

Total Bilirubin. DMSO.

Quantitative determination of total bilirubin total
Only for in vitro use in clinical laboratory
Store at 2-8°C

Ref.: BTC-021

2x125 mL R1
1x10 mL R2

TOTAL BILIRRUBIN**PRINCIPLE OF THE METHOD**

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically. Of the two fractions presents in serum, bilirubin-glucuronide and free bilirubin loosely bound to albumin, only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulphoxide (DMSO) to react (bilirubin indirect). In the determination of indirect bilirubin the direct is also determined, the results correspond to total bilirubin.
The intensity of the color formed is proportional to the bilirubin concentration in the sample^{1,2,3}.

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^{1,6,7}.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REACTIVOS

R 1	Sulfanilic acid	30 mmol/L
	Hydrochloric acid (HCl)	50 mmol/L
	Dimethylsulphoxide (DMSO)	7 mol/L
R 2	Sodium nitrite	29 mmol/L
Optional	BILIRUBIN CAL	

PRECAUTIONS

R1/RT: Corrosive (C):R35:Causes severe burns.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

PREPARATION

All the reagents are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Color development in R 2.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 555 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma, free of hemolysis¹. Protect samples from direct light.
Stability: Bilirubin is stable at 2-8°C for 4 days and 2 months at -20°C.

PROCEDURE

- Assay conditions:
Wavelength: 555 nm (530-580)
Cuvette: 1 cm light path
Temperature 15-25°C
- Adjust the instrument to zero with distilled water.
- Pipette into a cuvette:

	Blank	B. Total
R 1 (mL)	1.5	1.5
R 2 (µL)	--	50
Sample ^(Note 1) / Calibrator (µL)	100	100

- Mix and incubate for exactly **5 minutes** at room temperature.
- Read the absorbance (A).

CALCULATIONS**a. With Calibrator:**

$$\frac{(A) \text{ Sample} - (A) \text{ Sample Blank}}{(A) \text{ Calibrator} - (A) \text{ Calibrator Blank}} \times \text{Conc. Calibrator} = \text{mg/dL}$$

b. With Factor:

$$(A) \text{ Sample} - (A) \text{ Sample Blank} \times \text{Factor}^* = \text{mg/dL}$$

$$\text{*Factor: } \frac{\text{Concentration of Calibrator}}{(A) \text{ Calibrator} - (A) \text{ Calibrator Blank}}; \text{ Theoretical factor} = 19.1$$

Conversion factor: mg/dL x 17.1 = µmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES¹

Up to 1.10 mg/dL \cong Up to 18.81 µmol/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0.099 mg/L to linearity limit of 18 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	1.12	5.36	1.01	5.28
SD	0.02	0.12	0.03	0.12
CV (%)	2.33	2.27	2.70	2.32

Sensitivity: 1 mg/dL = 0.01540 A.

Accuracy: Results obtained using BSM reagents (y) did not show systematic differences when compared with other commercial reagents (x). The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.996.

Regression equation: y = 0.0208 + 0.9884 x.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemolysis causes decreased bilirubin values^{1,2,3}.

A list of drugs and other interfering substances with bilirubin has been reported by Young et. al^{4,5}.

NOTES

- For bilirubin determination in newborns, pipette 50 µL of sample. Multiply the result by 2.
- BSM has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

BIBLIOGRAPHY

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