Total Bilirubin

DPD. Colorimetric

Quantitative determination of total bilirubin

CE IVD

PACKAGING

Ref.: 101-0782	Cont.: 1 x 240 / 1 x 60 mL

Store at 2-8º C

CLINICAL SIGNIFICANCE

Bilirubin is caused by the degradation of hemoglobin and exists in two forms. Unconjugated bilirubin is transported to the liver bound by albumin where it becomes conjugated (direct) with glucuronic acid and excreted. Hyperbilirubinemia is the result of an increase of bilirubin in plasma. Possible causes:

Total bilirubin: Increase hemolysis, genetic alteration, neonatal anemia, erythropoiesis alterations and presence of drugs.

Direct Bilirubin: cholestasis liver, liver abnormalities and genetic**.**

Clinical diagnosis should not be made based on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Bilirubin (both conjugated and unconjugated) couples with the diazo reagent in the presence of a surfactant to form azobilirubin. The intensity of color formed is proportional to the bilirubin concentration in the sample tested. The increase of absorbance at 546 nm is directly proportional to the total bilirubin concentration.

REAGENTS

R 1	Surfactants	< 1 %
	Hydrochloric acid (HCl)	160 mM
R 2	2,4-DPD	≥2 mM
	Hydrochloric acid (HCl)	120 mM
	Surfactant	< 1 %

PRECAUTIONS

R1/ R2: H290- Corrosive to metals. H314 - Irritation or skin corrosion.
R1: contains HCl and Triton X-114. R2: contains HCl and 2,4-DPD.
Follow the safety advice given in MSDS and product label.

PREPARATION

The reagents are provided in a ready to use format.

STORAGE AND STABILITY

The reagents are stable until the expiry date stated on the label when stored at 2-8º C, protected from light and contaminations are prevented during their use. Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.

ADDITIONAL EQUIPMENT

- Spectrophotometer or analyzer capable of measuring absorbance at 546 nm.
- Cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma, free of hemolysis. Protect samples from light**.**
Stability of the sample: 4 days at 2-8º C or 2 month at –20º C.

PROCEDURE

1. Assay conditions:
   Wavelength:…….…….. 546 nm (530-580)
   Cuvette……1 cm light path
   Temperature: ……..…….37º C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Calibrator blank	Sample blank
R 1 (µL)	800	800
Calibrator (µL)	40	-
Sample (µL)	-	40

4. Mix and incubate for **5 minutes** at 37º C.
5. Read the absorbance (A1) of the sample and calibrator.
6. Add:

	Calibrator	Sample
R 2 (µL)	200	200

7. Mix and incubate for **5 minutes** at 37º C.
8. Read the absorbance (A2) of the sample and calibrator against the blank.
9. Calculate the increase of the absorbance: ΔA= A2–A1.

CALCULATIONS

– With calibrator:

$$\frac{(\Delta A)\ Sample}{(\Delta A)\ Calibrator}$$ x Calibrator conc. = mg/dL of bilirubin in the sample

– With Factor: (ΔA) Sample x **Factor*** = mg/dL bilirubin in the sample

***Factor:** $$\frac{Calibrator\ \ concentra\ tion}{(\Delta A)\ Calibrator}$$

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. 101-0252 and 101-0083) and Contro-P (Ref. 101-0253 and 101-0084). 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

Total bilirubin 0.2-1.2 mg/dL (3.4 – 20,5 µmol/L)

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

PERFORMANCE CHARACTERISTICS

Measuring range: From *quantification limit* of 0.1 mg/dL to *linearity limit* of 30 mg/dL.
If the results obtained were greater than the linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Inter assay (n= 40)		Intra assay (n= 80)	
Mean (mg/dL)	1.169	5.0485	1.1682	5.0485
SD	0.0285	0.0594	0.012	0.046
CV (%)	2.4	1.2	1.0	0.9

Sensitivity*:* 1 mg/dL = 0.033 Abs. units.
Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x) on a Spintech 240 analyzer. The results obtained using 61 samples ranging from 0.42 a 19.36 mg/dL (7.18 to 331.056 µmol/L) were:
Correlation coefficient (r): 0.996
Regression equation: y= 0.9836 x + 0.1644
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Interferences from hemolysis, lipemia and a. ascorbic were evaluated for this total bilirubin method on a Spintech 240 analyzer. Two concentrations of total bilirubin were evaluated. No interferences were observed for lipemia (Intralipid) up to 1800 mg/dL, hemoglobin up to 2000 mg/dL and ascorbic acid up to 40 mg/L.
A list of drugs and other interfering substances with bilirubin has been reported by Young et. al [4,5].

NOTES

1. **CHRONOLAB has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

BIBLIOGRAPHY

1. David G Levitt and Michael D Levitt. Quantitative assessment of the multiple processes responsible for bilirubin homeostasis in health and disease . Clin Exp Gastroenterol. 2014; 7: 307–328.
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.

CHRONOLAB SYSTEMS, S.L., Travessia Prat de la Riba 34 B, 08849 Sant Climent de Llobregat, Barcelona, Spain
Tel. +34 617722466, www.chronolab.com, e-mail: info@chronolab.com