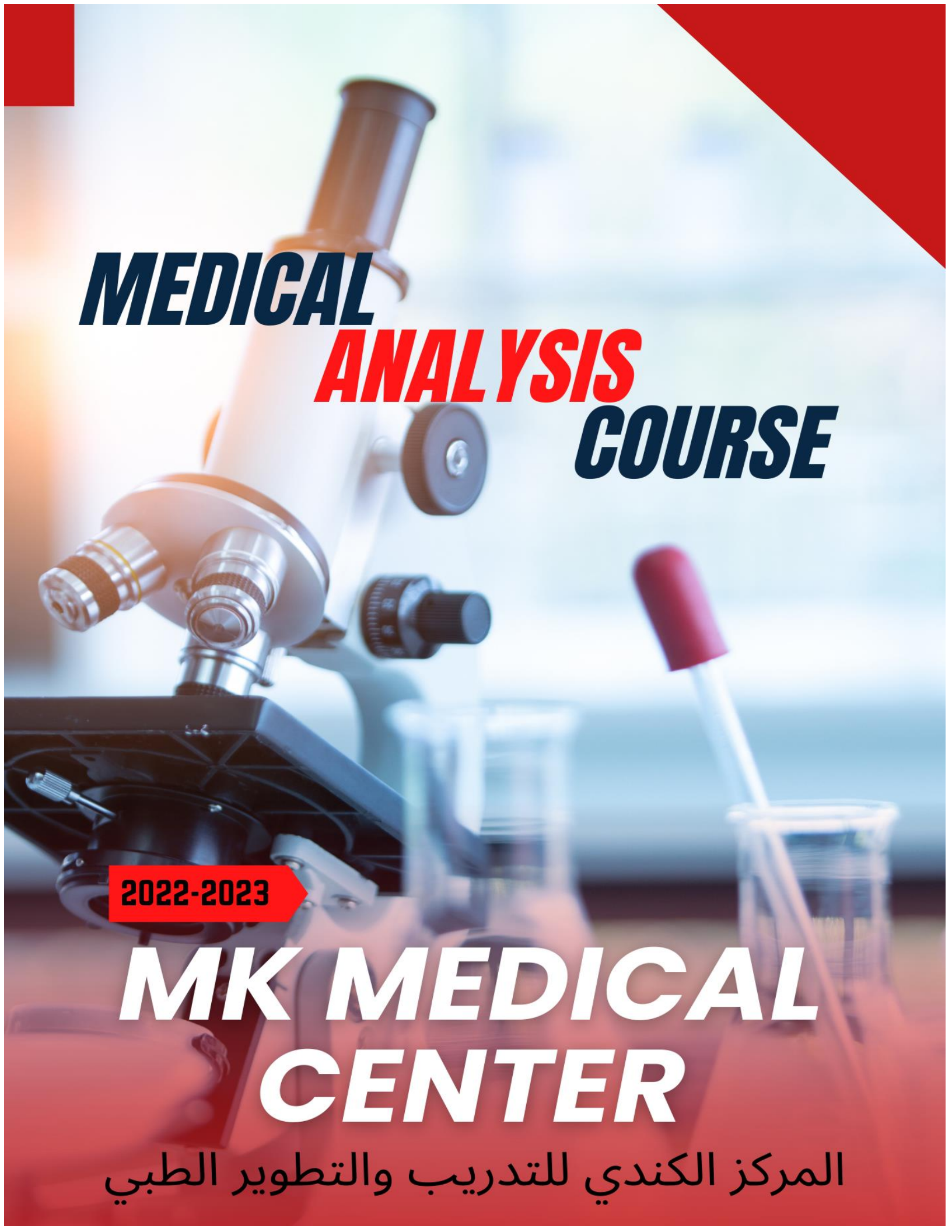


MEDICAL ANALYSIS COURSE

2022-2023

MK MEDICAL CENTER

المركز الكندي للتدريب والتطوير الطبي

المركز الكندي للتدريب والتطوير الطبي

بغداد/ كراده / شارع ٦٢ /
مقابل مطعم توست

07822103880

LABORATORY SAFETY

1. laboratory coveralls, gowns or uniforms must be worn at all times for work in the laboratory.

2. Appropriate gloves must be worn for all procedures that may involve direct or accidental contact with blood, body fluids and other potentially infectious materials or infected animals after use, gloves should be removed aseptically and hands must be washed.

3. personnel must wash their hands after their hands after handling infectious materials and animals, and before they leave the laboratory working areas.

4. Safety glasses, face shields (visors) or other protective devices must be worn when it is necessary to protect the eyes and face from splashes impacting objects and sources of artificial ultraviolet radiation.

5. It is prohibited to wear protective laboratory clothing outside the laboratory, e.g in canteens, coffee rooms, offices, libraries, staff rooms and toilets.

6. Open-toed foot wear must not be worn in laboratories.

7. Eating, drinking, smoking, applying cosmetics and handling contact lenses is prohibited in the laboratory working areas.

8. Storing human foods or drinks anywhere in the laboratory working areas is prohibited.

9. Protective laboratory clothing that has been used in the laboratory must not be stored in the same lockers or cupboards as street clothing

INTRODUCTION TO CLINICAL LABORATORIES

When a person is ill, diagnosis begins with physical examination by a doctor. It may not possible to diagnose disease only on the basis of physical examination. There are various diagnostic tests to confirm a suspected diagnosis. The clinical/pathological laboratory tests are extremely useful to find out the cause of disease.

The functional components of the clinical laboratory are:

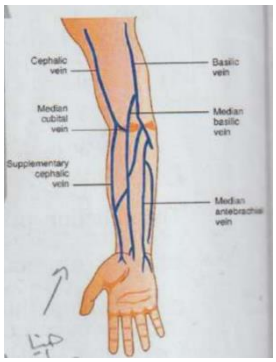
1. Clinical pathology
2. Hematology
3. Clinical biochemistry
4. Clinical microbiology
5. Serology
6. Blood bank
7. Histology and cytology.

Phlebotomy equipments:

The phlebotomist, the technician who collects blood, should be trained to:

1. Prepare specimen collection material.
2. Instruct patient appropriately.
3. Collect, preserve and transport specimen carefully.
4. Separate serum or plasma properly.
5. Maintain proper record of collection.
6. Handle the specimen carefully.
7. Analyze the specimen accurately.
8. Maintain proper recored of reports.
9. Work with appropriate safety precautions.

Blood collection:



Selecting vein site: For most venipuncture procedure on adults vein located in the arm are used. The median cubital vein is the one used for the patient. If the venipuncture of this vein is unsuccessful, one of cephalic or basilic veins may be used. The blood however usually flows more slowly from these veins.

Note: For the determination of blood pH, PCo2, PO2 and bicarbonate, arterial blood is used. It is usually performed by physicians.

LAB REQUEST:

The order or lab request contains a list of tests to be performed on one or more patient specimen, for example blood or urine. Each lab has its specific request that contains tests that performed in that lab only i.e. chemistry request, hematology request…ect.

The following items should be included on the lab requisition:

1. Full name: middle name should be included to avoid confusion in the event that there is another patient with the same first name and last name.

2. Location: inpatient, room unit, outpatient, address.

3. Patient's identification number: this identification can be very useful for instance in the blood bank.

4. Patient age and sex: in evaluating laboratory results, the reference values may differ for age and sex; disease prevalence may be age- or sex-linked.

5. Name(s) of the physician(s): name all of the physicians on the case; "panic values" should be called to the attention of the physician ordering the test; a physician may have some specific guidelines for his patients.

6. The date and time the test is to be done: some tests must be scheduled by the laboratory; patient preparation and diet regulations need to be considered.

7. Special notation: provide relevant information to assist the laboratory—e.g., medications taken; for hormone assay, the point in the menstrual cycle when the specimen was obtained.

Hemolysis of blood:

Hemolysis means liberation of hemoglobin from RBCs. Due to hemolysis, plasma or serum assumes pink to red color. It is important to avoid hemolysis during sampling, transporting and storage (too hot or too cold) because hemolysis causes changes in measurement of a number of analysis such as:

1- Serum K

2- Serum in.org P.

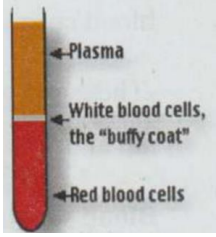
3- SGOT

4- SLDH

5- Acid phosphatase

Blood collection tubes:

- The tubes are covered with a color-coded plastic cap.
- They often include additives that mix with the blood when collected, and the color of the tube's plastic cap indicates which additives that tube contains.
- The tubes may contain additional substances that preserve the blood for processing in clinical laboratory.
- Using the wrong tube may therefore make the blood unusable.



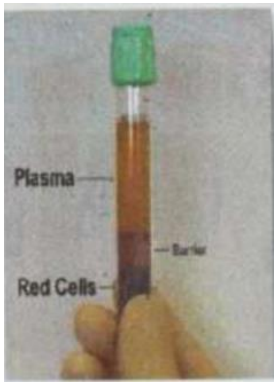
BLOOD

Blood is a liquid tissue. Suspended in the watery plasma are seven types of cells and cell fragments.

-Red blood cells (RBCs) -White blood cells (WBCs)

-Platelets-Five kinds of Leukocytes (lymphocytes, monocytes, neutrophils, eosinophils, basophils)

-After centrifugation of blood, the blood separate into three layers (see the figure)



BLOOD PLASMA:

- Plasma is the liquid component of blood.
- It is mainly composed of water, blood proteins and inorganic electrolytes.
- Roughly 92% water, mixed with organic and inorganic-substances.

- The most abundant plasma solute is the plasma protein, of which there are three groups: albumin, globulins, and fibrinogen.

Procedure of Plasma Preparation:

1. Draw blood from patient. Select vacutainer with an appropriate anticoagulant.
2. Mix well with anticoagulant.
3. Allow to stand for 10min.
4. Centrifuge the sample to speed separation and affect a greater packing of cells.
5. The supernatant is the plasma which can be now collected for testing purposes or stored (-20C to -80C) for subsequent analysis or use

Blood clot:

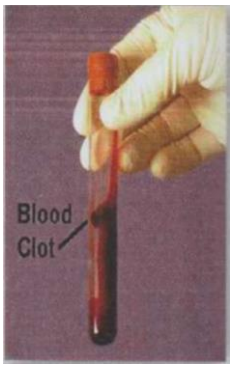
-When a blood sample is left standing without anticoagulant, it forms a coagulum or blood clot.

-One of the normal components of plasma is a soluble plasma protein called fibrinogen.

-On standing, this protein will be converted to insoluble substance called fibrin »» this occurrence is referred to as blood coagulation or clotting.

-The clot contains coagulation proteins, platelets, and entrapped red and white blood cells

Blood serum:



Serum is the same as plasma except that coagulation factors (such as fibrin) have been removed.

- For many biochemical laboratory tests, plasma and blood serum can be used interchangeably

Procedure of serum preparation:

1. Draw blood from patient. Select vacutainer with NO anticoagulant.

2. Allow to stand for 20 – 30min for clot formation.

3. Centrifuge the sample to speed separation and affect a greater packing of cells. Clot and cells with separate from clean serum and settle to the bottom of the vessel.

4. The supernatant is the serum which can be now collected by dropper or pipette for testing purposes or store (-20C to – 80C) for subsequent analysis or use.

General urine examination (GUE)

URINE

- Is an ultrafiltrate of plasma from which glucose, amino acids, water and other substances essential to body metabolism have been reabsorbed.
- The physiological processes by which approximately 170000 ml of filtered plasma is converted to the average daily urine output of 1200 ml is complex. Urine carries waste products, excess water or chemical substances from the body.
- In general, urine consists of urea (2%) and other organic and inorganic chemicals (2%) dissolved in water (96%).
- The concentration of these substances can be affected by various factors such as dietary intake, physical activity, body metabolism, endocrine function and others.
- Urea accounts for half of the total dissolved solids in urine. Other organic substances include primaryjcreahnine and uric acid (UA).

- The major inorganic solid dissolved in urine is chloride, followed by Na and K.
- Small or trace amounts of many additional inorganic chemicals (sulfate, carbonate, etc.) are also present in urine.

URINALYSIS

A urinalysis is a serial test of urine that used to detect and manage a wide range of disorders, such as urinary tract infections, kidney disease and diabetes.

Urinalysis involves checking the appearance, concentration and content of urine. Abnormal urinalysis results may point to a disease or illness. For example, a urinary tract infection can make urine look cloudy instead of clear. Increased levels of protein in urine can be a sign of kidney disease. Unusual urinalysis results often require more testing to uncover the source of the problem.

he purposes of performing a routine urine analysis are:

To aid in the diagnosis of disease

To screen for asymptomatic, congenital, or hereditary diseases

To monitor disease progression

To monitor therapy effectiveness or complications

Urinalysis

Physical examination

1. Volume.
2. color.
3. odor.
4. reaction(pH).
5. specific gravity.

Bipchemical Examination

1. Proteins
2. Sugers
3. Ketone bodies
4. Bile salts
5. Bile Pigments
6. Blood

Microscopic Tests

1. Cells
2. Crystals
3. Casts
4. Microorganism
5. Parasites
6. Contamination

Methodology:

A sample of well-mixed urine (usually 10-15 ml) is centrifuged in a test tube at relatively low speed (about 2-3,000 rpm) for 5-10 minutes until a moderately cohesive button is produced at the bottom of the tube. The supemate is decanted and a volume of 0.2 to 0.5 ml is left inside the tube. The sediment is resuspended in the remaining supmate by flicking the bottom of the tube several times. A drop of re-suspended sediment is poured onto a glass slide and cover slipped.

Physical Characteristics:

The first part of a urinalysis is direct visual observation Normal, fresh urine is pale to dark yellow or amber in color and clear. Normal urine volume is 750 to 2000 ml/24hr.

Examine the urine carefully by eye and… nose! Comment on the color of the urine. Try to use words like yellow, amber, dark and pale. Examine the sample for its odor(smell). Also note whether the sample is clear or cloudy.

1. Colour:

Color Typically yellow-amber, but varies according to recent diet and the concentration of the urine. Drinking more water generally tends to reduce the concentration of urine, and therefore causes it to have a lighter color.

CLOUDY OR MURKY

Cloudy or Murky urine color can be a sign of a urinary tract infection (UTI) or kidney stones.

RED

Reddish or Pinkish urine may indicate a tumor in the bladder or kidneys.

ORANGE

If your urine has a light orange shade, it could be due to mild dehydration. It could also be a sign of problem in liver or bile ducts.

BROWN

If your pee is dark brown or cola-colored, it can be due to diet, medication, or certain health issues.

NEON OR DARK YELLOW

Your urine may turn dark yellow due to severe dehydration or as a result of certain medicines.

GREEN

Consuming asparagus, green beer or artificial food coloring can change the color of your urine to a greenish tint.

BLUE

A rare genetic condition with excess calcium in blood can cause blue urine. Or, it could simply be due to medications containing blue dye.

2. Transparency: This is classified as clear and turbid.

The degree of cloudiness of urine depends on both its pH and its dissolved solids composition.

Turbidity may be due to gross bacteriuria, whereas smoky appearance is seen in hematouria.

Threadlike cloudiness is observed when the sample is full of mucus. In normal urine, the main cause of cloudiness is crystals and epithelial cells.

- In pathological urine, it is due to pus, blood and bacteria.

3. Odour:

Odour ordinarily has a little diagnostic significance.

1 - Aromatic odour------> Normal urine due to aromatic acids.

2- Ammonical odour----------> on standing due to decomposition of urea. 3- Fruity odour-------- > Diabetes due to the presence of ketones.

4. Volume:

- Urine volume measurements are part of the assessment for fluid balance and kidney functions.

- Most adults produce from 750ml-2500ml / 24h, with the average of about 1.5L per person.

- For RU A. a 10ml-12ml of sample is optimal for accurate of analysis.

5. Reaction (pH):

- The pH is a measure of how acidic or alkaline (basic) the urine is.

- Normal urine pH falls within the range of 4.5-8.

- Increased acidity in urine--------- > Diabetes mellitus and some medications.

- The urine must be fresh (why?), owing to the marked tenden of urine to be alkaline on standing as a result of ammonia liberation.

6. Specific Gravity (SG):

-This measures the amount of substances dissolved in the urine. It also indicates how well the kidneys are able to adjust the amount of water in urine. The higher the specific gravity, the more solid material is dissolved in the urine

Normal Chemical Constituents of Urine:

1-Urea

2- Uric acid (UA)

3- Creatinine

4- Chloride

5- Phosphate

6- Carbonate

7- Ammonia

8- Sulphates

Abnormal (Pathological) constituents of urine:

In determining whether pathological conditions exist, through urine analysis it is necessary to perform both physical and chemical tests. Of the physical tests that are available, only appearance of the urine will be observed, the chemical tests will be for pH, protein, glucose, ketones, hemoglobin. The significance of each abnormality will accompany the specific test. For detecting the presence of abnormal constituents, urine reagent strip will be used.

urine strip: The most cost-effective device used to screen urine is a paper or plastic dipstick. This microchemistry system has been available for many years and allows qualitative and semi-quantitative analysis within one minute by simple but careful observation. The color change occurring on each segment of the strip is compared to a color chart to obtain results.

Depending on the test performed, the results are reported as:

1. In concentration (mg/dl) .

2. As small, moderate, or large

3. Using the plus system (1+, 2+, 3+, 4+)

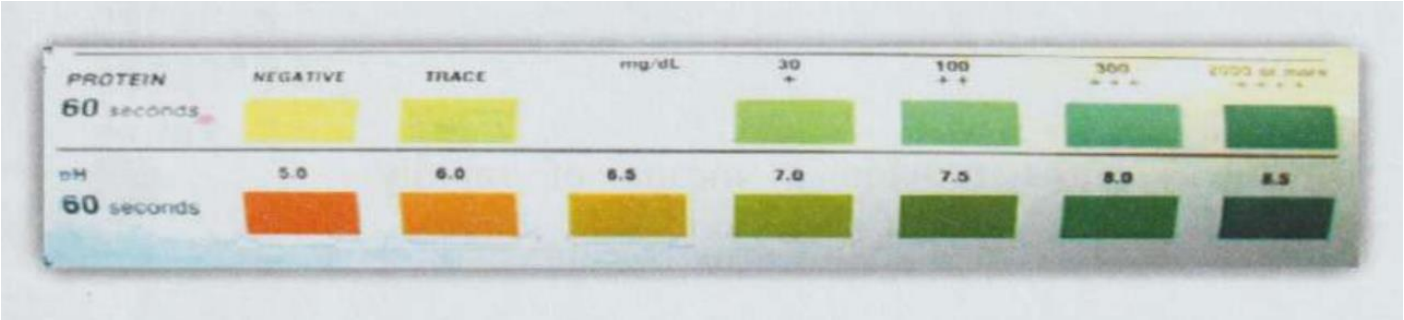
4. As positive, negative, or normal

Procedure:

1. Shake up the sample of urine and dip the test portion of a test strip into the urine. Tap the strip against the edge of the urine container to remove excess urine.

2. Immediately compare the test area with the color chart on the bottle. Note that the color scale runs from (negative) to (++++).

3. Record your results on the Urinalysis Worksheet.



Abnormal urine constituents include:

Substance	Name of Condition	Possible Causes
Glucose	Glucosuria	Excess sugary intake; diabetes mellitus
Proteins	Proteinuria	Physical exertion, pregnancy; glomerulonephritis, hypertension
Pus(WBCsand bacteria)	Pyuria	Urinary tract infection
RBCs	Hematuria	Bleeding in the urinary tract

Hemoglobin	Hemoglobinuria	Various: transfusion reaction, hemolytic anemia
Bile pigments	Bilirubinuria	Liver disease (hepatitis)

Microscopic Examination:

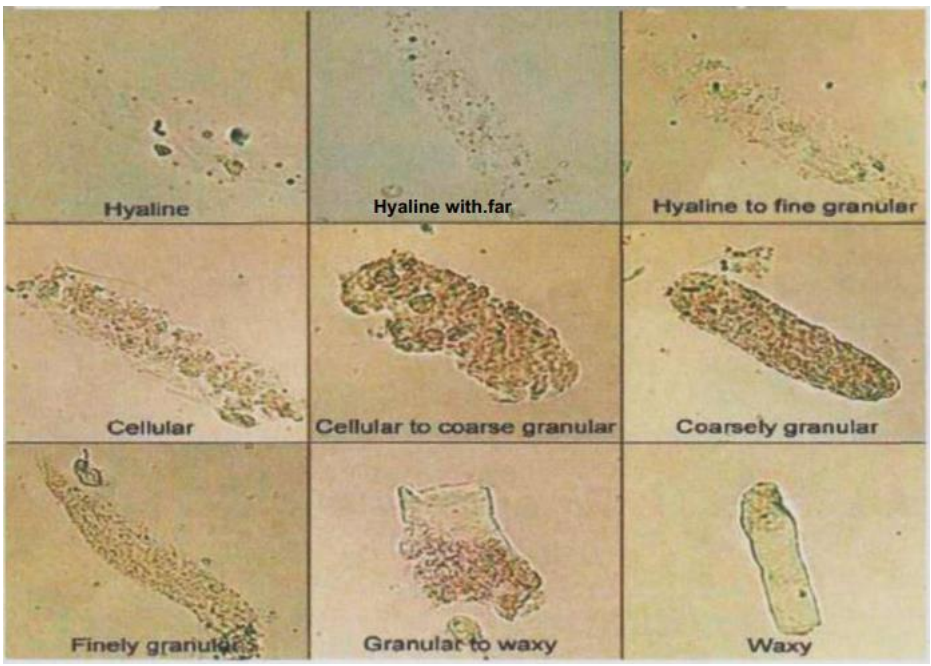
Specimens should be examined macroscopically and microscopically. Urine sedimentation may contain cells, casts and crystals and is examined microscopically after centrifugation of a urine sample.

A very small amount of all of the above sediments is normal. Concern begins when any of these components is significantly elevated. There are many different crystals, cell types, and casts that may be found in the urine of animals. Listed below are some common findings in the urine of small animals.

Crystals are common findings in urine especially if urine is allowed to stand long before examination. The type of crystal depends on the pH and constituents of the urine. Certain crystals like cystine, tyrosine, and leucine are always abnormal if detected. But other crystals are not abnormal, unless when associated with stone disease, in which case the type of crystal can give a clue to the origin of the stone. Exception is presence of large number of calcium oxalate crystals in association with ethylene glycol overdose.

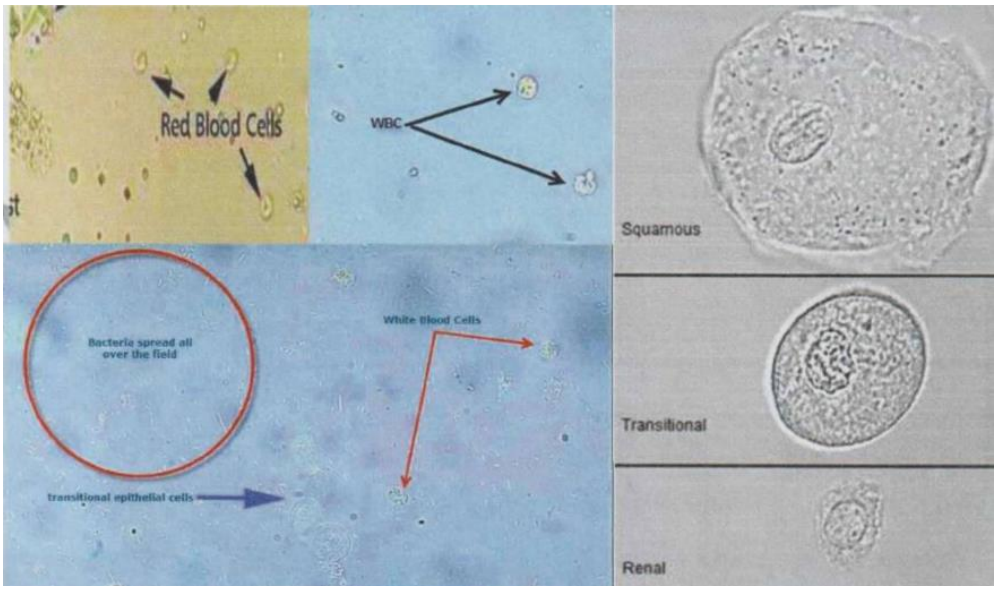
Urine under Microscopy

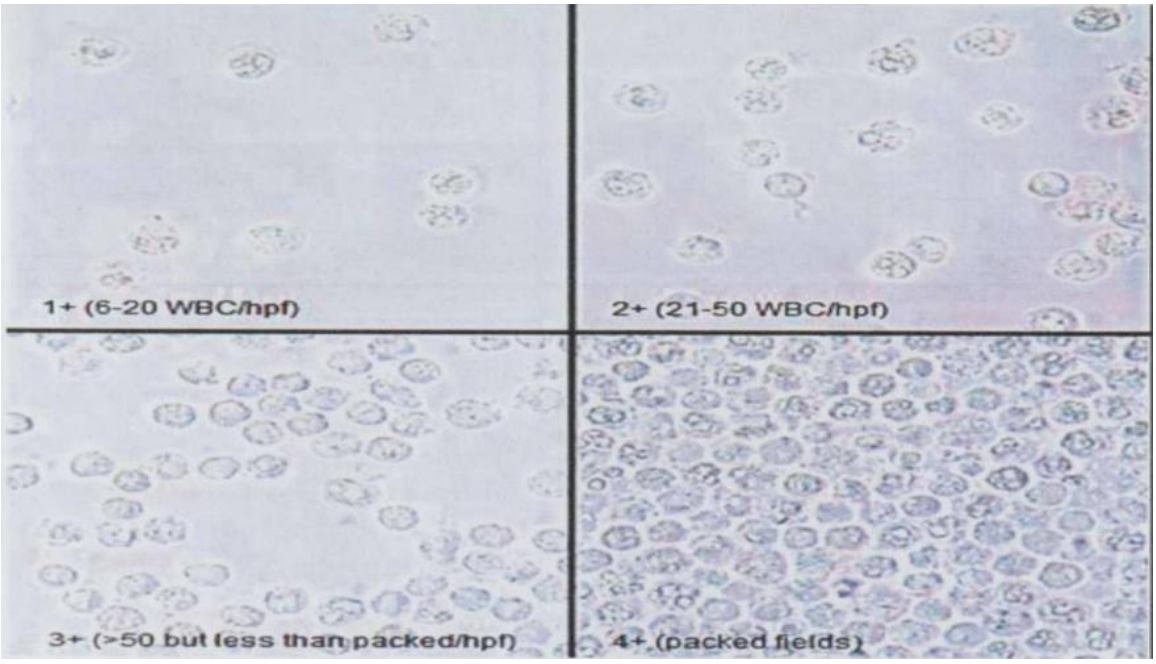
- Casts
- Hyaline Casts
- Cellular Casts
- White Cell Casts
- Red Cell Casts
- Renal Tubular Epithelial (RTE) Cell Casts
- Granular Casts
- Waxy Casts
- Fatty Cast
- Broad Cast



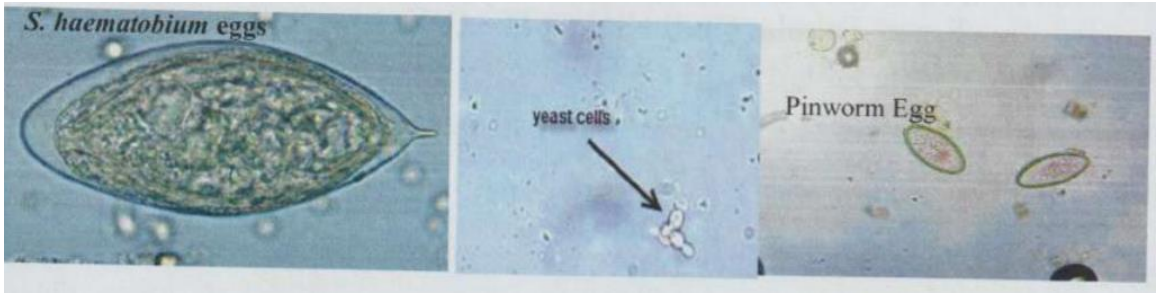
Cellular Elements

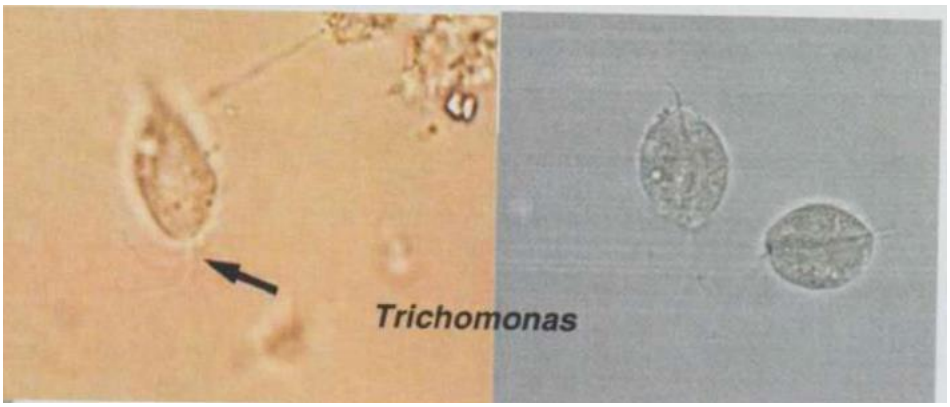
- Squamous Epithelial Cells
- Transitional Epithelial Cells
- Renal Tubular Epithelial (RTE) Cell
- White Blood Cells (WBCs)
- Red Blood Cells (RBCs)



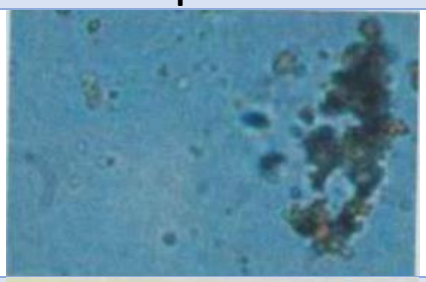
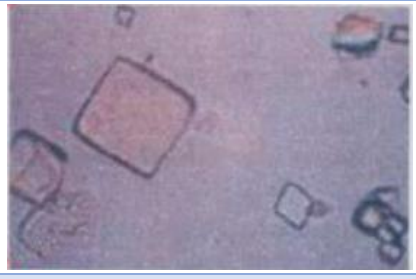


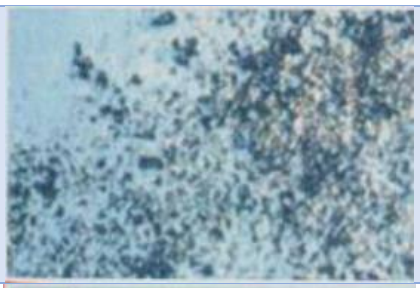
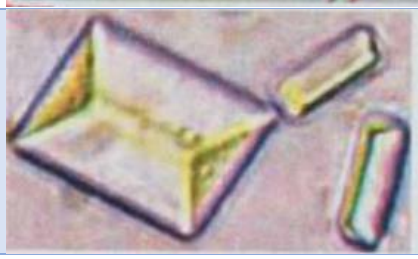
Bacteria, Yeast, and Parasites





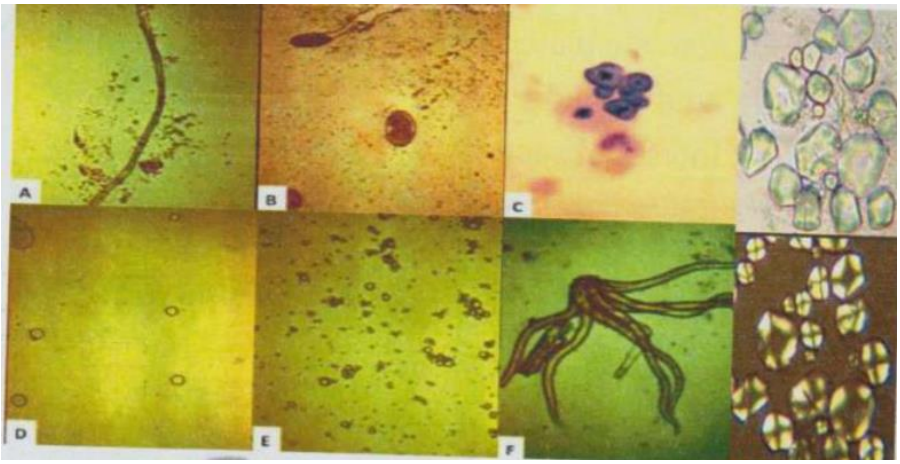
CRYSTALS

Crystal	Image	Description
Urine pH = Acidic		
Amorphous urates		Dark or yellow-red granules
Calcium oxalate	 Dihydrate  monohydrate	Envelope (dihydrate form) Oval, dumbbell (monohydrate form) Calcium oxalate crystals can be found in both normal and abnormal urine, and that they can be found in a range of urine pH from acidic to neutral.
Uric acid		Various shapes—rhombus, hexagonal plates. rosettes, rectangles, irregular shapes Colorless to yellow or brown
Urine pH = Alkaline		
Ammonium biurate		Round with thorny projections Dark yellow to brown

Amorphous phosphate		Amorphous granules
Calcium phosphate		Flat rectangles, prisms, rosettes
Triple phosphate		Four to six-sided prisms resembling coffin lids

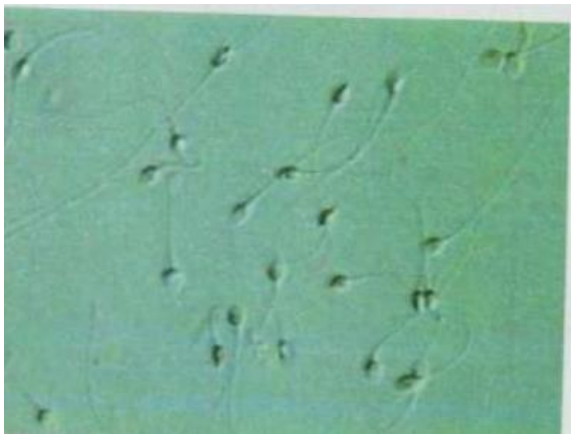
Artifacts in Urine Sediment

A. Hair
B. Pollen grain
C. Talcum powder
D. Air bubble
E. Fat droplets
F. Cloth fiber
G. Starch Granules

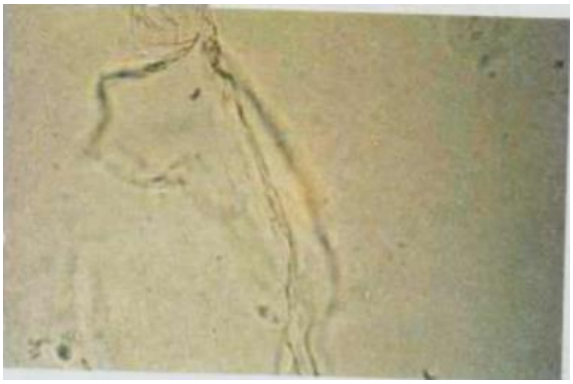


Miscellaneous Elements

Sperm



Mucous Threads



COMPLETE BLOOD COUNT (CBC)

The complete blood count (CBC) is a test that evaluates the cells that circulate in blood. Blood consists of three types of cells suspended in fluid called plasma: white blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs). They are produced and mature primarily in the bone marrow and, under normal circumstances, are released into the bloodstream as needed.

A CBC is typically performed using an automated instrument that measures various parameters, including counts of the cells that are present in a person's sample of blood. A standard CBC includes the following:

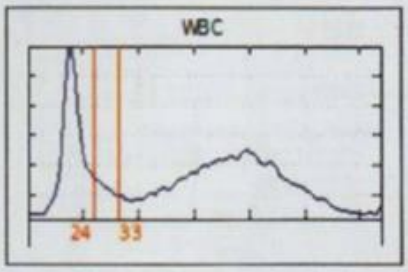
- Evaluation of white blood cells: WBC count; include a WBC differential
- Evaluation of red blood cells: RBC count, hemoglobin (Hb), hematocrit (Het) and RBC indices, which includes mean corpuscular volume (MCV), mean corpuscular hemoglobin J MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (ROW). The RBC evaluation may or may not include reticulocyte count.
- Evaluation of platelets: platelet count; may or may not include mean platelet volume (MPV) and/or platelet distribution width (PDW)

PATIENT RESULT REPORT

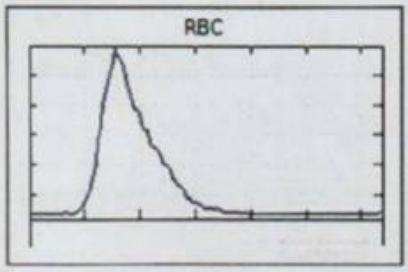
Patient ID	Name	First name
Type woman	Date of birth	
Sample ID Name?	Analysis date 2018/06/25 10:30:11	Operator labtech
Department		Physician

Analyser Alarms
Analysis Alarms

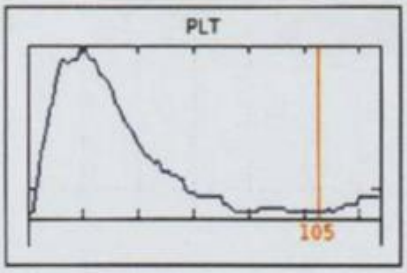
Name	Unit	value	Flag	Normal range
WBC	IO'/mm¹	7.3		4.0 -11.0
IYMS	%	28.8		20.0 -40.0
MONS	%	5.4		2.0-10.0
GRAS	%	65.8		40.0 - 75.0
LYM#	IO'/mm'	2.10		1.00-4.00
MON#	IO'/mm¹	0.30		0.10-1.00
GRA#	IO'/nvn'	4.90		2.00-7.50

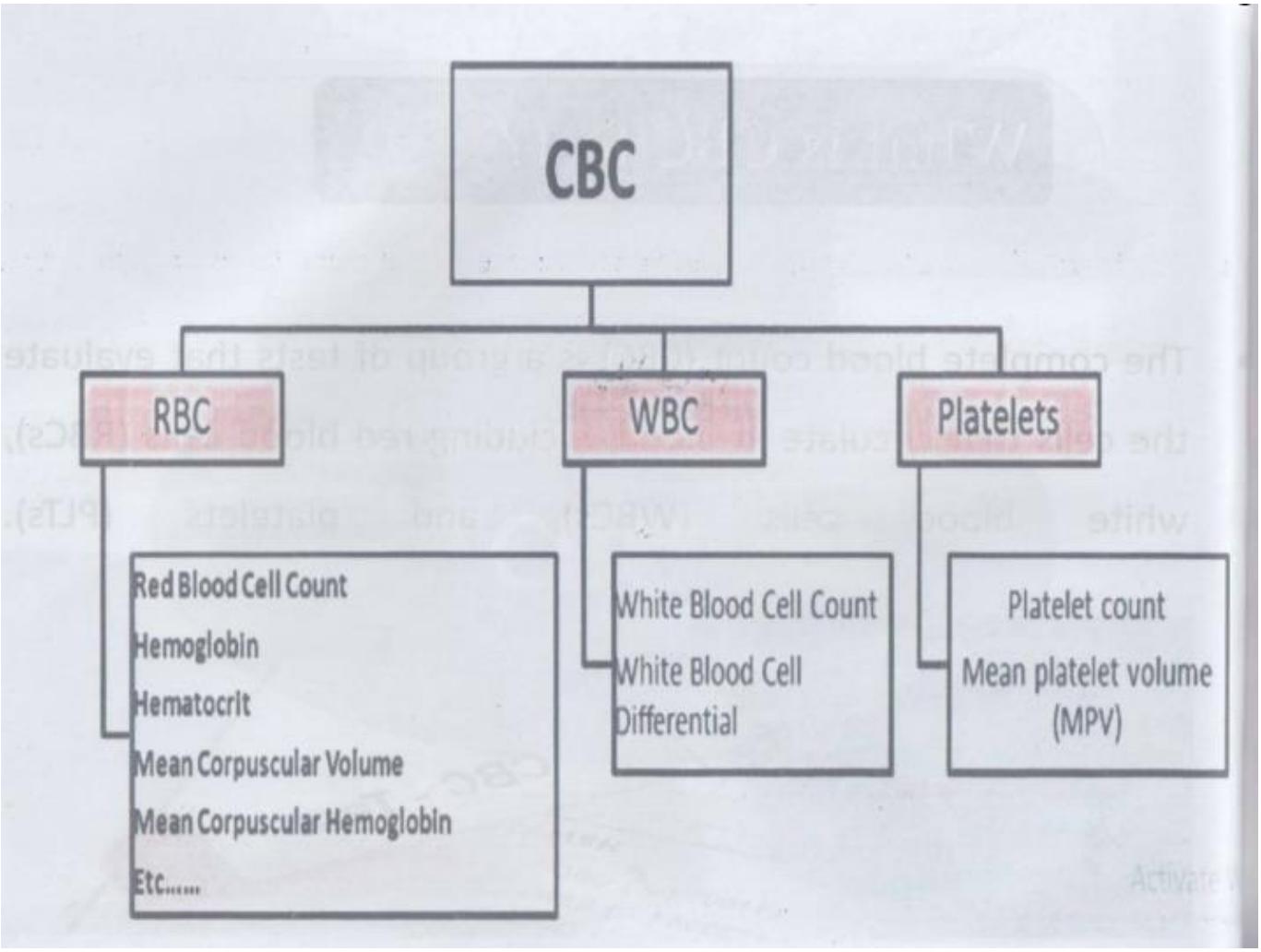


Name	Unit	value	Flag	Normal range
RBC	10'ffim¹	4.01		3.80 -4.80
HGB	g/dl	9.7	L	11.0-14.0
HCT	%	29.7	L	36.0 - 46.0
MCV	pm*	74	L	80-100
MCH	pg	24.3	L	27.0-31.0
MCHC	g/dl	32.8		31.0-34.0
RDW	%	14.7	H	11.0-14.0



Name	Unit	value	Flag	Normal range
PU	10¹/mm'	299		150 -400
MPV	pm¹	8.4		7.0-10.5
THT	%	0.250		0.150-0.500
PDW	%	15.8		11.0 -18.0





RED BLOOD CELLS

- Red blood cells, also called erythrocytes, are produced in the bone marrow and released into the bloodstream when they mature. They contain hemoglobin, a protein that transports oxygen throughout the body.

- A number of conditions can affect the production of new RBCs and/or
  their lifespan.

- RBCs normally are uniform in size and shape, but their appearance can be affected by a variety of conditions, such as vitamin B12 and folate deficiencies and iron deficiency.

Low RBC count	High RBC count
Anemia	**polycythemia**
Acute or chronic blood loss	**Congenital heart disease, causing low blood**
Malnutrition	**oxygen levels**
Chronic inflammation	**Renal problem**

WHITE BLOOD CELLS

- White blood cells, also called leukocytes, are cells that exist in the blood, the lymphatic system, and tissues and are an important part of the body's natural defense (immune) system.
- They help protect against infections and also have a role in inflammation, and allergic reactions. There are five different types of WBCs and each has a different function.
- They include neutrophils, lymphocytes, basophils, eosinophil and monocytes
- WBCs are present in the blood at relatively stable numbers infection can stimulate your bone marrow to produce a higher number of neutrophils to fight off a bacterial infection. With allergies, there may be an increased number of eosinophils.An

increased number of lymphocytes may be produced with a viral infection. In certain diseases, such as leukemia, abnormal (immature or mature) white cells may rapidly multiply.

Low WBC count -> Leukopenia

A Condition in which the number of leukocytes is abnormally low and which is most commonly due to sever infections (such as HIV) and radiation poisoning.

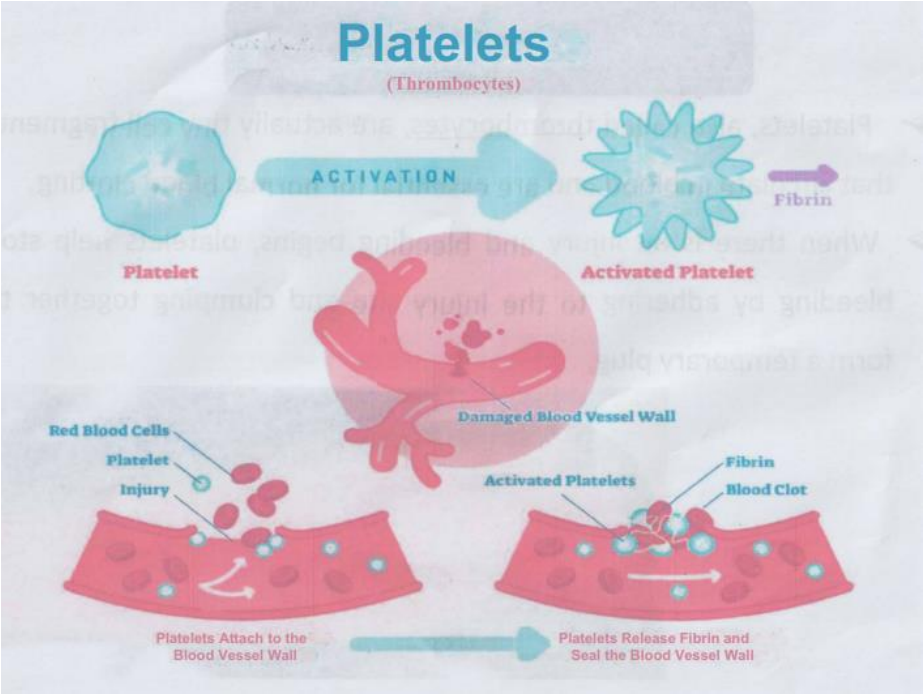
High WBC count -> Leukocytosis

A condition characterized by an elevated the number of WBC occur as a result of an infection, or cancer (Leukemia).

PLATELETS

Platelets, also called thrombocytes, are actually tiny cell fragments that circulate in blood and are essential for normal blood clotting.

When there is an injury and bleeding begins, platelets help stop bleeding by adhering to the injury site and clumping together to form a temporary plug.



WHAT IS INCLUDED IN A CBC?

A CBC is typically performed using an automated instrument that measures various parameters, including cell counts and the physical

features of some of the cells. A standard CBC includes:

Red blood cell (RBC) tests:

> Red blood cell (RBC) count is a count of the actual number of

red blood cells in your blood sample.

> Hemoglobin measures the total amount of the oxygen-carrying protein in the blood, which generally reflects the number of red blood cells in the blood.

> Hematocrit measures the percentage of your total blood

volume that consists of red blood cells.

White blood cell (WBC) tests:

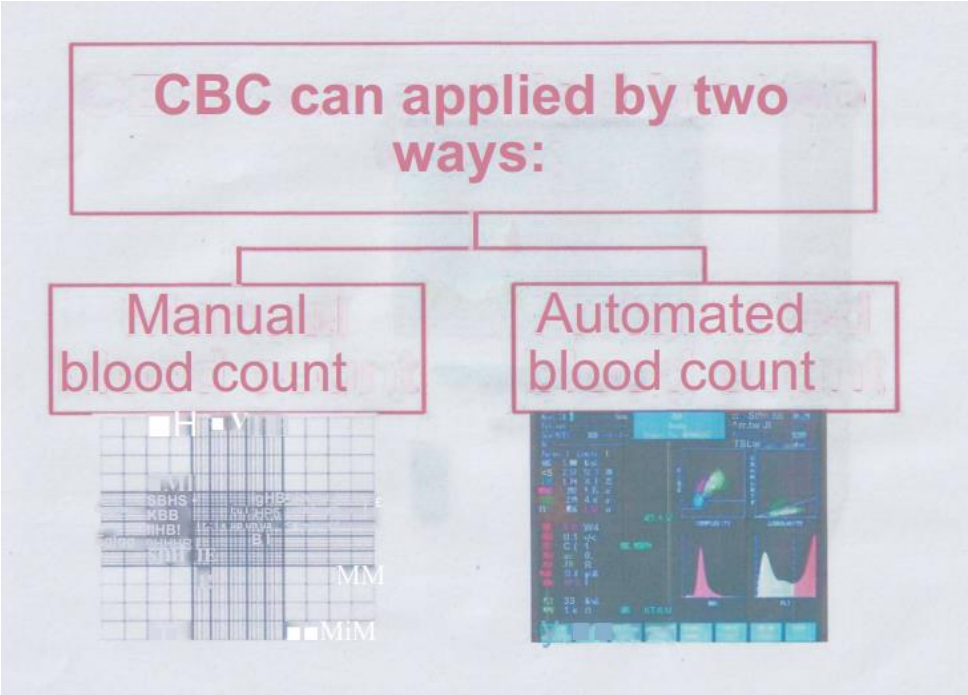
> White blood cell (WBC) count is a count of the total number of

white blood cells in your blood sample.

> White blood cell differential may be included as part of the CBC

or may be done in follow up if the WBC count is high or low.

. > The WBC differential identifies and counts the number of the five types of white blood cells present (neutrophils, lymphocytes, monocytes, eosinophils, and basophils)..



HAEMATOCRIT (PACKED CELL VOLUME - PCV)

Haematocrit or packed cell volume is the compact volume occupied by the red blood cells in a given volume of blood, expressed as a percentage. Materials required

- Well-mixed, anticoagulated whole blood
- Clay
- Reader card
- Centrifuge
- Capillary tubes.

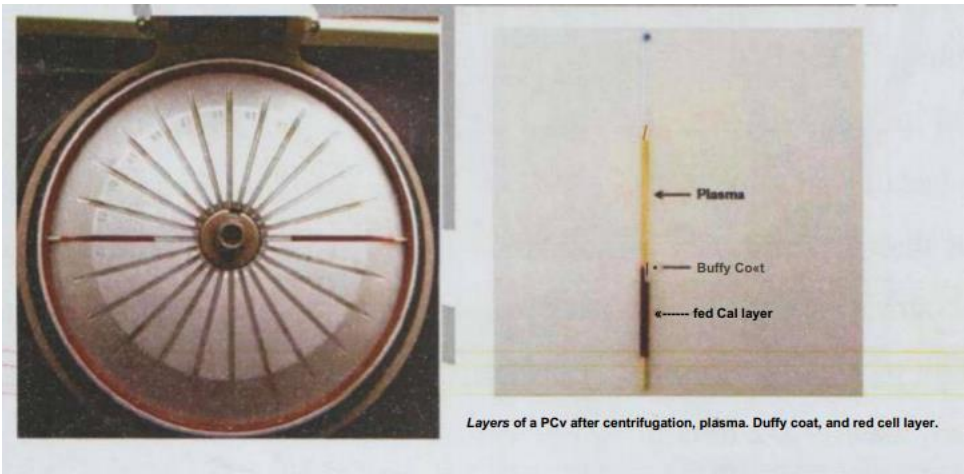
Procedure

1. Begin with well-mixed, anticoagulated blood.

2. Fill two capillary tubes 3/4 full with blood, and seal one end with clay.

3. Place tubes in the groves of the centrifuge, directly across from one another, with the clay pointing towards the outside.

4. Close centrifuge lid(s). Speed: 10.000 rpm Time: 5 minutes.



Layers of a PCv after centrifugation, plasma. Duffy coat, and red cell layer.

- 42-52% in men
- 36-48% in women
- 44-64% of newborn

CLINICAL IMPLICATIONS

A lower than normal hematocrit may indicate:

- An insufficient supply of healthy red blood cells (anemia)

• A large number of white blood cells — usually a very small portion of your blood — due to long-term illness, infection, leukemia, lymphoma or other disorders of white blood cells.

A higher than normal hematocrit may indicate:

- Abnormal increase in red blood cells (erythrocytosis)
- A disorder, such as polycythemia vera that causes your body to produce too many red blood cells (in polycythemia it may rise to as high as 70%).
- At higher altitudes, there is a lower oxygen supply in the air and thus hematocrit levels may increase over time.
- Low blood oxygen levels (hypoxia)

Erythrocyte Sedimentation Rate (ESR)g

Erythrocyte Sedimentation Rate is a nonspecific measure of inflammation that is commonly used as a medical “in vitro" screening test. Anticoagulated whole blood in tube separates into an upper layer of plasma and lower layer of plasma and lower layer of blood cells because of gravity. Erythrocytes are heavier and suspended in lighter plasma. The distance that cells fall (sedimentate) within a specified time interval (usually 1 hour) is defined as erythrocyte sedimentation rate (ESR). The rate of sedimentation of a single erythrocyte is 0.2 mm/h but in vivo the sedimentation rate is higher, usually 1-2 mm/h.

Procedure:

1. Collect approximately 1.2 mL of the venous blood and open the citrate tube.

2. Fill the tube with blood up to the level of 1.25 mL, close the tube firmly and mix.

3. Introduce Westergren tube into opened citrate tube. The blood meniscus should reach “0 mm” level Air bubbles should not be observed.

4. Place Westergren tube into the vertical stand (for an hour) or into the stand (For 1 hr) and then record the number of mm to which erythrocytes have settled.

Factors affecting ESR:

A- PHYSIOLOGICAL FACTORS:

a- ESR is increased in :

1- Old age

2- Females

3- Pregnancy

4- Menstruation.

b- ESR is decreased in:

1- New born

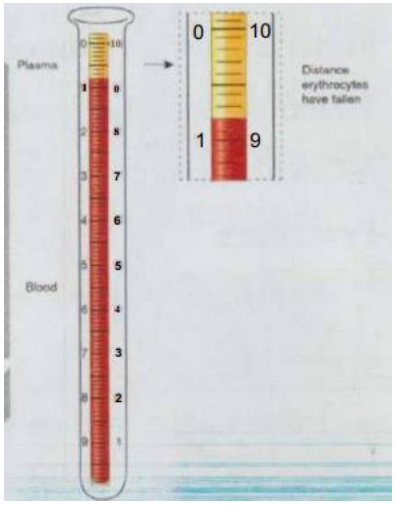
2- Males

الهاتف:0782210388
المركز الكندي للتدريب و التطوير الطبي

3- High altitude

B- PATHOLOGICAL FACTORS:

a- ESR is increased in:



1- Acute inflammation as tonsillitis.

2- Malignancy.

3- Chronic inflammation as T.B

4- Fevers

5- Rheumatic fever

6- Tissue trauma.

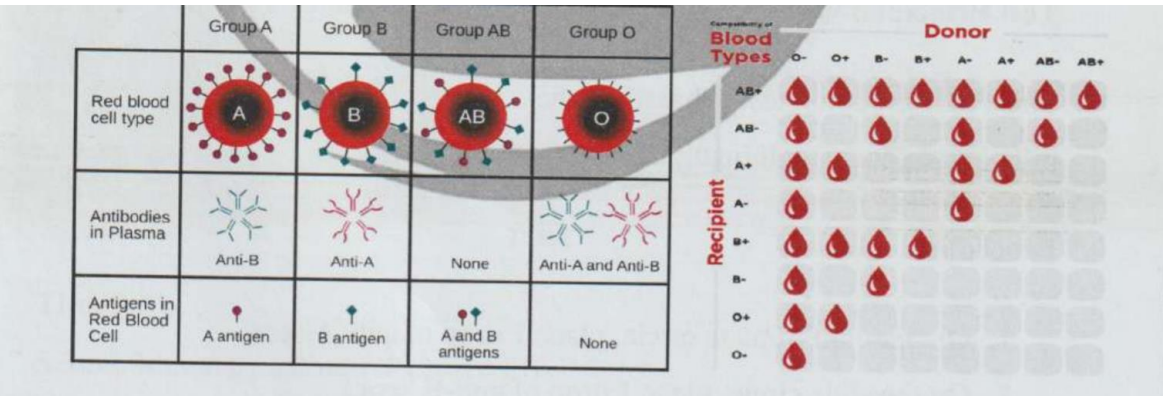
b- ESR is decreased in:

1 -polycythemia.

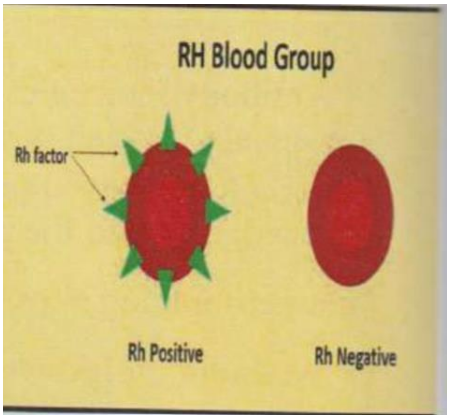
2-Hyperviscosity of plasma.

BLOOD GROUPING

The human blood consists of 4 main components, the red blood cells, the white blood cells, the plasma and the platelets. Groups are classified based on the presence or absence of a particular antigen on red blood cells. The ABO system is the main blood grouping system behind the principle that helps classify people into one of the following four groups, i.e., A, B, AB or O.



The Rh Factor: A person with the Rh factor on his or her red blood cells is said to be Rh-positive (Rh+).



Since this person has the factor, he or she will not make anti-Rh antibodies. A person without the Rh factor on their red blood cells is said to be Rh-Negative (Rh-).

This person WILL produce anti-Rh antibodies. Therefore, an Rh+ person may receive both an (Rh+J and an (Rh-) transfusion, but an (Rh-) person can only receive Rh- blood.

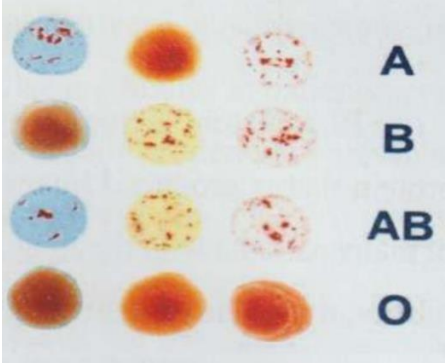
The Rh factor was first identified in the blood of a rhesus monkey. Also called Rhesus factor.

If an Rh – person receives Rh+ blood, hemolysis and anemia occur.

Rh factor is very important, especially in pregnancy. If mother is Rh- and the fetus Rh+, a condition called Erythroblastosis fetalis occurs, which can cause fetal death.

Test Principle:

This ABO grouping test is based on the principle of hemagglutination reaction.



Procedure:

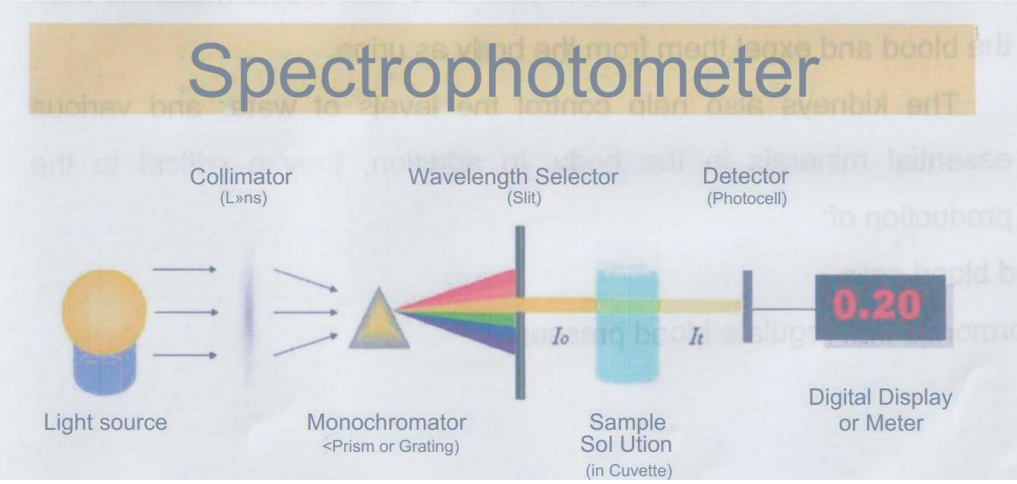
1. On one-half of plate circle, place 1 drop of anti-A sera.
2. On the slide circle, place 1 drop of anti-B sera.
3. On the slide circle, place 1 drop of anti-D sera.
4. Using Pastuer pipette add one drop of blood in front of reagent.
5. With separate applicator sticks, mix each cell- serum mixture well.
6. Observe and test agglutination.

Clinical Biochemistry

- Clinical Biochemistry is the division of laboratory medicine that deals with the measurement of chemicals (both natural and unnatural) in blood, urine and other body fluids.
- These test results are useful for detecting health problems, determining prognosis and guiding the therapy of a patient.

Principles of Spectrophotometry

The amount of light passing through the tube is measured by the photometer. The photometer delivers a voltage signal to a display device, The signal changes as the amount of light absorbed by the liquid changes.



الهاتف:0782210388
المركز الكندي للتدريب و التطوير الطبي

KIDNEY FUNCTION TESTS

- One of their most important jobs is to filter waste materials from the blood and expel them from the body as urine.
- The kidneys also help control the levels of water and various essential minerals in the body. In addition, they're critical to the production of:
- red blood cells
- hormones that regulate blood pressure Symptoms of kidney problems

Symptoms that may indicate a problem with your kidneys include: o high blood pressure

o blood in the urine

o frequent urges to urinate

o difficulty beginning urination o painful urination

o swelling of the hands and feet due to a buildup of fluids in the body

Types of kidney function tests

Serum creatinine test

This blood test examines whether creatinine is building up in your blood. The kidneys usually completely filter creatinine from the blood. A high level of creatinine suggests a kidney problem.

Type of specimen collection

Serum or plasma

Requirement and procedure

- Creating testing kit (R1 + R2)
  R1 = Picric acid
  R2 = Alkaline Reagent
- Spectrophotometer
- Timer
- Water bath/ heat block (37C)

	Blank	Standard	Sample
Std		100µl	
sample			100 µl

الهاتف:0782210388
المركز الكندي للتدريب و التطوير الطبي

Working slultion	1ml	1ml	1ml

$$\frac{A_s\ 2 - As\ 1}{A_{ST}2\ \text{“-A-grp* 1}} \times \text{con. of standard} = \text{mg/dl}$$

Normal value in serum

0.6-1.1 mg/dL Men

0.5-0.9 mg/dL women

URIC ACID

- Uric acid is the final product of purine metabolism , which found in the nucleic acid.

- Analysis or testing of uric acid levels is used for the diagnosis and treatment of numerous renal disorders including renal failure and kidney stones.

- Specimen collection

Serum or plasma

Requirements

- Water bath at 37°C , Timer
- Spectrophotometer at 520 nm
- Uric acid testing kit

Procedures For kit methods, follow manufacturer's instructions.

	Blank	Standard	Sample
Std		20µL	
sample			20 µL
Working slultion	1ml	1ml	1ml

Incubated at 37 gC for 5-10 minutes and read at 520 nm Result / Calculations of results

$$\frac{As}{Ast} \times \text{con. of standard} = \text{mg/dl}$$

	Blank	Standard	Sample
Standard		10 µL	
sample			10 µL
Working slultion	1ml	1ml	1ml

Incubated at 37 9C for 5-10 minutes and read at 500 nm Result I Calculations of results

$$\frac{As}{Ast} \times \text{con. of standard} = \text{mg/dl}$$

Normal values:

Fasting blood sugar : 70-100 mg/dl

Random Blood sugar : 70-120 mg/dl

- By measuring glycated haemoglobin (HbAic), clinicians are able to get an overall picture of what our average blood sugar levels have been over a period of weeks/months.
- For people with diabetes this is important as the higher the HbAic, the greater the risk of developing diabetes-related complications.
- The amount of glucose that combines with this protein is directly proportional to the total amount of sugar that is in your system at that time.
- Because red blood cells in the human body survive for 8-12 weeks before renewal, measuring glycated haemoglobin (or HbAic) can be used to reflect average blood glucose levels over that duration, providing a useful longer-term gauge of blood glucose control.
- If your blood sugar levels have been high in recent weeks, your HbAic will also be greater.

Lipid profile test

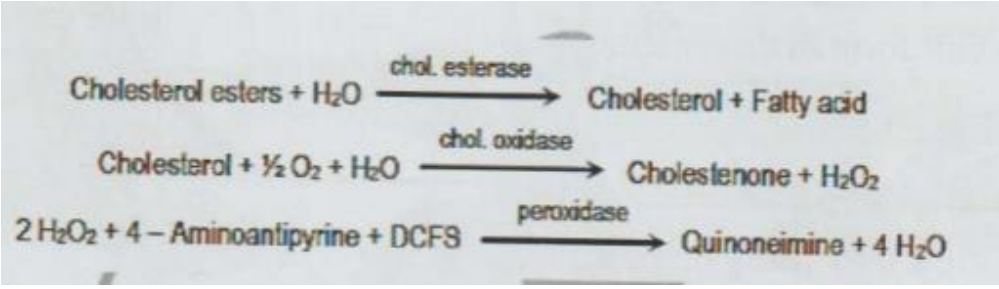
- Cholesterol
- Triglyceride
- HDL : High density lipoprotein
- LDL : low density lipoprotein
- VLDL very low density lipoprotein

HDL values:

• HDL less than 40 mg/dL ----- High risk of heart disease.

• A good level of HDL is > 60 mg/dL.

-Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the sample precipitate with phosphotungstate and magnesium ions then removed by centrifugation. The supernatant contains high density lipoproteins (HDL). HDL remain in the supernatant and can be determined enzymatically.



VLDL(Bad):

VLDL stands for very-low-density lipoprotein. Your liver makes CLDL cholesterol and releases it into your bloodstream. The VLDL particles carry triglycerides, another type of fat, to your tissues. VLDL is similar to LDL cholesterol, but LDL carries cholesterol to your tissues instead of triglycerides.

Triglyceride(TG):

The triglyceride molecule is a form of the chemical glycerol (tri=three molecules of fatty acid + glyceride=glycerol) that contains three fatty acids. To be absorbed, these parts are broken apart in the small intestine, and afterwards are reassembled with cholesterol to form chylomicrons. This is the source of energy for cells in the body. Fat cells and liver cells are used as storage sites and release chylomicrons when the body needs the energy.

- Triglycerides circulate in the blood to provide fuel for muscles to work.
- Extra triglycerides are found in the blood after eating a meal—when fat is being sent from the gut to adipose tissue for storage.

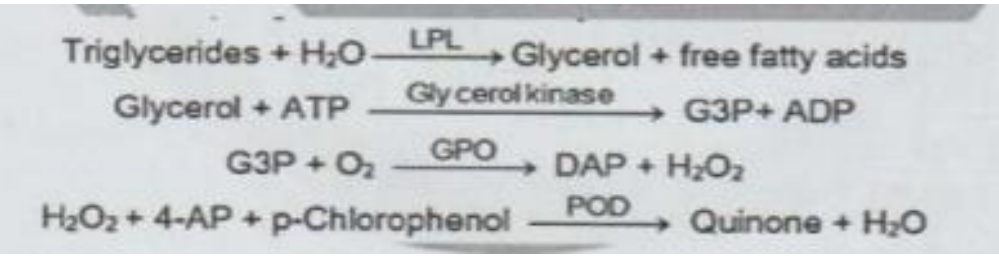
Certain diseases and conditions may cause elevated triglyceride blood levels, for example:

- Poorly-controlled diabetes
- Kidney disease
- Cirrhosis of the liver or other liver diseases
- Hypothyroidism
- Some medications

Principal (Triglyceride):

Sample triglycerides incubated with lipoprotein lipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H2O2).

In the last reaction, hydrogen peroxide (H2O2) reacts with 4-amino phenazone (4- % AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye:



The intensity of the color formed is proportional to the triglycerides concentration in the sample.

	Blank	Standard	Sample
R ml	1	1	1
Standard µl		10	
Sample µl			10

Incubated at 37 9C for 5-10 minutes and read at 510 nm

Result / Calculations of results

$$\frac{As}{Ast} \times \text{con. of standard} = \text{mg/dl}$$

Normal values :

Less than 200 mg/dl ----- normal

200-240 mg/dl ------- borderline

More than 240 mg/dl----- high

to the cholesterol concentration

The intensity of the color formed is proportional to the cholesterol concentration in the sample.

Samples:

Serum: Stability of the sample 7 days at 2-8C or freezing at – 20C will keep samples stable for 3 months.

Procedure:

1. Assay conditions:

Wavelength: 505 nm (500-550).

Cuvette: 1cm light path.

Temperature: 37C/ 15-25C

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	Standard	Sample
R ml	1	1	1
Standard µl		10	
Sample µl			10

4. Mix and incubate for 5 min at 37C or 10 min at 15-25C.

5. Read the absorbance (A) of the samples and calibrator, against the Blank. The color is stable for at least 60 minutes.

Calculations:

A (sample /A(standard)x 200 (Standard cone.) = mg/dL cholesterol in the sample

Conversion factor (cholesterol): mg/dL x 0.0258= mmol/L.

Conversion factor (TG): mg/dL x 0.0113= mmol/L.

Test	Result	Unit	Normal Range	
Cholesterol		mg/dl	Desirable	<200
			Borderline high	200 - 239
			High	>240
Triglycerides		mg/dl	Desirable	< 150
			Borderline high	150-199
			High	200-499
HDL		mg/dl	Male	
			No Risk	>55
			Moderate Risk	35 - 55
			High Risk	<35
			Female	
			No Risk	>65
			Moderate Risk	45-65
			High Risk	<45
LDL		mg/dl	optimal	< 100
			Near optimal / above optimal	100-129
			Borderline high	130-159
			High	160-189
			Very high	>=190

VLDL		mg/dl	13-60

C-REACTIVE PROTEIN (CRP)

• C-reactive protein (CRP) is a blood test marker for inflammation in the body. CRP is produced in the liver and its level is measured by testing the blood.

• CRP is classified as an acute phase reactant, which means that its levels will rise in response to inflammation. Other common acute phase reactants include the erythrocyte sedimentation rate (ESR) and blood platelet count.

ANTISTREPTOLYSIN O (ASO)

• Antistreptolysin O (ASO) is an antibody targeted against streptolysin O, a toxic enzyme produced by group A Streptococcus pyogenes bacteria.

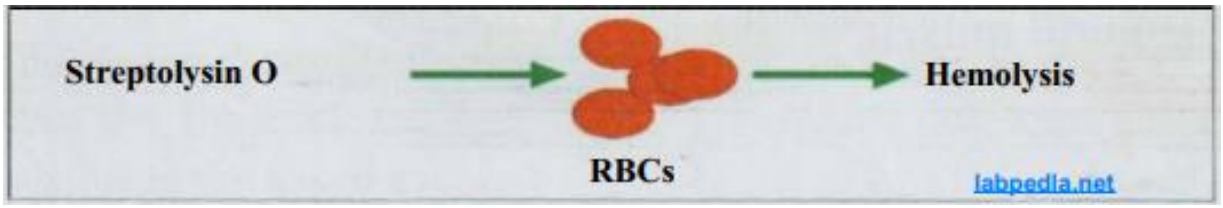
• Streptolysin O - lyses WBC. platelets, RBC — immunogenic.

Titer:

Serum diluted by saline 1:1 (20:20 µl)

If -v -> titer 200 IU/ml

if +v -> titer 400 IU/ml, and repeat by the dilution of last of last mix by retio 1:1 ,or. dilute the serum directly by the ratio 1 serum : 2 saline .and so on the result give - v. The titers in the test are 200-400-600-800 IU per ml.



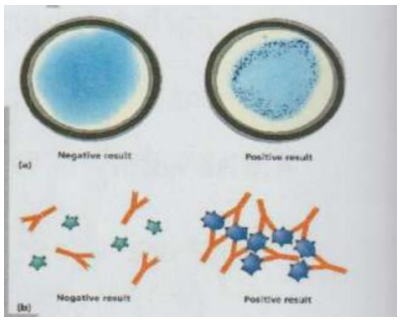
RHEUMATOID FACTOR (RF)

Rheumatoid factor (RF) is an autoantibody, an immunoglobulin M (IgM) protein that is produced by the body's immune system, and present in the blood of many patients with rheumatoid arthritis.. Autoantibodies attack a person's own tissues, mistakenly identifying the tissue as "foreign."

Test procedure

1. Allow the reagents and urine samples to reach room temperature.
2. slide.
3. Shake the regent vial and add one drop or reagent next to the drop of sample.
4. Mix both drops with stirrer covering the whole surface of the slide.
5. Rotate the slide for 2 min.
6. Observe for the presence or absence of agglutination.

Reading the results



Positive : Large clumping with clear background.
Negative: Absence of agglutination, uniform suspension.

Other test:

Anti nuclear antibodies (ANA) \ SLE

Anti mitochondrial antibodies (AMA)

Anti smooth muscle antibodies (ASMA)

Typhoid fever "Enteric fever"

Typhoid fever is a syndrome caused by Salmonellae Spp. This bacteria are often pathogenic for humans or animals when acquired by the oral route. They are transmitted from animals and animal products to humans where they cause enteritis, systemic infection and enteric fever.

The titer: It is the highest dilution that gives a positive (antigen-antibody reaction)

Viral Hepatitis

Hepatitis is an inflammation of the liver. Viruses cause most cases of hepatitis. There are several types of hepatitis viruses including types A, B, C, D, E, and possibly G. Types A, B, and C are the most common.

المركز الكندي للتدريب و التطوير الطبي
الهاتف:0782210388

WHAT ARE LIVER FUNCTION TESTS?

Liver function tests, also known as liver chemistries, help determine the health of your liver by measuring the levels of proteins, liver enzymes, and bilirubin in your blood.

Liver Function Tests (LFTs)

The liver is in the upper right part of the abdomen.

The functions of the liver include:

1 -Storing glycogen (fuel for the body) which is made from sugars. 2-Helping to process fats and proteins from digested food.

3-Making proteins that are essential for blood to clot (clotting factors). 4-Processing many medicines which you may take.

5- Helping to remove poisons and toxins from the body.

Liver function tests:

- LFTs are group of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver.

- As the liver performs it's various functions it makes a number of chemicals that pass into the bloodstream and bile. Various liver disorders alter the blood level of these chemicals. Some of these chemicals can be measured in a blood sample. Some tests that are commonly done on a blood sample are called 'LFTs' (liver function tests).

Liver function tests can be classified as:

a. Tests of excretion by the liver (ALP, Bilirubin).

b. Evaluation of synthesis in liver (Protein).

c. Evaluation of enzyme activity' (G PT,GOT, GGT).

Liver function tests are most often employed to determine:

i. The presence of liver disease.

ii. The type of liver disease.

iii. The extent and progression of liver disease. These usually measure the following:

Alanine Aminotransferase (ALT)=SGPT:

• ALT is the enzyme produced within the cells of the liver. The level of ALT abnormality is increased in conditions where cells of the liver have been inflamed or undergone cell death. As the cells are damaged, the ALT leaks into the bloodstream leading to a rise in the serum levels. Any form of hepatic cell damage can result in an elevation in the ALT.

• Clinical applications of ALT assays are confined mainly to evaluation of hepatic disorders.

Aspartate Aminotransferase (AST)= (SCOT);

-This enzyme also reflects damage to the hepatic cell. It is less specific for liver disease. It may be elevated and other conditions such as a myocardial infarct (heart attack)

Alkaline Phosphatase (ALP):

- Alkaline phosphatase is an enzyme, which is associated with the biliary tract. It is not specific to the biliary tract. It is also found in bone and the placenta. -If the alkaline phosphatase is elevated, biliary tract damage and inflammation should be considered.

-Alkaline phosphatase may be elevated in primary biliary cirrhosis, alcoholic

Gamma Glutamic Transpeptidase (GGT);

This enzyme is also produced by the bile ducts. However, it is not very specific to the liver or bile ducts. It is used often times to confirm that the alkaline phosphatase is of the hepatic etiology. Medications commonly cause GGT to be elevated. Liver toxins such as alcohol can cause increases in the GGT.

Measurement of Serum Bilirubin

Bilirubin is a yellow breakdown product of normal heme catabolism.

Production:

- After approximately 120 days in the circulation, red blood cells are taken up and degraded by the reticuloendothelial (RE) system, particularly in the liver, spleen and in the bone marrow.

- This releases hemoglobin which destroyed to the heme and the protein portion (globin).

- The globin parts are turned into amino acids.

- Iron is removed from the heme molecule, and the porphyrin ring is opened to form bilirubin.

Transport in plasma and hepatic uptake:

• Bilirubin is insoluble in water and is carried in plasma bound to albumin, and thus is not filtered at the glomerulus unless there is glomerular proteinuria.

• On reaching the liver, the bilirubin is taken into the hepatocyte.

Conjugation of bilirubin and secretion into bile:

In hepatocyte, water soluble bilirubin is produce and readily transported into bile.

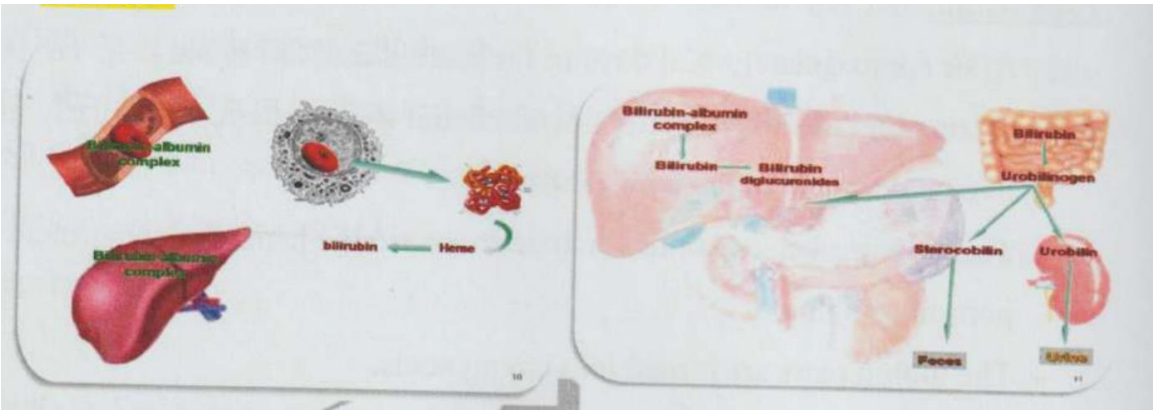
Further metabolism of bilirubin in the gut:

- In the intestine, glucouronic acid is removed by bacteria so, bilirubin is converted to urobilinogen.

- Most of urobilinogen is oxidized by intestinal bacteria to sterocobilin, which gives faeces its brown colour.

- Some of the urobilinogen is reabsorbed from the gut and enters the portal blood. Some urobilinogen participates in the enterohepatic urobilinogen cycle in which it is taken by the liver, and then re-exerted into the bile.

The remainder of the urobilinogen is transported by the blood to the kidney, where it is converted to yellow urobilin and exerted, giving urine its colour.



المركز الكندي للتدريب و التطوير الطبي
الهاتف:0782210388

TYPE OF BILIRUBIN:

Bilirubin is present in plasma as:

•Indirect Bilirubin (unconjugated bilirubin)

• Direct Bilirubin (conjugated bilirubin)

Jaundice:

• Is a term A used in c linical medicine to describe a condition in which the skin and sclera appear yellow.

Classification of the causes of Jaundice:

1. Prehepatic jaundice
2. Hepatic jaundice
3. Posthepatic jaundice

Prehepatic jaundice

•Results when excessive amount of bilirubin is presented to liver for metabolism, such as in hemolytic anemia.

• Characterized by unconjucated hyperbilirubinemia. Bilirubin not appear in urine. Why?

Hepatic jaundice

Results from:

• Impaired cellular uptake.
• Defective conjugation.
• Abnormal secretion of bilirubin by the liver cell.

Posthepatic jaundice

• Results from Impaired excretion of bilirubin caused by mechanical obstruction of the flow of bile into intestine.
• This may be due to gallstones or a tumor.
• Rise in the serum conjugated bilirubin level and stool becomes clay colored. Why?
• Conjugated bilirubin appears in the urine and the urine urobilinogen levels decrease.
• Too much bilirubin (hyperbilirubinemia) in a newborn baby can cause brain damage, hearing loss, problems with the muscles that move the eye, physical abnormalities, and even death.

Other tests include:

1 - Blood clotting tests
2- Total proteins (Globulin, albumin- alb, and fibrinogen).
3- Prothrombin time (PT)
4- Liver biopsy, ultrasound scan, other types of scan, etc, may be needed to clarify the cause of a liver disorder, and/or to monitor its progress.

Albumin test

Albumin is the main protein made by your liver. It performs many important bodily functions. For example, albumin:

• stops fluid from leaking out of your blood vessels
• nourishes your tissues
• transports hormones, vitamins, and other substances throughout your body An albumin test measures how well your liver is making this particular protein. A low result on this test can indicate that your liver isn't functioning properly. The normal range for albumin is 3.5-5.0 grams per deciliter (g/dL). However, low albumin can also be a result of poor nutrition, kidney disease, infection, and inflammation.

Test	Result	unit	Normal Range	
Bilirubin total		mg/dL	Male	Up to 1.4
			Female	Up to 0.9
			24 hours	<= 8.0
			48 hours	<=13.0
			84 hours	<=17.0
A.L.T(G.P.T)		U/L	Male	Up to 41
			Female	Up to 33
A.S.T(G.O.T)		U/L	Male	Up to 40
			Female	Up to 32
A.L.P		U/L	male	
			0 – 14 days	83 - 248
			15 – 365 days	122 - 469
			1 – 10 years	142 - 335
			10 – 13 years	129 - 417
			13 – 15 years	116 - 468
			15 -17 years	82 - 331
			17 – 19 years	55 - 149
			Adults	40 - 129
			female	
			0 – 14 days	83 - 248
			15 – 365 days	122 - 469
			1 – 10 years	142 - 335
			10 - 13 years	129 - 417
			13 – 15 years	57 - 254
			15 – 17 years	50 - 117

			17 – 19 years	45 - 78
			Adults	35 - 104

In males, FSH stimulates the Sertoli cells resulting in spermatogenesis and LH causes the interstitial Leydig ceils of the testes to produce testosterone.
2. The LH test measures the amount of luteinizing hormone, which is also secreted by the pituitary gland. In women, LH levels rise at mid-cycle; within 24 to 36 hours, ovulation occurs. Higher-than-normal levels of LH indicate several disorders, including ovarian failure and polycystic ovary disease.
3. Estradiol is the most important form of estrogen. It is primarily made in and released from the ovaries, adrenal cortex and the placenta, and it is responsible for the growth of the breasts, outer genitals, uterus, fallopian tubes and vagina.
These three hormones can all be checked with a simple performed on cycle Day 3.

- Testosterone is the principal male sex hormone (androgen) secreted by the test, starting at puberty (In women, the adrenal gland and ovaries produce small amounts.) Levels begin to plateau at around age 40, and gradually decrease. To one-fifth the peak level at age 80.

Purpose of Testosterone test:

- To evaluate male infertility or sexual dysfunction
- To help determine the cause of hypogonadism (decreased testosterone secretion)
- To aid in the evaluation of virilization (the development of male characteristics such as male-type baldness) in women

Prolactin

In females, prolactin stimulates the breasts to produce milk, after Oestrogen priming. During pregnancy, prolactin concentrations begin to increase at approximately six weeks gestation, peaking during late pregnancy.

Progesterone

Progesterone is the dominant ovarian hormone secreted during the luteal (second) phase of the menstrual cycle. Its main function is to prepare the uterus for implantation of an embryo, in the event that fertilisation occurs during theat cycle. If pregnancy occurs, human chorionic gonadotropin (hCG) is released which maintains the corpus luteum, which in turn allows progesterone levels to remain raised. At approximately twelve gestation weeks, the placenta begins to produce progesterone in place of the corpus luteum. Progesterone levels decrease after delivery and during breastfeeding. Progesterone levels are low in women after menopause. In males almost all progesterone is converted to testosterone in testes.

Test	Result	Unit	Normal Range	
follicle-stimulating hormone (FSH)		mIU/ml	Male	1.70 – 12.00
			female	
			Ovulation peak(D0)	6.30 – 24.00
			Follicular phase	
			First half (D-15 to D-9)	3.90 – 12.00
			Second half (D-8 to D-2)	2.90 – 9.00
			Luteal phase (D+3 to D+15)	1.50 – 7.00
			Menopause	17.00 – 95.00
luteinizing hormone (LH)		mIU/ml	Male	1.10 – 7.00
			Female	
			Ovulation peak(D0)	9.60 – 80.00
			Follicular phase	
			First half (D-15 to D-9)	1.50 – 8.00
			Second half (D-8 to D-2)	2.00 – 8.00
			Luteal phase (D+3 to D+15)	0.20 – 6.50
			Menopause	8.00 – 33.00

Prolactin (PRL)		ng/ml	Male	3.00 – 25.00
			Female	5.00 – 35.00
Testosterone		ng/ml	Male	2.27 – 10.30
			Female	
			>= 19 – 50 y	0.23 – 0.73
			>50 y	0.14 – 0.68
Progesterone		ng/ml	Male	0.11 – 0.56
			Female	
			Follicular phase	0.10 – 0.54
			Luteal phase	1.50 – 20.00
			Ovulation (D0)	0.12 – 6.22
			menopause	0.10 – 0.41
Human chorionic gonadotropin (HCG)		mIU/ml	Men:	< 3mIU/ml
			Cyclic women	<4 mIU/ml
			Menopausal women	<13mIU/ml
			Pregnant women	
			4 – 5w	1500 - 23000
			5 – 6w	3400 - 135300
			6 – 7 w	10500 - 161000
			7 – 8w	18000 - 209000
			8 – 9w	37500 - 219000
			9 – 10w	42800 - 218000
			10 – 11w	33700 - 218700
			11 – 12w	21800 - 193200
			12 – 13w	20300 - 166100
			13 - 14	15400 - 190000

			2nd trimester	2800 - 175100
			3rd trimester	2800 - 144400

THYROID FUNCTION TEST

HOW DOES THE THYROID GLAND FUNCTION?

The major thyroid hormone secreted by the thyroid gland is thyroxine, also called T4 because it contains four iodine atoms. To exert its effects, T4 is converted to triiodothyronine (T3) by the removal of an iodine atom. This occurs mainly in the liver and in certain tissues where T3 acts, such as in the brain. The amount of T4 produced by the thyroid gland is controlled by another hormone, which is made in the pituitary gland located at the base of the brain, called thyroid stimulating hormone (abbreviated TSH). The amount of TSH that the pituitary sends into the blood stream depends on the amount of T4 that the pituitary sees. If the pituitary sees very little T4, then it produces more TSH to tell the thyroid gland to produce more T4. Once the T4 in the blood stream goes above a certain level, the pituitary's production of TSH is shut off.

Test	result	unit	Normal range	
Triiodothyronine (T3)		nmol/L	Euthyroidism	0.92 - 2.33
			Hyperthyroidism	> 3.00
Thyroxine (T4)		nmol/L	Euthyroidism	60.00 - 120.00
			Hyperthyroidism	> 120.00
			Hypothyroidism	< 50.00
Thyroid stimulating hormone (TSH)		µUI/ml	Euthyroidism	0.25 - 5.00
			Hyperthyroidism	<0.15
			Hypothyroidism	>7

COMMON HORMONS

TEST SEX HORMONES

Measuring blood levels of the major sex hormones—estrogen and progesterone in women and testosterone in men—can aid in the evaluation of a variety of conditions, including fertility problems and certain cancers with sex-hormone- producing tumors. Because abnormal levels of sex hormones are sometimes associated with dysfunction elsewhere in the endocrine system—primarily the pituitary and adrenal glands—this test may be performed in conjunction with other hormone tests.

What is Cycle Day3?

What testing should I expect to have done?

A woman's menstrual cycle is measured from the first day of her period (blood flow, not spotting), so Cycle Day 3 is the third day of her period. When a woman is undergoing a fertility work up, cycle 3 is the day she has blood work performed to check the levels of three important levels: follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2)

The FSH blood test is used to help diagnose problems with sexual development, menstruation, and fertility. It can be used to diagnose or evaluate polycystic ovary disease, ovarian cysts, irregular vaginal bleeding and infertility.

In females, the combined action of FSH and LH stimulates growth of ovarian follicles and steroidogenesis, with the production of androgens, which are then converted to estrogens by the action of the enzyme aromatase. A mid-cycle surge in LH also triggers ovulation. FSH levels usually increase during menopause, because the ovaries become less responsive to FSH, which causes the pituitary gland to increase FSH production.