**Bilirubin (DIRECT)**

**Colorimetric**

REF: 224 001 120 Test

R1 Direct Bilirubin 2 x 90 ml
R2 Nitrite 1 x 7 ml

**Intended Use**

Spectrum 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 bilirubin produced in humans from different sources ranges 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**

Colorimetric method.

**Assay Principle**

Bilirubin is converted to colored diazotized sulfanilic acid and measured photometrically. Of the two fractions presents in serum, bilirubin glucuromide and free bilirubin loosely bound to albumin, only the former reacts directly in aqueous solution (bilirubin direct).

**Reagents**

Reagent 1 (R1) D- Bilirubin
- Sulfanilic acid 30 mmol/l
- HCL 150 mmol/l

Reagent 2 (R2)
- Sodium Nitrite 29 mmol/l

**Precautions and Warnings**

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**

Spectrum bilirubin reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when stored at 2 - 8 °C.

**Deterioration**

Do not use the Spectrum 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>Temperature</th>
<th>Direct Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20 °C</td>
<td>6 months</td>
</tr>
<tr>
<td>4 – 8 °C</td>
<td>7 days</td>
</tr>
<tr>
<td>20 – 25 °C</td>
<td>2 days</td>
</tr>
</tbody>
</table>

**Procedure**

**Direct Bilirubin**

<table>
<thead>
<tr>
<th>Sample blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 ml</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>-----</td>
<td>50 µl</td>
</tr>
<tr>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

Mix and incubate for 5 minutes at 20 – 25 °C. Measure absorbance of sample (Asample) against sample blank at 546 nm (530 - 580 nm).

**Calculation**

\[
\left(\frac{A_{\text{Sample}} - A_{\text{Sample blank}}}{\text{Factor}}\right) \times \text{mg/dl}
\]

*Theoretical Factor

Direct bilirubin = 14

Conversion Factor = mg/dl x 17.1 = µmol/l

**Note**

For bilirubin determination in newborns, pipette 50 µl 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
Within run (Repeatability)

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean (mg/dL)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>0.299</td>
<td>0.016</td>
<td>5.41</td>
</tr>
<tr>
<td>Level 2</td>
<td>0.77</td>
<td>0.057</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Run to run (Reproducibility)

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean (mg/dL)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>0.32</td>
<td>0.023</td>
<td>5.57</td>
</tr>
<tr>
<td>Level 2</td>
<td>0.82</td>
<td>0.062</td>
<td>8.1</td>
</tr>
</tbody>
</table>

Methods Comparison
A comparison between Spectrum 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 direct bilirubin concentration of 18 mg/dL (308 μmol/L). Specimens showing higher concentration 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

Direct Bilirubin
0 – 0.3 mg/dL (0 – 51 μmol/L)