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CLINICAL BIOCHEMISTRY

13.1 INTRODUCTION

Clinical Biochemistry deals with the study of biochemical events or parameters in the body.



OBJECTIVES

After reading this lesson, you will be able to:

- describe clinically important enzymes
- explain liver and renal functional test
- explain specimen collection & other pre-analytical variables.
- describe general laboratory techniques, procedures & safety measure

13.2 CLINICALLY IMPORTANT ENZYMES

Certain enzymes, proenzymes, and their substrates are present at all times in the circulation of normal individuals and perform a physiologic function in the blood. Examples of these **functional plasma enzymes** include lipoprotein lipase, pseudocholesterase, and the proenzymes of blood coagulation and blood clot dissolution. The majority of these enzymes are synthesized in and secreted by the liver.

Enzymes	Principle Sources of Enzymes in Blood	Clinical Applications
Alanine aminotransferase	Liver	Hepatic parenchymal diseases

Alkaline phosphatase	Liver, bone, intestinal mucosa, placenta	Bone diseases, hepatobiliary diseases
Amylase	Salivary glands, pancreas	Pancreatic diseases
Aspartate aminotransferase	Liver, skeletal muscle, heart erythrocytes	Hepatic parenchymal disease, muscle disease
Cholinesterase	Liver	Organophosphorus insecticide, hepatic parenchymal disease
Creatine kinase	Skeletal muscle, heart	Muscle diseases
γ -glutamyl transferase	Liver	Hepatobiliary diseases, marker of alcohol abuse.
Lactate dehydrogenase	Heart, liver, skeletal muscle, erythrocytes, platelets, lymph nodes	Hemolysis, hepatic parenchymal diseases, tumor marker
Lipase	Pancreas	Pancreatic diseases
5'-nucleotidase	Liver	Hepatobiliary diseases
Trypsin (Serine protease)	Pancreas	Pancreatic diseases



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Alanine aminotransferase (ALT)

Clinical applications of ALT assays are mainly used for the evaluation of hepatic disorders. Higher elevations are found in hepatocellular disorders than in extrahepatic or intrahepatic obstructive disorders. In acute inflammatory conditions of the liver, ALT elevations are frequently higher than those of AST and tend to remain longer half-life of ALT in serum (16 hours and 24 hours, respectively). ALT levels have historically been compared with levels of AST to help determine the source of an elevated AST level and to detect liver involvement with myocardial injury.

Normal values of ALT:

Male: $<45 \text{ U/L} = <0.77 \mu\text{kat/L}$

Female: $<34 \text{ U/L} = <0.58 \mu\text{kat/L}$



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Aspartate aminotransferase (AST)

The clinical use of AST is limited mainly to the evaluation of hepatocellular disorders and skeletal muscle involvement. In Acute myocardial infarction (AMI), AST levels begin to rise within 6-8 hours, peak at 24 hours, and generally return to normal within 5 days. However, because of the wide tissue distribution, AST levels are not useful in the diagnosis of AMI.

AST elevations are frequently seen in pulmonary embolism. Following congestive heart failure, AST levels also may be increased, probably reflecting liver involvement as a result of inadequate blood supply to that organ. AST levels are highest in acute hepatocellular disorders. Skeletal muscle disorders, such as the muscular dystrophies, and inflammatory conditions also cause increases in AST levels.

Normal values of AST:

Male: $<35\text{U/L} = <0.60\ \mu\text{kat/L}$

Female: $<31\ \text{U/L} = <0.53\ \mu\text{kat/L}$

 α -Amylase

It is an enzyme of the hydrolyase class that catalyzes the hydrolysis of 1,4- α -glycosidic linkages in polysaccharides. AMYs are calcium metalloenzymes, with the calcium absolutely required for functional integrity. AMYs normally occurring in human plasma are small molecules with molecular weights varying from 54 to 62 kDa. The enzyme is thus small enough to pass the glomeruli of the kidneys and AMY is the only plasma enzyme physiologically found in urine. The AMY activity present in normal serum and urine is of pancreatic (P-AMY) and salivary gland (S-AMY) origin. If the plasma amylase activity fails to fall after an attack of acute pancreatitis there may be leakage of pancreatic fluid into the lesser sac (a pancreatic pseudocyst). Urinary amylase levels are high, differentiating it from macroamylasaemia. This is one of the few indications for estimating urinary amylase activity, which is inappropriately low relative to the plasma activity if there is glomerular impairment or macroamylasaemia.

Normal values of amylase: $28\text{-}100\ \text{U/L} = 0.48\text{-}1.7\ \mu\text{kat/L}$

Lipase

It is a single-chain glycoprotein with molecular weight of 48 kDa. For full catalytic activity and greatest specificity the presence of bile salts and a cofactor called colipase, which is secreted by the pancreas, is required. LPS is a small molecule and is filtered through the glomerulus. It is totally reabsorbed by the

renal tubules, and it is not normally detected in urine. Plasma lipase levels are elevated in acute pancreatitis and carcinoma of the pancreas.

Normal values: 40-200 U/L

Gamma-glutamyl-transferase

It catalyzes the transfer of the γ -glutamyl group from peptides and compounds that contain it to an acceptor. Gamma-glutamyl transferase occurs mainly in the cells of liver, kidneys, pancreas and prostate. Plasma GGT activity is higher in men than in women.

Causes of raised plasma GGT activity

- Induction of enzyme synthesis, without cell damage, by drugs or alcohol.
- Hepatocellular damage, such as that due to infectious hepatitis: A patient should never be labeled an alcoholic because of a high plasma GGT activity alone.

Normal values for GGT

Male: <55 U/L = <0.94 μ kat/L

Female: <38 U/L = <0.65 μ kat/L

Lactate Dehydrogenase (LD)

It catalyses the reversible interconversion of lactate and pyruvate. The enzyme is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes: measurement of plasma total LD activity is therefore a non-specific marker of cell damage.

It is increased in plasma in Myocardial Injury, acute leukemias, generalized carcinomatosis and in acute hepatitis. Estimation of isoenzymes is more useful in clinical diagnosis between hepatic disease and M.I.

Normal range of total LDH: 180-360 U/L = 3.1-6.1 μ kat/L.

13.3 LIVER FUNCTION TESTS

Definition

Liver function tests, or LFTs, include tests that are routinely measured in all clinical laboratories. LFTs include bilirubin, a compound formed by the breakdown of hemoglobin; ammonia, a breakdown product of protein that is



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normally converted into urea by the liver before being excreted by the kidneys; proteins that are made by the liver including total protein, albumin, prothrombin, and fibrinogen; cholesterol and triglycerides, which are made and excreted via the liver; and the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH).

Other liver function tests include serological tests (to demonstrate antibodies) and DNA tests for hepatitis and other viruses; and tests for antimitochondrial and smooth muscle antibodies, transthyretin (prealbumin), protein electrophoresis, bile acids, alpha-fetoprotein, and a constellation of other enzymes that help differentiate necrotic (characterized by death of tissues) versus obstructive liver disease.

The hepatic function panel evaluates:

Alanine aminotransferase (ALT)**Alkaline phosphatase (ALP)****Aspartate aminotransferase (AST)**

Total bilirubin and direct bilirubin. Bilirubin is a byproduct of the normal breakdown of red blood cells. It usually passes through the liver and is excreted from the body. But if that doesn't happen due to a liver disease, bilirubin levels in the blood can rise and the skin can take on the yellow discoloration known as jaundice. Tests for bilirubin may be total (measuring the level of all of the bilirubin in the blood) or direct (measuring only bilirubin that has been processed by the liver and attached to other chemicals).

Albumin and total protein. Protein is needed to build and maintain muscles, bones, blood, and organ tissue. Sometimes when there's a problem with the liver, it can't make proteins as well, so protein levels decrease. Liver function tests measure albumin specifically (the major blood protein produced by the liver), as well as the total amount of all proteins in the blood.

Normal results

Reference ranges vary from laboratory to laboratory and also depend upon the method used. However, normal values are generally framed by the ranges shown below. Values for enzymes are based upon measurement at 37°C.

ALT: 5–35 IU/L. (Values for the elderly may be slightly higher, and values also may be higher in men and in African-Americans).

AST: 0–35 IU/L

ALP: 30–120 IU/LALP is higher in children, older adults and pregnant females

GGT: males 2–30 U/L; females 1–24 U/L

LDH: 12–60 years: 100–190 U/L

Bilirubin: (Adult, elderly, and child)

Total bilirubin: 0.1–1.0 mg/dL

Indirect bilirubin: 0.2–0.8 mg/dL

Direct bilirubin: 0.0–0.3 mg/dL. (Newborn)

Note: Critical values for adult: greater than 1.2 mg/dL

Critical values for newborn (requiring immediate treatment): greater than 15 mg/dL

Ammonia: 10–70 micrograms per dL (heparinized plasma). Normal values for this test vary widely, depending upon the age of the patient and the type of specimen.

Albumin: 3.2–5.4 g/L

13.4 RENAL FUNCTION TEST

Definition

Kidney function tests are a collective term for a variety of individual tests and procedures that can be done to evaluate how well the kidneys are functioning. A doctor who orders kidney function tests and uses the results to assess the functioning of the kidneys is called a nephrologist.

Laboratory tests

There are a number of urine tests that can be used to assess kidney function. A simple, inexpensive screening test a routine urinalysis is often the first test conducted if kidney problems are suspected. A small, randomly collected urine sample is examined physically for things like color, odor, appearance, and concentration (specific gravity); chemically, for substances such a protein, glucose, and pH (acidity/alkalinity); and microscopically for the presence of cellular elements (red blood cells [RBCs], white blood cells [WBCs], and epithelial cells), bacteria, crystals, and casts (structures formed by the deposit of protein, cells, and other substances in the kidneys's tubules). If results indicate a possibility of disease or impaired kidney function, one or more of the following additional tests is usually performed to pinpoint the cause and the level of decline in kidney function.



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**Notes****Creatinine clearance test**

This test evaluates how efficiently the kidneys clear a substance called creatinine from the blood. Creatinine, a waste product of muscle energy metabolism, is produced at a constant rate that is proportional to the individual's muscle mass. Because the body does not recycle it, all creatinine filtered by the kidneys in a given amount of time is excreted in the urine, making creatinine clearance a very specific measurement of kidney function. The test is performed on a timed urine specimen – a cumulative sample collected over a two to 24-hour period. Determination of the blood creatinine level is also required to calculate the urine clearance.

Urea clearance test

Urea is a waste product that is created by protein metabolism and excreted in the urine. The urea clearance test requires a blood sample to measure the amount of urea in the bloodstream and two urine specimens, collected one hour apart, to determine the amount of urea that is filtered, or cleared, by the kidneys into the urine.

Urine osmolality test

Urine osmolality is a measurement of the number of dissolved particles in urine. It is a more precise measurement than specific gravity for evaluating the ability of the kidneys to concentrate or dilute the urine. Kidneys that are functioning normally will excrete more water into the urine as fluid intake is increased, diluting the urine. If fluid intake is decreased, the kidneys excrete less water and the urine becomes more concentrated. The test may be done on a urine sample collected first thing in the morning, on multiple timed samples, or on a cumulative sample collected over a 24-hour period. The patient will typically be prescribed a high-protein diet for several days before the test and be asked to drink no fluids the night before the test.

Urine protein test

Healthy kidneys filter all proteins from the bloodstream and then reabsorb them, allowing no protein, or only slight amounts of protein, into the urine. The persistent presence of significant amounts of protein in the urine, then, is an important indicator of kidney disease. A positive screening test for protein (included in a routine urinalysis) on a random urine sample is usually followed up with a test on a 24-hour urine sample that more precisely measures the quantity of protein.

There are also several blood tests that can aid in evaluating kidney function. These include:

Blood urea nitrogen test (BUN)

Urea is a byproduct of protein metabolism. Formed in the liver, this waste product is then filtered from the blood and excreted in the urine by the kidneys. The BUN test measures the amount of nitrogen contained in the urea. High BUN levels can indicate kidney dysfunction, but because BUN is also affected by protein intake and liver function, the test is usually done together with a blood creatinine, a more specific indicator of kidney function.

Creatinine test

This test measures blood levels of creatinine, a by-product of muscle energy metabolism that, similar to urea, is filtered from the blood by the kidneys and excreted into the urine. Production of creatinine depends on a person's muscle mass, which usually fluctuates very little. With normal kidney function, then, the amount of creatinine in the blood remains relatively constant and normal. For this reason, and because creatinine is affected very little by liver function, an elevated blood creatinine level is a more sensitive indicator of impaired kidney function than the BUN.

Other blood tests

Measurement of the blood levels of other elements regulated in part by the kidneys can also be useful in evaluating kidney function. These include sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus, protein, uric acid, and glucose.

Results

Normal values for many tests are determined by the patient's age and gender. Reference values can also vary by laboratory, but are generally within the following ranges:

Urine tests

Creatinine clearance. For a 24-hour urine collection, normal results are 90 mL/min–139 mL/min for adult males younger than 40, and 80–125 mL/min for adult females younger than 40. For people over 40, values decrease by 6.5 mL/min for each decade of life.

Urine osmolality. With restricted fluid intake (concentration testing), osmolality should be greater than 800 mOsm/kg of water. With increased fluid intake (dilution testing), osmolality should be less than 100 mOsm/kg in at least one of the specimens collected. A 24-hour urine osmolality should average 300–900 mosm/kg. A random urine osmolality should average 500–800 mOsm/kg.



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Urine protein. A 24-hour urine collection should contain not more than 150 mg of protein.

Urine sodium. A 24-hour urine sodium should be within 75–200 mmol/day.

Blood tests

Blood urea nitrogen (BUN) should average 8–20 mg/dL.

Creatinine should be 0.8–1.2 mg/dL for males, and 0.6–0.9 mg/dL for females.

Uric acid levels for males should be 3.5–7.2 mg/dL and for females 2.6–6.0 mg/dL.

Low clearance values for creatinine indicate a diminished ability of the kidneys to filter waste products from the blood and excrete them in the urine. As clearance levels decrease, blood levels of creatinine, urea, and uric acid increase. Because it can be affected by other factors, an elevated BUN, alone, is suggestive, but not diagnostic for kidney dysfunction.

The inability of the kidneys to concentrate the urine in response to restricted fluid intake, or to dilute the urine in response to increased fluid intake during osmolality testing, may indicate decreased kidney function. Because the kidneys normally excrete almost no protein in the urine, its persistent presence, in amounts that exceed the normal 24-hour urine value, usually indicates some type of kidney disease.

13.5 COLLECTION OF SPECIMENS**Collection of Blood sample**

Blood and separated serum are the most common specimens taken to investigate outbreaks of communicable disease. Venous blood can be used for isolation and identification of the pathogen in culture.

Venous blood samples**Materials for collection**

- Skin disinfection: 70% alcohol (isopropyl alcohol, ethanol) or 10% povidone iodine, swabs, gauze pads, band aid
- Disposable latex or vinyl gloves
- Tourniquet, Vacutainer, Monovette, or similar vacuum blood collection devices, or disposable syringes and needles
- Vacutainer or sterile screw-cap tubes (or cryotubes if indicated), blood culture bottles (50ml for adults, 25ml for children) with appropriate media
- Labels and indelible marker pen.

Method of collection

- Place a tourniquet above the venepuncture site.
- Palpate and locate the vein. It is critical to disinfect the venepuncture site meticulously with 10% povidone iodine or 70% isopropyl alcohol by swabbing the skin concentrically from the centre of the venepuncture site outwards. Let the disinfectant evaporate. Do not repalpate the vein again. Perform venepuncture.
- If withdrawing with conventional disposable syringes, withdraw 5-10 ml of whole blood from adults, 2-5ml from children and 0.5-2ml for infants.
- If withdrawing with vacuum systems, withdraw the desired amount of blood directly into each transport tube and culture bottle.
- Remove the tourniquet. Apply pressure to site until bleeding stops, and apply sticking plaster (if desired).
- Using aseptic technique, transfer the specimen to relevant cap transport tubes and culture bottles. Secure caps tightly.
- Label the tube, including the unique patient identification number, using indelible marker pen.
- Discard the sharps into disposal container without recapping.
- Complete the case investigation and the laboratory request forms using the same identification number.

Handling and transport

- Blood culture bottles and blood sample tubes should be transported upright and secured in a screw cap container or in a rack in a transport box.
- Cushion or suspend bottles during transport over rough terrain to prevent lysis of red cells. They should have enough absorbent paper around them to soak up all the liquid in case of a spill.
- If the specimen will reach the laboratory within 24 hours, most bacterial pathogens can be recovered from blood cultures transported at ambient temperature.

Urine specimen collection**Materials for collection**

- Sterile plastic cup with lid (50 ml or more)
- Clean, screw-top specimen transport containers (“universal” containers are often used)
- Gauze pads
- Soap and clean water (or normal saline) if possible.



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Method of collection

- Give the patient clear instructions to pass urine for a few seconds, and then to hold the cup in the urine stream for a few seconds to catch a midstream urine sample. This should decrease the risk of contamination from organisms living in the urethra.
- To decrease the risk of contamination from skin organisms, the patient should be directed to avoid touching the inside or rim of the plastic cup with the skin of the hands, legs or external genitalia. Tighten the cap firmly when finished.
- For hospitalized or debilitated patients, it may be necessary to wash the external genitalia with soapy water to reduce the risk of contamination.
- Urine collection bags may be necessary for infants. If used, transfer urine from the urine bag to specimen containers as soon as possible to prevent contamination with skin bacteria. Use a disposable transfer pipette to transfer the urine and label it.

Handling and transport

- Transport to the laboratory within 2–3 hours of collection. If this is not possible, do not freeze but keep the specimen refrigerated at 4–8°C. Keeping the specimen refrigerated will decrease the risk of overgrowth of contaminating organisms.
- Ensure that transport containers are leak-proof and tightly sealed.

13.6 GENERAL LABORATORY TECHNIQUES

Laboratory services are an integral part of disease diagnosis, treatment, monitoring response to treatment, disease surveillance programmes and clinical research. Essential Health Technology as an important ingredient of Essential Clinical Services. Use of diagnostic techniques aid early diagnosis enabling appropriate and prompt intervention thereby reducing overall disease burden and promoting health. All laboratories are not equipped with facilities for carrying out complex investigations. The structure and function of a clinical laboratory varies according to the level of health care facility. Peripheral laboratories carry out simple tests like urine analysis and haemoglobin estimation whereas higher centers are equipped with sophisticated technology and trained manpower to carry out complex investigations. Establishing a network between peripheral and higher laboratories allows collection of specimen at periphery and their storage and transport for testing at higher centers and communicating report to the peripheral center efficiently without actually having to transfer the patient. In the event of patient transfer, the higher centers do not need to repeat

investigations carried out at the peripheral health center, thereby saving crucial time as well as cost and providing continuity in patient care. Networking between laboratories is also essential in disease surveillance programmes and outbreak investigations in order to obtain quick and reliable results.

Scope

Good Clinical Laboratory Practices should be used by all laboratories where tests are done on biological specimens for diagnosis, patient care, disease control and research such as:

- Microbiology & Serology
- Hematology & Blood Banking
- Molecular Biology and Molecular Pathology
- Clinical Pathology
- Clinical Biochemistry
- Immunology (Immunochemistry and Immunobiochemistry)
- Histopathology/Pathology and Cytology

Equipment

- Each laboratory should prepare an exhaustive list of equipment and consumables required and available for general functioning of the laboratory and specialized equipment for special tests.
- Laboratory equipment should be of adequate capacity to meet work load requirement.
- Equipment should be suitably located in the laboratory so as to allow accessibility and sequential utilization thus minimizing the need for frequent movement of specimens or reagents.
- All equipment should be in good working condition at all times. Periodic inspection, cleaning, maintenance of equipment should be done. An equipment log book should be maintained for all major equipment. Laboratories should maintain necessary instructions for operation and maintenance of equipment in the form of Standard Operating Procedures (SOPs). A copy of SOP should be readily available.
- Maintenance contracts including warranty cards, telephone numbers of staff to be contacted in case of equipment malfunction should be kept safely. User manual should be available readily for reference. The staff should be aware of trouble shooting measures to be adopted for preventing equipment

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malfunction. A format of the equipment log book provided in Annexure 1 can be used.

- New equipment should be calibrated and validated before routine use. AMR (Analytical Measurement range) should be verified, manufacturer can be consulted for verification and selection of range.
- Periodic performance check/calibration check for all equipment should be done using reference standard/reference material. The frequency of performance check should be based on the day-to-day performance of the equipment.
- Equipment performance should be verified from Internal Quality Control results and External Quality Assessment results. Outlier parameter trend analysis record should be maintained in respect of its effect on the equipment.
- All analytical equipment should be calibrated and calibration certificate provided by equipment company. Non-analytical equipment such as pipette, thermometer, weighing balance and centrifuge should be calibrated by accredited calibration laboratory or done in-house with traceability to National Physical Laboratory (NPL). For in-house calibration, laboratories should use :
 - Calibrated tachometer - for centrifuge
 - Calibrated digital temperature sensor - for checking temperature of refrigerator, incubator etc.
 - Calibrated glass thermometer- for temperature checking of oven, water bath etc.
 - Calibrated weights - for balance
 - National Institute of Science and Technology (NIST) buffer – for pH meter. Standard buffer solutions bought from reputed manufacturers with certifiable traceability can be used as alternative.

Standard Operating Procedure (SOP)

- SOP is a document, which contains detailed, written instructions describing the stepwise process and technique of performing a test or procedure in the laboratory.
- SOP helps to ensure uniformity, consistency and control over the processes carried out. It ensures that the procedures are done in exactly the same way each time irrespective of the operator.
- SOP should contain information on who can perform the test, their qualification and training, how to carry out the test including pre-analytical,

analytical and post-analytical stages of test/procedure, laboratory conditions required for the test/ procedure, routine care and maintenance of equipment, precautions and safety instructions, trouble shooting measures, waste disposal and linkage with reference laboratories.

- SOP should be simple and written in an easy to understand language.
- The procedure described in the SOP must be followed exactly by all staff members to ensure high quality results.
- It should be titled along with version number, dated and signed by an authorized person and updated regularly.
- It is important for the SOP document to be readily available in the working area and is therefore also referred to as '**laboratory bench work manual**'.
- SOPs are **controlled documents** and can be changed only with approval of the laboratory quality manager and/or Head of the laboratory.

Safety in Laboratories

Personnel working in laboratories may be exposed to risks from various chemicals, infectious materials, fire hazard, gas leak etc. The environment is also at risk of being contaminated by hazardous materials used and wastes generated in the laboratory. Safety in laboratories therefore includes **protection of both the staff and the environment** from hazardous materials.

General Safety Measures

- Documentation of Laboratory Safety Policies and Procedures.
- All laboratory personnel should be aware about the laboratory safety policies and procedures and follow these at all times.
- List of hazardous materials used in the laboratory should be prepared. All hazardous materials should be accounted for on a continuous basis.
- Laboratory personnel should follow safe hygienic practices which include hand washing, wearing protective clothing, gloves, eye protection etc.
- Eye wash facility should be available as “stand-alone” facility or attached to sink. Portable, sealed, refillable bottles should also be available.
- Biohazard symbol should be used on all container/equipment containing biohazardous material.
- Laboratories should ensure proper preservation and security of specimens.

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- Destruction/disposal of hazardous material should be authorized, supervised and handled according to standard procedures.
- Laboratory personnel should be thoroughly trained in managing fire, and nonfire emergencies such as large spillage, gas leakage etc.
- Adequate fire extinguishers should be readily available in the laboratory
- Periodic checking of all safety equipment and accessories should be ensured.
- Accident/incident/injuries record of laboratory personnel should be maintained and reported to the designated authority. The report should include description of the event, factors contributing to the event and information on first aid or other health care provided. This information can be analyzed periodically towards effectively controlling and preventing future events. The records should be checked periodically by the laboratory safety officer even in the absence of fresh entries.



INTEXT QUESTIONS 13.1

1. Fill in the blanks:

1. Clinical applications of ALT assays are mainly used for the evaluation of disorders.
2. The normal value of a-amylase in blood is
3. Bilirubin is a byproduct of the normal breakdown of
4. Urea is a waste product that is created by metabolism.
5. is a by-product of muscle energy metabolism that is similar to urea and is filtered from the blood by the kidneys.



TERMINAL QUESTIONS

1. Name some clinically important enzymes?
2. What is the normal value of ALT and AST?
3. What is Bilirubin?
4. What is Urea clearance test?
5. What is the normal range of creatinine in blood?



ANSWERS TO INTEXT QUESTIONS

13.1

1. hepatic
2. 28-100U/L
3. red blood cells
4. protein
5. creatinine



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