

PRINCIPLE:

Conjugated bilirubin (Direct Bilirubin) reacts with diazotized sulfanilic acid to form a red colored compound. The intensity of the colour is proportional to the bilirubin concentration in the sample.

Total Bilirubin reacts with diazotized sulfanilic acid in the presence of dimethyl sulfoxide (DMSO) to form a coloured compound. The intensity of the colour is proportional to the Bilirubin concentration in the sample.

REAGENT COMPOSITION:

1. Reagent I (Direct Bilirubin)
2. Reagent II (Total Bilirubin)
3. Reagent III (Colour reagent)

REAGENT PREPARATION:

Reagent is ready to use.

STORAGE & STABILITY:

Store at 2-8° C, and keep away from light. Unopened reagent is stable until expiry date stated on the label.

SAMPLE:

Unhemolysed serum or EDTA or heparinised plasma can be used.

AUTOMATED PARAMETERS:**DIRECT BILIRUBIN**

Parameter	Test
Reaction type	Endpoint (Jendrassik Gorf)
Wavelength	550 nm
Reaction temperature	37° C
Incubation	5 min.
Blank	Sample
Reagent blank limit	< 0.2 O.D
Sample volume	50 µl
Reagent volume	1020 µl
Linearity	20mg/dl

**AUTOMATED PARAMETERS:
TOTAL BILIRUBIN**

Parameter	Test
Reaction type	Endpoint
Wavelength	550 nm
Reaction temperature	37° C
Incubation	5 min.
Blank	Sample
Reagent blank limit	< 0.2 O.D
Sample volume	50 µl
Reagent volume	1020 µl
Linearity	20mg/dl

PROCEDURE:

Let stand reagents and specimens at room temperature.

Tube	T. Bilirubin		Direct Bilirubin	
	Blank (B)	Test (T)	Blank (B)	Test (T)
Reagent I	---		1000 µl	1000 µl
Reagent II	1000 µl	1000 µl	---	
Reagent III	-	20 µl	-	20 µl
Sample	50 µl	50 µl	50 µl	50 µl
Mix and Incubate @ 37 °C for 5min. Read the absorbance at 550 nm within 15 min.				

CALCULATIONS:

Calculate the result as follows:

$$\text{Direct Bilirubin} = \text{O.D (T)} - \text{O.D (B)} \times 20$$

$$\text{Total Bilirubin} = \text{O.D (T)} - \text{O.D (B)} \times 30$$

EXPECTED VALUE:

Direct bilirubin = upto 0.3mg/dl

Total Bilirubin

New born: upto 13.2mg/dl

Adult : upto 1.1mg/dl

Each lab should optimize its own normal range.

REFERENCE:

1. Malloy, H.T. and Evelyn, K.A, J. Biol.Chem. 119,481 (1937).
2. Walters M.I, Gerarde R.W. Microchem 15, 231 (1970).