

IN VITRO DIAGNOSTIC USE



Total Bilirubin				
REF	18014	2 x 160 ml (160 T)	R1: 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 icterus 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 icterus.

PRINCIPLE

Sulfanilic acid reacts with sodium nitrite to give diazotized sulfanilic acid. In the presence of dimethyl sulfoxide total bilirubin couples with diazotized sulfanilic acid to give azobilirubin.

REAGENT COMPOSITION

Reagent 1	Sulfanilic Acid Hydrochloric Acid Dimethylsulfoxide	30 mmol/l 150 mmol/l 7 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.

SAMPLE PREPARATION

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

The method is linear up to 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.

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

• Calibration

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

• Working Solution (T.B)

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.

CALCULATION

$$[\text{Total.Bil}] = \frac{\text{Abs (A) Sample}}{\text{Abs (A) Standard}} \times [\text{Conc. standard}] \quad [\text{Total.Bil}] = \text{Abs (A) sample} \times F$$

• NOTE :

$$\begin{array}{c} 0.585 \\ \mu\text{mol/l} \rightleftharpoons \text{mg/l} \\ 1.710 \end{array}$$

REFERENCE VALUES

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