**PRINCIPLE OF THE METHOD**

Total bilirubin is determined colorimetrically using diazotized sulfanilic acid and measured photometrically. Of the two fractions present in serum, bilirubin-glucuronide and free bilirubin, the former appears directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulphoxide (DMSO) to react (bilirubin indirect). The determination of indirect bilirubin is the same as total bilirubin, except that the latter has been corrected for the direct fraction. The intensity of the color formed is proportional to the bilirubin concentration in the sample.

**CLINICAL SIGNIFICANCE**

Total bilirubin is increased in conditions that either increase bilirubin formation or decrease its elimination. The most common causes of hyperbilirubinemia are chronic liver disease, hemolysis, some congenital and haemoglobinopathies. Bilirubin can cause jaundice when its concentration is higher than 2.32 µmol/L.

**STORAGE AND STABILITY**

All the reagents are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contamination prevented during their use. Do not use reagents over the expiration date.

**QUALITY CONTROL**

Control sera are recommended to monitor the performance of the assay procedures. If control values are found outside the defined range, check the instrument, reagents, and calibrator for problems. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

**REFERENCE VALUES**

- Up to 1.10 mg/dL.
- Up to 18.81 µmol/L.

These values are for orientation purpose; each laboratory should establish its own reference range.

**PERFORMANCE CHARACTERISTICS**

<table>
<thead>
<tr>
<th>Measuring range:</th>
<th>0.099 mg/L to 18 mg/dL.</th>
</tr>
</thead>
<tbody>
<tr>
<td>If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.</td>
<td></td>
</tr>
<tr>
<td>Precision:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intra-assay (n=20)</td>
</tr>
<tr>
<td>Mean (mg/dL)</td>
<td>1.12</td>
</tr>
<tr>
<td>SD</td>
<td>0.02</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.33</td>
</tr>
</tbody>
</table>

Sensitivity: 1 mg/dL = 0.01540 A.

**NOTES**

1. For bilirubin determination in newborns, pipette 50 µL of sample.
2. BSM has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

**BIBLIOGRAPHY**