**BILIRUBIN**
Modified Jendrassik & Grof’s Liquid

**Quantitative determination of Total and Direct Bilirubin 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 form of serum bilirubin is 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 and absence or deficiency of glucuronyl transferase. 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**
Total bilirubin (both bilirubin fractions) and conjugated (direct) bilirubin are stable and can be incubated for 48 hours. Direct and Total Bilirubin can be measured by means of a colored complex that is measured at 546 nm. The total bilirubin reaction takes place in the presence of an accelerating agent to facilitate reaction of albumin-bound bilirubin with the diazo reagent.

**Reagents**
Each Bilirubin kit contains:
- R 1(D)  Direct Reagent
- R 2 (D)  Direct Nitrite Reagent
- R I (T)  Total Reagent
- R II (T)  Total Nitrite Reagent

**Reagent Preparation**
Reagents are ready to use.

**Stability**
All the components of the kit are stable at room temperature until the expiration date on the label when protected from light and contamination prevented during their use. Do not use reagents over the expiration date.

**Signs of Reagent deterioration:**
- All reagents should be clear and colorless solution. Turbidity or formation of a precipitate would indicate deterioration.
- Development of a dark yellow color in the Activator reagent indicates that the reagent should not be used.
- Visible turbidity in reagent indicates reagent deterioration.

**Specimens**
**Serum**
- Collection: Obtain serum free from hemolysis.
- Additives: Not required.
- Stability: If serum is not immediately assayed, it can be stored up to 4-7 days in refrigerator (2°C-8°C). Samples must be protected from sunlight and white artificial light, as bilirubin is highly photo-labile. Reportedly about 50% of bilirubin can be lost in an hour of exposure to sunlight.
- Interfering Substances:
  - Grossly hemolyzed or lipemic samples cause false bilirubin values. For a comprehensive list of interfering substances.

**Procedure**
**Assay Procedure:**
- Wavelength : 546 nm / Yellow – Green
- Temperature : Room Temperature
- Light Path : 1 cm

**TOTAL BILIRUBIN**

<table>
<thead>
<tr>
<th></th>
<th>Blank (B)</th>
<th>Unknown (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R I (Total Reagent)</td>
<td>1000 µL</td>
<td>1000 µL</td>
</tr>
<tr>
<td>R II (Total Nitrite)</td>
<td>25 µL</td>
<td>25 µL</td>
</tr>
<tr>
<td>Unknown Sample</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
</tbody>
</table>

1. Mix thoroughly and incubate for 10 minute at room temperature or 5 minutes at 37°C.
2. Read at 546 nm against respective Blanks.

**DIRECT BILIRUBIN**

<table>
<thead>
<tr>
<th></th>
<th>Blank (B)</th>
<th>Unknown (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R 1 (Direct Reagent)</td>
<td>1000 µL</td>
<td>1000 µL</td>
</tr>
<tr>
<td>R 2 (Direct Nitrite)</td>
<td>~</td>
<td>25 µL</td>
</tr>
<tr>
<td>Unknown Sample</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
</tbody>
</table>

1. Mix thoroughly and incubate for 10 min at room temperature or 5 minutes at 37°C.
2. Read immediately at 546 nm against respective Blanks.

**Calculation**
Total Bilirubin of Unknown Sample = (Abs. T – Abs. B) X 26.30
Direct Bilirubin of Unknown Sample = (Abs. T – Abs. B) X 26.30
(Factor = 26.30)

**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 Standard for problems.

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

**Reference Values**
- Total Bilirubin: Adults up to 1.0 mg/dl  Newborn up to 12 mg/dl
- Direct Bilirubin: Adults and Infants (over 1 month) up to 0.2 mg/dl

(These values are for orientation purpose).

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

**Reagent Performance**

**Linearity Range**
Reaction is linear up to 25 mg/dL under the described assay conditions. If results obtained are greater than linearity limit, dilute the sample suitably with NaCl 9 g/L (Normal Saline) and multiply result by the dilution factor.

**Literatures**

**SYSTEM PARAMETER**

<table>
<thead>
<tr>
<th>Mode</th>
<th>Differential Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>546 nm</td>
</tr>
<tr>
<td>Reaction Direction</td>
<td>Increasing</td>
</tr>
<tr>
<td>Sample Volume</td>
<td>50 µL</td>
</tr>
<tr>
<td>Reagent Volume</td>
<td>1025 µL</td>
</tr>
<tr>
<td>Incubation Time</td>
<td>5 min at 37°C or 10 min at room temp</td>
</tr>
<tr>
<td>Read Time</td>
<td>5 sec</td>
</tr>
<tr>
<td>Normal High (mg/dL)</td>
<td>0.2 (Direct); 1.0 (Total)</td>
</tr>
<tr>
<td>Factor</td>
<td>26.30</td>
</tr>
<tr>
<td>Linearity</td>
<td>25 mg/dL</td>
</tr>
</tbody>
</table>

**Manufactured by**
Asritha Diatech India Pvt.Ltd. Hyderabad, India.

**Marketed by**
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