Reactivos GPL

Barcelona, España

CE

- Bilirubin T&D-DMSO -

BILIRUBIN TOTAL + DIRECT

DMSO. Colorimetric.

Store at: +2+8º C.

Presentation:
Cod. SU005-SP CONT: *R1 1 x 50 mL.+ R2 1 x 50 mL. + R3 1 x 10 ml.*
Cod. SU005 CONT: *R1 1 x 125 mL.+ R2 1 x 125 mL. + R3 1 x 10 ml.*
Cod. SU005-B CONT: *R1 4 x 125 mL.+ R2 4 x 125 mL. + R3 4 x 10 ml.*

Procedure

Quantitative determination of 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-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 bilirubin concentration in the sample[1,2,3].

REAGENTS COMPOSITION

R.1 (Direct)	Sulfanilic acid Hydrochloric acid (HCl)	30 mmol/L 150 mmol/L
R.2 (Total)	Dimethylsulphoxide (DMSO) Sulfanilic acid Hydrochloric acid (HCl)	7 mol/L 30 mmol/L 50 mmol/L
R.3	Sodium nitrite	29 mmol/L
Optional	Bilirubin Calibrator (Note 3)	20 mg/dL

PRECAUTIONS
R1/R2: 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.

REAGENT PREPARATION AND STABILITY
All the reagents are ready to use.
Signs of Reagent deterioration:
- Presence of particles and turbidity.
- Colour development in R 3.

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

SPECIMEN
Serum or plasma, free of hemolysis[1] (separated from red blood cells as soon as possible). Protect samples from direct light.
Stability: Bilirubin is stable at 2-8º C for 4 days and 2 months at –20º 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 ..... 15-25ºC.
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette (Note 2):

	Blank	Total B.	Blank	Direct B.
R 1 (D) (mL)	--	--	1.5	1.5
R 2 (T) (mL)	1.5	1.5	--	--
R 3 (µL)	--	50	--	50
Sample (Note 1) / Calibrator (µL)	100	100	100	100

4. Mix and incubate for exactly **5 minutes** at room temperature.
5. Read the absorbance (A).

CALCULATIONS
- **With Calibrator:**

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

- **With Factor:**

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

$$\textbf{Factor*} = \frac{\text{Concentration of Calibrator}}{(A)\text{Calibrator} - (A)\text{Calibrator Blank}}$$

Theoretical factor: Bilirubin (T) = 19,1 ; Bilirubin (D) = 14

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

QUALITY CONTROL
Control sera are recommended to monitor the performance of the procedure, Control H Normal Ref. QC003 and Control H Pathological Ref. QC004. 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 VALUES[1]

Direct Bilirubin	Up to 0.25 mg/dL ≅ 4.27 µmol/L
Total Bilirubin in adults	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).

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

CLINICAL SIGNIFICANCE
Bilirubin is a breakdown product of haemoglobin, 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 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.

REAGENT PERFORMANCE
- *Measuring Range*:
  Bilirubin Total: From detection limit of 0.00526 mg/dL. to linearity limit of 18 mg/dL., under the described assay conditions.
  Bilirubin Direct: From detection limit of 0.07 mg/dL. to linearity limit of 20 mg/dL., under the described assay conditions.
  If results obtained were greater than linearity limit, dilute the sample ½ with NaCl 9 g/L. and multiply result by 2.
- *Precision*:

Bilirubin T	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
Bilirubin D	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:*
  Bilirubin T: 1 mg/dL. = 0.05074 A
  Bilirubin D: 1 mg/dL. = 0.06856 A.
- *Accuracy:* Results obtained GPL reagents did not show systematic differences when compared with other commercial reagents.
  The results obtained using 50 samples for Bilirubin were the following:
  Correlation coefficient $(r)^2$: 0.96 (D) / 0.991 (T)
  Regression equation:
  y= 0.71177x – 0.05267 (D)
  y= 0.82743x – 0.0382 (T)
  The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES
Hemolysis causes decreased Bilirubin values[1,2].
A list of drugs and other interfering substances with bilirubin determination has been reported by Young et. Al[3,4].

NOTES
1. For bilirubin determination in newborns, pipette 50 µL of sample. Multiply the result by 2.
2. Use clean disposable pipette for the dispensation.
3. Only to be used in bilirubin total.

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