

BILIRUBIN (Total + Direct)

Colorimetric Method	Total	100 Tests
	Direct	100 Tests

PRINCIPLE :

The reaction between bilirubin and the diazonium salt of sulphanilic acid produced azobilirubin which shows a maximum absorption at 535 nm in an acid medium. In the presence of (DMSO) the total bilirubin participate in the reaction and in the absence of (DMSO) only conjugated bilirubin react .

SAMPLE :

Serum, plasma. Hemolysis will interfere with the test .Samples should be kept away from light.

NORMAL VALUES :

Total bilirubin up to 1.0 mg / dL (17 µmol/L)

Direct bilirubin up to 0.25 mg / dL (4.3 µmol/L)

REAGENTS :

1.	Direct Bilirubin :	
	Sulphanilic acid	60 mmol /L
	Hydrochloric acid	200 mmol /L
2.	Total bilirubin :	
	Sulphanilic acid	60 mmol /L
	Hydrochloric acid	200 mmol /L
	Dimethylsulfoxide (DMSO)	10 mol/L
3.	Sodium nitrite	90 mmol /L

STABILITY :

The reagents are stable up to the expiry date specified when stored at +4 to +8 °C .

PROCEDURE :

	Direct Bilirubin		Total Bilirubin	
	Blank (ml)	Sample (ml)	Blank (ml)	Sample (ml)
Reagent 1	1.0	1.0	-	-
Reagent 2	-	-	1.0	1.0
Reagent 3	-	0.05	-	0.05
Sample	0.1	0.1	0.1	0.1

Mix. Incubate at room temperature for 10 min. away from light. Read the absorbances of the sample (A_{Sample}) against its blank at 535 nm. (530 – 540 nm), using cuvettes 1 cm light path. Linearity up to 25 mg / dL (425 µmol/L). Color stable for one hour.

CALCULATION :

$$\text{Total bilirubin (mg /dl)} = A_{\text{Sample}} \times 14$$

$$\text{Direct bilirubin (mg /dl)} = A_{\text{Sample}} \times 14$$

$$\text{Indirect bilirubin (mg / dl)} = \text{Total} - \text{Direct}$$

REFERENCE :

Walter M. and Gerade H., (1970) Microchem. J. 15. 231

QUALITY CONTROL :

For accuracy and reproducibility control:-
Assayed Multi – Sera, Normal and Elevated

**BILIRUBIN
(Total + Direct)**

Colorimetric Method

+4 to +8°C **Direct 100 Tests**
 Total 100 Tests
In vitro diagnostic use

CAT. NO.

BR 11 10

REAGENTS

R1 Sulphanilic acid **100 ml**
R2 Sulphanilic acid –DMSO **100 ml**
R3 Sodium nitrite **10 ml**

CONTACTS

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