

Direct Bilirubin – Instructions for Use (IFU)

R1: 4 x 50 mL – R2: 4 x 20 mL • **REF** A-R010000201

R1: 3 x 18,5 mL – R2: 1 x 18,5 mL • **REF** R333000010



INTENDED USE

Product for use in the quantitative determination in vitro of the concentration of the Direct Bilirubin in human serum or plasma. The results of the test must always be interpreted in conjunction with the clinical context. For professional use only.

CLINICAL SIGNIFICANCE

Approximately 80-85 % of the bilirubin produced is derived from the heme moiety of the haemoglobin released from aging erythrocytes in the reticuloendothelial cells. Bilirubin, bound to albumin, is transported into the liver where it is rapidly conjugated with glucuronide to increase its solubility. Then it is excreted into biliary canaliculi, and hydrolyzed in the gastrointestinal tract.

Unconjugated bilirubin serum concentration increases in case of overproduction of bilirubin (acute or chronic haemolytic anemias) and in case of disorders of bilirubin metabolism and transport defects (impaired uptake by liver cells: Gilbert's syndrome; defects in the conjugation reaction: Crigler-Najjar syndrome). Reduced excretion (hepatocellular damage: hepatitis, cirrhosis, Dubin-Johnson and Rotor syndrome) and obstruction to the flow of bile (most often produced by gallstones or by tumors) induce an important elevation of conjugated bilirubin and in a minor extent an increase of unconjugated bilirubin (conjugated hyperbilirubinemia).

PRINCIPLE

Mally – Evelyn modified. End point. Sulphanilic acid reacts with sodium nitrite to form diazotized sulphanilic acid. In the presence of accelerator (centrimide), conjugated and unconjugated bilirubin react with diazotized sulphanilic acid to form azobilirubin (Bilirubin Total 4+1). In the absence of accelerator, only conjugated bilirubin reacts (Bilirubin direct 4+1). The increase of absorbance at 550 nm is proportional to bilirubin concentration.

Sulphanilic acid + NaNO₂ → Diazotized sulphanilic acid

Bilirubine + Diazotized sulphanilic acid → Azobilirubin

REAGENTS

A-R010000201 – R1: 4 x 50 mL – R2: 4 x 20 mL

Reagent 1: n° 4 vials x 46,0 mL ready to use

Reagent 2: n° 4 vials x 12,0 mL ready to use

R333000010 – R1: 3 x 18,5 mL – R2: 1 x 18,5 mL

Reagent 1: n° 3 vials x 12,6 mL ready to use

Reagent 2: n° 1 vial x 9,5 mL ready to use

Concentrations

| Reagent 1: | Conc. | U.M. |
|------------------|-------|--------|
| Sulphanilic Acid | 29.0 | mmol/L |
| Reagent 2: | | |
| Sodium Nitrite | 11.0 | mmol/L |

Precautions

Reagent 1 contains sulphanilic acid. May produce an allergic reaction. Consult the Safety Data Sheet. In addition to risk indications related to the active components, reagents may contain inactive components such as preservatives and detergents. The total concentration of these components is lower than the limits reported in the EC 1272/2008 regulation and subsequent amendments and additions. However, It is recommended to handle reagents according to the standards of good laboratory practice.

Reports of serious incidents

In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements. Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

Storage and stability

Store at 2-8°C and protect from direct light. When correctly stored, the reagents are stable up the expiry date reported on the label. A slight variation in the colour of the reagent may occur between batches, but this has no effect on the test results. After opening, the vial R1 and R2 are stable 28 days if recapped immediately and protected from contamination, evaporation, direct light and stored at correct temperature

SAMPLE COLLECTION

Type of sample and storage

Fresh, non-haemolyzed serum or plasma samples should be used. Store away from the light. The samples must be tested within 2 hours if stored at room temperature or within

12 hours if stored at 2 - 8°C. If frozen between - 15°C and - 20°C the samples are stable for 3 - 4 months (4).

Precaution

All human samples must be handled and disposed of as potentially infectious materials.

PROCEDURE

Quality control

Human control serum with known levels of Direct Bilirubin is commercially available for quality control purposes. Data sheets are included, listing the values and the confidence limits. Normal and abnormal control sera are available as "Normal control serum" code R040000006 and "Pathological control serum" code R040000106. The values obtained must be contained within the acceptability range. In case of incorrect results check the following points:

Cleanliness of glassware.

- Wavelength setting.
- Expiration date of reagents.

Automation

Although this device has been developed and manufactured to be used by manual method and instrumental systems, it can also be used in combination with other instrumental devices that are able to meet the requirements indicated in the paragraph "Reaction conditions / Technique".

All applications that are not explicitly approved cannot be guaranteed in terms of performance and will therefore have to be evaluated by the user

Calibration

For calibration use the "Multicalibrator code R030000006. For the use on ISE srl instruments Calibration every 10 days is recommended.

Traceability:

The Direct Bilirubin value is reported in the package insert supplied with the "Multicalibrator".

Reagents included in the kit

The reagent is described above.

Materials required but not supplied in the kit

Calibrators and controls.

Reaction conditions

Wavelength (primary): 550 nm

Temperature: 37°C

Technique

Bring the reagents to the reaction temperatures

| | U.M. | Calibration Serum | Sample | Blank |
|--|------|-------------------|--------|-------|
| Reagent A | µL | 800 | 800 | 800 |
| Calibration Serum | µL | 100 | - | - |
| Sample | µL | - | 100 | - |
| Blank | µL | - | - | 100 |
| Mix gently and incubate at 37°C for 5 min. Read absorbance A1 at 550 and 700 nm, then add. DA1= A1 550nm - A1 700nm | | | | |
| Reagent B | µL | 200 | 200 | 200 |
| Mix gently and incubate at 37°C for 50 sec. Read absorbance A2 at 550 and 700 nm, then add. DA2= A2 550nm - A2 700nm | | | | |

Reading

Read the absorbances at 550 nm and 700 nm of the sample and the calibrator, subtracting the absorbance of the reagent white. The final color is stable 1 hour away from direct light.

Results

The concentration of Direct Bilirubin is obtained through the following formula

$$\left[\frac{A_{\lambda 2} - A_{\lambda 1} \times F_{dil}}{N} \right]_{\text{sample}} \times n$$

$$\left[\frac{A_{\lambda 2} - A_{\lambda 1} \times F_{dil}}{N} \right]_{\text{calibrator}}$$

Fdil = Dilution factor (0.81)


N = Calibrator concentration

Conversion factor mg/ dl x 17.1 = µmol/L



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NORMAL VALUES

Serum or plasma:

Up to 0.2 mg/dL (3.4 µmol/L).

Each laboratory must establish its own normal values on the basis of its local population.

ANALYTICAL CHARACTERISTICS / PERFORMANCE

Linearity

The measurement range is 0.08 to 10.55 mg/dL (1,4 – 180,4 µmol/L) of Direct Bilirubin in the sample. For higher concentrations, repeat the test on the sample diluted 1:5 in physiological solution then multiply the result by the dilution factor. This procedure extends the measuring range up to 50 mg/dL (855,2µmol/L)

Interference

No significant interference was detected in the presence of triglycerides (up to 2000 mg/dl), Hemoglobin (up to 125 mg/dl); Acetaminophene (up to 30 mg/dl); Ascorbic acid (up to 0.5 mg/dl); Acetyl salicylic acid (up to 200 mg/dl). Do not use hemolyzed samples.

Accuracy-Recovery

Accuracy studies were conducted on normal samples to which amounts of pure bilirubin notes in the range 1 - 10 mg/dL were added. The data indicate a recovery of 99.95%.

Precision of the method

| Within-run precision | | | | |
|-----------------------|-------|------|----------|-----|
| Range | U.M. | Mean | C.V. (%) | No. |
| Low | mg/dL | 0.36 | 3.8 | 80 |
| Medium | mg/dL | 1.51 | 1.9 | 80 |
| High | mg/dL | 3.99 | 0.9 | 80 |
| Between-run precision | | | | |
| Range | U.M. | Mean | C.V. (%) | No. |
| Low | mg/dL | 0.36 | 5.2 | 80 |
| Medium | mg/dL | 1.51 | 5.3 | 80 |
| High | mg/dL | 3.99 | 4.7 | 80 |

Sensitivity

At λ 546 nm a concentration of about 0.08 mg/dL of Direct Bilirubin in the conditions established for this test.

Comparative method

The method was compared with a similar method on the market. Samples analysed n° = 100 (with values between 0.09 and 10.52 mg/dL); Regression line $y = 0.926x - 0.03$; $r = 0.998$.

Disposal of reagent

Disposal of reagents must be performed in accordance with the EC regulations regarding waste, or the local national or regional legislation.

Manufacturer:

Sclavo Diagnostics International

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Distributor:

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Symbols used in IFU and Packaging

| | |
|--|--|
|  In vitro diagnostic medical device vitro |  Manufacturer |
|  Catalogue Number |  Instruction for use |
|  Lot Number |  Temperature limitation |
|  Expiration date | |

References

1. Malloy HT, Evelyn KA. J Biol Chem 1937; 119: 481-490.
2. Colombo JP, Peheim E, Kyburz S, Hoffmann JP. Clin Chim Acta 1974; 51: 217-219.
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4. Martinek RG. Clin Chim Acta 1966; 13: 161-170.
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| REVISION | DATE | CHANGE |
|----------|---------|--|
| Rev.A | 12/2022 | New Issue for IVDR Regulation (UE) 2017/746 compliance |

