Total Bilirubin

**INTENDED FOR USE:**
Reagent for quantitative determination of total bilirubin in human serum or plasma.

**CLINICAL SIGNIFICANCE (1)**
At least four distinct bilirubin species exist in the serum: Direct-reacting bilirubin (DB) consists of mono and diconjugated bilirubin (beta and y-Bilirubin) and the d-fraction which is bilirubin tightly bound to albumin; unconjugated u-bilirubin which is water soluble and bound to albumin. Total bilirubin (TB) is the sum of these different species.

There are icteruses in which unconjugated bilirubin predominates (hemolytic icteruses, Biermer disease, Thalassemia...); icteruses in which conjugated bilirubin predominates (extra or intra-hepatic bile ducts obstruction, viral hepatitis...); finally, icteruses in which both species of bilirubin are present without any predominance (cirrhosis, Dubin-Johnson disease).

**PRINCIPLE (4) (5)**
Method based on Rand and Di Pasqua principle automated by Golub and al. The reaction between TB and diazotised dichloroaniline leads, when a solvent or a detergent is present, to a compound, azobilirubin which absorbance, directly proportional to the concentration of TB in the specimen, is measured at 550 nm (540-560).

**REAGENTS**

<table>
<thead>
<tr>
<th></th>
<th>TOTAL BILIRUBIN</th>
<th>NITRITE SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td></td>
<td></td>
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</tbody>
</table>

**SAFETY CAUTIONS**
Reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not petetise by mouth.
- In case of contact with skin and eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- For further information, Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

**REAGENT PREPARATION**
Reagents are ready for use.

**STABILITY AND STORAGE**
Store away from light, well cap in the original vial at 2-8°C
- Reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert and free from contamination.
- Discard any reagent if cloudy or if absorbance of the working reagent is > 0.100 at 550 nm.
- Don’t use reagent after expiry date stated on the label of the kit.

**SPECIMEN COLLECTION AND HANDLING (2) (6)**
Unhemolysed serum or plasma. Bilirubin is photolable. Store the specimen away from light.

- Stability in the specimen : 4 to 7 days at 2-8°C.
- 2 days at room temperature.
- Icteric or pediatric specimens : see at MANUAL PROCEDURE.

**INTERFERENCES (3) (4)**
Hemolysis leads to under-estimated results.
The bilirubin diazo reaction is temperature sensitive and should be carried out at a constant temperature.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

**MATERIAL REQUIRED BUT NOT PROVIDED**
1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.

**CALIBRATION (7)**
The calibration frequency depends on proper instrument functions and on the preservation of reagents.

It is recommended to calibrate in the following cases:
1. When using a new batch of reagent.
2. After maintenance operations on the instrument.
3. When control values obtained are out of ranges, even after using a new vial of fresh serum.

**QUALITY CONTROL**
- Assayed control sera referring to the same method and to the selected procedure.
- External quality control program.

It is recommended to control in the following cases:
- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument. If control is out of range, apply following actions:
  1. Repeat the test with the same control.
  2. If control is still out of range, prepare a fresh control serum and repeat the test.
  3. With factor : Verify analysis parameters (Wavelength, temperature, specimen/reagent ratio, time counting, calibration factor).
  4. Use a new vial of reagent and repeat the test.
  5. With a calibrator: If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
  6. If control is still out of range, calibrate again with a new vial of reagent and repeat the test.
  7. If control is still out of range, please contact BIOMED technical support or your local Agent.

**EXPECTED VALUES (2)**

<table>
<thead>
<tr>
<th>Total Bilirubin</th>
<th>mg/dL</th>
<th>[μmol/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full term</td>
<td>[≤ 34]</td>
<td>[≤ 2.0]</td>
</tr>
<tr>
<td>Premature</td>
<td>[≤ 34]</td>
<td></td>
</tr>
<tr>
<td>0-1 day</td>
<td>&lt; 8.0</td>
<td>1.4-8.7</td>
</tr>
<tr>
<td>1-2 days</td>
<td>&lt; 12.0</td>
<td>3.4-11.5</td>
</tr>
<tr>
<td>3-5 days</td>
<td>&lt; 16.0</td>
<td>1.5-12.0</td>
</tr>
</tbody>
</table>

**Total bilirubin**

<table>
<thead>
<tr>
<th>Adult (and child &gt; 5 days)</th>
<th>mg/dL</th>
<th>[μmol/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;5 days-60 years</td>
<td>0.3-1.2</td>
<td>[5-21]</td>
</tr>
<tr>
<td>60-90 years</td>
<td>0.2-1.1</td>
<td>[3-19]</td>
</tr>
<tr>
<td>&gt; 90 years</td>
<td>0.2-0.9</td>
<td>[3-15]</td>
</tr>
</tbody>
</table>

Each laboratory should establish its own normal ranges for the population that it serves.
LINEARITY
Procedure n°1 : to 20 mg/dL (342 Mmol/L).
Above, do not dilute the specimen : perform procedure n°2. Procedure n°2 : up to 100 mg/dL (1710 Mmol/L)
Pediatric specimen : perform procedure n°2

PERFORMANCES (PROCEDURE N°1)

<table>
<thead>
<tr>
<th>High level</th>
<th>Medium level</th>
<th>Within run N = 20</th>
<th>High level</th>
<th>Medium level</th>
<th>Between run N = 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.28</td>
<td>1.23</td>
<td>Mean mg/dL</td>
<td>3.48</td>
<td>1.20</td>
<td>Mean mg/dL</td>
</tr>
<tr>
<td>0.090</td>
<td>0.018</td>
<td>S.D. mg/dL</td>
<td>0.15</td>
<td>0.059</td>
<td>S.D. mg/dL</td>
</tr>
<tr>
<td>1.7</td>
<td>1.5</td>
<td>C.V. %</td>
<td>2.79</td>
<td>4.87</td>
<td>C.V. %</td>
</tr>
</tbody>
</table>

Detection limit: approximately 0.1 mg/dL Sensitivity for 1 mg/dL : 70 mAbs at 550 nm.
Comparison study with commercially available reagent : y = 1.0042 x + 0.015 r = 0.9980

MANUAL PROCEDURE
Let stand reagents and specimens at room temperature.

Procedure n1:
TOTAL BILIRUBIN
Blank
Assay
1 mL 1 ml Reagent R1
50 ul 50 ul Reagent R2
100 ul 100 ul Specimen
Pipette into well identified test tubes:
Mix and read absorbances at 550 nm (540-560) after 5 minutes at 37°C against blank.

Procedure n2: Icteric or Pediatric Specimens
TOTAL BILIRUBIN
Blank
Assay
1 mL 1 ml Reagent R1
50 ul 50 ul Reagent R2
20 ul 20 ul Specimen
Pipette into well identified test tubes:
Mix and read absorbances at 550 nm (540-560) after 15 minutes at room temperature against blank.

1. The colour developed by the reaction is stable for 1 hour away from light.
2. Specific procedures are available upon request for automated instruments. Please contact BIOMED technical support.
3. To take into account the colration of the working reagent, one should perform a reagent blank

CALCULATION
Calculate the result as follows:

With a calibrator (Procedure n1 only):
Result = \[ \frac{\text{Abs (Assay - Blank) specimen}}{\text{Abs (Assay - Blank) calibrator}} \] x calibrator concentration

with factor:
Procedure n°1: mg/dL = [Abs. Assay – Abs. Blank] x 11.8*
Mmol/L = [Abs. Assay – Abs. Blank] x 255*
Procedure n°2: mg/dL = [Abs. Assay – Abs. Blank] x 58.6*
Amol/L = [Abs. Assay – Abs. Blank] x 1173*

*These factors should be used as a guide only and may vary with instrument and the batch of reagent used. It is recommended to verify it with elevated control serum.

REFERENCES
(3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-90 to 3-110
(7) SRM: Standard Reference Material®