BILIRUBIN

INTENDED USE

Vitro bilirubin reagent is intended for the in vitro quantitative determination of total and direct bilirubin in serum or plasma on manual system.

BACKGROUND

Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from other-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. This process accounts for about 80% of bilirubin formed daily. Other sources of bilirubin include the breakdown of myoglobin and cytochromes and the catabolism of immature red cells in the bone marrow. In the liver, bilirubin is conjugated with glucuronic acid for solubilization to form conjugated or direct bilirubin for subsequent transport through the bile duct into the digestive tract where it is metabolized by bacteria to a group of products collectively known as stercobilinogen. Total bilirubin is the sum of the conjugated and unconjugated fractions. Pre-hepatic diseases or conditions such as hemolytic disease or liver diseases resulting in impaired entry, transport or conjugation within the liver cause elevation of unconjugated (indirect) bilirubin. Monitoring of bilirubin in newborns, particularly if premature, has special importance since the hepatic handling of bilirubin is immature leading to elevated unconjugated bilirubin. If not bound to albumin, unconjugated bilirubin is able to cross the blood brain barrier more easily, increasing the risk of cerebral damage. Total bilirubin is elevated in conditions causing obstruction of the bile duct, hepatitis, cirrhosis, in hemolytic disorders and several inherited enzyme deficiencies. There is information indicating elevated levels of direct bilirubin in patients with liver or biliary tract disease, even though, total bilirubin levels are normal. Therefore, the greatest clinical significance is for direct bilirubin assays stem from their ability to indicate occult liver disease.

EXPECTED VALUES

<table>
<thead>
<tr>
<th>Total Bilirubin</th>
<th>Direct Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults and infants &gt; 1 month</td>
<td>0.2 – 1.0 mg/dl</td>
</tr>
<tr>
<td>Newborns premature (3-5 days)</td>
<td>10 – 14 mg/dl</td>
</tr>
<tr>
<td>Newborns (3-5 days)</td>
<td>4.0 – 8.0 mg/dl</td>
</tr>
<tr>
<td>Newborns (&lt;48 hrs)</td>
<td>6.0 – 10.0 mg/dl</td>
</tr>
<tr>
<td>Newborns (&lt;24 hrs)</td>
<td>2.0 – 6.0 mg/dl</td>
</tr>
<tr>
<td>Direct Bilirubin</td>
<td>Up to 0.2 mg/dl</td>
</tr>
<tr>
<td>Adults and infants</td>
<td>Up to 3.4 μmol/l</td>
</tr>
</tbody>
</table>

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the bilirubin results should always be assessed in conjunction with the patient’s medical history, clinical examination, and other findings.

REAGENTS

| R1 | Sulfanilic acid | 30.0 mmol/l |
| R2 | Sodium nitrite | 0.20 N |
| R3 | Caffeine | 30.0 mmol/l |
| R4 | Sodium benzoate | 0.26 mol/l |
| R5 | Tartarate | 0.93 mol/l |
| R6 | Sodium hydroxide | 1.90 N |

● Reagent Preparation & Stability

All reagents are stable up to the expiry date given on label when stored at room temperature.

METHOD

Diazoo method according to Jendrassik-Graf.

PROCEDURE

Mix, and Incubate for 5 minutes at room temperature Measure the absorbance of specimen (A specimen) against specimen blank. The color is stable for 30 minutes.

Direct Bilirubin

Wavelength 546 nm
Cuvette 1 cm light path
Temperature 20-25 °C
Zero adjustment against specimen blank
Specimen Serum
R1 200 μl 200 μl
R2 One drop
R3 1000 μl 1000 μl
Specimen 200 μl 200 μl

Mix well, let stand 10 minutes at room temperature then add:

R2 1000 μl 1000 μl

Calculation

Calculate the bilirubin concentration by using the following formulae:

Total Bilirubin Concentration = Specimen absorbance X 10.8 =mg/dl

Direct Bilirubin Concentration = Specimen absorbance X 14.4 =μmol/l

Mix, and incubate for exactly 5 minutes at room temperature. Measure the absorbance of specimen (A specimen) against specimen blank.
QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:
- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commerically available control material with established bilirubin values may be routinely used for quality control. Failure to obtain the proper range of values in the assay of control material may indicate:
- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:
- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

1. Anticoagulants:
The only accepted anticoagulants are heparin and oxalate for direct bilirubin.

2. Drugs:
Young in 1990 has published a comprehensive list of drugs and substances, which may interfere with this assay.

3. Hemolysis:
Avoid hemolyzed specimens. Even slight hemolysis interferes with the test. Haemoglobin interference is dependent on both analyte and hemoglobin concentration. The interference becomes decreasingly significant with increasing concentration of total bilirubin.

4. Lipemia:
Avoid lipemic specimens. Even slight lipemia interferes with the test.

5. Procedure errors.

PRODUCIBILITY

Reproducibility was determined using in an internal protocol. The following results were obtained.

**Within Run**

<table>
<thead>
<tr>
<th>I. Total Bilirubin</th>
<th>Control</th>
<th>Level I</th>
<th>Level II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Mean (mg/dl)</td>
<td>1.15</td>
<td>4.22</td>
<td></td>
</tr>
<tr>
<td>SD (mg/dl)</td>
<td>0.02</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.02</td>
<td>1.05</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. Direct Bilirubin</th>
<th>Number of samples</th>
<th>Mean (mg/dl)</th>
<th>SD (mg/dl)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
<td>0.78</td>
<td>0.015</td>
<td>1.31</td>
</tr>
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</table>

**Between Day**

<table>
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<tr>
<th>I. Total Bilirubin</th>
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<tr>
<td>Mean (mg/dl)</td>
<td>1.15</td>
<td>4.27</td>
<td></td>
</tr>
<tr>
<td>SD (mg/dl)</td>
<td>0.02</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.31</td>
<td>3.2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
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<tr>
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<th>Mean (mg/dl)</th>
<th>SD (mg/dl)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
<td>0.8</td>
<td>0.01</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.8</td>
<td>0.01</td>
<td>1.83</td>
</tr>
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</table>

**Method Comparison**

Comparison studies were carried out using a similar commercially available Total Bilirubin reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

- Number of sample pairs: 929
- Range of sample results: 0.05-26 mg/dl
- Mean of reference results: 1.2 mg/dl
- Mean of Total Bilirubin results: 1.2 mg/dl
- Slope: 1.10
- Intercept: 0.12 mg/dl
- Correlation coefficient: 1.00

**Sensitivity**

The sensitivity is defined as the change of analytical response (ΔA/min) per unit change in analyte concentration at a pathlength of 1 cm.

When run as recommended the sensitivity of this assay is 0.01 mg/dl (0.17 mol/l).

**LINEARITY**

When run as recommended, the assay is linear up to 25 mg/dl (0.428 mmol/l). If result exceeds 25 mg/dl (0.428 mmol/l), specimen should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

**BIBLIOGRAPHY**


**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>REF</th>
<th>SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1081</td>
<td>265 ml</td>
</tr>
<tr>
<td>1082</td>
<td>625 ml</td>
</tr>
</tbody>
</table>

**SYMBOL DECLARATION**

- Manufacturer
- Consult instructions for use
- Batch code (Lot #)
- Catalog number
- Temperature limitation
- In vitro diagnostic medical device
- Use by
- Caution. Consult instructions
- Keep away from light

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