Bilirubin (TOTAL AND DIRECT)
Jendrassik Grof

REF: 222 001 (255ml) 100 Test REF: 222 002 (750ml) 300 Test

R1 Sulphanilic Acid 1 x 45 ml R1 Sulphanilic Acid 2 x 65 ml
R2 Nitrite 1 x 10 ml R2 Nitrite 2 x 15 ml
R3 Caffeine 1 x 100 ml R3 Caffeine 3 x 100 ml
R4 Tarrtarate 1 x 100 ml R4 Tarrtarate 3 x 100 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 the 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 by 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 Diazo method.

Assay Principle
The total bilirubin concentration is determined in presence of caffeine by the reaction with diazotized sulphanilic acid to produce an intensely colored diazo dye (560-600 nm). The intensity of color of this dye formed is proportional to the concentration of total bilirubin. Direct bilirubin is determined in absence of caffeine by the direct reaction with diazotized sulphanilic acid to form red-colored azobilirubin, the color intensity of which measured at 546 nm is proportional to the concentration of the direct bilirubin in the sample.

\[
\text{Sulphanilic acid} + \text{NaNO}_2 \xrightarrow{\text{HCL}} \text{Diazotized sulphanilic acid}
\]

\[
\text{Bilirubin + Diazotized sulphanilic acid (pH 1.4)} \xrightarrow{\text{Azobilirubin}}
\]

Reagents

Reagent 1 (R1)
- Sulphanilic acid
- HCL

Reagent 2 (R2)
- Sodium nitrite

Reagent 3 (R3)
- Caffeine
- Sodium benzoate

Reagent 4 (R4)
- Tartarate
- Sodium hydroxide

Reagent 4 contains caustic material. Corrosive (C)

K35 Causes severe burns.
K41 Risk of serious damage to eyes.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 After contact with skin, wash immediately with plenty of soap and water.

For further information, refer to the Bilirubin reagent material safety data sheet.

Precautions and Warnings
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 room temperature.

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>Procedure</th>
<th>Total Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample blank</td>
<td>Sample</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>200 ( \mu )l</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>1 drop</td>
</tr>
<tr>
<td>Reagent 3</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Sample</td>
<td>200 ( \mu )l</td>
</tr>
</tbody>
</table>

Mix and incubate for 10 minutes at 20 – 25 \( ^{\circ} \)C, then add;

| Reagent 4 | 1.0 ml | 1.0 ml |

Mix and incubate for 5 minutes at 20 – 25 \( ^{\circ} \)C. Measure absorbance of sample \( \lambda \text{sample} \) against sample blank at 578 nm (560 - 600 nm)

The color intensity is stable for 30 minutes.

Direct Bilirubin

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Sample blank</td>
<td>Sample</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>200 ( \mu )l</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>1 drop</td>
</tr>
<tr>
<td>Saline 0.9% NaCl</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>Sample</td>
<td>200 ( \mu )l</td>
</tr>
</tbody>
</table>

Mix and incubate for exactly 5 minutes at 20 – 25 \( ^{\circ} \)C. Measure absorbance of sample \( \lambda \text{sample} \) against sample blank at 546 nm (530 - 560 nm).

Calculation

Total bilirubin = \text{Sample} \times 10.8

Direct bilirubin = \text{Sample} \times 14.4

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></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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</tbody>
</table>

Run to run (Reproducibility)

<table>
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<tr>
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</tr>
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<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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</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 both total and direct bilirubin.

Linearity

The reaction is linear up to a total bilirubin concentration of 30 mg/dL (513 μmol/L) and a direct bilirubin concentration of 10 mg/dL (171 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 propanolol may cause artificially low total bilirubin levels.

Expected Values

Total Bilirubin
Adults and infants>1 month  ≤ 0.2-1.0 mg/dL (≤3.4-17 μmol/L)
Newborns premature (3-5 d) 10-14 mg/dL (171-239 μmol/L)

Newborns:
(3-5 d) 4.0 - 8.0 mg/dL (68-137 μmol/L)
(<48 h) 6.0 - 10.0 mg/dL (103-171 μmol/L)
(<24 h) 2.0-6.0 mg/dL (34-103 μmol/L)

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

Spectrum 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 – 30 mg/dL (0.17 – 513 μmol/L)
Direct bilirubin : 0.1 – 10 mg/dL (0.17 – 171 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

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
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<tr>
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