INTENDED USE
For the quantitative in vitro determination of Total Bilirubin in serum and plasma. This product is suitable for manual use.

Cat. No.
BW 2361  R1a. Sulphanilic acid/DMSO  2 x 250 ml
2 x 250 ml  R1b. Sodium Nitrite  1 x 15 ml

PRINCIPLE
Total bilirubin is determined in the presence of dimethylsulphoxide (DMSO) by the reaction with diazotised sulphanilic acid.

SAMPLE
Serum, heparinized plasma or EDTA-plasma. Haemolysis interferes with the test. Fresh samples should be kept out of direct light.

REAGENT COMPOSITION

<table>
<thead>
<tr>
<th>Contents</th>
<th>Concentrations of Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1a. Sulphanilic acid</td>
<td>29.0 mmol/l</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>164 mmol/l</td>
</tr>
<tr>
<td>Dimethylsulphoxide</td>
<td>5.6 mol/l</td>
</tr>
<tr>
<td>R1b. Sodium nitrite</td>
<td>0.35 mol/l</td>
</tr>
</tbody>
</table>

SAFETY PRECAUTIONS AND WARNINGS
For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents. Health and Safety Data Sheets are available on request. The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENT
Prepare diazo reagent by mixing 7.5 ml of Reagent R1a and 1 drop of Reagent R1b (or 15 ml of R1 and 100 μl of R2). Stable for 5 hrs at +15 to +25°C and 24 hr at +2 to +8°C protected from light.

MATERIALS PROVIDED
Sulphanilic acid
Sodium Nitrite

MATERIALS REQUIRED BUT NOT PROVIDED
Randox Assayed Multisera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532)
Randox Calibration Serum Level 3 (Cat. No. CAL 2351)
Randox Bilirubin Elevated Control (Cat. No. BE 454)

PROCEDURE NOTES
For bilirubin determination in newborns the sample volume in the procedure should be reduced to 20 μl.

PROCEDURE

Wavelength: 546 nm

Cuvette: 1 cm

Reaction Temperature: 20 - 25°C

Measurement: against reagent blank

Total Bilirubin

Pipette into tube:

<table>
<thead>
<tr>
<th>Reagent blank</th>
<th>Sample/Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazo Reagent (R1) 1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Sample/Standard</td>
<td>40 μl</td>
</tr>
</tbody>
</table>

Mix, and allow to stand for 5 min at 20-25°C and then read the absorbance of the sample/standard against the reagent blank (A).

CALCULATION

Concentration of Total Bilirubin in Sample

\[
\text{A of Sample} = \frac{\text{A of Sample}}{\text{A of Standard}} \times \text{standard concentration}
\]

CALIBRATION

We recommend Randox Calibration Serum Level 3.

QUALITY CONTROL
Randox Assayed Multisera, Level 2 and Level 3 and Randox Bilirubin Elevated Control are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Customer Technical Support, Northern Ireland (028) 94422413.

INTERFERENCES
A sample blank should be carried out with lipaemic samples. Reagent 1 is used as a blank reagent. The absorbance of the sample blank is subtracted from the sample absorbance before calculating the concentration in the sample.

NORMAL VALUES IN SERUM

Total bilirubin: up to 17 μmol/l (1 mg/dl)

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

LINEARITY
The method is linear up to 340 μmol/l (20 mg/dl).

REFERENCES

Revised 17 Sep '08 ne