Biomaghreb

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TOTAL and DIRECT BILIRUBIN

Reagents for the Quantitative Determination of Total Bilirubin (Accelerator: DMSO) and Direct Bilirubin in Human Plasma

IN VITRO DIAGNOSTIC USE

IVD

Total and Direct Bilirubin						
REF	**19011**	**2 x 160 ml** (160 T)	**R1:** 2 x 80 ml	**R2:** 2 x 80 ml	**R3:** 1 x 15 ml	**R4:** 1 lyophilisat
REF	**19028**	**2 x 250 ml** (250 T)	**R1:** 2 x 125 ml	**R2:** 2 x 125 ml	**R3:** 1 x 20 ml	**R4:**2 lyophilisats
Total Bilirubin						
REF	**18014**	**2 x 160 ml** (160 T)	**R1:** 4 x 80 ml	**R3:** 1 x 15 ml	**R4:**1 lyophilisat	
Direct Bilirubin						
REF	**17017**	**2 x 160 ml** (160 T)	**R2:** 4 x 80 ml	**R3:** 1 x 15 ml	**R4:** 1 lyophilisat	

CLINICAL SIGNIFICANCE

Bilirubin is a product of the degradation of hemoglobin. It is delivered in the blood in its «free» form, then transformed by the liver into «conjugated bilirubin» to be filtered by the kidney. The increase in bilirubin levels (bilirubinemia) causes jaundice, commonly called «jaundice», which can be caused by either unconjugated (free) bilirubin, conjugated bilirubin, or both fractions. Unconjugated bilirubin icters are primarily associated with increased red blood cell destruction (hemolysis) or decreased bile conjugation in the liver. Cholestasis, the reduction or cessation of bile secretion, is the most common cause of conjugated bilirubin icters.

PRINCIPLE

Sulfanilic acid reacts with sodium nitrite to give diazotized sulfanilic acid. In the absence of dimethyl sulfoxide only direct bilirubin couples with diazotized sulfanilic acid to give azobilirubin

REAGENT COMPOSITION

Reagent 1	Sulfanilic Acid Hydrochloric Acid Dimethylsulfoxyd	30 mmol/l 150 mmol/l 7 mmol/l
Reagent 2	Sulfanilic Acid Hydrochloric Acid	30 mmol/l 150 mmol/l
Reagent 3	Sodium Nitrite	20 mmol/l
Reagent 4 Standard	See Operating Procedure (preparation of standard R4)	

SAFETY CAUTIONS

Biomaghreb reagents are intended for use by qualified personnel for in vitro use (do not pipette by mouth).

- Refer to the current SDS available on request or at www.biomaghreb.com;
- Verify the integrity of the reagents before use; and
- Disposal of waste: comply with the legislation in force.

For safety reasons, treat any specimen or reagent of biological origin as potentially infectious. Respect the legislation in force.

SAMPLES

Serum or plasma collected on EDTA heparin, citrate or fluoride and stored in a dark place. Embarrassing hemolysis for Bilirubin.

ADDITIONAL EQUIPMENT

- Basic equipment of the medical analysis laboratory ;
- Spectrophotometer or Clinical Biochemistry Analyzer.

QUALITY CONTROL

External quality control program.
It is recommended to check in the following cases:

- At least one serial check;
- Changing the reagent bottle;
- After maintenance operations on the analyzer.

When a control value is outside the confidence limits, repeat the operation using the same control. Use normal and pathological control sera.

LINEARITY

TB: 20 mg/dl-200 mg/l (340 µmol/l) ;
DB: 10 mg/dl-100 mg/l (170 µmol/l).

PROCEDURE

- **Preparation of standard (R4)**

Reconstitute the lyophilisate R4 with exactly 3 ml of distilled water. Wait 15 minutes. Complete the dissolution of the lyophilisate by successively turning the vial over. The exact concentrations are indicated on each vial.
Stability in the dark after reconstitution is:
2 days at 20° - 25°C;
4 days at 2 - 8°C;
6 weeks at minus 20°C.
It is essential to establish a calibration factor under laboratory conditions as soon as the R4 is reconstituted.

$$F = \frac{\text{(Conc.Direct Bilirubin) standard}}{\text{(Abs (Standard) - Abs (Standard Blank)}}$$

- **Calibration**

Wavelength: 555 nm (546 Hg) ;
Temperature: 37°C ;
Tank : 1 cm thick ;
Analyzer zero: Standard Blank or sample Blank.

- **TOTAL BILIRUBIN**

Working Solution (TB)
Mix 20 volumes R1 with 1 volume R3.

Stability in the dark
6 Hours at 20 -25°C;
2 Days at 2-8°C.

	Standard		Sample	
	Blank	**Dosage**	**Blank**	**Dosage**
Standard R4	50 µl	50 µl		
Sample			50 µl	50 µl
Reagent R1	1 ml	1 ml		
Working Solution (T.B)			1 ml	1 ml
Mix and incubate exactly 5 minutes at 37°C. Read the absorbance (A) of the standard and the samples against their blanks				

N.B : Dilute newborn or very icteric samples 1/5 in a 9g/l NaCl solution.

- **DIRECT BILIRUBIN**

Working Solution (D.B)
Mix 20 volumes R2 with 1 volume R3.

Stability in the dark
6 Hours at 20 -25°C;
2 Days at 2-8°C.

	Standard		Sample	
	Blank	**Dosage**	**Blank**	**Dosage**
Standard R4	50 µl	50 µl		
Sample			50 µl	50 µl
Reagent R2	1 ml		1 ml	
Working Solution (D.B)		1 ml		1 ml
Mix and incubate exactly 5 minutes at 37°C. Read the absorbance (A) of the standard and the samples against their blanks				

- **CALCULATION (B.T et B.D.)**

$$[\text{Tot.or Dir.Bil.}] = \frac{\text{Abs (A) sample}}{\text{Abs (A) Standard}} \times [\text{Conc. standard}]$$

[Tot. or Dir.Bil.] = Abs (A) sample x F

NOTE :

µmol/l $\underset{1.710}{\overset{0.585}{\rightleftharpoons}}$ mg/l

REFERENCE VALUES

Total Bilirubin	0.2 - 1.0 mg/dl 2 - 10 mg/l 3.4 - 17 µmol/l
Direct Bilirubin	0.0 - 0.2 mg/dl 0.0 - 2 mg/l 0.0 - 3.4 µmol/l

REFERENCES

Hijmans Van den Bergh A.A., Muller P., Biochem, 77, 90 (1916) ;
Walters M.I., Gerarde R.W., Microchem 15, 231 (1970).

Manufacturer | Use by | In Vitro Diagnostic | Temperature Limitation | Catalogue number | See insert | Store away from light | Sufficient for < n > essais | Batch number