According to 1272/2008 regulation, this reagent is not classified as dangerous.

### INTENDED USE

I This reagent is designated for professional use in laboratory (automated method). It allows the quantification of Total and Direct Bilirubin in human serum and plasma.

### GENERALITIES (1) (6)

At least four distinct bilirubin species exist in the serum: Direct-reacting bilirubin (DB) consists of mono and diconjugated bilirubin (β and γ- Bilirubin) and the δ-fraction which is bilirubin tightly bound to albumin; unconjugated α-bilirubin which is water soluble and bound to albumin. Total bilirubin (TB) is the sum of these different species.

### PRINCIPLE (4) (5)

Reaction between bilirubin and diazotised sulfanilic acid which leads to a compound, the azobilirubin, coloured in very acid or basic medium. Malloy-Evelyn principle modified by Walters and al: in an aqueous solution, only DB reacts. To enable the assay of TB, it is necessary to break the link between unconjugated bilirubin and albumin. This step is done by adding dimethyl sulfoxide (DMSO). The absorbance of azobilirubin thus produced is proportional to the concentration of bilirubin and is measured at 550 nm (530-580).

### REAGENTS COMPOSITION

#### R1 TOTAL BILIRUBIN

- Sulfanilic acid: 30 mmol/L
- DMSO: 7 mol/L
- Hydrochloric acid: 130 mmol/L

EUH210: Safety Data Sheet available on request
EUH208: Contains sulfanilic acid. May produce an allergic reaction

#### R2 DIRECT BILIRUBIN

- Sulfanilic acid: 30 mmol/L
- Hydrochloric acid: 130 mmol/L

EUH210: Safety Data Sheet available on request
EUH208: Contains sulfanilic acid. May produce an allergic reaction

#### R3 TOTAL AND DIRECT BILIRUBIN

- Sodium Nitrite: 0.74 mmol/L

According to 1272/2008 regulation, this reagent is not classified as dangerous.

### SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

### REAGENTS PREPARATION

Ready for use.

### STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 2-8°C, reagent is stable when stored and used as described in the insert: Unopened and once opened (when free from contamination):
- Until expiry date stated on the label of the kit
- Discard any reagent if cloudy or if absorbance at 550 nm > 0.100.

### SPECIMEN COLLECTION AND HANDLING (2) (7)

Unhemolyzed serum or plasma.
Bilirubin is photo labile. Store the specimen away from light.

- Stability in the specimen: 4 to 7 days at 2-8°C, 2 days at RT.

### LIMITS (3)

The bilirubin diazo reaction is temperature sensitive and should be carried out at a constant temperature. For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

### MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Clinical Analyzer
CALIBRATION (8)

- Factor as indicated § CALCULATION or
- REF 95015 Multicalibrator traceable to internal master lot (issued from SRM 916).

The calibration frequency depends on proper instrument functions and on preservation of reagents.

EXPECTED VALUES (2)

<table>
<thead>
<tr>
<th>Total Bilirubin mg/dL</th>
<th>[µmol/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>New born</td>
<td></td>
</tr>
<tr>
<td>In cord</td>
<td></td>
</tr>
<tr>
<td>0-1 day</td>
<td></td>
</tr>
<tr>
<td>1-2 days</td>
<td></td>
</tr>
<tr>
<td>3-5 days</td>
<td></td>
</tr>
<tr>
<td>Adult (child &gt; 5 years)</td>
<td></td>
</tr>
<tr>
<td>&gt; 5 days-60 years</td>
<td></td>
</tr>
<tr>
<td>60-90 years</td>
<td></td>
</tr>
<tr>
<td>&gt; 90 years</td>
<td></td>
</tr>
</tbody>
</table>

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES

On Kenza 240TX, 546 nm, 37°C

- Detection limit:
  - TB: approx. 0.28 mg/dL, DB: approx. 0.01 mg/dL
- Linearity Range:
  - TB: between 0.45 and 20 mg/dL, DB: between 0.60 and 8.0 mg/dL
- Precision TB:
  - Between run:
    - Level 1
      - Mean (mg/dL): 1.06
      - S.D. mg/dL: 0.03
      - C.V. %: 2.5
    - Level 2
      - Mean (mg/dL): 3.14
      - S.D. mg/dL: 0.06
      - C.V. %: 2.0
    - Level 3
      - Mean (mg/dL): 14.14
      - S.D. mg/dL: 0.12
      - C.V. %: 0.9
- Precision DB:
  - Between run:
    - Level 1
      - Mean (mg/dL): 1.03
      - S.D. mg/dL: 0.05
      - C.V. %: 4.9
    - Level 2
      - Mean (mg/dL): 3.15
      - S.D. mg/dL: 0.10
      - C.V. %: 3.3
    - Level 3
      - Mean (mg/dL): 13.73
      - S.D. mg/dL: 0.37
      - C.V. %: 2.7

Analytical sensitivity:
- TB: approx. 0.0083 abs for 1 mg/dL
- DB: approx. 0.0019 abs for 1 mg/dL

For more information, please refer to the manual or contact the local support service.

QUALITY CONTROL

- REF 96010 EXATROL-N Level 1
- REF 95011 EXATROL-P Level 2

External quality control program.

It is recommended to control in the following cases:
- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.

After maintenance operations on the instrument. If it is out of range, apply following actions:
1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. With factor: Verify analysis parameters (Wavelength, temperature, specimen/reagent ratio, time counting, calibration factor).
4. Use a new vial of reagent and repeat the test.
5. With a calibrator: If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
6. If control is still out of range, calibrate again with a new vial of reagent and repeat the test.

If control is still out of range, please contact BIOLABO support or your local agent.

MANUAL PROCEDURE

Let stand reagents and specimen at room temperature.

- With Seric Multicalibrator
  - Result = Abs (Assay - Blank) Specimen x calibrator concentration
  - Abs (Assay - Blank) Calibrator

With Factor (Path length 1 cm, 37°C, 550 nm)

mg/dL = [Abs. assay – Abs. Blank] x 11.4*  
[µmol/L] = [Abs. assay – Abs. Blank] x 195*

With Paediatric method: multiply the factor by 5 (sample volume).

*This factors should be used as a guide only.

REFERENCES

(8) SRM: Standard Reference Material®