Liver Function Tests

The liver is of vital importance in intermediary metabolism and in the detoxification and elimination of toxic substances. Damage to the organ may not obviously affect its activity since the liver has considerable functional reserve and, as a consequence, simple tests of liver function (e.g. plasma bilirubin and albumin concentrations) are insensitive indicators of liver disease. Tests reflecting liver cell damage (particularly the measurement of the activities of hepatic enzymes in plasma) are often superior in this respect.

Serum bilirubin:

Objectives:

1. Define jaundice and outline its types
2. Differentiate between direct and indirect bilirubin
3. Outline the Patient Preparation, Specimen Collection and Storage, Reference Range for bilirubin analyses
4. Describe the principle of determination of total bilirubin:
5. Calculate the concentration serum bilirubin from reading the absorbance of standard and sample against their blank

Case scenario: A 50-year old women visited the nearby clinic complaining from an eight-day history of loss of appetite, nausea and flue like symptoms. She had noticed that her urine had been dark in color over the past two days. On examination she had tenderness in her right upper quadrant and ecteric sclera. Her physician ordered several laboratory tests and there results came as follows:
Serum total bilirubin= 4.5 mg%
Direct bilirubin = 2.5 mg%
Indirect bilirubin= 2.0 mg%
She was diagnosed with viral hepatitis. This is a case of hepatic jaundice

1. What is jaundice?
2. What are the types of jaundice
3. What is the difference between direct –reacting (conjugated) and indirect reacting (unconjugated jaundice)
4. Is there any basic patient preparations needed before testing serum bilirubin.
5. From which body liquids (specimens) could one analyze bilirubin? How must they be stored to avoid contamination before analysis?
6. What is the principle of determination of total bilirubin:
Bilirubin is derived mainly from the haem moiety of haemoglobin molecules and is liberated when senescent red cells are removed from the circulation by the reticuloendothelial system; the iron in haem is reutilized but the tetrapyrrole ring is degraded to bilirubin.

Unconjugated bilirubin is not water-soluble; it is transported in the blood stream bound to albumin. In the liver it is taken up by hepatocytes where it undergoes conjugation, principally with glucuronic acid. Conjugated bilirubin is water soluble and is secreted into the biliary canaliculi, reaching the small intestine via the duct of the biliary system. In the gut, bilirubin is converted by bacterial action into urobilinogen, a colourless compound. Some urobilinogen is absorbed from the gut into the portal blood. Hepatic uptake of this is incomplete; a small quantity reaches the systemic circulation and is excreted in the urine. Most of the urobilinogen in the gut is oxidized in the colon to a brown pigment, stercobilin, which is excreted in the stool.

The bilirubin normally present in the plasma is mainly unconjugated; since it is protein bound, it is not filtered by the renal glomeruli and, in health, bilirubin is not detectable in the urine.

Hyperbilirubinaemia can be caused by increase production of bilirubin, impaired metabolism, decrease excretion or a combination of these.
Jaundice:
Yellowish discoloration of skin, nail bed and sclera of the eye caused by deposition of bilirubin secondary to increase bilirubin level in the blood.

Types of jaundice:

1. pre-hepatic (hemolytic):
The liver has a capacity to conjugate and excrete over 3000mg of bilirubin per day, whereas the normal production is only 300mg/day. Massive lysis of RBC e.g. (sickle cell anemia, pyruvate kinase deficiency, G6PD, malaria) may produce bilirubin faster than it can conjugated which lead to increase level of bilirubin excreted into the bile.

2. hepatocellular:
Damage to the liver cell e.g. patients with hepatitis or cirrhosis. Unconjugated bilirubin level increase due to decrease conjugation and regurgitation of conjugated bilirubin to the blood because conjugated bilirubin not efficiently excreted.

3. post-hepatic (obstructive):
Due to obstruction of bile duct e.g. hepatic tumor, bile stone. Patient with obstructive jaundice experience gastrointestinal pain, nausea with pale clay color stool. Conjugated bilirubin regurgitates from the liver which increase in its level.

Patient Preparation, Specimen Collection and Storage, Reference Range

1. Patient Preparation
   - AM fasting specimen preferred (avoid lipemia) [for total and direct bilirubin]

2. Specimen collection
   - serum or heparinized plasma (no hemolysis or lipemia) [for total and direct bilirubin]
   - urine: fresh random specimen preferred [for total and direct bilirubin]

3. Specimen storage
   - serum/plasma: protect from exposure to light (bilirubin is photooxidized, causing unconjugated form to react with Diazo reagents as well as conjugated form)
   - stored at low temperature (minimized photooxidation)
   - stable 3 days at 1-6 C, 3 months at -70 C
   - urine: protect from light to avoid oxidation. Stable 1 day at 1-6 C.

Reference range:
   - serum/plasma [for total bilirubin] < 1.5 mg/dl
   - serum/plasma [for direct bilirubin] < 0.2 mg/dl
   - urine (random): negative.
Determination 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.

Procedure:
Total bilirubin:

<table>
<thead>
<tr>
<th>Standard</th>
<th>Blank</th>
<th>Reaction</th>
<th>Blank</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard R4</td>
<td>50 μl</td>
<td>50 μl</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sample</td>
<td>—</td>
<td>—</td>
<td>50 μl</td>
<td>50 μl</td>
</tr>
<tr>
<td>Reagent R1</td>
<td>1 ml</td>
<td>—</td>
<td>1 ml</td>
<td>—</td>
</tr>
<tr>
<td>Working solution</td>
<td>—</td>
<td>1 ml</td>
<td>—</td>
<td>1 ml</td>
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</tbody>
</table>

Wavelength: 555nm (Hg 546)
Cuvette: 1 cm light path
Temperature: 37°C
Zero adjustment: Reagent blank

Mix well and incubate exactly 5 minutes at 37°C.
Read the absorbance of standard and sample against their blank.

Study questions:
Q1 Choose the most appropriate answer
1. Which enzyme is responsible for the conjugation of bilirubin?
   a. β-Glucuronidase
   b. Uridine dihydropate (UDP)-glucuronyl transferase
   c. Bilirubin oxidase
   d. Bilirubin reductase
2. Which condition is caused by deficient secretion of bilirubin into the bile canaliculi?
   a. Gilbert's disease
   b. Neonatal hyperbilirubinemia
   c. Dubin-Johnson syndrome
   d. Crigler-Najjar syndrome
3. Which statement regarding total and direct bilirubin levels is true?
   a. Total; bilirubin level is less sensitive and specific marker of liver disease than the direct level
   b. Direct bilirubin level exceeds 3.5 mg/dL in most cases of hemolytic anemia
   c. Direct bilirubin is normal in cholestatic liver disease
   d. The ratio of direct to total bilirubin exceeds 0.4 in hemolytic anemia.
4. Which statement about colorimetric bilirubin methods is true?
   a. Direct bilirubin must react with diazo reagent under alkaline conditions
   b. Most methods are based upon reaction with diazotized sulfanilic acid
   c. Ascorbic acid can be used to eliminate interference caused by Hgb
   d. The color of the azobilirubin product independent pf pH.