



Store at: +2+8°C.

Presentation:

Cod. SU003 CONT: R 2 x 125 mL

Procedure

Quantitative determination of Direct Bilirubin.

Only for in vitro use in clinical laboratory (IVD)

TEST SUMMARY

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically. Of the two fractions presents in serum, bilirubin-gluconamide 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 bilirubin concentration in the sample^{1,2,3}.

REAGENTS COMPOSITION

R.1	Sulfanilic acid Hydrochloric acid (HCl)	30 mmol/L 150 mmol/L
R.2	Sodium nitrite	29 mmol/L
Optional	Bilirubin Calibrator.	20 mg/dl.

PRECAUTIONS

Hydrochloric acid (HCl): Irritant (Xi): R36/37/38 Irritating to eyes, respiratory system and skin. S26: S26: In case of contact with eyes, rinse immediately with plenty of water and sep medical advice.

REAGENT PREPARATION AND STABILITY

All the reagents (R.1) (R.2) are ready to use. 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.

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8°C. Do not use reagents over the expiration date.

SPECIMEN

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.

MATERIAL REQUIRED BUT NOT PROVIDED

- Spectrophotometer or colorimeter measuring at 555 nm.
- Matched cuvettes 1.0 cm. light path.

General laboratory equipment.

TEST PROCEDURE

1. Assay Conditions

- Wavelength : 555 nm. (530-580).
- Cuvette: 1 cm light path.
- Temperature 15-25°C.

- 2. Adjust the instrument to zero with distilled water.
- 3. Pipette into a cuvette:

	Blank	Sample
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:

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

With Factor:

$$\text{Bilirubin D (mg/dL)} = (A) \text{ Sample} - (A) \text{ Sample Blank} \times \text{Factor}^{*Note2}$$

Theoretical Factor = 14

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

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, Control Normal Ref. QC001 and Control Pathological Ref. QC002. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions

REFERENCE VALUES⁶

Serum or plasma:	
Bilirubin Direct	Up to 0.25 mg/dL \cong 4.27 µmol/L

(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE

Bilirubin is a breakdown product of hemoglobin, insoluble in water. 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 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.

REAGENT PERFORMANCE

- Measuring Range:

From detection limit of 0.04 mg/dL. to linearity limit of 25 mg/dL., under the described assay conditions.

If results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L. and multiply result by 2.

- Precision:

	Intra-assay n= 20		Inter-assay n= 20	
Mean (mg/dL)	0.63	2.28	0.68	2.53
SD	0.01	0.02	0.02	0.05
CV	1.91	1.10	2.51	1.95

- Sensitivity: 1 mg/dL. = 0.073 A/min.

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

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

INTERFERING SUBSTANCES

- Hemolysis causes decreased bilirubin values^{1,2,3}.
- A list of drugs and other interfering substances with bilirubin determination has been reported by Young et al. A^{4,5}.

NOTES

1. For bilirubin determination in newborns, pipette 50 µL of sample. Multiply the result by 2.
2. Factor = $\frac{\text{Concentration of Calibrator}}{(A)\text{Calibrator} - (A)\text{Calibrator Blank}}$

BIBLIOGRAPHY

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