

**PRINCIPLE:**

Conjugated bilirubin (direct bilirubin) reacts with diazotized sulphanilic acid to form a red azo colored compound.

The intensity of the color is proportional to the bilirubin concentration in the sample.

**SAMPLE:**

Fresh nonhemolyzed serum, EDTA- or heparinized plasma. Store sample in dark until use.

**REAGENTS:**

- |    |   |            |
|----|---|------------|
| 1. | Reagent 1 (Direct bilirubin) (3x100 ml) |            |
|    | Sulphanilic acid                        | 30 mmol/L  |
|    | HCl                                     | 150 mmol/L |
| 2. | Reagent 3 (Nitrite solution) (1x15 ml)  |            |
|    | Sodium nitrite                          | 29 mmol/L  |

Stable to expiry date when stored at +15 °C to +25 °C.

**PREPARATION OF REAGENTS:**

All reagents are ready for use.

**PROCEDURE:**

Wavelength:	555 (530- 580) nm
Cuvette:	1 cm light path
Temperature:	20 °C, 25 °C, 37 °C
Zero:	against H <sub>2</sub> O

Pipette into test tubes:		
	Direct bilirubin	
	Sample blank	Sample
Reagent 1	1000 µl	1000 µl
Reagent 3	-	50 µl
Sample	100 µl	100 µl
Mix and let stand for 5 minutes at room temperature. Measure the absorbance of the sample blank and sample against the distilled water.		

**CALCULATION:**

**Direct bilirubin**

$$(A_{\text{sample}} - A_{\text{sample blank}}) \times 239 = \mu\text{mol/L Direct bilirubin}$$

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

**EXPECTED VALUES:**

**Direct bilirubin:**

Children and adults up to 5 µmol/L (0.3 mg/dl)

**LINEARITY:**

up to 256 µmol/L (15 mg/dl)

**QUALITY CONTROL**

All control sera with mean values determined by this method can be used.

**NOTE:**

1. Incubation time must be strictly respected.
2. If is concentration of bilirubin greater than 256 µmol/L, dilute sample 1 : 5, with physiological NaCl and repeat the determination. The result multiplay by 5.
3. Avoid contamination of the opened reagent.
4. The reagent are stables up to the date of expiration as specified.

**REFERENCES:**

1. Malloy, H.T. and Evelyn, K.A., J.Biol.Chem. 119, 481 (1937).
2. Matimek, R.G. Clin.Chim.Acta., 13 161 (1966).
3. Walters M.I., Gerarde R.W., Microchem 15, 231 (1970)