Swemed DIAGNOSTICS

# BILIRUBIN KIT / TOTAL & DIRECT

**INTRODUCTION:**

Elevation of total serum Bilirubin may occur due to excessive hemolysis or destruction of the red blood cells e.g. hemolytic disease of the newborn,
liver diseases e.g. hepatitis and cirrhosis,
Obstruction of the biliary tract e.g., gallstones. There is literature indicating that elevation of direct Bilirubin levels in patients with liver or billiary tract diseases even though total Bilirubin levels are normal. Therefore, the greatest diagnostic value of direct Bilirubin assays from their ability to indicate occult liver disease.

**PRINCIPLE:**

Bilirubin reacts with diazotized sulfanilic acid to produce azoBilirubin which has maximum absorbance at 546 nm in the aqueous solution. The intensity of the color produced is directly proportional to the amount of Direct or Total Bilirubin concentration present in the sample.

**REAGENT PREPARATION:**

1. Total Bilirubin Reagent - R1 : Ready-To-Use
2. Direct Bilirubin Reagent - R2 : Ready-To-Use
3. Bilirubin -Activator : Ready-To-Use

**STABILITY:**

The unit is stable at 2°-8°c until the expiry mentioned on the label.

**SPECIMEN COLLECTION:**

1. Hemolysis interferes with the test i.e., haemolysed samples should be avoided since they may give falsely low values.
2. All specimens for this assay must be carefully protected from light.
3. Bilirubin in serum is stable for stable for 4-7 days when stored in the dark at 2-8$^0$ C.

**PROCEDURE:**

For DIRECT and TOTAL BILIRUBIN

**ASSAY PARAMETERS**

| | |
|---|---|
| METHOD | : End Point |
| REACTION SLOPE | : Increasing |
| BLANK | : Sample blank |
| WAVE LENGTH | : 546nm |
| UNITS | : mg/dl |
| LINEARITY | : 20 mg/dl |
| TOTAL BILIRUBIN FACTOR | : 15 |
| DIRECT BILIRUBIN FACTOR | : 15 |

**MANUAL ASSAY:**

| | Marco | | Semi -Micro | |
|---|---|---|---|---|
| Pipette into Cuvettes | Blank | Test | Blank | Test |
| Bilirubin Reagent Total/Direct | 1000 µl | 1000 µl | 500µl | 500 µl |
| Sample Total/Direct | 100 µl | 100 µl | 50 µl | 50 µl |
| Bilirubin-Activator | - | 100µl | - | 50 µl |

Mix & incubate for 10 minutes at room temperature and read the absorbance of the all cuvettes at 540 – 560 nm, against a serum blank immediately.

**CALCULATIONS:**

Total Bilirubin (mg / dl) = Abs of Sample – Abs of Sample Blank x 15

Direct Bilirubin (mg / dl) = Abs of sample – Abs of Sample Blank x 15

**EXPECTED VALUES :**

Direct Bilirubin – upto 0.49 mg/dl
Total Bilirubin – upto 1.0 mg/dl

**LINEARITY**

The Bilirubin unit is linear upto 20mg/dl.

**INTERFERENCES:**

1. Young, et al., give an exhaustive list of drugs and other substances known to affect the circulating level of Bilirubin.
2. In this assay, as in all laboratory procedures, materials which come in contact with specimens should be clean and free of contamination by heavy metals, detergents and other chemicals.
3. Direct sunlight may cause upto a 50% decrease in Bilirubin level within 1 hour.

**REFERENCES:**


1. tietz, N.W., Fundamentals of Clinical Chemistry. W.B. Saunders Co., Philadelphia, (1976), p. 1028.
2. Young, D.S., et. at. Clin. Chem. 21, 10 (1975).
3. Perry B., Darmas BT, Buffone G., et. all – Clin. Chem. 32, 329 (1986).



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