CHRONOLAB

CE
IVD

BILIRUBIN DIRECT
DMSO.
Colorimetric
Quantitative determination of bilirubin

PACKAGING

Ref.: 101-0447	Cont.: 3 x 100 mL
Ref.: 101-0598	Cont.: 8 x 100 mL

Store at 2-8º 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: 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.

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].

REAGENTS

R 1	Sulfanilic acid Hydrochloric acid (HCl)	30 mmol/L 150 mmol/L
R 2	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 2-8º 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 (530-580)
- 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 2-8º C for 4 days and 2 months at –20º C.

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.

PROCEDURE
Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.
For bilirubin determination in newborns, pipette 50 µL of sample. Multiply the result by 2.
Use clean disposable pipette for the dispension.

1. Assay conditions:
   Wavelength: ……555 nm (530-580)
   Cuvette: ……1 cm light path
   Temperature:……15-25º C
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 15-25º C.
5. Read the absorbance (A).

CALCULATIONS

– **With Calibrator:**

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

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

***Factor:**

$$\frac{Concentration\ of\ Calibrator}{(A)\,Calibrator - (A)\,Calibrator\,Blank}$$

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 VALUES [1]
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 0.07 mg/dL to linearity limit of 20 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)	0,96	2,48	0,96	2,50
SD	0,024	0,051	0,043	0,035
CV (%)	2,52	2,06	4,49	1,41

Sensitivity: 1 mg/dL = 0,006856 A.

Accuracy**:** Results obtained using CHRONOLAB 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): 0.96.
Regression equation: y= 0,71177x – 0,05267
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].

BIBLIOGRAPHY

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