ROBONIK®

CE

prietest™ ...Clinical Chemistry Reagents

BILIRUBIN TOTAL

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	BILT02 50
	4 X 100 ml	BILT04 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 glucoronic acid (mono and di glucuronides) to form conjugated bilirubin (direct bilirubin) by the enzyme gulcuronyl 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 and 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 546nm.

REAGENTS :

R1 : Sulphanilic acid	5 mmol/L
R2 : Sodium Nitrite	144 mmol/L
R3 : Caffeine	0.260 mol/l

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 2 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 : 37 °C
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 37°C for 5 minutes and read the absorbance of sample against the sample blank.

CALCULATION :
Concentration of sample = (Abs. of sample – Abs. of sample Blank) X 14

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 [%]
Sample 1	1.27	0.008	0.63
Sample 2	0.912	0.007	0.77
Sample 3	0.956	0.008	0.84

Inter-assay precision n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
Sample 1	2.26	0.03	1.33
Sample 2	4.04	0.04	0.99
Sample 3	4.35	0.05	1.15

METHOD COMPARISON :
A comparison between Robonik Prietest Bilirubin Total (y) and a commercially available test (x) using 20 samples gave following results:
Linear Regression : y = 1.054 x - 0.1668 mg/dl
Correlation Coefficient : r = 0.9948

REFERENCE RANGE :

Adults	Bilirubin Total Up to 1.1 mg/dl	(18.81 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 :

1. 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).
2. Tietz, N.W., Clinical guide to laboratory tests. 3rd Ed., (W.B. Saunders eds. Philadelphia USA).
3. Vassault, A., *et al., Protocole de validation de techniques.* (Document B, stade 3), Ann. Biol. Clin.,. (1986)
4. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed. AACC Press, 1995.

INSTRUMENT APPLICATION prietest TOUCH
Name : TBIL , Mod : DIFF
Pri.: 546 , Sec.: 0
Temp: 37C , 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	BILIRUBIN TOTAL
Reaction	End Point - Differential
Wavelength 1	546 nm
Temperature	37°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

ROBONIK®
An ISO 9001 : 2008 Certified Company
An ISO 13485 : 2012 Certified Company

Manufactured and Marketed by:
ROBONIK (INDIA) PVT. LTD.,
A-374, TTC Industrial Area, Mahape,
Navi Mumbai - 400 710, INDIA.
Tel. No.: + 91 (22) 67829700
Fax. No.: + 91 (22) 67829701
Orders : sales.reagents@robonikindia.com
Queries : feedback@robonikindia.com
Website : www.robonik.in

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