CHRONOLAB

CE

IVD

# *BILIRUBIN TOTAL*
## *DMSO.*
## *Colorimetric*

*Quantitative determination of bilirubin*

***PACKAGING***

| | |
|---|---|
| Ref.: 101-0448 | Cont.: 3 x 100 mL |
| Ref.: 101-0599 | 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<br>Hydrochloric acid (HCl)<br>Dimethylsulphoxide (DMSO) | 30 mmol/L<br>50 mmol/L<br>7 mol/L |
| **R 2** | Sodium nitrite | 29 mmol/L |
| **Optional** | **BILIRUBIN CAL** | Not included in the kit |

***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.
- 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.

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 room temperature.
5. Read the absorbance (A).

***CALCULATIONS***

– **With Calibrator:**

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

– **With Factor:**

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

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

***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.

***EFERENCE VALUES [1]***

| | |
|---|---|
| Total Bilirubin | Up to 1.10 mg/dL (18,81 µmol/L) |
| Total Bilirubin in newborns | <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 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): 0.991.
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].

***BIBLIOGRAPHY***


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