Tikrit University

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Bio Chemistry

Serum Bilirubin

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Serum Bilirubin

Billirubin (BR) is a yellow-orange pigment which gives the serum its characteristics yellow color. At the end of their life span red blood cells are broken down by the reticuloendothelial system, mainly in the spleen .

The released hemoglobin is split into globin, which enters the general protein pool and haem, which is converted to billirubin after removal of iron. The iron is reutilized.

About 80 percent of billirubin is derived from the breakdown of haem within the reticuloendothelial system. Other sources include breakdown of immature red cells in the bone marrow and of compounds chemically related to hemoglobin, such as myoglobin and the cytochromes.

The resulting BR is insoluble in water but soluble in lipid and in the organic solvents, it is carried by albumin and circulates in blood to reach the liver where conjugated with glucuronic acid. The conjugated billirubin is soluble in water and is excreted in the bile but some of it regurgitates into circulation and if exceeds 0.4 mg/100ml then appears in urine.

Conjugated BR reacts with diazo reagent (Diazotized sulphanlic acid) to give a purple color and said to have direct Van Den Berg reaction.

Two forms of bilirubin can be estimated by laboratory tests:

1- Unconjugated bilirubin is formed when heme is released from hemoglobin.

It is carried by proteins to the liver. In the liver, sugars are attached (conjugated) to bilirubin to form conjugated bilirubin.

2- Conjugated bilirubin enters the bile and passes from the liver to the small intestines, where it is further broken down by bacteria andeventually eliminated in the stool. Thus, the breakdown products of bilirubin give stool its characteristic brown color.

Normally, the level of conjugated bilirubin in the blood is very low

❖ Usually, an initial test measures the total bilirubin level (unconjugated plus conjugated bilirubin).

❖ If the total bilirubin level is increased, the laboratory can use a second test to detect water-soluble forms of bilirubin, called "direct" bilirubin.

The direct bilirubin test provides an estimate of the amount of conjugated bilirubin presen

❖ Subtracting the direct bilirubin level from the total bilirubin level helps estimate the "indirect" level of unconjugated bilirubin.

Functions of Bilirubin

1- Acts as uncoupler in Neonates and thus maintain body heat
2- 2- Bile pigments such as Biliverdin naturally possess significant antimutagenic and antioxidant properties The formation and metabolism of bilirubin and its excretion into the intestine

Clinical Significant:

Hyperbilirubinemia : there are two types

a- Unconjugated Hyperbilirubinemia: causes

1- Overproduction
2- Hemolysis (intra and extravascular)
3- Decreased hepatic uptake 4- Decreased bilirubin conjugation (Transferase deficiency)

a- Gilbert's Syndrome

b- Crigler-Najjar Syndrome c-Hepatocellular disease (hepatitis, cirrhosis).

Conjugated Hyper Bilirubinemia : Causes

1- Impaired hepatic excretion
2- Dubin-Johnson syndrome
3- cholestasis 3- Hepatocellular disease (e.g. Viral or drug induced

hepatitis, cirrhosis) 4- Biliary cirrhosis.

**Jaundice

Jaundice appears when the bilirubin level is above 2.5 mg/dl. When the

liver cannot conjugate bilirubin in the newborn, and if the level

increases, this indirect bilirubin can cross the blood-brain barrier and

leads to toxic injury to the brain and called Kernicterus.

Types of jaundice:

1- Pre-hepatic jaundice. The etiology is before the liver, like increased hemolysis of RBC.

2- Hepatic jaundice. Now the causes are in the liver, like hepatitis.

3- Post-hepatic jaundice. The cause is after the liver, like gallstone, cancers, and these are the obstructive type of jaundice

PRINCIPLE:

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and is measured photometrically. Of the two bilirubin fractions in serum –bilirubin-glucuronide and free

bilirubin which is bound to albumin– only the former reacts directly, while free albumin reacts after being displaced from protein by an accelerator. The difference of two measurements total bilirubin (with accelerator) and direct bilirubin (without

accelerator) enables the calculation of indirect bilirubin. The terms «direct» and «indirect» bilirubin refers exclusively to the reaction characteristics in the presence or absence of an accelerator or solubilizer and are only approximate equivalents of the two bilirubin fractions

PROCEDURE:

TOTAL BILIRUBIN

1. Pipette into labelled tubes:

TUBES	Reagent Blank	Sample Blank	Sample	CAL
Distilled water	100 µL	–	–	–
Sample	–	100 µL	100 µL	–
CAL	–	–	–	100 µL
RT	–	1.0 mL	–	–
Working reagent	1.0 mL	–	1.0 mL	1.0 mL

2- Mix thoroughly and let the tubes stand for 2 minutes at room temperature.

3- Read the absorbance (A) of the sample blanks at 540 nm against distilled water.

4- Read the absorbance (A) of the samples at 540 nm against the reagent blank

The color is stable for at least 60 minutes at room temperature

DIRECT BILIRUBIN

1. Pipette into labelled tubes:

TUBES	Reagent Blank	Sample Blank	Sample	CAL
Distilled water	100 µL	–	–	–
Sample	–	100 µL	100 µL	–
CAL	–	–	–	100 µL
RD	–	1.0 mL	–	–
Working reagent	1.0 mL	–	1.0 mL	1.0 mL

2- Mix thoroughly and let the tubes stand for exactly 5 minutes at 37°C.

3- Read the absorbance (A) of the sample blanks at 540 nm against distilled water.

4- Read the absorbance (A) of the samples at 540 nm against the reagent blank.

CALCULATIONS

$$\frac{A_{Sample} - A_{Sample\ blank}}{A_{Cal}} \times C_{Cal} = \text{mg/dL total or direct bilirubin}$$

Samples with concentrations higher than 20 mg/dL should be diluted 1:2 with saline and assayed again. Multiply results by 2. If results are to be expressed as SI units apply:

mg/dL x 17.1 = Mmol/L.