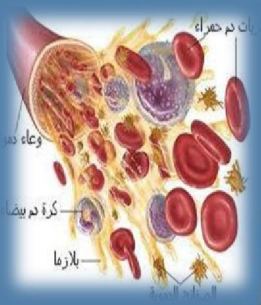
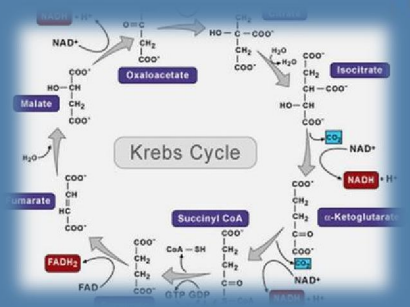
Al-Rasheed University College
Medical Analysis Department
Clinical Chemistry Lab.
Third Stage

كليـــــة الرشيد الجامعـــــــة
قســـــم التحليلات المرضيـــة
مختبر الكيميـــاء السريريـــة
المرحلـــــة الثالثـــــة





Lecture (7)

Liver Function: Estimation of Blood Bilirubin

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Blood Bilirubin Test

Bilirubin is a break down product of hemoglobin. It is transported from the spleen to the liver and excreted into bile. The increase the Bilirubin concentration in plasma causes **Hyperbilirubinemia**.

Bilirubin is made in the body when old red blood cells are broken down. The breakdown of old cells is a normal, healthy process. After circulating in your blood, bilirubin then travels to your liver. In the liver, bilirubin is excreted into the bile duct and stored in your gall bladder. Eventually, the bilirubin is released the small intestine as bile to help digest fats and ultimately excreted with your stool.

☯ Bilirubin Test Types:

Bilirubin attached to sugar is called "direct" or "conjugated" bilirubin, and bilirubin without sugar is called "indirect" or "unconjugated" bilirubin. All the bilirubin in your blood together is called "total" bilirubin.

A bilirubin blood test will get an accurate count of all three bilirubin levels in your blood: ***direct***, ***indirect***, ***and total***.

☯ High levels of bilirubin causes:

- ☯ If bilirubin is not being attached to sugars (conjugated) in the liver and/or is not being adequately removed from the blood, it can mean that there is **damage to your liver**. Testing for bilirubin in the blood is therefore a good test of damage to your liver.
- ☯ Newborn infants often have some **jaundice**, and bilirubin in the blood may be tested several times in the first few days of an infant's life to check that the liver is starting to work properly. Jaundice in a newborn can be very serious if left untreated.

- Other reasons for high bilirubin levels could be that more blood cells are being destroyed than normal. This is called **hemolysis**.

In an adult, high bilirubin may be due to problems with the liver, bile ducts, or gallbladder. Examples include:

- Drug toxicity
- Liver diseases like hepatitis
- Gilbert's disease (a genetic disease affecting some families)
- Cirrhosis (scarring of the liver)
- Biliary stricture (part of the bile duct is too narrow to allow fluid to pass)
- Cancer of the gallbladder or pancreas
- Gallstones

In an infant, high bilirubin and jaundice can be very dangerous, and may be caused by several factors. There are three common types:

- Physiological jaundice (at 2-4 days after birth, caused by a brief delay in the functioning of the liver, usually not serious)
- Breast feeding jaundice (during first week of life, caused by a baby not nursing well or low milk supply in the mother)
- Breast milk jaundice (after 2-3 weeks of life, caused by the processing of some substances in breast milk)

- Sometimes bilirubin is measured as part of a "panel" of tests. Often, the liver is evaluated with a group of tests that include bilirubin, alanine transaminase (ALT), asparate transaminase (AST), alkaline phosphatase (ALP), albumin, total protein, and others.

☯ Bilirubin Blood Test requirement:

For this test, you will need to fast (not eat or drink anything other than water) for four hours before you have the test performed. Drink a normal amount of water before going to the laboratory or collection site.

You may have to stop taking certain medications before the test is performed, but only if your doctor tells you to do this. Examples of drugs that can affect bilirubin levels include antibiotics like penicillin G, sedatives like phenobarbital, diuretics like furosemide, and asthma medications like theophylline.

Practical Estimation of Bilirubin in Blood

Principle of the method:

Bilirubin converted to color azobilirubin by diazotized sulfanilic acid and measured photometrically. Of the two fractions presents in serum, bilirubin glucuromide and free bilirubin loosely bound to albumin, only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with di-methyl sulfoxide (DMSO) to react (bilirubin indirect). In the determination of indirect bilirubin the direct is also determined, the results correspond to total bilirubin.

The intensity of the color formed is proportional to the bilirubin concentration in the sample.

Procedure:

➲ Assay conditions:

- Wavelength …… 555 nm (530-580).
- Cuvette ……... 1 cm light path.
- Temperature …… (15-25) ºC.

- Adjust the instrument (colorimeter) to zero with distilled water.
- Pipette into a cuvette:

	Blank	Total B	Blank	Direct B
R_1 (D) (ml)	**---**	**---**	**1.5 ml**	**1.5 ml**
R_2 (T) (ml)	**1.5 ml**	**1.5 ml**	**---**	**---**
R_3 (µL)	**---**	**50 µL**	**---**	**50 µL**
Sample / calibrator (µL)	**100 µL**	**100 µL**	**100 µL**	**100 µL**

- Mix and incubate exactly 5 minutes at (15-25) ^{o}C, then read the absorbance (A).
- Calculation:
- Calculation: $\frac{A^{o}\ \text{sample} - A^{o}\ \text{sample Blank}}{A^{o}\ \text{Calibrator} - A^{o}\ \text{Calibrator Blank}} \times \text{Conc}_{(\text{Calibrator})} = \text{mg / dl}$

$$A^{o}\ \text{sample} - A^{o}\ \text{sample Blank} \times \text{Factor} = \text{mg / dl}$$