Intended Use
BioMed Diagnostics bilirubin reagent is intended for the in-vitro Quantitative, diagnostic determination of bilirubin in human serum on both automated and manual systems.

Background
The average level of the bilirubin produced in humans from different Sources range between 250 to 300 mg/day, of which 85% is derived From the heme moiety of the haemoglobin released from senescent Erythrocytes that are destroyed in the reticuloendothelial system. The remaining 15% is produced from erythrocytes destroyed in the Bone marrow and from catabolism of other heme containing proteins such as cytochromes and myoglobin.

After it is produced in the peripheral tissues, bilirubin is transported to the liver in association with albumin. In the liver, bilirubin is Conjugated with glucuronic acid for solubilization and subsequent Transport through the bile duct and elimination via the digestive tract. Disease or conditions which, through hemolytic processes, Produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Bile duct obstruction or damage to hepatocellular structure causes Increases in the levels of both conjugated (direct) and unconjugated (Indirect) bilirubin in the circulation.

Method
DMSO. Colorimetric method.

Assay Principle
Bilirubin is converted to colored diazotized sulfanilic acid and Measured photometrically. Of the two fractions presents in serum, bilirubin glucuroamide and free bilirubin loosely bound to albumin. Only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulfoxide (DMSO) to react (bilirubin indirect). In the determination of indirect bilirubin the direct is also determined, the results correspond to total bilirubin.

Reagents
Reagent 1 (R1) D- Bilirubin
Sulfanilic acid 30 mmol/l
HCL 150 mmol/l
Reagent 2 (R2) T- Bilirubin
Sulfanilic acid 30 mmol/l
HCL 150 mmol/l
Dimethylsulfoxide(DMSO) 7 mol/l
Reagent 3 (R3) Sodium Nitrite 29 mmol/l

Precautions and Warnings
R1/R2/RT: Corrosive (C)
R3C Causes severe burns.
S28 After contact with skin, wash immediately with plenty of soap and water.
Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Reagent Preparation, Storage and Stability
BioMed bilirubin reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when stored at 2 - 8 oC

Deterioration
Do not use the BioMed bilirubin reagents if precipitate forms. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation
Avoid exposure of the specimen to light. If plasma is used, only Heparin and oxalate plasma are suitable. Other anticoagulants should Not be used. The average half-life of total bilirubin and direct bilirubin In serum is 17 days and few hours respectively.

Stability:
<table>
<thead>
<tr>
<th></th>
<th>-20 oC</th>
<th>4 – 8 oC</th>
<th>20 – 25 oC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>6 months</td>
<td>7 days</td>
<td>1 day</td>
</tr>
<tr>
<td>Direct</td>
<td>6 months</td>
<td>7 days</td>
<td>2 days</td>
</tr>
</tbody>
</table>

Procedure
Direct Bilirubin

Sample blank  Sample
Reagent 1 (D)  1 ml  1 ml
Reagent 3  50 µ  50 µ
Sample  50 µ  50 µ

dMix and incubate for 5 minutes at 20 – 25 oC. Measure absorbance of sample (A Sample) against sample blank at 546 nm (530 - 580 nm)

Total Bilirubin

Sample blank  Sample
Reagent 2 (T)  1 ml  1 ml
Reagent 3  50 µ  50 µ
Sample  50 µ  50 µ

Mix and incubate for exactly 5 minutes at 20 – 25 oC. Measure absorbance of sample (A Sample) against sample blank at 546 nm (530 - 580 nm).

Calculation

\[(A) \text{Sample} - (A) \text{Sample blank} \times \text{Factor} = \text{mg/dl}\]

*Theoretical Factor
Direct bilirubin = 14
Total bilirubin = 19.1
Conversion Factor = mg/dl \times 17.1 = \mu mol/L.

Note
For bilirubin determination in newborns, pipette 50 µ of sample. Multiply the result by 2.

Quality Control
Normal & abnormal commercial control serum of known concentrations Should be analyzed with each run.
Performance Characteristics

**Precision**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Direct</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level 1</td>
<td>Level 2</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (mg/dL)</td>
<td>0.79</td>
<td>4.37</td>
</tr>
<tr>
<td>SD</td>
<td>0.016</td>
<td>0.18</td>
</tr>
<tr>
<td>CV%</td>
<td>2.13</td>
<td>4.12</td>
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</table>

Run to run (Reproducibility)

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Direct</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Level 1</td>
<td>Level 2</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (mg/dL)</td>
<td>0.82</td>
<td>4.52</td>
</tr>
<tr>
<td>SD</td>
<td>0.02</td>
<td>0.27</td>
</tr>
<tr>
<td>CV%</td>
<td>2.24</td>
<td>4.21</td>
</tr>
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Methods Comparison

A comparison between BioMed Diagnostics Bilirubin and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.975 was obtained.

Sensitivity

When run as recommended, the sensitivity of this assay is 0.1 mg/dL (1.7 µmol/L) for total and 0.04 mg/dL (0.68 µmol/L) for direct bilirubin.

Linearity

The reaction is linear up to a total bilirubin concentration of 18 mg/dL (308 µmol/L) and a direct bilirubin concentration of 18 mg/dL (308 µmol/L). Specimens showing higher concentrations should be diluted 1+4 with physiological saline and repeat the assay (result × 5).

Interfering substances Serum, plasma

**Haemolysis**

Avoid haemolysis since it interferes with the test.

**Lipemia**

Lipemic specimens interfere with the test.

**Drugs**

Theophyllin and propranolol may cause artificially low total bilirubin levels.

Expected Values

<table>
<thead>
<tr>
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<th>(4.0 – 8.0 mg/dL)</th>
<th>(6.0 – 10.0 mg/dL)</th>
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<tbody>
<tr>
<td>Newborns:</td>
<td>3.4 - 17 µmol/L)</td>
<td>(103-171 µmol/L)</td>
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<tr>
<td>(&lt;48 h)</td>
<td>(34-103 µmol/L)</td>
<td></td>
</tr>
<tr>
<td>(&lt;24 h)</td>
<td>2.0-6.0 mg/dL</td>
<td></td>
</tr>
<tr>
<td>Direct Bilirubin</td>
<td>0 – 0.3 mg/dL</td>
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BioMed Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

**Analytical Range**

Total bilirubin : 0.1 – 18 mg/dL (1.7 – 308 µmol/L)
Direct bilirubin : 0.04 – 18 mg/dL (0.68 – 308 µmol/L)

**Waste Disposal**

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal. S56: dispose of this material and its container at hazardous or special waste collection point. S57: use appropriate container to avoid environmental contamination. S61: avoid release in environment. refer to special instructions/safety data sheets.

**References**


**ORDERING INFORMATION**

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<th>CATALOG NO.</th>
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<td>60 test</td>
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<tr>
<td>BIL099250</td>
<td>150 test</td>
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