King Abdulaziz University
Faculty of Science
Department of Biochemistry
Girls section

Clinical Biochemistry Lab
BIOC 416

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Contents

Lab (1): Introduction to clinical laboratories

Diagnosis of any disease is first done by physical examination by physician and confirmed by lab diagnostic tests. Lab values are very important in determination of disease severity, drug doses and in follow up.

The sections of clinical laboratory are:

- Clinical biochemistry
- Clinical microbiology and paracytology.
- Hematology
- Serology
- Blood bank
- Histology and cytology

Clinical biochemistry:

In this lab the concentration of one or more substances in biological specimen of patient are measured and compared with reference value obtained from healthy subjects.

Types of samples that are used in testing:

Body fluids: blood, serum, plasma, urine, cerebrospinal fluid (CSF), feces, and other body fluids or tissues.

Biochemical tests in clinical medicine

- Lipid profile
- Diabetic profile
- Kidney profile
- Liver profile
- Bone profile
- Electrolyte profile

Lab request and lab report forms:

Lab request form: is the form that contains all the tests that can be ordered. Each lab has its own request form for example, chemistry request, hematology request… etc. It is filled by the doctor and sent to the lab.

Lab report form: is the form contains the result of patient.

Laboratory work flow cycle:

The flow cycle includes the entire steps of laboratory test, starting from test ordering by a doctor until reporting the results.

Three phases of laboratory testing:

- **Pre-analytical:** test ordering, specimen collection, transport and processing
- **Analytical-testing**
- **Post-analytical:** results transmission, interpretation, follow-up, retesting.

Phlebotomy (blood collection or blood drawing) :

The term phlebotomy refers to blood draw from a vein, artery, or the capillary bed for lab analysis or blood transfusion.

The phlebotomy equipments:

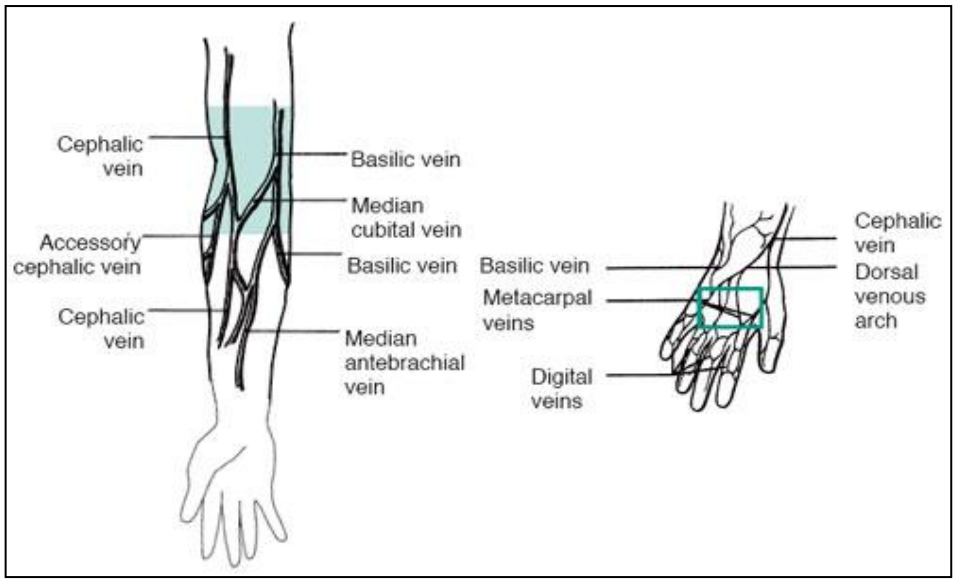
For blood collection, the following materials are required:

Disposable syringes 	**Vacationer systems** 	**Disposable lancets** 
Gauze pads 	**absorbent cotton** 	**Tourniquet** 
Alcohol swap 	**Plastic bandage**	**Waste container** 

Note: minimum use of tourniquet is advised because blood constituents may be changed due to prolonged venous occlusion.

Selection of vein site:

- Usually vein is used to collect blood by **veinpuncture procedure.**
- **On arm, there are three veins that can be used in blood collection: median cubital vein** "located on the middle"**, cephalic vein and basilic vein** "located on both sides".
- **Median cubital vein is the best choice (why?)** because it has good blood flow than cephalic and basilica which has slower blood flow.
- However, if veinpuncture procedure is unsuccessful in median capital; cephalic or basilic is used.
- **Artery blood** is rarely used in special cases as when blood gases, pH, CO_2, O_2 and bicarbonate is requested. It is usually performed by physicians.

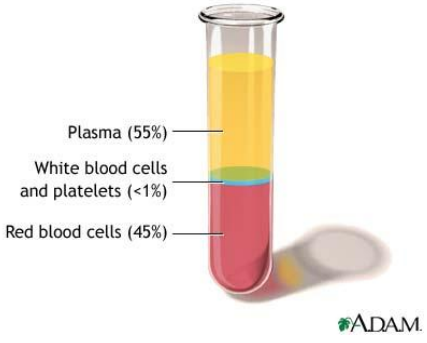


The image source from: http://www.unboundmedicine.com/

Preparation of Blood Sample:

One of three different specimens may be used: **whole blood**, **serum**, or **plasma**. Serum and plasma are prepared from whole blood by centrifugation.

After centrifugation of blood, it is separated into three layers (see the below figure).



Whole blood:

Whole-blood specimen must be analyzed within limited time (why?)

- Over time, cells will lyse in whole-blood which will change the conc. of some analytes as potassium, phosphate and lactate dehydrogenase.
- Some cellular metabolic processes will continuo which will alter analytes conc. like glucose and lactate.

Serum or plasma:

Differences between serum and plasma:

- Serum is the same as plasma except it doesn't contain clotting factors (as fibrin).
- Plasma contains all clotting factors.
- So, serum and plasma all has the same contents of electrolytes, enzymes proteins, hormones except clotting factors
- Serum is mainly use in chemistry lab & serology.

Blood collection tubes:

They are specific tubes evacuated from air called **vacutainer tubes. There are two major types of blood collection tubes:**

1. **Serum separating tubes (SST):** contain no anticoagulants**.**
2. **Plasma separating tubes (PST):** contain anticoagulant or preservative.

Blood anticoagulants:

- They are substances which prevent blood coagulation. They inhibit coagulation process by eliminating Ca2+ ions which are important in coagulation or by binding with thrombin and prevent conversion of fibrinogen to fibrin.
    - **EDTA, citrate and oxalate:** inhibits coagulation by binding with Ca^{2+} and prevent the activation of prothrombin to thrombin.
    - **Heparin**: inhibits coagulation by binding with thrombin which is important in activation of fibrinogen to fibrin.

Coagulation occurs in two major steps:

1- Prothrombin $\xrightarrow{Ca^{+2}}$ Thrombin

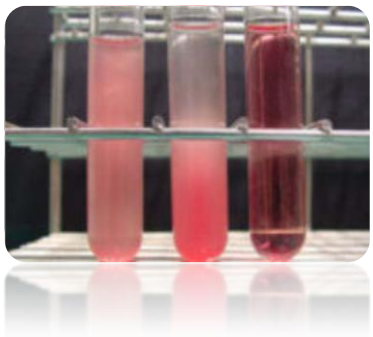
2- Fibrinogen $\xrightarrow{\text{Thrombin}}$ Fibrin (Clot)

- Choose the correct anticoagulant in blood collection is very important. It depends on the test to be performed; anticoagulant shouldn't interfere with the substance to be analyzed (measured).
- False positive or false negative results may be produced if blood collected in tube contains wrong anticoagulant. Here are some examples:
    - **Tubes contain sodium and potassium anticoagulants are not used in measuring concentration of electrolytes;** this will produce false positive result (Li, ammonia or heparin is used in electrolytes).
    - **Tubes contain Na^{+}-oxalate anticoagulant are not used for measuring Ca^{2+} level;** because oxalate will react with Ca^{+2} and precipitated as Ca^{2+}-oxalate.
    - **EDTA and citrate are not suitable for enzymatic assays**, because it binds with Ca^{2+} ion that is a cofactor for enzymes like alkaline phosphatase.
- Fluoridated **oxalate specimen is often recommended for glucose (Why?)**

See the last pages for the table of blood collection tubes.

Sample haemolysis:

- Haemolysis is the liberation of hemoglobin due to rupture of RBCs. Plasma or serum of haemolyzed sample appears pink to red color.
- Haemolysis must be avoided because it **interferes with the results** of some investigations. Usually it is associated with elevation in K^{+}, Ca^{2+}, phosphate, AST, LDH and acid phosphatases due to libration of RBCs contents.
- According to the degree of hemolysis it is classified as: H+, H++ and H+++. H+ may be accepted for some tests that are not affected by RBCs contents as glucose and lactate, H++ and H+++ not acceptable for any test.



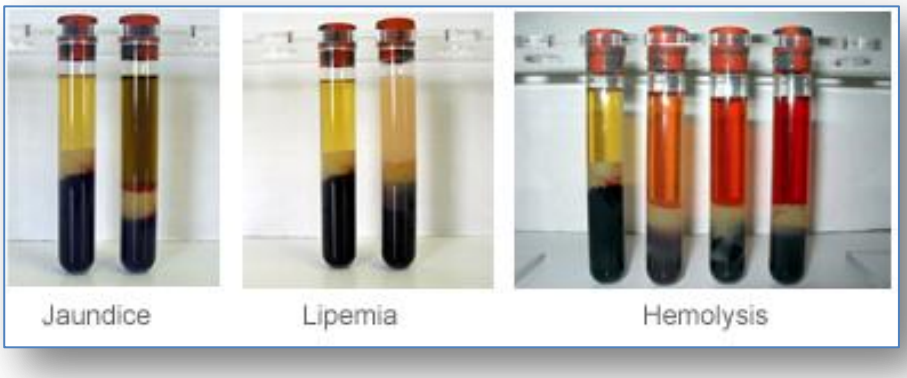
Causes of haemolysis:

Haemolysis may occur due to:

1. Dispense of blood directly from syringe through needle, the applied pressure may cause rupture of RBCs.
2. Refrigerate non-separated blood sample (too cold, too hot will also effects).
3. Mechanically due to blood sample shaking.

Changes in the serum color indicate one of the following:

- **Hemolyzed**: serum appears **pink** to red due to rupture of RBCs.
- **Icteric**: serum appears **yellow** due to high bilirubin (Jaundice).
- **Lipemic**: serum appears milky or turbid due to high lipid.



http://www.cdha.nshealth.ca/pathology-laboratory-medicine/clinical-chemistry/conditions-affecting-lab-results

Specimen rejection criteria:

- Specimen improperly labeled or unlabeled.
- Specimen improperly collected or preserved.
- Specimen submitted without properly completed request form.
- Hemolyzed sample.

Blood Collection Tubes

Plasma separating tubes (PST)			
Top Colors	**Additives**	**Principle**	**Uses**
Lavender	EDTA	➢ The strongest anti-coagulant ➢ Ca^{+2} chelating agent ➢ preserve RBCs components	➢ Hematology ➢ Blood bank ➢ ABO blood group ➢ HbA1C
Light blue	Sodium Citrate	➢ Ca^{+2} chelating agent ➢ must be completely filled and inverted immediately after filling.	➢ PT: Prothrombin Time ➢ PTT: Thromboplastin Time
Green	Sodium or Lithium Heparin	➢ Heparin binds acts as anti thrombin.	➢ Enzymes ➢ Hormones ➢ Electrolytes
Black	Sodium Citrate	➢ Ca^{+2} chelating agent	ESR (Erythrocyte Sedimentation Rate)
Gray	Na- Fluoride and K- Oxalate	Fluoride inhibits Glycolysis Oxalate is anti-Coagluant	➢ Glucose tests

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 will separate from clean serum and settle to the bottom of the vessel.
4. The supernatant is the serum which can be now collected by
5. Dropper or pipette for testing purposes or stored (-20°C to -80°C) for subsequent analysis or use.

Serum separating tubes (SST)			
Top Colors	**Additives**	**Principle**	**Uses**
Red	Sometimes it has gel or silicon at the bottom of tube to reduce hemolysis	Enhancing the formation of blood clot	➢ Serology, Antibodies ➢ Chemistry, Hormones, Drugs ➢ Virology ➢ Blood cross matching before blood transfusion
Yellow	It has gel at the bottom of the tube to separate serum from the blood	Serum separating from the blood through the gel in the tube	➢ Serology ➢ Chemistry

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
6. Purposes or stored (-20°C to -80°C) for subsequent analysis or use.

References:

- Springhouse Handbook of Diagnostic Tests, 2nd edition, Springhouse Co., 1999.
- Brunner, L.S. & Suddarth, D.S., The Lippincott Manual of Nursing Practice, JB Lippincott, 1999.
- Buckingham, Alan, Arterial Blood Gases Made Simple, Nursing Life, Nov/Dec 1994.
- Diagnostic Tests Handbook, Springhouse, Penn. Intermed Communications, 1994.
- Fischbach, F., A Manual of Laboratory diagnostic Tests, Philadelphia, Lippincott, 1998.
- Kee, Joyce LeFever; Laboratory and Diagnostic Tests with Nursing Implications, Appleton-Century-Crofts, 1991.
- Wikipedia

LAB. REQUEST

Patient Name: ..... Sex ..... Age ..... File No. .....

Date: ..... Company ..... Refering Dr. .....

Clinical Details: .....

BIOCHEMISTRY		HAEMATOLOGY		MICROBIOLOGY		SEROLOGY		HORMONES	
1	FBS	31	CBC	88	Tuberculin T.	67	RPR-(VDRL)	88	FSH
2	PPBS	32	Hb	54	AFB-Smear	68	TPHA	89	LH
3	RBS	35	HCT	160	AFB-Cult.	101	HIV	86	Prolactin
4	Blood Sugar Curve	39	T & D.L.C.	161	Gram Smear	206	HIV Screening	91	Estradiol
30	HBA1C	36	Plateles Dif.	59	Prostatic Smear	100	HEXAGON -TB	93	Oestriol
17	Triglycerides	37	Reticulocytes	162	Fungal Culture	66	CRP	87	Progesterone
14	Cholesterol	42	ESR	163	Fungal Scrapping	64	ASOT	90	Testosterone
15	HDL-C	34	T.L.C.	CULTURE & SENSITIVITY OF		65	RF	110	Free T3
16	LDL-C	33	RBC	55	Urine	105	ANF	111	Free T4
18	Lipid Profile	38	Film for Malaria	53	Ureth. Disharge	82	Febrile test	84	T4
12	T-Proteins	48	G-6 PD	58	Prost. Discharge	76	widal	83	T3
13	Albumin	22	Iron	57	Stool	77	brucella	85	TSH
141	Globulin	23	TIBC	56	Blood Culture	131	inf. Mono. Teat	196	Antithyro-globulin Ab
142	A/G Ratio	107	Transferin	60	Chlamydia	134	H. Pylori Ab	197	Antimicrosomal Ab
7	T. Bilirubin	121	Feritin	164	Ear Discharge	208	Urea Breath T	198	Antithyroid Ab Titre
8	D. Bilirubin	40	Sickling Test	304	Throat Swab	132	Anti HAV IgG	159	TSH Newborn
9	Alk. Phosph.	123	Folic Acid	165	Pus	133	Anti HAV IgM	199	Growth H.
114	GGT	124	Vit. B12	166	Conjuctival	183	HAV Total Ab.	92	Cortisol (AM.)
11	SGPT	46	BL Time & CT	167	Vaginal Discharge	72	Anti HBC	200	17-OH Progesterone
10	SGOT	44	Proth. Time	169	Sputum	70	HBs-Ag	201	DHEA-SO4
28	LDH	45	PTT	MISCELLANEOUS		71	HBe-Ag	202	Aldosterone
27	CK	126	PDP,S	52	Semen Analysis	75	HCV-Ab	214	11-Desoxy Cortisol
120	CKMB	43	ABO & Rh	168	Fructose in Semen	184	HCV-ab-Screening	203	Lipase
205	Troponin	128	L.E. Cells	102	Anti-Sporm Abs	207	HBS-Ag Screening T.	119	Calcitonin
228	CI	47	Coomb's T (D)	170	Lithium	73	Anti-HBe	204	A.C.T.H.
5	Urea	41	Coomb's T (ind.)	210008	فحص شامل للكبار	74	Anti-HBs	185	B. HCG-follow up
6	Creatinine	125	Fibrinogen	210009	VIP فحص شامل للكبار	140	Anti-HBc IgM	229	B. HCG-Quan
19	Uric Acid	109	Factor VIII	210033	فحص شامل للصغار	63	Pregn. T. Serum	172	Cortisol pm
20	Calcium	112	Factor Ix	210012	فحص شامل للصغار	78	Toxopl. IgG	211	Insuline F
21	Phosphorous	URINE EXAM		210014	VIP فحص شامل أطفال	79	Toxo. IgM	308	Insuline P.P.
122	Magnesium	50	Urine Analysis	210018	فحص توظيف عادي	95	CMV-IgG	210	C Peptide F
209	Bicarbonate	136	T . Urea	210019	فحص توظيف شامل	96	CMV-IgM	309	C Peptide P.P.
117	Amylase	182	Creatinine	210013	كشف رخصة	144	HSV II .IgG	310	Triple Test
118	Pancreatic Amylase	113	Cl. (24 h)	175	كشف إقامات	146	HSV II . IgM	334	T P H
213	T.Acid Phosph	116	Protein (24 h)	177	فحص ما قبل الزواج	186	HSV-Ag (PCR)	335	H C V P C R Q A.
212	Prostatic A. PH	137	Cortisol (24h)	178	VIP فحص ما قبل الزواج	187	Torch (ELISA)	336	H C V P C R Q L.
221	Na	94	VMA	179	كارت بلدية	188	Toech (Quality)	337	H B V P C R Q A.
222	K	138	Calcium	180	VIP كارت بلدية	106	Ig E total (Quantity)	225	H B V P C R Q L.
311	Lithium	143	Phosphate	154	فحص جامعي خاص	191	Allergy Screening Pane	333	H. Pylort In Stool
		145	Sodium	216	فحص مدرسي أو جامعي عادي	192	Allergy Vaccine	191	Mono Test
TUMOR MARKER		152	Potassium	210039	فحص فيروسيات الكبد	193	Pregnancy follow up		Others
147	AFP	215	Amylase	210015	فحص مدرسي أطفال	194	Newborn follow up		
148	PSA	153	Micro Albumin	180300	أخذ عينة من المنزل	195	ANA		
149	CA 15 - 3	302	Stone Analusis			104	Anti-DNA		
150	CA 125	STOOL EXAM				80	Rubella IgG		
151	CA 19 - 9	51	Stool analysis			81	Rubella IgM		
103	CEA	130	Occult Blood			220	Drug abuse		
ELECTROPHORESIS		156	Rota V. Ag			90	Bilharz Ab		
115	S.Protein	303	Stool 3 Time			301	Dengue Screening		
49	Hb					305	Dengue Elisa		
HISTOPATHOLOGY						306	Chlamydia Ig G		
139	Pap Smear					307	Chlamydia Ig M		
189	Biopsy Small								
190	Biopsy Large								

Lab (2): Measurement of liver Enzymes.

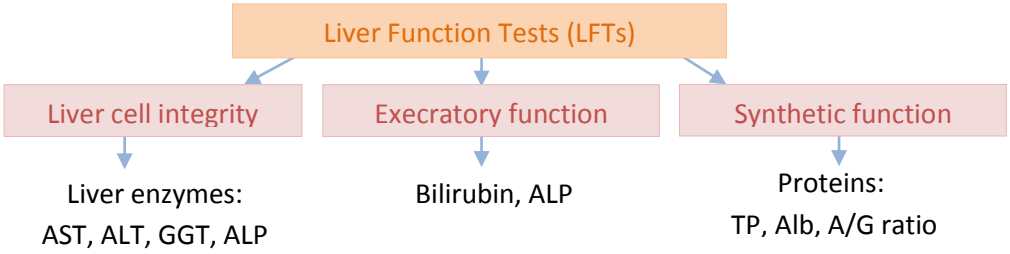
- Liver is an important organ in human body. Many vital functions occur in liver as: protein synthesis, glycogen storage, drug metabolism and detoxification process all are occurred in liver.
- Many diseases can affect on liver and its function as:
  - Hepatitis due to viruses (hepatitis A,B,C,D, G) or other causes
  - Cirrhosis
  - Jaundice
  - Fatty liver

Liver function tests can be classified as:

a. Tests evaluate the excretion function of the liver.

b. Tests evaluate the synthetic function of liver.

c. Tests evaluate the integrity of liver cells.



Liver enzymes:

1. **ALT (alanine aminotransferase) or GPT(Glutamate Pyruvate Transaminase)**
   - It is found mainly in the liver.
   - **High serum ALT is due to:**
     - **Liver cells damage due to inflammation, virus infection or cell death (why?)** when liver cells have damaged, ALT enzyme leaks to blood stream leads to raise its level in serum.
     - **Some medication may also elevate serum ALT,** because some drugs cause liver damage leads to raise ALT level.

- **ALT is the most sensitive marker for liver cell damage;** since it is only synthesized by liver cells but other liver enzymes may be also synthesized by other organs.
- **Used sample**: serum or plasma

2. AST (Aspartate aminotransferase) or GOT (Gglutamate Oxaloacetate Transaminase)

- **Is synthesized by:** liver cells, cardiac muscle and skeletal muscles.
- This enzyme also reflects damage of the hepatic cell but **less sensitive than ALT,** since it is synthesized by other organs. The ALT/AST ratio is useful in assessing the etiology of liver enzyme abnormalities.
- **High serum AST due to:**
  - Muscle damage, myocardial infarction (heart attack) and in chronic liver disease. To confirm that high AST is due to heart or muscle injury; another enzyme (creatinine kinase CK) which is specific for heart is also tested.
- **Used sample:** serum or plasma

  Fasting is preferred but not required for this test.

3. ALP (alkaline phosphatase)

- It is found high in liver, bile ducts and bone.
- It is **not specific for bile** because it is synthesized also by bone and placenta (isoenzymes).
- **High serum ALP may be due to:**
  - Bile duct damage, inflammation, cirrhosis or obstruction due to stones.
  - Alcoholic hepatitis.
- **Normal physiological elevation :**
  - During pregnancy
  - During child growth
- **To assess the etiology of ALP elevation,** GGT and bilirubin levels are also measured.
- **Used sample:** serum or plasma

  Fasting is preferred but not required for this test.

4. GGT (Gamma Glutamic Transpeptidase)

- Produced by liver, kidney and pancreas.
- Its level is elevated in toxins and alcoholic hepatitis.
- It is used to confirm hepatic etiology of ALP elevation.
- GGT is not helpful test in distinguishing between the different causes of liver damage
- **Used sample:** serum or plasma

  Eight hours fasting is recommended because GGT drops after eating.

References:

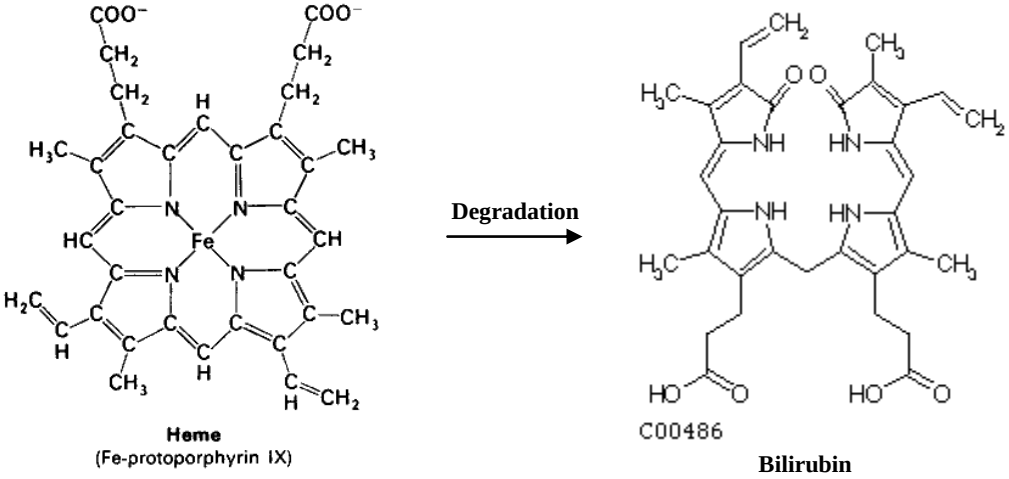
- American Association for clinical chemistry AACC. http://labtestonline.org/understanding/analytes/ggt/tab/test
- McClatchey, Kenneth D. (2002). Clinical laboratory medicine. Lippincott Williams & Wilkins. pp. 288–. ISBN 9780683307511. http://books.google.com/books?id=3PJVLH1NmQAC&pg=PA288. Retrieved 5 August 2011.
- Mengel, Mark B.; Schwiebert, L. Peter (2005). Family medicine: ambulatory care & prevention. McGraw-Hill Professional. pp. 268–. ISBN 9780071423229. http://books.google.com/books?id=XvLo7xvmFo0C&pg=PA268. Retrieved 5 August 2011. ^ a b c d[broken citation]
- Nyblom H, Björnsson E, Simrén M, Aldenborg F, Almer S, Olsson R (September 2006). "The AST/ALT ratio as an indicator of cirrhosis in patients with PBC". Liver Int. 26 (7): 840–845. doi:10.1111/j.1478-3231.2006.01304.x. PMID 16911467British Liver Trust 2011. All rights reserved. Registered Charity England and Wales 298858, Scotland SC042140

Lab (3): Bilirubin measurement.

Bilirubin:

Bilirubin produced from heme degradation (80% of heme come from RBCs hemoglobin, 20% from other hemo-protein as cytochrome, myoglobin). Bilirubin is transferred to liver to undergo further metabolic process called **conjugation** to make it more water soluble. Then conjugated bilirubin is excreted in bile to help in food digestion and the excess amount is excreted in urine and stool. Elevated levels of bilirubin in blood and urine indicate certain diseases.

Structure: Bilirubin consists of four open chain pyrrols, unlike heme which consists of four rings pyrrols called (porphyrin).



Heme
(Fe-protoporphyrin IX)

Bilirubin

Bilirubin metabolism:

Bilirubin is water insoluble and is carried in plasma bound to albumin (as carrier). When it reaches to the liver, bilirubin is taken by specific carrier meachanism.

In liver: Bilirubin is conjugated with glucouronic acid to produce bilirubin diglucuronides, which is water soluble and readily transported to bile. Further metabolic processes are occurred in intestine and kidney is illustrated in the last page.

Types of bilirubin in serum:

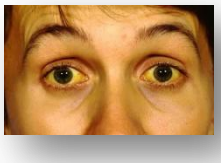
When serum bilirubin level is measured in lab, it is classified to:

- ✓ **Direct bilirubin:** is conjugated or water soluble bilirubin, in aqueous solution it reacts rapidly with reagent and said to be (direct reacting).
- ✓ **Indirect bilirubin:** is unconjugated or water insoluble bilirubin, because it is less soluble in it reacts more slowly with reagent therefore the reaction carried out in the presence of methanol, in this case both conjugated and unconjugated bilirubin are measured given **total bilirubin**. Unconjugated will calculated by subtracting direct from total and so called indirect.

Knowing the level of each type of bilirubin has diagnostic important.

Jaundice:

Is a medical term describes the elevation of bilirubin in blood result in yellow color of skin and sclera.

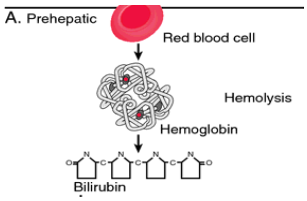
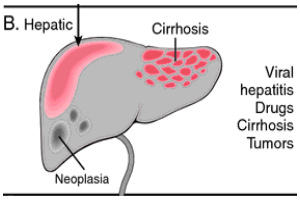
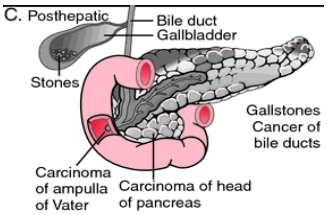


Types of Jaundice:

According to the cause of jaundice, it is classified to three main types:

1. Pre-hepatic jaundice (haemolytic jaundice).
2. Hepatic jaundice (hepato-cellular jaundice).
3. Post-hepatic or obstructive jaundice (most common type)

The differences between the three types are explained in table below.

	Pre-hepatic jaundice	Hepatic jaundice	Post-hepatic jaundice
Cause	➢ Due to increase in RBCs breakdown due to hemolytic anemia. ➢ The rate of RBCs lysis and bilirubin production more than ability of liver to convert it to the conjugated form. 	➢ Due to liver cell damage (cancer, cirrhosis or hepatitis) ➢ Conjugation of bilirubin decreased (D.Bil. ↑). ➢ Blilirubin that is conjugated is not efficiently secreted into bile but leaks to blood (ID.Bil. ↑) 	➢ Due to the obstruction of bile duct which prevents passage of bilirubin into intestine. ➢ D.Bil will back to liver and then to circulation elevating its level in blood and urine. 
Type of Bil.	ID.Bil > D.Bil	D.Bil, ID.Bil, T.Bil all (High)	D.Bil (High)
Conformational test	K^+ (High) Hematology: CBC (low Hb)	ALT, AST (High)	ALP (High)

Physiologic jaundice of the newborn (Neonatal Jaundice):

- High bilirubin levels are common in newborns (1-3 days old).
- It is happened because after birth the newborns breaking down the excess RBCs they are born with it and because the newborn's liver is not fully mature, it is unable to process the extra bilirubin, leads to elevate its level in blood and other body tissues.
- This situation usually resolves itself within a few days.
- Usually newborn is treated by **phototherapy** which breakdown bilirubin (ID ⟶ D) and convert it to the photo isomer form which is more soluble.
- Very high bilirubin is danger and toxic. It may cause brain damage and affect on muscles, eyes and even death.

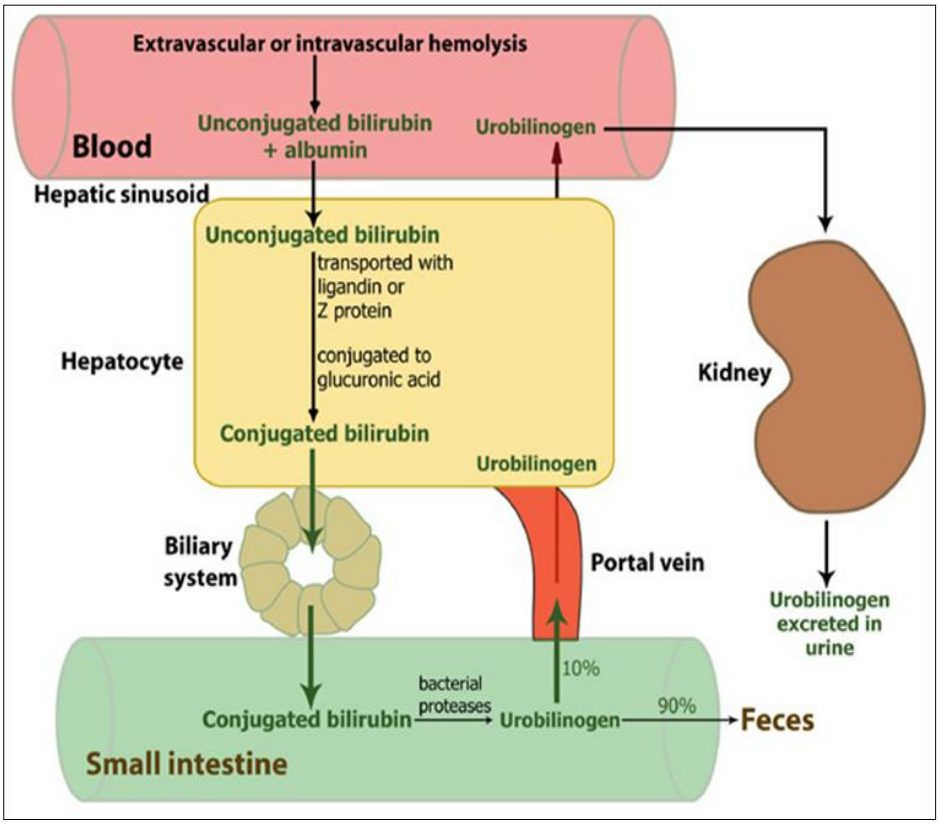


Figure: Bilirubin metabolism

References:

- http://www.docstoc.com/docs/75552457/Jaundice
- The image source from: http://nursingcrib.com/nursing-notes-reviewer/maternal-child-health/bilirubin-conjugation/
- http://www.porphyria.uct.ac.za/professional/pim-porphyrin-structures.htm
- http://omlc.ogi.edu/spectra/hemoglobin/hemestruct/index.html

Lab (4): Blood Urea Nitrogen (BUN)

Kidney is important organ in body. It has many vital functions as:

* Blood filtration is a major function.
* Eliminates products of metabolism such as creatinine, uric acid and urea.
* Regulates the balance of water, Na^+, K^+, Cl^-, Ca^{2+} and PO_4^{-3}.
* Maintains blood volume, pressure and pH (acidity/alkalinity).

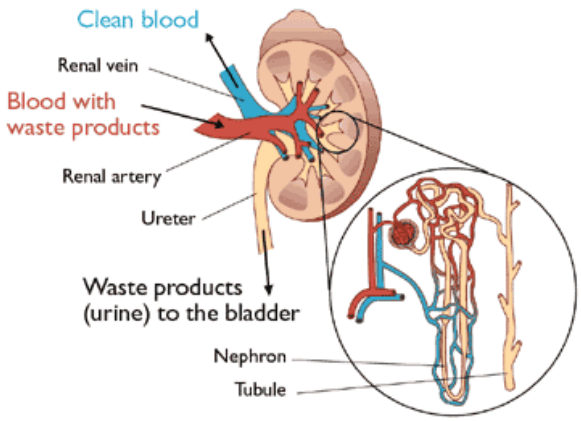
Kidney damage occurs in stages that can early detect. Many factors can affect on kidney function leads to kidneys damage. It occurs in stages that can early detect. Diabetes and high blood pressure are the most common causes of kidneys damage.

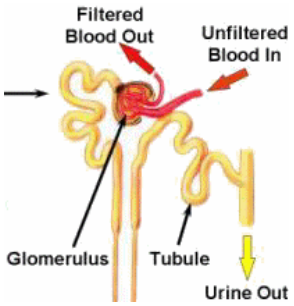
Kidney filtration function:

The functional filtration unit of kidney is ***nephrone***. Each kidney contains million nephrones.

Nephrone consists from two major parts:

1. **Glomerular**: responsible for filtration. It is defined as a high pressure mass of capillaries that filter blood. Filtration occurs through glomulus tri-layered membrane which depends on molecular weight and charge of compounds to be filtrated. Blood cells and proteins are large and can't pass though this membrane.
2. **System of tubules:** responsible on re-absorption of important material.

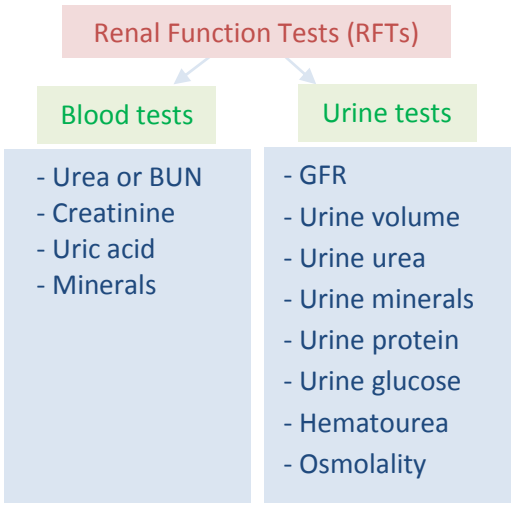




Kidney dysfunction occurs when large number of theses nephrons loss their function. As a result the filtration process occurs less efficiently and some of large molecular weight compounds, as proteins, appear in urine.

Renal Function Tests:

See the diagram.



1- Urea or Blood Urea nitrogen test (BUN):

- Urea is waste product of protein metabolism, it is synthesized in liver via urea cycle from ammonia which is produced from amino acids by deamination. Then it is transported by blood to kidney to be excreted in urine.
- **Blood urea level is sensitive but not specific indicator for renal dysfunction, because:**
  - Its level is affected by dietary protein.
  - Other non renal causes such as heart failure and blood pressure may raise its level.
  - Its level is elevated in last stages of renal failure after 50% of renal function is lost.

  Therefore, other more accurate test, creatinie, which is specific for kidney is performed. (Creatinine test will be discussed in the next lab)

- **High blood urea can indicates:**
  - Renal insufficiency due to obstruction or cancer.
  - Blockage of the urinary tract (by a kidney stone or tumor).
  - Low blood flow to the kidneys caused by dehydration or heart failure.
  - High-protein diet.

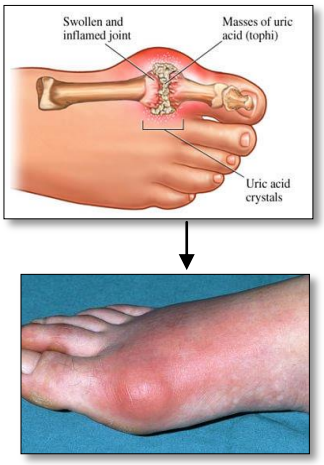
- **Low blood urea may be due to:**
  - Very low protein diet as in malnutrition.
  - Severe liver damage inhibits urea cycle, decrease urea formation and increase free ammonia leads to hepatic comma.

  Note: BUN= 50% urea

2- Uric acid:

Is the end product of purine metabolism and excreted in urine. Purine in body comes from food and body cells break down. Elevated level of uric acid in blood is one of the markers of kidney dysfunction.

- **High blood uric acid occurs in:**
  - Gout (is a disease characterized by high level of uric acid which deposited in as crystals in joins causing arthritis).
  - Renal failure (due to decreased excretion in urine).
  - Leukemia (increased turnover of cells).
  - Alcoholism
  - Toxaemia of pregnancy.
  - Diabetes Mellitues.
  - Starvation.
  - Drugs like diuretics.



- **Low blood uric acid occurs in:**
  - Liver diseases (cirrhosis).
  - Renal disease that decrease renal tubular re-absorption.
  - Some drugs.

References:

- Marsh, W., Fingerhut, B. and Miller, H. (1965), Clin. Chem. 11, 624.
- Wybenga, D.R. (1971), Clin. Chem. 17, 891.
- http://sitik.wordpress.com/2010/07/16/mengenali-gout/
- http://www.medic4health.com/2011/08/gout/
- http://www.kidneydialysis.org.uk/kidneys.htm

Lab (5): Creatinine and Creatinine Clearance

Creatinine:

Creatinine is by-product of muscle energy metabolism (break down of creatine). It is a waste product and filtered from blood by kidneys then it is excreted into the urine. Production of creatinine depends on an individual's muscle mass, which is usually constant.

Serum creatinine:

- In renal failure, the level of creatinine is elevated in blood and decreased in urine because kidney unable to excrete creatinine in urine.
- **Elevated blood creatinine is a more sensitive indication of impaired kidney function than the BUN because:**
  - Its level only effected by muscle mass which is usually constant.
  - Creatinine level is very little affected by liver function.

The BUN/Creatinine ratio:

BUN-creatinine ratios increase with renal disease. Other factors such as liver function, muscle mass, and dietary protein can affect on this ratio.

- **High BUN-to-creatinine ratio occurs in:**
  - Sudden (acute) kidney failure.
  - Urinary tract blockage (as a kidney stone).
- **Very high BUN-to-creatinine ratio** may be caused by
  - Bleeding in the digestive tract or respiratory tract.
- **A low BUN-to-creatinine ratio may be caused by:**
  - Low protein diet,
  - Severe muscle injury.

Glomerular filtration rate (GFR):

GFR is a useful assessment for glomerlaur function of kidney. It measures the rate of blood filtration through the glomerular. It is defined as the rate at which blood is filtered through Glmulus and transferred to Bowman's space. It is determined by measuring creatinine clearance.

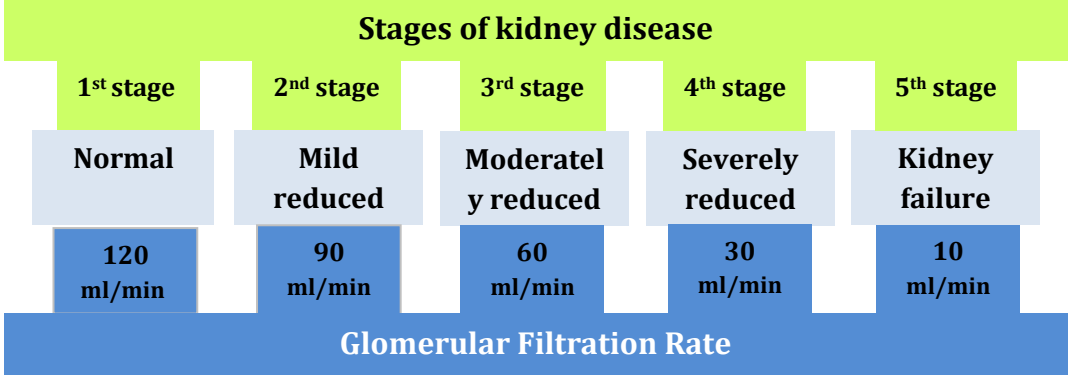
Creatinine clearance:

- **Definition:** Creatinine clearance is the removal of creatinine from the body by kidney. More accurately, it is the **volume of blood plasma that is cleared from creatinine per unit time.**
- **Clinical important:** creatinine clearance is a useful measure for estimating the glomerular filtration rate (GFR) of the kidney. It is usually expressed in ml/min.
  - **Normal range:** man: 120 ml/min, women: 100 ml/min.
  - **If GFR below 60 ml/min** for three months the patient has chronic kidney disease CKD.
  - **If GFR is less than 10 ml/min.** this stage is called end-stage kidney disease or end-stage renal disease (ESRD). End stage means kidneys cannot filter chemicals and minerals out of blood. In this stage patient needs dialysis or kidney transplant.

Note:

Serum creatinine is not suitable for detecting early stage of kidney disease because high serum creatinine is observed only with marked damage in nephrons function; therefore, creatinine clearance is better for detecting early stage of kidney disease.

- **According to the value of GFR, kidney disease is classified to five stages as following figure:**



- **Estimated creatinine clearance rate (eCcr):** creatinine clearance can be calculated in two ways.

  <u>The first way</u>:

  This method not required urine creatinine. It depends only in serum creatinine and some parameters as: sex, age, and weight (kg).

$$\text{Creatinine clearance} = \frac{(140 - \text{age}) \times \text{weight} \times \text{constant}}{\text{serum creatinine}}$$

Cockcroft-Gault formula

  * **Sample:** serum

  * **If serum creatinine is measured in mg/dl:**
    Serum creatinine must be multiplied by factor 72
    Constant = 1 for men, constant = 0.85 for women.

  * **If serum creatinine is measured in µmol/l:**
    Constant = 1.23 for men, constant = 1.04 for women

  <u>The second way:</u>

  This way is using 24-hour urine collection.

$$\text{Creatinine clearance (mL/min)} = \frac{\text{urine creatinine (mg/dl)} \times \text{urine volume (ml)}}{\text{serum creatinine (mg/dl)} \times 1440 \text{ min}}$$

  * **Sample:** serum and urine

  * Urine Volume is the total urine volume in 24 hour by ml and 1440 is a number of
    minutes in 24 hours.

Cystatin C:

- It is a ubiquitous protein secreted by most cells in the body and small amounts excreted in the urine.
- It may be used as an alternative to creatinine and creatinine clearance for monitoring kidney dysfunction.
- It is a better marker of the GFR. A reduction in GFR causes a rise in the concentration of cystatin C.

Further reading

Read more information about cystatin C by this link:

http://labtestsonline.org/understanding/analytes/cystatin-c/tab/test

References:

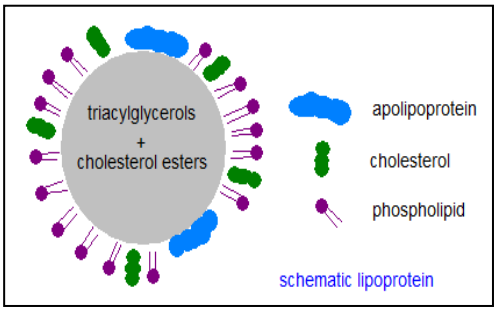
- Murray R.L. Creatinine. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis.Toronto. Princeton 1984; 1261-1266 and 418.
- Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
- Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
- Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
- Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.
- http://www.fpnotebook.com/renal/lab/CrtnClrnc.htm

Lab (6): Total cholesterol, LDL-C, HDL-C, and TG

Lipid profile test:

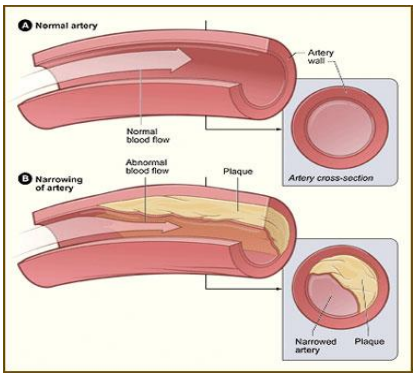
It is a group of tests used together to determine the risk of cardiovascular disease. These tests include:

* Total cholesterol (TC)
* Low-density lipoprotein (LDL)
* High-density lipoprotein (HDL)



Triglyceride and cholesterol are carried internally by lipoprotein in the blood. There are different types of lipoproteins in blood. They differ from each other by protein and cholesterol content. For example; LDL has high cholesterol content and HDL has the lowest cholesterol content. (See the figure).

1. LDL-C (bad cholesterol):



- It is type of lipoprotein that carries cholesterol from liver to the rest of the body.
- It is considered to be **BAD** cholesterol because it can form deposits (plaque) in the walls of arteries throughout the body. Such deposits can narrow arteries and limit blood flow.
- **LDL cholesterol levels:**

  Normal

  * LDL less than 100 mg/dl if you have heart disease or diabetes.
  * LDL less than 130 mg/dl if you have 2 or more risk factors.
  * LDL less than 160 mg/dL if you have 0 or 1 risk factor.

    (Risk factors: smoking, life style, diabetes, obesity, alcohol consumption).

  High (abnormal) is 160-189 mg/dl

- **Measuring LDL-C level:**
  - Direct LDL-C (using method that measures it directly).
  - Calculated LDL-C (using the below equation)

    LDL = Total cholesterol - (HDL + TG/5)

2. HDL-C (GOOD CHOLESTEROL):

- It is type of lipoprotein that carries cholesterol from arteries to the liver.
- It is considered to be **beneficial** because it removes excess cholesterol from the blood, prevent fatty build up and the formation of plaque.
- **HDL values:**
  - Poor level: less than 40 mg/dl means high risk of heart disease.
  - Acceptable: 40- 59
  - Good level of HDL is ≥ 60 mg/dL.

3. Cholesterol:

- Cholesterol is the most commonly occurring steroid.
- Cholesterol itself is not bad but it is required in specific amount. It is an important precursor of cholesterol esters, bile acids and steroid hormones.
- It is obtained from dietary sources such as meat, eggs and dairy products.
- A small amount of the body's cholesterol circulates in the blood in complex particles called lipoproteins; LDL and HDL.
- **Cholesterol levels:**
  - Desirable: Cholesterol below 200 mg/dL is considered desirable and reflects a low risk of heart disease.
  - High Risk: Cholesterol above 240 mg/dL is considered high risk.
- **Causes of hypercholesterolemia:**
  - Inherited disorders of lipid metabolism
  - Diabetes mellitus
  - Hypothyroidism
  - Pancreatitis
  - High cholesterol may be seen due to inhibition of lipoprotein lipase.
  - Some drugs as anabolic steroids, oral contraceptives and vitamin D.

- **Causes of abnormally low cholesterol levels:**

  There are many factors but the most common causes are:

  * Decreased absorption in malabsorption and maldigestion problems.
  * Decreased production of cholesterol or lipoprotein by liver due to chronic liver diseases (cirrhosis) or synthetic liver failure (acute or chronic).

  Important notes:

  * Fasting is NOT required for the cholesterol test because cholesterol does not change in response to a single meal; it is only affected by long term changes in eating pattern.
  * Cholesterol level may be elevated during pregnancy; more reliable value could be obtained after six weeks of delivery.

4. Triglyceride (TG):

- Triglycerides are the body's storage form of fat in the adipose tissue. The body uses TG for energy supply.
- **Triglyceride level:**

  Normal: below 150 mg/dl High: above 200mg/dl Very High: 500mg/dl

- **Causes of High Triglycerides (Hypertriglyceridemia):**
  * Obesity and alcohol consumption
  * Kidney disease
  * Hypothyroidism
- **Causes of low Triglycerides (Hypotriglyceridemia):**
  * Very low carbohydrate, low-fat diets
  * Inherited conditions
  * Fat metabolism disorders
  * Liver or thyroid diseases

  Important Note:

  Fasting is required (12-14 h) for TG test to allow TG from diet to be completely eliminated in order to test the TG that being made in the body.

References:

- Naito H.K. Cholesterol. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1194-11206 and 437.
- Meiattini F. et al. The 4-hydroxybenzoate/4-aminophenazone Chromogenic System. Clin Chem 1978; 24 (12): 2161-2165.
- Buccolo G et al. Quantitative determination of serum triglycerides by use of enzimes. Clin Chem 1973; 19 (5): 476-482.
- Kaplan A et al. Tryglycerides. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 437 and Lipids 1194-1206
- http://www.chp.edu/CHP/p01593
- http://www.umm.edu/altmed/articles/hypercholestrolemia-000084.htm
- www.med.ucla.edu/modules/wfsection/article.php?articleid=497
- http://ahdc.vet.cornell.edu/clinpath/modules/chem/cholest.htm

Lab (7): Measurement of Blood Glucose

Diabetes mellitus:

It is a chronic disease associated with hyperglycemia (increased blood glucose level) & glucourea (presence of glucose in urine) due to insulin deficiency.

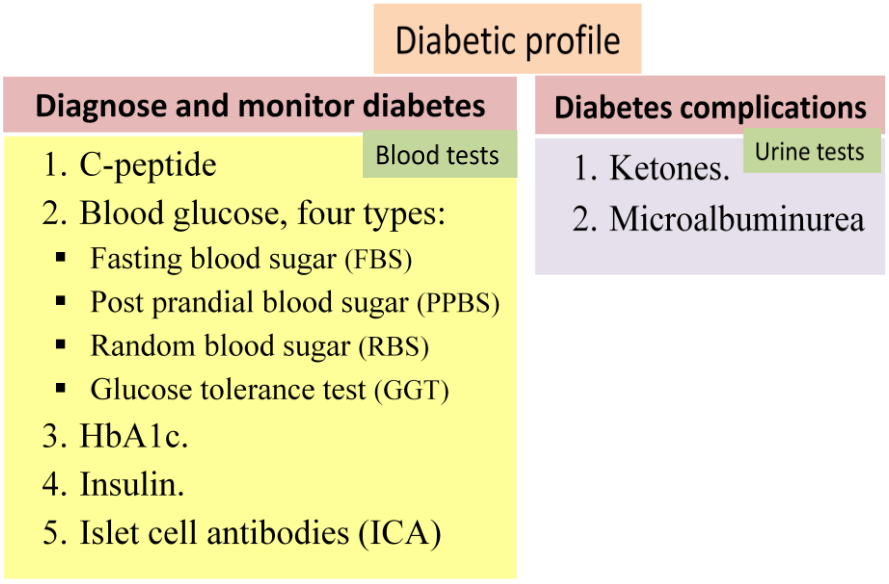
It characterized by several changes in metabolism mainly in carbohydrates, proteins and fat metabolism.

It is classified into four clinical classes:

- Type I diabetes mellitus (TIDM)
- Type 2 diabetes mellitus (TIIDM)
- Gestational diabetes mellitus (GDM)
- Other specific types due to other causes e.g. Drugs.

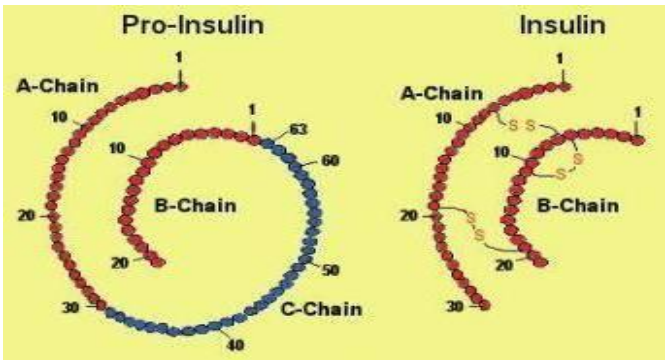
Diabetic Profile Tests:

It is a group of tests that are used to diagnose and monitor diabetes or estimates its complications. These Tests are classified as:



1- C-peptide:

a) Background on c-peptide: insulin is produced by pancreas beta cells as pro-insulin which consist of: inactive A and B chains held together by C-peptide chain. Pro-insulin is converted to active insulin by cleaving c-peptide chain, and then active insulin is secreted into blood stream. C-peptide is also secreted as inactive peptide (it has no biological function). So, for every molecule of insulin in blood there is one c-peptide molecule. Insulin has short half life (does not stay for long in blood), unlike c-peptide which has long half life. Therefore c-peptide measurement is used as indirect test to estimate insulin level produced by pancreas.



b) Advantages of measuring C-peptide than insulin:

- Is considered as better indicator for insulin level in blood than insulin itself (why?)
- It is used to estimates pancreatic beta cells activity and capability over time.

c) C-peptide is also used to differentiate between type 1 and type 2 diabetes.

1. C-peptide in Type 1 diabetes:

- Low level of insulin and C-peptide are observed in TIDM because:
  - Type I diabetes (TIDM) involves complete destruction of beta cells with disabilities to produce insulin.
- C-peptide is useful measurement in patient newly diagnosed with TIDM to evaluate the residual beta cell function.

2. **C-peptide in type 2 diabetes:**

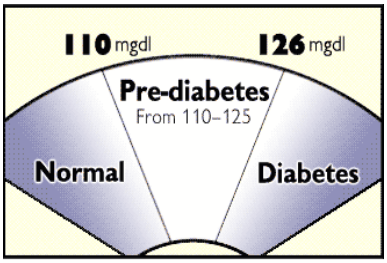
- Normal or high level of C-peptide will be detected.
- C-peptide test is useful in type-2 diabetes:
  - To monitor the status of beta cell production of insulin.
  - To determine if the insulin injection is required for the patient.

2- Blood glucose:

It is a vital component of diabetes management. Types of blood glucose tests are:

1. **Fasting blood sugar (FBS)**

- Definition: it measures blood glucose after fasting.
- Sample preparation: fasting at least for 8 hours.
- Conformation Tests: glucose tolerance test (GTT) and tests to diagnose other diseases that usually associated with elevated blood Glucose levels, such as overactive thyroid gland and pancreatitis.
- Reference value:
  - Normal: 60-110mg/dl
  - Pre-diabetic: 110-126 mg/dl
  - Diabetic: above 126mg/dl



Fasting Plasma Glucose Test

2. **Two-hour postprandial blood sugar (2-hour PP)**

- It measures blood glucose exactly 2 hours after eating a meal.
- Sample Preparation: For a 2-hour postprandial test.
- Home blood sugar test is the most common way to check 2-hour postprandial blood sugar levels.

3. Random blood sugar (RBS)

- Definition**:** it measures blood glucose randomly throughout the day without patient fasting. In healthy individuals, glucose levels do not vary widely throughout the day. If blood glucose levels vary widely this indicate a problem.
- Sample preparation**:** No special preparation is required.

4. Oral glucose tolerance test (OGTT)

- Glucose Tolerance: is defined as the capacity of the body to tolerate an extra load of glucose or it measures the body's ability to use glucose.
- Oral glucose tolerance test: is a series of blood glucose measurements taken after drink glucose solution.
- This test is recommended when fasting blood glucose test is between 100 - 126 mg/dl (5.5-7 mmol/l)
- Uses:
  - It is considered as definitive diagnostic test for DM.
  - Confirm the diagnosis in pre-diabetic.
  - To diagnose gestational diabetes (most commonly).
- Sample preparation: it needs specific preparation as following:
  1. Patient should be fast for 12-16hr, blood and urine sample are taken for FBS.
  2. Then patient asked to drink: 75-100g glucose (pregnant women 100g).
  3. After drink: blood and urine samples are collected every 30 min for 3 hrs (0.5 hr, 1 hr, 1.5 hr , 2 hr, 2.5 hr, 3 hr).
  4. A curve between time and blood glucose concentration is plotted.
- Other types of OGTT:

  a. Extended GTT:
  - Glucose measured is extended for 4-5 hrs instead of 3 hours after drink glucose to see how the curve behaves below the normal fasting glucose limits.
  - Done in some conditions causing hypoglycaemia.

b. Cortisone stressed GTT :

It measures glucose level after injection with cortisone, to evaluate body response to cortisone.

c. Intrevenous GTT :

* Is done if oral glucose is not tolerated or oral GTT curve is flat.
* In these cases 20% glucose as 0.5g glucose/Kg body weight.
* Usually peak occurs within 30 min after infusion and returns to normal after 90 min.

- Interpretation:

* **Normal Response :**

FBS is normal.

After 1 hr it will raise, returns to normal fasting level within 2 hours.

* **Diabetic curve :**

FBS: more than 130mg/dl or 7.8 mmol/L.

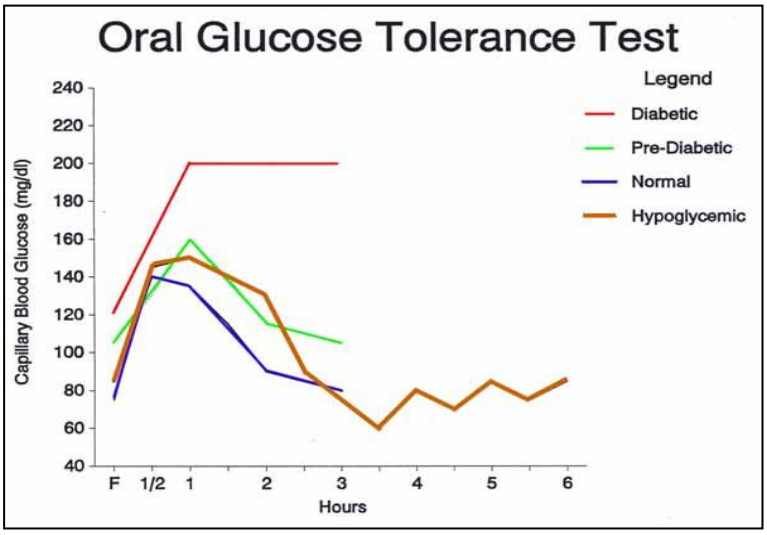
After 2 hr: more than 180mg/dl (11 mmol/L) and didn't back to the fasting level. Glucosuria is usually seen

* **Impaired GTT:**

FBS: is normal but doesn't back to its fasting level during 2-3 hrs.

(during 2hrs glucose level between 100mg/dl–120mg/dl).

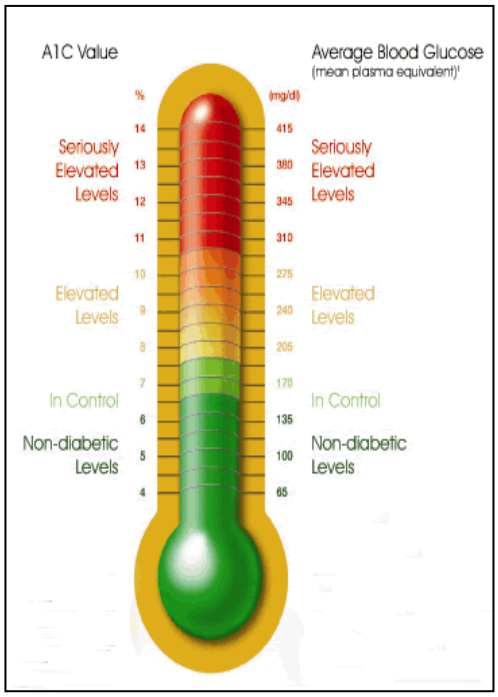
It is not abnormal but must be followed up for DM.



3- Glycosylated hemoglobin HbA1c:

- Definition**:** is glucose bound to RBCs hemoglobin to give glycosylated hemoglobin or HbA1c. In normal case about 2-5% of total hemoglobin is bound to glucose, but when glucose level is elevated in blood, more hemoglobin will bind to glucose.

- It indicates how well diabetes has been controlled in the 2-3 months before the test. (because it measures blood glucose conc. over a longer period of time).
- The A1C level is directly related to complications from diabetes (the lower the A1C level, the lower risk for complications)
- Sample preparation**:** whole blood collected in EDTA tube.
- Reference values**:**
  - Normal: 4.5%-5.7% of total hemoglobin
  - Abnormal: >7% (associated with higher risks of diabetes complications)
- Expected blood glucose level in HbA1c ranges:
  - Sugar: 90-150 mg/dl 5-0% to 7.0%
  - Sugar: 150-180 mg/dl 7.0% to 8.0%
  - Sugar: 180-360 mg/dl 9.0% to 14.0%



References:

- Kaplan L.A. Glucose. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1032-1036.
- Trinder P. Ann Clin Biochem 1969; 6: 24-33.
- www.webmed.com.
- www.medicdirect.co.uk
- http://diabetes.webmed.com/guide/glycated-hemoglobin-test-hba1c.
- http://diabetes.about.com/od/symptomsdiagnosis/a/ogtt.htm
- http://www.nlm.nih.gov/medlineplus/ency/article/003466.htm
- http://labtestsonline.org/understanding/analytes/glucose/tab/test

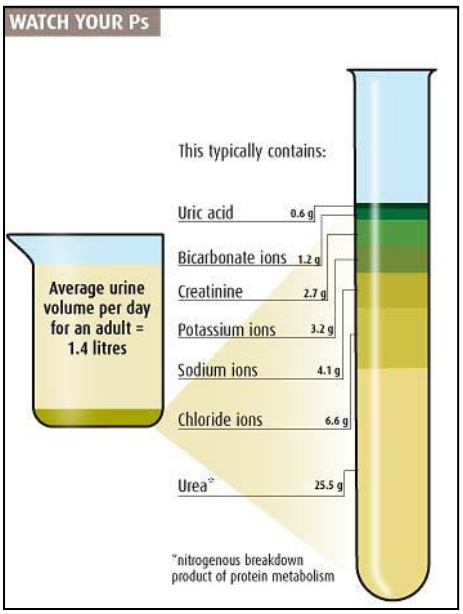
Lab (8): Identification of Normal Physical and Chemical Urine Constituents

Urine is ultrafiltration of plasma which contains waste products and chemical substances excreted by the kidney. Normally, urine is clear, transparent and contains many constituents. From urine sample you can determine all metabolites in body.

Normal constituents of urine:

- In general, urine consists of:
  95 % water and 5% other dissolved chemicals.

- **Factors affect on urine constituents are:**
  dietary intake, physical activity, body metabolism, endocrine function and others.



(Image Credit: New Scientist online.)

Urine Sample collection:

Types of urine specimens:

The types of specimen and collection procedure are depending on the test to be performed.

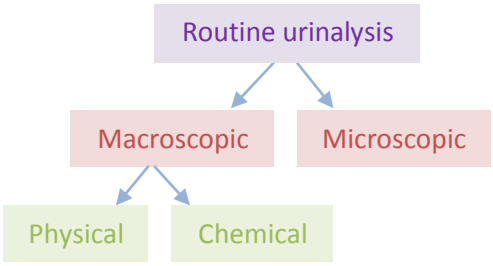
- Qualitative "Para + microbiology"
- Quantitative "conc." in chemistry lab.
  - First morning.
  - Random.
  - 24 hour urine collection container.
  - Timed collection after use of medication.

Note:

- **Initial morning sample is preferred**, particularly for protein analysis, because it is more concentrated due to overnight retention in the bladder.
- **Urine sample must be analyzed within 1h of collection or kept in 2-8°C not more than 8h;** because on standing urea in urine decompose to ammonia and change urine pH (make it more alkaline) and sample become more susceptible to bacteria.

Routine urinalysis:

It includes both macroscopic and microscopic analysis.



Macroscopic Examination:

A. Physical Characteristics:

The first part of a urinalysis is direct visual observation like color, smell, volume, transparency, pH, and specific gravity.

Appearance

1. Color:

Normally, Urine color ranges from pale yellow to deep amber depends on how diluted or concentrated it is, this color is due to pigment called urochrome.

- Amber yellow ------> Urochrome (derivative of urobilin, the end product of bilirubin degradation) a pigment found in normal urine.
- Colourless ------> High dilution.

Abnormal colors:

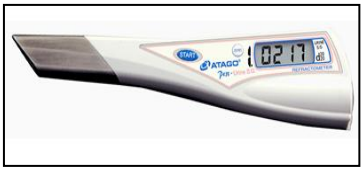
- **Silvery sheen or milky appearance** ------> Pus, bacteria or epithelial cells
- **Reddish brown** ------> Blood (Hemoglobinuria)
- **Yellow foam** -------> due to Bile pigments in jaundice disease or medications.
- **Orange**, **green**, **blue** or red ------> due to some medications.

2. **Transparency or clarity:**
    - This is classified as clear or turbid.
    - The degree of cloudiness of urine depends on both its pH and dissolved solids. In normal urine, the main cause of cloudiness is crystals and epithelial cells.
    - 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.

3. **Odour:**
    - Odour has a little diagnostic significance.
        * Aromatic odour------> Normal urine due to aromatic acids.
        * Ammonical odour ---------> On standing due to decomposition of urea.
        * 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.
    - Urine volume in adult: 750ml-2500ml/ 24h, (average: 1.5L/ 24h)
    - For RUA, a 10ml-12ml of sample is optimal for accurate of analysis.

5. **Specific Gravity (SG):**
    - It measures the amount of dissolved substances in urine (urine density) or the ability of the kidney to concentrate or dilute substances of plasma.
    - This test is used to measure kidney tubular function.
    - It is measured by strip or refractometer (most laboratories use it).
    - **False positive** may be occurred when measuring SG with strip due to:
        * High protein concentration
        * pH of urine.
        * High lipid in urine may increase or decrease SG.
    - Normal values: (1.002 - 1.035) on random sample.



Urine SG refractometer.

6. **Reaction (pH):**

- The pH is a measure how acidic or alkaline (basic) the urine is.
- Normal urine pH falls within the range of 4.5-8.
- Increased acidity in urine occurs in diabetic (due to ketosis) and some medications.
- The urine must be fresh (why?) because tendency of urine to be alkaline (as a result of ammonia liberation) on standing.

B. Chemical analysis:

Name	Procedure	Observation
Urea	1ml urine + 3ml NaOCl (sodium hypochlorite)	Evolution of N_2 gas
Uric acid	1ml urine + 0.5 ml 10% NaOH + 1ml Folin`s reagent	Blue colour.
Creatinine	1ml urine + drops of sat. picric acid + drops of NaOH 10%	Deep red color of creatinine ppt.
Chloride	1 ml urine + drops dil. HNO3 + 1 ml $AgNO_3$ $Cl^- + AgNO_3 \Longrightarrow AgCl + NO_3$	White ppt. of AgCl
Phosphate	1 ml urine + 1 ml conc. HNO_3 + 1 ml ammonium molybdate	Yellow colour
Carbonate	1 ml urine + drops conc. HCl $Na_2CO_3 + 2HCl \Longrightarrow H_2O + 2NaCl + CO_2$	Effervescence
Ammonia	1ml urine + 1ml phenol + 1ml NaBr	Blue color
Sulphates	1ml urine + 2 drops conc. HCl + few drops $BaCl_2$ $\Longrightarrow SO_4 + BaCl_2 \Longrightarrow BaSO_4 + 2Cl^-$	White ppt. of $BaSO_4$

References:

- www.mayoclinic.com
- http://library.med.utah.edu/WebPath/TUTORIAL/URINE/URINE.html
- http://www.vet.uga.edu/vpp/clerk/sine/index.php
- http://csm.jmu.edu/biology/danie2jc/urinalysis.htm
- http://www.webmd.com/a-to-z-guides/urine-test

Lab (9): Identification of Pathological Physical and Chemical Urine Constituents

Pathological urine constituents are substances which are not usually present in urine such as glucose, protein, ketones, RBCs, Hb, bilirubin…. etc.

Urine strip:

Strip is filter paper or plastic which has different test pads, each pad has chemical substance (reagent) coated on it which is specific for certain test.

- It gives color when react with substance in urine.
- The produced color is visually compared with chart color.
- **Depending on the test performed, the results are reported as:**
  - In concentration (mg/dl)
  - As small, moderate, or large
  - Using the plus system (1+, 2+, 3+, 4+)
  - As positive, negative, or normal
- This method is rapid, easy, give early indication, qualitative and semi quantitative.
- Therefore, usually there are other confirmatory tests: chemistry, microbiology and microscopic analysis.
- To reduce timing errors (because reaction in strip is effected by time) and to limit variations in color interpretation; **automated instrument** is used to read the reaction color on each test pad.


Urinalysis test strip


Automated Urine Testing Machine

Abnormal urine constituents include:

1- Proteinurea:

Proteinuria is the presence of abnormal amount of protein in urine.

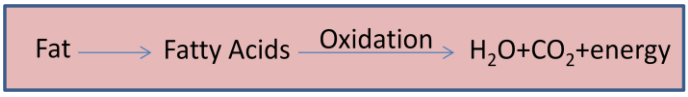
- Urine of healthy individual contains no protein or only traces amounts, due to:
  - In normal physiology, protein is reabsorbed by kidney tubules (proximal tubule).
  - Protein has large molecular weight so it can't pass through kidney tubule to urine unless kidney tubule has damage.
- The main protein in urine is albumin; therefore, proteinurea=albumin urea.
- **Microalbumin urea:**
  - Is the presence of small amounts of albumin in urine.
  - It is very important in detection of early stage of nephronpathy and in diagnosis of DM complication (nephropathy).
- High protein in urine makes urine looks foamy.
- It also associated with face or feet abnormal odema; due to disturbance in liquid balance in the body caused by protein loss.

2- Glucoseurea:

- Glucosuria is the presence of abnormal concentration of glucose in urine.
- Normally, glucose is reabsorbed by active transport in proximal tubule and therefore it doesn't appear in urine.
- If the blood glucose level exceeds the reabsorption capacity of kidney tubules (renal threshold), glucose will appear in urine. (this is indication for high blood glucose).
- **Renal threshold of glucose:** is around 160 mg/100 ml.
  - Glucosuria indicates that glucose concentration in blood exceeds this amount and the kidneys are unable to reabsorb it efficiently.
- **Glucosuria occurs in diabetes mellitus, which characterized by:** hyperglycemia, polyurea (increased volume of urine), high SG and urine may be light in color.

3- Ketourea:

- Ketourea is the presence of abnormal amount of ketone bodies in urine.
- Body normally uses carbohydrates as source of energy. If carbohydrate source is depleted or if there is a defect in carbohydrate metabolism; body use fat as a source of energy.



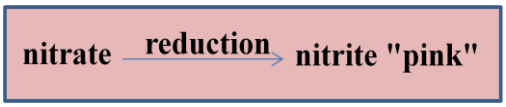
- Fat metabolism is occurred for certain time. At certain point, fatty acid utilization occurs incompletely results in production of intermediate substances (keton bodies : acetone, acetoacetate and ß- hydroxybutayric acid).
- Elevated ketone bodies in blood and urine cause acidosis which leads to coma and death.
- **Ketourea is common in uncontrolled DM (why?)** because uncontrolled diabetic patient use lipids as source of energy although they have high blood glucose but they can't use it (body cells can't uptake glucose).
- **Causes of ketourea:** Some diseases, diet low in carbohydrates and high in lipids and proteins or vomiting for long time.
- Results effected by: diet and drugs.

4- Bilirubin (Bile) and Urobilinogen:

- Urine normally does not contain detectable amounts of bilirubin. Presence of high concentration of bilirubin in urine indicates liver dysfunction.
- Normal urine contains only small amounts of urobilinogen. Hemolysis and hepatocellular disease can elevate urobilinogen levels.

5- Nitrite:

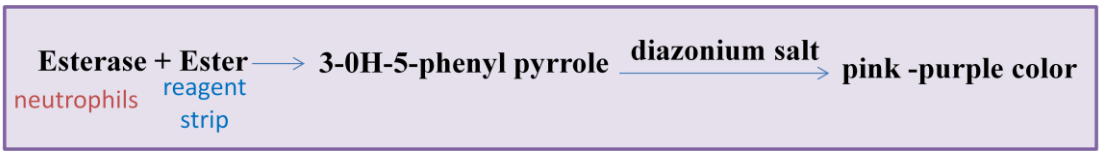
- It is used for bacteria screening.
- Normal urine contains nitrate but not contain nitrites.
- In the presence of bacteria, the normally present nitrate is reduced to nitrite.



- Positive test indicates presence of more than 10 microrganisms/ml.

6- Urine leucocytes:

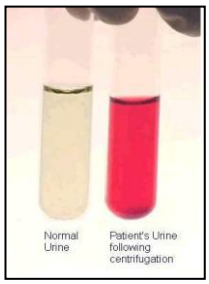
- This test detects any microbial infection in the body.
- It depends on esterase method:

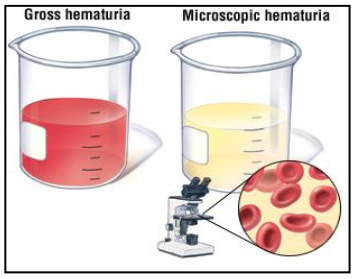


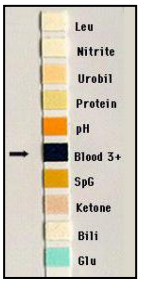
- Positive test indicates presence of more than 5 leucocytes/hpf.
- **False positive:** occurs in vaginal contamination, presence of glucose, albumin, ascorbic acid, tetracycline.
- **False negative:** due to large amounts of oxalic acid which inhibit the reaction.

7- Haematourea:

- It is the presence of red blood cells (RBCs) in urine, it can be either gross (visible) or microscopic.
- It can't detected by the naked eye. Detection occurs by strip or microscope examination as anucleated cells.
- **Positive result may be** normal or pathological due to stones or tuners.



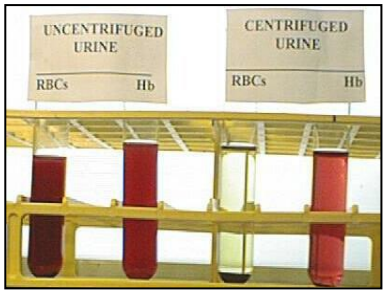




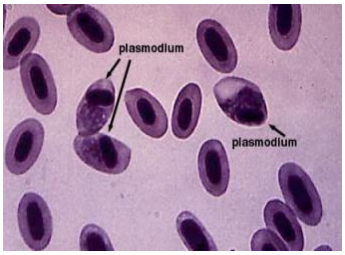
Hematourea may be gross or microscopic.

8- Hemoglobinuria:

- Presence of heamoglobin in urine due to rupturing of RBCs.
- This may occur in malaria, typhoid, yellow fever, hemolytic jaundice or other hemolytic diseases.



Difference between hematourea and hemoglobinuria



Palsmoduim malaria which cause hemolysis of RBCs and release of Hb.

References:

1. Corwin HL. Urinalysis in Diseases of the Kidney (6th Ed) Ed : Schrier RW, Gottschalk, Boston Little Brown and Company. 1996; 1: 295-306.
2. Wise KA, Sagert LA, Grammens GL. Urine leucocyte esterase and nitrite tests as an aide to predict urine culture results. Lab Med 1984; 15: 186.
3. Guide to clinical preventive services, 2nd ed. Williams & Wilkins, Baltimore, 1996; pp 181-6.
4. Kasiske BL, Keane WF. Laboratory assessment of renal disease : Clearance, urinalysis and renal biopsy in the kidney (6th ed) Ed : Brenner BM, WB Saunders, Philadelphia 2000; pp 1142-53.
5. Norvin Peter AF. Urinary sediment in the interpretation of proteinuria. Annals of Internal Medicine 1983; 98: 254-5.
6. Schumann GB, Greenberg NF. Usefulness of macroscopic urinalysis as a screening procedure. A preliminary report. Am J Clin Pathol 1979; 71: 452-6.
7. Dinda AK. Supravital staining and bright field microscopy. A simple technique for urine sediment. The India J of Path And Micro 1999; 42 (3): 391-2.

Lab (10): Urine Microscopic Examination

Background:

Urine specimens required macroscopically and microscopically examination. After centrifugation of a urine sample, small amount of urine will sedimented. This sediment contains different types of crystals, cells, casts, and other constituents. Here some common components that may be found in urine sediment.

Microscopic analysis:

1. Crystals (defined structure)

- The crystal is formed when the urine sample stands and cools before examination.
- The type of crystal depends on the pH, temperature, and constituents of the urine.
- There are common crystals and pathological crystals.
- Presence of large amounts of crystals in urine for long period of time large may lead to kidney stone formation.

a) Common crystals:

It is crystals normally present in urine of healthy individuals such as calcium oxalate dehydrate, triple phosphate (magnesium ammonium phosphate), bilirubin, calcium carbonate, ammonium biurate, and amorphous.

b) Pathological crystals:

It is crystals abnormally present in urine it may indicate an abnormal metabolic process such as uric acid, cystine, cholesterol, leucine, calcium oxalate monohydrate, ammonium biurate, and tyrosine, 2,8-dihydroxyadenine.

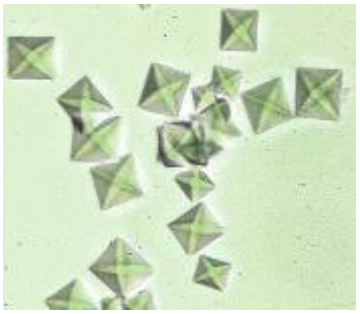
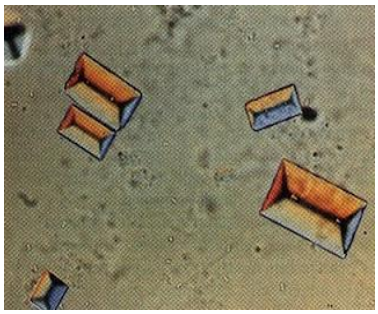
a. Common crystals:

1. Calcium oxalate dehydrate

- Colourless squares resembling an envelope.
- It is formed in urine of any pH.
- It takes different sizes from very small to quite large.

2. Triple phosphate (magnesium ammonium phosphate)

- It is formed in urine whose pH is neutral to alkaline.
- The primary factor of the triple phosphate crystals formation is the ammonia concentration.
- It can indicate urinary tract infection.
- Triple phosphates are usually associated with bacterial growth. With a first-morning fresh specimen.

Calcium oxalate dehydrate	**Triple phosphate**
	

3. Amorphous (irregularly shaped crystals)

It appears as aggregates of finely granular.

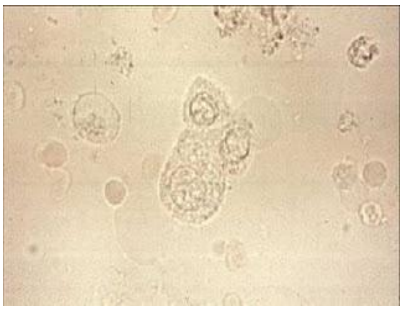
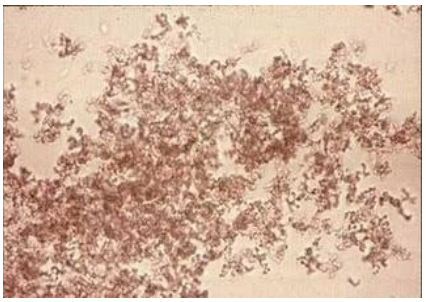
a. Amorphous urates

- It is salts of Na, K, Mg, or Ca.
- It is formed in acidic urine.
- They have a yellow or yellow-brown color.

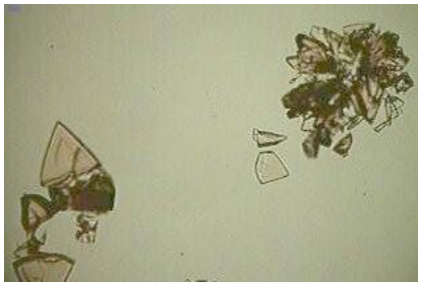
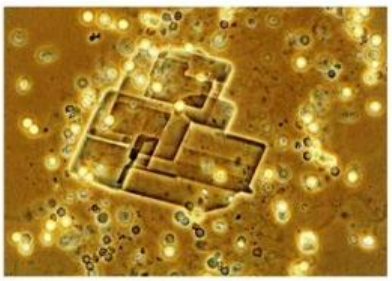
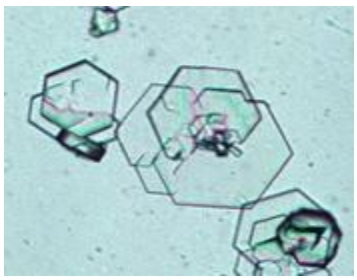
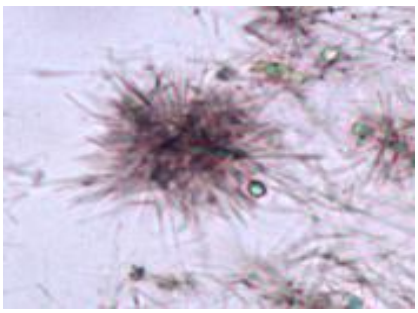
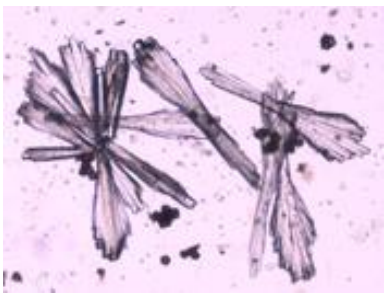
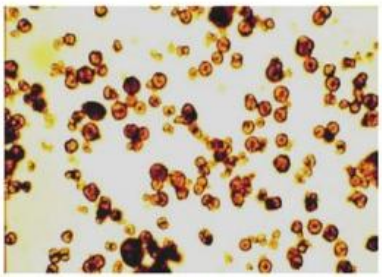
b. Amorphous phosphates

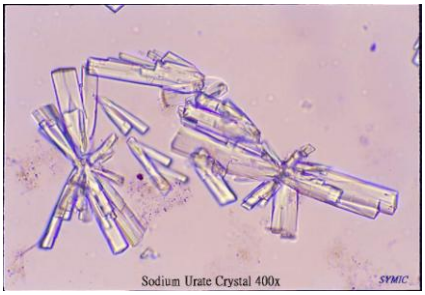
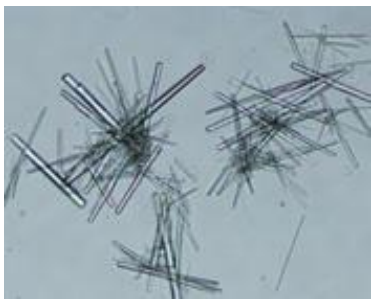
- It contains precipitate of calcium and phosphate.

* It is formed in alkaline urine. It caused by the diet and an also represent a pathological situation.
* The different between amorphous urates and amorphous phosphates is pH and the color of the centrifuge pellet (the precipitate of calcium phosphate is white, but the amorphous urate is pink).

Amorphous urates	Amorphous phosphates
	

b. Uncommon crystals:

Uric acid	Cholesterol	Cystine
		
Tyrosine	**Calcium phosphate**	**2,8-di-hydroxy adnenine**
		

Sodium urates crystal	Unknown crystals Needles
	

2. Cells:

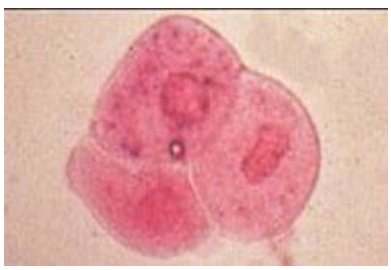
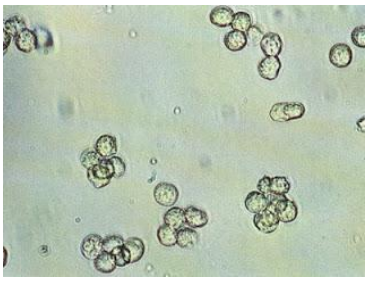
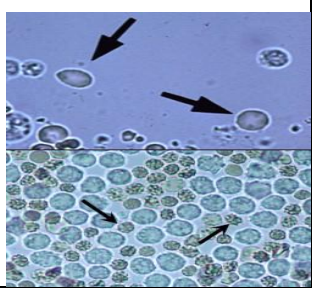
Epithelial cell, RBCs, and WBCs are the most common types of cells found in urine.

- **Epithelial cell**
  - Cells lining the urinary tract are seen in the urine.
  - Little amount normally appear in female due to reproductive period.
- **WBCs**

  Normal urine contain up to 5/HPF. More than five WBCs may indicate an acute infection in the urinary tract. It needs more conformation test like uine culture test. Bacteria may be insignificant contaminants, but the pathologic bactiuria usually lead to increased numbers of leukocytes that are referred to pyuria.

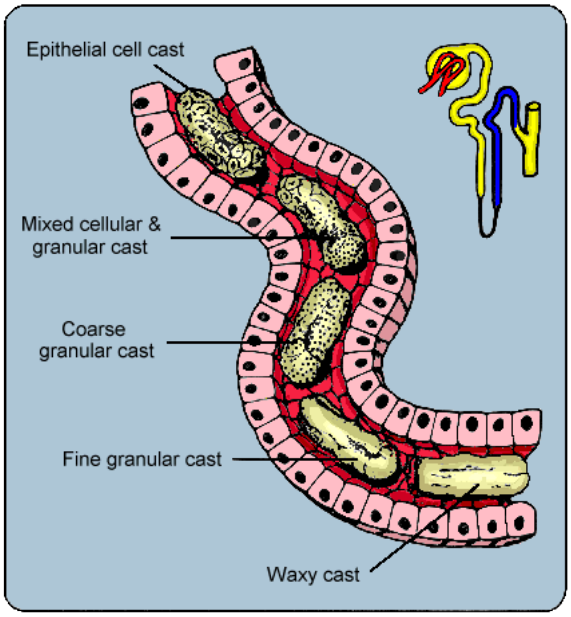
- **RBCs**

  Normal urine contain up to 5/HPF. More than five RBCs is termed hematuria, which are caused such as hemorrhage, inflammation, or necrosis in the urinary tract.

Epithelial cell	Pus cells	RBCs
		

3. Urinary casts

Casts are cylindrical structures composed mainly of mucoprotein. There are different types of casts like granular, cellular, hyaline, fatty cast, and waxy casts.



Schematic illustration of transition between epithelial cell, coarse granular and waxy casts formed in the loops of Henle, distal tubules, and collecting ducts (blue area of nephron in upper right-hand corner). From Osborne, et. al.

1. Granular cast

It ranges from fine to coarse. Since they usually form as a stage in the degeneration of cellular casts.

2. Cellular casts

* **Epithelial cast**

  It normally found in men and women in the urine sediment as a few cells. Kidney cells are less common. More epithelial cells are present in urinary tract disease such as infections, inflammation ... etc.

* **Red blood cell casts:**

  The presence of red blood cells within the cast (protein matrix) is always pathological, and is strongly indicative of glomerular damage. They can also be associated with renal infarction.

* **White blood cell casts:**

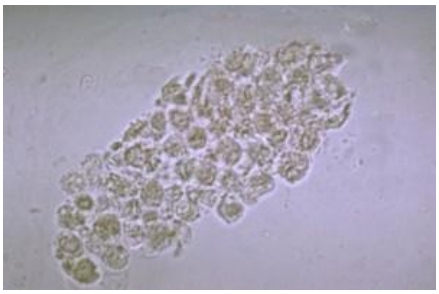
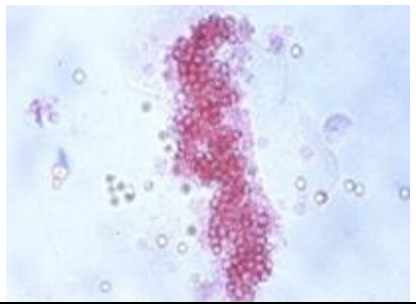
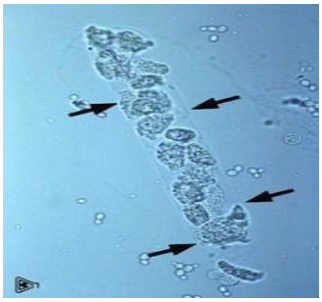
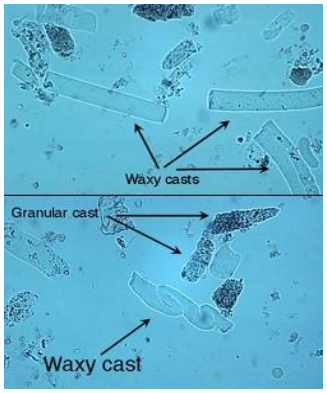
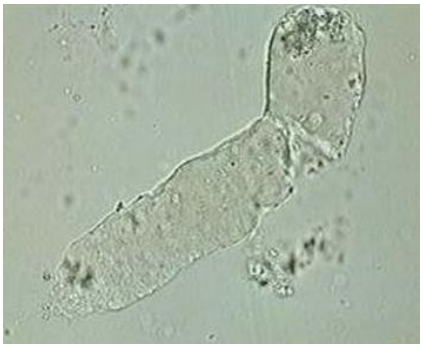
  The presence of white blood cells within casts strongly relevant a direct infection of the kidney.

3. Waxy casts

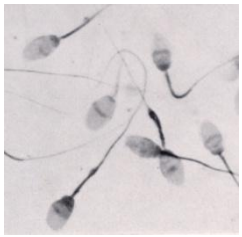
* It represents the different stages of degeneration of cells in a cast.
* It is originating from cellular and granular casts.
* It is smooth and not contains internal texture compared hyaline casts.
* It is formed in kidney disease such as renal failure lead to form waxy casts.

4. Hyaline cast

* It formed in acidic environment.
* We can see this cast in healthy person like dehydration or vigorous exercise.
* The greater numbers of this cast are associated with proteinuria.

Granular cast	**Red blood cell casts**	**White blood cell cast**
		
Epithelial cast	**Waxy cast**	**Hyaline cast**
		

4. Sperm



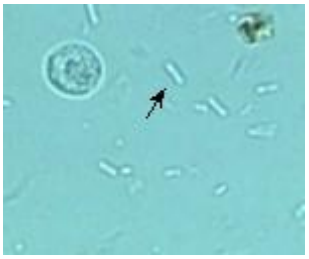
Under microscopic examination we can check the shape and movement speed of sperm.

5. Contaminants or other constituents

Parasites , fungi, fibers , mucus, micro-organism, pollen, yeasts, and starch.

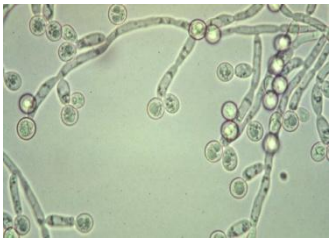
6. Micro-organisms:

- **Bacteria:**
  - It the commonest organism seen and are commonly contaminants.
  - The concomitant presence of WBC's; however, suggests infection.



- **Fungi:**
  - It can be seen, especially in females as contaminants.
  - They may be pathogenic also Protozoan: Trichomonas vaginalis can be seen in women.

- **Yeasts**
  - It indicates of a contamination with vaginal secretion.
  - It observed with diabetic patients because their urine has sugar.
  - There are casts containing yeast. These are pathognomonic of pyelonephritis



Procedure:

- Collect urine in a clean container.
- Run microscopic examination on the sample
- A sample of well-mixed urine (usually 10-15 ml) is centrifuged in a test tube about 2-3,000 r.p.m for 5-10 minutes.
- The supernatant is decanted and a volume of 0.2 to 0.5 ml is left inside the tube.
- The sediment is resuspended in the remaining supernatant by flicking the bottom of the tube several times.
- A drop of resuspended sediment is poured onto a glass slide and cover-slipped.
- The sediment is first examined under low power to identify most crystals, casts, squamous cells, and other large objects. Next, examination is carried out at high power.
- Record the results in the lab report of urinalysis.

References:

- Corwin HL. Urinalysis in Diseases of the Kidney (6th Ed) Ed : Schrier RW, Gottschalk, Boston Little Brown and Company. 1996; 1: 295-306.
- Wise KA, Sagert LA, Grammens GL. Urine leucocyte esterase and nitrite tests as an aide to predict urine culture results. Lab Med 1984; 15: 186.
- Guide to clinical preventive services, 2nd ed. Williams & Wilkins, Baltimore, 1996; pp 181-6.
- Kasiske BL, Keane WF. Laboratory assessment of renal disease : Clearance, urinalysis and renal biopsy in the kidney (6th ed) Ed : Brenner BM, WB Saunders, Philadelphia 2000; pp 1142-53.
- Norvin Peter AF. Urinary sediment in the interpretation of proteinuria. Annals of Internal Medicine 1983; 98: 254-5.
- Schumann GB, Greenberg NF. Usefulness of macroscopic urinalysis as a screening procedure. A preliminary report. Am J Clin Pathol 1979; 71: 452-6.
- Dinda AK. Supravital staining and bright field microscopy. A simple technique for urine sediment. The India J of Path And Micro 1999; 42 (3): 391-2.
- http://ahdc.vet.cornell.edu/clinpath/modules/

Lab Report for Routine Urine Analysis

(Identification of Normal & Phathological Physical and Chemical Urine Constituents)

Patient Name:	**Age/Sex:**
Patient ID:	**Sample Date:**

Macroscopic Examination	Observation	Comment
A) Identification of Normal Physical and Chemical Urine Constituents		
1. Physical urine constituents		
Volume		
Color		
Odor		
Transparency		
SG		
pH		
2. Chemical urine constituents		
Urea		
Uric acid		
Creatinine		
Chloride		
Phosphate		
Carbonate		
Ammonia		
Sulphates		

Macroscopic Examination	Observation	Comment
B) Identification of Pathological Physical and Chemical Urine Constituents		
1. Physical urine constituents		
SG		
pH		
2. Chemical urine constituents		
Nitrite		
Protein		
Glucose		
Ketones		
Urobilinogen		
Bilirubin		
Blood		
Leucocytes		
Ascorbic acid		

Microscopic Examination	Observation	Comment
Calcium oxalate crystal		
Uric acid crystal		
Amorphous urates		
Amorphous phosphates		
Triple phoshpate		
Epithelial cell		
WBCs cast		
RBCs cast		
Hyaline cast		
Waxy cast		
Sperm		
Schistosoma /Hematoglobin Hematobium		
Tricochomonas vaginal		