Bilirubin Jendrassik-Gróf FS*

For in vitro determination of direct and total bilirubin with the Jendrassik-Gróf method on photometric systems

Order Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 0849 99 90 336</td>
<td>1 x 90 mL Reagent 1 Sulfanilic acid solution</td>
</tr>
<tr>
<td></td>
<td>1 x 25 mL Reagent 2 Sodium nitrite solution</td>
</tr>
<tr>
<td></td>
<td>2 x 100 mL Reagent 3 Accelerator</td>
</tr>
<tr>
<td></td>
<td>2 x 100 mL Reagent 4 Fehling’s solution II</td>
</tr>
</tbody>
</table>

Principle

Bilirubin reacts with diazotized sulfanilic acid to form an azo dye which is red in neutral and blue in alkaline solutions. Whereas the water-soluble bilirubin glucuronides react "directly", the free "indirect" bilirubin reacts only in the presence of an accelerator. The total bilirubin in serum or plasma is determined using the method of Jendrassik and Gróf by coupling with diazotized sulfanilic acid after the addition of caffeine, sodium benzoate and sodium acetate. A blue azobilirubin is formed in alkaline Fehling’s solution II. This blue compound can also be determined selectively in the presence of yellow by-products (green mixed coloration) by photometry at 578 nm. Direct bilirubin is measured as the red azo dye at 546 nm using the method of Schellong and Wende without the addition of alkali. Indirect bilirubin is calculated from the difference between the total and direct bilirubin.

Reagents

Concentrations of the Reagents

- **R1:** Sulfanilic acid 29 mmol/L
- **R2:** Sodium nitrite 29 mmol/L
- **R3:** Caffeine 130 mmol/L
- **R4:** Sodium benzoate 156 mmol/L
- **R5:** Sodium acetate 460 mmol/L
- Potassium sodium tartrate 930 mmol/L
- Sodium hydroxide 1.9 mol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 15 – 25°C, protected from light and contamination is avoided. Do not freeze the reagents!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.

Material required but not provided

NaCl-Solution 9 g/L

General laboratory equipment

Specimen

Serum

It is very important to store the sample protected from light.

Stability [5]:

- Direct bilirubin: 2 days at 20 – 25°C
- 7 months at 4 – 8°C
- 6 months at –20°C
  - if stored immediately. Freeze only once!

- Total bilirubin: 1 day at 20 – 25°C
- 7 days at 4 – 8°C
- 6 months at –20°C
  - if stored immediately. Freeze only once!

- Discard contaminated specimens!

Assay Procedure

Optical path 1 cm

Temperature 15 – 25°C

Measurement Against sample blank

**Determination of total bilirubin**

(Refer to note 1)

<table>
<thead>
<tr>
<th>Wavelength:</th>
<th>Hg 578 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample blank</td>
<td>Sample</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>200 µL 200 µL</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>200 µL 200 µL</td>
</tr>
<tr>
<td>Reagent 3</td>
<td>1000 µL 1000 µL</td>
</tr>
<tr>
<td>Sample</td>
<td>200 µL 200 µL</td>
</tr>
</tbody>
</table>

Mix and allow to stand for 10 to 60 min. at 15 to 25°C, then add:

- **Reagent 4:** 1000 µL 1000 µL
- **NaCl solution:** 2000 µL 2000 µL
- **Sample:** Mix immediately and allow standing at 15 to 25°C. Exactly 5 min. after the addition of serum measure the absorbance of the sample against the sample blank.

Calculation

Concentration total bilirubin: [mg/dL] = A x 10.5

<table>
<thead>
<tr>
<th>µmol/L</th>
<th>= A x 180</th>
</tr>
</thead>
</table>

**Determination of direct bilirubin**

(Refer to notes 1 and 2)

<table>
<thead>
<tr>
<th>Wavelength:</th>
<th>Hg 546 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample blank</td>
<td>Sample</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>- 50 µL</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>200 µL 200 µL</td>
</tr>
<tr>
<td>NaCl solution</td>
<td>2000 µL 2000 µL</td>
</tr>
<tr>
<td>Sample</td>
<td>200 µL 200 µL</td>
</tr>
</tbody>
</table>

Mix immediately and allow standing at 15 to 25°C. Exactly 5 min. after the addition of serum measure the absorbance against the sample blank.

3. In very rare cases, samples of patients with gammapathy might give falsified results [7].

4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.

5. For professional use only!
Notes for manual use

1. With larger series it is possible to mix 4 parts of sulfanilic acid and one part of sodium nitrite in advance. Instead of sulfanilic acid and sodium nitrite, 200 µL of this diazotization solution are pipetted into the sample. This solution can be used for up to 2 hours at 15 to 25 °C. The conversion factors in this case are:

   \[
   [\text{mg/dL}] = A \times 14.0
   \]

   \[
   [\text{umol/L}] = A \times 240
   \]

   For total bilirubin:

   \[
   [\text{mg/dL}] = A \times 10.3
   \]

   \[
   [\text{umol/L}] = A \times 177
   \]

   For directly reacting bilirubin:

   \[
   [\text{mg/dL}] = A \times 13.7
   \]

   \[
   [\text{umol/L}] = A \times 235
   \]

2. It is also possible to measure direct bilirubin at 578 nm. For this purpose, add only 1000 µL of isotonic saline to the sample and the blank. Add 1000 µL of Fehling’s solution II 5 min. after the addition of serum. Mix well and after 5 min. measure the absorbance of the sample against the blank at 578 nm using an optical path of 1 cm. This factor must then be included in the calculation:

   Concentration of directly reacting bilirubin: \[
   [\text{mg/dL}] = A \times 10.5
   \]

   \[
   [\text{umol/L}] = A \times 180
   \]

Performance Characteristics

Measuring Range
The test has been developed to determine bilirubin concentrations within a measuring range from 0.03 to 10 mg/dL. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity/Interferences
No interference was observed by hemoglobin up to 400 mg/dL, naproxen up to 0.4 mmol/L and lipemia up to 800 mg/dL triglycerides for total bilirubin. For further information on interfering substances refer to Young DS [6].

Limit of Detection
The lower limit of detection is 0.03 mg/dL.

Precision Bilirubin direct

<table>
<thead>
<tr>
<th>Intra assay</th>
<th>Mean [mg/dL]</th>
<th>SD [mg/dL]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.33</td>
<td>0.00</td>
<td>1.44</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.71</td>
<td>0.01</td>
<td>0.93</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0.15</td>
<td>0.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>

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</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.77</td>
<td>0.02</td>
<td>2.47</td>
</tr>
<tr>
<td>Sample 2</td>
<td>1.99</td>
<td>0.06</td>
<td>2.82</td>
</tr>
<tr>
<td>Sample 3</td>
<td>3.44</td>
<td>0.13</td>
<td>3.64</td>
</tr>
</tbody>
</table>

Precision Bilirubin total

<table>
<thead>
<tr>
<th>Intra assay</th>
<th>Mean [mg/dL]</th>
<th>SD [mg/dL]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.35</td>
<td>0.01</td>
<td>2.15</td>
</tr>
<tr>
<td>Sample 2</td>
<td>1.79</td>
<td>0.01</td>
<td>0.45</td>
</tr>
<tr>
<td>Sample 3</td>
<td>3.86</td>
<td>0.03</td>
<td>0.79</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter assay</th>
<th>Mean [mg/dL]</th>
<th>SD [mg/dL]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>1.37</td>
<td>0.05</td>
<td>3.32</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.76</td>
<td>0.03</td>
<td>3.33</td>
</tr>
<tr>
<td>Sample 3</td>
<td>5.96</td>
<td>0.09</td>
<td>1.43</td>
</tr>
</tbody>
</table>

Method Comparison
A comparison of DiaSys Bilirubin Total FS according to Jendrassik-Gróf (y) with a commercially available test (x) using 38 samples gave following results:

\[
y = 1.01 x - 0.08 \text{ mg/dL}; r = 0.999
\]

A comparison of DiaSys Bilirubin Direct FS according to Jendrassik-Gróf (y) with a commercially available test (x) using 27 samples gave following results:

\[
y = 0.98 x - 0.01 \text{ mg/dL}; r = 0.991
\]

Reference Range [1]

**Bilirubin total**

- **Neonates**
  - 24 h: \(< 8.8 \text{ mg/dL} < 150 \mu\text{mol/L}
  - 2nd day: \(1.3 – 11.3 \text{ mg/dL} < 22 – 193 \mu\text{mol/L}
  - 3rd day: \(0.7 – 12.7 \text{ mg/dL} < 12 – 217 \mu\text{mol/L}
  - 4th – 6th day: \(0.1 – 12.6 \text{ mg/dL} < 1.7 – 216 \mu\text{mol/L}

- **Children**
  - >1 month: \(0.2 – 1.0 \text{ mg/dL} < 3.4 – 17 \mu\text{mol/L}

- **Adults**
  - \(0.1 – 1.2 \text{ mg/dL} < 1.7 – 21 \mu\text{mol/L}

**Bilirubin direct**

- Adults and children: \(\leq 0.2 \text{ mg/dL} < 3.4 \mu\text{mol/L}

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature


Manufacturer

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany