**ORDER INFORMATION**

<table>
<thead>
<tr>
<th>REF</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA4256 00</td>
<td>10x15 + 1x10 ml</td>
</tr>
<tr>
<td>KL4256 00</td>
<td>10x15 + 1x10 ml</td>
</tr>
<tr>
<td>BK4256 00</td>
<td>5x(50+5 ml)</td>
</tr>
</tbody>
</table>

**INDICATION**

Determination of total and direct bilirubin is used for the diagnosis and monitoring of hepatic (hepatitis, cirrhosis) and hemolytic and biliary disorders. In particular, high levels of direct bilirubin (or "conjugated" bilirubin), generally absent or present only in negligible quantities, are relieved in the following cases:

- extra hepatic biliar disorders (e.g.: gallbladder and choledochal calculi, pancreas tumor);
- inter hepatic biliar disorders (e.g.: cirrhosis, hepatitis and liver tumor).

**METHOD PRINCIPLE**

In the present Jendrassik modified method, total bilirubin, in the presence of diazosulphanilic acid, forms a coloured compound (azobilirubin). The colour intensity is proportional to the direct bilirubin concentration present in the sample.

**COMPOSITION**

**REAGENT A (liquid):**

- Sulphanilic acid 30 mmol/l
- Hydrochloric acid 0.25 mol/l

**REAGENT B (liquid):**

- Sodium nitrite ≤ 10 mmol/l

**Preparation**

Mix 15 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (e.g. 30 ml of RA + 2 ml of RB).

**Storage and stability**

Store at room temperature (15-25 °C). Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 7 days at 2-8 °C.

**ANCILLARY EQUIPMENT**

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l
- Calibrator (GD CAL Ref. GD8577 00)

**SAMPLES**

Serum, sodium heparin or EDTA-Na2 plasma. Do not use hemolyzed samples. For hyperlipemic, not limpid sample it is necessary to use sample blank. As bilirubin is a photosensitive pigment, samples must be stored protected from light and from heat. Samples must be analyzed immediately.

**Specimen collection / Preanalytical factors**

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

**INTERNAL QUALITY CONTROL**

It is recommended to use commercial Quality Control sera with known direct bilirubin concentration. Check that the values obtained are within the reference range provided.

**ANALYTICAL PROCEDURE**

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Blank</th>
<th>Calibrator</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100 µl</td>
<td></td>
</tr>
<tr>
<td>Distilled H2O</td>
<td>100 µl</td>
<td>-</td>
<td>100 µl</td>
</tr>
<tr>
<td>Calibrator</td>
<td>-</td>
<td>100 µl</td>
<td>-</td>
</tr>
<tr>
<td>Working reagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Mix and incubate for 10 minutes at room temperature (20-25 °C). Read absorbance A for each cuvette at 570 (550-580) nm against Blank cuvette. The colour is stable 30 minutes at room temperature.

**Note:**

- Reaction volumes can be proportionally changed.
- For concentration > 20 mg/dl, the sample should be diluted 1+9 with NaCl solution (0.9 g/l) and result multiplied by 10.

**CALCULATION OF RESULTS**

Direct bilirubin, mg/dl = \( \frac{A \text{ sample}}{A \text{ calibrator}} \times \text{mg/dl calibrator} \)

**Conversion factor**

Direct bilirubin [mg/dl] x 17.1 = Direct bilirubin [µmol/l]

**REFERENCE VALUES**

Adults: up to 0.25 mg/dl (4.3 µmol/l)

Each laboratory should establish reference ranges for its own patients population.

**ANALYTICAL PERFORMANCES**

**Precision**

Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different direct Bilirubin concentrations. The obtained results are reported in the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (mg/dl)</th>
<th>Within Run SD</th>
<th>%CV</th>
<th>Between Run SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>0.84</td>
<td>0.01</td>
<td>1.2</td>
<td>0.05</td>
<td>5.9</td>
</tr>
<tr>
<td>Serum 2</td>
<td>1.43</td>
<td>0.02</td>
<td>1.4</td>
<td>0.11</td>
<td>7.7</td>
</tr>
<tr>
<td>Serum 3</td>
<td>1.95</td>
<td>0.01</td>
<td>0.5</td>
<td>0.14</td>
<td>7.2</td>
</tr>
</tbody>
</table>

**Linearity**

The assay is linear up to 20 mg/dl (342 µmol/l).

**Sensitivity**

Test sensitivity, in terms of limit of detection, is 0.03 mg/dl (0.51 µmol/l).

**Correlation**

A correlation study comparing the present method and a commercial one gave the following results:

\[
y = 0.9705x + 0.0778\text{mg/dl} \\
\text{r} = 0.9585
\]
Interferences
Hemoglobin and lipids interfere with the results: hemoglobin interference may cause false low results, while lipemic samples may cause an increase in the values due to the turbidity.

PRECAUTIONS IN USE
The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

BIBLIOGRAPHY