**BILIRUBIN TOTAL & DIRECT 4+1**

In vitro diagnostic reagent, for professional use only

**CLINICAL SIGNIFICANCE (1-3)**

Approximately 80-85% of the bilirubin produced is derived from the heme moiety of the haemoglobin released from aging erythrocytes in the reticuloendothelial cells. Bilirubin, bound to albumin, is transported into the liver where it is rapidly conjugated with glucuronide to increase its solubility. Then it is excreted into the biliary canalliculi, and hydrolyzed in the gastrointestinal tract. Unconjugated bilirubin serum concentration increases in case of overproduction of bilirubin (acute or chronic haemolytic anemias) and in case of disorders of bilirubin metabolism and transport defects (impaired uptake by liver cells; Gilbert’s syndrome; defects in the conjugation reaction: Crigler-Najjar syndrome). Reduced excretion (hepatocellular damage: hepatitis, cirrhosis...; Dubin-Johnson andRotor syndrome) and obstruction to the flow of bile (most often produced by gallstones or by tumours) induce an important elevation of conjugated bilirubin and in a minor extent an increase of unconjugated bilirubin (conjugated hyperbilirubinemia).

**METHOD (3)**

Malloy-Evelyn modified. End point.

**PRINCIPLE (3)**

Sulfanilic acid reacts with sodium nitrite to form diazotized Sulfanilic acid. In the presence of accelerator (cetrimide), conjugated and unconjugated bilirubin reacts with diazotized Sulfanilic acid to form azobilirubin (Bilirubin Total 4+1). In the absence of accelerator, only conjugated bilirubin reacts (Bilirubin Direct 4+1). The increase of absorbance at 550 nm is proportional to bilirubin concentration.

- Sulfanilic acid + NaNO₂ → Diazoactive Sulfanilic acid
- Bilirubin + Diazoactive Sulfanilic acid → Azobilirubin

**REAGENTS COMPOSITION**

<table>
<thead>
<tr>
<th>Bilirubin</th>
<th>Bilirubin</th>
<th>Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total 4+1</td>
<td>Direct 4+1</td>
<td>Total &amp; Direct 4+1</td>
</tr>
<tr>
<td>Reagent R1</td>
<td>Reagent R1</td>
<td>Reagent R2</td>
</tr>
<tr>
<td>Sulfanilic acid</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>Cetrimide</td>
<td>37</td>
<td>-</td>
</tr>
<tr>
<td>Sodium nitrite</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Note:** Bilirubin Total 4+1 reagent R1 can be slightly cloudy. It contains a detergent that can lead to the formation of foam in washing units of some equipments. These two characteristics are without consequences on the product performances.

**PRECAUTIONS**
- Bilirubin Direct 4+1 reagent R1 is irritant (Xi).
- R36/37/38: Irritating to eyes, respiratory system and skin.
- S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- S37/39: Wear suitable gloves and eye/face protection.
- - Sulfanilic acid contained in reagents R1 may produce an allergic reaction.
- - Use clean or single use laboratory equipment only to avoid contaminations.

**STABILITY OF REAGENTS**

To store at 2-8°C and protected from light. The reagents are stable until the expiry date stated on the label. On board stability:

- **Note:** The stability is specific for each analyser (for Selectra refer to § PERFORMANCE DATA)

**PREPARATION AND STABILITY WORKING REAGENT**

The reagents are ready for use.

**SAMPLES (0, 4)**

- **Specimen**
  - Serum free of hemolysys. Heparinized plasma.
  - Care must be taken to fill heparinized tubes according to the manufacturer’s instructions. An insufficient filling may lead to erroneous results.
  - Protect the samples from light before and during the analysis.
- **Conservation and storage**
  - If plasma and serum are protected from light, samples are stable 2 days at room temperature and 4 days at 4°C. For a longer storage, freeze the samples at -20°C.

**REFERENCE VALUES (4)**

- Serum, plasma:
  - Total bilirubin:
    - Adults and children over 10 days: 0.3-1.2 mg/dL (3-12 mg/L; 5-21 µmol/L)
  - Direct bilirubin:
    - < 0.2 mg/dL (2 mg/L; 3.4 µmol/L)

**PROCEDURE**

These reagents can be used on most analysers, semi-automated analysers and manual method.

The applications are available on request.

**CALCULATION**

\[
\text{TEST} = \frac{(A2 - A1) \times n}{n} = \text{Calibrator concentration}
\]

**QUALITY CONTROL**

To ensure adequate quality, control sera such as ELITROL I (normal control) and ELITROL II (abnormal control) are recommended.

**PERFORMANCE DATA at 37°C on Selectra**

- **Total bilirubin**
  - **Analytical range**
    - The reagent is linear from 0.3 to 20 mg/dL (3 to 200 mg/L; 5 to 340 µmol/L).
  - **Detection limit**
    - Determined according to SFBC protocol, the detection limit is equal to 0.05 mg/dL (0.5 mg/L; 0.9 µmol/L).
**BILIRUBIN**

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**-Analytical Sensitivity**
The average variation of the analytical signal is 66 mÅA per mg/dL of bilirubin (6.6 mÅA per mg/L or 3.9 mÅA per µmol/L) for a light path of 1 cm.

**- Precision**
**Within-run reproducibility on serum**

- Low level:  n = 20  m = 0.83 mg/dL  CV = 0.9 %
- Medium level:  n = 20  m = 2.81 mg/dL  CV = 1.3 %
- High level:  n = 20  m = 8.74 mg/dL  CV = 1.2 %

**Between-run reproducibility on serum**

- Low level:  n = 84  m = 0.99 mg/dL  CV = 3.3 %
- Medium level:  n = 87  m = 3.64 mg/dL  CV = 3.3 %
- High level:  n = 88  m = 10.42 mg/dL  CV = 2.8 %

**- Correlation**

**Analytical range**
- B) Direct bilirubin
  - Analytical range
    - The reagent is linear from 0.15 to 18 mg/dL (1.5 to 180 mg/L; 3 to 310 µmol/L).
  - Detection limit [5]
    - Determined according to SFBC protocol, the detection limit is equal to 0.02 mg/dL (0.2 mg/L; 0.3 µmol/L).

**- Analytical Sensitivity**
The average variation of the analytical signal is 66 mÅA per mg/dL of conjugated bilirubin (6.6 mÅA per mg/L or 3.9 mÅA per µmol/L) for a light path of 1 cm.

**- Precision**
**Within-run reproducibility on serum**

- Low level:  n = 20  m = 0.60 mg/dL  CV = 2.0 %
- Medium level:  n = 20  m = 2.03 mg/dL  CV = 2.1 %
- High level:  n = 20  m = 6.14 mg/dL  CV = 1.5 %

**Between-run reproducibility on serum**

- Low level:  n = 84  m = 0.65 mg/dL  CV = 3.5 %
- Medium level:  n = 88  m = 2.52 mg/dL  CV = 3.6 %
- High level:  n = 87  m = 6.91 mg/dL  CV = 2.8 %

**- Interferences [5,6]**

According to SFBC recommendations, studies have been performed to determine the level of interference from different compounds:

- **Turbidity:**
  - No significant interference up to 600 mg/dL (6 g/L; 6.9 mmol/L) triglyceride equivalent.

- **Haemoglobin:**
  - No significant interference up to 500 mg/dL (5 g/L).

- **Ascorbic acid:**
  - No significant interference up to 2 mg/dL (20 mg/L).

Other compounds may interfere. [7,9]

**- Correlation**

A comparative study has been performed between ELITech method and another commercial reagent (DCA method) on 62 human serum samples. The sample concentrations range from 0.24 to 25.32 mg/dL. The parameters of linear regression are as follows:

**Correlation coefficient:**  (r) = 0.9988

**Linear regression:**  y = 1.007 x + 0.01 mg/dL

The parameters of linear regression are as follows:

- High level:  n = 87  m = 8.74 mg/dL  CV = 1.2 %
- Medium level:  n = 88  m = 2.52 mg/dL  CV = 1.3 %
- Low level:  n = 84  m = 0.99 mg/dL  CV = 3.3 %

**Within-run reproducibility on serum**

- High level:  n = 20  m = 6.14 mg/dL  CV = 1.5 %
- Medium level:  n = 20  m = 2.03 mg/dL  CV = 2.1 %
- Low level:  n = 20  m = 0.60 mg/dL  CV = 2.0 %

**- Interferences [5,6]**

According to SFBC recommendations, studies have been performed to determine the level of interference from different compounds:

- **Turbidity:**
  - No significant interference up to 500 mg/dL (5 g/L; 5.75 mmol/L) triglyceride equivalent.

- **Haemoglobin:**
  - Negative bias from 250 mg/dL (2.5 g/L).

- **Ascorbic acid:**
  - Positive bias from 2 mg/dL (20 mg/L).

Other compounds may interfere. [7,9]

**- On board stability/calibration frequency on Selectra (no refrigered)**

On-board Stability: 14 days (Capped vials and stored at 2-8 °C during the night)

Calibration Frequency: 14 days

Make a new calibration when reagent lots change, when quality control results fall outside the established range, and after a maintenance operation.

**BIBLIOGRAPHY**


**SYMBOLS USED ON LABELS**

- IVD: In vitro diagnostic medical device
- : Consult Instruction for use
- : Manufacturer’s address
- REF: Catalogue number
- : Temperature limitation
- LOT: LOT number
- : Expiration date

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