TOTAL BILIRUBIN

For quantitative determination of total bilirubin in serum and plasma by colorimetric Jendrassik modified method

Diagnostic implication
Bilirubin is a bile yellow-orange pigment, a metabolite of waste derived from the breakdown of aged red blood cells. Increasing level of bilirubin are found in the liver diseases and in the presence of gallstones.

Principle
The bilirubin in an acid medium reacts with sulphanilic acid and sodium nitrite, forming a diazo-red colored compound, whose intensity, measured at 550 nm, is directly proportional to the concentration of bilirubin in the sample. In the reagent are present accelerators substances to allow, in a few minutes, the development of the reaction.

Reagents/composition and supplied material
R1 – Reagent 1 (5 x 80mL)
Sulphanilic acid 12 mmol/L; Hydrochloric acid 1,00 mol/L; Citric acid 1,24 mol/L; Chloramphenicol 2,01 mmol/L; Urea 3,0 mol/L; Caffeine 0,6 mol/L; Surfactants

R2 - Reagent 2 (1 x 100mL)
Sodium Nitrite 12,6 mmol/L

Reagent preparation and stability
Reagents: Liquid and ready to use
Stability and storage: reagents are stable until the expiration date indicated on the label if stored at room temperature.
Working solution: mix 4 parts of reagent 1 with one part of reagent 2. The working solution is stable 1 day if stored at room temperature.

Material required but not provided
-Spectrophotometer or photometer
-General laboratory equipment
-Saline solution (9 g/L)
-Multiparametric control ONE-TROL 2 (REF. CC02225) and ONE-TROL 3 (REF. CC02226)

Reference value

<table>
<thead>
<tr>
<th></th>
<th>2-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>0.2 – 1.2 mg/dL</td>
</tr>
<tr>
<td>Newborn (1° week)</td>
<td>1.0 – 12.0 mg/dL</td>
</tr>
</tbody>
</table>
These values are a guide. Each laboratory should establish its own reference values.

Sample collection
Use fresh serum and/or EDTA plasma

Manual procedure
- Wavelength: 550 nm
- Temperature: 37 °C
- Optical path: 1 cm
- Zero the spectrophotometer with distilled water
- Pipette into tube

<table>
<thead>
<tr>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water 100µL</td>
<td>100µL</td>
</tr>
<tr>
<td>Reagent 1 800µL</td>
<td>800µL</td>
</tr>
</tbody>
</table>

Incubate for 5 minutes and read the absorbance of the sample against white.

Calculation
Total Bilirubin (mg/dL) = O.D. Sample x 10,5
Conversion value: mg/dL x 17.1 = µmol/L

Quality control
For quality control use Clinical Chemistry Multiparametric Control of CPM SAS (ONE-TROL 2 REF. CC02225 and ONE-TROL 3 REF. CC02226) or other suitable material. The limits of controls intervals should be individually tailored to each laboratory reference one. The values must be within the limits. Each laboratory should make appropriate corrective action if the values are out of bounds. The panel must be tested and evaluated in the same manner as patient samples.

Linearity
The reaction is linear up to 10 mg/dL. Samples with values above 10 mg/dL should be diluted with saline solution and retested. Multiply the result by the dilution factor.

Interference
There are no significant interference with
- Hemoglobin (up to 0.05 g /L or 0.03 mmol/L)
- Triglycerides (up to 5 g /L or 5.65 mmol/L)

Sensibility
Change of mAbs for 0.01 mg/dL: 5.00

Detection and Quantification Limit
The minimum detectable limit for this reagent is 0.05 mg/dL. The quantification limit is equal to 0.08 mg/dL.

Precision (intra-assay)
Determined with 20 replicates for each level of control (LEV1; LEV2)
Obtained results (mg/dL):

<table>
<thead>
<tr>
<th>Mean n=20</th>
<th>Lev1 (1,08)</th>
<th>Lev2 (3,85)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.D.</td>
<td>0,011</td>
<td>0,072</td>
</tr>
<tr>
<td>C.V. %</td>
<td>1,03</td>
<td>1,88</td>
</tr>
</tbody>
</table>

Precision (inter-assay)
Determined with 20 replicates for each level of control (LEV1; LEV2) within three days.
Obtained results (mg/dL):

<table>
<thead>
<tr>
<th>Mean n=20</th>
<th>Lev1 (1,10)</th>
<th>Lev2 (3,82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.D.</td>
<td>0,012</td>
<td>0,085</td>
</tr>
<tr>
<td>C.V. %</td>
<td>1,16</td>
<td>2,23</td>
</tr>
</tbody>
</table>

Correlation
The correlation study, determined with 20 samples, between our reagent and a reference reagent commercially available gave the following results:

r = 0.9984     y=0.9751x + 2.156
Accuracy
The accuracy is evaluated for each production lot. For the results of the batch in use to refer to the equivalent certificate of quality control.

Caution
- For in vitro professional use
- Do not use reagents after the expiration date printed on the label
- Handle all serum samples as potentially infectious

Bibliography

Symbology
- REF Reference
- Cont. Content of the kit
- LOT Batch number
- Exp. Expiry date
- IVD in vitro medical device
- Read instruction for use
- Manufacturer:

Conformity with the requirements of European Directive 98/79 EC on in vitro diagnostic medical devices