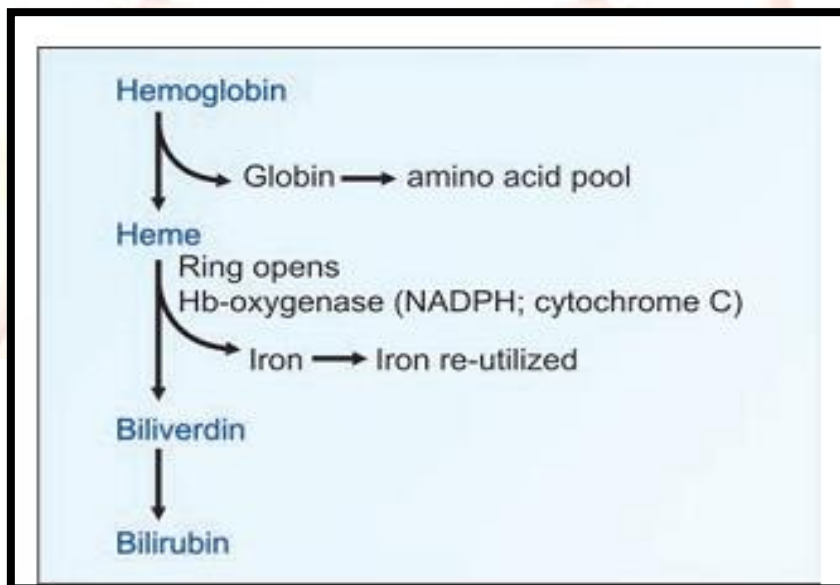


Determination of serum Bilirubin level

Generation of Bilirubin

The end-products of heme catabolism is bilirubin. Bilirubin has no function in the body and is excreted through bile. The senescent RBCs breakdown and liberating the hemoglobin, the globin chains are separated, and the amino acids are channelled into the body amino acid pool. The iron liberated from heme is reutilized. Lastly, the porphyrin ring is broken down cells of liver, spleen and bone marrow, to form biliverdin which is green in color. In mammals biliverdin is further reduced to bilirubin, a red-yellow pigment, by an NADPH dependent biliverdin reductase as figure below reveals.



Types of Bilirubin

- ✚ **Direct bilirubin:** Conjugated with glucuronic acid by means of Bilirubin-UDP Glucuronosyl Transferase.
- ✚ **Indirect bilirubin:** unconjugated, insoluble in water



✚ **Total bilirubin:** sum of the direct and indirect of bilirubin.

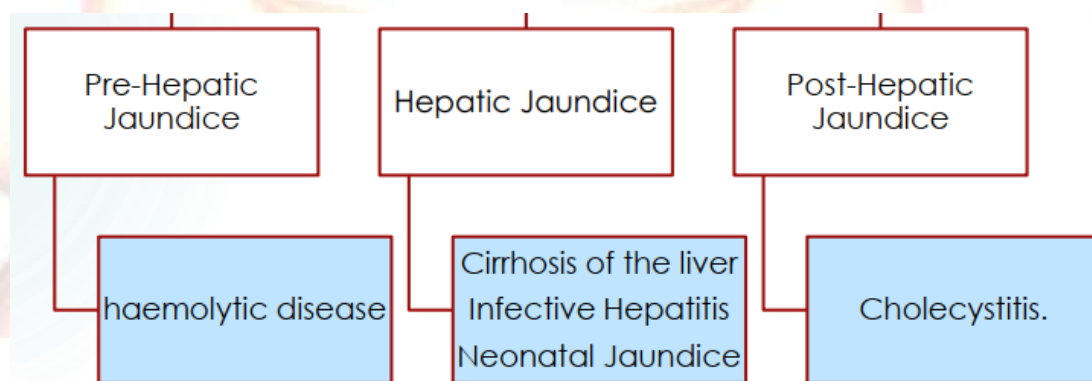
Normal Plasma Bilirubin Level

ranges from **0.2–0.8 mg/dl**. The unconjugated bilirubin is about 0.2–0.6 mg/dl, while conjugated bilirubin is only 0–0.2 mg/dl. If the plasma bilirubin level exceeds 1 mg/dl, the condition is called **hyperbilirubinemia**.

When the bilirubin level exceeds 2 mg/dl, it diffuses into tissues producing yellowish discoloration of sclera, conjunctiva, skin and mucous membrane resulting in **jaundice**. Icterus is the Greek term for jaundice.

Types of Jaundice

Jaundice is the discoloration of skin and sclera of the eye. The causes of jaundice may be classified as



HYPERBILIRUBINEMIAS

Depending on the nature of the bilirubin that elevated, the condition may be grouped into conjugated or unconjugated hyperbilirubinemia .

Based on the cause it may also be classified into congenital and acquired.



Neonatal Jaundice

- ✓ Conjugating enzymes in the liver are often absent at birth.
- ✓ Raised serum level of indirect (and total) bilirubin is to be expected o Low level of direct bilirubin.
- ✓ The other liver functions are normal. The indirect bilirubin level will rise for the first few days after birth until the conjugating enzymes begin to synthesize.
- ✓ If the latter process is delayed and the serum level of indirect bilirubin rises towards 20 mg/dl, an ultraviolet therapy or an exchange blood transfusion should be carried out owing to the
- ✓ danger of deposition of the insoluble unconjugated bilirubin in the
- ✓ basal ganglia of the brain leading permanent Brain Damage.

Method

Label 4 tubes as TT (total test), TB (total Blank), DT (direct test), DB (direct Blank).

Total bilirubin

	TB	TT
Solution-1	0.2 ml	0.2 ml
Solution-2	--	0.05 ml
Solution-3	1 ml	1 ml
Sample	0.2 ml	0.2 ml

stand for 30 min at 20-25°C.

Solution 4	1 ml	1 ml
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Mix and let stand for 15 min and read the absorbance at 546 nm against blank (ATB).

Direct bilirubin

	DB	DT
Solution-1	0.2 ml	0.2 ml
Solution-2	--	0.05 ml
0.9 % NaCl	2 ml	2 ml
Sample	0.2 ml	0.2 ml

Mix, let stand for 5 min. at 20-25°C. Read absorbance of test against blank (A_{DB}) **for direct only** at 546 nm.



Calculation

- Concentration of direct bilirubin in mg/dl serum
 - = (abs. DT - abs. DB) X 14.4 = mg /dl
 - Normal range Up to: 0.25 mg/dl
- Concentration of total bilirubin in mg/dl serum
 - = (abs. TT - abs. TB) X 10.8 = mg /dl
 - Normal range Up to 1 mg/dl
- Concentration of indirect bilirubin in mg/dl serum
 - = Conc of total bilirubin – Conc of direct bilirubin = mg /dl
 - Normal range 0.1-0.4 mg/dl

