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

**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>Pack Size</th>
<th>Cat No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 X 50 ml</td>
<td>BIL TD Q4 100</td>
</tr>
</tbody>
</table>

**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 haemoglobin, 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 glucuronide (mono and di glucoronic acids) to form conjugated bilirubin (direct bilirubin) in 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 and other abnormalities of the biliary tract e.g. gallstones.

**METHOD:** Modified Jendrassik & Grof.

**PRINCIPLE:** In the determination of total bilirubin, bilirubin is coupled with diazotized sulfanilic acid in the presence of caffeine benzene solution to produce azobilirubin which has maximum absorbance at 546 nm. Direct Bilirubin in presence of diazotized sulfanilic acid forms a red coloredazo compound in acidic medium which has maximum absorbance at 546 nm.

**REAGENTS**

- **R1:** Sulfanilic acid : 5 mmol/L
- **R2:** Sodium Nitrite : 1.44 mmol/L
- **R3:** Caffeine : 0.260 mol/L
- **R4:** Sodium Chloride : 0.9%

**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.

- **Wave length** : 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:

<table>
<thead>
<tr>
<th>Sample Blank / Control Blank</th>
<th>Sample / Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

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 4 (mg/dl)

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

**CONVERSION FACTOR:** 1 mg/dl = 17.1 µmol/L

**CALIBRATION:**

For the calibration of automated photometric systems use 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 AND 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:**

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean (mg/dl)</th>
<th>SD (mg/dl)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>1.021</td>
<td>1.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.912</td>
<td>2.26</td>
<td>0.07</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0.955</td>
<td>0.54</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**METHOD COMPARISON:**

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

- **Bilirubin Total**
  - Linear Regression: y = 1.054x - 0.1668 mg/dl, r = 0.9948
  - Correlation Coefficient = 0.9918

- **Bilirubin Direct**
  - Linear Regression: y = 0.260x - 0.1668 mg/dl, r = 0.9948
  - Correlation Coefficient = 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 2 days < 8.0 mg/dl (136.8 µmol/L)
- 1 to 2 days < 12 mg/dl (205.2 µmol/L)
- 3 to 6 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 normal range.

**LITERATURE:**


**INSTRUMENT APPLICATION**

**prietest TOUCH**

**PARAMETERS FOR INSTRUMENT SETTING**

<table>
<thead>
<tr>
<th>TEST NAME</th>
<th>TOTAL BILIRUBIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction</td>
<td>End Point - Differential</td>
</tr>
<tr>
<td>Wavelength</td>
<td>546 nm</td>
</tr>
<tr>
<td>Temperature</td>
<td>30°C</td>
</tr>
<tr>
<td>Zero Setting</td>
<td>Distilled Water</td>
</tr>
<tr>
<td>Factor</td>
<td>14</td>
</tr>
<tr>
<td>Units</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Sample Volume</td>
<td>100 µl</td>
</tr>
<tr>
<td>Reagent Volume</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Incubation Time</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Reference Range</td>
<td>0 to 1.1 mg/dl</td>
</tr>
<tr>
<td>Reagent Linearity</td>
<td>25</td>
</tr>
</tbody>
</table>

**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.

**prietest** is the Trade Mark of ROBONIK (INDIA) PVT. LTD., for Clinical Chemistry Reagents.

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

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Queries: feedback@robonikindia.com
Website: www.robonik.in

**AN ISO 9001 : 2008 Certified Company**

**AN ISO 13485 : 2003 Certified Company**

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**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)**

**Concentration in Sample (mg/dl) = (Abs. of Sample – Abs. of Sample Blank) X 1.4 (mg/dl) x 17.1 = µmol/L**

**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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**REFERENCE:**

1. Jendrassik & Grof.