Introduction To Laboratory Testing

- Eye disease can be diagnosed with history, PE, and in office procedures
- Systemic diseases with ocular manifestations require use of laboratory medicine
  - Thyroid disease, cholesterol, uveitis, GCA, MS, tumors, TB, syphilis, Lyme, Sarcoid, Lupus, gonorrhea, chlamydia, toxoplasmosis, toxocariasis, diabetes, cytomegalovirus, HTN, DM, AD, vascular diseases, HIV/AIDS
Laboratory Structure

- Anatomic pathology – examines tissue biopsy
- Blood bank – transfusions
- Chemistry – examines myriad of blood compounds
- Hematology – examines cells and plasma of blood
- Immunology – detects infections and inflammations
- Microbiology – identify infectious agents
- Nuclear medicine – scans tissues and organs with radiopharmaceuticals

Blood Appropriation

- Fingerstick – blood sampled from finger capillary is of small volume and most useful for single chemical tests
  - Alcohol to fingertip, air dry, lancet to puncture, micropipette to collect
  - Heel in infants and earlobes in adults
- Venipuncture – large samples obtained from superficial veins of midarm, wrist and back of hand
  - Tourniquet above site, clean skin, insert needle into vein and collect to an evacuation container attached to needle
  - Tourniquet removed, needle removed and gauze pad applied

Hematology

- Complete Blood Count (CBC)
  - Red Blood Count (RBC)
    - Indices and morphology
    - Differential
  - White Blood Cell Count (WBC)
    - Hematocrit and Hemoglobin
    - Platelet Count
- Cost - $30.00
- Collection – venipuncture
- Availability – few hours
Hematology

- Red Blood Count (RBC)
  - Men: 4.3-5.4 mil/cu mm (av 4.8)
  - Women: 3.6-5.0 mil/cu mm (av 4.3)
  - Back: carry oxygen to tissue and transfer carbon dioxide via biconcave and flexible shape. RBC lifespan is 120 days. 2 million RBCs produced per second, removed by spleen, liver, marrow. 1/120 total erythrocyte mass replaced each day.
  - Interfering factors – exercise, dehydration, age, altitude, pregnancy
  - Anemia – decrease number of RBCs
    - Due to excessive blood loss or deficient red cell production

- Hematocrit (HCT)
  - Men: 40-54%
  - Women: 37-47%
  - Infants: 50-62%
  - Back: hematocrit means “separate blood”, test determines space occupied by packed red cells expressed as a percentage of RBCs in whole blood
  - Interfering factors – age (normally lower in both sexes after age 50), infants have macrocytic RBCs so always higher, pregnancy (lower), living at higher altitudes yields higher values
**Hematology**

- **Hematocrit (HCT)**
  - Decreased values – anemia (30 or less means moderate anemia, leukemia, hyperthyroidism, cirrhosis, massive blood loss
  - Increased values – polycythemia vera, severe diarrhea, dehydration, acute poisoning, shock
  - HCT usually follows the RBC count when cell are of normal size
  - Cost: $17.00

- **Hemoglobin (Hgb)**
  - Men: 14-16.5g/100ml
  - Women: 12-15g/100ml
  - Infants: 14-20g/100ml
  - Backg: hemoglobin is main component of erythrocytes, serves as vehicle for the transportation of O2 and CO2. The iron component is the portion which combines readily with oxygen and gives blood characteristic red color. Each RBC carries 200-300 million molecules of Hgb, and is more important than RBC tests in anemia evaluations. Hgb also serves as an important buffer in extracellular fluid by exchanging chloride for bicarbonate ions in RBCs
  - Test used for screening for diseases associated with anemia, determine severity of anemia, follow response to treatment for anemias, determining acid-base balance
  - Decreased values – anemias, hyperthyroidism, cirrhosis, severe hemorrhage, Lymphoma, Leukemia, lupus, sarcoidosis, CA
  - Increased values – COPD, congestive heart failure (CHF), polycythemia, severe burns
  - HCT and Hgb together with history and other lab tests are very useful
  - Interfering factors – living at higher altitudes will increase the values, excessive fluid intake will decrease the values, pregnancy (lower), many systemic drugs
  - Cost: $17.00
Hematology

- Red Blood Cell Indices
  - Defines size and hemoglobin content of RBCs
  - Macrocytic, microcytic, or normocytic
  - Hypochromic or normochromic
  - AIDS in differentiating anemias
  - Mean Corpuscular Volume (MCV)
  - Mean Corpuscular Hemoglobin (MCH)
  - Mean Corpuscular Hemoglobin Concentration (MCHC)

Mean Corpuscular Volume (MCV)
- Normal values: 87-103 cu um/red cell
  - <87 = microcytic
  - >103 = macrocytic
  - Measures volume occupied by a single red cell
  - Decreased values – iron deficiency anemias, pernicious anemia, Thalassemia
  - Increased values – Alcoholism, liver disease, deficiency of folate
  - Interfering factors – sickle cell and other anemias of abnormal cell shape are likely to cause unreliable tests of MCV

Mean Corpuscular Hemoglobin (MCH)
- Normal values: 27-32 pg
- MCH is a measure of the average weight of hemoglobin in red cells and is useful in diagnosing severely anemic patients
  - Not as useful as MCHC because it uses RBC count which is not always accurate
  - Decreased values – Microcytic anemia
  - Increased values – Macrocytic anemia
**Hematology**

- **Mean Corpuscular Hemoglobin Concentration (MCHC)**
  - Normal values: 32-36%
  - Decreased values – iron deficiency, macrocytic anemias, Thalassemia
  - Increased values – indicates spherocytosis
  - Procedure – MCHC is a calculated value. Average concentration of hemoglobin in red cells is a ratio of weight of hemoglobin to the volume of red cell, or the ratio of Hgb/HCT

- **White Blood Count (WBC)**
  - Normal Values: 5,000-10,000/cu mm
  - Background: Main function of WBC is to fight infection, phagocytosis of invading organisms and produce and transport antibodies in the immune response. WBC lifespan is 13-20 days, removed by lymphatic system. All WBCs are produced in bone marrow except lymphocytes which are made in the lymphatic system (spleen, thymus, tonsils)
  - **Types of Leukocytes**
    - Granulocytes (aka PMNs) – Neutrophils (60-70%), Eosinophils (1-4%), Basophils (0.5-1%)
    - Agranulocytes – Lymphocytes (20-40%), Monocytes (2-6%)

- **Staining Properties of Leukocytes**
  - Differentiating cell types by staining properties of the granules in their cytoplasm
  - Neutrophils – neutral staining reaction
  - Eosinophils – acid stain reaction
  - Basophils – basic stain reaction

- **Explanation of Test** – WBC count indicates the severity of disease process, differential count will identify susceptibilities certain to infections
- **Procedure** – 7ml blood venous sample
**Hematology**

- **Differential White Blood Count (DIFF)**
  - Neutrophils – bacterial infections, stress, inflammatory disorders
  - Eosinophils – allergic disorders and parasitic infestations
  - Basophils – Blood dyscrasias and myeloproliferative diseases
  - Lymphocytes – Viral infections (measles, rubella, chicken pox, bacterial infections)
  - Monocytes – Severe infections as the infection is controlled

The differential count has limited value; it must be interpreted in relation to the total leukocyte count

Cost: $21.00

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**Hematology**

- **Neutrophils (PMNs, Polys)**
  - Normal Values: 50-60% total white count
  - 3,000-7,000/cu mm
  - Backg: Most numerous and important type of white cell in reaction to inflammation, and microbial invasion with phagocytosis.

Interfering factors – children over-respond to infection and elderly under-respond, near death causes large decrease, steroids reduce expected response, myelosuppressive chemotherapy, bone marrow efficiency and reserve

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**Hematology**

- **Neutrophils (PMN)**
  - Decreased values / Neutropenia – acute viral infections like influenza, infectious hepatitis, measles, mumps, poliomyelitis, blood dyscrasias like aplastic and pernicious anemia, hormonal diseases like addison’s disease, acromegaly, HIV
  - Increased values / Neutrophilia – bacterial and parasitic infections, metabolic changes such as diabetic or uremic coma, gout and eclampsia, tissue breakdown as in burns, MI, gangrene, after surgery, venoms
**Hematology**

- **Eosinophils**
  - Normal Values: 1-4% total white count
  - 50-250/cu mm
  - Backg: Capable of phagocytosis. Active in latter stages of inflammation and ingest antigen-antibody complexes, active in allergic and parasitic infections
  - Interfering factors – hourly rhythm (lowest in morning, rises from noon until midnight), stress (labor, eclampsia, burns, postoperative status) causes decrease, steroid therapy will mask eosinophilia

- **Eosinopenia** – increases in steroid production that accompanies most bodily stress, infectious mononucleosis, CHF, Cushing’s syndrome, aplastic and pernicious anemia, drugs (ACTH, epinephrine, thyroxin)
- **Eosinophilia** – response to hyper-immune, allergic or degenerative reactions, parasitic disease, Addison’s disease, lung and bone cancer, chronic skin infections such as psoriasis, Hodgkin’s disease, polycythemia, many tumors

- **Basophils**
  - Normal Values: 0.5-1.0% total white count
  - 25-100/cu mm
  - Backg: Small percentage of white cells with uncertain function. Phagocytic and contain heparin, histamines, serotonin. Tissue basophils are also called mast cells and are not seen in peripheral blood.
  - Explanation of test – basophil counts are used to study allergic reactions. Direct correlation between high basophil counts and high concentration of blood histamines
Hematology
- Basopenia – Acute allergic reactions, hyperthyroidism, stress reactions, prolonged steroid use, hypersensitivity reactions like urticaria, and anaphylactic shock
- Basophilia – Basophilic and granulocytic leukemia, chronic inflammation, polycythemia, chronic hemolytic anemia, following radiation, healing phase of inflammation.

Hematology
- Monocytes
  - Normal Values: 2-6% total white count
  - 100-600/cu mm
  - Background: Agranulocytes, the body’s second line of defense against infection and the largest cells in the normal blood act via phagocytosis to remove injured and dead cells, microorganisms, and insoluble particles from circulating blood. Some escape from the upper and lower respiratory tracts and the gastrointestinal and genitourinary organs performing a scavenger function, clearing debris. These phagocytic cells produce the antiviral agent called interferon.

Hematology
- Monocytosis – viral infections (infectious mononucleosis, chicken pox, mumps, bacterial and parasitic infections (TB, subacute bacterial endocarditis, malaria, ulcerative colitis, enteritis, amebic dysentery), collagen disease, blood disorders (leukemia, lymphoma, myeloma), lupus, agranulocytosis, thromocytic purpura
- Decreased monocyte count – not usually identified with specific diseases
Hematology

- **Erythrocyte Sedimentation Rate (ESR)**
  - Normal Values (Westegren method)
    - Males: 0-15mm/hr
    - Females: 0-20mm/hr
  - Backg: rate at which RBCs settle out of unclotted blood in one hour. Inflammation and necrosis cause alterations in blood plasma proteins resulting in aggregation (rouleau formation) of red cells, which makes them heavier and fall rapidly in a vertical tube. Useful test to diagnose occult disease, follow cases of disease. It is a non-specific test (not considered diagnostic for particular disorder)!

- Increased ESR – all collagen diseases, infections, inflammatory diseases, carcinoma, metal poisoning, tissue destruction, syphilis, nephritis, pneumonia, rheumatoid arthritis (only slightly increased in OA), severe anemia, MI, giant cell arteritis
- Decreased ESR – polycythemia, sickle cell anemia, CHF

- Interfering factors – time from sampling, temperature
  - Increases - pregnancy or menstruation, cholesterol, Oral contraceptives, theophylline, vitamin A, methyldopa
  - Decreases - high albumin, sugar, ACTH, salicylates, Quinine, ethambutol

- Cost: $12.00

Autoimmune Studies

- **C-Reactive Protein Test; (CRP)**
  - Normal: Trace amounts, abnormal = any titre
  - During the course of an inflammatory process an abnormal specific protein appears in the blood. It is absent in the blood of healthy persons. It is considered a transport protein for polysaccharides and interacts with the complement system. CRP is an antigen-antibody test that is a non-specific method of evaluating the severity and course of inflammatory diseases and conditions of necrosis, cancer, or infarction.
  - Positive in giant cell arteritis, rheumatoid arthritis, colon CA, ARMD, rheumatic fever, malignancy, MI after surgery. CRP rises sooner than ESR and decreases sooner than ESR
Hematology

- Hemoglobin S (Sickle Cell Test)
  - Normal Values: 0
  - Backg: Sickle cell anemia is caused by an abnormal form of hemoglobin called “S”, which is not soluble when unoxygenated, causing precipitation and changing RBC shape to a sickle. This shape prevents normal circulation. This disorder is genetically transmitted by a recessive gene with one gene giving “trait” and two genes giving sickle cell disease. The gene can be tracked to the African continent. Approximately 12% of American population has the gene.

- Positives tests are 99% accurate

- Sickle cell trait – abnormal S gene inherited from one parent, with no affect on longevity or no disease is present

- Sickle cell anemia – abnormal S gene inherited from each parent, with all clinical manifestations of disease
  - Positives tests must be confirmed with electrophoresis
  - Genetic implications
  - Care traveling to high altitude, strenuous exercise, general anesthesia.

Hematology

- Platelet Count
  - Normal Values: 150,000-350,000/cu mm
  - Backg: Platelets (thrombocytes) are the smallest of the formed elements of the blood. Activity is necessary for blood clotting. Deficiency yields to prolonged bleeding times or impaired clot formation. Formation is in bone marrow, with 7.5 days lifespan. 2/3rds are circulating in blood and 1/3rd are in spleen.

- Interfering factors – increases at high altitude, strenuous exercise and in winter
**Hematology**

- **Platelet Count**
  - Decreased values / Thrombocytopenia – pernicious, aplastic and hemolytic anemias, pneumonia, cancer chemotherapy, after massive blood transfusion, lesions of bone marrow and MANY drugs
  - Increased values / Thrombocythemia – cancer, leukemia, polycythemia, splenectomy, R.A, iron deficiency anemia, cirrhosis, pancreatitis, TB
    - Unexpected increase in platelets suggests an advanced, disseminated malignancy
  - Cost: $16.00

**Hematology**

- **Prothrombin Time (Pro Time, PT)**
  - Normal Values: 11-16 seconds
  - Backg: Prothrombin is a protein produced in the liver and is used to clot blood. Production of prothrombin depends on adequate intake of vitamin K (green, leafy vegetables). In clot formation, prothrombin is converted to thrombin. Reduced in liver disease.
  - Anticoagulant therapy – normally accomplished by heparin followed by coumadin
    - Cardiac patients maintained at 16-24 sec (2-2.5 times normal)
    - Blood clots are treated to maintain 28-40 sec
    - Pro times above 40 may result in hemorrhages
Laboratory Structure
- Anatomic pathology – examines tissue biopsy
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Blood Chemistry
- Electrolytes – calcium, chloride, potassium, sodium
- Blood sugars – Oral glucose tolerance, fasting blood sugar, 2hr post-prandial, glycosolated hemoglobin
- End products of metabolism – ammonia, bilirubin, blood urea nitrogen, creatinine, uric acid
- Hormone tests – Cortisol, growth hormone, prolactin
- Enzyme tests – alkaline phosphatase, angiotensin converting enzyme, glutamic-oxaloacetic transaminase, creatine kinase, lactic acid dehydrogenase
- Protein – ceruloplasmin, protein, albumin

Blood Chemistry
- Lipoprotein test – cholesterol, free fatty acids, HDL, LDL, VLDL, total lipids, triglycerides
- Thyroid function tests – Thyroxin, triiodothyronine, thyroid stimulating hormone
- Vitamin and mineral tests – folic acid, B-12
Blood Chemistry

- Biochemical profile – aids in diagnosis and management of disease states
- Simultaneous Multiple Analysis (SMA-12) – twelve of the most meaningful and common tests performed by ordering chemistry profile
  - May include blood chemicals such as protein, albumin, calcium, phosphorus, cholesterol, glucose, bilirubin, blood urea nitrogen, creatinine, uric acid, alkaline phosphatase, lactic acid dehydrogenase and serum glutamic-oxaloacetic transaminase
  - Cost: $58.00

Blood Chemistry

- Total serum protein – proteins are used as co-transporters and buffers in the blood. Protein with albumin together is used to evaluate nutritional status, liver function, and nephrotic syndromes
  - Normal: 6.0-8.5g/dl
  - Indicated in alcoholism, nutritional amblyopia, or anorexia
  - Decreased – mal-absorption, burns, severe liver disease
  - Increased – lupus, RA, acute liver disease, myloma, sarcoid
  - Cost - $20.00

Blood Chemistry

- Serum albumin – specific protein whose level is good indicator of nutritional status
  - Normal: 3.2-5.2g/dl
  - Indicated in Liver disease, chronic alcoholism, Kidney disease, Crohn’s disease, burns, and heart disease
  - Decreased – liver disease, burns, starvation, poor iron intake, mal-absorption, diarrhea, nephrosis, eclampsia
  - Increased – not observed
  - Cost - $20.00
**Blood Chemistry**

- **Calcium** – essential for heart, muscle, and nerve function, as well as blood coagulation. Bulk stored in the skeleton. 50% of Ca is protein bound
  - Normal: 8.5-10.6 g/dl
  - Elevated – in carcinoma, hyperparathyroidism, alcoholic dehydration, sarcoidosis, tuberculosis, histoplasmosis, leukemia and hyperthyroidism
  - Decreased – in malnutrition and low protein levels
  - Indicated in eye patients with band keratopathy, lithiasis of the conjunctiva and corneal arcus juvenilis
  - Cost - $25.00

- **Phosphorus** – an inorganic blood compound may be found in some patients with sarcoidosis and diabetic ketosis. Functions to transfer energy, generation of bone, maintenance of acid-base balance, metabolism of glucose and lipids.
  - Normal: 2.5-4.5 mg/dl
  - Elevated – in relation to calcium level, renal insufficiency, excessive intake of alkaline, fractures healing, acromegaly, Addison’s.
  - Decreased – in acute alcoholism, rickets, osteomalacia, diabetic coma, malabsorption syndrome
  - Cost - $20.00

- **Cholesterol** – not considered adequate in evaluating lipid levels, need to consider HDL-C, LDL-C, triglycerides
  - Normal: 200 mg/dl
  - Elevated – in cardiovascular disease and atherosclerosis, obstructive jaundice, hypothyroidism, uncontrolled diabetes
  - Decreased – terminal stages of cancer, anemia, sepsis, stress, hemolytic jaundice
  - Indicated in pronounced arcus juvenilis and Hollenurst plaques, carotid artery bruits, heart disease, MI, stroke
  - Cost - $13.00
**Blood Chemistry**

**Glucose** – useful in diagnosis of disorder of glucose metabolism, mainly diabetes mellitus
- Normal: 70-100 mg/dl
- Elevated – in diabetes mellitus, Cushing’s, acute stress (MI), pheochromocytoma, hyperthyroidism, pancreatitis, chronic liver disease, chronic malnutrition, many drugs (steroids)
- Decreased – overdose of insulin, hepatic necrosis, Addison’s, sepsis, islet cell carcinoma of the pancreas
- Indicated in pronounced refractive changes, polyuria, polydipsia, polyphagia, possible diabetic retinopathy or neuropathy
- Cost - $16.00

**Bilirubin** – useful in testing liver function. Bilirubin is a bile pigment, the breakdown of erythrocyte hemoglobin. It circulate in the plasma bound to albumin. It is a waste product and must be removed
- Normal: 0.1-1.2mg/dl 
  Newborn: 1-12mg/dl
- Elevated – in hepatitis, cirrhosis, alcoholism, some anemias, mononucleosis, obstructive jaundice,
- Indicated in jaundice, yellowing of the conjunctiva
- Cost - $35.00

**Blood urea nitrogen (BUN)** – useful in testing renal function. The formation of urea, the end product of protein catabolism, accounts for most nitrogen excretion in the urine.
- Normal: 8-20 mg/dl
- Elevated – in renal insufficiency, shock, or heart failure, gout, diabetes, dehydration, excessive protein intake
- Decreased - in malnutrition, protein deficiency, or liver disease
- Indicated in jaundice, yellowing of the conjunctiva
- Cost - $12.00
**Blood Chemistry**

- **Creatinine** – useful in testing renal function. It is more specific and sensitive indicator for renal function than the BUN. Creatinine is a nitrogenous waste product from breakdown of muscle creatine phosphate.
  - Normal: 0.6-1.4 mg/dl
  - Elevated – in renal disease with nephron destruction, muscle diseases of gigantism, acromegaly.
  - Decreased – muscular dystrophy
  - Interfering factors – diet high in roast meat, high levels ascorbic acid
  - Cost - $27.00

- **Uric Acid** – is a major end product of metabolism (breakdown of nucleonic acids), 2/3rds cleared from the plasma by the kidney, 1/3rd cleared in the stool.
  - Normal:
    - Males: 3.8-8.5 mg/dl
    - Females: 2.2-7.7 mg/dl
  - Elevated – in gout, diabetes, hypertension, atherosclerosis, and MI, leukemia, chemotherapy for cancer, shock, alcoholism
  - Decreased – in Wilson’s disease
  - Cost - $25.00

- **Alkaline phosphatase (AP)** – is an enzyme found in serum, bone, kidneys, spleen, lung, and other organs where it catalyzes chemical reactions. AP mediates bone formation, functions best at pH 9.
  - Normal: 38-126 IU/L
  - Elevated – in liver problems (hepatitis, cirrhosis, jaundice, space occupying lesions). If there is no liver disease, than elevated AP is probably due to bone pathology, such as carcinoma, Paget’s disease or fracture. Elevated in ocular trauma such as orbital floor fracture.
  - Cost - $32.00
Blood Chemistry

- Lactic acid dehydrogenase (LDH) – is an enzyme found in many tissues including heart, brain, liver, kidney and skeletal muscles. LDH is elevated when one of these tissues is damaged or killed
  - Normal: 300-610 U/L
  - Elevated – in myocardial infarction (12-24 hrs), acute leukemia, pulmonary infarction, shock with necrosis, hepatic disease, skeletal muscle necrosis, cancer, anemias
  - Decreased – in response to cancer therapy
  - Cost - $25.00

Blood Chemistry

- Serum glutamic-oxaloacetic transaminase (SGOT) – is an enzyme found in bone, kidney, heart, spleen, liver, and lung. SGOT is most useful as a liver function test. The enzyme is released into circulation following injury or death to cells (12hrs-5days)
  - Normal: 8-40 U/L
  - Elevated – in liver disease (cirrhosis), Myocardial infarction (4-10Xs normal, peaks at 24hrs) curve follows CPK, congestive heart failure, alcoholism and shock, burns, pancreatitis
  - Decreased – Beriberi, uncontrolled diabetes
  - Cost - $27.00

Urine Formation

- Composition – 95% water and 5% solids
  - End product of metabolism
  - Output of 1-1.5L per day
  - Water, urea and sodium chloride main constituents
- Formation – kidneys, 25% of blood flows through glomerulus / tubule apparatus
  - 1L urine results from 1000L of blood passing through kidney
- Testing – single, random or timed, long term specimen (24hr)
- Macroscopic testing – appearance, specific gravity, color, pH, protein, glucose, ketones, bilirubin, urobilinogen
- Microscopic testing – examines sediment after centrifugation looking for bacteria, RBCs, WBCs, casts and crystals
Urinalysis - Macroscopic

- Appearance – normal color results from metabolic breakdown products such as bile and pigments from patient’s diet
  - Yellow is normal color (SG 1.011-1.019)
  - Straw colored is normal (SG <1.010)
  - Amber colored is normal (SG >1.020)
- Specific gravity – 1.020 is normal, indicates degree of hydration, an indicator of renal function
- Urine volume – increases in uncontrolled diabetes mellitus, called polyuria

- Orange – concentrated urine, restricted fluids, excess sweating, fever
- Brownish-yellow – bilirubin in urine
- Red – hemoglobinuria or beets
- Port wine color – porphyrins or mixture of methemoglobin and oxyhemoglobin
- Dark brown – Addison’s, melanotic tumor, porphyrins, rhubarb
- Brown-black – great deal of hemoglobin, lysol poisoning, or melanin
- Black – alkaptonuria (tyrosine metabolic disorder)
- Smoky – may be due to red cells

Urinalysis - Macroscopic

- Dipstick testing – collect urine in clean, disposable container after fasting or other instructions
  - Numerous tests on each stick expedites testing of glucose, protein, ketones, blood, pH, bile, bilirubin, nitrite, urobilinogen, leukocyte esterase
- Glucose – positive finding is abnormal, ruling out diabetes is mandatory. Serum glucose is a more meaningful test.
- Protein – indicator of renal disease. Usually the protein is albumin. May be sign of renal failure, glomerulonephritis, lupus
**Urinalysis - Macroscopic**

- Ketone bodies – intermediates of fat metabolism formed in the liver, important screening test in diabetes, children and pregnancy. Large amounts occur in diabetic ketoacidosis.
- Blood – hematuria is visually detected, dipstick detected, or microscopically detected.
- pH – 5.0-8.5 with wide variation. UTI yields alkaline shift (i.e., 9.0) and calculi form in basic environment
  - Diet rich in citrus fruit and legumes yields alkaline urine.
  - Diet rich in animal meat and cranberry juice yields acidic urine.
- Bile – Bilirubin is formed from hemoglobin bound to serum protein carried to liver for processing. Bile is then produced and excreted into intestines. Increase when chemicals or viruses interfere with liver function.

**Urinalysis - Macroscopic**

- Urine urobilinogen and bilirubin – form from hemoglobin metabolism and are both considered bile pigments. Tested by dipstick. Bilirubin can appear in the urine in hepatitis, bile duct obstruction, and chemical injury to the liver. Elevated urobilinogen occurs in jaundice and cirrhosis.

**Urinalysis - Microscopic**

- Centrifuged urine yields sediment which can be examined for casts, cells, crystals and bacteria.
- Casts – Bits of concealed protein form a plug in the kidney tubule and are then washed into the urine. Casts of RBCs are indicate glomerular inflammation or renal vascular disease.
- Cells – RBCs may indicate kidney trauma, exercise, passage of stones, tumors. WBCs are indicative of infection within the urinary tract.
- Urinalysis in eyecare – dipstick technique is effective for screening; any positive results should be referred to the primary care provider.
Cerebrospinal Fluid Studies

- CSF is clear, colorless liquid formed in the ventricles of the brain by choroid plexus. 500ml produced per day, replaced three times per day. Functions as a shock absorber, regulates intracranial pressure, transport nutrients and waste products. Constituents of CSF are similar to or lower concentration than blood plasma. CSF contain few cells and little protein. Disease may alter blood-brain barrier and change composition. Normal CSF pressure is 100-200mmH2O in lateral decubitus position. Elevated pressure causes hydrocephalus in children and increased intracranial pressure, papilledema in adults. Removal causes headache in most.

Cerebrospinal Fluid Studies

- CSF examination
  - General appearance, color consistency
  - Pressure (manometer)
  - Cell count
  - Protein, chloride, sugar concentrations
  - Serologic and bacteriologic tests
- Reasons to examine CSF
  - Multiple sclerosis, meningitis, intracranial hemorrhage, elevated CSF
  - Introduction of drugs, anesthetics and contrast material

Procedure for Lumbar Puncture

- Side lying position with head flexed into chest, knees down up to but not compressing abdomen
  - Increases space between lumbar vertebrae for needle insertion
- Select puncture site between L4-L5
- Local anesthetic injected into dermis
- Spinal needle with stylet into midline into subarachnoid space until a “pop” is felt by patient of needle entry into dura mater
- Attach manometer, measure pressure, remove specimen
- Aftercare – lie prone 4-8 hours, fluids, observe for changes in neurologic state (pupils changes, HTN, irritability, numbness), administer analgesics and longer bedrest for headache
Cerebrospinal Fluid Studies

- Pressure of CSF
  - 100-200 mm H2O
  - Increased – in intracranial tumors, purulent meningitis, encephalitis, pseudotumor cerebri
  - Decreased – diabetic coma, obstructing tumors of spinal cord

- Protein electrophoresis; Albumin and IgG
  - Albumin: <43.2mg/dl
  - IgG: <8.3mg/dl
  - Increased – MS, neurosyphilis, TB meningitis, Guillain-Barre
  - Decreased – blockage of flow of CSF

Hormone Studies

- Prolactin
  - Normal Male 0-20ng/ml
  - Normal Female 0-23ng/ml
  - Prolactin is a pituitary hormone essential for initiating and maintaining lactation
  - Increased – galactorrhea, prolactin secreting pituitary tumor, diseases of pituitary stalk, hypothyroidism, renal failure

- Cortisol
  - Normal Male 0-20ng/ml
  - Normal Female 0-23ng/ml
  - Cortisol is a glucocorticoid of the adrenal cortex and affects metabolism of proteins, carbohydrates and lipids. It is the most potent of the glucocorticoids and inhibits the effect of insulin. Higher levels in morning (6-8AM) and lower in the evening (4-6pm). It is a test of adrenal hormone function.
  - Increased – Cushing’s syndrome, stress, hyperthyroidism, obesity
  - Decreased – Addison’s, liver disease, therapy with steroids
  - Interfering factors – pregnancy, oral contraceptives increase
Protein Tests

- Ceruloplasmin
  - Normal 22.9-43.1 mg/dl
  - Ceruloplasmin is a protein that transports copper. 95% of blood copper is in ceruloplasmin
  - Increased – Rheumatoid arthritis, cancer, biliary cirrhosis
  - Decreased – Wilson's disease

Enzyme Tests

- Creatine phosphokinase (CPK), Creatine Kinase (CK)
  - Normal 50-180IU/L
  - Creatine kinase is an enzyme found in high concentration in heart and skeletal muscle, small concentration in brain. Specific index of injury to myocardium and muscle. Divided into three CK iso-enzymes; MM or CK3 (skeletal muscle), BB or CK1 (brain), and MB or CK2 (cardiac muscle)
  - Increased – MI (rise starts 4-6hrs after attack, peaks at 30hrs, returns to normal 2-3days after attack) and muscular dystrophies (even before signs appear), acute cerebrovascular disease, electric shock, cardiac surgery, pulmonary infarction

- Angiotensin Converting Enzyme (ACE)
  - Normal 23-57 units/ml
  - Angiotensin I is produced by the action of renin on angiotensinogen. Angiotensin converting enzyme catalyzes the conversion of angiotensin I to the vasoactive peptide angiotensin II. Angiotensin I is concentrated in the proximal tubules of the kidney. The test is used primarily to evaluate the severity and activity of patients with sarcoidosis. Serum ACE levels are significantly higher in 79% of patients with active sarcoidosis. 5% of normal population have elevated levels.
  - Increased – sarcoidosis 68% positivity in stage I, 86% in stage II, and 92% in stage III, leprosy
  - Decreased – in those treated with prednisone
Blood Sugars

Glucose; Fasting Blood Sugar (FBS)
- Normal: 70-100mg/dl
- Glucose is formed from the digestion of carbohydrates and conversion of glycogen by the liver. This is regulated by insulin and glucagon. Glucagon accelerates the breakdown of glycogen in the liver, causing blood glucose to rise. Insulin increases the permeability of the cellular membranes to glucose, transports glucose into cells for metabolism.
- Screening is done to detect disorders of metabolism from:
  - Inability of the islet cells of pancreas to produce insulin
  - Inability of the liver to accumulate and breakdown glycogen
  - Inability of the intestines to absorb glucose
  - Presence of increased ACTH

Blood Sugars

2-hr Post-Prandial Blood Sugar (2hr PPBS)
- Normal: <140mg/dl
- Taken after a meal and is an excellent screening test for diabetes. Glucose concentration in a fasting specimen obtained 2 hrs after a meal is rarely elevated in normals, but is significantly elevated in diabetic patients.
- Increased – MI, malignancies, pregnancy, malnutrition, advanced cirrhosis, Cushing’s, pheochromocytoma
- Decreased – Islet cell adenoma, Addison’s, anterior pituitary insufficiency

Blood Sugars

Glycosolated Hemoglobin (HbA1c)
- Normal: 2.2-4.8% of total hemoglobin
- Hemoglobin A1 undergoes glycosylation to hemoglobin A1a, A1b, A1c by a slow process within the red blood cells during their circulating lifespan. Glyco-hemoglobin is blood glucose bound to hemoglobin. The red cell binds with some of the glucose in the blood in a one-way reaction. The amount of glyco-hemoglobin depends on the amount of glucose available to the cell over its 120 day life span. This glycosolation is irreversible. The test is an index of long term glucose control or an average of the last 2-3-months.
**Blood Sugars**

- **Glycosolated Hemoglobin (HbA1c)**
  - Tests are not affected by time of day, meal intake, exercise, just-administered diabetic drugs, emotional stress
  - Particular value in diabetic children, unstable insulin-dependent diabetics with markedly variable sugars, or those who change usual habits so their control appears better than it actually is.

- **Oral Glucose Tolerance Test (OGTT)**
  - Normal: FBS<115mg/100ml
  - 30min, 1 hr, and 16 hrs:<200mg/dl
  - 2hrs:<140mg/dl
  - 3hrs:<125mg/dl
  - All three values must be met to be considered normal with negative urine for glucose
  - Timed test of the blood and urine to rule out diabetes by determining the rate of removal of a concentrated dose of glucose from the blood stream. Test the morning after an overnight fast. Normals peak at 30-60minutes and return to normal within three hours.

- **GTT instead of 2hr post-prandial is indicated in certain pts:**
  - Family history of diabetes
  - Obesity
  - Unexplained episodes of hypoglycemia
  - History of recurrent infections, boils, abscesses
  - Transitory glycosuria or hyperglycemia in pregnancy, surgery, stress, MI, ACTH administration
  - Timed at 2hrs for diabetes detection, 3hrs for pregnant females, 5hr test for hypoglycemia
  - Drink a very sweet commercial preparation of liquid containing 75g of glucose, all at once.
  - Blood samples at 30min, 1 hr, 2 hrs, sometimes at 3 hours
**Blood Sugars**

- **Oral Glucose Tolerance Test (OGTT)**
  - In adult onset diabetes, secretion of insulin is delayed until the 2hr point
  - In overt diabetes, there is no secretion of insulin resulting in above normal values throughout the test
  - In hypoglycemia, glucose is below normal after 2hrs point and remains up to 4-5 hrs because of high insulin levels
  - If fasting glucose is >200, GTT is usually not done; if done monitor patient for severe reactions or coma

**Lipoprotein Tests**

- Lipids are fat substances which consist of cholesterol, cholesterol esters, triglycerides, non-etherified fatty acids and phospholipids. Lipoproteins are macromolecular complexes of unique plasma proteins known as apoproteins and lipids that serve in the plasma to transport otherwise insoluble lipids. They are divided into groups based on density, flotation characteristics and electrophoresis mobility. These groups are chylomicrons, beta lipoprotein (low density lipoprotein (LDL)), prebeta lipoprotein (very low density lipoprotein VLDL), and alpha lipoprotein (high density lipoprotein HDL)

- Lipids provide energy for metabolism and serve as precursors of steroids hormones (adrenal, ovaries, and testes), and bile acids. They also make up cell membranes

**Lipids**

- **Cholesterol**
  - Normal <175mg/dl
  - Cholesterol is used by the body to form steroid hormones, bile acids, and cell membranes.
  - Increased – cardiovascular disease and atherosclerosis, jaundice, uncontrolled diabetes
  - Decreased – malabsorption syndromes, stress, sepsis, liver disease, hyperthyroidism
  - Interfering factors – pregnancy, many drugs

- **Framingham Heart Studies** – 1/3rd of all MI patients have cholesterol <200. The implication is that “normal” levels are probably not normal at all
Lipids

- **High Density Lipoproteins (HDL)**
  - Normal: 45mg/dl
  - HDL is the cholesterol carried by alpha lipoproteins. A high HDL is an indication of a healthy metabolic system in a person free of liver disease. HDL serve as transporters of cholesterol and carry it from peripheral tissues to liver for catabolism and excretion. HDL probably inhibit uptake of LDLs.
  - Increased – chronic liver disorders
  - Decreased – in coronary artery disease, chronic physical inactivity, long distance runners
  - Modifications – losing weight, moderate alcohol consumption, lecithin supplements, exercise, less red meat may all increase HDL

Lipids

- **Low Density Lipoproteins (LDL)**
  - Normal: 130mg/dl, High risk: 100mg/dl
  - LDL is the cholesterol rich remnants of the lipid transport vehicle, VLDL.
  - Increased – coronary heart disease, atherosclerosis
  - Modifications – losing weight, moderate alcohol consumption, niacin supplements, exercise, less red meat, less dairy, limit saturated fat, no fried foods, may all decrease LDL

Lipids

- **Triglycerides**
  - Normal: 0-150/dl, lower in females, higher with age
  - Triglycerides are produced in the liver from glycerol and fatty acids. They are used for production of energy. Excess levels of triglycerides are stored in adipose tissue.
  - Increased – atherosclerosis, liver disease, pancreatitis, MI, hyperlipoproteinemias, toxemias, nephrotic syndromes
  - Decreased – malnutrition, congenital lipoproteinemias
  - Modifications – losing weight, low fat diet, exercise
Thyroid Function Tests

- Function of the thyroid gland is to take iodine from circulating blood and combine it with amino acid tyrosine, convert it to thyroid hormone thyroxin (T4), and triiodothyronine (T3). Gland also functions to store T3 and T4 until they are released into blood stream under influence of TSH from pituitary gland. Only a small portion of the hormone is not bound by protein, but it is the free portion that is the true determinant of the thyroid status of the patient.

- Free Thyroxin - T4
  - Normal: 1-2.3ng/dl
  - Free T4 is the metabolically active form of this hormone.
  - Increased – Graves disease, thyrotoxicosis
  - Decreased – hypothyroidism
  - Interfering factors – infant values are higher, heparin falsely elevates

- Free Triiodothyronine - T3
  - Normal: 250-390pg/dl
  - Free T3 is a measure of the free circulating triiodothyronine unbound to protein in circulation.
  - Increased – hyperthyroidism, and T3 toxicosis
  - Decreased – hypothyroidism
  - Interfering factors – radiation
**Thyroid Function Tests**

- **Thyroid Stimulating Hormone – (TSH)**
  - Normal: 1.9-5.4uIU/ml
  - Stimulation of the thyroid gland by thyroid stimulating hormone, which is produced by the anterior pituitary gland causes the release of stored thyroid hormones.
  - Increased – hypothyroidism
  - Decreased – hyperthyroidism
  - Interfering factors – values are lower in aspirin, corticosteroid and heparin treatment

- **Long Acting Thyroid Stimulator – (LATS)**
  - Normal: Present in only 5% of healthy people
  - LATS does not have origin in the pituitary gland. This factor has a longer effect than the TSH and is found in the blood of some hyperthyroid patients. This test is important in the evaluation of any person with thyroid disease especially in identifying persons with malignant exophthalmos and Grave’s disease
  - Increased – exophthalmos, Grave’s disease

**Microbiologic Studies**

- **Tuberculin Skin Test**
  - Intradermal skin test used to detect tuberculin infection. Tuberculin is a protein fraction of tubercule bacilli and when introduced into the skin of a person with active or dormant tuberculosis infection causes a localized thickening of the skin because of an accumulation of small sensitized lymphocytes.
  - Methods of testing
    - Purified protein derivative tubercule antigen test (PPD), read at 48-72 hours after injection. It is intermediate strength.
    - Mantoux test – small needle into volar aspect of forearm
    - Tine test – stainless steel disc with four tines impregnated with PPD pressed into skin. Practical for mass screenings
Microbiologic Studies

- **Tuberculin Skin Test Results**
  - Read within 48-72 hours
  - Examine in good light, inspect for induration by rubbing finger lightly over area of injected skin
  - Measure area of induration
    - Negative: <5mm
    - Doubtful: 5-10mm
    - Positive: >10mm
  - Clinical implications – positive PPD test indicates the presence of TB infection without distinguishing between active or dormant infection; sputum cultures, bronchial washings and chest X-rays are always indicated

Immunodiagnostic Studies

- **Overview** – Immunology studies antigen-antibody reactions in vitro. Aids in diagnosis of immune disorders, infectious disease and allergic reactions. Involves testing of serum proteins such as antigens, immunoglobulins, antibodies. Methods may be based on the rise in titres (reciprocal of the highest dilution serum which causes agglutination in the presence of a specific antigen) of a specific antibody between acute phase and convalescent phase (2-4 weeks later).
- **Purpose of antigen-antibody tests** – demonstrate a change over time, diagnose a condition when biological testing has been ineffective, confirm diagnosis

Immunodiagnostic Studies

- **Types of immunologic Testing**
  - Immunofluorescence – fluorescent antibody testing. Useful in treponemal testing
  - Precipitation – reaction between a soluble antigen and its antiserum leads to a visible form of precipitation. Useful in C-reactive protein testing.
  - Agglutination – antigen mixes with a homologous antiserum causing cells to clump and settle to the bottom. Useful in thyroid testing
  - Compliment – compliment will “fix” to the antigen-antibody complex if it forms in a patient’s serum. Useful in histoplasmosis
  - Enzyme linked immunosorbant assay (ELISA) – useful in hepatitis, rubella and toxocariasis
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**Immunodiagnostic Studies - Bacterial**
- **Syphilis Testing**
  - Normal values: non-reactive, negative.
  - Fluorescent antibody, flocculation or hemagglutination.
  - Background: syphilis is a venereal disease caused by the Trepanema pallidum, a spirochete. Untreated disease progresses through three stages.
    - Primary – 3-6 weeks after exposure a chancre forms at site of inoculation, healing spontaneously.
    - Secondary stage – 2-10 weeks after the disappearance of the chancre, red papular lesions erupt on the skin or pharynx along with granulomatous uveitis.
    - Tertiary stage – granulomatous gummas, cardiovascular disease (aortic arch), deafness, CNS (tabetic gait, AR pupils, insanity).
Immunodiagnostic Studies - Bacterial

- Serologic Tests for Syphilis
  - FTA-ABS: fluorescent Treponema antibody absorption
    - Fluorescent antibody
    - Detects treponemal antibodies, differentiates biologic false positives from true syphilis positives and diagnoses syphilis when clinical signs are present, but other tests are negative
  - TP-MHA: microhemagglutination assay for Treponema pallidum antibodies
    - Hemagglutination
    - Shows presence of treponema antibody. More specific than FTA-ABS

- Non-Syphilitic Conditions Giving False Positives
  - Malaria
  - Leprosy
  - Active immunizations in children
  - Mononucleosis
  - Lupus erythematosus
  - Vaccinia
  - Hepatitis
  - Chickenpox
  - Measles
  - Rheumatoid arthritis, and others
**Immunodiagnostic Studies - Bacterial**

- Biological False Positives (BFPs) – a positive reaction does not necessarily mean the patient has syphilis. Several conditions can cause BFPs. BFPs are by no means “false”, they indicate the presence of serious diseases other than syphilis. Positive tests are always confirmed and correlated with clinical signs. If necessary, other non-screening tests are also ordered.
- Treatment of syphilis will change the clinical course and the serologic patterns of the disease
  - The longer the patient goes untreated, the longer the screening tests will remain reactive

**Immunodiagnostic Studies - Viral**

- Infectious Mononucleosis Tests
  - Screening: Monoscreen
  - Confirmatory: Mono-diff, Monospot, Epstein-Barr virus (specific indirect IgM fluorescent antibody)
  - 1:56 suspicious, 1:224 is diagnostic, with positive reactions lasting 4-8 weeks after symptoms appear
  - High titres are also seen in patients with Burkitt’s lymphoma, nasopharyngeal carcinoma, lymphocytic leukemia, SLE, sarcoidosis

- Rubella Antibody Tests
  - ELISA: negative, not immune
  - ELISA: positive, immune
  - Rubella virus is causative agent of German measles, characterized by fever and rash. Infection in the first trimester of pregnancy is associated with congenital abnormalities, miscarriage or stillbirth. Tests determine susceptibility or immunity to rubella virus. All pregnant women should have antibody screening test during first antepartum visit and at risk persons like doctors, nurses, midwives, dentists etc
Immunodiagnostic Studies - Fungal

- Histoplasmosis, Coccidioidomycosis Antibody Tests
  - Normal: negative
  - Fungi associated with respiratory diseases are acquired by inhalation of spores from sources like dust, soil and bird droppings. Coccidioidomycosis (valley fever, San Joaquin fever) is caused by coccidioides immitis. Histoplasmosis is a granulomatous infection caused by histoplasma capsulatum. Antibodies appear from first to fourth weeks then disappear.
  - Clinical diseases include uveitis, chorioretinitis

- Cryptococcus Antibody Tests
  - Normal: negative
  - Cryptococcus neoformans is a fungi associated with respiratory diseases are acquired by inhalation of spores from pigeon droppings. Symptoms include fever, HA, dizziness, ataxia, somnolescence.
  - Clinical diseases include uveitis, chorioretinitis, lymphoma, sarcoidosis, or steroid therapy

Immunodiagnostic Studies - Parasitic

- Toxoplasmosis Antibody Test
  - Normal: negative, IFA titer positive>1:128
  - Toxoplasmosis is a disease caused by protozoan Toxoplasmosis gondii. It is congenital or acquired. It may be a severe generalized infection or a granulomatous disease of the CNS. Clinical diseases include uveitis, chorioretinitis, intracranial calcifications, convulsions, HIV or steroid therapy. Exposure to cat litter, uncooked meats, or farm animals are risk factors. Titer of 1:256 or higher is positive.
Autoimmune Studies

- **Sjogren’s Antibody Test**
  - Normal: negative for SS-A and SS-B antibodies
  - Sjogren’s syndrome cannot be diagnosed with any single test and has symptoms similar to connective tissue disease like RA, SLE and scleroderma. SS_B antibodies are associated with primary Sjogren’s disease, an immunologic abnormality associated with decreased secretion of exocrine glands. 50% have RA. SS_A antibodies found in Sjogren’s syndrome alone or in Sjogren’s syndrome associated with SLE. Patients with Sjogrens and RA have neither SS-A or SS-B antibodies, they develop antibodies to Epstein-Barr virus.

Autoimmune Studies

- **Allergic Antibody or hypersensitivity; (RAST)**
  - Normal: no detectable specific IgE antibody
  - Allergen specific antibodies can be identified only by radioallergosorbent test (RAST). RAST tests measure the increase and quantity of allergen specific immunoglobin-E antibodies and are an accurate and conventional alternative to skin testing (which spares patient of hypersensitivity reactions)
  - Allergens available include many grasses, molds, trees, weeds, animal epithelia, foods house dust, house dust mites, antibiotics and insects.

Autoimmune Studies

- **Human Leukocyte Antigen Test; (HLA)**
  - Normals are not applicable.
  - The major histocompatibility antigens of man belong to the HLA system, are present on all nucleated cells and are easily identifiable on leukocytes. Each antigen is produced under genetic control by a gene that shares a locus on the chromosome with another gene, one paternal and one maternal. The HLA complex located on the short arm of chromosome 6 is a major histocompatibility complex and controls important immune functions.
  - Useful in tissue matching for transplants, diagnosing certain diseases such as RA, AS, Reiter’s syndrome.
Autoimmune Studies

- **Human Leukocyte Antigen Test; (HLA)**
  - The presence of certain HLA antigens may be associated with an increased susceptibility to a specific disease but does not mandate the development of the disease.
  - Association of HLA to disease states include Ankylosing spondylitis (HLA-B27 90%), multiple sclerosis (HLA-B27, Dw2, A3, B18), Reiters (HLA-B-27), Acute anterior uveitis (HLA-B-27), Juvenile rheumatoid arthritis (HLA-B-27), Myasthenia gravis (HLA-B8), IDDM HLA Bw15, B8
  - HLA typing is used adjunctively to diagnose disease!

Autoimmune Studies

- **Rheumatoid Factor; (RA Factor)**
  - Normal: Negative (<1:20)
  - Blood of patients with rheumatoid arthritis contains a macroglobulin type of antibody called rheumatoid factor. The exact role of RF is unknown in the disease process. RF can be seen the blood of patients with other diseases, such as SLE, endocarditis, TB, syphilis, sarcoid, cancer, Sjogrens. RA remains a clinical diagnosis with morning stiffness, pain on motion, joint swelling, subcutaneous nodules, symmetry of joint involvement, positive RF

Autoimmune Studies

- **Antinuclear Antibody Test; (ANA)**
  - Normal: Negative, positive >1:20
  - Antinuclear antibodies are globulins that react to specific antigens when mixed in the lab. It is used to detect presence of antinucleoprotein factors associated with certain autoimmune diseases. A pattern is associated with systemic lupus erythematosus; another with scleroderma Raynards disease, Sjogrens, TB, mixed connective tissue disease, chronic hepatitis.
  - A negative test is strong evidence against the diagnosis of SLE.
Autoimmune Studies

- C-Reactive Protein Test; (CRP)
  - Normal: Trace amounts, abnormal = any titre
  - During the course of an inflammatory process an abnormal specific protein appears in the blood. It is absent in the blood of healthy persons. It is considered a transport protein for polysaccharides and interacts with the compliment system. CRP is an antigen-antibody test that is a non-specific method of evaluating the severity and course of inflammatory diseases and conditions of necrosis, cancer, or infarction.
  - Positive in giant cell arteritis, rheumatoid arthritis, colon CA, ARMD, rheumatic fever, malignancy, MI after surgery. CRP rises sooner than ESR and decreases sooner than ESR.

Thank you

McGreal Educational Institute

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