Quantitative determination of Bilirubin Total and Bilirubin Direct by means of photometric method.

Only for in vitro use in clinical laboratory (IVD).

Clinical Significance
Bilirubin is produced from the catabolism of the heme molecule. In the liver, a major part of the Bilirubin is converted to Conjugated Bilirubin (Direct Bilirubin) which is water soluble. Unconjugated Bilirubin (Indirect Bilirubin) is not water soluble. Direct and Indirect forms of serum bilirubin are elevated in acute or chronic hepatitis, biliary tract obstruction, toxic reactions to many drugs, chemicals, toxins. Indirect serum bilirubin is elevated in hemolytic disease or reactions or absence or deficiency of glucoronyl trasferase. Direct and Total Bilirubin can be significantly elevated in normal and jaundiced subjects due to fasting 24-48 hours (in some instances even 12 hours) or due to prolonged caloric restriction.

Principle
Direct Bilirubin reacts in an acid medium with diazonium salt of sulphanilic acid to form a diazo compound (azobilirubin). Color intensity is proportional to the concentration of direct bilirubin present in the sample. Total Bilirubin reacts in presence of a Quaternary Ammonium salt in an acid medium with diazonium salt of sulphanilic acid to form a diazo compound (azobilirubin). Color intensity is proportional to the concentration of total Bilirubin present in the sample.

Reagents
Each Bilirubin Total-Direct Kit contains
R-1: Diazol-A
R-2: Diazol-B
R-3: Caffeine
R-4: Methyl Red Standard (10 mg/dL)
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. Please read carefully the MSDS information.

Reagent Preparation
Reagents are ready for Use.

Stability
The reagents are ready to use. The expiry date of each reagent stored at 2-8°C and protected from the direct light is printed on the label. Keep the bottles closed when not in use to avoid air oxidation and evaporation. Don’t use if the reagents present turbidity.

Specimens
Serum, heparin plasma or EDTA plasma.
Stability: 7 days at 20 - 25°C
7 days at 4 - 8°C
3 months at -20°C
Discard contaminated specimens.

Procedure
Monochromatic Method
1. Assay Conditions
   - Wavelength: 546 nm.
   - Cuvette: 1 cm. light path.
   - Constant temperature 20-25°C.
2. Measurement Against sample blank

<table>
<thead>
<tr>
<th>Dispense</th>
<th>Direct Bilirubin</th>
<th>Total Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>Sample</td>
<td>Blank</td>
</tr>
<tr>
<td>Diazol-A</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
<tr>
<td>Diazol-B</td>
<td>----</td>
<td>50 µL</td>
</tr>
<tr>
<td>Caffeine</td>
<td>----</td>
<td>50 µL</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>1000 µL</td>
<td>1000 µL</td>
</tr>
<tr>
<td>Sample</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
</tbody>
</table>

3. Mix and incubate for 2 min at RT. Then read the absorbance of the sample (Abs S) against the Distilled water blank (Abs SB ). Final color is stable for at least 90 minutes away from light. Reaction volumes can be proportionally varied without change in calculation.

Calculation
Factor: 26.0
Read the absorbance of Methyl Red Standard against distilled water and discard the Standard. It is recommended to check the Factor with Standard. If it differs, use new Factor.

Concentration of Bilirubin (Total or Direct) in Sample (mg/dL)
(Abs. Sample – Abs. of Blank) x 10
(Abs. of Standard – Abs. of Blank)

Bichromatic Method
1. Assay Conditions
   - Wavelength: 546 nm.
   - Cuvette: 1 cm. light path.
   - Constant temperature 20-25°C.
2. Measurement Against distilled water

<table>
<thead>
<tr>
<th>Dispense</th>
<th>Direct Bilirubin</th>
<th>Total Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>Sample</td>
<td>Blank</td>
</tr>
<tr>
<td>Diazol-A</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
<tr>
<td>Diazol-B</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
<tr>
<td>Caffeine</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>1000 µL</td>
<td>1000 µL</td>
</tr>
<tr>
<td>Sample</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
</tbody>
</table>

3. Mix and incubate for 2 min at RT. Then read the absorbance of the sample (Abs S) against the Distilled water blank using bichromatic method in automated analyzer at 546 & 630 nm. Reaction volumes can be proportionally varied without change in calculation.

Calculation
Factor: 26.0
Read the absorbance of Methyl Red Standard against distilled water and discard the Standard. It is recommended to check the Factor with Standard. If it differs, use new Factor.

Concentration of Bilirubin (Total or Direct) in Sample (mg/dL) is calculated automatically by

Abs. Sample x Factor

Quality Control
Commercially available normal and pathological control sera are recommended to monitor the performance of the procedure. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.

Reference Values
Direct Bilirubin: up to 0.1 - 0.4 mg/dL
Total Bilirubin: 0.4 - 1.1 mg/dL
(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

Reagent Performance
Sensitivity
The sensitivity of the method is found as average of determination on reagent blank + 3 standard deviation and is equal to: 0.05 mg/dL.
Linearity
Up to 25 mg/dL (427 µmol/l). When the concentration exceeds this limit, dilute the sample with Saline Solution (Sodium Chloride 9 g/lt.) and repeat the assay. The result is multiplied by dilution factor.

Test Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode</td>
<td>End Point</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>546 nm</td>
</tr>
<tr>
<td>Reaction Direction</td>
<td>Increasing</td>
</tr>
<tr>
<td>Sample Volume (µL)</td>
<td>50 µL</td>
</tr>
<tr>
<td>Working Reagent Volume (µL)</td>
<td>1100 µL</td>
</tr>
<tr>
<td>Incubation Time</td>
<td>2 minute</td>
</tr>
<tr>
<td>Read Time</td>
<td>5 sec</td>
</tr>
<tr>
<td>Normal Low (mg/dL)</td>
<td>0.1 (Direct); 0.4 (Total)</td>
</tr>
<tr>
<td>Normal High (mg/dL)</td>
<td>0.4 (Direct); 1.1 (Total)</td>
</tr>
<tr>
<td>Linearity</td>
<td>25 mg/dL</td>
</tr>
<tr>
<td>Units</td>
<td>mg/dL</td>
</tr>
</tbody>
</table>

Literatures

Manufactured by: JK Diagnostics, India

Marketed by

A Division of
Euro Diagnostic Systems Pvt. Ltd.
'Millennium House' M. K. Srinivasan Nagar Main Road
No. 144, Old Mahabalipuram Road, Perungudi, Chennai-600 096
Tamil Nadu, India, Phone: 044-2496 1064, 044-2496 0541
Fax: 044-2496 2825, email: eurods@vsnl.net, www.europsychs.in