



ESTIMATION OF SERUM SERUM BILIRUBIN

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Estimation of Serum Bilirubin

Types of Bilirubin:

- Total Bilirubin:

direct bilirubin + indirect bilirubin

- Indirect bilirubin:

Unconjugated bilirubin (water insoluble, produced in the spleen)

- Direct Bilirubin:

Conjugate (water soluble, converted in the liver).

Principle

- Bilirubin reacts with **diazo reagent** to produce **azobilirubin**.
- **Azobilirubin is purple colored compound.**
- Intensity of color is **proportional to amount of bilirubin in serum.**

❖ **Reactions:**

▪ **Direct Reaction**

Involve direct or conjugated bilirubin

The reaction occurs in an aqueous medium

▪ **Indirect Reaction**

Carried out in methyl alcohol.

Methyl alcohol solubilizes unconjugated bilirubin.

Reagents

○ R1 (Total Bilirubin)

- Sulfanillic acid 30 mmol/L
- DMSO 7 mol/L
- Hydrochloric acid 130 mmol/L

○ R2 (Direct Bilirubin)

- Sulfanillic acid 30 mmol/L
- Hydrochloric acid 130 mmol/L

○ R3 (Total and Direct Bilirubin)

- Sodium Nitrite 0.74 mmol/L

Procedure

Total Bilirubin

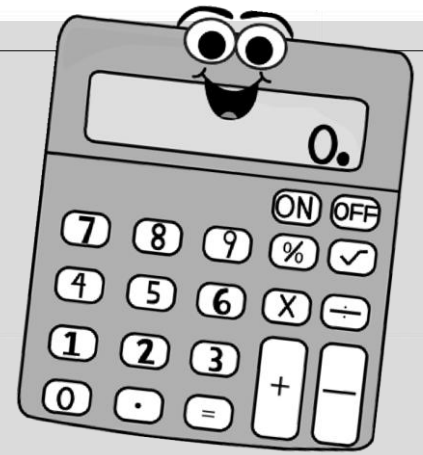
Reagent/ Tubes	Assay tube (μL)	Blank tube (μL)
R1	1000	1000
R3	50	-
dH ₂ O	-	50
Serum sample	100	100

Direct Bilirubin

Reagent/ Tubes	Assay tube (μL)	Blank tube (μL)
R2	1000	1000
R3	50	-
dH ₂ O	-	50
Serum sample	100	100

Measure the Absorbance at 550 nm immediately after adding the serum sample

Calculations



$$\text{Conc. (mg/dL)} = \text{Absorbance of sample} - \text{Absorbance of Blank} \times 11.4$$

11.4 is a **standard factor** under the condition that absorbance was taken in a cuvette of 1 cm path length, at 550 nm wavelength and 37 °C

Normal Range

Total bilirubin (5 days – 60 years) =
0.3 – 1.2 or 0.2 – 1.0 (> 60 years) mg/dL

Direct bilirubin (5 days – 90 years) =
<0.2 mg/dL

