Direct Bilirubin – Instructions for Use (IFU)

Manufactured exclusively for: ISE S.r.l. CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

R1: 4 x 46 mL – R2: 4 x 12 mL • REF A-R0100000201

R1: 3 x 12,6 mL – R2: 1 x 9,5 mL • REF R3330000010

IVD CE

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 aniemias) and in case of disorders of bilirubin metabolism and transport defects (impaired uptake by liver cells: Gilbert's syndrome; defects in the conjucation 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**.** Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. In the presence of accelerator (centrimide), conjugated and unconjugated bilirubin react with diazotized sulfanilic acid to form azobiliburin (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.
Sulfanilic acid + $NaNO_2$ → Diazotized sulfanilic acid
Bilirubine + Diazotized sulfanilic acid → Azobilirubin

REAGENTS
A-R0100000201 – R1: 4 x 46 mL – R2: 4 x 12 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
R3330000010 – R1: 3 x 12,6 mL – R2: 1 x 9,5 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 R0400000006 and "Pathological control serum" code R0400000106. 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 R0300000006. 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
$$\frac{[\Delta A2 - \Delta A1 \times Fdil]\ sample}{[\Delta A2 - \Delta A1 \times Fdil]\ calibrator} \times n$$
Fdil = Dilution factor (0.81)
N = Calibrator concentration
Conversion factor mg/ dl x 17.1 = µmol/L

Sclavo Diagnostics International S.p.A
Via Po 26-28 – Loc. Pian dei Mori – 53018 Sovicille (SI) (Italy)
Phone +39 0577 39041 - Fax +39 0577 390 444

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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); Acetaminephene (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 = 0.926 x - 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
Via Po 26-28 – Loc. Pian dei Mori – 53018 Sovicille (SI) (Italy)
Phone +39 0577 39041 - Fax +39 0577 390 444

Distributor:
I.S.E S.r.l.
Via Delle Driadi, 45 – 00133 Roma
Tel.+39 077 4579365; FAX +39 077 4579305
E-mail: info@logotech-ise.com
www.logotech-ise.com

Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

References

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REVISION	DATE	CHANGE
Rev.C	01/2024	Edit header and “Reagents” paragraph.