


Total Bilirubin – Instructions for Use (IFU)

Manufactured exclusively for:

R1: 4 x 41 mL – R2: 4 x 10,5 mL •  A-R0100000101

R1: 3 x 12,6 mL – R2: 1 x 9,5 mL •  R3330000011



INTENDED USE

Product for use in the quantitative determination in vitro of the concentration of the Total 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

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-R0100000101 – R1: 4 x 41 mL – R2: 4 x 10,5 mL

Reagent 1: n° 4 vials x 41,0 mL ready to use

Reagent 2: n° 4 vials x 10,5 mL ready to use

R3330000011 – 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	mmol/L
Cetrimide	29	mmol/L
Reagent 2:		
Sodium Nitrite	11	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 composition 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-hemolyzed 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 Total 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 Total Bilirubin value is reported in the package insert supplied with the "Multicalibrator".

Reaction conditions

Wavelength (primary): 550 nm
Temperature: 37°C

Technique – Procedure with Reagent B as Starter

Bring the reagents to the reaction temperatures

	U.M.	Calibration Serum	Sample	Blank
Reagent A	µL	800	800	800
Calibration Serum	µL	50	-	-
Sample	µL	-	50	-
Blank	µL	-	-	50
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 Total Bilirubin is obtained through the following formula

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

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

Fdil = Dilution factor (0.81)

N = Calibrator concentration

Conversion factor mg/ dl x 17.1 = µmol/L

Reagents included in the kit

The reagent is described above.

Materials required but not supplied in the kit

Calibrators and controls.



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ISE S.r.l.
CUSTOMISED SOLUTIONS
FOR YOUR LABORATORY

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NORMAL VALUES

Serum or plasma:

Up to 1.2 mg/dL (21.0 mmol/L).

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

ANALYTICAL CHARACTERISTICS / PERFORMANCE

Linearity

The measurement range is 0.25 to 25.0 mg/dL Total 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 60 mg/dL (1026.3 µmol/L)

Interference

No significant interference was detected in the presence of triglycerides (up to 2100 mg/dl). Hemoglobin (up to 500 mg/dl); Acetaminophene (up to 30 mg/dl); Ascorbic acid (up to 4 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	1.15	1.8	80
Medium	mg/dL	4.08	0.4	80
High	mg/dL	14.61	0.5	80
Between-run precision				
Range	U.M.	Mean	C.V. (%)	No.
Low	mg/dL	1.15	5.0	80
Medium	mg/dL	4.08	3.1	80
High	mg/dL	14.61	2.9	80

Sensitivity

At λ 546 nm a concentration of about 0.15 mg/dL of Total 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 0.32 and 23.02 mg/dL); Regression line $y = 0.948x - 0.11$; $r = 0.999$.

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:

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Distributor:

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


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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

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



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