

PACKAGING

Ref.: 101-0422	Cont.: 3 x 100 mL
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Store at 2 - 8° C.

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 (T): Increase hemolysis, genetic errors, neonatal jaundice, ineffective erythropoiesis, and drugs. Direct bilirubin (D): Hepatic cholestasis, genetic errors, hepatocellular damage^{1,5,6}. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

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 dimethylsulfoxide (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}.

REAGENTS

R 1 (D)	Sulfanilic acid	30 mmol/L
	Hydrochloric acid (HCl)	150 mmol/L
R 2 (T)	Sulfanilic acid	30 mmol/L
	Hydrochloric acid (HCl)	50 mmol/L
	Dimethylsulfoxide (DMSO)	7 mol/L
R 3	Sodium nitrite	29 mmol/L

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 haemolysis (separated from red blood cells as soon as possible). Protect samples from direct light.
 Sample Stability (without red blood cells): 2-8° C for 4 days and 2 months at -20° C.

PRECAUTIONS

R1/R2: H290-May be corrosive to metals. H314-Causes severe burns and eye damage. EUH208-Contains sulphanic acid. May produce an allergic reaction. Follow the precautionary statements given in MSDS and label of the product.

PROCEDURE

Notes: For bilirubin determination in newborns, pipette 50 µL of sample. Multiply the result by 2.

CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers.

Instructions for many of them are available on request

- 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	Total B.	Blank	Direct B.
R 1 (D) (mL)	--	--	1,5	1,5
R 2 (T) (mL)	1,5	1,5	--	--
R 3 (µL)	--	50	--	50
Sample ^(Note 1) / Calibrator (µL)	100	100	100	100

- Mix and incubate exactly for **5 minutes** at 15-25° C.
- 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:**

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

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

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

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: Contro-N (Ref. 101-0083, 101-0252) and Contro-P (Ref. 101-0084, 101-0253). 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

Total Bilirubin	Up to 1.10 mg/dL ≅ (18,81 µmol/L)
Total Bilirubin in newborns	<12 mg/dL ≅ < 205,2 µmol/L
Bilirubin Direct	Up to 0.25 mg/dL ≅ (4,27 µmol/L)

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

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of (T) 0,00526 mg/dL (D) 0,07 mg/dL to linearity limit of 18 mg/dL (T) and 20 mg/dL (D).
 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:

Bilirubin T	Intra-assay (n=20)		Inter-assay (n=20)	
	Mean (mg/dL)	1,53	5,06	1,53
SD	0,03	0,05	0,03	0,11
CV (%)	1,73	1,01	1,92	2,18

Bilirubin D	Intra-assay (n=20)		Inter-assay (n=20)	
	Mean (mg/dL)	0,96	2,48	0,96
SD	0,024	0,051	0,043	0,035
CV (%)	2,52	2,06	4,49	1,41

Sensitivity: 1 mg/dL = 0,05074 A (T).

1 mg/dL = 0,06856 A (D).

Accuracy: Results obtained using CHRONOLAB reagents did not show systematic differences when compared with other commercial reagents.

The results obtained using 50 samples for Bilirubin D were the following:

Correlation coefficient (r): 0,96
 Regression equation: y=0,71177x - 0,05267

The results obtained using 50 samples for Bilirubin T were the following:

Correlation coefficient (r): 0,991
 Regression equation: y=82743x - 0,0382

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

INTERFERENCES

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

A list of drugs and other interfering substances with bilirubin determination has been reported^{3,4}.

BIBLIOGRAPHY

- Kaplan A et al. Bilirubin. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1238-1241. 436 and 650.
- Malloy H T. et al. The determination of bilirubin with the photoelectric colorimeter. J. Biol Chem 1937; 112, 2; 481-491.
- Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
- Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
- Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
- Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995