Total BILIRUBIN

DPD Colorimetric method

R1: 2 x 100 ml + R2: 2 x 25 ml CL11-250

INTENDED USE

Kit for quantitative determination of Total Bilirubin in serum.

CLINICAL MEANING

The measurement of total and fractionated bilirubin is mainly carried out to determine the existence of any liver damage and disorders, in particular: obstruction of the biliary tract, haemolytic anaemia, metabolic problems, stones. Characteristic of people with high bilirubinemia is jaundice, which manifests itself with the typical yellow color of the skin and the whites of the eyes.

PRINCIPLE

In an acid medium, total bilirubin reacts with 3.5-DPD forming a red-colored diazo compound (azobilirubin), whose intensity is proportional to the concentration of direct bilirubin present in the sample.
While direct bilirubin, that is conjugated with glucuronic acid, is water-soluble and reacts directly, total bilirubin is obtained through the presence of an accelerator that splits the bond with albumin.
Indirect bilirubin can be calculated by the difference between total bilirubin and direct bilirubin.

SAMPLE

Non hemolyzed fresh serum.
Analyze samples within 2 hours after collection. Protect samples from light.
STABILITY: 12 hours in the fridge at 2-8°C, 3 months at -20°C if protected from light.

REAGENTS

Only for in Vitro diagnostic use. Liquid mono-reagent ready to use.

Package content		**CL11-250**
REAGENT 1 Hydrochloric acid 170 mM		**CL11-250R1** 2 x 100 ml
REAGENT 2 Surfactant Hydrochloric acid 170 mM 3,5-DPD 3 mM		**CL11-250R2** 2 x 25 ml

Stability: Store at 2-8°C and protect from light to keep the reagents stable up to the expiration date on the label. Keep bottles closed when not in use.

PREPARATION OF THE WORKING REAGENT

The reagents, clear and colorless, must be brought to room temperature before use.

MANUAL ASSAY PROCEDURE

Primary wavelength:	545-580 nm
Reference wavelength:	800 nm
Optical Path:	1 cm
Temperature:	37 °C
Reading:	Against Sample Blank
Reaction:	two final points
Ratio:	1/20/5

Bring reagents to the chosen temperature for the analysis.

Pipette in cuvette:

	Calibrator	Sample
Reagent 1	1 ml	1 ml
Sample	---	50 µl
Calibrator	50 µl	---

Stir carefully. After exactly 5 minutes of incubation at 37°C, read the Blank Calibrator (AbsBCal) and the blank sample (AbsBS) against water.

Add:

Reagent 2	250 µl	250 µl

Stir carefully. After exactly 5 minutes of incubation at 37°C, read the Calibrator (AbsCal) and the Sample (AbsS) against water

Reaction volumes can be proportionally varied without any change in calculation.

CALCULATION

Calculate the concentration in the sample using the following formula:

$$\frac{AbsS - AbsBS}{AbsCal - AbsBCal} \times Val.\ Cal = BIL\text{-}T\ (mg/dl)$$

REFERENCE VALUES

Total Bilirubin: 0,12-1,2 mg/dl (3,42-20,52 µmol/L).

Each laboratory should define its own reference values for this method.

QUALITY CONTROL – CALIBRATION

All Clinical Chemistry laboratories should implement a quality control program. Control serums of human origin are available for this purpose on request:
PRE-NORM (REF 7526) serums with normal values
PRE-PATH (REF 7528) serums with pathological values
If the method requires it, a multi-parameter calibrator of human origin is available (REF 7532).

PERFORMANCE CHARACTERISTICS

Measuring range/Linearity: 11 mg/dl.

Measurable limit: up to 20 mg/dl.
For higher values, dilute the samples with saline solution and multiply the result by the dilution factor.

Precision:

Within run (n=30)	Average [mg/dl]	CV %
Sample 1	0.71	2.7
Sample 2	5,6	1,2

Between run (n=20)	Average [mg/dl]	CV %
Sample 1	0.71	5,8
Sample 2	4.0	1.9

Correlation against a reference method: the correlation of FAR method (Y) against a reference method (X) gives this equation:

Y = 1,002x – 1,4 0 (n=30) r = 0.987 (n=30)

Interferences:

- up to 1000 mg/dl triglycerides do not interfere.
- up to 500 mg/dl of hemoglobin does not interfere.

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

The reagents, according to current legislation, are not classified as dangerous. The total concentration of inactive components (preservatives, detergents, stabilizers) is lower than the limits required for citation. However, handle the product with caution, according to good laboratory practice, avoiding ingestion, contact with skin, eyes and mucous membranes. All specimens should be treated as potentially HIV or hepatitis infected material.

REFERENCES

1. Ehrlich, P., Zeitschr, Sur Anal. Chemie 23: 275 (1884).
2. Vassault, A. et al. Ann. Biol. Clin.,44, 686 (1986).
3. Young, D.S., et al. Clin. Chem. 21:1D (1975).

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalog number
	temperature limits
	use by
	caution
	consult accompanying documents

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