Bilirubin Total MonlabTest®

DMSO. Colorimetric.

IVD

Quantitative determination of bilirubin total

Only for professional *in vitro* diagnostic use. Store at 2-8°C.

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-glucuromide 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 bilirrubin concentration in the sample[1,2,3].

CLINICAL SIGNIFICANCE

Bilirubin is a breakdown product of hemoglobin.

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 erythrpoiesis, 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.

REAGENTS

R 1	Sulfanilic acid Hydrochloric acid (HCl) Dimethylsulphoxide (DMSO)	30 mmol/L 50 mmol/L 7 mol/L
R 2	Sodium nitrite	29 mmol/L
Optional	**BILIRUBIN CAL**	Ref: MO-165109

PRECAUTIONS

R1: H290-May be corrosive to metals. H314-Causes severe burns and eye damage. EUH208-Contains sulphanilic acid. May produce an allergic reaction.

Follow the precautionary statements given in MSDS and label of the product.

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 2-8ºC, 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 hemolysis[1]. Protect samples from direct light.

Stability: Bilirubin is stable at 2-8ºC for 4 days and 2 months at –20ºC.

PROCEDURE

1. Assay conditions:
   Wavelength: ..... 555 nm (530-580)
   Cuvette: ..... 1 cm light path
   Temperature:..... 15-25ºC
2. Adjust the instrument to zero with distilled water.
3. 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

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

CALCULATIONS

– **With Calibrator:**

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

– **With Factor:**

(A) Sample - (A) Sample Blank x **Factor*** =mg/dL bilirubin in the sample

***Factor:** $\frac{\text{Concentration of Calibrator}}{(A)\text{Calibrator} - (A)\text{ Calibrator Blank}}$

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

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: CONTROL Normal and Pathologic (MO-165107 and MO-165108).

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 VALUES[1]

Total Bilirubin in adult	Up to 1.10 mg/dL	≅ Up to 18.81 µmol/L
Total Bilirubin in newborn	<12 mg/dL	< 205,2 µmol/L

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

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0.00526 mg/dL 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)	1.53	5.06	1.53	5.02
SD	0.03	0.05	0.03	0.11
CV (%)	1.73	1.01	1.92	2.18

Sensitivity: 1 mg/dL = 0.05074 A.

Accuracy: Results obtained using MONLABTEST 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 $(r)^2$: 0.991.

Regression equation: y= 0.82743x – 0.0382.

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[4,5].

NOTES

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

BIBLIOGRAPHY

1. Kaplan A et al. Bilirubin. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1238-1241. 436 and 650.
2. Malloy H T. et al. The determination of bilirubin with the photoelectric colorimeter. J. Biol Chem 1937; 112, 2; 481-491.
3. Martinek R. Improved micro-method for determination of serum bilirubin. Clin Chim 1966: Acta 13: 61-170.
4. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
5. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
6. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
7. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

PACKAGING

Ref.: MO-165073	Ref.: MO-165190
R1: 2 x 125 mL	R1: 4 x 250 mL
R2: 1 x 10 mL	R2: 3 x 10 mL

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

	Manufacturer	IVD	For i*n vitro* diagnostic use only
	Don't re-use		Consult instructions for use
Σ n	Contains sufficient for <n> tests		Keep dry
REF	Catalogue Code		Temperature limitation
LOT	Lot Number		Use by

Monlab SL Selva de Mar 48 08019 Barcelona (Spain) Tel. + 34 93 433 58 60 Fax +34 93 436 38 94 pedidos@monlab.com www.monlab.com