lab guide
Foreword by the Director of Health

Medical laboratory services are one of the core components of UNRWA health services. Therefore, quality and conformity of laboratory results are of the utmost importance for the provision of good primary health care services to the Palestine Refugees.

Improvement of laboratory quality services is a continuous responsibility of UNRWA’s health department and its physicians, nurses, and laboratory personnel. Health staff need the support to order proper laboratory tests; to make the selection of the appropriate specimen and proper collection; to ensure the preservation and transportation of the specimen to the laboratory.

On a daily basis, the laboratory personnel implement a laboratory quality management system which includes performing internal quality control, and on a monthly basis external quality control by participating in an international quality assessment scheme. Additionally, laboratory personnel exercise control on the post analytical phase to ensure validation of laboratory tests and releasing laboratory results in a timely manner.

This guideline is to help our doctors and nurses when they are requesting various tests. It contains the test names, normal values, specimen types and sizes as well as descriptions for the patient preparation, special precautions, test descriptions and clinical significance.

I hope you will find this guide useful and that it will support the excellent work the laboratory personnel have been doing in the clinics.

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Director of Health
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Vision and Mission

Vision
To ensure and sustain medical laboratories with competent staff, high quality equipment, instruments and reagents which contribute to the provision of high quality health care services.

Mission
To provide high quality services in the right place and at the right time in respect to the need of healthcare providers and clients.
# Test Information Index

**Test name**

**Normal (reference) values**

**Specimen**

**Patient preparation and special precautions**

**Test description**

**Clinical significance**
Lab Guide
Biochemistry tests section
Fasting Plasma Glucose (FPG)

**Normal Values:**
70-100 mg/dl (3.9-5.6 mmol/L).

**Specimen:**
Size: 1 ml serum or 1 ml, NAF (preferable)/EDTA / Heparin Plasma.
Stability: 24 hours at 2-8 °C and 8 hours at room temperature if serum or plasma is separated within 20 minutes.

**Patient preparation and special precautions:**
Fasting for 8 to 12 hours, water intake is not restricted during the fasting period, but smoking is not allowed.

**Precautions:**
1. Weight reduction diets before testing can reduce carbohydrate tolerance and suggest “false diabetes.”
2. Prolonged oral contraceptive use causes significantly higher glucose levels in the second hour or in later blood specimen.
3. Operative procedures and infectious diseases affect glucose tolerance. Hence, two weeks of recovery should pass before performing the test.
4. Certain drugs impair glucose tolerance levels. These drugs include large doses of salicylates, thiazide diuretics, oral contraceptives, corticosteroids, estrogens, heparin, nicotinic acid, phenothiazine, lithium, and metopirone. If possible these drugs should be discontinued for at least 3 days before testing.
5. The bed rest for long periods influences glucose tolerance results. A glucose tolerance on a hospitalized patient has limited value.

**Test description:**
Glucose is the end product of carbohydrate digestion and glycogen conversion in the liver. Two hormones glucagon and insulin regulate blood glucose.

The detection of glucose in body fluids is important in the diagnosis of diabetes and in the investigation of hypoglycaemia. The increase in blood glucose levels may be caused by pancreatic islet β-cells inability to produce insulin, deficiency in insulin receptors, inability to metabolize the glucagon by the liver, or alteration in the levels of hormones that play a role in glucose metabolism (e.g., ACTH).

**Clinical significance:**
Blood glucose is elevated in the following conditions: diabetes mellitus, cushing’s disease, pancreatitis, pheochromocytoma, pituitary adenoma, advanced liver disease, chronic renal disease, glucagonoma, and acute emotional or physical stress.

Decreased blood glucose (hypoglycemia) can be observed in the following conditions: pancreatic islet cell carcinoma, Addison’s disease, starvation, mal-absorption, hypopituitarism, hypothyroid, liver damage, and enzyme deficiency diseases (e.g., galactosemia, inherited maple syrup urine disease).
Oral Glucose Tolerance test

Normal values:
70 to < 140 mg/dl (3.9 - < 7.8 mmol/L)

Specimen:
Size: 1 ml serum or 1 ml, NAF (preferable)/EDTA / Heparin Plasma.
Stability: 24 hours at 2-8 °C and 8 hours at room temperature if serum or plasma is separated within 20 minutes.

Patient preparation and special precautions:
Fasting for 8 to 12 hours, water intake is not restricted during the fasting period, but smoking is not allowed.

Precautions: similar to FPG above, vomiting after ingestion of the glucose affects the test result

Test description:
In healthy people, the body responds to a large oral glucose dose by immediate releasing of insulin. It peaks in 30 to 60 minutes and returns to normal levels within 3 hours when sufficient insulin is present to metabolize the glucose ingested at the beginning of the test. A fasting blood sample is taken after an overnight fast of 10-12 hours.

Another sample should be collected 2 hours after the oral glucose load of 75 g of glucose in 250 ml of water. Water intake is not restricted during the fasting period, but smoking is not permitted. If OGTT is performed in children, 1.75g of glucose per kg body weight is given, up to a total of 75g.

Clinical significance:
OGTT is performed to confirm the diagnosis of diabetes. The OGTT should be done for patients with the following conditions:
1. Family history of diabetes
2. Obesity
3. Hypoglycemia of unexplained causes
4. History of recurrent infections (boils and abscesses)
5. In women with history of still birth, neonatal death, spontaneous abortions, and premature labor
6. In women who have been diagnosed with gestational diabetes or have delivered a baby weighing more than 4 kg
7. Patients with FPG between 100 – 125 mg/dl
8. Glycosuria or hyperglycemia during myocardial infarction, ACTH administration, surgery, trauma, stress, and pregnancy

Elevated levels:
1. 140-199 mg/dl indicate impaired glucose tolerance;
2. 200 mg/dl is diagnostic for diabetes mellitus;
3. 140 mg/dl in a pregnant woman indicates gestational diabetes;
4. In normal individuals, glucose level return to baseline levels 2 hours after eating; patients taking insulin have lower values;
5. After GI surgery postprandial hypoglycemia may occur. It is also described with hereditary fructose intolerance, galactosemia, and leucine sensitivity;
6. See Technical Diagnostic Criteria in Appendix1.
Two Hours Postprandial Plasma Glucose (2-h PPG)

Normal Values:
< 180 mg/dl (< 10 mmol/L)

Specimen:
Size: 1 ml serum or 1 ml, NAF (preferable)/EDTA / Heparin Plasma.
Stability: 24 hours at 2-8 °C and 8 hours at room temperature if serum or plasma is separated within 20 minutes.

Patient preparation and special precautions:
2 hours after breakfast, water intake is not restricted during the 2 hours after breakfast, but smoking is not allowed.
Drugs should be taken as usual.
Precautions: similar to FPG

Test description:
A postprandial test is performed 2 hours after a meal. Non diabetic patients rarely show elevated glucose concentration in a blood specimen collected 2 hours after meal, but glucose level is significantly increased in diabetic patients.

Clinical significance:
Levels < 140 mg/dl indicate good glycemic control
Levels 140 - 180 mg/dl indicate acceptable glycemic control
Levels > 180 mg/dl indicates poor glycemic control
In normal individuals, glucose level return to baseline levels 2 hours after eating; patients taking insulin have lower values.
Blood Urea

Normal Values:
15 - 50 mg/dl (2.5 - 8.3 mmol/L)

Specimen:
Size: 1 ml serum or 1 ml EDTA / Heparin Plasma
Stability: 3 days at room temperature, 7 days at 4-8 °C and 6 months at - 20°C
Do not use lipemic sera.

Patient preparation and special precautions:
1. A low-protein and high-carbohydrate diet can cause a decreased blood urea levels.
2. Children and women normally have low urea serum levels because they have less muscle mass than a male adult.
3. In late pregnancy, urea serum values normally decreases due to plasma dilution.
4. In older persons, kidney may not be able to concentrate urine adequately; hence they have an increased urea serum.

Test description:
Urea is a major by-product of protein metabolism. Liver synthesizes urea from ammonia by deamination of amino acids. Urea blood concentrations vary with diet, hepatic function and other disease states.

The determination of serum urea is presently the most widely used screening test for the evaluation of kidney function. The test is frequently requested along with the serum creatinine test since simultaneous determination of these two compounds appears to aid in the differential diagnosis of pre renal, renal and post renal hyper uremia. Hyper uremia may also indicate liver disease or dehydration.

Clinical significance:
Increased urea level in blood occurs in the following conditions: congestive heart failure, chronic renal disease such as glomerulonephritis and pyelonephritis, diabetes mellitus with ketoacidosis, protein intake or protein catabolism as occurs in burns or cancers, salt and water depletion, shock, stress, acute MI, urinary tract obstruction, and hemorrhage into GI tract.

Decreased level of blood urea occurs in the following conditions: malnutrition, low protein diets, impaired absorption (celiac disease), nephrotic syndrome, liver failure, and anabolic steroid use.
Creatinine

**Normal values:**
Men: 0.6 – 1.1 mg/dl (53 – 97 µmol/L)
Women: 0.5 – 0.9 mg/dl (44 – 80 µmol/L)

**Specimen:**
Size: 1 ml serum or 1 ml EDTA / Heparin Plasma
Stability: 3 days at room temperature, 7 days at 4-8 °C and 3 months at -20°C
Avoid hemolysis, ecteric and lipemic specimens.
Diluted urine 1+49 with distilled water.

**Patient preparation and special precautions:**
1. Creatinine levels are affected by age and gender.
2. High levels of ascorbic acid and cephalosporin antibiotics can cause a falsely increased creatinine level.
3. Drugs that influence kidney function plus other medications can cause a change in the blood creatinine level.
4. A rich meat diet can cause increased creatinine levels.
5. Creatinine is falsely decreased by bilirubin, glucose, histidine, and quinidine compounds.
6. Ketoacidosis may increase the creatinine serum level.

**Test description:**
Creatinine is produced as a byproduct in the breakdown of muscle creatine phosphate resulting from energy metabolism. Creatinine produced at a constant rate that depends on the individual’s muscle mass and is removed from the body by the kidneys. If the kidney function is affected, the excretion of creatinine decreases. This results in increased blood creatinine levels. Creatinine fulfills many requirements for a perfect filtration marker. It is not protein bound, it is freely filtered. It is not metabolised by the kidney, and it is physiologically inert.

**Clinical significance:**
Determination of blood creatinine is performed in connection with blood urea to assess the kidney function.
Increased levels of creatinine are found in renal failure, urinary tract obstruction, shock, dehydration and reduced renal blood flow.
Uric Acid

**Normal values:**
- Men: 3.7 – 7.8 mg/dl (226 – 468 µmol/L)
- Women: 2.7 – 7.3 mg/dl (160 – 430 µmol/L)

**Specimen:**
- Size: 1 ml serum or 1 ml EDTA / Heparin Plasma
- Stability: 3 days at room temperature, 7 days at 4-8 °C and 6 months at - 20°C
- Diluted urine 1:10 with distilled water.

**Patient preparation and special precautions:**
1. Uric acid is falsely elevated by stress and strenuous exercise.
2. Purine-rich diet (e.g. liver, kidney, sweetbreads) increases uric acid levels.
3. High levels of aspirin decrease uric acid levels.

**Test description:**
Uric acid is the end product of purine metabolism and is formed from the breakdown of nucleonic acids. This poor soluble substance accumulates in body fluids when the enzyme uricase decreases. Two thirds of the uric acid produced daily is excreted by the kidneys, whereas the remaining one third exits by the stool. The overproduction of uric acids occurs when there is either excessive cell breakdown and catabolism of nucleonic acids (as in gout), excessive production and destruction of cells (as in leukemia), or an inability to excrete the substance produced (as in renal failure).

**Clinical significance:**
Elevated uric acid levels (hyperuricemia) occur in the following conditions: gout, renal disease and renal failure, liver disease, hyperlipidemia, obesity, hypothyroidism, hemolytic anemia, sickle cell anemia, leukemia, lymphoma, multiple myeloma, alcoholism, Down syndrome, lead poisoning, starvation, metabolic acidosis, toxemia of pregnancy, and following excessive cell destruction as in chemotherapy and radiation treatment.
Decreased levels of uric acid occur in Fanconi’s syndrome, Wilson’s disease, Hodgkin’s disease, and Xanthinurea (deficiency of xanthine oxidase).
Cholesterol

**Normal values:**
Adults: 150 – 250 mg/dl (3.9 – 6.5 mmol/L)
Children: 120 – 200 mg/dl (3.1 – 5.2 mmol/L)
NCD patients: <200 mg/dl (< 5.2 mmol/L)

**Specimen:**
Size: 1 ml serum or 1 ml EDTA / Heparin Plasma
Stability: 3 days at room temperature, 7 days at 4-8 °C and 3 months at - 20°C

**Patient preparation and special precautions:**
1. Cholesterol levels have seasonal variations. Levels are higher in fall and winter and lower in spring and summer.
2. External estrogens decrease plasma cholesterol levels while pregnancy increases these levels.
3. Cholesterol levels have also positional variations. Levels are lower when sitting versus standing and lower when recumbent versus sitting.

**Test description:**
Cholesterol test helps in evaluating the risk of atherosclerosis, myocardial occlusion, and coronary arterial occlusion as cholesterol test is considered an important screening test for risk factors. Elevated cholesterol levels are a major component in the hereditary hyperlipoproteinemias. It is a part of the lipid profiles. It is also considered sometimes a part of thyroid and liver function studies.

**Clinical significance:**
Cholesterol is a sterol (steroid alcohol) found in animal fats and oils. It is widely present all through the body, in the blood, brain, liver, kidneys, and nerve fiber myelin sheaths. It is the precursor of the biosynthesis of bile acids as well as adrenal, pituitary and sex hormones. It is also the essential component of the bile acids production, in addition to cell membrane development.
HDL – Cholesterol

Normal values:
Men: 35 – 60 mg/dl (0.97 – 1.55 mmol/L)
Women: 45 – 70 mg/dl (1.18 – 1.92 mmol/L)

Specimen:
Size: 1 ml serum or 1 ml EDTA / Heparin Plasma
Stability: 2 days at room temperature, 7 days at 4-8 °C and 3 months at - 20°C

Patient preparation and special precautions:
Estrogen therapy causes an increase in HDL level. Other increasing factors are moderate intake of alcohol and other drugs (especially androgenic and related steroids), and insulin therapy.
HDL levels decrease in the following conditions:
1. Smoking
2. Certain drugs such as steroids, antihypertensive agents, diuretics, and β-blockers
3. Recent illness and stress
4. Anorexia and starvation
5. Lack of exercise, obesity

Test description:
HDL-Cholesterol is a class of lipoproteins produced by the liver and intestines. Both the HDL and LDL are the cholesterol-rich lipoprotein fractions. HDL is consisted of phospholipids and 1 or 2 polipoproteins. It is responsible for the metabolism of the other lipoproteins in addition to cholesterol transport from peripheral tissues to the liver. LDL and HDL act jointly to maintain cellular cholesterol balance by LDL moving cholesterol into the arteries and HDL removing it from the arteries. HDL has also anti-oxidative effect and endothelial function.

Clinical significance:
Decreased HDL levels are atherogenic, whereas elevated HDL levels protect against atherosclerosis by removing cholesterol from vessel walls and transporting it to the liver where it is removed from the body.
HDL is a protecting lipid component against coronary heart disease (CHD). Therefore, it has a diagnostic importance to evaluate the risk for CHD together with LDL cholesterol.
Triglycerides

Normal values:
Men: 40 – 160 mg/dl (0.45 – 1.8 mmol/L)
Women: 40 – 140 mg/dl (0.45 – 1.6 mmol/L)

Specimen:
Size: 1 ml serum or 1 ml EDTA / Heparin Plasma
Stability: 2 days at room temperature, 7 days at 4-8 °C and 6 months at - 20°C

Patient preparation and special precautions:
1. After a heavy meal or alcohol ingestion, triglyceride levels increases. A transient decrease occurs after strenuous exercise.
2. Pregnancy and oral contraceptive use are associated with increased levels.
3. Values may be increased in acute illness, colds, or flu.
4. Fasting for 12-14 Hrs is preferred.

Test description:
Triglycerides are synthesized in the intestinal mucosa by the esterification of glycerol and free fatty acids. Then released into the mesenteric lymphatics and distributed for storage in most body tissues. They are part of the lipid group found in plasma and make up to 95% of adipose tissue in humans as glycerol, fatty acids, and monoglycerides. Because they are insoluble in water, they are the main plasma glycerol esters. Of the total, 80% of triglycerides are in VLDL, and 15% are in LDL.

Clinical significance:
Increased triglycerides serum levels are found in atherosclerosis, secondary hyperlipoproteinemia, glycogen storage diseases, and nephrotic syndrome. They are greatly elevated in chronic hepatitis, alcoholism and diabetes mellitus.
Decreased levels are found in congenital α-β-lipoproteinemia, chronic obstructive lung disease, hyperthyroidism, malnutrition and recent weight loss.
Bilirubin, Total and Direct

Normal values:
Total Bilirubin:  
At birth: up to 5 mg/dl or (85.5 µmol/L)  
At 5 days: up to 12 mg/dl or (205 µmol/L)  
Adult: 1.1 mg/dl or (18.8 µmol/L)  
Direct Bilirubin: Up to 0.25mg/dl or (4.3 µmol/L)

Specimen:
Size: 1 ml serum or 1 ml EDTA / Heparin Plasma  
Stability: 1 day at room temperature, 7 days at 4-8 °C and 6 months at -20°C  
Avoid haemolysis, specimens must be protected from light (kept in dark).

Patient preparation and special precautions:
1. High fat meals should be avoided before taking the specimen. Also contrast media should not be administered 24 hours before measurement; these cause decreased bilirubin levels by interfering with the chemical reactions.  
2. Bilirubin levels can be falsely increased by the yellow color of serum caused by certain foods (eg. carrots, yams) and drugs when tests are done using colorimetric methods (e.g. spectrophotometer).  
3. Prolonged fasting and anorexia raise the bilirubin levels.

Test description:
Bilirubin is produced from the breakdown of haemoglobin in the red blood cells. It is a metabolite of the haem portion of hemoglobin by the reticuloendothelial system. Bilirubin is then released into the bloodstream where it binds tightly to albumin. Then it is removed from the body by the liver, which excretes it into the bile giving the bile its major pigmentation.

Two forms of bilirubin are present in the body: indirect or unconjugated bilirubin, which is albumin bound, and direct or conjugated bilirubin, which circulates freely in the blood until it reaches the liver, where it is conjugated with glucuronide transferase and then excreted into the bile. An increase in protein-bound bilirubin (unconjugated bilirubin) is more frequently related to destruction of red blood cells (haemolysis). An increase in free flowing bilirubin is more likely seen in dysfunction or blockage of the liver.

Clinical significance:
In the normal condition, a small amount of bilirubin is found in the serum. Serum bilirubin levels raise when there is either an excessive destruction of red cells or the liver is unable to excrete the normal amounts of bilirubin produced.

The routine examination measures only the total bilirubin. If the total bilirubin level is normal, it rules out any significant impairment of the excretory function of the liver or excessive hemolysis of red cells. If total bilirubin levels are elevated there will be a need for differentiation of the bilirubin levels by conjugated and unconjugated types.
Hepatocellular jaundice results from injury or disease of the parenchymal cells of the liver caused by: viral hepatitis, infectious mononucleosis, reactions of certain drugs such as chloromazine.

Obstructive jaundice is caused by obstruction of the common bile or hepatic ducts due to bile regurgitation.

Haemolytic jaundice is due to overproduction of bilirubin resulting from haemolytic processes that produce high levels of unconjugated bilirubin. This could happen in the following conditions: transfusion reactions (ABO or Rh incompatibility), pernicious anaemia, Crigler-Najjar syndrome, sickle cell anaemia and erythroblastosis fetalis.

Increased levels of indirect unconjugated bilirubin occur in the following conditions: haemolytic anaemia due to a large haematoma, haemorrhagic pulmonary infarcts, Crigler – Najjar syndrome and Gilbert’s disease.

Increased levels of direct conjugated bilirubin occur in the following conditions: Choledocholithiasis, Dubin-Jonson syndrome, and cancer of the head of the pancreas.
Aspartate Transaminase - (AST), (SGOT)

Normal values:
Men: up to 37 U/L at 37°C or up to 18 U/L at 25°C
Women: up to 31 U/L at 37°C or up to 15 U/L at 25°C

Specimen:
Size: 1 ml serum or 1 ml EDTA / Heparin Plasma
Stability: 3 day at room temperature, 7 days at 4-8 °C and 3 months at - 20°C
Avoid haemolysis.

Patient preparation and special precautions:
1. During pregnancy a slight decrease occurs.
2. Many drugs can cause elevated or decreased levels as can alcohol ingestion.
3. Results are not affected by exercise and IM injections.
4. False decrease is noticed in diabetic ketoacidosis, severe liver disease, and uremia.

Test description:
AST is an enzyme present in tissue of high metabolic activity. It is present in the heart, liver, skeletal muscle, kidney, brain, pancreas, spleen, and lungs. When an injury or death of cells occurs, the enzyme is released into the circulation causing high levels of blood enzyme. Any disease that causes change in these high metabolic tissues will result in a rise in AST level. The increase of AST level in the blood is directly related to the number of damaged cells and the time that passes between injury to the tissue and the test.

Clinical significance:
After severe cell damage the blood AST level will rise in 12 hours and remain elevated for 5 days.
In myocardial infarction, the AST level may be increased to 4 to 10 times the normal values. The AST level reaches a peak in 24 hours and returns to normal by day 3 or 4 post-MI. If a secondary rise in AST levels occurs, this suggests an extension or recurrence of MI. The last curve in MI parallels that of creatinine phosphokinase (CPK).
In liver disease, the increase in AST level may reach 10 to 100 times normal.
Alanine Transaminase - (ALT), (SGPT)

**Normal values:**
Men: up to 42 U/L at 37°C or up to 22 U/L at 25°C  
Women: up to 32 U/L at 37°C or up to 17 U/L at 25°C

**Specimen:**
Size: 1 ml serum or 1 ml EDTA / Heparin Plasma  
Stability: 3 day at room temperature, 7 days at 4-8 °C and 3 months at -20°C  
Avoid haemolysis.

**Patient preparation and special precautions:**
1. Falsely increased and decreased ALT levels may be caused by many drugs.  
2. Salicylates may cause decreased or increased ALT levels.

**Test description:**
ALT is an enzyme found in tissue of high metabolic activity such as the heart, liver, skeletal muscles and red cells. In cases of acute cellular destruction, the enzyme is released into the blood stream from damaged cells. Elevated values usually appear 8 hours after injury and remain for more than 5 days.

**Clinical significance:**
ALT is increased in liver disease, congestive heart failure, acute myocardial infarction, infectious mononucleosis, renal infarcts, acute pancreatitis, drug toxicity, skeletal muscle disease, and heparin therapy.  
AST/ALT comparison: although the AST level is always increased in acute MI, the ALT level does not always increase proportionately. The ALT is usually increased more than the AST in acute extrahepatic biliary obstruction. ALT is less sensitive than AST to alcoholic liver disease.  
ALT levels are decreased in the following conditions: malnutrition and genitourinary tract infection.
Alkaline Phosphatase

Normal values:
Men: 80-306 U/L at 37°C or 50-190 U/L at 25°C  
Women: 64-306 U/L at 37°C or 40-190 U/L at 25°C  
Children: up to 644 U/L at 37°C or /up to 400 U/L at 25 °C

Specimen:
Size: 1 ml serum or 1 ml EDTA / Heparin Plasma  
Stability: 3 day at room temperature, 7 days at 4-8 °C and 2 month at - 20°C  
Avoid haemolysis.

Patient preparation and special precautions:
1. ALP levels are age and gender dependent.  
2. Many drugs produce mild to moderate increases or decreases in ALP levels.  
3. Physiological changes, which increase the ALP levels, are found in young children, those experiencing rapid growth, pregnant women, and post-menopausal women; this level is slightly increased in older persons.  
4. After IV administration of albumin, there is sometimes a marked increase in ALP for several days.  
5. Anti-coagulated blood samples show decreases in ALP levels.

Test description:
Alkaline phosphatase is an enzyme originating mainly in the bone, liver and placenta, with some activity in the kidney and intestines. It functions best at a pH of 9, therefore it is called alkaline.

Alkaline phosphatase (ALP) catalyses the hydrolysis of phosphate monoesters. There are four genes, which encode ALP, the first is the liver-bone-kidney form. This form constitutes the majority of serum ALP activity and differs from one another only in their post translational glycosylation.

When correlated with other clinical findings, alkaline phosphatase is used as an index of liver and bone disease.

Clinical significance:
Increased alkaline phosphatase activity may be related to hepatobiliary and bone disease. Very high alkaline phosphatase activity in serum is seen in patients with bone cancer and marked increase also occurs in obstructive jaundice and biliary cirrhosis. Moderate elevations have been noted in case of Hodgkin's disease, congestive heart failure, infective hepatitis and abdominal problems.
Lab Guide

Haematology tests section
Haemoglobin

**Normal values:**
- Adult Male: 13.5 – 18 g/dl
- Adult Female: 12 – 16 g/dl
- Newborn: 16 – 20 g/dl

**Specimen:**
- Size: 2 ml EDTA whole blood or direct fingers puncture blood
- Stability: 7 days at 4-8 °C

**Patient preparation and special precautions:**
1. High altitudes cause increased Hb values, as well as increased Haematocrit and RBC.
2. Excessive fluid intake causes decreased hemoglobin.
3. In infants, the immature erythropoiesis causes higher haemoglobin levels.
4. In pregnancy, the increased plasma volume causes decrease in haemoglobin levels.
5. Drugs such as gentamicin and methyldopa cause an increased Hb level.
6. Haemoglobin is increased by the extreme physical exercise.

**Test description:**
Haemoglobin is the main constituent of erythrocytes. It works as a vehicle for the transportation of oxygen and carbon dioxide. Haemoglobin is consisted of a protein called globin, and a compound called haem, which contains a red pigment porphyrin and iron atoms. This iron pigment combines readily with oxygen and gives blood its characteristics red color. The oxygen binding capacity is directly proportional to the concentration of haemoglobin rather than to the count of red blood cells because some RBCs contain more Hb than others. The binding capacity of haemoglobin can be expressed as, “each gram of Hb can carry 1.34 ml of oxygen.” This is the reason why Hb determinations are more important than other blood variables in the evaluation of anaemia.

**Clinical significance:**
Haemoglobin levels are decreased in anaemia states. The Hb must be evaluated in the following conditions:
1. Iron deficiency, thalassemia, pernicious anemia, and haemoglobinopathies
2. Hemorrhage (chronic or acute)
3. Liver disease, hypothyroidism
4. Hemolytic anemia caused by:
   a. Transfusions of incompatible blood
   b. Reactions to drugs, infections, physical agents (artificial heart valves, severe burns)
   c. Certain systemic diseases (Hodgkin’s disease, renal cortical necrosis, leukaemia, sarcoidosis, lymphoma, SLE, carcinomatosis)
Levels are increased in:
1. Polycythemia vera
2. Chronic obstructive pulmonary disease
3. Congestive heart failure

Some incidences cause variation in Hb levels such as transfusions, burns, hemorrhages, (where both Hb and Hct are high during and immediately after hemorrhage).
Mean Cell Volume (MCV)

**Normal values:**
76-96 fl

**Specimen:**
Size: 2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

**Patient preparation and special precautions:**
1. Mixed population of macrocytes can result in a normal MCV. Examination of the blood film is necessary to exclude the mixed volume.
2. MCV could be increased by the presence of reticulocytes.
3. MCV could be increased in a marked leukocytosis.

**Test description:**
The mean cell volume is the average volume of all erythrocytes. It is calculated by the formula:

\[
\text{MCV (fl)} = \frac{(\text{Hct} \times 10)}{\text{RBC} \times (10^{12}/L)}
\]

**Clinical significance:**
The mean cell volume is decreased in all anaemias with microcytic erythrocytes.
Mean Cell Haemoglobin (MCH)

Normal values:
27 - 32 pg

Specimen:
Size: 2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

Patient preparation and special precautions:
1. MCH is falsely elevated in hyperlipidemia.
2. Haemoglobin is falsely elevated when the WBC counts more than 50,000/mm3 and therefore falsely elevates the MCH.
3. MCH is falsely elevated by high heparin concentrations.

Test description:
The mean cell haemoglobin is the average load of haemoglobin in erythrocyte. It is used in the diagnosis of severely anemic patients. It is calculated by the formula:

\[
MCH \text{ (pg)} = \frac{(Hb \text{ (g/dl)} \times 10)}{RBC \text{ (10}^{12}/L)}
\]

Clinical significance:
MCH increase is associated with macrocytic anaemia, while a decrease of the MCH is associated with microcytic anaemia.
Mean Cell Haemoglobin Concentration (MCHC)

Normal values:
30%–36%

Specimen:
Size: 2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

Patient preparation and special precautions:
1. Lipemia, rouleaux, cold agglutinins, and high heparin concentrations may cause false increase in the MCHC value.
2. The RBCs cannot accommodate more than 37g/dl of haemoglobin. Therefore, the MCHC value cannot be greater than 37 g/dl. If any, check for errors in haemoglobin measurement or in calculations.

Test description:
The MCHC assess the average concentration of haemoglobin in the red blood cells. This indicator is considered the most important indicator in monitoring therapy for anaemia because it involves the use of the two most accurate haematologic determinations (haemoglobin and haematocrit).
MCHC represents the ratio of the concentration of Hb to the volume of the erythrocyte:

\[
\text{MCHC (g/dl) = } \frac{\text{Hb (g/dl)} \times 100}{\text{Hct} \text{ (%)}}
\]

Clinical significance:
MCHC decreased value means that a unit volume of packed RBCs contains less haemoglobin than normal.
Hypochromic anaemia (MCHC < 30) occurs in: iron deficiency, some thalassemia, chronic blood loss anaemia, and all kinds of microcytic anaemias.
MCHC increased values occur in: newborns and infants, and in spherocytosis.
Red Cell Size Distribution Width (RDW)

**Normal values:**
11.5 - 14.5 coefficient of variation of red cell size (CV)

**Specimen:**
Size: 2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

**Patient preparation and special precautions:**
None

**Test description:**
The RDW is an automated method of measurement and it is helpful in diagnosing some haematologic disorders and in monitoring the response to therapy.
The RDW reflects the degree of anisocytosis (abnormal variation in size of RBCs). Normal RBCs have a slight degree of variation:

\[
\text{RDW (CV %)} = \frac{\text{Standard deviation of RBC size} \times 100}{\text{MCV}}
\]

**Clinical significance:**
The measurement of RDW is necessary to distinguish the iron-deficiency anaemia (low MCV, high RDW) from the uncomplicated heterozygous thalassemia (low MCV, normal RDW).
The RDW can also help in distinguishing early iron-deficiency anaemia (low-normal MCV, elevated RDW) from anaemia of chronic disease (low normal MCV, normal RDW).
RDW increases in: marked reticulocytosis, iron deficiency, vitamin B12 or folate deficiency (pernicious anaemia), abnormal haemoglobin (S, S-C, H), immune haemolytic anaemia, and post- haemorrhagic anaemia.
Blood film examination

Normal values:
Size: Normocytic
Color: Normochromic
Shape: Normocytes
Structure: Normocytes

Specimen:
Size: 2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

Patient preparation and special precautions:
None

Test description:
The stained blood film examination shows the variations and abnormalities in the size, structure, shape, haemoglobin, and staining properties of the erythrocytes. It is used in the diagnosis of blood disorders such as anaemia, thalassemia, and other haemoglobinopathies. Blood film examination is also helpful as a guide to therapy and as an indicator of chemotherapy and radiation therapy. The leukocytes are also examined at this time.

Clinical significance:
The presence of variations in shape, staining, color, and RBC inclusions are indicative of RBC abnormalities.
See Appendix 2: Table of Peripheral Blood Red Cell abnormalities.
Complete Blood Count (CBC)

Normal values:
As per print out of result generated by the automated haematology analyzer

Specimen:
2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

Patient preparation and special precautions:
None

Test description:
The CBC is one of the basic tests that are frequently ordered in medical laboratory. It is consisted of a series of tests that determine number, percentage, concentrations, quality, and diversity of blood cells:

1. White blood cell count (WBC)
2. Differential white blood cell count (Diff)
3. Red blood cell count (RBC)
4. Hematocrit (Hct)
5. Hemoglobin (Hb)
6. Red blood cell indices
7. Platelets count

Clinical significance:
The findings in the CBC are helpful in the diagnosis of haematologic and other systems disorders. It is used in monitoring disease prognosis, response to treatment, and recovery.
White Blood Cells (WBCs) count

Normal values:
4 - 10 $\times 10^3$/cu.mm

Specimen:
Size: 2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

Patient preparation and special precautions:
1. White blood cells have an hourly rhythm. They are present an early-morning low level and late-afternoon high peak.
2. The white blood cells count is high in newborns and infants (10,000/mm3 to 20,000/mm3) and gradually decreases in children until the adult values are reached at about age 21 years.
3. WBCs count increase rapidly with any stressful situation as a result of an increase in endogenous epinephrine production.

Test description:
White blood cells (leukocytes) are part of the immune system. They play the role of defending the body against foreign materials and infectious diseases. White blood cells are divided into two major groups: granulocytes and agranulocytes. The granulocytes have distinctive granules in their cytoplasm and they include neutrophils, basophils, and eosinophils. The agranulocytes have non-lobular nuclei and they do not contain distinctive granules, they include the lymphocytes and the monocytes.

Clinical significance:
The purpose of this test is to determine the white cells quantitative variations associated with infectious diseases, inflammation and leukocytes disorders such as leukaemia and leucopenia.
Differential leukocytes count

Normal values:
- Neutrophils: 50-60% of total WBC
- Eosinophils: 1-4% of total WBC
- Basophils: 0.5-1% of total WBC
- Monocytes: 3-7% of total WBC
- Lymphocytes: 25-40% of total WBC

Specimen:
- Size: 2 ml EDTA whole blood
- Stability: 7 days at 4-8 °C

Patient preparation and special precautions:
None

Test description:
The white blood cells are consisted of five types of leukocytes, each of which performs a specific function:
1. Neutrophils: Pyogenic infections (bacterial)
2. Eosinophils: Allergic disorders and parasitic infestations
3. Basophils: Parasitic infections
4. Lymphocytes: Viral infections (measles, rubella, chickenpox infectious mononucleosis)
5. Monocytes: Sever infections by phagocytosis

Clinical significance:
The WBCs differential count assesses the count of each type of white blood cell, present in the blood. It can be expressed as an absolute value or as a percentage. The absolute value is much more commonly used than the relative value. Such values are calculated automatically by the haematology autoanalyzer.
The test is used to determine the variations in white blood cells in terms of quantity and type.
Neutrophils

Normal values:
50-60% of the total WBC

Specimen:
Size: 2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

Patient preparation and special precautions:
1. Steroid administration causes a neutrophilia peak in 4 to 6 hours and returns to normal by 24 hours.
2. Myelo-suppressive chemotherapy.
3. In children, the response to infection involves a higher degree of neutrophilia, while elderly patients show weak response.
4. When the patient resistance is exhausted or debilitated the body fail to respond with high degree of neutrophilia.
5. A specimen with a clot should not be tested.

Test description:
Neutrophils are the most abundant and important type of leukocytes in the body’s fight against inflammation. They represent a secondary defense after microbial invasion through the process of phagocytosis. On the other hand, they can damage the body tissue by releasing enzymes and endogenous pyogenes. Immature neutrophils are referred to as “band” cells. The term band comes from the nucleus appearance which has not taken the lobed shape of the mature cell yet.

Clinical significance:
Neutrophilia: the increase in relative percentage of the neutrophils for more than 70% occurs in: bacterial infections, inflammation, acute hemolysis of RBCs, tissue necrosis, intoxications by chemicals or drugs, and acute hemorrhage.

Ratio of segmented neutrophils to band neutrophils: the normal ratio is 1 – 3 % of the neutrophils are immature band forms. This ratio changes in the following conditions:
1. Degenerative shift to left: an increase in band forma with no leukocytosis in some massive infections.
2. Regenerative shift to left: an increase in band forms with leukocytosis in bacterial infections.
3. Shift to the right: few band cells with leukocytosis in allergies, liver disease, hemolysis, and cancer.
4. Hyper-segmentation with no band cells in megaloblastic anaemia.

Neutropenia: the decrease of neutrophils for less than 40% occurs in the following conditions: Massive bacterial infections, viral infections, drugs, toxic agents, radiation, Rickettsial diseases, drugs, aplastic anaemia, vitamin B12 deficiency, anaphylactic shock,
Severe renal shock, Addison's disease, and thyrotoxicosis.

Neutropenia due to decreased neutrophils survival occurs in systemic lupus erythematosus (SLE), splenic sequestration, drug-induced, autoimmune-mediated, and infections especially in Escherichia coli sepsis.

Neutropenia of neonates can be caused by: inborn error of metabolism, immune deficits, disorders of myeloid stem cell, and factors of maternal origin.
Eosinophils

**Normal values:**
1-4% of the total WBC

**Specimen:**
Size: 2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

**Patient preparation and special precautions:**
1. Serial eosinophilic counts should be repeated at the same time of the day because they have daily rhythm. They are lowest in the morning and begin to rise from noon until midnight.
2. Eosinophils decrease markedly after administration of corticosteroids.
3. Eosinophils are decreased after stressful situations such as labor, burns, and postoperative states.
4. A specimen with a clot should not be tested.

**Test description:**
Eosinophils are one of the WBCs that can carry out phagocytosis. They become active in the later stages of inflammation and ingest antigen-antibody complexes. Eosinophils especially respond to allergic and parasitic diseases. The granules inside the eosinophils contain histamine as one third of all the histamine in the body exists in these cells.

**Clinical significance:**

**Eosinophilia:** the increase in relative percentage of the eosinophils of more than 5% occurs in: allergies, asthma, hay fever, parasitic diseases, tropical eosinophilia (related to filariasis), chronic skin diseases, pulmonary infiltration, Hodgkin’s disease, lymphomas, eosinophilic gastrointestinal disease, Addison's disease, hypopituitarism, drug reactions, immunodeficiency disorders, and acute renal allograft syndrome.

**Eosinopenia:** the decrease in eosinophils in the circulation is caused by the increased production of adrenal steroid. This is especially associated with the use of ACTH, thyroxin, prostaglandins, and epinephrine as drugs, Cushing's syndrome, bacterial infections with bands increased.

Eosinophilic myelocytes are found only in leukaemia so they have a great significance.
Basophils

Normal values:
0.5-1% of the total WBC

Specimen:
Size: 2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

Patient preparation and special precautions:
1. Drugs such as procainamide and thiopental affect the counts of basophils.
2. A specimen with a clot should not be tested.

Test description:
Basophils constitute a small percentage of the total leukocyte count. They also carry out phagocytosis. The granules in the basophiles contain heparin, histamines, and serotonin. Basophils are found also in the tissue and are called mast cells. These cells are similar to blood basophils. In the normal condition, mast cells are not found in peripheral blood and are rarely seen in healthy bone marrow.

Clinical significance:
Basophilia: the increase in relative percentage of the basophils for more than 1% is commonly associated with Hodgkin’s disease, granulocytic leukaemia, myeloid metaplasia and acute basophilic leukaemia. This increase is found less frequently in allergy, sinusitis, polycythemia vera, after splenectomy, hypothyroidism, and infections such as tuberculosis, smallpox, influenza, and chicken box.

Basopenia: the decrease of basophils for less than 0.2% occurs in the following conditions:
After prolonged therapy with steroids, chemotherapy, and radiation, hereditary absence of basophils, hyperthyroidism, acute rheumatic fever in children, and as a stress reaction for example to pregnancy or myocardial infarction.

The presence of tissue basophilic (tissue mast cells) is found in: anaphylactic shock, asthma, rheumatoid arthritis, hypoadrenalism, macroglobulinemia, urticaria pigmentosa, osteoporosis, chronic liver or renal disease, mast cell leukemia, lymphoma invading bone marrow, and systemic mastocytosis.
Monocytes

Normal values:
3-7% of the total WBC

Specimen:
Size: 2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

Patient preparation and special precautions:
1. Increased false values are caused by drugs such as: ampicillin, carbencillin, griseofulvin, haloperidol, interleukin-3, methsuximide, colony stimulating factor, phosphorus, pipercillin, propylthiouracil, and tumor necrosis factor.
2. Decreased false values are associated with administration of glucocorticoids such as prednisone.
3. A specimen with a clot should not be tested.

Test description:
Monocytes are the largest cells of normal blood. They constitute the body’s second line of defense against infection. The large macrophagic phagocytes are called histiocytes and are classified as monocytes in a differential leukocyte count. Both cells, histiocytes and monocytes, can reverse from one to the other.

The main function of the phagocytic cells is to remove injured and dead cells, microorganisms, and insoluble particles from the circulating blood.

A scavenger function to clear the body of debris is performed by the monocytes that escape from the gastrointestinal and genitourinary organs and from the upper and lower respiratory tracts. These cells generate a substance that has an antiviral agent called interferon.

Clinical significance:
Monocytosis: an increase in the relative percentage of monocytes for more than 10%. It is found mostly in bacterial infections, sub-acute bacterial endocarditis, tuberculosis, and syphilis. Other causes could be monocytic leukaemia, Hodgkin’s disease, carcinoma of stomach and ovary, lipid storage diseases, recovery state of neutropenia, surgical trauma, chronic ulcerative colitis, and tetrachloroethane.

The macrophages (phagocytic monocytes) occur in haemolytic anaemias, severe infections, and lupus erythmatosus.
Lymphocytes

**Normal values:**
25-40 % of the total WBC

**Specimen:**
Size: 2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

**Patient preparation and special precautions:**
1. In newborns, a physiologic lymphocytosis may occur. It includes an elevation in WBCs count, and abnormal lymphocytes, which may be mixed up with malignant cells.
2. A relative increase in lymphocyte count normally seen in African American people.
3. Lymphocytes can be slightly increased in conditions of exercise, menstruation, and emotional stress.
4. Some drugs cause increases in lymphocyte values. Some of these are: amino salicylic acid,
5. chlorpropamide, dexamethasone, grisoefulvin, levodopa, narcotics, niacinamide, propylthiouracil, spironolactone. Other drugs cause a decrease in lymphocyte values such as: antineoplastics, cyclosporine A, folic acid, furosemide, glucocorticoids, ibuprofen, lithium, niacin, phenytoin, pyridoxine, thiamine, tumor necrosis factor, and x-ray therapy.
6. A specimen with a clot should not be tested.

**Test description:**
Lymphocytes are small, mononuclear cells without specific granules. These cells are mobile cells and can migrate to inflammation areas in both early and late stages of the process. They represent the source of serum immunoglobulins. This makes them play a crucial role in cellular immune response and immunologic reactions.

Lymphocytes are produced in the bone marrow and they are consisted of two types: B-lymphocytes that mature in the bone marrow, and T lymphocytes that mature in the thymus gland. B cells are responsible for controlling the antigen-antibody response specific to the offending antigen. It is said to have “memory”. The T cells include CD4+ T-helper cells, killer cells, cytotoxic cells, and CD8+ T-suppressor cells.

Plasma cells are other cells that are similar in appearance to lymphocytes, but they have abundant blue cytoplasm and an eccentric, round nucleus. Plasma cells are not normally present in blood.
**Clinical significance:**

**Lymphocytosis:** the increase in lymphocytes' count is mainly found in acute and chronic lymphoma, infectious lymphocytosis, and the infectious mononucleosis caused by Epstein-Barr virus. It is also increased in other viral diseases such as cytomegalovirus, mumps, measles, chicken pox, and infectious hepatitis and in toxoplasmosis. However, some bacterial diseases cause lymphocytosis such as tuberculosis, brucellosis, and pertussis. Other causes are Crohn’s disease, drug hypersensitivity, hypoadrenalism, Addison’s disease, and thyrotoxicosis.

**Lymphopenia:** occurs in chemotherapy, radiation treatment, after administration of ACTH or cortisone, aplastic anaemia, Hodgkin’s disease, inherited immune disorders, AIDS, congestive heart failure, renal failure, and advanced tuberculosis.
Red Blood Cell (RBCs) count

Normal values:
Adult Males: \(4.70 - 6.10 \times 10^{12}/L\)
Adult Female: \(4.20 - 5.4 \times 10^{12}/L\)

Specimen:
Size: 2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

Patient preparation and special precautions:
1. Posture: recumbent position of a healthy person cause decrease in RBCs of 5%.
2. Dehydration: haemconcentration caused by severe burns, severe persistent vomiting, diuretic abuse, or untreated intestinal obstruction may hide the presence of significant anaemia.
3. The RBC count of a newborn is higher than that of adults. At age 14 years old, the normal adult level is reached and maintained until old age when there is a gradual drop.
4. Altitude: the higher the altitude, the greater the increase in RBC. Decreased oxygen content of the air stimulates the RBC to rise (erythrocytosis).
5. A specimen with a clot should not be tested. The EDTA blood sample tube must be at least three-fourths filled or values will be invalid because of cell shrinkage.

Test description:
Red blood cells main function is to carry oxygen from the lungs to the body tissues and to transfer carbon dioxide from the tissue to the lungs. The haemoglobin present in the red blood cells is responsible for this process. Haemoglobin binds easily with oxygen and carbon dioxide and gives arterial blood a bright red appearance.

Clinical significance:
RBC values decrease in:
1. Anaemia of any origin such as blood loss, cell destruction, iron deficiency or deficiency of any other vitamins essential in the production of RBCs.
2. Disorders such as lupus erythematosus, lymphomas and leukaemia, rheumatic fever, Addison's disease, sub-acute endocarditis, and chronic infections.

RBC values increase erythrocytosis in:
1. Primary erythrocytosis (polycythemia vera, erythemic erythrocytosis);
2. Secondary erythrocytosis (renal disease, extra-renal tumors, high altitudes, pulmonary disease, cardiovascular disease, haemoglobinopathy, and tobacco/carboxyhaemoglobin);
3. Decrease in plasma volume that causes relative erythrocytosis that occurs in dehydration, vomiting, diarrhea and Gaisbock's syndrome.
Platelet count

Normal values:
(150 – 400) x 10³/cu.mm

Specimen:
Size: 2 ml EDTA whole blood
Stability: 7 days at 4-8 °C

Patient preparation and special precautions:
1. Platelet counts normally decrease during pregnancy and before menstruation.
2. Falsely lowered results could be caused by clumping of platelets.
3. Platelet count is slightly increased by oral contraceptives.
4. Platelet counts normally increase at high altitudes, after strenuous exercise, trauma and in winter.
5. A specimen with a clot should not be tested.

Test description:
Platelets are the smallest elements in the blood. These cells are non-nucleated, round or oval shaped. Platelets activity is necessary for blood clotting. A deficiency of platelets leads to prolonged bleeding time. The life span of a platelet is approximately 5-7 days.

Clinical significance:
Platelets count is increased in cancer, splenectomy, iron deficiency anaemia, cirrhosis, polycythemia vera, rheumatoid arthritis, rapid blood regeneration caused by acute blood loss, haemolytic anaemia, acute infections, inflammatory diseases, chronic pancreatitis, tuberculosis, and renal failure. Platelet count is decreased in idiopathic thrombocytopenic purpura (ITP), pneumonia, allergic conditions, toxic effects of many drugs, congestive heart failure, HIV infection, DIC, eclampsia, alcohol toxicity and hypersplenism.
Reticulocytes count

**Normal values:**
- Adults & children: 0.2 - 2.0%
- Infants: 2-6%

**Specimen:**
- Size: 2 ml EDTA whole blood
- Stability: 1 day at 4-8 °C

**Patient preparation and special precautions:**
1. Pregnancy and infancy cause a normal increase in count.
2. When Howell-Jolly bodies are present, they falsely elevate reticulocytes count when automated methods are used.
3. Recently transfused patients have a blood dilution that causes lower count.
4. A specimen with a clot should not be tested.

**Test description:**
Reticulocytes are immature red cells that pass into the blood stream from the bone marrow. The number of reticulocytes in the blood indicates the degree of activity of the bone marrow. The number increases when the marrow is very active.

**Clinical significance:**
The count of reticulocytes increases when the RBC production increases as the bone marrow replaces cells lost or prematurely destroyed. These elevations are observed after treatment of anaemias where the increased reticulocyte count may be used as an index of the effectiveness of treatment. The rise in reticulocytes may exceed 20% after adequate doses of iron in iron-deficiency anaemia. There is a proportional increase when pernicious anaemia is treated by transfusion or vitamin B12 therapy.

Reticulocyte counts also rise in hemolytic anaemia, haemoglobinopathies and sickle cell disease, RBC enzyme deficits, and malaria. The count also increases after 3 to 4 days after hemorrhage.

Decreased reticulocyte count means that bone marrow is not producing enough erythrocytes. This occurs in untreated iron deficiency anaemia, aplastic anaemia (a persistent deficiency of reticulocytes suggests a poor prognosis), anaemia of chronic disease, untreated pernicious anaemia, endocrine problems, radiation therapy, myelodysplastic syndromes, tumor in marrow and alcoholism.
Haematocrit (Packed Cell Volume, PCV)

Normal values:
Men: 40% - 54%
Women: 37% - 47%
Children (5) years: 38% - 44%
Infants (3 months): 35% - 40%
Newborn: 44% - 64%

Specimen:
Size: 2 ml EDTA whole blood or direct fingers puncture blood
Stability: 7 days at 4-8 °C

Patient preparation and special precautions:
1. Hct values are higher in people living at high altitudes, as are Hb and RBC.
2. A slight decrease in Hct is caused by pregnancy due to the physiologic haemodilution.
3. Age and gender cause variations in Hct values. The infants Hct normal value is higher due to the high percentage of macrocytic red cells. Hct values in females are slightly lower than in males.
4. Men and women older than 60 years of age have lower Hct values corresponding to lower RBC values.
5. Hct values are falsely increased by severe dehydration from any cause.

Test description:
The word haematocrit means “to separate blood”, this highlights the mechanism of the test. The test depends on separating the plasma and blood cells by centrifugation. This is one of the simplest, most accurate and most valuable of all haematological investigations. By means of haematocrit, haemoglobin and red cell count the absolute indices can be calculated.

Clinical significance:
Decreased Hct values are an indicator of anaemia as a percentage of less or equal to 33% indicates a moderate to severe anaemia. Other conditions are associated with decreased values also: Adrenal insufficiency, leukaemias, lymphomas, acute and chronic blood loss.

In decreased Hct values several points have to be taken into consideration:
1. After acute hemorrhage the Hct may be normal, but during the recovery phase, both the Hct and the RBC drop markedly. This indicates that Hct may not be reliable in cases of moderate blood loss and blood transfusion.
2. When the RBCs are of a normal size, a relative relation exists between HCT and RBCs. However, this relationship does not hold true in patients with microcytic or macrocytic anaemia.
3. In iron-deficiency anaemia, the RBCs are small. This makes Hct decrease because the microcytic cells pack to a smaller volume. However, the RBCs may be normal or higher than normal.

Hct values increase in: erythrocytosis, polycythemia vera, and shock when haem concentration rise considerably.
Erythrocyte Sedimentation Rate (ESR)

Normal values:
Men: 1-10 mm/hr  
Women: 3-15 mm/hr

Specimen:
Size: 2 ml EDTA whole blood (use the EDTA blood to complete the 3.2% sodium citrate ESR tube) 
Stability: 2 hours at room temperature

Patient preparation and special precautions:
1. ESR decreases if the blood sample stands more than 24 hours before the test.
2. Refrigerated blood should be allowed to return to room temperature before the test is performed otherwise, the ESR decreases.
3. ESR is increased in several conditions including: anaemia, menstruation, young children, pregnancy, drug such as heparin and oral contraceptives, and the presence of CRP, fibrinogen, and globulins.
4. Apparently healthy women over 70 to 89 years old may have very high ESR (up to 60mm/h).
5. ESR is decreased in people with: 
   a. High Hb and RBC count 
   b. High blood glucose, high albumin level, high phospholipids 
   c. In the newborns with decreased fibrinogen levels 
   d. Certain drugs (steroids, high dose aspirin)

Test description:
The ESR is the rate at which erythrocytes settle in anti-coagulated blood in 1 hour. Sedimentation is the clumping of the erythrocytes in a column-like manner (Rouleau formation). These changes are caused by alterations in the plasma proteins concentrations. The inflammatory and necrotic processes alter the concentrations of blood proteins, resulting in aggregation of RBCs. These RBC clumps are heavy and more likely to fall rapidly when placed in a special vertical test tube. The ESR is higher when the settling of cells is faster. The sedimentation rate is not a specific test and is not diagnostic of any particular disease but rather is an indication of the continuity of the disease that must be investigated. It is also useful in monitoring the progression of inflammatory disease.

Clinical significance:
ESR is increased in all collagen diseases, acute heavy metal poisoning, rheumatoid arthritis infections, inflammatory diseases, carcinomas, nephritis, nephrosis, cell or tissue destruction, toxemia, anaemia, and gout.
ESR is decreased in spherocytosis, polycythaemia, sickle cell anaemia, and hypofibrinogenaemia.
Clotting time

**Normal values:**
5 - 12 minutes

**Specimen:**
Whole blood without anticoagulant
Stability: to be performed immediately

**Patient preparation and special precautions:**
The test is not currently reliable because of poor reproducibility

**Test description:**
Clotting time is the time required for the solid clot to form. The basis for this test is that whole blood will form a solid clot when exposed to a foreign surface such as a glass test tube.

**Clinical significance:**
Clotting time is increased in the following conditions: factor V deficiency, vitamin K deficiency, factor VII deficiency, heparin therapy, haemophilia (factor VIII deficiency), dicumarol therapy, factor IX deficiency (Christmas disease), factor XI deficiency, factor XII deficiency afibrinogenemia, haemorrhagic disease of newborn, pneumonia, anaemia and leukaemia.
Bleeding time

Normal values:
1 - 5 minutes

Specimen:
Blood from puncture from the lobe of the ear

Patient preparation and special precautions:
1. A uniformed depth and width of the puncture site is necessary to avoid variations in the normal value.
2. Fibrin particles could be damaged by touching the puncture site while this test is performed causing prolonged bleeding time.
3. Alcoholic people may have increased bleeding time.
4. The ingestion of 10 g of aspirin as long as 5 days before the test causes prolonged bleeding time.
5. Drugs such as dextran, streptokinase-streptodornase (fibrinolytic agents), and mithramycin, pantothenyl alcohol may cause increased bleeding times.
6. Results may be altered due to extreme hot or cold conditions.
7. Invalid results could be obtained if edema is present in patient’s hands.

Test description:
Bleeding time measures the primary phase of haemostasis, the interaction of the platelets with the blood vessel wall and the formation of the haemostatic plug.

This test is of significant value in detecting vascular abnormalities and of moderate value in detecting platelet abnormalities or deficiencies.

Clinical significance:
Low platelet count for any reason causes prolonged bleeding time in conditions such as: Thrombocytopenia, platelets dysfunction syndromes, leukaemia, a decrease in plasma factors (von Willebrand’s factor, fibrinogen), severe liver disease, DIC disease, scurvy, and abnormalities in the walls of the small blood vessels, vascular disease.

Bleeding time can be variable in Von Willebrand’s disease.

Other than platelet dysfunction, bleeding time is normal in the presence of coagulation disorders, vascular disease, or von Willebrand’s disease.

In some cases, a larger vessel can be punctured. This indicates that a single prolonged bleeding time does not prove the existence of haemorrhagic disease. In these cases, the puncture should be repeated on another body site, and an average value should be obtained.
Haemoglobin electrophoresis

Normal values:
HbA: 96.5 – 98.5 %
Hb S: Negative
Hb A2: 1.5 – 3.5 %
Hb C: Negative
Hb F: < 2.0 %
Hb D: Negative

Specimen:
2 ml EDTA whole blood
Stability: 3 days at room temperature, 7 days at 4-8 °C and 6 months at -20°C

Patient preparation and special precautions:
Blood transfusion may give false or inconsistent results

Test description:
Haemoglobin electrophoresis detects normal and abnormal haemoglobins by matching haemolyzed RBC material against standard bands for the various haemoglobins known. The normal forms of haemoglobins are HbA1, HbA2, and Hb F (fetal haemoglobin).

Clinical significance:
The most common types of abnormal Hb (haemoglobinopathies), are Hb S (responsible for sickle cell anemia) and Hb C (results in a mild haemolytic anaemia). The significant increase in Hb A2 is the most common quantitative abnormality and is diagnostic of the thalassemias, especially β-thalassemia trait. More than 350 variants of Hb have been described and identified.
Fetal haemoglobin (haemoglobin F; Alkali-resistant haemoglobin)

Normal values:
Adults: 0 – 2 %
Newborn: 60 – 90 %
By 6 months of age: 2 %

Specimen:
Size: 2 ml EDTA whole blood
Stability: 3 days at room temperature, 7 days at 4-8 °C and 6 months at - 20°C

Patient preparation and special precautions:
1. Infants with chronic intrauterine anoxia or small for gestational age have persistently elevated Hb F.
2. Hb F is increased during anticonvulsant drug therapy.

Test description:
Fetal haemoglobin (Hb F) is a normal Hb manufactured in the RBCs of the fetus and infants; it makes up 50% to 90% of the Hb in the newborn. The remaining portion of the Hb in the newborn is made up of Hb A1 and Hb A2, the adult types.
Under normal conditions, Hb F is replaced by the adult haemoglobin Hb A during the first year of life. But if Hb F persists and constitutes more than 5% of the haemoglobin after 6 months of age, an abnormality should be expected.

Clinical significance:
Hb F is increased in the following conditions: major and minor thalassemias, hereditary familial fetal haemoglobinemia (persistence of Hb F), sickle cell disease, haemoglobin H disease, leakage of fetal blood into the maternal bloodstream, hyperthyroidism, as compensatory mechanism to anaemias (pernicious anemia, PNH, sideroblastic anaemia), acute or chronic leukaemia, multiple myeloma and lymphoma.
Hb F production may slightly continue (5-10 %) in thalassemia minor and the patient usually lives. In thalassemia major, the value may reach 40% to 90% leading to a severe anaemia and death may occur.
Haemoglobin A2

**Normal values:**
Newborn: 0% - 1.8%
Adult: 1.5% - 3.5%

**Specimen:**
Size: 2 ml EDTA whole blood
Stability: 3 days at room temperature, 7 days at 4-8 °C and 6 months at -20°C

**Patient preparation and special precautions:**
Blood transfusion may give false or inconsistent results.

**Test description:**
The identification of Hb A2 levels is especially important in the differential diagnosis of \( \beta \)-thalassemia trait from iron deficiency. On the contrary, low mean corpuscular volume (MCV) may exist in the majority of patients with \( \beta \)-thalassemia trait, but it does not differentiate iron-deficient patients.

**Clinical significance:**
Increased Hb A2 levels are described in:
1. \( \beta \)-thalassemia major (3 – 11%)
2. Thalassemia minor (3.5 – 7.5%)
3. Thalassemia intermediate (6 – 8%)
4. Hb A/S (sickle cell trait) (15 – 45 %)
5. Hb S/S (sickle cell disease) (2 – 6 %)
6. S-\( \beta \)-thalassemia (3.0 – 8.5%)
7. Megaloblastic anaemia

Decreased Hb A2 occurs in untreated iron - deficiency anaemia, sideroblastic anaemia, erythroleukaemia, and Hb H disease.
Sickle Cell test

Normal values:
Negative

Specimen:
Capillary or EDTA whole blood

Patient preparation and special precautions:
None

Test description:
In sickle cell anaemia the haemoglobin has an abnormal structure called haemoglobin S (Hb S). The red blood cells containing Hb S don’t last as long as “normal” red blood cells. They also lose their normal disc shape and become rigid and deformed as a sickle or crescent shape resulting in chronic anaemia. The sickle shaped cells are not flexible enough to squeeze through small blood vessels which may block the blood vessels. The tissues served by those blood vessels will then be damaged and cause pain.

The test is performed by depriving the erythrocytes’ oxygen. In normal erythrocytes the normal shape is retained, but erythrocytes containing hemoglobin S will take a sickle shape. However, this test cannot distinguish between sickle cell trait and sickle cell disease. This has to be done by electrophoresis, which identifies a haemoglobin pattern.

The presence of hemoglobin S (a positive test) means that most of the erythrocytes have taken the typical sickle cell shape. Positive tests are 99% accurate.

Clinical significance:
Haemoglobin S exists in different percentages in the following conditions:

1. Sickle cell trait: the heterozygous (A/S) pattern: Hb S 20% - 40%; Hb A1 60% - 80%; Hb F small amount. The sickle cell trait does not affect longevity and the symptoms of sickle cell anaemia do not exist. Sickle cell trait may cause haematuria, renal papillary necrosis, and an increased risk of pulmonary embolus.
2. Sickle cell anaemia homozygous pattern (S/S): Hb S 80% - 100%; Hb F most of the rest; Hb A1 0% or small amount. It is confirmed by haemoglobin electrophoresis. Patients have all the clinical symptoms of the disease.
3. Hb C-Harlem (rare).
4. Hb S can coexist with other disorders, such as thalassemia or Hb S-C.
Glycosylated haemoglobin (HbA1c)

Normal values:
Normal individuals: < 6.5 %
Controlled diabetic patients: < 7.0 %
Non-controlled diabetic patients: ≥ 7.0 %

Specimen:
2 ml EDTA whole blood
Stability: 3 days at room temperature, 7 days at 4-8 °C and 6 months at -20°C

Patient preparation and special precautions:
The presence of Hb F and H causes falsely elevated values, while the presence of Hb S, C, E, D, G and Hb Lepore causes falsely decreased HbA1c values.

Test description:
Glycohaemoglobin (Glycosylated haemoglobin) is a normal, minor type of haemoglobin. It is formed at a proportional rate to the average glucose concentration by a slow, non-enzymatic process within the red blood cells during their 120- day circulating life span.

Clinical significance:
Glycohaemoglobin is formed by the bound of haemoglobin to blood glucose. In the presence of hyperglycemia, an increase in glycohaemoglobin causes an increase in HbA1C. This glycosylation is irreversible and persists for the 120 day life span of the red cells.

Haemoglobin A1c is recommended for patients with diabetes to monitor the glycaemia control at initial assessment and as a part of continuing care. In addition, this test is used as a measure of risk for the development of diabetes complications.
ABO grouping

Normal values:
Group A    Group B    Group AB    Group O

Specimen:
2 ml EDTA whole blood
Stability: 3 days at room temperature, 7 days at 4-8 °C

Patient preparation and special precautions:
Test may be affected by abnormal plasma proteins, potent cold autoagglutinins, positive direct antiglobulin test, and in bacteraemia.

Test description:
Human blood is divided into groups according to the presence or absence of specific blood group antigens (ABO). These antigens are located on the surface of the red blood cells. They can induce the body to produce antibodies. More than 300 distinct antigens have been identified. Compatibility of the ABO group is the foundation for all other pre-transfusion testing. The antigen either A or B is determined by specifically linked sugars.

The table below lists the blood groups and their ABO antigens.

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>ABO Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>AB</td>
<td>A and B</td>
</tr>
<tr>
<td>O</td>
<td>None</td>
</tr>
</tbody>
</table>

Clinical significance:
The test is essential for blood transfusion.
Rh typing

Normal values:
Both Rh + or Rh – are normal

Specimen:
2 ml EDTA whole blood
Stability: 3 days at room temperature, 7 days at 4-8 °C

Patient preparation and special precautions:
Test may be affected by abnormal plasma proteins, potent cold autoagglutinins, positive direct antiglobulin test, and in bacteraemia.

Test description:
Human blood is grouped as Rh-positive or Rh-negative. This relates to the presence or the absence of the D antigen on the red cell membrane. The D antigen (Rh1) is considered the second most important antigen after A and B antigens in transfusion practice.

Clinical significance:
The Rh antigens have the capacity to immunise when entering the body. This is especially significant when receiving a transfusion or becoming pregnant.
**Coombs’s Test, direct**

**Normal values:**
Direct Coomb’s test: negative for red blood cells

**Specimen:**
2 ml EDTA whole blood
Stability: stable at room temperature

**Patient preparation and special precautions:**
Contaminated reagents may cause false positive results. Other factors such as over-centrifugation, presence of strong cold agglutinins or saline stored in metal or glass containers also cause false positive results. False negative reactions are caused by factors such as reagent infidelity, improper washing, and improper centrifugation, failure to add antiglobulin reagents, low serum/cell ratio or delayed washing in presence of already eluted weakly attached antibody.

**Test description:**
The anti-human globulin is added to the patient’s erythrocytes to detect incomplete antibodies coating erythrocytes. The test aims at detecting antibodies tagged on red cells in vivo.

**Clinical significance:**
Direct Coomb’s test is used in the diagnosis of haemolytic transfusion reactions, haemolytic disease of the newborn, investigation of cold or warm auto-antibodies as well as in drug induced haemolytic anaemia.
**Coomb’s Test, indirect**

**Normal values:**
Indirect Coomb’s test: negative for serum

**Specimen:**
1 ml serum  
Stability: stable at room temperature

**Patient preparation and special precautions:**
Contaminated reagents may cause false positive results. Other factors such as over-centrifugation, presence of strong cold agglutinins or saline stored in metal or glass containers also cause false positive results. False negative reactions are caused by factors such as reagent infidelity, improper washing, and improper centrifugation, failure to add antiglobulin reagents, low serum/cell ratio or delayed washing in presence of already eluted weakly attached antibody.

**Test description:**
Indirect Coomb’s is used to detect anti erythrocyte (free antibodies) in the serum of the patient, which could be against patient’s own erythrocyte antigen or other antigens.

**Clinical significance:**
Indirect Coomb’s test is used in the detection of erythrocyte antibodies in serum, typing red cell antigens and in cross-matching.
Lab Guide

Serology tests section
Brucella Antibodies, total, titer

**Normal values:**
Negative

**Specimen:**
1 ml serum  
Stability: 2 days at 4-8 °C and 1 month at -20°C

**Patient preparation and special precautions:**
Sample has to be drawn during the first week of illness and then 3-4 weeks later.

**Test description:**
The Brucella bacterium is a gram-negative, non-spore forming, non-motile short bacillus. It causes the zoonotic infection brucellosis. The clinical symptoms of the disease affect a wide range of systems and organs. In some cases, the disease gets complicated and requires continuous medical treatment. However, mortality rate is relatively low and somehow limited to disease complications affecting central nervous system and cardiovascular system. The disease is endemic especially in the Mediterranean region and the Middle East, also in Central Asia and parts of Africa and Latin America.

**Clinical significance:**
Brucellosis is a common disease, which must be considered in the differential diagnosis of fevers of unknown origin. It is often transmitted to man by digesting infected milk or milk products. The rise in Brucella Antibody titer indicates current infection or relapse. It is an occupational disease of those working with infected animals or their tissues.
**C-Reactive Protein (CRP)**

**Normal values:**
< 6.0 mg/L

**Specimen:**
1 ml serum
Stability: 2 days at 4-8 °C and 1 month at -20°C

**Patient preparation and special precautions:**
Contaminated and markedly lipemic serum may cause non-specific reaction and therefore should not be tested.

**Test description:**
C-reactive protein is an abnormal protein that appears in the blood during any inflammatory process. This protein does not exist in blood and body fluids of healthy persons but it rapidly appears in response to injurious stimuli.

CRP is mainly synthesized in the liver. Large amounts appear in peritoneal, pleural, pericardial, and synovial body fluids. CRP is the most remarkable acute-phase reactant. Its levels increase up to 1000-fold and then decline rapidly when the inflammatory process move back. This test is nonspecific in conditions of myocardial infarction, rheumatoid arthritis, or malignancy where there is tissue necrosis.

**Clinical significance:**
Blood serum CRP rises after the onset of tissue damage within 18 to 24 hours. CRP is used to follow the progress of rheumatic fever therapy and to interpret the sedimentation rate that could be influenced by altered physiologic state. It is also used in monitoring the wound healing process, burns and organ transplantation. The test is also positive in bacterial and viral infections, myocardial infarction, malignancy and post-surgically where it declines after the fourth day of surgery.

CRP’s other advantage is that it tends to rise before antibody titer and ESR rise and also tend to fall earlier than ESR levels.
Anti Streptolysin O (ASO)

Normal values:
<200 IU/ml

Specimen:
1 ml serum
Stability: 2 days at 4-8 °C and 1 month at -20°C

Patient preparation and special precautions:
1. High levels of serum β-lipoprotein in liver diseases cause false-positive ASO titres.
2. Healthy carriers can occasionally have increased titre.
3. Treatment with antibiotics suppresses streptococcal antibody response.

Test Description:
Streptolysin A is produced by Group A β-haemolytic streptococci. This type also produces several other enzymes such as hyaluronidase and DNase B. Several serologic tests that detect these enzyme antibodies are available and include antistreptolysin O titer (ASO), which detects streptolysin O; and anti-DNase B (ADB), which detects DNase B; and streptozyme, which detects antibodies to multiple enzymes.

Clinical significance:
The ASO test is useful in the diagnosis of several diseases related to streptococcal infections such as rheumatic fever, scarlet fever, glomerulonephritis, post streptococcal glomerulonephritis, and endocarditis. More significant results can be obtained by serial rising titres over several weeks rather than a single result. The rise over 3 folds in the titer is consistent with an immunologic response to Group A streptococcus. In streptococcal pharyngitis, anti DNase B antibodies may appear earlier than ASO and are more sensitive for streptococcal pyoderma.

Normally, the titre of ASO falls within 6-12 months. If the titre persists this indicates a continuity of streptococcal infection or complications.
Rheumatoid Factor (RF)

Normal values:
<20 IU/L

Specimen:
1 ml serum
Stability: 2 days at 4-8 °C and 1 month at -20°C

Patient preparation and special precautions:
Older patients usually have higher serum values of RF. Also, patients who have received multiple vaccinations and transfusions have high RF serum levels.

Test description:
Rheumatoid factor (RF) is a macroglobulin-type antibody found in people with rheumatoid arthritis. It is almost confirmed that rheumatoid factor is an anti-gamma globulin antibody. However, until discovering the antigen that produces RF, the nature of this factor will remain uncertain. The role of RF in rheumatoid arthritis is also not clear enough. RF is supposed to be responsible for the destructive change associated with rheumatoid arthritis. Yet it can be found sometimes in patients with other diseases in lower values than rheumatoid arthritis.

The clinical significance of RF determination consists in differentiating between rheumatoid arthritis, in which the rheumatoid factor has been demonstrated in the serum of approximately 80% of the cases examined, and rheumatic fever, in which the rheumatoid factor is almost always absent. The RF test is more frequently positive in long term active processes than in diseases which are less active or are still in early stages.

Clinical significance:
After a patient with a positive test improves, the subsequent tests will remain positive unless titres were initially low.

A positive RF test result indicates a tentative diagnosis of rheumatoid arthritis rather than rheumatic fever.

Rheumatoid factors are also found in a variety of other diseases such as endocarditis, tuberculosis, systemic lupus erythematosus, syphilis, cancer, sarcoidosis, viral infections, liver diseases, Sjogren’s syndrome and in skin and renal allograft patients.

The absence of RF does not exclude the diagnosis of rheumatoid arthritis.
Lab Guide

Bacteriology tests section
Diagnostic procedures in bacteriology

The basic flow of procedures involved in the diagnosis of bacterial infectious disease is: direct microscopy, growth and cultivation of bacteria, and analysis of the cultivated bacteria for identification and establishing a susceptibility profile. Appendix 4 lists bacterial diseases and their laboratory diagnosis. The bacteria whether direct from sample or cultivated is differentiated under microscope based on its shape, arrangement of cells and staining properties.

**Direct microscopy:**
The bacteria whether direct from sample or cultivated is differentiated under microscope based on its shape, arrangement of cells and staining properties.

**Growth and cultivation of bacteria:**
Bacterial culture involves growing of microorganisms on a special medium that supports the growth of suspected bacteria.

**Analysis of the cultivated bacteria:**
Different types of stains are used for preliminary identification of bacteria: simple, gram stain (the most widely used), and Ziehle-Neelson stain. Chemical tests and serological tests are all used for definitive identification and characterization of infectious bacteria.

**Collection and transport of specimen:**
The specimen obtained should be representative of the disease process, and cultured only for specific pathogenic bacteria of the disease process. Specimens should be collected without contamination. Fluids, tissues, skin scrapings, and urine should be collected in sterile containers with tight fitting lids.

**Collection of specimen:**
Specimen should be collected and obtained:
1. Before antimicrobial agents have been administered.
2. Where and when the suspected pathogen is most likely to be found avoiding external contamination.
3. With sufficient amount to assure complete and accurate examination. When only a small quantity is available, swabs should be moistened with sterile saline just before sample collection.
4. Proper labeling of the sample with: patient’s name, age, sex, specimen source, time of collection, clinical diagnosis, suspected microorganisms, patient’s history, patient’s immune state, previous and current infections, and previous or current antibiotic therapy.

**Transport and preservation of specimen:**
Prompt delivery of specimen to the bacteriology laboratory is desirable to prevent deterioration of sample and death of pathogenic bacteria, or overgrowth of contaminants. Urine specimens is preserved at 2-8°C for up to 24hrs until examination.
Culture swabs are preserved using cotton (calcium alginate or polyester) swabs containing buffered semi-solid agar that maintains
Urine culture

General information:
Urine is typically sterile, yet usually contaminated while passing through a contaminated milieu. Urine is an excellent culture and growth medium for most pathogenic bacteria that infect the urinary tract.

Urinary tract infections are predominantly a disease of females mainly due to the anatomy of the female urethra.

Urine cultures are the most frequently used to diagnose bacterial urinary tract infection (kidneys, ureter, bladder, and urethra). The combination of pyuria and significant bacteriuria strongly suggests the presence of urinary tract infection.

Common pathogens:
Gram negative: escherichia coli, enterococci, pseudomonas and proteus
Gram positive: staphylococcus aprophytics
Candida albicans, and mycobacterium tuberculosis

Specimen collection:
1. A clean-catch, midstream first morning urine specimen of at least 3 to 5 ml in sterile container is the most frequently received specimen. Other alternative methods for collection of urine include: direct catheterized urine, supra pubic bladder aspiration, or indwelling catheter.
2. Urine specimen should be collected when the patient is ill and suggestive of suffering of UTI.
3. Urine collection bag that is a part of an indwelling catheter drainage system must not be a source of specimens for culture.
4. Urine should be examined and cultured immediately. If this is not possible, it can be refrigerated for up to 24 hours.
5. To establish whenever true bacteriuria is present, two successive clean-voided or midstream urine specimens should be collected.

Interferences:
Bacterial contamination comes from sources such as bacteria beneath the prepuce in male patients, perineal hair, bacteria from vaginal secretions, from the vulva, or from the distal urethra in female patients, and bacteria from the hands, skin or clothing.

Patients who receive forced fluids may have urine that is sufficiently dilute to reduce the bacterial count to insignificant counts.

Negative results do not necessarily rule out the diagnosis. Close cooperation is needed between clinician and microbiologist.
Ear culture

General information:
Ear infections include: otitis externa (external ear infection), and otitis media (middle ear infection)

Acute localized otitis externa is often occurs in the form of a pustule. Acute diffuse otitis externa (Swimmer’s ear) is related to maceration of the ear from swimming or hot, humid weather. Otitis media is the most common ear infection in children often begins as a viral infection, with a bacterial infection occurring soon afterward.

Common pathogens:
pseudomonas aeruginosa, staphylococcus aureus, proteus species, streptococcus pneumonia, haemophilus influenza and streprococcus pyogenes, fungi

Specimen collection:

1. In cases of external otitis, the ear should be cleansed with a mild germicide to minimize the contaminating skin flora before taking the swab for culture.
2. Specimens from the ear, specially obtained after spontaneous perforation of the ear drum or by needle aspiration, should be collected by sterile equipment and sterile cotton swab. Cultures from the mastoid usually are taken during surgery.
3. Specimens should be delivered to the laboratory as soon as possible after collection, and transported anaerobically if anaerobes are suspected as the pathogens.
4. Discharges from the ear in chronic otitis media usually reveal the presence of proteus aueriginosa and proteus species.
Eye culture

General information:
The eye and its associated structures are predisposed to infections by various microorganisms. Major infections of the eye are: blepharitis, conjunctivitis, keratitis and keratoconjunctivitis. Bacterial conjunctivitis is the most common type of ocular infection characterized by swelling of the conjunctiva and inflammatory exudates.

Common pathogens:
Staphylococcus aureus, haemophilus spp. streptococcus pneumonia, neisseria gonorrhea, chlamydia trachomatis, beta-hemolytic streptococci and pseudomonas aeuroginosa

Specimen collection:
1. Purulent material from the lower conjunctival sac or inner canthus of the eye is collected with calcium alginate sterile swab and placed in transport medium. For confirmation, both eyes should be cultured separately.
2. In cases of keratitis, scrapings of the cornea with a heat-sterilized platinum spatula are made directly onto the medium (blood or chocolate agar or thioglycollate broth).
Lower Respiratory Tract Infection: sputum culture

General information:
Diseases of the lower respiratory tract include: bronchitis that is the inflammation of the tracheobronchial tree, pneumonia which is inflammation of the lungs' airways and supporting structure, and pleural infections.

Common pathogens:
Mycobacterium tuberculosis, streptococcus pneumonia, haemophilus influenza, staphylcoccus aureus, and klebsiella pneumonia

Specimen collection:
1. Sputum is the specimen of choice for Lower Respiratory Tract Infections (LRTI). However Lower respiratory tract secretions will be contaminated when they pass through upper respiratory tract normal flora unless collected using invasive technique.
2. The sputum cultures are used to diagnose pulmonary TB, bacterial pneumonia, bronchiectasis, mycoplasmal pneumonia, suspected viral pneumonia, and suspected pulmonary mycotic infections. Sputum that is green or yellowish in color (indicating its content of pus) may be cultured and examined grossly and microscopically.
3. Patients should be instructed to provide deep-coughed sputum into a wide-mouthed sterile container. Sputum volume of 1 to 3 ml is sufficient for most examinations. Sputum specimens should not be refrigerated but should be delivered to the laboratory as soon as possible, as even a moderate amount of time at room temperature may lead to overgrowth of contaminants and lose of etiologic agent.

Interferences:
Sputum specimens passing through the upper respiratory tract secretions can give misleading results because of contamination with the normal bacterial flora present in the mouth and throat. For this reason sputum specimens are one of the fewest specimens received for culture in the microbiology laboratory.
Upper Respiratory Tract Infections: throat culture

General information:
Pharyngitis (sore throat) and tonsillitis are common upper respiratory tract infections. By visualization of the pharynx affected tissues appear red and swollen.

Common pathogens:
Streptococcus pyogenes is the most clearly associated with acute bacterial pharyngitis. Diagnostic endeavor is to be directed towards isolation and proper identification of streptococcus pyogenes (beta haemolytic streptococci). Routine susceptibility test on throat isolate is not required as Erythromycin and Benzyl penicillin are considered the drug of choice to treat.

Specimen collection:
1. Cotton swabs (either dacron or calcium alginate-tipped swabs) are used for collecting the throat specimen by a physician or other well-trained personnel.
2. With a very good visual light, the patient’s tongue is depressed down with a tongue depressor. The cotton swab is firmly and gently rotated over the back of the throat, around both tonsils and fossae, and on areas of inflammation, exudation, or ulceration.
3. If specimen is not processed within 4 hours, then the swab is kept in transport medium and refrigerated if examination is delayed.
Nasal and nasopharyngeal culture (swab)

General information:
The sinuses are normally sterile, and most cases of sinusitis are believed to be bacterial complications of primarily viral infections.

Common pathogens:
Haemophilus influenza, streptococcus pneumonia, streptococcus pyogenes, and moraxilla catarrhalis

Specimen collection:
1. Specimens are obtained by otolaryngologist from the maxillary sinus by puncture and aspiration or during surgery. Sinus drainage is unacceptable as it is contaminated with normal upper respiratory tract flora.
2. Diagnosis can be made based on physical findings, history, radiograph studies and magnetic resonance imaging.
Wound and abscess culture

General information:
Wound infections and abscesses occur as complications of surgery, trauma, or disease that interrupts a skin surface. The nature of infecting flora depends mostly on the location of surgery or trauma.

Pathogenicity depends on the quantity of the organisms present. Quantitative or semi-quantitative reporting of culture results helps in providing information on the relative importance of the various organisms present in the lesion and also the response of the infection to antibiotic therapy.

Common pathogens:
Staphylococcus aureus, streprococcus pyogenes, escherichia coli, pseudomonas auroginosa, bacteroides clostridium

Specimen collection:
1. No single procedure for specimen collection can be formulated.
2. During specimen collection extreme cautions is needed as many of these lesions, wounds and abscesses are open and so colonized by either normal flora of that site or by nosocomial bacteria.
3. After cleaning of the site, the clinician should look beneath the surface for collection of pus, devitalized tissue, or oozing gas and place the specimen in sterile tube using sterile swabs.
4. The specimen should be processed as soon as possible. After the preliminary examinations have been completed, the remaining of specimen is refrigerated until no additional tests are ordered and needed.
5. Abscess samples are aspirated using syringe and needle. Then the specimen is aseptically, transferred to sterile specimen containers. If such containers are not available, the specimen should be kept in the syringe with the needle capped, and the syringe itself should be transported to the laboratory.
Stool and rectal swab culture and smears

General information:
The gastrointestinal tract contains a vast and diverse normal flora. The upper small intestine contains up to $10^3$/ml of normal flora, but in the distal ileum the count reaches up to $10^7$/ml. The most dominant bacteria of the gastrointestinal tract are: enterobacteracea and bacteroides.

Common pathogens:
campylobacter, salmonella, and shigella species

Specimen collection:
1. The specimen should be collected in a clean, dry container. The specimen is better to be freshly collected. A stool amount in the size of a walnut is usually adequate; however, the entire passed stool should be sent for examination.
2. A single negative stool culture should not confirm or exclude the diagnosis. At least 3 stool cultures are recommended if the patient’s clinical picture suggests bacterial involvement, despite previous negative cultures. Moreover, once a positive diagnosis has been made, the patient’s personal contacts should also be tested to prevent a potential spread of infection. Stool specimens received for culture should be processed within two hours, otherwise should be placed in a transport media that enhance the growth of suspected pathogen and inhibits the growth of normal flora.

Interfering factors:
Feces from patients receiving barium, bismuth, mineral oil, or antibiotics are not satisfactory specimens to proceed.
Cervical culture

General information:
Urethral discharge occurs in both males and females who are infected with bacteria considered as pathogens of the genital tract. Infections in females are more likely to be asymptomatic, because the discharges are less profuse and can be masked by vaginal discharges. In addition to cervical samples, vaginal discharge samples are also collected for the detection of pathogens causing vaginal discharge.

Common pathogens:
The vaginal flora of pre-menopausal women normally consist predominantly of lactobacilli and of a wide variety of facultative aerobic and anaerobic bacteria.

Abnormal vaginal discharge may be due to:
- Vaginitis: trichomonas vaginalis, candida albicans;
- Bacterial vaginosis: overgrowth of anaerobes and gardnella vaginalis;
- Cervicitis: neisseria gonorrhoeae, chlamydia trachomatis.

Specimen collection:
1. Specimens are to be collected during pelvic examination using moistened not lubricated speculum. The minimum diagnostic criteria for bacterial vaginosis are the presence of at least three of the following: vaginal pH > 4.5, abnormal vaginal discharge with fishy or amine like odor when a drop of 10% KOH is added, and the visualization of clue cells under direct microscopic examination.
2. Specimens collected should be processed as soon as possible. Because trichomonas vaginalis may be present in urethral or vaginal discharge, an additional swab should be placed in a tube containing 0.5 ml of sterile saline and be delivered to the laboratory immediately for direct microscopic examinations.
3. Swabs that are held for longer time should be transported to the laboratory in a transport medium and should be held at room temperature until processed. If specimens are not processed within 12 hours, they should be refrigerated but not frozen.
Sensitivity of bacteria to antimicrobial agents

Isolation of an infectious agent from a diseased patient is not sufficient for determining proper therapy. Since the susceptibility of bacteria to antimicrobial agents cannot be predicted, antibacterial susceptibility tests are needed to measure the ability of an antibacterial agent to kill or inhibit bacterial growth in vitro.

The result of the sensitivity test is reported as either:

1. Sensitive: when the infection caused by that pathogen is likely to respond to treatment with this drug.
2. Resistant: when the pathogen do not respond to the drug irrespective of the dosage or the location of infection.

Antimicrobial agents act either as a bactericidal (killing the organism) or bacteriostatic (inhibiting the organism growth) action.

The basic sets of antibiotics for routine susceptibility testing are: Cefuroxime, Cephalexin, Ciprofloxacin, amoxicillin and clavulanic acid, Co-trimoxazole, Gentamicin, Norfloxacin, Doxycyclin, Azithromycin, Erythromycin and Amoxicillin.
Lab Guide
Urine examination section
Urine examination

Test description:
Urine analysis is a diagnostic tool used to screen for metabolic and kidney disorders and for urinary tract infections. When a patient has symptoms of urinary tract infection, such as abdominal pain, back pain, frequent or painful urination, as part of a pregnancy checkup or pre-surgical work up.

Specimen required:
20 to 25 ml of urine are needed. Midstream, clean catch first morning specimen considered as the most suitable specimen for the test. Urine is sterile under normal health conditions.

Patient's preparation for the test:
Urine for a urinalysis can be collected at any time. The first morning specimen is considered the most valuable because it is more concentrated and more likely to yield abnormalities if present. It is important to clean the genitalia before collecting urine. Bacteria and cells from the surrounding skin can contaminate the specimen and interfere with the interpretation of test results. In women, menstrual blood and vaginal secretions can also be a source of contamination. Women should spread the labia of the vagina and clean from front to back. Men should wipe the tip of the penis. As patient starts to urinate, he/she should let some urine fall into the toilet, then collect one to two ounces of urine in the container provided, then void the rest into the toilet. This type of collection is called a “midstream collection” or a “clean catch.”

A urine specimen will only be useful for a urinalysis if taken to the doctor’s office or laboratory for processing within a short period of time. If it will be longer than an hour between collection and transport time, then the urine should be refrigerated or a preservative may be added.

Transport and stability of specimen:
Delivery of specimens to the laboratory is desirable as they are not recommended for transportation, but if transporting urine was considered as a must, then urine is to be transported in a tightly closed and leak proof containers under cold storage to avoid deterioration of specimen.

Urine specimens are stable for 24 hours if stored at 2 - 8ºC.

What is being tested in a urine sample?
A urinalysis is a group of chemical and microscopic tests. They detect the byproducts of normal and abnormal metabolism, cells, cellular fragments, and bacteria in urine. Urine is produced by the kidneys, located on either side of the spine at the bottom of the ribcage. The kidneys filter wastes out of the blood, help regulate the amount of water in the body, and conserve proteins, electrolytes, and other compounds that the body can
reuse. Anything that is not needed is excreted in the urine, passing from the kidneys to the bladder and then through the urethra and out of the body. Urine is generally yellow and relatively clear, but each time someone urinates, the color, quantity, concentration, and content of the urine will be slightly different because of varying constituents. Many disorders can be diagnosed in their early stages by detecting abnormalities in the urine. Abnormalities include increased concentrations of constituents that are not usually found in significant quantities in the urine, such as: glucose, protein, bilirubin, red blood cells, white blood cells, crystals, and bacteria. They may be present because:

1. there are elevated concentrations of the substance in the blood and the body is trying to decrease blood levels by “dumping” them in the urine,
2. kidney disease has made the kidneys less effective at filtering or,
3. of an infection, as in the case of bacteria and white blood cells.

A complete urinalysis consists of three distinct testing phases:

4. Visual examination, which evaluates the urine’s color, clarity, and concentration;
5. Chemical examination, which tests chemically for 9 substances that provide valuable information about health and disease; and
6. Microscopic examination, which identifies and counts the type of cells, casts, crystals, and other components, such as bacteria and mucus that can be present in urine.

A routine urinalysis usually consists of the visual and the chemical examinations. These two phases may be completed in the laboratory or doctor’s office. A microscopic examination is then performed if there is an abnormal finding on the visual or chemical examination, or if the doctor specifically orders it.

Interpretation of Urine Test Result:

Urinalysis results can have many interpretations. Abnormal findings are a warning that something may be wrong and should be evaluated further. Generally, the greater the concentration of the atypical substance, such as greatly increased amounts of glucose, protein, or red blood cells, the more likely it is that there is a problem that needs to be addressed. But the results do not tell the doctor exactly what the cause of the finding is or whether it is a temporary or chronic condition.

A normal urinalysis does not guarantee that there is no illness. Some people will not release elevated amounts of a substance early in a disease process, and some will release them sporadically during the day, which means that they may be missed by a single urine sample. In highly diluted urine, small quantities of chemicals may be undetectable.

Visual examination:

Urine can be a variety of colours, most often shades of yellow, from very pale or colourless to very dark or amber.

Unusual or abnormal urine colours can be the result of a disease process, some medications, or the result of eating certain foods. For example, some people excrete red-coloured urine after eating beets. The colour is from the natural pigment of beets and is not a cause for worry. However, red-colored urine can also occur when blood is present in the urine and can be an indicator of disease or damage to some
part of the urinary system. Blood can also be a contaminant that gets into the urine unintentionally during collection, such as from hemorrhoids or from menstruation. Once this contaminating blood is in the urine, it will be detected during the chemical phase of a urinalysis, and doctors will initially assume that it came from the urinary tract.

The density of urine color is also a crude indicator of urine concentration:

1. Pale yellow or colourless urine indicates dilute urine where lots of water is being excreted.
2. Dark yellow urine indicates concentrated urine and the excretion of waste products in a smaller quantity of water, such as is seen with the first morning urine, with dehydration, and during a fever.

Urine clarity refers to how clear the urine is. Usually, laboratories report the clarity of the urine using one of the following terms: clear, slightly cloudy, cloudy, or turbid. “Normal” urine can be clear or cloudy. Substances that cause cloudiness but that are not considered unhealthy include mucus, sperm and prostatic fluid, cells from the skin, normal urine crystals, and contaminants such as body lotions and powders. Other substances that can make urine cloudy, like red blood cells, white blood cells, or bacteria, indicate a condition that requires attention.

Urine colour and clarity can be a sign of what substances may be present in urine. However, confirmation of suspected substances is obtained during the chemical and microscopic examinations.

**Chemical examination:**

To perform the chemical examination, most clinical laboratories use commercially prepared test strips. These are thin plastic strips that hold small squares of paper called test pads, arranged in a row. The test pads have chemicals impregnated into them. When a strip is briefly, but completely, dipped into urine, the test pads absorb the urine and a chemical reaction changes the colour of the pad within seconds to minutes.

The laboratorian compares the colour change for each reaction pad to a colour chart provided with the test strips to determine the result for each test. Each reaction pad must be evaluated at the appropriate time for that chemical. If too short time or too much time has passed since the reaction, the laboratorian may get incorrect results. To reduce timing errors and eliminate variations in colour interpretation, automated instruments are frequently used to “read” the reaction colour on each test pad.

The degree of colour change on a test pad can also give an approximation of the amount of substance present. For example, a slight colour change in the test pad for protein may indicate a small amount of protein present in the urine whereas a deep colour change may indicate a large amount.

The most frequently performed chemical tests using reagent test strips are:

1. **Specific gravity:** there are no “abnormal” specific gravity values. This test simply indicates how concentrated the urine is. Specific gravity measurements
are actually a comparison of the amount of solutes (substances dissolved) in urine as compared to pure water.

2. **pH**: The kidneys play an important role in maintaining the acid-base balance of the body. Therefore, any condition that produces acids or bases in the body such as acidosis or alkalosis, or the ingestion of acidic or basic foods, can directly affect urine pH. Diet can be used to modify urine pH. A high-protein diet or consuming cranberries will make the urine more acidic. A vegetarian diet, a low-carbohydrate diet, or the ingestion of citrus fruits will tend to make the urine more alkaline.

3. **Protein**: the protein test pad measures the amount of albumin in the urine. Normally, there will not be detectable quantities. When urine protein is elevated, you have a condition called proteinuria which can be an early sign of kidney disease. Albumin molecule is smaller than most other proteins and is typically the first protein that is seen in the urine when kidney dysfunction begins to develop.

4. **Glucose**: glucose is normally not present in urine. When glucose is present, the condition is called glucosuria. It results from either: an excessively high glucose concentration in the blood, as may be seen in patients who have uncontrolled diabetes mellitus or a reduction in the “renal threshold.” When blood glucose levels reach a certain concentration, the kidneys begin to excrete glucose into the urine to decrease blood concentrations. Sometimes the threshold concentration is reduced and glucose enters the urine sooner, at a lower blood glucose concentration.

5. **Ketones**: ketones are not normally found in the urine. They are intermediate products of fat metabolism. They can form when a person does not eat enough carbohydrates (for example, in cases of starvation or high-protein diets) or when a person’s body cannot use carbohydrates properly. Ketones in urine can give an early indication of insufficient insulin in a person who has diabetes. Severe exercise, exposure to cold, and loss of carbohydrates, such as with frequent vomiting, can also increase fat metabolism, resulting in ketonuria.

6. **Blood (haemoglobin)**: this test is used to detect haemoglobin in the urine (haemoglobinuria). Its presence in the urine indicates blood in the urine (known as haematuria). The small number of RBCs normally present in urine usually results in a “negative” test. However, when the number of RBCs increases, they are detected as a “positive” test result. Even small increases in the amount of RBCs in urine can be significant. Numerous diseases of the kidney and urinary tract, as well as trauma, medications, smoking, or strenuous exercise can cause haematuria or haemoglobinuria. This test cannot determine the severity of disease nor be used to identify where the blood is coming from. For instance, contamination of urine with blood from hemorrhoids or vaginal bleeding cannot be distinguished from a bleed in the urinary tract. This is why it is important to collect a urine specimen correctly and for women to tell their health care provider that they are menstruating when asked to collect a urine specimen. Sometimes a chemical test for blood in the urine is negative, but microscopic exam shows increased numbers of RBCs. When this happens, the
laboratorian may test the sample for ascorbic acid (vitamin C) because vitamin C has been known to interfere with the accuracy of urine blood test results, causing them to be falsely low or falsely negative.

7. **Leukocyte**: esterase is an enzyme present in most white blood cells (WBCs). Normally, a few white blood cells (see microscopic examination) are present in urine and this test is negative. When the number of WBCs in urine increases significantly, this screening test will become positive.

8. **Nitrite**: this test detects nitrite and is based upon the fact that many bacteria can convert nitrate to nitrite in urine. When bacteria find their way into the urinary tract, they can cause a urinary tract infection (UTI). A positive nitrite test result can indicate a UTI. However, since not all bacteria are capable of converting nitrate to nitrite, a patient can still have a UTI despite a negative nitrite test.

9. **Bilirubin**: bilirubin is not present in the urine of normal, healthy individuals. In certain liver diseases, such as biliary obstruction or hepatitis, bilirubin leaks back into the blood stream and is excreted in urine. The presence of bilirubin in urine is an early indicator of liver disease and can occur before clinical symptoms such as jaundice develop.

10. **Urobilinogen**: urobilinogen is normally present in urine in low concentrations. It is formed in the intestine from bilirubin, and a portion of it is absorbed back into the bloodstream. Positive test results help detect liver diseases such as hepatitis and cirrhosis and conditions associated with increased RBC destruction (hemolytic anemia). When urine urobilinogen is low or absent in a patient with urine bilirubin and/or signs of liver dysfunction, it can indicate the presence of hepatic or biliary obstruction.

**Microscopic examination:**

Microscopic examination is performed on urine sediments. Cells, crystals, and other substances are counted and reported as the number observed “per high power field” (HPF). In addition, some entities, if present, are estimated as “few,” “moderate,” or “many,” such as epithelial cells, bacteria, and crystals.

1. **Red Blood Cells (RBCs)**: normally, a few RBCs are present in urine sediment. Inflammation, injury, or disease in the kidneys or elsewhere in the urinary tract, for example, in the bladder, ureter, or urethra, can cause RBCs to leak out of the blood vessels into the urine. RBCs can also be a contaminant due to an improper sample collection and blood from hemorrhoids or menstruation.

2. **White Blood Cells (WBCs)**: the number of WBCs in urine sediment is normally low. When the number is high, it indicates an infection or inflammation somewhere in the urinary tract. WBCs can also be a contaminant, such as those from vaginal secretions.

3. **Epithelial Cells**: normally in men and women, a few epithelial cells from the bladder (transitional epithelial cells) or from the external urethra (squamous epithelial cells) can be found in the urine sediment. Cells from the kidney (kidney cells) are less common. In urinary tract conditions such as infections, inflammation, and malignancies, more epithelial cells are present. Determining the kinds of cells present helps the health care provider pinpoint where the
condition is located. For example, a bladder infection may result in large numbers of transitional epithelial cells in urine sediment. Epithelial cells are usually reported as “few,” “moderate,” or “numerous” present per low power field (LPF).

4. **Microorganisms (bacteria, trichomonas, yeast):** In health, the urinary tract is sterile. There will be no microorganisms seen in the urine sediment. Microorganisms are usually reported as “none,” “few,” “moderate” or “numerous” present per high power field (HPF). Bacteria from the surrounding skin can enter the urinary tract at the urethra and move up to the bladder, causing a urinary tract infection (ascending UTI). If the infection is not treated, it can eventually move to the kidneys and cause pyelonephritis. Less frequently, bacteria from a blood infection (septicemia) may move into the urinary tract. This also results in a descending UTI. Special care must be taken during specimen collection, particularly in women, to prevent bacteria that normally live on the skin or in vaginal secretions from contaminating the urine. A urine culture may be performed if a UTI is suspected.

Yeast can also be present in urine. They are most often present in women who have a vaginal yeast infection, because the urine has been contaminated with vaginal secretions during collection. If yeast are observed in urine, then tests for a yeast (fungal) infection may be performed on vaginal secretions. Trichomonas are parasites that may be found in the urine of women or men (rarely). As with trichomonas are actually infecting the vaginal canal and their presence in urine is due to contamination. If found during a urinalysis, then follow-up testing for trichomonas vaginalis may be performed to look for a vaginal infection. The sexual partner should also be examined.

5. **Casts:** Normally, healthy people may have a few (0–5) hyaline casts per low power field (LPF). After strenuous exercise, more hyaline casts may be detected. Cellular casts, such as RBC and WBC casts, indicate kidney disorder. There are other types of casts formed, which may indicate other conditions.

6. **Crystals:** Urine contains many dissolved substances (solute) – waste chemicals that the body needs to eliminate. These solutes can form crystals, solid forms of a particular substance, in the urine if:
   a. the urine pH is increasingly acidic or basic;
   b. the concentration of dissolved substances is increased; and
   c. the urine temperature promotes their formation.

Crystals are identified by their shape and colour, and by the urine pH. They may be small, sand-like particles with no specific shape (amorphous) or have specific shapes, such as needle-like. Crystals are considered “normal” if they are from solutes that are typically found in the urine. Some examples of crystals that can be found in the urine of healthy individuals include: amorphous urates, crystalline uric acid, calcium oxalates, amorphous phosphates and calcium carbonate.

If the crystals are from solutes that are not normally in the urine, they are considered “abnormal.” Abnormal crystals may indicate an abnormal metabolic process. Some of these include: Cystine, Tyrosine and Leucine.
Crystals may group together to form kidney “stones” or calculi. These stones can become lodged in the kidney itself or in the ureters (the tubes that pass the urine from kidney to the bladder) causing extreme pain.
Lab Guide

Stool examination section
Stool examination

1. Ova, Cyst and Trophozoites

Stool analysis is a diagnostic tool used to test if the patient has a parasitic infection in his/her digestive tract.

The test is indicated when the patient complains from diarrhea that lasts for few days and/or having blood or mucous in loose stools, especially if the patient drinks unpurified water.

Specimen required:

A fresh stool specimen is collected in a clean container. The stool specimen should not be contaminated with urine or water. Once it has been collected, the stool should either be taken to the laboratory within an hour after collection or transferred into special transport container containing preservative solutions.

Often, multiple specimens are collected and tested. These should be collected at different times on different days because parasites are shed intermittently and may not be in the stool at all times. Multiple specimens can increase the likelihood that parasites will be detected.

Ova are hardy and can exist for some time in the environment without living in a host and remain infectious and that is why a fresh specimen is asked for.

Patient’s preparation for the test:

No special preparations are needed before giving the specimen.

General but necessary information:

Parasitic infections are especially a concern for certain groups such as infants, the elderly, and people with weakened immune systems such as those with HIV/AIDS. In these populations, a parasitic infection can result in serious symptoms and complications.

The most common symptoms of a parasitic infection are prolonged diarrhea, bloody diarrhea, mucus in stool, abdominal pain, and nausea. These symptoms typically arise days to weeks after exposure and persist. Some people may also have headaches and fever. Others may have few or no noticeable symptoms. If diarrhea lasts more than a few days, it may lead to weight loss, dehydration and electrolyte imbalance, dangerous conditions in children, the elderly and those with weak immune systems.
2. Fecal Occult Blood

Fecal occult blood test is intended to screen for digestive tract bleeding, which may be an indicator of silent peptic ulcer or colon cancer.

UNRWA adopts guaiac smear method, which uses a chemical indicator that shows a color change in the presence of blood.

**Preparation for the test:**

There are special dental, dietary and drug restrictions. The test detects any blood that enters the digestive tract. Therefore, steps that are taken to avoid introducing blood into the digestive tract will increase the quality of the test specimen.

1. Blood that arises from bleeding gums (following dental procedures or gum disease) may be detected by these tests. Patient should be advised to avoid having any dental procedures up to three days before submitting stool specimens.
2. Bleeding in the stomach that may be triggered by use of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, naproxen, and ibuprofen. Patient should be advised to stop taking these drugs for seven days prior to testing, if clinically possible.
3. Foods such as red meat, broccoli, turnips, cauliflower, apples, oranges, mushrooms, and horseradish, and drugs such as colchicine and oxidizing drugs (like iodine and boric acid) may also trigger the same chemical reaction and produce a false positive result. Patients may be instructed to avoid these foods and drugs three days prior to and during the testing period.
4. Vitamin C, on the other hand, interferes with the chemical reaction and prevents the color formation that is expected to develop when blood is present and produces a false negative result. Vitamin C supplements and fruit juices that contain vitamin C should be avoided three days prior to and during testing.

**Interpretation of Test Result:**

1. The fecal occult blood test is normally negative.
2. A positive test result indicates that abnormal bleeding is occurring somewhere in the digestive tract. This blood loss could be due to ulcers, diverticulosis, bleeding polyps, inflammatory bowel disease, hemorrhoids, blood swallowed due to bleeding gums or nose bleeds or due to benign or cancerous tumors.
Appendix 1: Diagnostic criteria for diabetes mellitus

Persons presenting with clinical manifestations that are normally associated with diabetes (such as polyuria, polydipsia, weight loss and blurred vision) and/or major risk factors for diabetes, should be referred to the laboratory for Fasting Plasma Glucose (FPG) testing. Fasting is defined as no consumption of food or drink other than water 8-12 hours before specimen collection.

1. The cut-off value for confirmation of diagnosis of diabetes is a FPG level 126mg/dl on at least two consecutive tests within one week.
2. A FPG falling between 100-125mg/dl would be classified as Impaired Fasting Glucose (IFG).
3. In order to establish or exclude the diagnosis of diabetes, patients should be required to perform another FPG test within a week. If the value is still between 100-125mg/dl, then a glucose tolerance test (OGTT) should be performed using a load containing the equivalent of 75gms of oral anhydrous glucose solution. Diabetes is diagnosed if plasma glucose is evaluated to be 200mg/dl two hours after the challenge.
## Appendix 2: Peripheral blood red cell abnormalities

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Description</th>
<th>Associated diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tear drop cells</td>
<td>Cells shaped like teardrops</td>
<td>Myeloproliferative syndrome, myeloplastic anaemia, thalassemia</td>
</tr>
<tr>
<td>Nucleated red cells</td>
<td>Erythrocytes with nuclei still present, normoblastic or megaloblastic</td>
<td>Haemolytic anaemias, leukaemias, myeloproliferative syndrome, polycythemia vera, myeloplastic anaemia, multiple myeloma, extra medullary haematopoiesis, megaloplastic anaemia, any severe anaemia</td>
</tr>
<tr>
<td>Howell-Jolly bodies</td>
<td>Spherical purple bodies (wright stain) within or on erythrocytes, nuclear debris</td>
<td>Hyposplenism, pernicious anaemia, thalassemia</td>
</tr>
<tr>
<td>Heinz inclusion bodies</td>
<td>Small round inclusions of denatured haemoglobin</td>
<td>Congenital hemolytic anaemias, hemolytic anaemia secondary to drugs, thalassemia (Hb H), haemoglobinopathies (Hb Zurich, Koln, and I)</td>
</tr>
<tr>
<td>Pappenheimer bodies (sidrocytes)</td>
<td>Sidrotic granules, staining blue with Wright or Prussian blue stain</td>
<td>Iron loading anaemias, Hyposplenism.</td>
</tr>
<tr>
<td>Cabot's rings</td>
<td>Purple, fine ring-like intra erythrocytic structure</td>
<td>Pernicious anaemia, lead poisoning.</td>
</tr>
<tr>
<td>Basophilic stippling</td>
<td>Punctate stippling with Wright stain</td>
<td>Hemolytic anaemia, lead poisoning, (mitochondrial RNA), and thalassemia.</td>
</tr>
<tr>
<td>Spherocytes</td>
<td>Spherical cell without pale centers; often small</td>
<td>Hereditary spherocytosis, Coomb's positive hemolytic anaemia, after transfusion of stored blood</td>
</tr>
<tr>
<td>Ovalocytes</td>
<td>Oval cells</td>
<td>Hereditary elliptocytosis, iron deficiency</td>
</tr>
<tr>
<td>Stomatocytosis</td>
<td>Red cells with slit-like(instead of circular) areas of central pallor</td>
<td>Congenital hemolytic anemia, thalassemia, burns, lupus erythematosus, lead poisoning, liver disease</td>
</tr>
<tr>
<td>Sickle cells</td>
<td>Crescent-shaped cells</td>
<td>Sickle cell, haemoglobinopathies.</td>
</tr>
<tr>
<td>Target cells</td>
<td>Cells with a dark centre and periphery and a clear ring in between.</td>
<td>Liver disease, thalassemia, haemoglobinopathies. (S, C, S-C, S-thalassemia).</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Schistocytes</th>
<th>Irregular contracted cells (severe poikilocytosis), fragmented cells.</th>
<th>Uremia, carcinoma, haemolytic uremic syndrome, disseminated intravascular coagulation, micro-angiopathic haemolytic anaemia, toxins, burns, thrombotic thrombocytopenic purpura.</th>
</tr>
</thead>
</table>

|--------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------|

<table>
<thead>
<tr>
<th>Acanthocytes</th>
<th>Small cells with thorny projections</th>
<th>A beta-lipoproteinemia (hereditary acanthocytosis or Bassen-Kornzweig disease), after splenectomy.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Anisocytosis (diameter)</th>
<th>Abnormal variation in size (normal diameter 6-8 µm)</th>
<th>Any severe anaemia</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Microcytes</th>
<th>Small cells&lt; 6 µm (MCV &lt; 80 fl)</th>
<th>Iron-deficiency and iron loading (sideroblastic) anaemia, thalassemia, lead poisoning, vitamin B6 deficiency</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Macrocytes</th>
<th>Large cells &gt; 8 µm (MCV &gt; 100 fl), MCV &gt; 94 fl male, &gt; 98 fl female.</th>
<th>Megaloblastic anaemia, liver disease, hemolytic anaemia (reticulocytes), physiologic macrocytosis of newborn, myelophthisis, hypothyroidism</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Megalocytes</th>
<th>Large(&gt; 8 µm) oval cells</th>
<th>Megaloblastic anaemia, pernicious anaemia</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Hypochromia</th>
<th>Pale cells with decreased concentration of hemoglobin (MCHC &lt; 31 g/dl)</th>
<th>Severe-iron deficiency and iron loading (sideroblastic) anaemia, thalassemia, lead poisoning, transferring deficiency</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Poikilocytes</th>
<th>Abnormal variation in shape</th>
<th>Any severe anemia</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Rouleaux</th>
<th>Aggregated erythrocytes regularly stacked on one another</th>
<th>Multiple myeloma, Waldenstrom’s macroglobulinemia</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Polychromatophilia</th>
<th>RBCs containing RNA, staining a pinkish-blue color; stains supravitaly as reticular network with new methylene blue</th>
<th>Hemolytic anemia, blood loss, uremia, after treatment of iron-deficiency or megaloblastic anaemia</th>
</tr>
</thead>
</table>

### Appendix 3: White blood cells differentiation (reference: manual)

<table>
<thead>
<tr>
<th>Neutrophillic Segmented Cell</th>
<th>Neutrophillic Band Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size:</strong> Medium</td>
<td><strong>Size:</strong> Medium</td>
</tr>
<tr>
<td><strong>Nucleus:</strong> Broken up into segments</td>
<td><strong>Nucleus:</strong> Shaped like a band</td>
</tr>
<tr>
<td><strong>Cytoplasm:</strong> Contains small pink or brownish granules</td>
<td><strong>Cytoplasm:</strong> Contains small pink or brownish granules</td>
</tr>
<tr>
<td><strong>Comments:</strong> May be confused with a neutrophillic band cell. When in doubt, call cell a neutrophillic segmented cell</td>
<td><strong>Comments:</strong> May be confused with neutrophillic segmental cell. When in doubt, call cell a neutrophillic segmented cell.</td>
</tr>
<tr>
<td><strong>When found:</strong> 55 to 75% in normal blood; increase in appendicitis, pneumonia</td>
<td><strong>When found:</strong> 2 to 6% in normal blood; increased in appendicitis, pneumonia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Eosinophillic Segmented Cell</th>
<th>Basophillic Segmented Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size:</strong> Medium</td>
<td><strong>Size:</strong> Small and medium</td>
</tr>
<tr>
<td><strong>Nucleus:</strong> Usually has 2 lobes or segments</td>
<td><strong>Nucleus:</strong> Usually indistinct; appears buried under large purple or purplish-black granules.</td>
</tr>
<tr>
<td><strong>Cytoplasm:</strong> Contains large red granules</td>
<td><strong>Cytoplasm:</strong> contains large purple or purplish-black granules</td>
</tr>
<tr>
<td><strong>Comments:</strong> Eosinophillic segmented cell has large red granules whereas neutrophillic segmented cell has small pink or brownish granules</td>
<td><strong>Comments:</strong> Easily identified by the large purple or purplish-black granules scattered throughout the cell.</td>
</tr>
<tr>
<td><strong>When found:</strong> 1 to 3% in normal blood; increased in asthma, hay fever, etc.</td>
<td><strong>When found:</strong> 0 to 1% in normal blood</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lymphocyte Cell</th>
<th>Monocyte Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size:</strong> Small, medium, or large</td>
<td><strong>Size:</strong> Large</td>
</tr>
<tr>
<td><strong>Nucleus:</strong> Closely knit and usually round</td>
<td><strong>Nucleus:</strong> Spongy and sprawling</td>
</tr>
<tr>
<td><strong>Cytoplasm:</strong> Light blue; may contain a few reddish granules, cytoplasm may be sparse and even absent in some small lymphocytes</td>
<td><strong>Cytoplasm:</strong> Light grey; may contain very tiny reddish granules</td>
</tr>
<tr>
<td><strong>Comments:</strong> Large lymphocytes may confused with monocyte. Numbers of large lymphocyte is closely knit and usually round. Nucleus of monocyte is spongy and sprawling.</td>
<td><strong>Comments:</strong> Monocyte may be confused with large lymphocyte. Nucleus of monocyte is spongy and sprawling. Nucleus of lymphocyte is closely knit and usually round.</td>
</tr>
<tr>
<td><strong>When found:</strong> 20 to 35% in normal blood; increased in infectious mononucleosis, lymphocytic leukaemia, and many other diseases.</td>
<td><strong>When found:</strong> 2 to 6% in normal blood; increased in tuberculosis and monocytic leukaemia</td>
</tr>
</tbody>
</table>
## Appendix 4: Bacteria diseases and their laboratory diagnosis

<table>
<thead>
<tr>
<th>Disease</th>
<th>Causative Organism</th>
<th>Source of Specimen</th>
<th>Diagnostic Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucellosis</td>
<td>Brucella melitensis, Brucella abortus, Brucella suis</td>
<td>Blood, bone marrow, CSF, tissue, lymph node, urine</td>
<td>Culture, specific serologic test</td>
</tr>
<tr>
<td>Cholera</td>
<td>Vibrio cholera</td>
<td>Feces</td>
<td>Stool smear and culture</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Haemophilus influenza, Klebsiella pneumonia,</td>
<td>Bronchoscopy secretions, sputum, blood, lung aspirate or</td>
<td>Smear and culture</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus, Streptococcus pneumonia</td>
<td>biopsy, pleural fluid</td>
<td></td>
</tr>
<tr>
<td>Strep throat, scarlet</td>
<td>Streptococcus pyogenes</td>
<td>Throat, lesion</td>
<td>Culture, serology</td>
</tr>
<tr>
<td>fever, impetigo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetanus</td>
<td>Clostridium tetani</td>
<td>Wound</td>
<td>Wound smear and culture</td>
</tr>
<tr>
<td>Toxic shock syndrome</td>
<td>Staphylococcus aureus</td>
<td>Tissue</td>
<td>Culture, latex agglutination</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>Mycobacterium tuberculosis</td>
<td>Sputum, gastric washings, urine, CSF, bronchial washing</td>
<td>Smear and culture of sputum, gastric</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>washings, urine, and CSF, skin test</td>
</tr>
<tr>
<td>Typhoid</td>
<td>Salmonella typhi</td>
<td>Blood (after first week of infection; feces after second</td>
<td>Culture and serologic testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>week of infection)</td>
<td></td>
</tr>
<tr>
<td>Shigellosis</td>
<td>Shigella species</td>
<td>stool</td>
<td>Culture, latex agglutination</td>
</tr>
<tr>
<td>Cholera</td>
<td>Vibrio cholera</td>
<td>stool</td>
<td>Culture, latex agglutination</td>
</tr>
</tbody>
</table>

## Appendix 5: List of microorganisms according to their gram stain

<table>
<thead>
<tr>
<th>Gram negative organisms</th>
<th>Gram positive organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td><strong>Klebsiella species</strong></td>
<td>Staphylococcus epidermides</td>
</tr>
<tr>
<td><strong>Enterobacter species</strong></td>
<td>Alpha-haemolytic streptococci (viridans)</td>
</tr>
<tr>
<td><strong>Proteus species</strong></td>
<td>Streptococcus pneumonia</td>
</tr>
<tr>
<td><strong>Salmonella typhi</strong></td>
<td>Streptococcus faecalis (group D)</td>
</tr>
<tr>
<td><strong>Salmonella species</strong></td>
<td>Streptococcus pyogenes (group A)</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>Streptococcus agaiactia (group B)</td>
</tr>
<tr>
<td><strong>Neisseria meningitides</strong></td>
<td>Listeria monocytogenes</td>
</tr>
<tr>
<td><strong>Hemophilus influenza</strong></td>
<td>Clostridium perfringens</td>
</tr>
<tr>
<td><strong>Bacteroides fragilis (anaerobe)</strong></td>
<td>Peptococcus species (anaerobes)</td>
</tr>
<tr>
<td><strong>Brucella species</strong></td>
<td>Peptostreptococcus species</td>
</tr>
<tr>
<td><strong>Pseudomonas pseudomallei</strong></td>
<td>Candida albicans and other yeast-like fungi (e.g. Cryptococcus)</td>
</tr>
</tbody>
</table>

Appendix 6: Packaging and transport of biological specimens

Local transportation: transport of specimens from clinics, hospital wards or any health facility to a laboratory or from laboratory to another laboratory within the country.

1. Packaging:
   a. Primary specimen container should be leak & water-proof and should be sealed with parafilm, then foiled with absorbent paper and again sealed with tape.
   b. Specimen container should be located in one of double-pocket biohazard bag.
   c. The request form or any papers accompanies the specimen should be located in the other pocket.

2. Transport:
   a. The specimen should be in upright position.
   b. The container should be located in a leak-proof unbreakable box with biohazard label.
   c. The vehicle should be equipped with disinfectant, gloves and absorbent papers.
   d. A person assigned for transportation should be trained to tackle any accident that may happen during transportation.

International transportation: It should comply with the international regulation issued by IATA & ICAO.
Appendix 7: **Criteria for rejection of specimen**

1. Missing or inadequate identification or discrepancy between identity on specimen container and request form
2. Insufficient quantity of specimen
3. Specimen collected in inappropriate container
4. Inappropriate transport or storage of specimen leading to specimen deterioration
5. Deeply haemolysed blood
6. Deeply lipemic serum
7. Dry swab
Appendix 8: **Critical /panic values**

A critical or panic value is a test result falling significantly outside the normal range and may represent life-threatening value that requires urgent medical intervention. Panic values are summarized in the following table:

<table>
<thead>
<tr>
<th>Test</th>
<th>Unit</th>
<th>Patient age</th>
<th>Critical value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower limit</td>
<td>Upper limit</td>
</tr>
<tr>
<td>Glucose</td>
<td>mmol/L</td>
<td>All</td>
<td>≤ 2.8</td>
<td>≥ 30</td>
</tr>
<tr>
<td>Bilirubin total</td>
<td>mg/dL</td>
<td>0-7 days 1-4 weeks</td>
<td>≥15</td>
<td>20</td>
</tr>
<tr>
<td>Calcium</td>
<td>mmol/L</td>
<td>All</td>
<td>&lt; 1.7</td>
<td>≥ 3.2</td>
</tr>
<tr>
<td>Potassium</td>
<td>mmol/L</td>
<td>All</td>
<td>≤ 2.8</td>
<td>≥ 6.2</td>
</tr>
<tr>
<td>Sodium</td>
<td>mmol/L</td>
<td>All</td>
<td>≤ 120</td>
<td>≥ 160</td>
</tr>
<tr>
<td>Magnesium</td>
<td>mg/dL</td>
<td>All</td>
<td>&lt; 1</td>
<td>≥ 4.5</td>
</tr>
<tr>
<td>Creatinine</td>
<td>mg/dL</td>
<td>0-18 years 19 years</td>
<td>≥4</td>
<td>15</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>g/dL</td>
<td>All</td>
<td>&lt; 7</td>
<td>≥ 18</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>%</td>
<td>All</td>
<td>&lt; 24</td>
<td>≥ 54</td>
</tr>
<tr>
<td>WBCs</td>
<td>X 10³</td>
<td>Adult</td>
<td>≤ 2.5</td>
<td>≥ 20</td>
</tr>
<tr>
<td>Platelets</td>
<td>X 10³</td>
<td>6 months</td>
<td>≤ 50</td>
<td>≥ 700</td>
</tr>
</tbody>
</table>

*Any positive smear / culture from CSF, blood or Body fluids.
*Positive AFB(Acid Fast Bacteria (for tuberculosis)) smear or culture.
*Positive stool culture for salmonella, shigella, campylobacter & E.coli 0157
References

General:


Biochemistry:

c. International Federation of Clinical Chemistry:

Haematology:

e. Production of Basic Diagnostic Laboratory Reagents, WHO, 1995.

Serology:

b. Brad street CM and others: Intradermal test and serological tests in suspected Brucella

**Bacteriology:**

**Urine examination:**

**Stool examination:**