel surgery - due to loss of gastric acidity; increased transit time for food and decreased absorption of iron. **In infants: Decreased** total body iron at birth - linear relation between body iron and body weight. **Low** birth weight. **Prematurity. Twins. Early** clamping of umbilical cord - up to 100 mL blood left in the placenta; delayed clamping may increase RBC volume by 58%. **Fetomaternal** hemorrhage. **Inadequate** diet - iron in human milk more accessible than in cow's milk. **Blood** loss - may be caused by early introduction of cow's milk into infant's diet.

Rare causes: Factitious anemia: self-induced blood letting. Hereditary hemorrhagic telangiectasia: recurrent hemorrhage from the nose, gastrointestinal tract, and other sites. **Intravascular** hemolysis - such as paroxysmal nocturnal hemoglobinuria.

Serious causes: Gastrointestinal bleeding: may be occult and indicative of gastric or colon cancer. May increase risk of life-threatening hemorrhage, such as with peptic ulcer disease or diverticular bleeding.

Contributory or predisposing factors

Infants and menstrual-aged women are predisposed to iron-deficiency anemia due to increased demand. **Peptic ulcer disease** and its treatment - antacid therapy may decrease iron absorption rate in cases of iron deficiency. ***H. pylori*** and *H. pylori*-related gastritis. **Crohn's disease. Celiac disease.**

Epidemiology

Incidence and prevalence Most common form of anemia seen in general medical and clinical hematology practice.

Prevalence: 50/1000 adult men.**140/1000** adult women. **470/1000** children between 12 and 24 months of age

Frequency: Prevalence in low-income pregnant women 9, 14, and 37% in first, second, and third trimesters, respectively.

Demographics

Age :More common in infants with unsupplemented milk diets. **Common** in women during reproductive years (menses, pregnancy)
Gender : Adults: female > male. **Infants:** female= male.
Race: Common to all races, other factors being the same.
Socioeconomic status: Prevalence higher in those living in chronic poverty.

Diagnosis

Clinical presentation Most commonly, patients are asymptomatic with mild anemia, and the condition is picked up on a routine complete blood count. When symptoms are present, they are related to the severity of the anemia, with constitutional symptoms most common.

Symptoms: Fatigue. Dizziness. Exertional dyspnea. Anorexia. Pagophagia. Pica. Melena. Hematochezia. Hemoptysis.

Signs: Patient may have no signs. **Skin** and conjunctival pallor. **Koilonychia. Angular** stomatitis. **Glossitis**

Differential diagnosis

Anemia of chronic disease

Anemia of chronic disease is present in chronic inflammation such as rheumatoid arthritis, Crohn's disease (may also cause iron-deficiency anemia), and cancer.

Features: Characterized by decreased iron, decreased total iron-binding capacity, increased ferritin, and increased marrow iron stores, red cells that are slightly hypochromic and microcytic, hemoglobin that is normally 7-11g/dL rarely < 7g/dL. **Symptoms** are those of the underlying disease

Sideroblastic anemia

Sideroblastic anemia includes a heterogeneous group of disorders. It is

characterized by ferric phosphate and ferric hydroxide deposits in the erythroblast mitochondria.

Features: **Signs** and symptoms of anemia. **Congenital** (rare, X-linked) and acquired (most commonly idiopathic) forms of the disease present. **Severe** forms recognized in childhood. **Mild** hepatosplenomegaly. **Cardiac** arrhythmias, congestive heart failure. **Impaired** growth and development in infants and children. **Acquired** form may be treated with high-dose pyridoxine.

Thalassemia trait

Inherited disorder that affects synthesis of alpha- or beta-hemoglobin. Thalassemia trait is most commonly seen in the Mediterranean region, Middle East, and Southeast Asia.

Features: **Mild** anemia with microcytosis and hypochromia and poikilocytosis. **Hemoglobin** electrophoresis is diagnostic.

Workup

Diagnostic decision

Diagnosis is suspected on the basis of the history and examination. **Laboratory** testing confirms the diagnosis. **MCV** is an indicator of hypochromic microcytic anemia (a hallmark of iron-deficiency anemia). **Anisocytosis** and increased red cell distribution width (RDW) are the earliest signs of iron deficiency.

Examination

Physical examination is often normal. **Inspect** skin, conjunctiva, mucosa for pallor, blue sclerae. **Inspect** oral cavity for glossitis, angular stomatitis. **Inspect** fingernails for koilonychia - spoon-shaped fingernails that are flat and thin. **Auscultate** heart for tachycardia, arrhythmia. **Perform** a rectal examination and a hemoccult test to determine if rectal bleeding (occult or frank blood) is the cause of possible anemia. A mass may be felt. **Pelvic** examination may reveal uterine cause of excess bleeding. **Urine** analysis to rule out hematuria/hemoglobinuria.

Summary of tests

Complete blood count (CBC). Hemoglobin and hematocrit are used to confirm anemia. **Red blood cell indices** - low MCV confirms microcytic anemia. **Serum ferritin concentration** - low serum ferritin is the most specific indicator of low iron stores. **Total iron-binding capacity** reflects the availability of iron-binding sites on transferrin, which is increased in iron deficiency. **Serum iron concentration** measures the total amount of iron in the serum, which is decreased in iron deficiency. **Bone marrow biopsy** is rarely needed for the diagnosis of iron-deficiency anemia but would typically show absent iron stores. **Erythrocyte protoporphyrin concentration** increases when there is insufficient iron for hemoglobin production. Serum transferrin receptor concentration - increases in iron-deficiency anemia, reflects index of tissue needs; this is no longer recommended since it is less sensitive and less specific than ferritin. **Peripheral blood smear** - shows a microcytic hypochromic anemia, can be useful to rule out other diseases.

Tests

Complete blood count (CBC):

Description **Venous** blood sample taken for laboratory analysis. **Measures** RBCs, hemoglobin/hematocrit, RBC indexes, white blood cells (WBCs), and platelet count, but specifically interested in hemoglobin concentration (information provided for hemoglobin only here)

Advantages/disadvantages

Advantages: Widely available/ Specimen easy to obtain / Rapid results/ Confirms anemia/ Test can be run on capillary tube of blood .**Disadvantages:** Late indicator of iron deficiency/ Not diagnostic of the cause of the anemia.

Normal **Newborn:** 15.0-24.0g/dL , **1-23 months:** 10.5-14.0g/dL , **2-9 years**: 11.5-14.5g/dL **10-17 years (male):** 12.5-16.1g/dL ,**10-17 years (female):** 12.0-15.0g/dL, **>18 years (male):** 13.5-18.0g/dL,**>18 years (female):** 12.5-16.0g/dL. **Abnormal** Any value less than lower limit of normal for age and sex of the patient. **Cause of abnormal result** Inadequate production or excessive loss of blood.

Medications, disorders and other factors that may alter results. Acute blood loss (hemoglobin/hematocrit and RBC morphology changes may lag acute blood loss) / NSAIDs / Aspirin.

Hematocrit

Description Peripheral blood sample.

Advantages/disadvantages

Advantages: Widely available, Specimen easy to obtain, Rapid results, Test can be run on capillary tube of blood. **Disadvantage:** Late indicator of iron deficiency.

Normal. **Newborn:** 44-70%, **1-23 months:** 32-42%, **2-9 years:** 33-43%, **10-17 years (male):** 36-47%, **10-17 years (female):** 35-45%, **>18 years (male):** 42-52% **>18 years (female):** 37-47%. **Abnormal** Any value less than lower limit of normal for age and sex of the patient. **Keep** in mind possibility of a false-positive result. **Cause of abnormal result:** Decreased hemoglobin or increased plasma volume.

Medications, disorders and other factors that may alter results
Acute blood loss (hemoglobin/hematocrit and RBC morphology changes may lag acute blood loss) / NSAIDs / Aspirin

Red blood cell indices

Description: Peripheral blood sample.

Advantages/disadvantages

Advantages: **Specimen** easily obtained. **Low** MCV indicates iron deficiency. **Can** be calculated by manual methods if automated instruments not available. **Disadvantages:** **Nonspecific** to disorder. MCH concentration is poor indicator of iron-deficiency anemia because decrease occurs only when anemia severe.

Normal **MCV:** 82-92 mcm^3 (82-92fL) (hematocrit divided by RBC count). **MCH**: 27-31pg (hemoglobin divided by RBC count). **MCH** concentration (MCHC) 32-36g/dL (320-360g/L) (hemoglobin divided by hematocrit). **Abnormal:** Any value less than lower limit of normal for age and sex of the patient. Keep in mind the possibility of a false-positive result. **Cause of abnormal result:** Changes of intracellular hemoglobin concentration relative to RBC size.

Medications, disorders and other factors that may alter results: Acute blood loss (hemoglobin/hematocrit and RBC morphology changes may lag acute blood loss) / NSAIDs / Aspirin

Serum ferritin concentration

Description: Peripheral blood sample.

Advantages/disadvantages

Advantages: **Single** best indicator of iron storage. **Specimen** easily obtained. **Test** widely available. **Can** be used to measure compliance with therapy. **Disadvantage:** **Not** sensitive - normal value does not rule out iron deficiency

Normal: **Newborns:** 25-200ng/mL (25-200mcg/L). **One month**: 200-600ng/mL (200-600mcg/L). **2-5 months**: 50-200ng/mL (50-200mcg/L).**6 months to 15 years:** 7-142ng/mL (7-142mcg/L). **Adult male:** 20-300ng/mL (20-300mcg/L). **Adult female:** 12-120ng/mL (12-120mcg/L). **Abnormal:** Any value less than lower limit of normal for age and sex of the patient. **Cause of abnormal result:** **Decrease** is indicative of iron-deficiency anemia.

Medications, disorders and other factors that may alter results. Increase can be seen in acute and chronic liver disease, alcoholism, infection and inflammation, iron overload, other anemias, end-stage renal disease.

Total iron-binding capacity

Description: Peripheral blood sample.

Advantages/disadvantages

Advantage: Test is widely available and specimen easily obtained. **Disadvantages:** **Not** useful alone in diagnosing iron deficiency. **May** be elevated in liver damage.

Normal: 250-450mcg/dL (45-81mcmol/L). **Abnormal:** > 450mcg/dL (>81mcmol/L). Keep in mind the possibility of a false-positive result due to factors outlined below . **Cause of abnormal result:** Lack of functional iron.

Medications, disorders and other factors that may alter results. Elevated in liver disease, acute and chronic blood loss, late pregnancy, use of progesterone birth control pills. **Decreased** in hemochromatosis, cirrhosis of liver, thalassemia, anemia related to infection, anemia of chronic disease, nephrosis, hyperthyroidism

Serum iron

Description: Venous blood sample.

Advantages/disadvantages:

Advantages: Test is widely available. **Specimen** easily obtained. **A** measure of the amount of iron bound to transferrin

Normal: 60-170mcg/dL. **Abnormal:** Results below the normal reference range. **Cause of abnormal result:** Lack of functional iron.

Erythrocyte protoporphyrin

Description : Peripheral blood sample.

Advantages/disadvantages

Advantages: This test is not frequently performed but can be very helpful. The RBC zinc protoporphyrin level is increased in both iron deficiency and the anemia of chronic disease in which iron is present but trapped in macrophages and therefore not 'available' for heme synthesis. RBC zinc protoporphyrin is also elevated in lead poisoning. Thus measurement of zinc protoporphyrin may be doubly useful in screening pediatric populations for both iron deficiency and lead poisoning. **Determines** availability of iron for heme synthesis. **Disadvantages: Not** specific. **Sensitivity** and specificity for iron deficiency in adults are significantly less than serum ferritin. **Most** laboratories do not have a hematofluorometer with which to conduct test

Abnormal: >10mcg/dL packed RBCs. **Keep** in mind the possibility of a false-positive result. **Cause of abnormal result.** Lack of iron for last step in heme synthesis.

Medications, disorders and other factors that may alter results: Chronic infections. **Noninfectious** inflammation. **Malignancies**. **Chronic** liver disease. **Moderate** or severe hemolytic anemias

Peripheral blood smear

Description: Peripheral blood sample, smeared on glass slide and stained with Wright-Giemsa stain.

Advantages/disadvantages:

Advantages: Simple, inexpensive test . **Useful** in ruling out additional causes of anemia. **Disadvantage: Subjective,** requires knowledge of red blood cell morphology, expert interpretation

Normal: Qualitatively, red blood cells should be smaller than the nucleus of a normal lymphocyte (small and mature lymphocyte). **The** central clear area should not be overly prominent. **Abnormal: Hypochromic**, microcytic red blood cells. **Anisocytosis**, poikilocytosis . **Cause of abnormal result: Hypochromic**, microcytic red blood cells are associated with iron deficiency anemia, **The** presence of additional abnormalities, such as basophilic stipling of nucleated red blood cells can indicate other disorders, such as lead poisoning

Medications, disorders and other factors that may alter results. Numerous disorders can cause abnormal peripheral blood smears, including infection, bone marrow toxicity (associated with many medications), and hematopoietic malignancy.

Clinical Hallmarks

If ferritin is low (<12ng/mL), it is virtually certain that the patient has iron-r deficiency anemia. **In** male or nonmenstruating woman, assume gastrointestinal bleeding as a cause of anemia until proven otherwise

Treatment

Goals: Replenish iron stores. **Identify** underlying causes for iron deficiency

Immediate action: Iron-deficiency anemia is rarely life-threatening, but blood transfusion may occasionally be needed, especially in patients with cardiac decompensation or in the elderly.

Therapeutic options

Summary of therapies: Treatment of iron deficiency anemia requires the identification and correction of its cause where possible. The anemia itself is usually correctable with oral iron supplements. Ferrous salts only show marginal differences between one another with regards absorption efficency. Hemoglobin regeneration is little affected by the salt given, providing the dose is sufficient. In most patients the speed of response is not critical. **Ferrous sulfate is** the first-choice oral therapy. Gastrointestinal irritation can occur, however this can be minimized by reducing the dose with a longer treatment course. **Ferrous gluconate** is the second-choice oral therapy. Gastrointestinal irritation is less likely, however this is due to a reduced ferrous iron content. Treatment would have to be for a longer period. **Iron dextran** is a parenteral therapy. It is indicated when oral therapy has failed to produce an adequate response. Parenteral use of iron-carbohydrate complexes has caused anaphylactic reactions, and its use should be restricted to patients with an established diagnosis of iron deficiency anemia whose anemia is not corrected with oral therapy. Indications for use may include noncompliance, gastrointestinal disorders exacerbated by oral iron, continued blood loss too severe for oral therapy, and those unable to absorb iron effectively from the gastrointestinal tract.

Efficacy of therapies: Supplemental iron therapy is generally successful in replenishing iron stores that are low in iron-deficiency anemia. **It** is important to find the underlying cause of iron-deficiency anemia to ensure adequate treatment

Outcomes

Prognosis: Curable with iron therapy and if underlying condition can be discovered and treated.

Clinical Hallmarks

Prognosis depends on the underlying cause.

Prevention

Primary prevention:

Modifiable risk factors.

Diet: Inclusion of dietary sources of iron, including meat, beans, leafy green vegetables . **Limit** milk to one pint daily in adults. **Eliminate** pica. **Physical activity:** In runners, decrease the impact by reduced speed or change in technique (less pounding of body weight) or switch to different physical exercise. **Medication history: Use** of antacids to treat peptic ulcer disease may contribute to decreased iron absorption and may indicate possible cause of gastrointestinal blood loss. **NSAID** use may predispose to gastrointestinal blood loss.**Other:** Reassess coexisting disease - gastric surgery may contribute to malabsorption of iron. **Chemoprophylaxis:** Prophylactic iron supplementation in pregnancy.

Secondary prevention: Recurrence is usually related to noncompliance with treatment or recurrence of underlying disorder.

Screening: Screening is recommended for: **Pregnant** women at their first prenatal visit. High-risk infants, preferably at 6-12 months of age. High-risk infants include: **Infants** living in poverty, African-Americans, Native Americans, native Alaskans. **Infants** living in and/or immigrating from developing countries. **Preterm** and low-birth-weight infants. **Infants** whose major dietary intake is unfortified cow's milk .

Hemoglobin and hematocrit: May be measured from capillary tube sample, but venous blood is more accurate.

Serum ferritin: Sensitive indicator for assessing body stores of iron.

INTERPRETATION

IRON INDICES AND FERRITIN

Synonyms Fe and TIBC ; Iron Indices ; Iron Profile ; TIBC ; TIBC and Iron ; Total Iron Binding Capacity ; Transferrin Saturation ; UIBC ; Unsaturated Iron Binding Capacity

Includes: Percent of saturation; serum iron; total iron binding capacity; unsaturated iron binding capacity

Normal Values:

Total serum iron: 60 - 170 g/dL

TIBC: 250 - 450 g/dL

Iron saturation: 20 - 55%

(SI Conversion g/dL x 0.179 mol/L)

Patient Preparation: Patient should be drawn fasting in the morning (circadian rhythm affects Fe). Have sample drawn before patient is given therapeutic iron or blood transfusion. Iron determinations on patients who have had blood transfusions should be delayed for at least 4 days

Reject specimens with Hemolysis; specimen clotted on anticoagulants (these bind iron); improper labeling

Usage: Differential diagnosis of anemia, especially with hypochromia and/or low MCV. The percent saturation sometimes is more helpful than is the iron result for iron deficiency anemia. Evaluate thalassemia and possible sideroblastic anemia; work-up hemochromatosis, in which iron is increased and iron saturation is high. Decrease in iron level after performance of Schilling supports the diagnosis of vitamin B_{12} deficiency, *vide infra*. Evaluate iron poisoning (toxicity) and overload in renal dialysis patients, or patients with transfusion dependent anemias. Use of TIBC in iron toxicity may be less useful than previous believed. TIBC or transferrin is a useful index of nutritional status. Uncomplicated iron deficiency: Serum transferrin (and TIBC) high, serum iron low, saturation low. Usual causes of depleted iron stores include blood loss, inadequate dietary iron. RBCs in moderately severe iron deficiency are hypochromic and microcytic. Stainable marrow iron is absent. Serum ferritin decrease is the earliest indicator of iron deficiency if inflammation is absent. Anemia of chronic disease: Serum transferrin (and TIBC) low to normal, serum iron low, saturation low or normal. Transferrin decreases with many inflammatory diseases. With chronic disease there is a block in movement to and utilization of iron by marrow. This leads to low serum iron and decreased erythropoiesis. Examples include acute and chronic infections, malignancy and renal failure. Sideroblastic anemia: Serum transferrin (and TIBC) normal to low, serum iron normal to high, saturation high. Hemolytic anemias: Serum transferrin (and TIBC) normal to low, serum iron high, saturation high. Hemochromatosis: Serum transferrin (and TIBC) slightly low, serum iron high, saturation very high. Protein depletion: Serum transferrin (and TIBC) may be low, serum iron normal or low (if patient also is iron deficient). This may occur as a result of malnutrition, liver disease, renal disease (eg, nephrosis) or other entities. Liver disease: Serum transferrin variable; with acute viral hepatitis, high along with serum iron and ferritin. With chronic liver disease (eg, cirrhosis), transferrin may be low. Patients who have cirrhosis and portacaval shunting have saturated TIBC/transferrin as well as high ferritin. Chronic dialysis for renal failure: monitor iron levels in patients undergoing dialysis. To follow treatment of iron overload with deferoxamine or with regimen of recombinant human erythropoietin and phlebotomy.

Limitations: Ferritin levels are also useful for iron deficiency. Low iron level may not indicate iron deficiency in acute infection with leukocytosis. Low iron levels may be misleading in chronic infection, inflammation and malignancy; high ferritin levels occur in many such states. However, the most sensitive test for iron deficiency is bone marrow examination. TIBC and transferrin are increased in patients on oral contraceptives, with normal saturation. Gross hemolysis may interfere with serum iron.

Contraindications: Parenteral iron before sample is drawn will cause misleading high iron results. Recent blood transfusion may have only a small positive effect on iron.

Additional Information: **Serum iron is increased** in hemosiderosis, hemolytic anemias especially thalassemia, sideroachrestic anemias, hepatitis, acute hepatic necrosis, hemochromatosis, and with inappropriate iron therapy. Iron may reach high levels with iron poisoning. Some patients who receive multiple transfusions (eg, some hemolytic anemias, thalassemia, renal dialysis patients) will have increased serum iron levels. **Serum iron is decreased** with insufficient dietary iron, chronic blood loss (including the hemolytic anemias paroxysmal nocturnal hemoglobinuria), inadequate absorption of iron and impaired release of iron stores as in inflammation, infection and chronic diseases. The combination of low iron, high TIBC and/or transferrin and low saturation indicates iron deficiency. Without all of these findings together, iron deficiency is unproven. Low ferritin supports the diagnosis of iron deficiency. **Detection of iron deficiency may lead to detection of adenocarcinoma of gastrointestinal tract, a point which cannot be overemphasized.** In recovery from pernicious anemia, especially just after B_{12} dose, iron levels are low. In fact, the drop in serum iron 1 to several days after the Schilling test flushing dose of vitamin B_{12} may be more useful in diagnosis than the radioactivity of the 24-hour urine collection. Serum iron is reported to drop with acute infarct of myocardium.

TIBC is increased in iron-deficiency, use of oral contraceptives, and in pregnancy. **TIBC is decreased** in hypoproteinemia due to many causes, and is decreased in a number of inflammatory states.

Increased saturation occurs with HLA-related (classical) hemochromatosis before ferritin is greatly increased, and also with iron overload (eg, cirrhosis and portacaval shunt), in hemolytic anemias and with iron therapy. Saturation >70% in females, >80% in males is described as prerequisite for parenchymal loading. However, sample contamination and the vagaries of fluctuation in serum iron levels can make such criteria misleading on occasion. The serum ferritin is a more sensitive test than the serum iron or TIBC for iron deficiency and for iron overload. When all these tests are used together, as is often necessary, they usually can distinguish between iron deficiency anemia and the anemia of chronic disease. The best and most reliable evaluation of total body iron stores is by bone marrow aspiration and biopsy. The best evaluation of iron deficiency in childhood (unless lead toxicity is suspected) is free erythrocyte porphyrins. With recombinant erythropoietin therapy serum iron, transferrin saturation, and ferritin levels decline due to rapid utilization by stimulated erythropoiesis with resultant decrease in storage iron. While iron is usually considered in relation to hematopoiesis and oxygen transport functions of red cells, it is also of prime import to the lymphomyeloid systems.

Ferritin

Ferritin is the major storage protein for iron. It is present in virtually all cells where it sequesters iron in a soluble form providing accessible reserves for synthesis of iron containing compounds such as hemoglobin. It is present in particularly large amounts in macrophages and hepatocytes for storage and for metabolic purposes in erythroblasts. Although not a transport protein like transferrin, ferritin is present in small concentrations in the plasma which are directly proportional to the body's total iron stores. This relationship makes the serum or plasma assay for ferritin an ideal non invasive test of iron status.

Serum Ferritin Values:

Age	Values (in ng/mL)	Age	Values (in ng/mL)
Newborns	25 - 200	9 months	14 -103
15 days	90 - 628	1 - 15 years	07 -142
1 month	200 -600	Adult males (20-50 years)	34 - 310
2 months	87 - 430	Adult males (65-87years)	04 - 665
4 months	37 - 223	Adult females (20-50 years)	22 - 112
6 months	19 - 142	Adult females (65-90 years)	13 - 651

Serum Ferritin is increased in: Anemia (chronic , hemolytic, megaloblastic, pernicious, sideroblastic), carcinoma (generalized, hepatic), cirrhosis, hemochromatosis (idiopathic), hepatic disease (acute, chronic), hepatic necrosis, hepatitis, hepatoma, Hodgkin's disease, hyperthroidism, inflammation (chronic), iron intake (excessive dietary or by blood transfusion), leukemia, jaundice, (obstructive), multiple myeloma, polycythemia, renal disease (chronic), rheumatoid arthritis, siderosis, thalassemia (major and minor), tissue trauma, drug intake iron. **Serum Ferritin is reduced in:** Anemia (iron deficiency), hemodialysis, inflammatory bowel disease, pregnancy and surgery (gastrointestinal).

TROUBLESHOOTING

SPECIMEN PREPARATION (...continued)

Directions for Obtaining a Routine Cervical Smear

Do not use lubricating gel. **Do** not use Q-tip to obtain endocervical cells; consider using endocervical brush or a specially designed wooden/plastic spatula. **Do** not obtain during menstruation. **Obtain** a direct scrape of the cervix, preferably at the junction between exocervix and endocervix; consider adding a vaginal pool sample to the cervical sample in women over 40 years of age. **Follow** directions for making Direct Smears.

Note: In order to comply with the standards established internationally, the following guidelines should be followed. The pap smear will be reported as unsatisfactory with the following conditions: **Smears** unlabeled. **Scant** cellularity **Poor** fixation or preservation. Cells are obscured by inflammation, foreign material or blood. **Not** representative of anatomic site. **Slides** broken beyond repair.
A Gyn pap smear will be considered less than optimal when there is no endocervical component present.

Directions for Obtaining a Vaginal Smear for Hormonal Evaluation (Maturation Index)

Do not use lubricating gel. **Obtain** a lateral vaginal wall scrape. **Follow** directions for making Direct Smears.

Directions for Obtaining Gastrointestinal Tract Washings

Collect fasting specimen and put in labeled container. **Inject** 300 mL of normal saline rapidly. **Aspirate** as much as possible of the injected saline and place in labelled container. **If** possible, repeat steps 2. and 3. with patient in different positions. **Specimens** may be pooled or collected separately in containers of 95% alcohol. **Send** immediately to lab.

Directions for Obtaining Urine for Cytology

Specimen can be randomly collected anytime. **Female** patients should be instructed to wash their genitalia with soap and water prior to collection. **Void** directly into container with 50% alcohol. **Send** immediately to the laboratory in securely closed container.

Note: INCLUDE PATIENT AGE AND PERTINENT CLINICAL DATA ON THE REQUEST FORM. If there are any questions about specimen collection or if you need to order the container of fixative, call the Laboratory's Cytology Department.

Pathology, Anatomic (Biopsies and Surgical Specimens)

General Instructions: Use Surgical Pathology requisition form for all biopsies and surgical specimens. Relevant clinical information should be written down in the space provided. **Write** patient's name on specimen container. **Place** all tissues immediately in 10% buffered formalin at ten times the volume of the specimen. Specimen containers with 10% formalin are provided by the laboratory. **Send** immediately to the laboratory in securely closed container. For additional information, please contact the laboratory.

Collection and Transport of Microbiology Specimens:

Correct specimen collection and transport of clinical specimens to the laboratory are extremely important for rapid and accurate identification of significant microorganisms from patient samples. **Please send separate test requisitions for each culture.**
General Consideration for Collection and Transport: Use sterile technique and transport to the laboratory as soon as possible. **Close** collection containers securely to prevent leaking of sample during transport. These specimens are biohazards. Whenever possible obtain specimens prior to the administration of antibiotics. **Do** not use expired tubes or media for specimen collection. **Please** write the patient's name on each specimen container. **Send** specimens in one of the following transport systems: **Swabs** with transport media (culturettes): eye, ear, nose, stool, strep screen, throat, wounds- give site. (Hold at room temperature or refrigerate). **Non sterile** containers: sputum. **Sterile** containers: body fluids (except blood and urine, see below). **Special** transport systems: **Blood** - 2, 20 mL vacutainer tubes with Supplemented Peptone Broth (or any other system). **G.C. Specimens** - Urethral discharge or any source: JEMBEC plates with bag. **Urine** - Container with preservative (boric acid) "Boricon" or even ordinary sterile urine culture container could do. **Parasitology** - Special collection kits with Formalin and PVA.

Body Fluid Culture (For PD fluid see Peritoneal Fluid)

Pleural, pericardial, and synovial fluids must be aspirated aseptically. The body site should be disinfected with an iodophor/povidone prior to aspiration. Use sterile technique. Inoculate into sterile tube or container or blood culture media.

Blood Culture

Disinfect body site with iodophor/povidone prior to venipuncture. Use sterile technique. Inoculate 2 vacutainer tubes containing 18 mL Supplemented Peptone Broth (SPB) - 2 mL draw. disinfect top of tubes with alcohol prior to inoculation. Two sets from separate venipuncture sites recommended. If appropriate Hartleys' broth and Glucose broth may suffice.

Eye, Ear

Collect specimen with a culturette swab. After collection, place swab back into plastic tube. REFRIGERATE OR LEAVE AT ROOM TEMPERATURE.

Fluid

See Body Fluid or Peritoneal Fluid

Environmental (Water and Dialysate) Cultures

See separate instructions for use of Millipore Sampler

Genital Culture

Collect these specimens using a Culturette swab. The swab may be used to culture urethral exudate or inflammation of the vaginal area. While these specimens are not optimal for gonococcal isolation, the diagnosis of vaginitis or urethritis may be made by the recovery of other pathogens. Swabs must be stored at room temperature until transported to the laboratory. If Gonorrhoeae is suspected, do mention that the purpose of the investigation is for Gonorrhea Culture.

Gonorrhea Cultures

JEMBEC (or any other system) plates are provided for the isolation of Neisseria gonorrhoeae from rectal, pharyngeal, and genital sites. JEMBEC plates contain antibiotics to allow the isolation of N. gonorrhoeae from these heavily contaminated areas and must be stored at refrigerated temperatures before use. However, it is important that they be at room temperature at the time of inoculation because cold temperatures inhibit growth of gonococci. Exudate is obtained with a sterile swab and immediately inoculated on the JEMBEC agar. Urethral discharge may be collected with a swab which is then immediately streaked onto the agar surface. A CO_2 atmosphere is necessary to recover neisseria gonorrhoeae. This may be performed by placing a CO_2 generating tablet in the small well present in the plate. The plate is then sealed in a plastic bag. No water is needed to activate the tablet. ONCE SEALED, THE BAG SHOULD BE STORED AT ROOM TEMPERATURE UNTIL TRANSPORTED TO THE LABORATORY.

Gonorrhea/Chlamydia DNA Probe

Specimen collection swabs and transport media are supplied by the laboratory. Remove excess mucus from the cervical os and surrounding mucosa using one of the swabs provided; discard this swab. **Insert** the second swab from the collection kit 1.0 - 1.5 cm into the endocervical canal. **Rotate** the swab 30 seconds in the endocervical canal to ensure adequate sampling. **Withdraw** the swab, avoiding any contact with the vaginal mucosa. **Insert** this swab into the Gen-Probe transport tube, snap off the shaft at the score-line, cap the tube, and store at 2 - 25°C until tested.

Mycology (Yeast)

Cultures for yeast can be submitted on a Culturette. For fluids and sputum, best results are obtained by submitting the entire specimen. If dermatophytes are suspected, the specimen should be submitted in a dry sterile tube.

Nasal Culture

A Culturette swab is gently inserted through the nose to the posterior nasopharynx where it is gently rotated. It should remain in this position for several seconds. The withdrawal should be slow to minimize irritation. Place

the inoculated swab into the sterile plastic tube and crush the ampule.

Parasitology Specimens

Stools for Ova and Parasites should be shipped in Ova and Parasite kits- 5 gm minimum of stool in EACH of the paired vials. CLEAR tape preparation or pinworm paddle is appropriate for submission of specimens for pinworm examination. Submit intact parasites (insects or worms) in 70% alcohol.

Peritoneal Fluid or Dialysate Culture

Disinfect bag's injection sampling port. Use sterile collection technique. Inoculate 3 mL each into four vacutainer tubes with SPB (Supplemented Peptone Broth). Disinfect top of tubes with alcohol prior to inoculation.

Sputum Culture

Instruct the patient to obtain material from a deep cough which is expectorated into a sterile container. Sputum containers are best suited for this collection. The volume of specimen need not be large (3 mL). Once collected, sputum should be refrigerated until transport. Be sure that the cap is tightly sealed on the container once the specimen is collected. A leaky container is a biohazard.

Stool Culture

Use Culturette swab. Obtain pea-size feces on swab and place in culturette.

Throat swab Culture

Use Culturette swab to obtain all types of throat specimens. Rub the sterile swab firmly over the back of the throat (posterior pharynx), both tonsils or tonsillar fossa, and any area of inflammation. Once the specimen is collected, the swab should be replaced in the plastic tube and the ampule at the base crushed to wet the swab.

Throat, Group A Strep Screen

Use Culturette to obtain specimen for Group A Strep only. Results take one day versus 2 - 3 days for complete culture.

Urine Culture Screen

Collect a "clean-catch" mid stream urine into a paper cup. Immediately transfer to Boricon tube with Bacteriostatic preservative. A first morning specimen is preferable and should be refrigerated until pick-up.

Note: It is not necessary to collect a specimen for culture in a sterile cup if the urine is immediately transferred to the Boricon tube.

Wound Culture

A superficial wound culture should be collected with a Culturette swab. After collection, place swab back into plastic tube, and refrigerate or leave at room temperature until transport. If the lesion is not open, a sterile needle and syringe should be employed to remove material. the culturette may be inoculated with this sample.

Important

All test requests are to be signed by the ordering physician and the *time and date* of the specimen collection are to be clearly added to the request form.

Note: Reference Ranges are method dependent and may change if a methodology changes. Check the final report for all reference ranges.

BOUQUET

In Lighter Vein

In a psychiatrist's waiting room two patients are having a conversation. One says to the other, "Why are you here?

The second answers, "I'm Napoleon, so the doctor told me to come here." The first is curious and asks, "How do you know that you're Napoleon?"

The second responds, "God told me I was."At this point, a patient on the other side of the room shouts, "NO I DIDN'T!"

Two psychologists meet at their twentieth college reunion. One of them looks like he just graduated, while the other psychologist looks old, worried and withered

The older looking one asks the other, "What's your secret? Listening to other people's problems every day, all day long, for years on end, has made an old man of me. The younger looking one replies, "Who listens?"

THE MONITOR confirmed cardiac arrest as an elderly man suddenly lost consciousness. After about 20 seconds of resuscitation, he came to consciousness. Explaining to him that his heart has momentarily stopped, I asked if he remembered anything unusual during that time."I saw a bright light," he said, "and in front of me a man dressed in white."Zeroing in on this near-death impression, I inquired if he could describe the figure."Sure, doctor," he replied. "It was you."

Questionnaire

1) How do you tell the difference between psychiatrists and patients at the mental hospital?

A. The patients are the ones that eventually get better and go home.

2) Why do psychiatrists give patients shock treatment?

A. To prepare them for the bill.

Wisdom Whispers

- Love begins with a smile, grows with a kiss, and ends with a teardrop.
- Wind is to fire like distance is to love; it extinguishes the small and enflames the great
- And in the end the love you take is equal to the love you make.
- By all means marry. If you get a good wife you will become happy, and if you get a bad one you will become a philosopher.
- Marital Freedom: The liberty that allows a husband to do exactly that which his wife pleases.
- True happiness arises, in the first place, from the enjoyment of one's self, and in the next, from the friendship and conversation of a few select companions."
- I don't want to get to the end of my life and find that I have just lived the length of it. I want to have lived the width of it as well."
- "A man sooner or later discovers that he is the master-gardener of his soul, the director of his life."

Brain Teasers

1. Shrinkage in cell size by loss of substance is
   (A). Aplasia (B). Atrophy
   (C). Dysplasia (D). Hypoplasia

2. Hypertrophy refers to
   (A). Increase in cell numbers (B). Decrease in cell numbers.
   (C). Increase in cell size (D). Increase in cell size and number

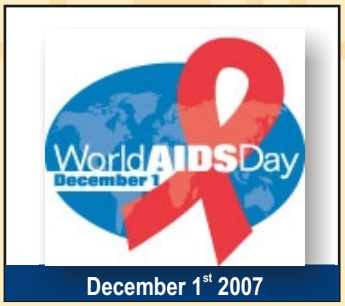
3. Lipofuscin is
   (A). Normally present in m cells. (B). Denatured chlorophyll in intestine
   (C). Stainable by iodine (D). Wear and tear pigment

4. In a plasma cell, the peri-nuclear hoff represents
   (A).Is occupied by ingested bacteria (B).Represents active Golgi apparatus
   (C).Is caused by nuclear degeneration. (D).Represents the extruded nucleus

Answers : 1. (B), 2. (C), 3. (D), 4. (B)

TULIP NEWS

Tulip Group releases an informative poster on HIV testing



December 1st is World AIDS Day. Established by the World Health Organization in 1988, World AIDS Day serves to focus global attention on the devastating impact of the HIV/AIDS epidemic. Observance of this day provides an opportunity for governments, national AIDS programs, faith organizations, community organizations and individuals to demonstrate the importance of the fight against HIV/AIDS. The annual theme for 2007 is : **Stop AIDS: Keep the Promise-Leadership**

Tulip Group has taken this opportunity to highlight the critical issues in HIV testing that includes UNAIDS/WHO testing strategy, false positives, false negatives, generations of HIV assays and their delectability etc., through an informative poster which will be released on the World AIDS Day on 1st December 2007.

Immunoassays for HIV Testing

RETROQUIC
Rapid immunoconcentration assay to detect & differentiate HIV 1&2 antibodies in human serum/plasma
Presentation
10Tests/25Tests/50Tests/75Tests/100Tests

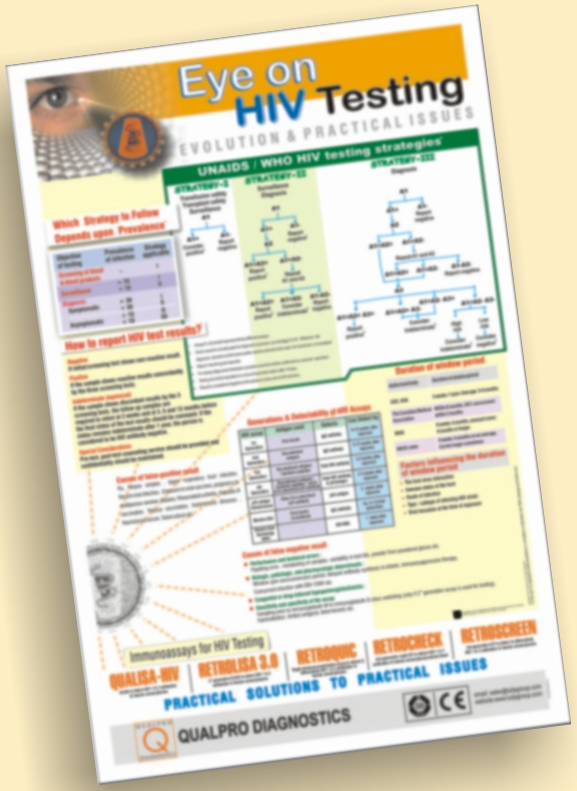
QUALISA-HIV
ELISA to detect HIV1&2 antibodies in human serum/plasma
Presentation
24Tests/96Tests/192Tests/480Tests

RETROSCREEN
3rd generation ICT to detect & differentiate HIV1&2 antibodies in human serum/plasma
Presentation
25Tests/50Tests

RETROLISA 3.0
3rd generation ELISA to detect HIV 1&2 antibodies in human serum/plasma
Presentation
24Tests/96Tests/192Tests/480Tests

RETROCHECK
3rd generation rapid ICT to detect HIV 1&2 antibodies in human serum/plasma/whole blood
Presentation
1Test/10Tests/25Tests/ 50Tests/100Tests

QUALPRO DIAGNOSTICS



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