

Manual Procedure

Cat. No. 12141

Bilirubin Total & Direct	R1	2 x 80 ml
For 80 test Total	R2	2 x 80 ml
80 test Direct	R3	1 x 16 ml

Bilirubin Total & Direct

Colorimetric method

Liquid Reagents

Test principle

Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid in the presence of dimethylsulfoxide accelerator. Total bilirubin couples with diazotized sulfanilic acid to give an azo dye, the color intensity of which is proportional to the bilirubin concentration. For direct bilirubin dimethylsulfoxide is not used.

Concentrations in the test

Reagent R1		
Sulfanilic acid	25	mmol/L
hydrochloric acid	74	mmol/L
dimethylsulfoxide	7	mol/L
Reagent R2		
Sulfanilic acid	25	mmol/L
hydrochloric acid	87	mmol/L
Reagent R3		
Sodium nitrite	25	mmol/L

Stability and preparation of working reagent

Reagent R1: liquid, ready to use.

Reagent R2: liquid, ready to use.

Reagent R3: liquid, ready to use.

Note: Don't use if Sodium nitrite reagent develops to a dark yellow discoloration.

Specimen collection and handling

- Fresh non-hemolyzed serum is recommended.
- Plasma collected in EDTA, heparin, citrate or fluoride.
- Sample stability: 2 hour at 20 - 25 °C, 12 hours at 2 - 8 °C, and 3 months at - 20°C.
- Keep sample away from light and sunlight.

Calibrator

MediCal U Cat. No. 15011

Quality control

Meditrol N Cat. No. 15171

Meditrol P Cat. No. 15181

Procedure

Wavelength	T-Bil & D-Bil Hg 546 nm
Spectrophotometer	T-Bil & D-Bil 550 nm
Cuvette	1 cm light path
Temperature	37°C / 20 - 25 °C
Measurement	against assay blank
Reaction	End point

Assay

1. Total Bilirubin

	Blank	Assay
Reagent R1	1000 µl	1000 µl
Reagent R3	--	50 µl
Mix well, then add...		
Sample / Calibrator	100 µl	100 µl
Mix, incubate for 5 min. at 20 - 25 °C, Read absorbance against assay blank (A _{assay}). The final color is stable for 1 hour in the dark.		

2. Direct Bilirubin

	Blank	Assay
Reagent R2	1000 µl	1000 µl
Reagent R3	--	50 µl
Mix well, then add...		
Sample / Calibrator	100 µl	100 µl
Mix, Read absorbance against assay blank (A _{assay}) exactly after 5 min. at 20 - 25 °C or 4 min. at 37 °C.		

Calculation

Calculation using Calibrator:

$$\text{Conc. T-Bilirubin (mg/dl)} = \frac{A_{\text{Sample}}}{A_{\text{Calibrator}}} \times \text{Conc. Calibrator (mg/dl)}$$

$$\text{Conc. D-Bilirubin (mg/dl)} = \frac{A_{\text{Sample}}}{A_{\text{Calibrator}}} \times \text{Conc. Calibrator (mg/dl)}$$

Calculation using Factor:

$$\text{Conc. Bilirubin (mg/dl)} = A_{\text{Sample}} \times \text{Factor}$$

Factor	T. Bilirubin	D. Bilirubin
	12.4	11.5

Note: Each laboratory could make its own factor, under standardized conditions, for each lot of the reagent by using the following formula :

$$F = \frac{\text{Conc. Calibrator}}{A_{\text{Calibrator}}} \times 0.0585$$

µmol/L \longleftrightarrow mg/dl
17.1 X

Linearity

T.Bil. up to 20 mg/dl (340 μ mol/L).

D.Bil. up to 10 mg/dl (170 μ mol/L).

If the result exceeds 20 mg/dl, repeat the test by using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Interference

1. A number of drugs and substances affect bilirubin results. See Young, et al.
2. Hemolysis interferes with the test. Usually low values are obtained.
3. Lipemia causes false high values.
4. Light and sunlight cause false low value. Direct sunlight may cause up to a 50 % decrease in bilirubin within one hour.
5. Hepatotoxic drugs which cause cholestasis and hemolysis produce elevated recoveries.

Precautions

Reagents are toxic and corrosive.

Don't pipette by mouth.

Avoid contact with skin and clothing.

Reference range

Total Bilirubin

1 d.	< 5.0	mg/dl
2 d.	< 9.0	mg/dl
3 – 5 d.	< 12	mg/dl
Children	< 1.5	mg/dl
Adults	< 1.1	mg/dl

Direct Bilirubin

Adults	< 0.3	mg/dl
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References

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6. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C