SPINREACT

CE BILIRUBIN T- DMSO

Bilirubin Total

DMSO. Colorimetric

Quantitative determination of bilirubin
IVD

Store at 2-8ºC

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 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]

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 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.

REAGENTS

R 1	sulfanilic acid Hydrochloric acid (HCl) Dimethylsulphoxide (DMSO)	30 mmol/L 50 mmol/L 7 mol/L
R 2	Sodium nitrite	20 mmol/L
Optional	**BILIRUBIN CAL**	Ref. 1002250

PRECAUTIONS

R1/R7: Corrosive (C) R35: Causes severe burns.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

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 2-8ºC, 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 R2.

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 2-8ºC for 4 days and 2 months at –20ºC.

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:

	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:

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

With Factor:

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

***Factor:** $\frac{\text{Concentration of Calibrator}}{(A)\text{Calibrator} - (A)\text{Calibrator Blank}}$; **Theoretical factor** = 19.1

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

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: SPINTROL H Normal and Pathologic (Ref. 1002120 and 1002130).
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 = Up to 18.81 µmol/L

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

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 0.099 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)	1.12	5.36	1.31	5.59
SD	0.02	0.12	0.03	0.12
CV (%)	2.33	2.27	2.70	2.32

Sensitivity: 1 mg/dL = 0.01540 A.
Accuracy: Results obtained using SPINREACT 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]: 0.998.
Regression equation: y= 0.0206 + 0.9684 x.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemolysis causes decreased bilirubin values[1,2].
A list of drugs and other interfering substances with bilirubin has been reported[4,5].

NOTES

1. For bilirubin determination in newborns, pipette 50 µL of sample. Multiply the result by 2.
2. **SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

BIBLIOGRAPHY

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2. Malloy H T et al. The determination of bilirubin with the photoelectric colorimeter. J. Biol Chem 1937; 112, 2: 481-491.
3. Marlinek R. Improved micro-method for determination of serum bilirubin. Clin Chim 1966; Acta 13: 65-170.
4. Young DS. Effects of drugs on Clinical Lab. Tests. 4th ed AACC Press, 1995.
5. Young DS. Effects of disease on Clinical Lab. Tests. 4th ed AACC 2001.
6. Burtis A et al. Tietz Textbook of Clinical Chemistry. 3rd ed AACC 1999.
7. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

PACKAGING

Ref: 1001042	Cont.	R 1: 2 x 150 mL R 2: 1 x 10 mL

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