

## **Experiment no.: 13**

**Experiment name: determination of Serum Bilirubin in blood serum.**

### **The aim of the Experiment:**

Determination of Serum Bilirubin using MALLOY AND EVELYN METHOD:

### **Principle**

Bilirubin couples with diazotized sulphanilic acid to form a purple coloured azobilirubin complex . Direct bilirubin reacts with the diazo reagent in aqueous solution to form a coloured diazo compound within 1 min. the indirect bilirubin is diazotized only in the presence of methanol. The subsequent addition of methanol accelerates the reaction of indirect bilirubin . The value of total bilirubin is obtained after letting the specimen stand for 30 min. The absorbance values of coloured solution are taken at 540 nm.

### **Equipment and materials used in the Experiment:**

#### **Reagents**

- Diazo reagent A and B
- Methanol
- Conc. HCl
- Working bilirubin std. (10 mg %)
- Diazo blank reagent (1.5 ml Conc. HCl diluted to make 100 ml

with distilled water )

- –Pipettes to measure reagent and samples.

### **Property of the machine:**

Normal UV-Vis spectrophotometer:

### **Machine usage:**

25- Wavelength set up step.

26- Blank against the solvent solution using a proper cuvette.

27- Reach O.D.

### **Experiment procedure or protocol:**

1. Prepare Diazo mixture by adding 5 ml Diazo A and 0.15 ml of Diazo B.

2. Take four test tubes. Label them as, TT (total test), TB (total blank), DT (direct test), and DB (direct blank)
3. Add the reagents as ——
  - a. 0.1 ml serum and 0.9 ml distilled water in each tube.
  - b. 0.25 ml Diazo blank in TB and DB
  - c. 0.25 ml Diazo reagent in TT and TB
  - d. 1.25 ml distilled water to DT and DB
  - e. 1.25 ml methanol to TT and TB.
4. Mix well and read the OD of DT and DB after one min. against distilled water at 540 nm.
5. Mix well and keep the tubes TT and TB in dark at room temperature for 30 min. and read the OD against distilled water at 540 nm.
6. Read OD of bilirubin std. (reagent 4) against distilled water.

Reagent	TT	TB	DT	DB
Serum	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Distilled water	0.9 ml	0.9 ml	0.9 ml	0.9 ml
Diazo blank	—	0.25 ml	—	0.25 ml
Diazo reagent	0.25 ml	—	0.25 ml	—
Distilled water	—	—	1.25 ml	1.25 ml
Methanol	1.25 ml	1.25 ml	—	—

Table showing Malloy and Evelyn method

### Calculation

$$\text{Total bilirubin (A)} = \frac{\text{O.D. of TT} - \text{O.D. of TB}}{\text{O.D. of Std.}} \times 10$$

$$\text{Direct bilirubin (B)} = \frac{\text{O.D. of DT} - \text{O.D. of DB}}{\text{O.D. of Std.}} \times 10$$

$$\text{Indirect bilirubin} = A - B.$$

### Experiment data and results:

Normal values:

Total bilirubin	Age	Value
	Newborn	up to 5.8 mg/dl
	1-2 days	up to 8.2 mg/dl
	3-5 days	up to 11.7 mg/dl
	Above one month to adult	up to 1.0 mg/dl

Direct bilirubin                      0.0 to 0.2 mg /dl

Indirect bilirubin                    0.4 to 0.8 mg /dl

### Conclusion:

- What is the role of this experiment?
- How does its deficiency affect the health?
- What are the normal level values?
- How can you determine its quantity on the blood?
- Discuss the methodology?