

Bilirubin

Liquid stable reagent Colorimetric method (T & D)

PRINCIPLE

In the presence of caffeine, Total Bilirubin reacts with Diazotized sulfanilic acid. In the absence of caffeine, only the Direct Bilirubin reacts.

REFERENCE VALUES

Total Bilirubin	Up to 1.0 mg/dl (17 µmol/L)
Direct Bilirubin	Up to 0.25 mg/dl (4.3µmol/L)

These ranges are given for orientation only, each laboratory should establish its own normal ranges.

SAMPLES

Serum, heparinized or EDTA plasma. Bilirubin is light sensitive and it is recommended that serum be stored in the dark.

REAGENTS

R ₁	Sulfanilic acid	29 mmol/l
	Hydrochloric acid	0.17 mol/l
R ₂	Sodium nitrite	25 mmol/l
R ₃	Caffeine	0.26 mol/l
	Sodium benzoate	0.52 mol/l
R ₄	Tartrate	0.93 mol/l
	Sodium Hydroxide	1.9 mol/l

All reagents are ready to use and stable at 15-25°C until the expiry date stated on each label.

PROCEDURE

Total Bilirubin	578 nm
Direct Bilirubin	546 nm
Temperature	Room temperature
Method	Endpoint - increasing

Total Bilirubin

	Adult		Pediatric	
	Blank	Sample	Blank	Sample
R ₁	0.1 ml	0.1 ml	0.1 ml	0.1 ml
R ₂	-	25 µl	-	25 µl
Mix well before the addition of R3				
R ₃	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Sample	100 µl	100 µl	20 µl	20 µl
Mix and incubate 5 minutes at 15-25°C				
R ₄	0.5 ml 0.5 ml		.5 ml	

Mix and read the optical density (O.D) after 5 minutes incubation against the Blank Sample .

The final color is stable for at least 30 minutes.

Direct Bilirubin

	Blank	Sample
R ₁	0.1 ml	0.1 ml
R ₂	-	25 µl
Nacl (9 g/L)	1 ml	1 ml
Sample	100u1	100µ1

Mix and read the optical density (O.D) after 5 minutes incubation against the Blank Sample.

CALCULATION Total Bilirubin

Adult assay:

 $(\mu mol/L) = OD \times 185$ $(mg/dl) = OD \times 50$ Pediatric assay:

Direct Bilirubin

 $(mg/dl) = OD \times 14.4$ $(\mu mol/L) = OD \times 246$

 $(\mu mol/L) = OD \times 856$

 $(mg/dl) = OD \times 10.8$

Up to 25 mg/dl.

LINEARITY

SPECIFICATION

Lipid 4g/l, glucose 10g/l and ascorbic acid 0.5g/l don't interfere with the assay up to the given levels.

NOTES

- Bilirubine in serum stable for 4 days at 2-8°C and

1 days at 15-25°C (avoid direct exposure to light). - R4 contains sodium hydroxide which is caustic.

PRESENTATION			
2 X 120 ml	Cat No 0901	240 tests x2	

BIBLIOGRAPHY

- Jendrassik, L. and Grof. P., Biochem. Z. 81. 297(1938).

- Matimck R.G. Clin. Chim. Acta 13. 161 (1966).

The following symbols are used on labels

For in vitro diagnostic use

- Use day (last day of the month)
- LOT

IVD

- **Temperature limitation**
- **Batch code**
- REF Code