King Saud University
Collage of Applied Medical Sciences
Department of Clinical Laboratory Sciences
(CLS)

Practical Biochemistry
CLS 432

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Clinical Chemistry is a branch of laboratory medicine which uses chemical analysis to measure the level of various body components during health and diseases.

Specimen Handling:

= **First;** Sample Information (check all samples for the following):

1. Patient's complete name.
2. Medical record numbers.
3. Date collected.
4. Time collected.
5. Name or badge number of person drawing specimen.
6. Specimen computer accession number.

= **Second;** Sample logging processing (enter in the computer or write in the receiving Master Log Book the following):

1. Date and time received.
2. Medical record numbers.
3. Patient's complete name.
4. Location.
5. Status of the request.
6. Number of the tubes received.

Special Specimen Handling For Clinical Chemistry:

1. Allow specimen to clot for 15-30 min at R.T.
2. Centrifuge all specimens at 3500 R.P.M. for 5-15 min.
3. Use refrigerator centrifuge as needed.
4. Don't centrifuge cyclosporine or alcohol samples.
5. Put Ammonia samples in ice and tightly capped.
6. For Routine Test; Aliquot into plastic tubes, label and store in chemistry freezer.

Specimen Priorities:

STAT	Done within one hour from collection.
EXPEDITE	Done within two hours from collection.
ROUTINE	General chemistry (6-8 hrs). Special chemistry (1-7 hrs).

EDTA Contamination:

* EDTA: Ethylene Diamine Tetra-Acetic Acid.
* Occurs when Lavender top filled before serum/plasma tube.
* May be seen with as little as one drop.
* Increased K (May be more than 20 mmol/L).
* Decreased Ca and Mg (Often to 0 level).
* Decreased CK and ALP.

Glycolysis:

* Since the rate of Glycolysis is around 7% per hour; a Glycolysis inhibitor, e.g. Sodium fluoride must be added to sample prior to determination of glucose level.

Collection Tube Colors and Additives (Type of Tubes):

Color	Additive	Additives Function	Considerations	Laboratory Use
Red (plain tube)	None.	Contains no anticoagulants and no additives.	Sterile with no additive best choice for blood banking. Clot formation takes 30 min.	* Serum chemistries. * Serology. * Blood bank.
Lavender (EDTA)	EDTA	Remove calcium to prevent clotting.	Should be well mixed; invert 6-8 times.	* CBC. * Whole blood.
Light Blue	Sodium citrate.	Remove calcium to prevent clotting.	Should be full and mixed well. Blood to anticoagulant ratio very important.	* Coagulation studies. * PT. * PTT. * Factor assay.
Dark (Royal) Blue	= None. = Sodium heparin EDTA.	= Contains no anticoagulants. = Anticoagulants.	Chemically cleaned and the rubber stoppers contain low levels of metals.	* Toxicology. * Trace metals.
Green	Sodium heparin. Lithium heparin. Ammonium heparin.	Inhibits thrombin formation to prevent clotting.	Be careful of the type of heparin that is being used of what type of testing.	Plasma chemistry.
Gray	Sodium fluoride. Potassium oxalate.	Inhibits Glycolysis. Removes calcium to prevent clotting.	Should not be used for other chemistries.	* Glucose testing usually. * Glucose tolerance. * Alcohol levels.
Brown	Sodium heparin.	Inhibits thrombin formation.	Less than 0.1 ug/ml of lead.	Lead determinations.
Orange (Marbled Yellow)	Thrombin.	Thrombin is a clot activator for aster clot formation, usually within 5 min.	Should be inverted 8 times.	STAT Chemistries.

Types of Blood Specimens:

Types	Description	Uses
Clotted Blood	No anticoagulant added.	For separation; to use the serum portion.
Serum	Clotted blood is allowed to stand for 20 min and then centrifuge. The upper portion is termed serum. Doesn't contain fibrinogen.	Chemistry testing. Serology testing. Blood banking.
Whole blood	Obtained by having a tube containing an anticoagulant that prevents the blood from clotting. The tube contains cells and plasma. * Important that tube is mixed well.	Can be used for testing in Haematology (CBC). * For separation to use the plasma portion.
Plasma	Whole blood is centrifuged and the upper layer is termed plasma. * Plasma contains fibrinogen.	Coagulation. Plasma chemistries.
Capillary Blood	A combination of venous, arterial blood and tissue fluid obtained through a skin puncture.	When a venipuncture can't be performed on newborns and children; a heel stick or finger stick procedure is performed.
Buffy Coat	The middle layer between plasma and red cells contain white blood cells and platelets.	Special stains. Haematology studies.

Sources of Blood Samples:

* Arterial blood = Standard.
* Capillary blood similar to arterial, especially if tissue warmed.
* Venous = Arterial – Nutrients extracted + Metabolites added.
* A-V differences dependent on tissue perfusion (e.g. Glucose meters depend on oxygen content).
* Avoid air bubbles in blood gases testing as it increases pO2 and decreases pCO2.

Transportation and Storage of Sample:

* Centrifugation should generally take place on longer than one hour from sample collection.
* Electrolytes and enzymes are stable for 4 days at 4C' and for one day at R.T. (Exception: ACP, Ammonia and Lactate).
* For storage: separate samples then freeze at -20C'.
* Avoid hemolysed samples.
* Urine sediment should be evaluated within 2-3hrs.
* CSF cells must be counted within one hour.

Minimizing Variation:

* Develop procedure manuals with detailed methods and specimens for correct collection.
* Educate all staffs on physiologic factors affecting results.
* Use Delta Check to detect unlikely changes in results.

Delta Check:

* Compare results to those of previous sample.
* Use only tests with relatively small individual variation.
* If three results fail Delta Check, the error is likely to be present, usually at mislabeled or contaminated tube.

Criteria For Specimen Rejection:

1. Mislabeled specimen; (If specimen doesn't match with the request).
2. Broken, leaking, contaminated specimen.
3. Specimen collected in wrong tube or container.
4. Specimen collected using inappropriate collection procedures (e.g. Not kept on ice).
5. No label or no complete information.
6. Wrong specimen submitted.
7. Rejection of hemolysed specimen.

Haemolysis:

* Occurs with turbulent flow during the process of collection, often with slow fill or high pressure on syringe.
* Hgb interferes in many photometric assays.
* Substances released from RBC (Bilirubin, K, Mg, LDH, other enzymes and ACP).

Evaporation:

* It is Increased concentration of all substances.
* Dependent of surface area/volume ratio.
* Impeded by column of air.
* Rate is increased by temperature, air flow and low humidity.

Hematological Disease Effects:

* Increased of WBC will decrease Glucose levels in the blood.
* Platelets release K on clotting (As the sample clotted in the tube). Therefore, serum K 0.1-0.15 mmol/mm3 rise in platelet count.
* Heparin causes release of K from lymphocytes when higher.

Laboratory Information System (LIS):

LIS is used in the laboratory for:

1. Patient data entry.
2. Patient data inquiry.
3. Patient results inquiry.
4. Reporting results.
5. Verification of results.
6. Checking of pending tests.
7. Entering QC data, Levy-Jennings Chart, etc.
8. Statistics for medical research and workload.

Quality Control (QC):

* **QC** is a statistical process used to monitor and evaluated the analytical process which produce patients' results.
* This statistical process requires:
1. Regular testing of QC materials along with patient samples.
2. Comparison of QC results to specific ranges.

 # QC Statistics:
* The mean or average (X) of QC is the arithmetic sum of the QC data divided by its number.
* Standard Deviation (S) is a statistic which quantities how close numerical values (i.e. QC) are in relation to each other.
* The term precision of often used interchangeably with Standard Deviation.
* Coefficient of variation (CV) = "(S/X)*100%", is used to compare the relative variability between two different sets of values.
* Standard Deviation (S) is comparing:
1. The instrument manual or test method, e.g. Between run precision of manufacturer and the laboratory performance should be closed to each other.
2. Proficiency surveys.
3. Inter laboratory QC programs; combine and compare monthly QC data from each laboratory.
* Coefficient of variation (CV) is the percentage ratio of Standard Deviation (S) to the mean/ average (X).
* Coefficient of variation (CV) allows to make easier comparisons of the overall precision.

 # Levey-Jennings Chart:
* It is used to graph QC values for each test and level of control.
* Mean +/- 2sS.
* Westgrad rules (1 2s,1 3s, 2 2s, R4s, 3 1s, 4 1s & 10x) are used to evaluate the quality of analytical runs.

Dietary Effects:

* Substances in food increase after meals (Triglycerides, Glucose).
* Hormones released by food (Insulin, Gastrin, ACTH, GH).
* Electrolyte shifts (K, PO4 shift into cells under the influence of Insulin).
* Blood GGT and MCV levels increase in alcoholic drinkers.
* Smokers have elevated CO-Hgb and CEA levels.
* Fasting:
  - 12hrs overnight fast is recommended for lipid test, since increases in Triglycerides persist up to 9hrs after fatty meal.
  - After 48hrs fast, 240% increase in Bilirubin level and decreased levels of Pre-albumin, Albumin and C3.

Postprandial (After meals) Increases:

1. Gastrin.
2. Glucose.
3. Growth Hormone (GH).
4. Insulin.
5. Ionized Calcium (Ca++).
6. Triglycerides.
7. Glucagon.

Postprandial (After meals) Decreases:

1. Potassium (K).
2. Phosphorous (PHOS).
3. Chloride (CL).

Cyclic Variation:

* Ultradian (Less than one day) ----- "Most pituitary hormones".
* Diurnal (Daily).
* Menstrual Cycle.
* Circadian Variation/Seasonal.

Higher in A.M.:

1. Cortisol.
2. ACTH.
3. Catecholamines.
4. Glucose Tolerance.
5. Rennin and Aldosterone.

Higher in P.M.:

1. Acid Phosphatase (ACP).
2. Parathyroid Hormone.
3. Gastrin.
4. Growth Hormone (GH).
5. Osteocalcin.
6. Prolactin.
7. TSH.

Menstrual Cycle:

* 20% of Cholesterols are lower in late phase; and highest in mid-cycle.
Adrenal steroid (e.g. Aldosterone and Cortisol) and Catecholamines (e.g. Epinephrine and Norepinephrine) are higher after ovulation.
* Prolactin and PTH are higher at time of ovulation.

Seasonal Effects:

= **Summer**:
  * Vitamin-D level is higher.
  * 20% of Tri-Iodothyronine (T3) levels are lower.
= **Winter:**
  * Triglyceride levels are lower slightly (2.5%), but total cholesterol level is increased.

Altitude Effects:

* At 1400 M; Haemoglobin (Hgb) and Haematocrit (Hct) values are 8% higher.
* At 3600 M; an increase of about 65% in CRP levels.
* At higher altitude; levels of Rennin, Transferrin and Oestradiol are increased, but Creatinine clearance decrease.

Physical Exercise:

* In short term exercise, muscle contents are released (e.g. CK and Lactate).
* In long term exercise, Gonadotropins and sex steroids are decreased and HDL-cholesterol is increased.
* Even moderate exercise (e.g. Running 5 M/Week) causes changes in ovarian function and progesterone.

Acute Illness Effects:

* Acute illness increases stress hormones; such as ACTH, Cortisol and Catecholamines. But most other hormones are decreased.
* Fall in LDL-C, HDL-C, rise in VLDL are observed in acute illness.
* Changes are seen with acute phase reactant proteins; such as Haptoglobin and Fibrinogen.

Pregnancy Effects:

* Increased volume will decrease proteins.
* Hyperfiltration will decrease Blood Urea Nitrogen (BUN) and Creatinine, but increase in Creatinine clearance by 50% or more.
* Increased TBG, Total T3 and Total T4 due to Estrogen effect (Also similar contraceptives).
* Increased placental proteins (ALP).
* Decreased Glucose tolerance (GGT).
* Increased in urine volume by 25% during third trimester.
* Cortisol, AFP, Amylase, Cholesterol and Triglycerides may also increase.
* ESR level Increases due to acute phase proteins.
* Decreased the levels of iron and ferritin.

Drugs Effects:

* Pharmacological effects; direct changes in physiology is predictable and dose related.
* Cross-reaction; loss common, usually photometric assays or immunoassays.
* Idiosyncratic effects; allergic reactions, metabolic changes.
*Oral contraceptives increase of T4 (in RIA method) and decrease of T3-U.
* They are also reported to increase of AAT, Fe, TG, ALT, GGT and decrease of Albumin.
* It affects as many as 100 tests.

Relative Variation of Drugs Effects:

* Small effects (Less than 5%) on Electrolytes, Ca, T4, Protein, Osmolilaity and Creatinine.
* Moderate effects (5-10%) on ALP, LDH, Cholesterol, Ferritin, Glucose, BUN and Uric Acid.
* High effects (More than 10%) on AST, ALT, CK, Cortisol, Fe, TSH, Triglyceride, Tumor markers and Total Bilirubin.

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Liver Function Tests (LFT):

1. **Alkaline phosphatase (ALP).**
2. **Alanine Aminotransferase (ALT).**
3. **Aspartate Aminotransferase (AST).**
4. **Gamma Glutamyl Transferase (GGT).**
5. **Total Bilirubin (BILT2).**
6. **Total protein (TP).**
7. **Albumin (ALB2).**
8. **Globulin.**
9. **Ammonia (NH3).**

Alkaline phosphatase (ALP)	
Type:	Enzyme.
Location:	Mainly in Liver and Bone.
Function:	Hydrolyses phosphate at high pH in vitro.
Disease associated:	**Increased**: In Liver, Bone and Malignant diseases.
Method:	Colorimetric: P-nitrophenyl phosphate.
Note:	ALP increases during bone growth and in pregnant women.

Alanine Aminotransferase (ALT)	
Type:	Enzyme.
Location:	Mainly in Liver.
Function:	Catalyzes the transformation of amino acids from ketoglutrate to Alanine to form Pyruvate.
Disease associated:	**Increased:** In Liver diseases.
Method:	Enzymatic, Pyruvate-NADH, LDH.

Method of Alanine Aminotransferase (ALT):

* Pyruvate is reduced to lactate in association with oxidation of NADH (Reduced form) to NAD+ (Oxidized form). The later reaction is catalyzed by LDH.
* (**NAD**: Nicotinamide-Adenine Dinucleotide).
* **Oxidation-Reduction Reaction**: it is a chemical reaction whereby electrons are removed (Oxidation) from atoms of the substance being oxidized and transfer to those being reduced (Reduction).

Aspartate Aminotransferase (AST)	
Type:	Enzyme.
Location:	Liver, Heart and Skeletal Muscles
Function:	Catalyzes the transformation of amino acids from ketoglutrate to Aspartate to form Oxaloacetate.
Disease associated:	**Increased:** In Heart and Liver diseases
Method:	Color-Enzymatic NADH → NAD+ reaction by Malate Dehydrogenase (MDH).

Gamma Glutamyl Transferase (GGT)	
Type:	Enzyme.
Location:	Liver.
Function:	Catalyzes the transfer of gamma Glutamyl group from one peptide to another or to an amino acid.
Disease associated:	**Increased:** 1. Cholestatic Liver Diseases. 2. Acute Hepatitis. 3. Cirrhosis of the Liver. 4. Cancer of the Liver.

	5. Acute Pancreatitis. 6. Gross alcohol abuse.
Method:	Color-Enzymatic @ 410 nm.
Note:	GGT may be induced by anticonvulsant drugs such as phenytoin.

Total Bilirubin (BILT2)	
Type:	Organic Compound (conjugated and unconjugated forms).
Location:	Liver.
Function:	Bilirubin is a Bile pigment formed from the breakdown of Heme group of hemeprotiens (e.g. hemoglobin).
Disease associated:	**Increased:** In Acute hemolysis and Liver diseases. Increase levels of Bilirubin may cause Jaundice.
Method:	Colorimetric DPD, Diazo reaction.

Bilirubin (Conjugated or Direct)	
Type:	Conjugated Bilirubin is a Water soluble form of Bilirubin.
Location:	Conjugation with Glucuronic acid takes place in the Liver by Uridyl-Diphosphate Glucuronyl Transferase (UDGT) and passes into the Bile system.
Function:	Conjugated Bilirubin is broken down in the gut o form stercoblinogen and accounts for the normal color of faeces.
Disease associated:	**Increased in**: Choletasis (Obstruction of Bile Flow).
Method:	Colorimetric reaction by coupling of direct Bilirubin with diazotized sulphanilic acid to form an Azo dye (Jendrassik).

Bilirubin Fractions

1. Unconjugated "Indirect"; (Alpha-Bili).
2. Monoconjugated; (Beta-Bili).
3. Diconjugated "Direct"; Gamma-Bili).
4. Protein; (Sigma-Bili).

Methods For Determination of Bilirubin:

1. Diazo Reagent.
2. Spectrophotometric.
3. Dry Slide.
4. High Performance Liquid Chromatography (HPLC)..

Total protein (TP)	
Type:	The sum of all proteins and globulins.
Location:	Most proteins are synthesized in the Liver and released into biological fluids.
Function:	It measures the sum of circulating proteins: Albumin, Alpha-1, Alpha-2, Beta and Gamma Globulins. Albumin must be measured at the same time to distinguish any increase or decrease in fractions
Disease associated:	**Increased in:** Multiple Myeloma and Immunoglobulin Disorders. Decreased in: Liver and Kidney Diseases.
Method:	Colorimetric (Biuret Method) in which protein forms a violet/purple colored complex with copper ions in an alkaline medium proportional to its concentration.

Ammonia (NH3)	
Type:	Organic Molecule.
Location:	Liver.
Function:	Toxic waste of degradation of amino acids.
Disease associated:	**Increased**: In Sever Liver diseases such as Cirrhosis, Hepatitis and Reye's Syndrome.
Method:	Color-Enzymatic (GLDH) oxidizes; NADPH (reduced form) to NADP+ (oxidized form).
Note:	NADP= Nicotinamide-Adenine Dinucleotide Phosphate.

Albumin (ALB2)	
Type:	Protein.
Location:	Synthesized in the Liver.
Function:	1. Carries ions and molecules (e.g. Bili & Ca). 2. Maintains oncotic pressure. 3. Regulates plasma volume.
Disease associated:	**Increased:** In Dehydration. **Decreased:** In Oedema, Nephrotic syndrome and Burns.
Method:	Color, Bromo-Cresol Green (BCG) at pH 4.1
Note:	Low levels also affect binding site availability and can give falsely low levels of total Calcium (Ca).

Electrolytes (E,U,C):

1. **Bicarbonate (HCO3- & CO2).**
2. **Chloride (CL-).**
3. **Potassium (K+).**
4. **Sodium (Na+).**

Bicarbonate (HCO3- & CO2)	
Type:	HCO3-: is the second largest organic anion in plasma. CO2: is an organic gas.
Location:	CO2 transports from tissue to the lungs in the form of HCO3- in the plasma.
Function:	HCO3- regulates acid-base balance and CO2 is the end products of metabolism of foodstuffs.
Disease associated:	**Increase** of HCO3- with decrease of pH is screen in respiratory acidosis. Increases of both are seen in metabolic alkalosis. **Decrease** of HCO3- with increase of pH is seen in respiratory alkalosis. Decreases of both are seen in metabolic acidosis.
Method:	Color-Enzymatic phospho-Enol Pyruvate Carboxylase (PEPC).

Chloride (CL-)	
Type:	Anion Ion.
Location:	Extracellular Fluid (ECF).
Function:	Maintain electrical neutrality of ECF.
Disease associated:	**Increased in:** 1. Hyperventilation (Excess breathing). 2. Drugs (NH3CL or KCL). 3. Dehydration. **Decreased in:** 1. Hypoventilation (Inadequate removal of CO2 from the blood by lungs). 2. Vomiting. 3. Diarrhea. 4. Diabetic ketoacidosis (Ketone bodies in blood displace of both HCO3 & CL) 5. Lactic acidosis. 6. Adrenal diseases. 7. Renal failure.
Method:	Potentio-metric electrode (e.g. ISE).
Note:	When (HCO3) ions increase; (CL) ions decrease, and when (Na) ions increase; (CL) ions increase.

Potassium (K+)	
Type:	Cation Ion.
Location:	Intracellular Fluid (ICF) inside cells.
Function:	It helps in nerve impulse transmission, contractility of cardiac and skeletal muscles. A pump mechanism in the cell membrane transfers Na from the inside cell to outside in exchange for K.
Disease associated:	**Increased in:** 1. Kidney failure. 2. Urinary obstruction. 3. Tissue damage (Crush injuries and massive hemolysis). 4. Addison's disease. 5. DM (Low Na and high K serum levels are seen in the last two conditions). **Decreased in:** 1. Diarrhea. 2. Vomiting. 3. Diuretic medications. 4. Cushing's Syndrome where hypokalemia in the rule. 5. Renal Tubular Acidosis (RTA). 6. Barter's Syndrome.
Method:	Potentio-metric electrode (e.g. ISE).
Note:	1. K and H ions are secreted into distal tubule in exchange for Na ions 2. If (K) is high, the heart stops beating in Diastole (The fully relaxed state). 3. If (K) is low, the heart stops beating in Systole (The fully contracted state).

Sodium (Na+)	
Type:	Cation Ion.
Location:	Extracellular Fluid (ECF) and regulated by aldosterone.
Function:	1. Maintain water balance and osmotic pressure. 2. Maintain the pH of plasma.
Disease associated:	**Increased in** (= increase of body Sodium or decrease of body water in the following): 1. Dehydration due to sweating. 2. Diabetes insipidus (Either lack of ADH or nephrogenic). 3. Increase of glucose and urea in urine which contribute to osmolilaity of urine and increase water excretion. 4. Excess of Na solution by IV (e.g. NaCL or NaHCO). 5. Excessive production of adrenal gland hormones as in primary hyperaldesteronism or Cushing's Syndrome. **Decreased in:** 1. Diuretic medications in CHF, RD and HPT. 2. Kidney diseases. 3. Congestive Heart Failure. 4. Hepatic Cirrhosis. 5. Addison's disease (Primary adrenal insufficiency). 6. Diabetes Mellitus. 7. Sever diarrhea and/or vomiting. 8. Inappropriate ADH secretion. 9. Pseudo-hyponatremia.
Method:	Potentio-metric electrode (e.g. ISE).
Note:	Artificially low levels of (Na) are observed with analyzer that uses diluted sample (Indirect ISE) of sera that contain elevated levels of protein and lipid.

Renal Function Tests (RFT):

1. **Albumin (ALB).**
2. **Creatinine (CREj).**
3. **Urea.**
4. **Uric Acid.**

Albumin (ALB)	
Type:	Protein.
Location:	Synthesized in the Liver.
Function:	4. Carries ions and molecules (e.g. Bili & Ca). 5. Maintains oncotic pressure. 6. Regulates plasma volume.
Disease associated:	**Increased:** In Dehydration. **Decreased:** In Oedema, Nephrotic syndrome and Burns.
Method:	Color, Bromo-Cresol Green (BCG) at pH 4.1
Note:	Low levels also affect binding site availability and can give falsely low levels of total Calcium (Ca).

Creatinine (CREj)	
Type:	Organic Compound.
Location:	Muscle tissues and then released into blood.
Function:	Plasma Creatinine is mostly derived from breakdown of Creatinine and then excreted by kidney into urine. Therefore, it uses to assess the kidney function.
Disease associated:	**Increased in:** Kidney diseases.
Method:	Colorimetric: Jaffe, alkaline pirate without deproteinisation.
Note:	Creatinine is generally used for the measurement of Glomerulur Filtration Rate (GFR) by estimating the Creatinine from the plasma and urine.

Urea	
Type:	Organic Compound of breakdown of amino acids in the Liver.
Location:	Plasma and Urine.
Function:	Urea is used as a marker of renal function and as an indication of reduced blood flow. (Congestive Cardiac Failure).
Disease associated:	**Increased in:** 1. Renal Failure. 2. High Protein Diet (Increased catabolism due to Starvation or Tissue damage may cause moderate elevation). 3. Dehydration. 4. Heart Failure. **Decreased in:** 1. Advanced Liver Disease. 2. Hemodialysis.
Method:	Color-Enzymatic, Urease NADH → NAD+.

Uric Acid	
Type:	Organic Molecule of end products of purine (Adenine and Guanine) catabolism.
Location:	Purines can be synthesized in the body or can be ingested from foodstuff.
Function:	Man is therefore prone to clinical gout and renal damage, whereas other mammals.
Disease associated:	**Increased in:** 1. Gout in which hyperuricaemia may be genetically determined or could be secondary to increased turn over of nucleic acids (Malignant tissue, tissue damage). 2. Uremia due to kidney disease. 3. Diabetic Acidosis. 4. Reduced excretion may be due to renal glomerular dysfunction or acidosis.
Method:	Color-Enzymatic

Bone Profile/Mineral Tests:

1. **Calcium (Ca++).**
2. **Phosphorous (PHOS).**
3. **Magnesium (Mg++).**

Calcium (Ca++)	
Type:	Inorganic Cation Element.
Location:	99% in Bone and 1% in Plasma.
Function:	Ca is essential for the normal function of heart, nerves impulses, muscle contractility and blood coagulation.
Disease associated:	**Increased in:** Hyperparathyroidism, Carcinoma metastatic to bone and multiple myeloma. **Decreased in:** Hypoparathyroidism, Vitamin D deficiency, Malabsorption, Kidney disease and Muscle Tetany.
Method:	Colorimetric: O-Cresolphathalein.
Note:	Bone releases Ca to prevent hypokalemia or to control high level with assisting of the kidney and parathyroid gland.

Phosphorous (PHOS)	
Type:	Inorganic Element.
Location:	Bone.
Function:	1. Give the bone its rigidity as calcium-phosphate salts. 2. Part of ATP structure. 3. Part of phospholipids structure. 4. Part of DNA and RNA structures.
Disease associated:	**Increased in:** 1. Renal diseases with secondary low calcium. 2. Hypoparathyroidism. 3. Hyperthyroidism. **Decreased in:** 1. Hyperparathyroidism. 2. Malabsorption that may leads osteomalacia (Soft bones; Low Ca & PHOS levels).
Method:	Colorimetric: Ammonium Molybdate (Acidified) conversion to Ammonium Phosphomolybdate.
Note:	1. When the level of Ca is high, the PHOS level is low and vice versa. 2. After the meal, PHOS level decrease because it moves into the cell along with glucose.

Magnesium (Mg++)	
Type:	Intracellular Cation.
Location:	Inside the cell.
Function:	All ATP-dependent enzymatic reactions require Mg.
Disease associated:	**Increased in:** Kidney diseases. **Decreased in:** 1. Prolonged IV feeding. 2. Acute alcohol intoxication. 3. Primary hyperaldesteronism. 4. Diabetic coma. 5. Hyperparathyroidism. 6. Alcoholic Cirrhosis. 7. Low serum Mg levels produce muscle irritability which leads to Tetany.
Method:	Colorimetric: Mg reacts with xylidyl blue in alkaline solution to form purple color. EGTA is used to prevent interference of the calcium in the sample.

Lipid Profile Tests:

1. **Cholesterol (CHOL).**
2. **Triglycerides (TRIG).**
3. **High Density Lipoprotein (HDL).**
4. **Low Density Lipoprotein (LDL).**

Cholesterol (CHOL)	
Type:	Cyclic Aliphatic Organic Hydrocarbons
Location:	Adrenal gland, Liver and Gallstone.
Function:	1. Precursor of steroid hormone. 2. Part of lipoproteins structure (LDH). 3. Part of cell membrane.
Disease associated:	**Increased in:** Atherosclerosis, Hypothyroidism, Nephrosis Diabetes Mellitus, Obstruction of Bile flow. **Decreased in:** During Starvation and in Hyperthyroidism.
Method:	Colorimetric: Chol-Esterase, Chol-Oxidase, Peroxiase.
Note:	2/3 of cholesterol is esterified with fatty acids (In the Liver). Routine assays measure the total cholesterol level (i.e. Ester and free cholesterol).
Triglycerides (TRIG)	
Type:	Triglycerides consists of glycerol esterified with 3 fatty acids that may reaches the Liver from fat sources or synthesizes from glucose.
Location:	Adipose Tissues.
Function:	In case of Starvation or Hypoglycemia; Triglycerides release from adipose tissues to give energy.
Disease associated:	**Increased in:** 1. Diabetes Mellitus. 2. Nephrosis. 3. Liver Destruction. 4. Lipid metabolism disorders.
Method:	Color-Enzymatic.

High Density Lipoprotein (HDL)	
Type:	Macromolecules that contain of a combination of 50% proteins, 28% phospholipids and 20% cholesterol and apoprotien A-I & A-II.
Location:	HDL is a catabolic product of chylomicrons and VLDL. It is synthesized in the Liver and intestine.
Function:	HDL removes cholesterol from peripheral cells to the Liver. This stats by the activation of lecithin- cholesterol acyltransferase (LCAT) by apoprotiens of HDL LCAT.
Disease associated:	**Increased in:** 1. protective against atherosclerosis and coronary heart diseases. 2. Physical activity. **Decreased in:** 1. Atherosclerosis and coronary heart diseases. 2. Smoking. 3. Obesity.
Method:	Color-Enzymatic: PEG; Polyethylene Glycol.

Cardiac Enzymes:

1. **Creatine Kinase (CK).**
2. **Lactate Dehydrogenase (LDH).**

Creatine Kinase (CK)	
Type:	Enzyme.
Location:	Skeletal Muscle (MM), Heart Muscle (MB) and Brain (BB).
Function:	Synthesizes and degrades Creatine phosphate which is an important molecule for storage and transfer of chemical energy within cell to other energy molecule such as adenosine triphosphate (ATP).
Disease associated:	**Increases** of CK-MB is seen in the cardiac muscle damage or in myocardial infarction (MI). CK-MM is found in muscle trauma and exercise.
Method:	Color-Enzymatic: HK/G6PDH to catalyze; G6PD + NADP → NADPH + H.

Lactate Dehydrogenase (LDH)	
Type:	Enzyme.
Location:	Heart, Skeletal muscle, Liver, Kidney, Brain and Erythrocytes (RBCs).
Function:	Catalyzes the interconvertion of lactate and Pyruvate (Reversible).
Disease associated:	**Increased in:** 1. Myocardial Infarction (MI). 2. Liver Diseases. 3. Megaloplastic Anaemia. 4. Acute Leukemia. 5. Metastasis but NOT localized Cancer. 6. Muscle Diseases. 7. Chronic Myelocyctic Leukemia but NOT chronic Lymphocotic Leukemia.
Method:	Color-Enzymatic: NADH2 → NAD+ and converting Pyruvate to Lactate.

Carbohydrate/Diabetic Tests:

1. **Glucose.**
2. **Lactate or Lactic Acid.**
3. **Pyruvate.**
4. **Hgb A1C.**

Glucose	
Type:	The simplest organic form of Carbohydrate.
Location:	In all Cells.
Function:	1. The major source of energy. 2. At low levels of blood glucose, the Liver synthesizes it from glycogen, adrenalin, cortisol, glucagons, thyroxin and growth hormone and raise blood glucose levels.
Disease associated:	**Increased in:** 1. DM. 2. Cushing's Syndrome (Excess of cortisol which produces more glucose via gluconegenesis). 3. Acromegaly (Excess of growth hormone). 4. Hyperadrenalinism (Excess of adrenalin causes the conversion of glycogen to glucose in the Liver). **Decreased in:** 1. Glycogen storage disease (Inability of the Liver to convert glycogen back to glucose). 2. Galctosemia (Increase of galactose in the blood due to deficiency of the enzyme Galactose-1-Phosphate Uridyl Transferase). 3. Hereditary fructose intolerance. 4. Tyrosinemia. 5. Islet cell adenoma "Insuloma" of the Pancreas (Excess of insulin). 6. Addison's disease or Hypoadrenalinism (Low cortisol level). 7. Liver diseases.
Method:	Color-Enzymatic: Hexokinase; NADP → NADPH + H by G6PDH.

Lactate or Lactic Acid	
Type:	An organic acid produced during the breakdown of glucose.
Location:	Human tissues and cells.
Function:	Formed in anaerobic metabolism of carbohydrate and also produced by the bacterial action in milk.
Disease associated:	**Increased in:** 1. Physical exercise where an anaerobic Glycolysis markedly increase blood lactate. 2. Shock. 3. Pneumonia. 4. Congestive Heart failure. 5. Renal failure. 6. Leukemia. 7. diabetic ketoacidosis. 8. In CSF levels with bacterial meningitis. Hydrocephalus, brain abscess and cerebral ischemia.
Method:	Color-Enzymatic: L-Lactate Oxidized to Pyruvate by Lactate Oxidase (LOD).

Special Chemistry Tests:

1. **Acid Phosphatase (ACP).**
2. **Amylase (AMY).**
3. **Cholinesterase.**
4. **Iron (Fe).**
5. **Urine Protein.**
6. **Cerebrospinal Fluid (CSF) Protein.**

Acid Phosphatase (ACP)	
Type:	Enzyme.
Location:	Prostate Gland that located just below the urinary bladder which surrounds the urethra.
Function:	It secreted into the serum to help in Fertilization.
Disease associated:	**Increased in:** Prostate Carcinoma.
Method:	Color-Enzymatic.
Note:	The tartrate is used as a specific inhibitor for prostatic acid phosphatase. (ACP Prostatic) = (Total ACP) – (ACP Non-Prostatic).

Amylase (AMY)	
Type:	Enzyme.
Location:	Saliva and Pancreas.
Function:	Breakdowns starch and glycogen to maltose (Di-sugar).
Disease associated:	**Increased in:** Acute Pancreatitis, other intra/extra abdominal conditions, Mumps and Renal failure.
Method:	Color-Enzymatic assay that uses P-Nitrophenyl Maltohepatoside that hydrolyzes to P-Nitrophenol and Glucose by Alpha-Glucosidase.

Cholinesterase	
Type:	Enzyme.
Location:	Liver, Pancreas, Heart, Serum and White matter of Brain Tissue.
Function:	Catalyzes the hydrolysis of acetylcholine ester which is a chemical compound that released as apart of the transmission of nerve impulses.
Disease associated:	**Decreased in:** 1. Organo-Phosphorous "insecticide" Poisoning. 2. Liver Diseases such as Hepatitis and Cirrhosis, but NOT Obstructive jaundice. 3. Patients with abnormal genetic alleles of PCHE that causing prolonged sleep apnea due to slow elimination of muscle relaxants)e.g. Succinyl Dicholine).
Method:	Color-Enzymatic: Butyrythiocholine Iodide/DTNB.
Note:	True Cholinesterase and Pseudo Cholinesterase (PCHE).

Iron (Fe)	
Type:	Trace Element.
Location:	70% in Hemoglobin, 25% in Liver, Spleen and Bone Marrow. It is stored in the Liver complexes to proteins ferritin and hemosiderine. It is transported in the blood by protein Transferrin.
Function:	It is binding site for O2 and CO2 in the hemoglobin and myoglobin. Many enzymes require its presence for their activities.
Disease associated:	**Increased in:** 1. Overdose Conditions either as iron salts or in blood transfusion. 2. Hemochromatosis (Excessive absorption of iron from GIT) which may complicated to DM and Cirrhosis. 3. Active Cirrhosis or Acute Hepatitis Temporary. **Decreased in:** 1. Iron Deficiency Anaemia. 2. Chronic Infection. 3. Chronic Kidney Diseases. 4. Malignant Diseases.
Method:	Colorimetric: Ferrozine without deproteinisation; using ascorbic acid (Vitamin-C) as a reducing agent.

Urine Protein	
Type:	All Proteins in Urine.
Location:	Urine.
Function:	2000 liters of blood flow through the Kidney everyday. The final urine output is 1-2 liters. While plasma contains about 70 g/L, the normal urine contains only 0.14 g/L or less of protein.
Disease associated:	**Increased in:** Proteinuria which occurs in nearly all Kidney diseases.
Method:	Colorimetric.

Cerebrospinal Fluid (CSF) Protein	
Type:	CSF is an ultra-filtration of blood from which the cells and proteins have been excluded.
Location:	Brain and Spinal cord (CNS).
Function:	Na, K, CL and HCO are present in CSF at the same concentration of plasma. Protein level in CSF if very low (0.15-0.45 g/L).
Disease associated:	**Increased in:** Nearly all CNS Disorders. **Decreased in:** NCS.
Method:	Colorimetric
Note:	Additional useful tests to be measured in CSF include Glucose, WBC and electrophoresis.

Functions of Cerebrospinal Fluid (CSF):

1. Supports and protects the Brain and Spinal cord.
2. Maintains a uniform pressure around these delicate structures.
3. Acts as a cushion and a shock absorber between Brain and Cranial Bones.
4. Keeps Brain and Spinal cord moist and there may be interchange of substances between CSF and nerve cells; such as nutrients and waste products.

King Saud University
Collage of Applied Medical Sciences
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Practical Biochemistry

Appreviation of Chemistry Tests:

- TSH = Thyrotrophin Stimulating Hormone.
- Neo TSH = Neonatal Thyrotrophin Stimulating Hormone.
- FT4 = Free Thyroxin.
- B-HCG = Beta HCG; Human Chronic Gonadotrophin, beta subunit.
- ERC = Cardiac Enzymes; Cardiac-4 (CK-MB, Troponin-1).
- CP III = Cardiac Enzymes; Cardiac-3 (CK-MB, Troponin-1, Myoglobin).
- PRL = Prolactin.
- FSH = Follicle Stimulating Hormone.
- LH = Luteinisng Hormone.
- PROG = Progesterone.
- AFP = Alpha- Fetoprotein.
- CEA = Carcinoembryonic Antigen.
- FERRITIN.
- OESTRADIOL = ESTRADIOL.
- TESTO = Testosterone.
- MA-FP = Male Fertility Profile (FSH, LH, Prolactin & Testosterone).
- FM-FP = Female Fertility Profile (FSH, LH, Prolactin, Progesterone & Oestradiol).
- TFT = Thyroid Function Test (TSH, FT4).
- PSA = Prostate Specific Antigen.
- CYCLO = Cyclosporine.
- ATA =
- HOMOC =
- INSULIN.
- INSULIN PEPTIDE.
- IGE = Immunoglobulin E.
- CORTISOL.
- DHEAS = Dehydroepiandrosterone Sulphate.
- PTH = Parathyroid Hormone.
- CA-19.9.
- CA-N.3. } Tumor Markers.
- CA15-3 2.
- CA125 1.
- VIT-B12. } Megalp;astic Anaemia.
- FOLATE.
- DIGOXIN.
- TG.
- E, U, C = Electrolyte:
  - :: UREL = Urea.
  - :: CREJ = Creatinine.
  - :: NA-I =Sodium.
  - :: K-I = Potassium.
  - :: CL-I = Chloride.
  - :: CO2-I = Carbon Dioxide.
- BONE PROFILE:
  - :: CA = Calcium.
  - :: ALB2 = Albumin.
  - :: PHOS2 = Phosphorous.
  - :: TP2 = Total Protein.
  - :: ALP2S = Alkaline Phosphate.

- LIPID PROFILE:
  - :: TRIGL = Triglyceride.
  - :: HDL-C = High Density Lipoprotein (Cholesterol).
  - :: CHOL2 = Cholesterol.
- LFT = Liver Function Test:
  - :: ALB2 = Albumin.
  - :: BILTS = Total Bilirubin.
  - :: TP2 = Total Protein.
  - :: ASTL = Aspartate Transaminase = Aspartate Aminotransferase.
  - :: ALTL = Alanine Transaminase = Alanine Aminotransferase.
  - :: ALP2S = Alkaline Phosphate.
  - :: GGT2 = Gamma-Glutamyl Transferase.
- CA = Calcium.
- FE = Iron.
- TRSF2 = Transferrin.
- FE-TRF = Total Iron.
- CRP = C=Reactive Protein.
- ASO = Anti-Streptolysin O.
- NEON = NEONATAL PROFILE:
  - :: UREL = Urea.
  - :: CREJ = Creatinine.
  - :: NA-I =Sodium.
  - :: K-I = Potassium.
  - :: CL-I = Chloride.
  - :: CO2-I = Carbon Dioxide.
  - :: MG = Magnesium.
  - :: CA = Calcium.
  - :: ALB2 = Albumin.
  - :: PHOS2 = Phosphorous.
  - :: TP2 = Total Protein.
  - :: GLUC3 = Glucose = Blood Sugar.
- NEBIL = NEONATAL BILIRUBIN:
  - :: BILTS = Total Bilirubin.
  - :: BIL-C = Bilirubin Conjugated.
- RF = Rheumatoid Factor.
- URINE ELECTROLYTE.
- FLUID ANALYSIS PROFILE:
  - :: AMYL2 = Amylase.
  - :: UREL = Urea.
  - :: CREJ = Creatinine.
  - :: NA-I =Sodium.
  - :: K-I = Potassium.
  - :: CL-I = Chloride.
  - :: ALB2 = Albumin.
  - :: TP2 = Total Protein.
  - :: CHOL2 = Cholesterol.
  - :: LDH = Lactate Dehydrogenase.
  - :: GLU3C = Sugar Glucose (FLUID).
  - :: UA2 = Uric Acid.
- AMYLASE-URINE.
- IG = IMMUNOGLOBULIN:
  - :: IGA.
  - :: IGM.
  - :: IGGT.
- MICROALBUMIN-URINE.
- C3.
- C4.
- CAERULOSMIN.

- ANTIBIOTICS = DRUGS (Except DIGOXIN):
  - :: GENTM = Gentamycin.
  - :: VANC = Vancomycin.
  - :: PHENO = Phendbarb.
  - :: PHENY = Phenytoin.
  - :: AMIKI = Amikimycin.
  - :: THEO = Theophylin.
  - :: CARB =Carbime.
- GL-HB = Glycoselected HB (HBA1C):
  - :: HEM1W
  - :: HEM2W
- ISEIN = Ion Selective Electrode-Indirect (BLOOD):
  - :: NA-I =Sodium.
  - :: K-I = Potassium.
  - :: CL-I = Chloride.
- ISEU = Ion Selective Electrode (URINE) = Urine Electrolyte:
  - :: NA-U =Sodium.
  - :: K-U = Potassium.
  - :: CL-U = Chloride.
- K = ----------:
  - :: UREL-U = Urea.
  - :: MG = Magnesium.
  - :: CA-U = Calcium.
  - :: CREJ-U = Creatinine.

(*) PROTEINS:

:: IGA.
:: IGM.
:: IGGT.
:: ALBU.
:: ASO.
:: HEM1W.
:: HEM2W.
:: CRP.
:: TRSF2.
:: RF.
:: CERU2.

(*) ENZYMES:

:: ASTL.
:: ALTL.
:: GGT2.
:: ALP2S.
:: AMY2 = Amylase.
:: LDH = Lactate Dehydrogenase.

(*) ELECTROLYTES:

:: NA-I =Sodium.
:: K-I = Potassium.
:: CL-I = Chloride.
:: NA-U =Sodium.
:: K-U = Potassium.
:: CL-U = Chloride.
:: LI = Lithium.

(*) SUBSTRATES:

:: UREL.
:: UREL-U.
:: TRIGL.
:: CHOL3.
:: HDL-C.
:: CA.
:: CA-U.
:: MG-U.
:: UA2 = Uric Acid.
:: UA-U = Uric Acid (URINE).
:: ALB2.
:: BILT2.
:: BIL-C.
:: IRON.
:: PHOS2.
:: PHOS-U.
:: TP2.
:: TPU3.
:: TPC3.
:: NH3L =Ammonia.
:: LACT2 = Lactate.
:: CREJ.
:: CREJ-U.
:: CRE2 = ----------.
:: CO2-L.
:: UUC = ----------.
:: GLUC3 = Sugar Glucose (BLOOD).
:: GLU3C = Sugar Glucose (FLUID).

= SPECIAL TESTS:

- OSMO = Osmolarity.
- BLOOD GAS.
- G6PDH = Glucose-6-Phosphate Dehydrogenase.
- ADA = Adenosine Deaminase.
- URINARY CALCULAI.
- PREGNANCY TEST.
- BENCE-JONSE PROTEIN (URINE).
- ELECTROPHORESIS:
  - :: SPE = Serum Protein Electrophoresis.
  - :: UPE = Urine Protein Electrophoresis.
- IMMUNOELECTROPHOESIS.
- REDUCE-SUB = Reducing Substances (URINE or FAECES).

Liver Function Tests (LFT):

The typical Liver Profile test includes ALT, AST, Alkaline Phosphatase, GGTP, Bilirubin, Prothrombin time, Protein, LDL, Albumin, and Globulin.

A Liver Health Panel, also known as Liver (hepatic) Function Tests or LFT, is used to detect liver damage or disease. It usually includes seven tests that are run at the same time on a blood sample. These include:

Alkaline phosphatase (ALP).
Alanine Aminotransferase (ALT).
Aspartate Aminotransferase (AST).
Gamma Glutamyl Transferase (GGT).
Total Bilirubin (BILT2).
Total protein (TP).
Albumin (ALB2).
Globulin.
Ammonia (NH3).

Alkaline Phosphatase (ALP):

Investigation	: Alkaline Phosphatase
Specimen type	: Plasma
Spec container	: Lithium Heparin
Volume required	: 5ml
Reference range	: 35 - 125 U/l
Turnround	: <24 hours

This enzyme level is elevated in a large number of disorders that affect the drainage of bile, such as a gallstone or tumor blocking the common bile duct, or alcoholic liver disease, or drug-induced hepatitis, blocking the flow of bile in smaller bile channels within the liver. The alkaline phosphatase is also found in other organs, such as bone, placenta, and intestine. For this reason, the GGT is utilized as a supplementary test to be sure that the elevation of alkaline phosphatase is indeed coming from the liver or the biliary tract.

Alanine Aminotransferase (ALT):

Investigation	: Alanine Aminotransferase (ALT)
Specimen type	: Plasma
Spec container	: Lithium Heparin
Volume required	: 5ml
Reference range	: <35 U/l
Turnround	: <24 hours

This enzyme used to be called Serum Glutamate Pyruvate Transaminase (SGPT), hence the two names. The normal range is 5-40 IU/L (International Units per Liter). Some doctors think that anything under 50 is still OK.

Aspartate Aminotransferase (ALT):

Investigation	: Aspartate Aminotransferase (ALT)
Specimen type	: Plasma
Spec container	: Lithium Heparin
Volume required	: 5ml
Reference range	: ----------
Turnround	: <24 hours

This enzyme used to be called Serum Glutamic-Oxaloaceti Transaminase (SGOT). The normal range is 5-40 IU/L. Some doctors think that anything under 50 is still OK.

Gamma Glutamyl Transferase (GGT) (or GGTP):

Investigation	: g-Glutamyl Transferase (GGT)
Specimen type	: Plasma
Spec container	: Lithium Heparin
Volume required	: 5ml
MALE Ref range	: <50 U/l
FEMALE Ref range	: <35 U/l
Turnround	: <24 hours

This enzyme level is elevated in case of liver disorders. In contrast to the alkaline phosphatase, the GGT tends not to be elevated in diseases of bone, placenta, or intestine.

NOTE:

Different cells have different enzymes inside them, depending on the function of the cell. Liver cells happen to have lots of AST, ALT, and GGTP inside them. When cells die or are damaged, the enzymes leak out causing the blood level of these enzymes to rise; that is why the levels of these enzymes in the blood are considered good indicators of liver cell damage. ALT is more specific for liver disease than AST because AST is found in more types of cell (e.g. heart, intestine, muscle). The AST, for instance, will rise after a heart attack or bruised kidney. GGTP and AP are said to be more specific for evaluating biliary disease since they are made in bile duct cells. In liver disease caused by excess alcohol ingestion, the AST tends to exceed the ALT, while the reverse is true to for viral hepatitis. However, this particular generalization is often wrong. There are several things to remember: These tests have a meaning, but they generally cannot be interpreted without clinical information. They are probably most useful to track, or follow a particular problem, but even then they often "bounce around" greatly.

These numbers are not linear. An AST that is 300 is not twice as bad as 150 (normal is less than 40), and an AST of 94 and 80 are essentially the same to a liver specialist.

These numbers do not always detect all liver disease. Some patients with severe advanced liver disease will have normal or nearly normal enzyme levels.

Despite the fact that they are often called "liver function tests" or "LFT's", these tests do not in fact measure the liver function per se. In order to assess the liver function they must be corroborated with other tests, including albumin, Bilirubin, and Prothrombin time. But clinical factors should be considered as well.

Total Bilirubin (BILT2):

Investigation	: Bilirubin (total)
Specimen type	: Plasma
Spec container	: Lithium Heparin
Volume required	: 5ml
Reference range	: 2 - 17 µmol/l
Turnround	: <24 hours

Bilirubin is the main bile pigment in humans which, when elevated, causes the yellow discoloration of the skin and eyes called jaundice. Bilirubin is formed primarily from the breakdown of a substance in red blood cells called "Heme." It is taken up from blood processed through the liver, and then secreted into the bile by the liver. Normal individuals have only a small amount of Bilirubin circulating in blood (less than 1.2 mg/dL). Conditions which cause increased formation of Bilirubin, such as destruction of red blood cells, or decrease its removal from the blood stream, such as liver disease may result in an increase in the level of serum Bilirubin. Levels greater than 3 mg/dL are usually noticeable as jaundice. The Bilirubin may be elevated in many forms of liver or biliary tract disease, and thus it is also relatively nonspecific. However, serum Bilirubin is generally considered a true test of liver function (LFT), since it reflects the liver's ability to take up, process, and secrete Bilirubin into the bile.
Unconjugated Bilirubin is carried to the liver, where sugars are attached to it, producing conjugated Bilirubin. This conjugated Bilirubin is passed to the bile by the liver and is further broken down by bacteria in the small intestines and eventually excreted in the feces, of which the characteristic color is due to the break down of Bilirubin. Some bile is stored in the gall bladder. As Bilirubin levels increase, the appearance of jaundice becomes more evident. Normally, almost all Bilirubin in the blood is unconjugated.

Total protein (TP):

Investigation	: Protein (Total)
Specimen type	: Plasma
Spec container	: Lithium Heparin
Volume required	: 5ml
Reference range	: 64 - 83 g/l
Turnround	: <24 hours

The total protein test is a rough measure of all of the proteins in the plasma portion of your blood. Proteins are important building blocks of all cells and tissues; they are important for body growth and health. Total protein measures the combined amount of two classes of proteins, albumin and globulin. Albumin is a carrier of many small molecules, but its main purpose is to keep fluid from leaking out of blood vessels, while globulin proteins include enzymes, antibodies, and more than 500 other proteins. The ratio of albumin to globulin (A/G ratio) is calculated from values obtained by direct measurement of total protein and albumin. It represents the relative amounts of albumin and globulins.

Albumin (ALB2):

Investigation	: Albumin
Specimen type	: Plasma
Spec container	: Lithium Heparin
Volume required	: 5ml
Reference range	: 34 - 45 g/l
Turnround	: <24 hours

Albumin is a major protein which is produced by the liver, and chronic liver disease causes a decrease in the amount of albumin produced. Therefore, in liver disease, and particularly more advanced liver disease, the level of the serum albumin is reduced (less than 3.5 mg/dL).

Globulin:

Investigation	: Globulin
Specimen type	: Plasma
Spec container	: Lithium Heparin
Volume required	: 5ml
Reference range	: 26 - 44 g/l
Turnround	: <24 hours

Total protein measures the combined amount of two classes of proteins, albumin and globulin. Albumin is a carrier of many small molecules, but its main purpose is to keep fluid from leaking out of blood vessels, while globulin proteins include enzymes, antibodies, and more than 500 other proteins. The immunoglobulin are the globulins of our immune systems and of antibodies while many other globulin are carriers of hormones or important components of enzymes.

Prothrombin Time (PT):

Also called protime or PT, is a test that is used to assess blood clotting. Blood clotting factors are proteins made by the liver. When the liver is significantly injured, the production of these proteins is not normal. The Prothrombin time is also a useful test, since there is a good correlation between abnormalities in coagulation measured by the Prothrombin time and the degree of liver dysfunction. Prothrombin time is usually expressed in seconds and compared to a normal control patient's blood.
Finally, specific and specialized tests may be used to make a precise diagnosis of the cause of liver disease. Elevations in serum iron, the percent of iron saturated in blood, or the storage protein ferritin may indicate the presence of Hemochromatosis, a liver disease associated with excess iron storage. In another disease involving abnormal metabolism of metals, Wilson's disease, there is an accumulation of copper in the liver, a deficiency of serum ceruloplasmin and excessive excretion of copper into the urine. Low levels of serum alpha1-antitrypsin may indicate the presence of lung and/or liver disease in children or adults with alpha1-antitrypsin deficiency. A positive antemitochondrial antibody indicates the underlying condition of primary biliary cirrhosis. Striking elevations of serum globulin, another protein in blood, and the presence of antinuclear antibodies or antismooth muscle antibodies are clues to the diagnosis of autoimmune chronic hepatitis. Finally, there are specific blood tests that allow the precise diagnosis of hepatitis A, hepatitis B, hepatitis C, and hepatitis D.

In summary, blood tests are used to diagnose or monitor liver disease. They may be simply markers of disease (e.g., ALT, AST, alkaline phosphatase, and GGT), more true indicators of overall liver function (serum Bilirubin, serum albumin, and Prothrombin time) or specific tests that allow the diagnosis of an underlying cause of liver disease. Interpretation of these liver tests is a sophisticated process that your physician will utilize in the context of your medical history, physical examination, and other tests such as X-rays or other imaging studies of the liver.

Lipid Profile

a blood test that measures total cholesterol, triglycerides, and HDL cholesterol. LDL cholesterol is then calculated from the results. A lipid profile is one measure of a person's risk of cardiovascular disease.

Related tests:
Triglycerides, LDL, HDL, Cholesterol

What is a lipid profile?

The lipid profile is a group of tests that are often ordered together to determine risk of coronary heart disease. The tests that make up a lipid profile are tests that have been shown to be good indicators of whether someone is likely to have a heart attack or stroke caused by blockage of blood vessels ("hardening of the arteries").

What tests are included in a lipid profile?

The lipid profile includes total cholesterol, HDL-cholesterol (often called good cholesterol), LDL-cholesterol (often called bad cholesterol), and triglycerides. Sometimes the report will include additional calculated values such as HDL/Cholesterol ratio or a risk score based on lipid profile results, age, sex, and other risk factors.

How is a lipid profile used?

The lipid profile is used to guide providers in deciding how a person at risk should be treated. The results of the lipid profile are considered along with other known risk factors of heart disease to develop a plan of treatment and follow-up.

How is treatment determined?

Treatment is based on your overall risk of coronary heart disease. A target LDL is identified. If your LDL is above the target value, you will be treated. Target LDL values are:

- 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.

The first step in treating high LDL is targeted at changes in lifestyle – specifically adopting diets low in saturated fat and participating in moderate exercise. You may be referred to a dietician for advice in making dietary changes.

If low-fat diets and exercise are not adequate to lower LDL-cholesterol to the target value, drug therapy would be the next step. There are several classes of drugs that are effective in lowering LDL. You will prescribed one of these. Your LDL will be checked at regular intervals to assure that the drug is working. If the drug does not result in reaching your target LDL-cholesterol, your doctor may increase the amount of drug or possibly add a second drug.

Cardiac Markers

There are several enzymes that are released when heart cells are damaged. A specific, sensitive marker that is present in 1-2 hours after the cardiac muscle injury continues to be sought.

- **Troponin T.**
- **CK-MB.**

Troponin T:

Investigation : **Troponin T**
Specimen type : **Serum**
Spec container : **Serum\serum gel**
Volume required : **5ml**
Reference range : **<0.01** µg/l
Turnround : **On arrival**

Knowledge of the time of on-set of chest pain is essential for interpretation and sampling. Samples must be taken at **LEAST** 12 hours post chest pain.

Sampling Requirements:-

Serum Gel Tube:
To be used when the sample is being transported using the pneumatic tube system (POD).

Serum Tube:
Not suitable for transportation using the pneumatic tube system (POD) because these samples are prone to haemolysis. Haemolysis invalidates Troponin T analysis.

Note: The serum-gel tube should **not** be confused with the lithium heparin plasma tube

Interpretation of Troponin T Results		
Troponin T ug/l	**NO** Features of acute ischaemia (clinical, ECG)	**WITH** Features of acute ischaemia (clinical, ECG)
<0.01	Normal/low risk	
0.01 - 0.09	LOW risk	HIGH risk
0.10 - 0.19	HIGH risk cardiac event	HIGH risk cardiac event
>0.20	No MI but marker of poor prognosis	Probable MI

Reminder note: please would all users at the RLUH and BGH use **BROWN GEL TUBES** for Troponin T's. These can be delivered to the laboratory via the pneumatic tube, thus helping to minimise the number of times the blood porters are called to the wards.

Troponin T and I are contractile proteins of the myofibril. The cardiac isoforms are very specific for cardiac injury and are not present in serum from healthy people. Current guidelines from the American College of Cardiology Committee state that cardiac troponins are the prefered markers for detecting myocardial cell injury.

Troponin I (cTnI) or T (cTnT) are the forms frequently assessed.

- Rises 2 - 6 hours after injury.
- Peaks in 12 - 16 hours.
- cTnI stays elevated for 5-10 days, cTnT for 5-14 days.

Creatine Kinase Isoenzyme (CK-MB):

Investigation : **Creatine Kinase Isoenzyme (CK-MB)**
Specimen type : **Plasma**
Spec container : **Lithium Heparin**
Volume required : **5ml**

Reference range : **<5** µg/l
Turnround : **<24 hours**

Only available if recent positive Troponin T exists and re-infarct suspected. Contact Duty Biochemist for information.

This enzyme is found in heart muscle (CK-MB), skeletal muscle (CK-MM), and brain (CK-BB). Creatine kinase is increased in over 90% of myocardial infarctions. However, it can be increased in muscle trauma, physical exertion, postoperatively, convulsions, delirium tremens and other conditions.

Time sequence after myocardial infarction

- begins to rise 4-6 hours
- peaks 24 hours
- returns to normal in 3-4 days

Creatine Phosphokinase Isoenzymes

- MM fraction - skeletal muscle
- MB fraction - heart muscle
- BB fraction - brain

MB fraction

- Rises and returns to normal sooner than total CK
- Rises in 3-4 hours
- Returns to normal in 2 days

CK - MB subforms

This test is becoming more popular. MB_2 is released from heart muscle and converted in blood to MB_1. A level of MB_2 equal or greater than 1.0 U/L and an MB_2/MB_1 ratio equal or greater than 1.5 indicates myocardial infarction.

Myoglobin

Found in striated muscle. Damage to skeletal or cardiac muscle releases myoglobin into circulation.

Time sequence after myocardial infarction

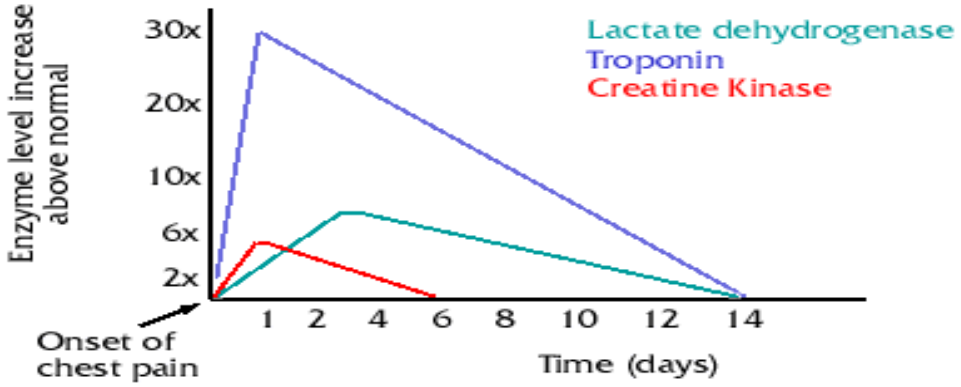
- Rises fast (2 hours) after myocardial infarction
- Peaks at 6 - 8 hours
- Returns to normal in 20 - 36 hours

Have false positives with skeletal muscle injury and renal failure.

Lactic Dehydrogenase (LDH):

This enzyme is no longer used to to diagnose myocardial infarction.

Cardiac enzyme changes with MI



Myocardial markers for infarction (MI) and ischaemia

specimen: serum

Because a patient with the typical clinical picture of an MI is admitted forthwith to a coronary care unit, community laboratories do not often have to make the diagnosis. There are, however, a few patientes who want to stay wherethey are or have had an unreported episode of chest pain during the past week and in these tests will be required.

The markers of myocardial damage are, in decreasing order of specificity, troponins I & T, CK - MB, total CK, myoglobin, AST and LD.
AST and LD no longer have a useful role.
Myoglobin, though relatively nonspecific, is the earliest to rise and can appear within the first 6 hours.
The remaining four typically start to rise 4-12 hours after infarction, reach a peak and return to baseline over a period which differs between the four.

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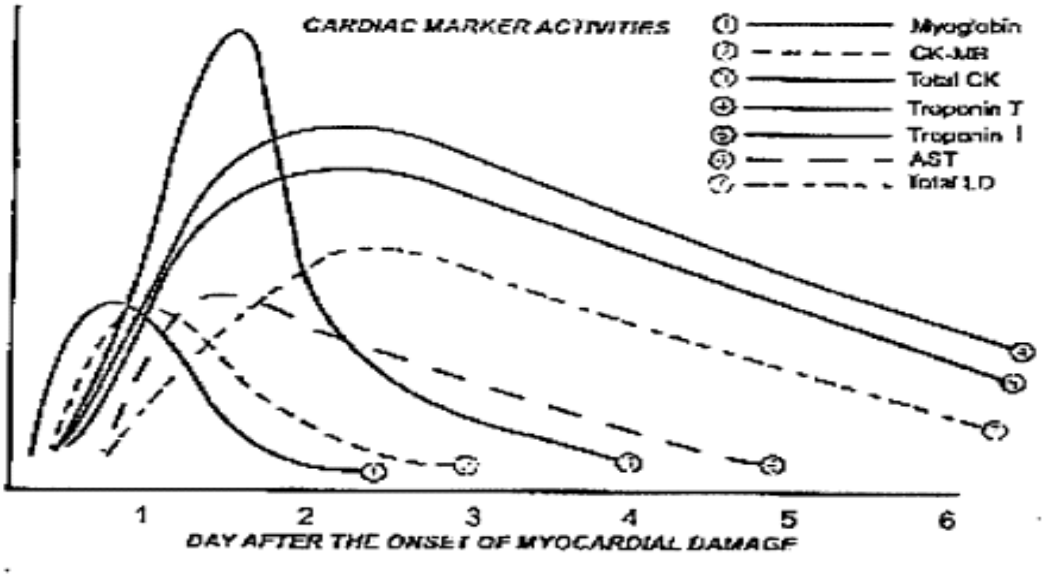
	elevated for
CK - MB	2-3 days
total CK	2-3 days
troponin I	4-7 days
troponin T	4-10 days



Of these, the troponins are the most specific and usually, when elevated, indicate infarction. Small elevations found in unstable angina may indicate ischaemic damage and carry a poorer prognosis than a normal level.
Troponins do not rise earlier than CK but their longer period of elevation is useful, e.g. when chest pain occurred several days earlier.

CK-MB is falling into disuse since the troponins arrived, though it may be useful in plotting successive reinfarctions.

An elevated total CK is non-specific but if serial CK measurements follow the typical time-course of an MI, they support the diagnosis. A small MI may occasionally be suggested by CK changes that stay within reference range but show a typical peak over 2-3 days.

CK (Creatine Kinase) Total:

specimen:	serum	
ref. range:	female	30-180 U/L
	male	60-220 U/L

Elevations of CK:

These are common and most arise from skeletal muscle. Clinically the elevations in myocardial infarction are important.

From skeletal muscle:

- Muscular exercise – the commonest cause. Well-muscled individuals, with or without regular exercise, tend to have levels at or above the upper end of the reference range. Acute bouts of more severe exercise will give elevations of variable extent and duration which may last a week after one episode. There is wide individual variation but levels can rise as high as 4000 U/L in severe, unaccustomed exercise.
- Muscle trauma – IM injections (even 1ml), accidents, bruising, physical violence
- Acute viral infections – particularly those with myalgia
- Alcoholism – even when there is no overt myopathy
- Hypothyroidism – and other endocrinopathies , muscular dystrophies
- Polymyositis, dermatomyositis, connective tissue disorders
- Tumours – some malignant tumours cause massive increases in CK
- Surgery – levels return to normal in 1 week
- Parturition – elevated for up to 6 weeks
- Acute psychoses
- Drugs – fibrates, statins, captopril, colchicine, isotretinoin, lithium, beta blockers.
- Renal Failure.

From cardiac muscle:

In MI (myocardial infarction), CK typically starts to rise 4-12 hours after chest pain commences and returns to normal in 2-3 days. Myocarditis can cause more prolonged elevations of CK.

Troponin I (TnI) and Troponin T (TnT):

specimen: serum

ref. ranges:		
	Troponin I	<2.0 µg/L
	Troponin T	<0.1 µg/L

Elevation of one of the cardiac troponins, which are almost identical in their clinical usage, is more sensitive and specific for myocardial infarction than CKMB. Concentrations rise within 4 - 12 hours of commencement of cardiac pain and remain elevated for 7 days in the case of TnI or 10 days for TnT.

Unstable angina can also cause elevations due to minimal myocardial damage not detected by CKMB. It is important to recognise that angina without myocardial necrosis will not elevate troponins.

If the specimen was obtained less than 12 hours after commencement of chest pain, a follow up should be collected 6-12 hours after the first. TnT can be elevated in chronic renal failure in the absence of known myocardial damage whereas TnI is largely unaffected. Absence of rise doesn't exclude ischaemic cause for chest pain — may warrant specialist follow-up.

Protein Electrophoresis

* **Electrophoresis** is a movement or separating of changes biological molecules (Mainly Proteins) under the influence of an electric field.
* The direction of migration of a protein in an electric field depends on
    1. The pH of the buffer.
    2. The isoelectric point (pI) of the protein.
* Other parameters that affecting Electrophoresis:
    1. Physical properties of proteins
    2. Buffer; pH, composition, concentration, ionic strength.
    3. Electrical Field (Voltage, Current and Time).
    4. Electroendosmosis.
    5. Wick Flow.
    6. Nature of the support medium (e.g. Agarose, Cellulose acetate, Starch and Polyacrylamide).

 # Isoelectric Point (pI):

* There is a pH value at which the number of positive charges and negative charges the molecule balance each other and this point is called the isoelectric point.
* If a protein is placed in a solution at pH of its isoelectric point, that protein has a zero net charge and will not migrate in electric field.
* The higher pH value above the isoelectric point, the greater will be the net negative charge on the protein and the further it will migrate toward the positive electrode (Anode).
* The lower pH value of the solution below the isoelectric point of the protein, the greater the net positive charge on the protein and further it will migrate toward the negative electrode (Cathode) when placed into a direct current electrical field.

 # Electroendosmosis:

* Support medium (gel) becomes negative charged.
* The negatively charged surface attracts positive ions from buffer.
* When current is applied, the positive ions move toward the cathode and against the flow of negative charged molecules (e.g. Protein) moving toward anode in which they can be slowed.
* The effect can be reduced in Agarose Gel by addition of sucrose that increases osmolality and makes gel almost free of endosmosis.

 # Wick Flow:

* Heat generated during Electrophoresis enhances evaporation of liquid from support medium (Gel).
* Wick flow is the upward movement of buffer through both immersed ends of the membrane to replaced lost moisture.
* This flow adds distance to molecules moving toward the center of the membrane and retards molecules moving away from the center.
* The net results can be reduced separation and lead to compression of final band patterns.

 # Specimens in Electrophoresis:

* **Serum**: 500ul minimum (Plasma or hemolysed samples are unacceptable).
* **Urine**: At least 6.0ml of a random specimen or aliquot of a 24hrs collection without preservative refrigerate.
* **CSF**: At least 3.0ml of CSF, with refrigerate.

 # Equipments & Supplies:

1. Templates and gel blotters.
2. Microdispenser, 5ul displacement pipetter with disposal glass tips.
3. Electrophoresis cell, with graphite electrodes, bridge assembly and lid.
4. Power Supply.
5. Dryer.
6. Wet Processor station, Gel Frame and Gel Frame Holder,
7. Densitometer.
8. Concentration Device; e.g. Amicon.

 # Reagents in Electrophoresis:

1. Barbital Buffer at pH: 8.4-8.8.
2. Blue Stain.
3. Acetic Acid (5%).
4. Acid Alcohol; 20% acetic acid – 30% methanol. This is to remove the stain.
5. Agarose Gel.

Serum Protein Electrophoresis (SPE):

* Standard Agarose gel electrophoresis system separates serum into five zones designated Albumin, Alpha-1, Alpha-2, Beta and Gamma.
* The protein content of each zone can be calculated after measuring the total serum protein.
* 13 proteins represent more than 90% of the total protein mass of serum.

Albumin Region:

* It is the most prominent band on a normal SPE.
* Modest hypoalbuminemia is an indication of chronic inflammatory conditions.
* Sever hypoalbuminemia occurs with protein losing syndromes such as:
    1. Sever Malabsorption.
    2. Glomerulonephritis.
    3. Protein-losing enteropathies.
    4. Burns.
    5. Sever acquired Liver diseases.

Alpha-1 Region:

* Alpha-1 zone contains mostly Alpha-1 antitrypsin.
* A marked decrease in the Alpha-1 band may suggest Alpha-1 antitrypsin deficiency, as low levels predispose to early onest emphysema.
* Quantitative level can be measured by nephelometry or turbidometry methods.
* The phenotyping can be determined through electrophoresis techniques.
* In acute phase reaction; Alpha-1 antitrypsin level is increased in response to inflammation and tissue necrosis.

Alpha-2 Region:

* The principal components of the Alpha-2 zone are:
    1. Alpha-2 Macroglobulin.
    2. Haptoglobin.
* Marked evaluation of Alpha-2 Macroglobulin is seen in the Nephrotic Syndrome.
* Haptoglobin levels increase in response to inflammation and tissue necrosis.
* Decreased levels of both may be seen in acquired Liver disorders.
* When hemolysis occurs; Hemoglobin-Haptoglobin complex form and migrate in a broad band between the Alpha and Beta regions.

Beta Regions:

* The Beta globulin zone contains three major protein components:
    1. Complement-3 (C3).
    2. Beta Lipoprotein (LDL).
    3. Transferrin.
* The Complement-3 (C3) band isn't stable and is often absent unless fresh serum is used. A more reliable method for measuring Complement-3 (C3) is Nephelometry Technique.

* Beta Lipoprotein (LDL) appears as a wavy band that can increase in intensity in a number of conditions, including:

- Liver Diseases.
- Nephrotic Syndrome.
- Some familial hyperlipidemia syndromes.

* Transferrin is elevated in iron deficiency anaemia and decreases during the acute phase response and by sever acquired Liver diseases.

Gamma Regions:

* The majority of immunoglobulins are found in the Gamma region zone.
* The finding of a homogenous band in the Gamma region should alert the physician to the possibility of a Monoclonal Gammopathy (MG).
* Gammopathy (MG) is confirmed by the Immunofixation Technique.
* Other proteins that may mimic the pattern of Gammopathy (MG) are:

1. C-Reactive Protein (CRP).
2. Lysozyme.
3. Fibrinogen.

* It should be noted that most serum IgA is found in the Alpha-2 and Beta fractions, and small amounts of IgM and IgG are also present in three fractions.

* Identification of a monoclonal immunoglobulin is suggestive of B-cell malignancy, particularly in concert with other clinical and laboratory evidence of Multiple Myeloma or B-cell Lymphoma.
* Following diagnosis, monitoring of serum levels of the paraprotein may sever as a useful measure of tumor load, while identification of monoclonal immunoglobulin fragments in urine may be of prognostic significance.
* Marked hypogammaglobulinemia in an older individual, with no indication of serum immunoglobulin abnormalities, should be followed up with a urine protein electrophoresis to screen for monoclonal free light chains.
* Hypergammaglobulinemia can be due to:

1. Polyclonal Gammopathy.
2. Oligoclonal Gammopathy.
3. Monoclonal Gammopathy.

Monoclonal Gammopathies:

* Monoclonal Gammopathies are characterized by an uncontrolled proliferation of a single clone of plasma cells at the expense of other clones. This dysfunction often leads to the synthesis of large amount of one homogeneous immunoglobulin or immunoglobulin subunit with decreased levels of levels of normal immunoglobulins.
* There are 6 cases of Monoclonal Gammopathies illustrating the differences in electrophoretic mobility and band appearance from case to case (L-C indicates light chain disease).
* Monoclonal Gammopathies are disorders of immunoglobulin synthesis consisting of a proliferation of B-cell clones. This increase plasma cells results in a single homogenous spike (M Protein) in the Beta-Gamma region. When M Protein is present, there is usually a decrease in normal immunoglobulins.
* High M Protein levels and decreased levels of other immunoglobulins may be associated with a malignant clinical course.

Polyclonal Gammopathies:

* Polyclonal Gammopathies are a secondary disease state characterized by a broad diffuse increase of the Gamma fraction. Usually all three major immunoglobulins (IgG, IgA and IgM) are increased in variable concentrations. Polyclonal Gammopathy is the second most commonly seen abnormality often hypoalbuminemia.
* Continued evaluation of Polyclonal Gammopathies has some prognostic. Clinical improvement in a primary disease state is marked by a decrease of the Gamma fraction.
* Polyclonal Gammopathy is seen in a wide variety of disorders:

1. Chronic Liver Diseases.
2. Collagen Disorders.
3. Chronic Infections.
4. Metastatic Carcinoma.
5. Cystic Fibrosis.
6. Thermal Burns.

Oligoclonal Banding:

* Oligoclonal bands appear in the Gamma globulin fraction in patients with multiple sclerosis. Banding can appear very early in the disease, and generally persists during subsequent remission and exacerbation cycles.

M-Protein:

* Monoclonal protein (M Protein = Myeloma Protein = Paraprotein = B-cell Malignancy) are individual antibodies produced by plasma cells from a single clone of cells.
* Each M Protein consists of two heavy polypeptide chains and two light polypeptide chains of the same type.
* All individual plasma cells contain Kappa or Lambda light chains, but not both.

Urine Protein Electrophoresis:

* It is indicated during the workup of any patient with Proteinuria.
* Urine dipstick tests for protein detect mainly Albumin and may grossly underestimate amounts of other proteins present such as monoclonal free light chains (Bence-Jones Proteins).
* Urine Protein Electrophoresis is useful for differentiating glomerular from tubular Proteinuria.
* The presence of Bence-Jones Proteinuria in patients with multiple myeloma usually indicates poor prognosis.
* Quantitative measurement of the daily excretion of Bence-Jones Protein provides an objective index of response to therapy.
* Glomerular Proteinuria is characterized by the passage of high molecular weight plasma proteins into the urine;
    - Albumin (The bulk constituents).
    - Transferrin (Trace).
    - Alpha-1 antitrypsin (Trace).
    - IgG (Trace).
* The hallmark of tubular in Proteinuria is the increase excretion of proteins of small size and an Albumin content of 10-20%.
* These proteins appear on Urine Electrophoresis as a heterogeneous group of bands; mostly of Alpha-2 and Beta region.
* Some Urine Electrophoresis patterns will exhibit features of both Proteinuria.

Immunoglobulins:

* Decreased levels of one or more immunoglobulins due to deficiencies in humoral anybody response are often associated with recurrent infections.
* Immunodeficiency Disorders with Abnormal Immunoglobulins:
    - Isolated IgA Deficiency.
    - Isolated IgM Deficiency.
    - X-Linked Immunodeficiency with Increased IgM.
    - Wiskott-Aldrich Syndrome.
    - Transient Hypogammaglobulinemia of Infancy.
    - Sever Combined Immunodeficiency.
    - Common Variable Immunodeficiency.
        1. Pan Hypogammaglobulinemia.
        2. IgG and IgA Deficiency.
        3. Isolated IgG Deficiency.

Inflammation Response:

* The inflammatory response is accompanied by characteristic plasma protein changes known as the acute phase pattern. The acute phase pattern varies with the duration of the inflammatory process, and may be used to differentiate between acute, subacute and chronic pathological conditions.

Liver Diseases:

* The Liver is the primary organ for synthesis of all plasma proteins and plays a role in immunoglobulin production by processing antigens from the gut before they are presented to the immune system. Advanced Liver disease can lead to abnormalities in acute phase reactions, transport proteins, complement components and immunoglobulins.

Clinical Manifestations of Multiple Sclerosis:

Psychological Disturbances	Ataxia	Vertigo	Dysarthia.
Nystagmus	Optic Neuritis	Bladder Dysfunction	Diplopic Ophthalmoplgia
Numbness	Tingling of Extremities Weakness of a limb or one side of the body or Weakness of a limb or one side of the body		

Glucose-6-phosphate Dehydrogenase (G6PD) deficiency

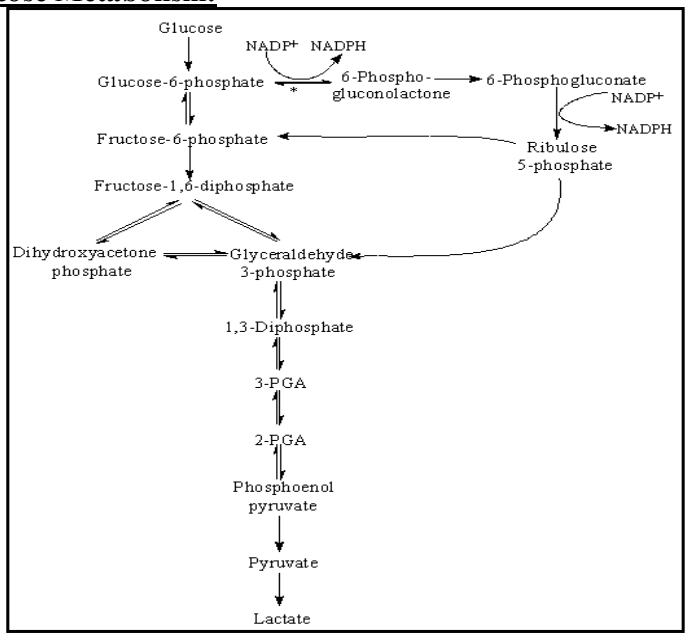
G6PDH: Glucose-6-Phosphate Dehydrogenase (G6PD) is the most common human enzyme which is critical to human in oxidation/reduction reaction.

Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency is an X-linked recessive hereditary disease featuring non-immune hemolytic anemia in response to a number of causes. Its classic association to consumption of fava beans has led to the alternative name fauvism. Also, G-6-PD deficiency is an inheritable, X-linked recessive disorder whose primary effect is the reduction of the enzyme G-6-PD in red blood cells.

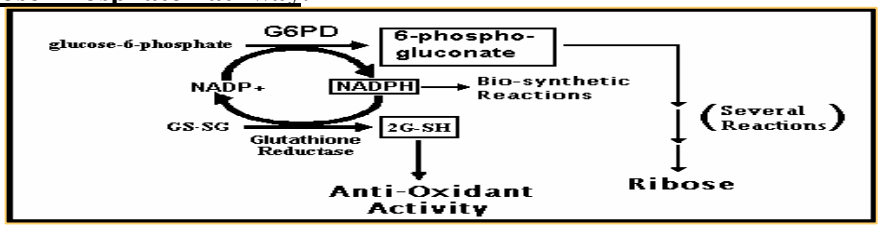
Biochemical Characteristics of G6PD:

G6PD is an enzyme in the pentose phosphate pathway, a metabolic pathway that supplies energy to a number of cells and maintains the level of the co-enzyme Nicotinamide adenine Dinucleotide phosphate (NADPH).

Pathways of Glucose Metabolism:



The Pentose Phosphate Pathway:



The Pentose Phosphate Pathway. Note the importance of G6PD in the production of reduced G-SH, ribose, and NADPH.
NADP+ = Nicotinamide adenine Dinucleotide phosphate
NADPH = reduced Nicotinamide adenine Dinucleotide phosphate
GS-SG = oxidized glutathione
G-SH = reduced glutathione

Diagnosis and Differential Diagnosis:

Blood test:
- By measuring the G6PD enzyme activity between episodes.
- Or by measuring Bilirubin during an episode.

Differential Diagnosis:

A specific diagnosis done by made by measuring of the amount of G6PD enzyme activity in red blood cells. to tell whether the amount of G6PD is abnormally low. In some cases, a blood test called protein electrophoresis may need to be done to confirm the diagnosis.

Others:

Complete Blood Count
Normochromic Normocytic Anemia
Reticulocyte Index >3% (Reticulocytosis)
Liver Function Tests
Serum Bilirubin Elevated
Urinalysis
Hemoglobinuria.

Special Tests:

Elevated Bilirubin Levels
Low Serum Haptoglobin
Hemoglobinuria
Elevated Absolute Reticulocyte Count
Low Red Blood Cell Count And Hemoglobinuria
Heinz Bodies Present On Examination Of The Peripheral Blood Smear Using Stains
Methylene Blue Test
Methemoglobin Reduction Test.