



Blood Biochemistry BCH 471[Practical]

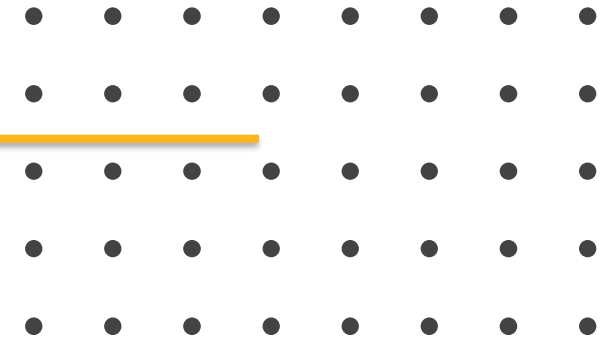
**Lab (7) Estimation of Serum Bilirubin (Total & Direct)**



## Objective

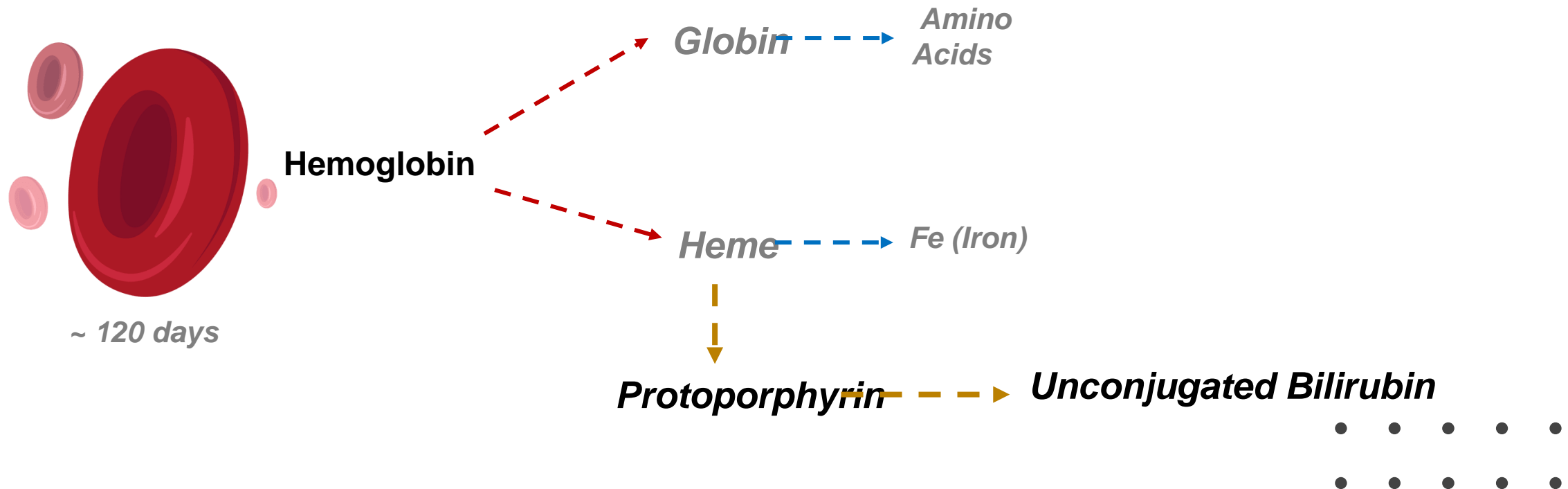
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- To estimate the amount of bilirubin in serum.



# Bilirubin

- It is the **yellow** breakdown product of normal heme catabolism.
- Heme is formed from **hemoglobin**, a principal component of red blood cells.
- Bilirubin is excreted in bile, and its levels are elevated in certain diseases.
- It is responsible for the yellow color of bruises and the yellow discoloration in **jaundice**.

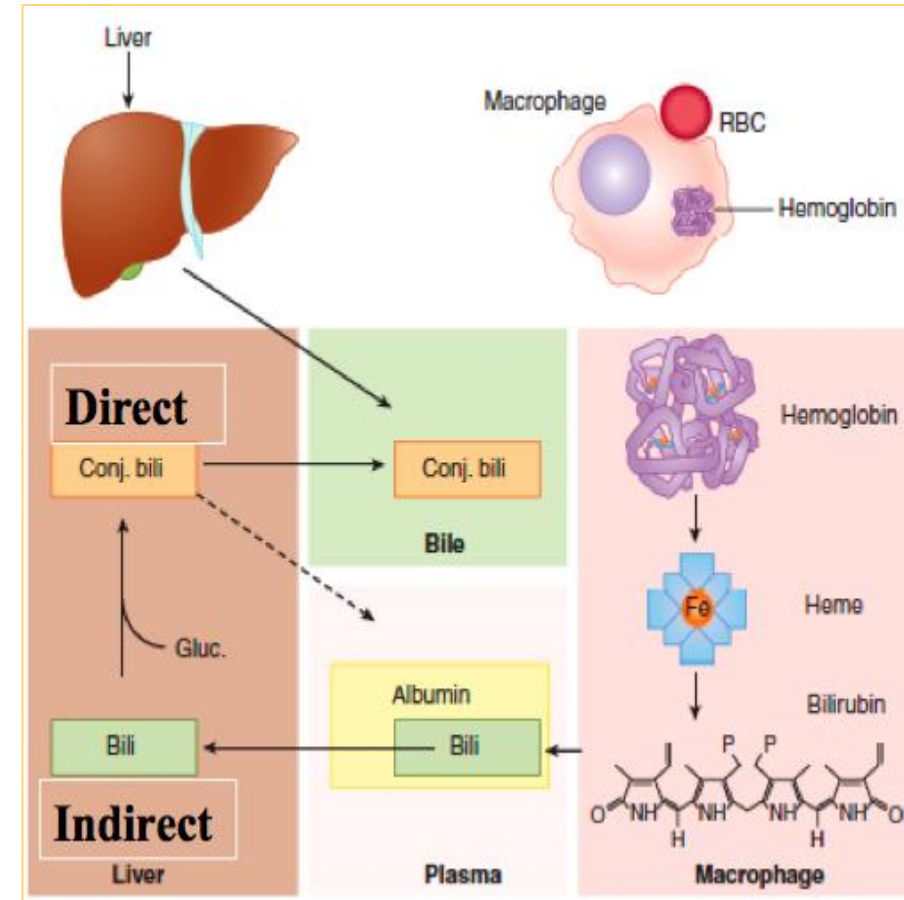


# Types of Bilirubin

- **Direct bilirubin:** Conjugated with glucuronic acid, water soluble.
- **Indirect bilirubin:** unconjugated, water insoluble.
- **Total bilirubin:** sum of the direct and indirect of bilirubin.

## Notes:

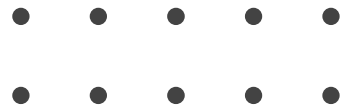
1. About 200 mg per day of unconjugated bilirubin are transported to the liver.
2. Disturbances in the powers of conjugated and/or excretion of the liver of this yellow compound will lead to raised levels in serum.



# Bilirubin and Jaundice

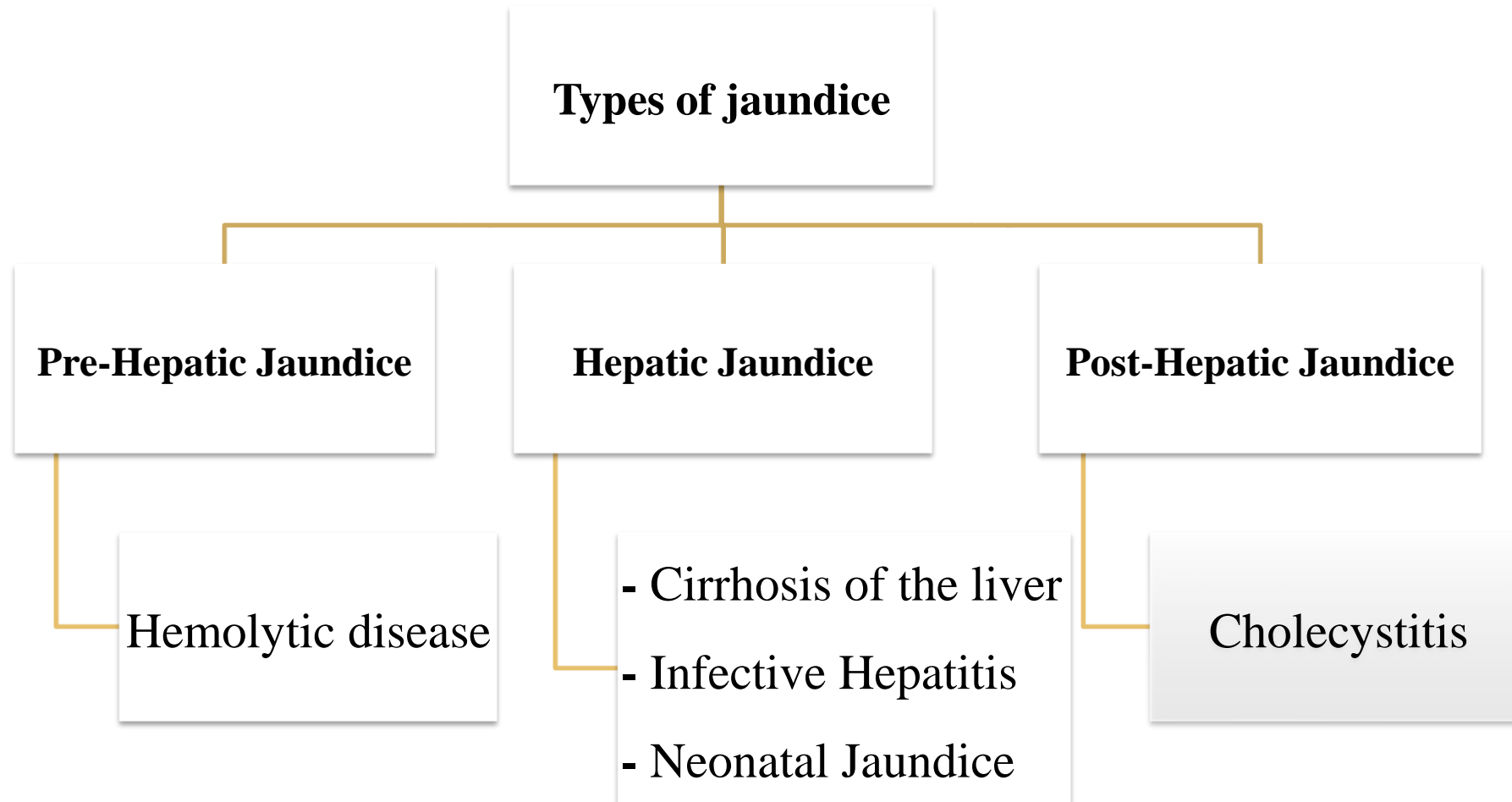
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- Above about 2 mg/dl in the blood, leads to disease called **Jaundice**.
- Jaundice is caused by a **build-up of bilirubin (yellow color)** in the blood and tissues of the body.
- Jaundice is the **discoloration** of skin and sclera of the eye caused by high concentration of bilirubin.



# Causes of Jaundice

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# Pre-Hepatic Jaundice

## Hemolytic disease (excess hemolysis)

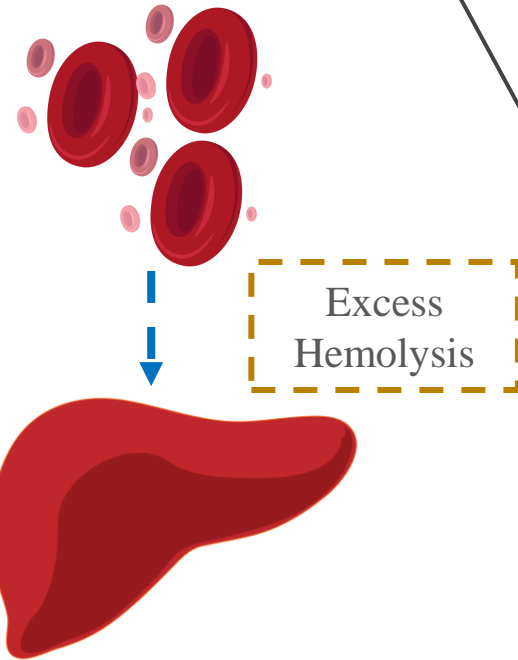
- The production of un-conjugated bilirubin may **exceed the conjugating capacity of the liver.**
- Direct bilirubin in the upper normal range or just a little elevated.
- The serum levels of indirect (and of total) bilirubin **will be raised.**
- The other liver function tests will usually give **normal results.**

Indirect bilirubin ► increased

Direct bilirubin ► Slightly increased

Total bilirubin ► increased

$$\uparrow\uparrow\uparrow \text{UCB} + \uparrow \text{CB} = \uparrow\uparrow\uparrow \text{TB}$$



- ↑ Unconjugated bilirubin (in blood)
- Upper normal range conjugated bilirubin (released to bile duct)

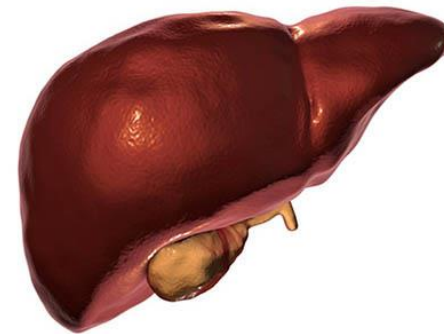
# Hepatic Jaundice

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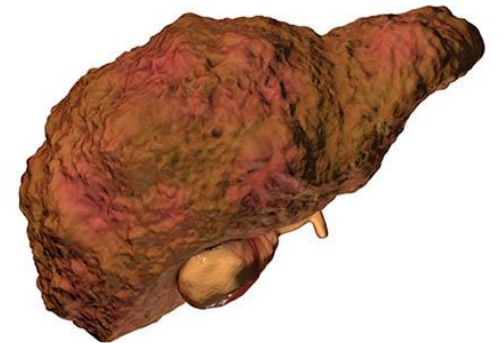
## 1- Cirrhosis (in the absence of infection)

- Destruction of liver cells will lead to a reduced conjugating capacity.
- **Raised** serum level of indirect (and of total) bilirubin.
- **Low** level of direct bilirubin.
- An **abnormally high release**, into the blood, of the enzymes: AST, ALT and ALP.
- Synthesizing power of liver will be **diminished** and hence low levels of total protein, albumin and cholesterol.

$$\uparrow\uparrow\uparrow \text{UCB} + \downarrow \text{CB} = \uparrow\uparrow\uparrow \text{TB}$$



Healthy liver



Cirrhotic liver

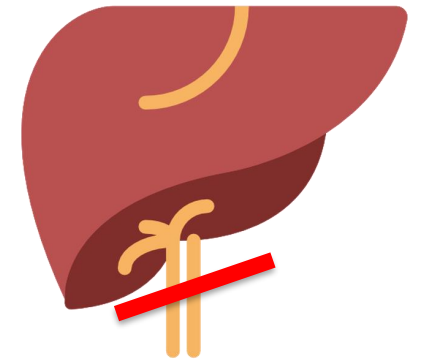


# Hepatic Jaundice

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## 2- Hepatitis (in the presence of infection)

- The conjugative capacity of the liver is approximately normal, but there is the inability to transport the conjugated bilirubin from the liver cells to the biliary system, and it will be regurgitated back into the blood.
- The serum level of **unconjugated** bilirubin will be **normal**.
- **Conjugated** (and total) bilirubin will be **raised**.
- Synthesizing power is diminished leading to **low** serum levels of proteins but the **raising** of antibodies to infection usually leads to raised total proteins level.



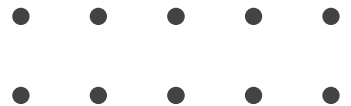
- Normal unconjugated bilirubin (in blood)
- ↑ conjugated bilirubin (in blood)

# Hepatic Jaundice

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## 3- Neonatal Jaundice

- Conjugating enzymes in the liver are often **absent at birth**.
- **Raised** serum level of indirect (and total) bilirubin is to be expected.
- **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 conjugation process is delayed and the serum level of indirect bilirubin rises towards **20 mg/dl**
- Can be treated by **Phototherapy** or an exchange **blood transfusion**.
- Deposition of the insoluble unconjugated bilirubin into basal ganglia of the brain leads to **permanent Brain Damage**.

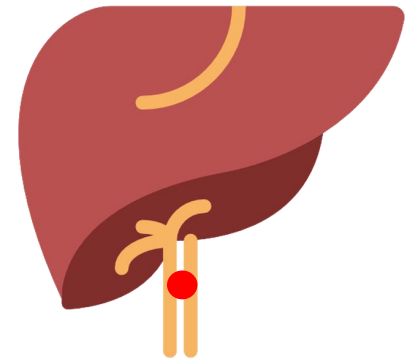


# Post-hepatic Jaundice

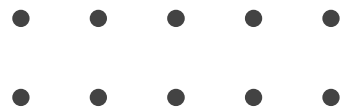
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## Cholecystitis

- The bile duct is **blocked**.
- The indirect bilirubin level is **normal** but conjugated bilirubin is regurgitated into the blood and excreted into the urine (**raised conjugated and total bilirubin**).
- Enzymes will be regurgitated into the blood giving **raised** levels.
- The other liver function tests are **normal**.



- Normal unconjugated bilirubin (in blood)
- ↑ conjugated bilirubin (in blood)



# Practical Part



# Principle

- Bilirubin in serum is coupled with **diazotized sulfanilic acid** to form **azobilirubin**. The intensity of the **purple color** that is formed is proportional to the bilirubin concentration in serum. The color is measured spectrophotometrically at 540nm.
- The **water soluble conjugated bilirubin** (direct bilirubin) reacts easily with **diazotized sulfanilic acid** to form a colored compound within 1 minute.
- The subsequent addition of **Methanol** (solubilizing agent) accelerates the reaction of the water insoluble unconjugated bilirubin (indirect bilirubin) in serum, and a value of **total bilirubin** is obtained after letting the sample stand for 5 minutes.
- The indirect or unconjugated bilirubin is then found by difference.

**Bilirubin in serum sample + diazotized sulfanilic acid → estimation of direct bilirubin+ Methanol → estimation of total bilirubin(the remained indirect bilirubin reacts with the reagent).**

# Method

- Label 2 tubes as Test Blank and Test.

	Test Blank	Test
Sulfanilic acid reagent	1.4 ml	1.4 ml
Sodium nitrate reagent	--	0.025 ml
Distilled water	0.025ml	--
Sample	0.1 ml	0.1 ml
Mix and let stand for 1 min. After exactly 1 min, read the absorbance of <b>test and test blank</b> at 540nm <u>against distilled water</u> . Use this to calculate <b>Direct Bilirubin</b> , then add		
Methanol reagent	1.5 ml	1.5 ml
Mix by inversion and let stand for 5 min. Read the absorbance of <b>test and test blank</b> at 540nm against distilled water. Use this to calculate <b>Total Bilirubin</b> .		

💡 **Standard** : Carefully pour **Bilirubin Equivalent Standard** in to a clean cuvette, read and record its absorbance against distilled water at 540 nm. Pour back to its original container.

= (0.166)

# Calculations

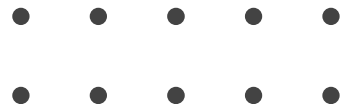
The concentration of direct and total bilirubin in Bilirubin Equivalent Standard :

- Direct Bilirubin = 2.5 mg/dL
- Total Bilirubin = 5.0 mg/dL

● **Concentration of direct bilirubin (mg /dL)** =  $\frac{Abs (Test) - Abs (Test Blank)}{Abs (Bilirubin Equivalent Standard)} \times 2.5 \text{ mg /dL}$

● **Concentration of total bilirubin (mg /dL)** =  $\frac{Abs (Test) - Abs (Test Blank)}{Abs (Bilirubin Equivalent Standard)} \times 5.0 \text{ mg /dL}$

● **Concentration of indirect bilirubin (mg /dL)** = *Concentration of total bilirubin - Concentration of direct bilirubin*



# Normal range

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The expected values in serum :

- **Direct Bilirubin** → up to 0.5 mg/dL
- **Total Bilirubin** → up to 1.0 mg/dL
- **Indirect Bilirubin** → 0.1 – 0.4 mg/dL

