Direct Bilirrubin. DMSO. Ref.: BDI-020
Quantitative determination of direct bilirrubin
Only for in vitro use in clinical laboratory
Store at 2-8°C 2x125 mL R1
1x10 mL R2

DIRECT BILIRRUBIN

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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-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].

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.

REAGENTS
R1 Sulfanílic Acid 30 mmol/L, Hydrochloric Acid 50 mmol/L.
R2 Sodium Nitrite 29 mmol/L.
Optional: **Bilirrubin Cal**. Bilirrubin 20 mg/dL.
PRECAUTIONS: Hydrochloric Acid: Irritant (Xi). R36/37/38: Irritating to eyes, respiratory system and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

PREPARATION
Reagents R1 & R2 are ready to be used.

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.

ADDITIONAL EQUIPMENT
- Spectrophotometer or colorimeter measuring at 555 ± 20 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 2-8°C for 4 days and 2 months at –20°C.

PROCEDURE
1. Assay conditions:
   Wavelength: ..... 555 ± 20 nm.
   Cuvette: ..... 1 cm light path
   Temperature ..... 15-25°C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette or in assay tubes:

	Blank	Sample
R1 (mL)	1,05	1,0
R2 (µL)	--	50
Distilled water / Sample / S (µL)	100	100

4. Mix and incubate for exactly 5 minutes at 15-25°C.
5. Read the absorbance (A).

CALCULATIONS
-With Calibrator:

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

-With Factor:
((A) Sample - (A) Sample Blank) x **Factor*** = mg/dL

$$\text{*Factor:} \frac{\text{Concentration of Calibrator}}{(A)\text{Calibrator} - (A)\text{ Calibrator Blank}} \text{ ; Theoretical factor = 14}$$

Conversion factor: mg/dL x 17.1 = µmol/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]
Up to 0.25 mg/dL ≈ 4.27 µmol/L.
These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
Measuring range: From detection limit of 0.04 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:	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	0.85	2.49	0.78	2.73
CV (%)	1.40	1.70	5.0	4.0

Accuracy: Results obtained using *BSM* reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained are available under request.

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].

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

NOTES
1. For bilirubin determination in newborns, pipette 50 µL of sample. Multiply the result by 2.
2. Aqueous calibrator calibration can give us some problems in automatic analyzers. In this case it is better using serum calibrators. (Biochemistry Calibrator. Ref. CAL-101)
3. Use clean dispensable tips for each determination.
4. BSM has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

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