

**Quantitative determination of Total & Direct Bilirubin in serum**  
**Only for *In Vitro* Diagnostic use**

**ORDER INFORMATION**

REF	Cont.
TDBIL 100	2 X 50 ML
TDBIL 200	2 X 100 ML
TDBIL 1000	2 X 500 ML

**CLINICAL SIGNIFICANCE**

Bilirubin is a breakdown product of hemoglobin, insoluble in water. It is transported from the spleen to the liver and excreted into bile. Hyperbilirubinemia results from the increase of bilirubin concentrations in plasma. Causes of hyperbilirubinemia:

Total bilirubin: Increase hemolysis, genetic errors, neonatal jaundice, ineffective erythropoiesis, and drugs.

Direct bilirubin: Hepatic cholestasis, genetic errors, hepatocellular damage.

**PRINCIPLE**

Direct bilirubin in the sample reacts with diazotized sulfanilic acid forming a coloured complex that can be measured by spectrophotometry. Both direct and indirect bilirubin couple with diazo in the presence of Accelerator. The terms "direct" and "total" refer to the reaction characteristics of serum bilirubin in the absence or presence of solubilising (accelerating) reagents. The "direct" and "indirect" bilirubin is only approximately equivalent to the conjugated and unconjugated fractions.

**REAGENT COMPONENT**

Reagent 1 : Bilirubin Total Reagent.  
Reagent 2 : Bilirubin Direct Reagent.  
Reagent 3 : Total Nitrite Reagent.  
Reagent 4 : Direct Nitrite Reagent,

**SAMPLE COLLECTION AND STORAGE**

**Serum:** Fresh non-hemolized serum is the recommended sample. Separate serum from cells promptly to minimize Hemolysis.

**Storage:** Serum samples should be protected from light. Direct sunlight or white light exposure may cause a 50% decrease in bilirubin within one hour. Serum bilirubin is stable up to one week if stored at 2-8°C and for approximately three months if stored frozen and protected from light exposure.

**REAGENT PREPARATION**

**Working Reagent: Total bilirubin & Direct Reagent** – Mix Total/Direct reagent and Total nitrite/Direct nitrite in the ratio of 1:50 i.e for making 5 ml working reagent add 100 µl of respective nitrite reagent to 5.0 ml of Total/Direct reagent. This working reagent is stable for 7 days at 2-8°C. The reagent may develop slight yellow color upon storage but this does not affect the performance.

**REAGENT STORAGE AND STABILITY**

The reagents included are stable until the expiry date stated on the labels at 15-30°C.

**LINEARITY**

This method is linear to a concentration of 25 mg/dl. Samples exceeding linearity should be diluted with normal saline and repeated. Multiply the concentration by the dilution factor when calculating the unknown

AUTOMATED ARAMETERS	TOTAL	DIRECT
Wavelength I	546 nm	546 nm
Wavelength II	630 nm	630 nm
Cuvette Light Path	1 cm	1 cm
Reaction Type	End Point	End Point
Reaction Temperature	37°C	37°C
Measurement	Against Reagent blank	
Sample Volume	50 µl	50 µl
Reagent Volume	1000 µl	1000 µl
Incubation	5 mins.	5 mins.
Factor	24.00	20.00
Low Normal	0.0 mg/dl	0.0 mg/dl
High Normal	1.2 mg/dl	0.5 mg/dl
Linearity	25 mg/dl	25 mg/dl

**MANUAL ASSAY PROCEDURE**

**PIPETTE INTO TEST TUBES**

Working reagent	1000 µl
Sample	50 µl

Mix & Incubate for 05 min. at 37°C or 10 min. at R.T. Measure absorbance at 546/630 nm.

**CALCULATION**

Total Bilirubin (mg/dl) = Abs of test X 24
Direct Bilirubin (mg/dl) = Abs of test X 20

**REFERENCE VALUES**

Adults:	
Total	Up to 1.5 mg/dL = 17 mmol/L
Direct	Up to 0.5 mg/dL = 3.4 mmol/L

Newborns (total bilirubin) :		
Age	premature	full-term
Up to 24 h	1.0-8.0 mg/dL = 17-137 mmol/L	2.0-6.0mg/dL= 34-103 mmol/L
Up to 48 h	6.0-12.0 mg/dL = 103-205 mmol/L	6.0-10 mg/dL = 103-171 mmol/L
3-5 days	10-14 mg/dL = 171-239 mmol/L	4.0-8.0mg/dL= 68-137 mmol/L

**INTERFERENCES**

Hemolysis causes decreased bilirubin values

**Lipemia:** No interference from lipaemia, measured as triglycerides up to 23 mmol/L (2000 mg/dL).

**QUALITY CONTROL**

It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.

**BIBLIOGRAPHY**

1. Pearlman FC and Lee RTY. Detection and measurement of total bilirubin in serum, with use of surfactants as solubilizing agents. Clin Chem 1974; 20: 447-453.