# Reactivos GPL

**Barcelona, España**

CE

**-Bilirubin T-DMSO-**

# BILIRUBIN TOTAL

DMSO - Colorimetric.

*Store at: +2+8ºC.*

*Presentatión:*
*Cod. SU004* CONT: *R 2 x 125 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 bilirrubin concentration in the sample[1,2,3].

***REAGENTS COMPOSITION***

| | | |
|---|---|---|
| **R.1** | **Dimethylsulphoxide (DMSO)**<br>**Sulfanilic acid**<br>**Hydrochloric acid (HCl)** | **7 mol/L**<br>**30 mmol/L**<br>**50 mmol/L** |
| **R.2** | **Sodium nitrite** | **29 mmol/L** |
| **Calibrator Optional** | **Bilirubin Calibrator.** | **20 mg/dL** |

***PRECAUTIONS***

Hydrochloric acid (hcl): irritant (xi) r36/37/38 irritate eyes, skin and respiratory system. s26: in case of contact with eyes, rinse immediately with plenty of water and seek medical advice. seek medical advice.

***REAGENT PREPARATION AND STABILITY***

All the reagents are ready to use.
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, 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. Stored at tightly closed at 2-8ºC,. Do not use reagents over the expiration date.***

***SPECIMEN***

Serum or plasma, free of hemolysis[1]. Protect samples from direct light.
Stability: Bilirubin is stable at 2-8ºC for 4 days and 2 months at –20ºC.

***MATERIAL REQUIRED BUT NOT PROVIDED***

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

***General laboratory equipment.***

***TEST PROCEDURE***

1. Assay Conditions
- Wavelenght : ..... 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:

| | Blank | Sample |
|---|---|---|
| R.1 (mL.) | 1.5 | 1.5 |
| R.2 (µL.) | -- | 50 |
| Sample / Calibrator (µL.) (Note 1) | 100 | 100 |

4. Mix and incubate for exactly 5 minutes at room temperature.
5. Read the absorbance (A)
6.

***CALCULATIONS***

With Calibrator:

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

With Factor:

Bilirubin(mg/dL) =(A) Sample - (A) Sample Blank x **Factor***[Note2]

***THEORETICAL FACTOR = 19.1**

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 VALUES[1]**

| | |
|---|---|
| Bilirubin Total | Up to 1.10 mg/dL ≅ Up to 18,81 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 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*:
From detection limit of 0.5 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 ½ with NaCl 9 g/L. and multiply result by 2.

– *Precision*:

| | Intra-assay n= 20 | | Inter-assay n= 20 | |
|---|---|---|---|---|
| Mean (mg/dL) | 1.12 | 5.36 | 1.01 | 5.28 |
| SD | 0.02 | 0.12 | 0.04 | 0.12 |
| CV | 2.16 | 2.27 | 4.77 | 2.38 |

- Sensitivity:
1 mg/dL. = 0.0588 A
- 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***

– Interference:
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[2,3].

***NOTES***

1. For bilirubin determination in newborns, pipette 50 µL of sample. Multiply the result by 2.

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

***BIBLIOGRAPHY***


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