

PACKAGING

| | |
|----------------|----------------------|
| Ref.: 101-0508 | Cont.: 2 x 150 tests |
|----------------|----------------------|

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: Increased hemolysis, genetic errors, neonatal jaundice, ineffective erythropoiesis, 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-glucuronide and free bilirubin loosely bound to albumin, only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with caffeine 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^{1,2,3}.

REAGENTS

| | | |
|-----------------|-------------------------|-------------------------|
| R 1 | Sulfanilic acid | 30 mmol/L |
| | Hydrochloric acid (HCl) | 400 mmol/L |
| R 2 | Sodium nitrite | 50 mmol/L |
| R 3 | Caffeine | 100 mmol/L |
| Optional | BILIRUBIN CAL | Not included in the kit |

Optional (not included in the kit)

| | | | |
|----------|----------------|-----------|---------------------------------|
| Contro-N | Ref.: 101-0252 | 4 x 5 mL | Lyophilized human control serum |
| | Ref.: 101-0083 | 20 x 5 mL | |
| Contro-P | Ref.: 101-0253 | 4 x 5 mL | Lyophilized human control serum |
| | Ref.: 101-0084 | 20 x 5 mL | |

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 540 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma, free of hemolysis¹. Protect samples from direct light. Stability: Bilirubin is stable at 2-8° C for 4 days and 2 months at – 20° C.

PRECAUTIONS

R1: Corrosive (C). R35: Causes severe burns.

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 4.

- Assay conditions:
 Wavelength: 540 nm
 Cuvette: 1 cm light path
 Temperature 15-25° C
- Adjust the instrument to zero with distilled water.
- Pipette into a cuvette:

| | B. Total | B. Direct | Blank |
|-----------------------------------|----------|-----------|-------|
| R 1 (µL) | 200 | 200 | 200 |
| R 2 (drop) | 1 | 1 | -- |
| NaCl 9 g/L (mL) | -- | 2.0 | 2.0 |
| R 3 (mL) | 2.0 | -- | -- |
| Sample / Calibrator (µL) (Note 1) | 200 | 200 | 200 |

- Mix and incubate for exactly **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:**

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

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

17.5

$$\text{Conversion factor: mg/dL} \times 17.1 = \mu\text{mol/L.}$$

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.

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 Total Up to 1.10 mg/dL (18.81 μ mol/L)

Bilirubin Direct Up to 0.25 mg/dL (4.27 μ mol/L)

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

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0.1 mg/L 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:

| Bilirubin D | Intra-assay (n=20) | | Inter-assay (n=20) | |
|-------------|--------------------|------|--------------------|------|
| | Mean (mg/dL) | 0.78 | 2.28 | 0.80 |
| SD | 0.01 | 0.01 | 0.01 | 0.03 |
| CV (%) | 1.28 | 0.65 | 1.63 | 1.53 |

| Bilirubin T | Intraserie (n= 20) | | Interserie (n= 20) | |
|-------------|--------------------|------|--------------------|------|
| | Mean (mg/dL) | 1.16 | 4.21 | 1.15 |
| SD | 0.02 | 0.04 | 0.02 | 0.13 |
| CV (%) | 2.03 | 1.06 | 1.91 | 3.10 |

Sensitivity: : (T) 1 mg/dL = 0,079 A. (D) 1 mg/dL = 0.087 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:

DIRECT BILIRUBIN

Correlation coefficient (r): 0.99.

Regression equation: $y = 0.9923x + 0.0048$.

TOTAL BILIRUBIN

Correlation coefficient (r): 0.99.

Regression equation: $y = 0.9832x + 0.0224$.

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 by Young et. al^{4,5}.

BIBLIOGRAPHY

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