Lab 4: "Liver function tests"

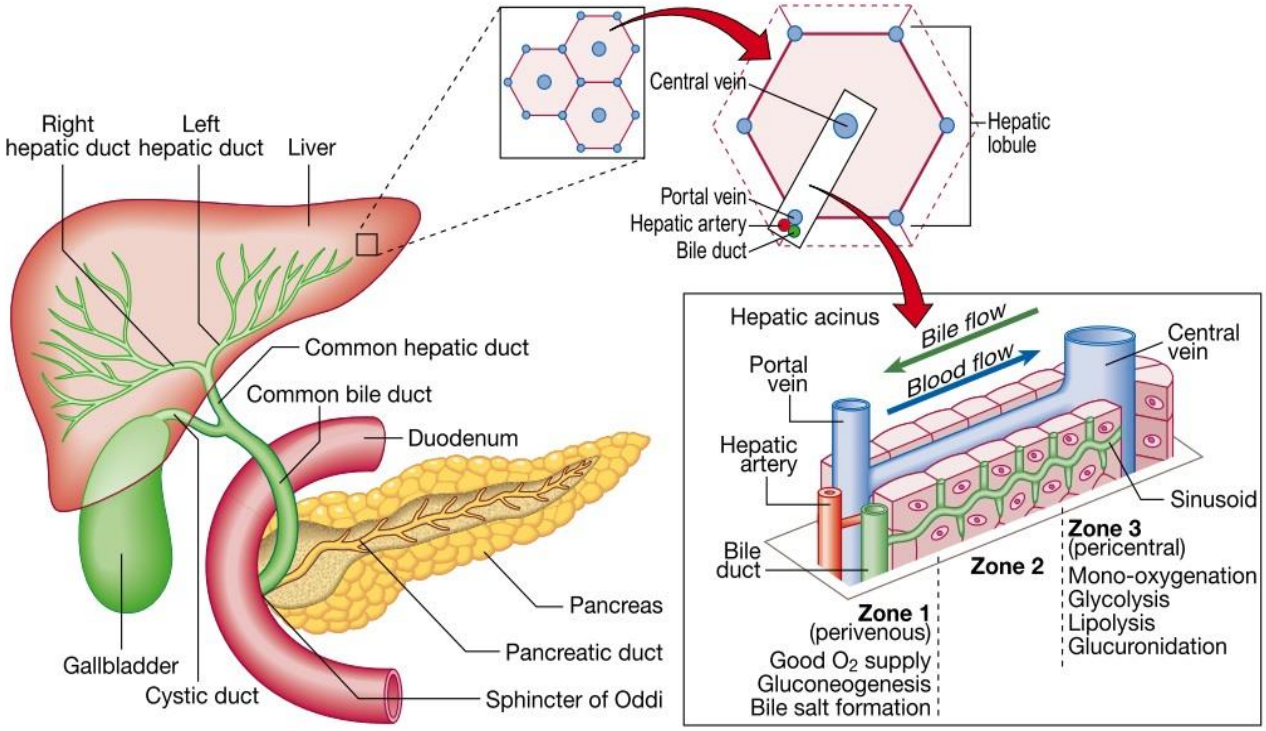
Functions of the liver:

The liver is the largest internal organ of the human body. It is a functionally complex organ that plays a critical biochemical role in the metabolism, digestion, detoxification, and elimination of substances from the body. The liver is involved in a number of excretory, synthetic, and metabolic functions, all of which are essential to life.

Note: The liver has a very large functional reserve. Deficiencies in synthetic function can be detected only if liver disease is extensive.

Structure of the liver:

The main blood supply to the liver is via the portal vein. The liver is made up of hexagonal lobules of cells. Rows of hepatocytes radiate from the central hepatic vein and are separated by sinusoidal spaces, along the walls of which are interspersed hepatic macrophages, the Kupffer cells. At the corners of each lobule are the portal tracts that contain branches of the hepatic artery, the portal vein and bile ducts. Blood flows from the portal tracts towards the central hepatic vein. As shown in figure.



Liver function tests:

Most laboratories perform a standard group of tests (table 1) which do not assess genuine liver function but are useful for:

1. *Detecting the presence of liver disease.*

2. *Placing the liver disease in the appropriate broad diagnostic category. This then allows the selection of further, more expensive and time-consuming investigations such as ultrasound, CT scanning, magnetic resonance spectroscopy, endoscopy and liver biopsy.*

3. *Following the progress of liver disease.*

Table 1 Routine liver function tests (examples of widely performed groups of serum measurements).

Standard group of tests	Property being assessed
Serum albumin, PT	Protein synthesis
Serum bilirubin (total)	Hepatic anion transport
Serum enzyme activities:	
ALT, AST	Hepatocellular integrity
ALP, GGT	Presence of cholestasis

Bilirubin:

Bilirubin production and metabolism:

Production

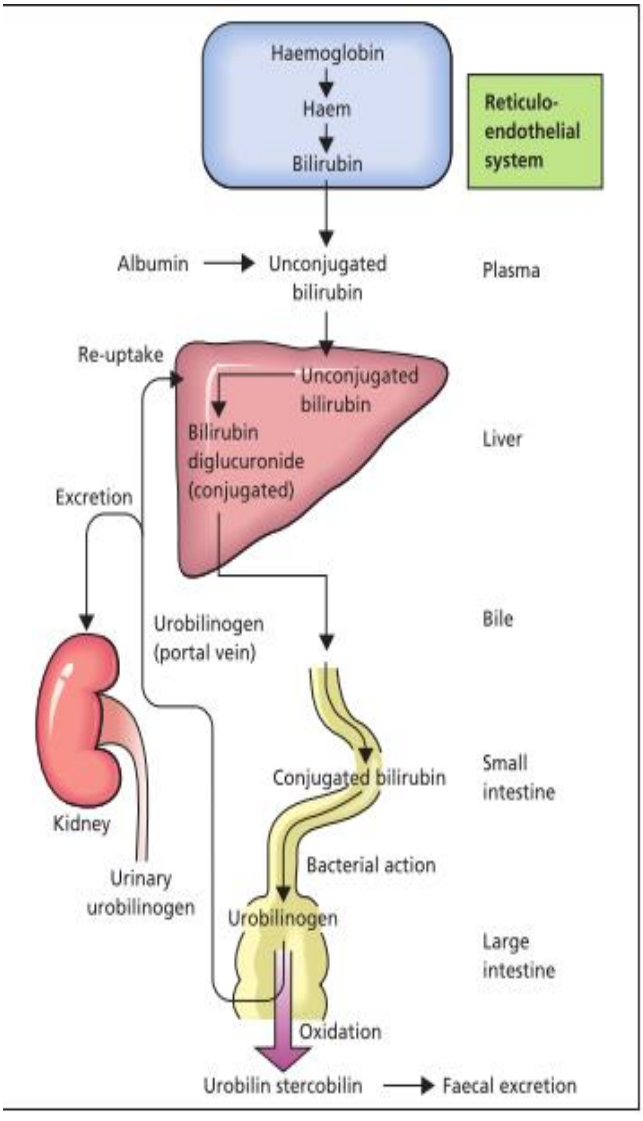
The body usually produces about 300 mg of bilirubin per day as a breakdown product of haem. About 80% arises from red cells, with the remainder coming from red cell precursors destroyed in the bone marrow, and from other haem proteins such as myoglobin and the cytochromes. Iron is removed from the haem molecule, and the porphyrin ring is opened to form bilirubin.

Transport in plasma and hepatic uptake

Bilirubin is insoluble in water and is carried in plasma bound to albumin, and is thus not filtered at the glomerulus unless there is glomerular proteinuria.
On reaching the liver, the bilirubin is taken into the hepatocyte by a specific carrier mechanism.

Conjugation of bilirubin and secretion into bile

In the endoplasmic reticulum of the hepatocyte, the enzyme bilirubin UDP-glucuronyltransferase conjugates bilirubin with glucuronic acid to produce bilirubin glucuronides which are water soluble and readily transported into bile.



Further metabolism of bilirubin in the gut

Bilirubin glucuronides cannot be reabsorbed from the gut and are degraded by bacterial action, mainly in the colon, to a mixture of colourless, water-soluble compounds collectively termed urobilinogen. These compounds oxidise to brown compounds known as urobilins and stercobilins and are excreted in the faeces. A small percentage of urobilinogen is absorbed and carried to the liver in the portal blood supply, that is, it undergoes an enterohepatic circulation. Most of this urobilinogen is cleared by the liver, but a proportion escapes clearance and is filtered at the kidney and appears in the urine, where it can be detected using point of care urine dipsticks.

Jaundice:

Jaundice is a yellow discoloration of the skin or sclera. This is due to the presence of bilirubin in the plasma and is not usually detectable until the concentration is greater than about 50 µmol/L.
Note: There are three main reasons why bilirubin levels in the blood may rise:

- *An increased rate of bilirubin production exceeds normal excretory capacity of the liver* ***(pre-hepatic jaundice)****. As in haemolytic anemia.*

- *The normal load of bilirubin cannot be conjugated and/or excreted by damaged liver cells* ***(hepatic jaundice)****. As in Crigler –Najjar syndrome, or in hepatocellular damage caused by infective agents.*

- *The biliary flow is obstructed, so that conjugated bilirubin cannot be excreted into the intestine and is regurgitated into the systemic circulation* ***(post-hepatic jaundice)****. As in gallstones.*

Newborn jaundice:

- *Red cell destruction, together with immature hepatic processing of bilirubin, may cause a high plasma level of unconjugated bilirubin in the newborn infant; so called* ***physiological jaundice*** *is common. Normal full term babies may show jaundice between days 2 and 8 of life.*
- *Jaundice during the first 24 hr. of life is more likely to be* ***pathological*** *than physiological. Like in maternofetal Rh or ABO blood group incompatibility, this occur in infants born from multiparous mothers or in intrauterine infections that affect the liver, such as syphilis, and toxoplasmosis.*
- *Sometimes, plasma unconjugated bilirubin levels may increase so much that they exceed the protein-binding capacity. The lipid-soluble, unbound bilirubin cross blood brain barrier and damages brain cells (kernicterus).*

Haemolytic jaundice:

There are many causes of haemolysis, including sickle cell anaemia, thalassaemia and spherocytosis, and it can also be drug or autoimmune induced. In adults, unconjugated hyperbilirubinaemia is usually mild because of the large reserve of hepatic secretory capacity. The plasma bilirubin concentration is usually less than 70 µmol/L. Erythrocytes contain high amounts of AST and lactate dehydrogenase (LDH_1 and LDH_2). Blood reticulocytes may be raised, with abnormal blood film and a low plasma haptoglobin concentration.

(Inherited) hyperbilirubinaemias:

Unconjugated hyperbilirubinaemia:	***Conjugated hyperbilirubinaemia***
Gilbert's syndrome	*Dubin–Johnson syndrome*
Crigler–Najjar syndrome	*Rotor's syndrome*

Notes:

- *Total serum bilirubin level is the sum of the conjugated (direct) and unconjugated (indirect) bilirubin.*
- *Although the terms jaundice and icterus are used interchangeably, the term icterus is most commonly used in the clinical laboratory to refer to a serum or plasma sample with a yellow discoloration due to an elevated bilirubin level.*
- *Unconjugated bilirubin is normally all protein bound and is not water soluble and therefore cannot be excreted in the urine. Patients with unconjugated hyperbilirubinaemia do not have bilirubinuria ('acholuric jaundice') such as Gilbert's syndrome.*
- *Conjugated bilirubin is water soluble and is less strongly protein bound than the unconjugated form, and therefore can be excreted in the urine. Bilirubinuria is always pathological. Dark urine may be an early sign of some forms of hepatobiliary disease.*
- *Urobilinogen, unlike bilirubin, is often detectable in the urine of normal people by testing with commercial strip tests, particularly if the urine, and therefore the urobilinogen, is concentrated.*
- *Urinary urobilinogen concentration is increased in case of severe haemolysis and when liver damage impairs re-excretion of normal amounts of urobilinogen into the bile.*
- *Pale stools suggest biliary obstruction associated with an absence of urinary urobilinogen.*

Estimation of bilirubin:

Principle:

Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. In the presence of dimethyl sulfoxide, total bilirubin reacts with diazotized sulfanilic acid to form azobilirubin. In the absence of dimethyl-sulfoxide, only direct bilirubin reacts with diazotized sulfanilic acid to form azobilirubin.

Specimen: Serum is used for analysis.

Fasting is preferred.

Avoid hemolysis during sample collection.

Interfering factors:

1- Drugs that may increase levels of total bilirubin include:

- *Allopurinol*
- *Morphine*
- *Diuretics*
- *oral contraceptives*
- *rifampicin*

2- Drugs that may decrease levels of total bilirubin:

- *Barbiturates*
- *Caffeine*
- *Penicillin*

3- *Avoid the exposure of the specimen to sunlight or high intensity artificial light at room temperature because this will decrease bilirubin content.*
4- *Blood hemolysis.*
5- *Certain food (e.g. Carrots), may increase yellow hue in serum, it may lead to false results.*

Hepatocellular damage:

ENZYMES: *Liver enzymes play an important role in the assessment of liver function because injury to the liver resulting cytolysis or necrosis will cause the release of enzymes into circulation. Enzymes also play an important role in differentiating hepatocellular (functional) from obstructive (mechanical) liver disease, which is an important clinical distinction because failure to identify an obstruction will result in liver failure if the obstruction is not rapidly treated.*
Note: It is important to note that the diagnosis of disease depends on a combination of patient history, physical examination, laboratory testing, and sometimes radiologic studies and biopsy and therefore abnormalities in liver enzymes alone are not diagnostic in and of themselves.

Aminotransferase measurements:

The two most common aminotransferases measured in the clinical laboratory are:

***AST** (formerly referred to as serum glutamic-oxaloacetic transaminase **[SGOT]**) and **ALT** (formerly referred to as serum glutamic-pyruvic transaminase **[SGPT]**). The aminotransferases are responsible for catalyzing the conversion of aspartate and alanine to oxaloacetate and pyruvate, respectively. In the absence of acute necrosis or ischemia of other organs, these enzymes are most useful in the detection of hepatocellular (functional) damage to the liver.*

ALT is found mainly in the liver (lesser amounts in skeletal muscle and kidney), whereas AST is widely distributed in equal amounts in (the heart, skeletal muscle, and liver), making ALT a more "liver-specific" marker than AST.

Regardless, the serum activity of both transaminases rises rapidly in almost all diseases of the liver and may remain elevated for up to 2–6 weeks. The highest levels of AST and ALT are found in acute conditions such as viral hepatitis, drug- and toxin-induced liver necrosis, and hepatic ischemia. The increase in ALT activity is usually greater than that for AST. Only moderate increases are found in less severe conditions. AST and ALT are found to be normal or only mildly elevated in cases of obstructive liver damage.

Notes:

- *A rise in plasma aminotransferase activities is a sensitive indicator of damage to cytoplasmic and/ or mitochondrial membranes. Plasma enzyme activities rise when the membranes of only very few cells are damaged.*
- *In inflammatory or infective conditions, such as viral hepatitis, the cytoplasmic membrane sustains the main damage; leakage of cytoplasmic contents causes a relatively greater increase in plasma ALT than AST activities. In infiltrative disorders in which there is damage to both mitochondrial and cytoplasmic membranes, there is a proportionally greater increase in plasma AST than ALT activity.*
- *A plasma AST: ALT ratio of > 2 is suggestive but not diagnostic of alcoholic liver disease and a ratio < 1 suggests chronic viral hepatitis or hepatic steatosis.*

Note: AST and ALT are present in other tissues besides the liver, elevations in these enzymes may be a result of other organ dysfunction or failure.

Examples of abnormal findings of AST Increased levels:	*Examples of abnormal findings of ALT Increased levels:*
Liver: • *Acute viral or toxic hepatitis* • *Cirrhosis (may be normal sometimes)* • *Malignant infiltration of the liver (may be normal sometimes)* • *Hepatic steatosis* • *Cholestatic jaundice*	*Liver:* • *Acute viral or toxic hepatitis* • *Cirrhosis (may be normal sometimes)* • *Hepatic steatosis* • *Cholestatic jaundice*
Others: • *Myocardial infarction* • *Skeletal muscle disease* • *Severe haemolytic episodes (of erythrocyte origin)*	*Others:* • *Surgery or extensive trauma and skeletal muscle disease*

Principle of measurement of ALT:

(Colorimetric method)(Serum)

2–Oxoglutarate + L- Alanine $\xrightarrow{\text{ALT}}$ L- Glutamate + Pyruvate

ALT activity is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Interfering factors:

1. Drugs that may cause increased levels: e.g. acetaminophen.
2. Previous intramuscular injections may cause elevated levels.

Principle of estimation of AST activity:

(Colorimetric method)(Serum)

2– Oxoglutarate + L-Aspartate $\xrightarrow{\text{AST}}$ L- Glutamate + Oxaloacetate

AST activity is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

Interfering factors:

1. Exercise. 2. Drugs that increased levels: e.g. erythromycin.

3. Decreased levels could be seen in severe long standing liver disease.

Cholestasis: alkaline phosphatase and γ-glutamyltransferase:

Alkaline phosphatase: is derived from a number of different tissues, including the liver, the osteoblasts in bone and the placenta. Plasma activities rise in cholestatic liver disease because ALP synthesis is increased and the enzyme within the biliary tract is regurgitated into plasma. A raised ALP concentration in the presence of a raised γ-glutamyl transferase (GGT) concentration implies that the ALP is of hepatic origin.

γ-Glutamyl transferase: is a membrane-localized enzyme found in high concentrations in the kidney, liver, pancreas, intestine, and prostate.

GGT is derived from the endoplasmic reticulum of the cells of the hepatobiliary tract. As this reticulum proliferates, for example in response to the prolonged intake of alcohol and of drugs such as phenobarbital and phenytoin, synthesis of the enzyme is induced and plasma GGT activity increases. Therefore, raised plasma activities do not necessarily indicate hepatocellular damage, but may reflect enzyme induction or cholestasis.

***Note: Intrahepatic Cholestasis**: in which bile secretion from the hepatocytes into the canaliculi is impaired, due to different causes like viral hepatitis, or drugs such as chlorpromazine or toxins such as alcohol, or due to autoimmune disease (primary biliary cirrhosis).*

***Extrahepatic Cholestasis**: due to obstruction to the flow of bile through the biliary tract, by different causes like biliary stones, and inflammation of the biliary tract.*

Examples of Increased levels of ALP:
Liver: • *Intrahepatic or extrahepatic cholestasis.* • *Primary or metastatic liver tumor.*
Others: • ***Bone disease*** • ***Physiological,** during the last trimester of pregnancy.* • ***In children**, the total activity increases by about two to five times during the pubertal bone growth spurt.*

Principle of Colorimetric determination of ALP activity:

(Colorimetric method) (Fasting is preferred, Serum is used for this test).

Phenylphosphate $\xrightarrow[\text{pH 10}]{\text{ALP}}$ Phenol + Phosphate

Phenol liberated is measured in the presence of 4-aminoantipyrine and Potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction.

Interfering factors:

1. Recent ingestion of a meal can increase the ALP level.
2. Drugs that may cause elevated levels: e.g. allopurinol, methyldopa.

Acid phosphatase (ACP) *is found in cells of the prostate, liver, erythrocytes, platelets and bone. The main indication for estimation was to help diagnose prostatic carcinoma and to monitor its treatment.*

Hepatic protein synthesis:

The measurement of certain plasma proteins provides an index of the liver's ability to synthesis protein.

Albumin: In chronic hepatocellular damage, there is impaired albumin synthesis with an accompanying fall in serum [albumin]. In acute liver disease, however, there may be little or no reduction in serum [albumin], as the biological half-life of albumin is about 20 days and the fractional clearance rate is therefore low.

Note: There are many other causes of a low plasma albumin concentration that are not due to hepatic disease.

Prothrombin time:

The prothrombin time may be prolonged by cholestasis: fat-soluble vitamin K cannot be absorbed normally if fat absorption is impaired due to intestinal bile salt deficiency. The abnormality is then corrected by parenteral administration of the vitamin. A prolonged prothrombin time may also result from severe impairment of synthetic ability if the liver cell mass is greatly reduced; in such cases it is not corrected by parenteral administration of vitamin K.

Other tests: *Immunoglobulins, Serological tests, and Markers of fibrosis.*