**Bilirubin (Total) Reagent**

Catalog #: 80132

for use with the

**LIASYS-330 CLINICAL CHEMISTRY SYSTEM**

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**INTENDED USE**

For the quantitative in vitro determination of total bilirubin in serum.

**CLINICAL SIGNIFICANCE**

Serum total bilirubin is elevated in cirrhosis, hepatitis, and in obstructive and hemolytic jaundice.

**METHOD HISTORY**

The most common method for the clinical determination of bilirubin is the coupling of serum bilirubin with diazotized sulfanilic acid (p-diazobenzenesulfonic acid) to produce an azobilirubin dye. The reaction was first described by Ehrlich in 1884 and was used by van den Bergh and Snapper to demonstrate the presence of bilirubin in normal serum. Von den Bergh further observed that there were two types of serum bilirubin which can be distinguished using the diazo reaction. The direct form reacted with diazo without the presence of alcohol (accelerator). This is bilirubin which has been conjugated with glucuronic acid by the liver and is then water-soluble. The indirect form of bilirubin is unconjugated and exists in serum tightly bound to albumin. This bilirubin-albumin complex is not water-soluble, and therefore requires an accelerator, or solubilizing agent, to remove the bilirubin from the albumin for it to react with the diazotized sulfanilic acid. The total bilirubin in serum is the sum of the direct and indirect forms.

Many substances have been used as accelerators for the reaction of unconjugated bilirubin with diazo reagent. Malloy and Evelyn first introduced methanol in 1937. Jendrassik and Grof introduced the use of caffeine and sodium benzoate in 1938. Subsequently, there have been many modifications to these two methods. 4,5

The LIASYS total bilirubin method is based on a modification of the Pearlmen and Lee method in which a surfactant is used as a solubiliser. Sodium nitrite is added to sulfanilic acid to form diazotized sulfanilic acid. Bilirubin in the sample reacts with the diazotized sulfanilic acid to produce azobilirubin which absorbs strongly at 550 nm. The absorbance measured at 550 nm is directly proportional to the total bilirubin concentration in the sample.

**REAGENT COMPOSITION**

<table>
<thead>
<tr>
<th>Active ingredients</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCL</td>
<td>4.9 mM</td>
</tr>
<tr>
<td>Sulfanilic acid</td>
<td>104 mM</td>
</tr>
<tr>
<td>Sodium nitrite</td>
<td>145 mM</td>
</tr>
</tbody>
</table>

**Precautions:**

1. Reagents are toxic and corrosive. Do not pipette by mouth. Avoid contact with skin and clothing.

2. This reagent is for in vitro diagnostic use only.

**REAGENT PREPARATION**

To prepare a working total Bilirubin reagent mix 1 part of Sodium Nitrite reagent with 50 parts of Total Bilirubin reagent. Example: Add 0.2mL sodium nitrite to 10mL total Bilirubin reagent.

**REAGENT STORAGE**

1. Packaged reagents may be stored at 2 - 25°C.

2. Combined working reagent can be stored for up to 21 days when stored at 2-8°C.

3. Do not freeze reagents.

4. Avoid exposure to direct sunlight.

**REAGENT DESTRUCTION**

Do not use the reagent if:

1. Reagent has an absorbance greater than 0.100 when measured against water at 550 nm.

2. Turbid.

3. The reagent fails to meet stated parameters of performance.

**NOTE:** Working reagent will normally develop a light yellow/orange color upon standing.

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**SPECIMEN COLLECTION AND STORAGE**

1. Fresh, unhemolysed serum is recommended.

2. Samples should be analyzed within two hours of collection if kept at room temperature in the dark and within twelve hours if kept refrigerated (2-8°C) and protected from light.

3. Bilirubin in serum is stable for three months when stored frozen (-20°C) and protected from light.

4. Direct sunlight may cause up to a 50% decrease in bilirubin within one hour.

**INTERFERENCES**

Studies to determine the level of interference for hemoglobin, and lipemia were carried out, the following results were obtained:

**Hemoglobin:** Do not use sera with visible hemolysis.

**Lipemia:** Do not use sera with visible turbidity.

**ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED**

1. A LIASYS 330 Clinical Chemistry System.

2. Deionized water and related equipment, e.g.: pipettes

3. Analyzer specific consumables, e.g.: sample cups

4. Control, and Calibrator materials such as those provided by AMS.

**ASSAY PROCEDURE**

**System Parameters**

**Total Bilirubin**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TEMPERATURE:</strong></td>
<td>37°C</td>
</tr>
<tr>
<td><strong>WAVELENGTH:</strong></td>
<td>550 nm</td>
</tr>
<tr>
<td><strong>ASSAY TYPE:</strong></td>
<td>End Point</td>
</tr>
<tr>
<td><strong>DIRECTION:</strong></td>
<td>Increase</td>
</tr>
<tr>
<td><strong>SAMPLE / RGT RATIO:</strong></td>
<td>1 : 20</td>
</tr>
<tr>
<td>e.g. Sample Vol.</td>
<td>0.05mL (50mL)</td>
</tr>
<tr>
<td>Reagent Vol.</td>
<td>1.0 mL</td>
</tr>
<tr>
<td><strong>INCUBATION TIME:</strong></td>
<td>10 min</td>
</tr>
</tbody>
</table>

**Procedure Notes:**

1. The reagent and sample volumes may be altered proportionally to accommodate various instrument requirements.

2. Bilirubin is extremely light sensitive. All samples should be stored protected from light sources.

**Calculations:**

\[
\text{Concentration} \times \text{Absorbance at } 550 \text{ nm} = \text{Total Bilirubin (mg/dL)}
\]

**Example:**

\[
\text{A patient} \times 0.350 = \text{Total Bilirubin (mg/dL)}
\]

**EXPECTED VALUES**

Adults and infants over 1 month old 0.2 – 1.5 mg/dL.

It is recommended each laboratory verify this range or derives a reference interval for the population that it serves.

**REFERENCE**


