TOTAL BILIRUBIN

Colorimetric Method

Liquid Reagents ready to use

IVD

REF 22101160 R1 8X20mL
REF 11210020 R2 4X5mL

PRODUCT CODE: BIL-8D25

Company with Quality System ISO 9001 – ISO 13485Certified by LMS

INTENDED USE

Quantitative determination of Total Bilirubin in the serum.

CLINICAL SIGNIFICANCE

Bilirubin is a yellow pigment derived from the catabolism of the heme group (mainly from senescent red blood cells). It exists in two forms: indirect (unconjugated) bilirubin, which is transported to the liver bound to albumin, and direct (conjugated) bilirubin, which in the liver is conjugated with glucuronic acid, is water-soluble, and excreted with bile. The total of direct and indirect bilirubin is called total bilirubin, and the human body produces about 250 to 300 mg daily. An increase in total bilirubin in the blood may indicate alterations in the hemolysis process or genetic disorders.

PRINCIPLE

In an acid medium, total bilirubin reacts with diazotized sulphanilic acid to form a pink diazo compound (azobilirubin), whose intensity is proportional to the concentration of bilirubin present in the sample.
Direct bilirubin consists of glucoronic acid conjugated derivates, is water soluble and it reacts directly. Total bilirubin is obtained by the presence of a solubilizing agent that cleaves the bond with albumin.

SAMPLE

Not hemolyzed fresh serum.
Analyze samples within 2 hours after collection. Protect samples from light. Direct sunlight may cause up to 50% decrease in direct bilirubin within 1hour.
The samples are stable 12 hours at 2-8°C in the dark and one month at -20°C.

KIT COMPONENTS

Reagent (A) BIL-T Volume = 100 ml	Sulphanilic Acid Hydrochloric acid Solubillizing agent	46 mmol/l 270 mmol/l 10 mmol/l
Reagent (B) BIL-T Volume = 50 ml	Sodium nitrite	143 mmol/l

The reagents are stable until the expiration date when stored at temperatures as indicated on the label and protected from light. Do not use over expiry date.
Once opened reagents are stable for 2 months if contamination is avoided. Keep bottles closed when not in use.

REAGENT PREPARATION

Liquid Reagents, ready to use.

PRECAUTIONS AND WARNINGS

Reagent (A) :
EUH210 Safety data sheet available on request.
EUH208 Contains: SULFHANILIC ACID
May produce an allergic reaction.

Reagents may contain some non-reactive and preservative components. Use the normal precautions required in the laboratory.
Dispose of waste according to local laws.

PROCEDURE

Wavelength:	546 nm (520 - 550)
Lightpath :	1 cm
Temperature:	25, 37°C
Reading:	against blank reagent and blank sample
Method:	EndPoint with Sample Blank

Use as bi-reagent:

pipette:	blank	sample	calibrator
Reagent (A)	280 µl	280 µl	280 µl
Water	25 µl		
Sample		25 µl	
Calibrator			25 µl
Reagent (B)	20 µl	20 µl	20 µl

Incubate for 5 minutes at 37°C and read the absorbance of the sample (Ax) and the calibrator (Ac).

RESULTS CALCULATION WITH FACTOR (BI-REAGENT)

Total bilirubin mg/dl = (Ax – Abx) x 20 at 546 nm

RESULTS CALCULATION WITH CALIBRATOR

Total bilirubin mg/dl = (Ax – Abx)/(Ac – Abc) x Value Calibrator

Factor to convert mg/dl in µmol/l = 17.1

EXPECTED VALUES

adults: 0.2 – 1.2 mg/dl (3.4 – 20.5 µmol/l)

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL

You must perform the controls at each kit's use and verify that the values obtained are within the reference range reported in the operating instructions. For this purpose we recommend the use of Sicurobio control sera: ACUTROL 1 (REF. SB-8334) and ACUTROL 2 (REF. SB-8335).

PERFORMANCE

Sensitivity: the sensitivity of the method is: 0.06 mg/dl.
Linearity: the method is linear up to 25 mg/dl. For higher values, dilute with saline 1:2 the sample and multiply the result by 2.

Precision intra-assay:

	Level 1	Level 2	Level 3
Mean (mg/dl)	0.58	1.03	6.00
DS	0.019	0.020	0.052
CV %	3.27	1.93	0.86

Precision inter-assay:

	Level 1	Level 2	Level 3
Mean (mg/dl)	0.55	1.05	5.82
DS	0.011	0.029	0.104
CV %	1.98	2.75	1.78

Interferences: triglycerides up to 2000 mg/dl do not interfere. Hemoglobin up to 150 mg/dl does not interfere.
Correlation against a reference method: Y = 0.975x + 0.042
r = 0.9994

REFERENCES

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