



Clinical Biochemistry

Lab. 6

Measurement of Serum Bilirubin

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Laboratory Tests for Bilirubin



- There are two types of bilirubin:
 - **Conjugated bilirubin (Direct)**
 - **Unconjugated bilirubin (indirect)**
- **Total bilirubin** is composed of :**The conjugated bilirubin + The unconjugated bilirubin**
- The total bilirubin level increases **with any type of jaundice.**
- **Types of Jaundice:**
 - **Pre hepatic:** pathology is occurring prior the liver
 - **Hepatic:** pathology is located within the liver
 - **Post hepatic:** pathology is located after the conjugation of bilirubin in the liver

Laboratory Tests for Bilirubin

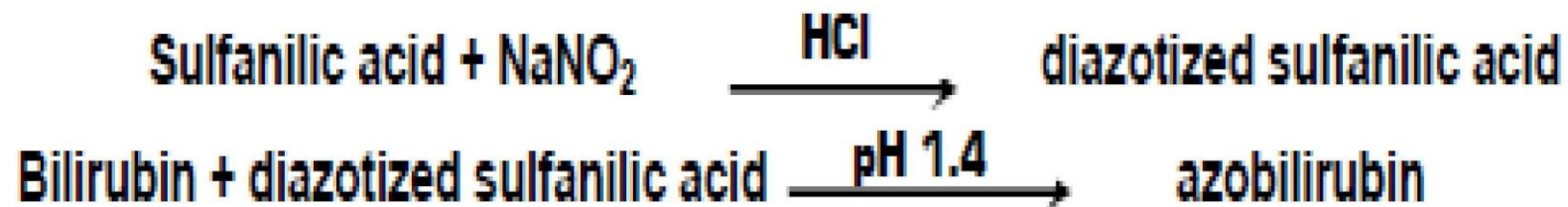


| | Pre-hepatic Jaundice | Hepatic Jaundice | Post-hepatic Jaundice |
|------------------------|----------------------|--------------------|-----------------------|
| Total bilirubin | Increased | Increased | Increased |
| Conjugated bilirubin | Normal | Normal / Decreased | Increased |
| Unconjugated bilirubin | Increased | Normal / Increased | Normal |

Principle of Bilirubin Measurement



- ❑ Bilirubin is typically measured using **diazotized sulfanilic acid**, which forms a **conjugated azo compound** with the **porphyrin rings of bilirubin**, resulting in reaction products that **absorb strongly at 540 nm**.
- ❑ Because **unconjugated bilirubin reacts slowly**, accelerants such as **caffeine** or **methanol** are used to measure total bilirubin.
- ❑ Deletion of **these accelerants** allows determination of direct, bilirubin.



Specimen & Storage



- Total bilirubin determinations using a **diazo method** require either **serum or plasma**.
- Bilirubin is very **sensitive to and is destroyed by light and heat**; therefore specimens should be **protected from ambient light prior to and during analysis**.
- If separated and stored **in the dark**, serum or plasma is stable for:
 - **1 day at 15 - 25 °C**
 - **7 days at 2 - 8 °C**
 - **3 months at - 20 °C (if frozen immediately)**

Serum bilirubin levels



- **Normal total Bilirubin in Adults**

- 0.2 – 1 mg/dL

- **Unconjugated (indirect):**

- 0.2 – 0.7 mg/dL

- **Conjugated (direct):**

- 0.0 – 0.2 mg/dL

- **Latent jaundice:**

- Above 1 mg/dL

- **Jaundice:**

- Above 2 mg/dL

- **Neonates: usually bilirubin level less than or equal 8 mg/dL is normal**