

prietest™...Clinical Chemistry Reagents
BILIRUBIN TOTAL & DIRECT

In vitro diagnostic test kit, for professional use only

INTENDED USE : Quantitative in vitro determination of concentration of Bilirubin in serum on photometric systems.

ORDERING INFORMATION

Pack Size	Cat No.
2 X 50 ml	BIL TD 02 50
4 X 100 ml	BIL TD 04 100

CLINICAL SIGNIFICANCE : Red blood cells at the end of their circulating life are broken down in the reticulo-endothelial systems, mainly the spleen. The resulting haem, once the iron is removed, is then converted to bilirubin (yellow orange bile pigment). Water insoluble bilirubin is called indirect or un-conjugated bilirubin is then released into the blood stream where it binds tightly to albumin and is transported to the liver. In the liver un-conjugated bilirubin binds with glucuronic acid (mono and di glucuronides) to form conjugated bilirubin (direct bilirubin) by the enzyme glucuronyl transferase. Conjugated bilirubin or direct bilirubin is excreted via the biliary system into the intestine.

Total bilirubin is the sum of un-conjugated bilirubin and conjugated fractions. Bilirubin is elevated in the hemolysis or lysis of increased breakdown of red blood cells, hepatitis, cirrhosis azo obstruction of the biliary tract e.g. Gallstone.

METHOD : Modified Jendrassik & Grof.

PRINCIPLE :

In the determination of total bilirubin, bilirubin is coupled with diazotized sulphanilic acid in the presence of caffeine benzoate solution to produce azobilirubin which has maximum absorbance at 546 nm. Direct Bilirubin in presence of diazotized sulphanilic acid forms a red colored azo compound in acidic medium which has maximum absorbance at 546 nm.

REAGENTS

R1 : Sulphanilic acid	: 5 mmol/L
R2 : Sodium Nitrite	: 1.44 mmol/L
R3 : Caffeine	: 0.260 mol/l
R4 : Sodium Chloride	: 0.9%

Preservative & Stabilizer

STORAGE INSTRUCTIONS AND REAGENT STABILITY :

The reagents are stable up to the end of the indicated date of expiry on the vial label, if stored at 15 to 30°C, protected from light and contamination is avoided. Do not freeze the reagent!

WARNINGS AND PRECAUTIONS :

Take the necessary precautions for the use of laboratory reagents. Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water, flush with plenty of water while disposing. Do not use mouth pipette.

WASTE MANAGEMENT :

Please refer to local regulation requirements.

REAGENT PREPARATION :

The reagents are ready-to-use.

MATERIAL REQUIRED BUT NOT PROVIDED :

NaCl solution 9 g/l, General laboratory equipment, Analyser / Photometer, pipettes etc.

SPECIMEN :

Serum free from hemolysis.

Storage: Specimen should be protected from bright light as bilirubin is photo labile. Specimen may be stored refrigerated for 3 days.

ASSAY PROCEDURE : 1 - Total Bilirubin End Point (Differential)

Application sheets for automated systems are available on request.

Wavelength	: Hg 546 nm
Temperature	: Room Temperature
Cuvette	: 1 cm light path
Mode	: End Point, Differential

Bring all the contents of the kit to Room Temperature prior to use.

Read absorbance of Sample against Sample Blank.

Label the test tube as Sample Blank, Sample for every patient sample. Control Blank and control sample for every control sample. Pipette into respective test tube the reagent, sample, control sample as per the table given below :

	Sample Blank / Control Blank	Sample/ Control
Reagent R1	100 µl	100 µl
Reagent R2	—	50 µl
Reagent R3	1000 µl	1000 µl
Sample / Control	100 µl	100 µl

Mix, let stand at room temperature for 5 minutes and read the absorbance of sample against the sample blank.

CALCULATION :

Concentration in Sample (mg/dl) = (Abs. of Sample – Abs. of Sample Blank) X 1.4 (mg/dl)

Concentration in Sample (µmol/L) = (Abs. of Sample – Abs. of Sample Blank) X 239 (µmol/L)

ASSAY PROCEDURE : 2 - Direct Bilirubin End Point (Differential)

Wavelength	: Hg 546 nm
Temperature	: Room Temperature
Cuvette	: 1 cm light path
Mode	: End Point, Differential

Read absorbance of Sample against sample blank.

Label the test tube as Sample Blank, Sample for every patient sample. Control Blank and control sample for every control sample. Pipette into respective test tube the reagent, sample, control sample as per the table given below :

	Sample Blank / Control Blank	Sample/ Control
Reagent R1	100 µl	100 µl
Reagent R2	—	50 µl
Reagent R4	1000 µl	1000 µl
Sample / Control	100 µl	100 µl

Mix, let stand at room temperature for **EXACTLY 5 minutes** and read the absorbance of sample against the sample blank.

CALCULATION :

Concentration in Sample (mg/dl) = (Abs. of Sample – Abs. of Sample Blank) X 1.4 (mg/dl)

Concentration in Sample (µmol/L) = (Abs. of Sample – Abs. of Sample Blank) X 239 (µmol/L)

CONVERSION FACTOR : mg/dl X 17.1 = µmol/L

CALIBRATION :

For the calibration of automated photometric systems use of the commercially available calibrator is recommended.

QUALITY CONTROL :

To ensure adequate quality, use of the commercially available control sera is recommended.

PERFORMANCE CHARACTERISTICS:

MEASURING RANGE:

The test has been developed to determine Bilirubin concentrations within a measuring range from 0.05 to 25 mg/dl (0.85 to 427.5 µmol/L). When values exceed higher limit of the range, such samples should be diluted 1 + 1 with NaCl solution (9 g/l) and the result multiplied by 2.

SPECIFICITY / INTERFERENCE :

No interference was observed by Glucose upto 300 mg/dl (16.65 mmol/L), Hemoglobin up to 0.5 g/dl (5 g/L) and lipemia up to 600 mg/dl (6.78 mmol/L) of Triglycerides. A list of drugs and other interfering substances with Bilirubin determination has been reported by Young et al.

SENSITIVITY / LIMIT OF DETECTION :

The lower limit of detection is 0.05 mg/dl (0.85 µmol/L).

PRECISION :

Intra-assay precision n = 20	Mean [mg/dl]		SD [mg/dl]		CV [%]	
	T. Bil.	D. Bil.	T. Bil.	D. Bil.	T. Bil.	D. Bil.
Sample 1	1.021	1.03	0.01	0.01	0.97	0.97
Sample 2	0.912	2.26	0.007	0.03	0.77	1.33
Sample 3	0.956	0.54	0.008	0.01	0.84	1.85

METHOD COMPARISON :

A comparison between Robonik Prietest Bilirubin (y) and a commercially available test (x) using 20 samples gave following results:

Bilirubin Total	Bilirubin Direct
Linear Regression : y = 1.054x - 0.1668 mg/dl	Linear Regression : y = 1.0147x + 0.3825 mg/dl
Correlation Coefficient : r = 0.9948	Correlation Coefficient : r = 0.9939

REFERENCE RANGE :

Adults:	Bilirubin Total Up to 1.1 mg/dl (18.81 µmol/L)
	Bilirubin Direct Up to 0.25 mg/dl (4.27 µmol/L)
Premature:	0 to 1 day < 8.0 mg/dl (136.8 µmol/L)
	1 to 2 days < 12 mg/dl (205.2 µmol/L)
	3 to 5 days < 16 mg/dl (273.6 µmol/L)
Above 5 days	0.3 to 1.2 mg/dl (5.13 to 20.52 µmol/L)

It is recommended that each laboratory should assign its own reference range.

LITERATURE :

- Nuttall, K.L., Klee, G.G., *Analytes of haemoglobin metabolism-Porphyrins, Iron and Bilirubin*, Tietz Fundamentals of Clinical Chemistry, 5th Ed., Burtis, C. A. & Ashwood, E.R. (W.B. Saunders eds. Philadelphia USA).
- Tietz, N.W., *Clinical guide to laboratory tests*. 3rd Ed., (W.B. Saunders eds. Philadelphia USA).
- Vassault, A., et al., *Protocole de validation de techniques*. (Document B, stade 3), Ann. Biol. Clin., (1986)
- Young DS. *Effects of drugs on Clinical Lab. Tests*, 4th ed. AACCPress, 1995.

INSTRUMENT APPLICATION prietest TOUCH	
Name : T-BIL	Mod : DIFF
Pri.: 546	Sec.: 0
Temp: 30C	KF : 1.000
Vol : 500ul	Unit : mg/dl
Lag : 5	Read : NA
Blk : N, QC : Y, Norm : Y	
Std : N	Factor: 14
Normal HI = 1.1	
Normal LO = 0	
QCNH : *	
QCNL : *	
QCABH = *	
QCABL = *	
Rgnt. Linearity : 25	
NOTE :	
* Indicates user definable parameter.	
NA Implies Not Applicable	

PARAMETERS FOR INSTRUMENT SETTING	
TEST NAME	TOTAL BILIRUBIN
Reaction	End Point - Differential
Wavelength 1	546 nm
Temperature	30°C
Zero Setting	Distilled Water
Factor	14
Units	mg/dl
Sample Volume	100 µl
Reagent Volume	1000 µl
Incubation Time	5 minutes
Reference Range	0 to 1.1
Reagent Linearity	25

NOTE : Instrument application is similar for Direct Bilirubin Test except name of the test & Normal / Reference range. "Assay procedure 2" to be followed for Direct Bilirubin Test.

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prietest TOUCH is the Trade Mark of ROBONIK (INDIA) PVT. LTD., for Biochemistry Analyser.



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