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

Direct Bilirubin – Instructions for use

R1: 4 x 50 mL • REF A-R0100000201
R2: 4 x 20 mL

CE IVD

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).

METHOD

Mally – Evelyn modified. End point.

PRINCIPLE

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

Reagent 1: n° 4 vials x 44.5 mL liquid reagent
Reagent 2: n° 4 vials x 11.5 mL liquid reagent

REAGENT COMPOSITION

Concentrations

Reagent 1:		
	Conc.	**U.M.**
Sulphanilic Acid	29	mmol/L
Hydrochloric Acid	67	mmol/L
Reagent 2:		
Sodium Nitrite	5.8	mmol/L

REAGENT 1

Xi

R36/37/38/43 : IRRITATING to eyes, respiratory system and skin
S26: in case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S37/39: Wear suitable gloves and eye/face protection.

H315 Skin Irrit.. 2
H317 Skin Sens. 1
H319 Eye Irrit. 2
H335 Stot SE 3

H314 Skin Corr. 1B

Sulfanic acid contained in reagent R1 may produce an allergic reaction.
Use clean or single use laboratory equipment only to avoid contamination.
For more information, Material Safety Data Sheet (MSDS) is available on request for professional user.

Precautions

In addition to the eventual risk indications regarding the active components, the reagents may contain inactive components such as preservatives (e.g. sodium azide or others) and detergents. The total concentrations of these components is lower than the limits reported by the 67/548/EEC and 1999/45/EC directives and following modification and amendments. However, it is recommended to handle reagents carefully, to avoid ingestion and contact with eyes, skin and mucos membranes and to use laboratory reagents according to good laboratory practice.

Storage

The components of the kit, stored at 2-8 °C in unopened vials, are stable up to the expiry date indicated on the package.

SAMPLE COLLECTION

Specimen

Serum free of hemolysis. Heparinized plasma.
Care must be taken to fill heparinised tubes according to the manufacturer's instructions. An insufficient filling may lead to erroneous results.
Protect the samples from light before and during the analysis.

Conservation and storage

If plasma and serum are protected from light, samples are stable 2 days at room temperature and 4 days at 4°C. For a longer storage, freeze the samples at -20°C.

Precautions

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 from I.S.E. S.r.l. "Normal control serum" code R0400000006 and "Pathological control serum" code R0400000106. Obtained values must be within the range of acceptability. If erratic results occur, the following points should be checked:

- Cleanliness of glassware.
- Wavelength setting.
- Expiration date of reagents.

Automation

This kit, though developed and manufactured to be used as manual assay and with I.S.E. S.r.l. analyzer, can be used also with other analyzers able to meet the specifications indicated in section "Reaction conditions - Test procedure" Application sheets are available for automatic instruments.
All applications not explicitly approved by I.S.E. S.r.l. cannot be guaranteed in terms of performance, and must therefore be established by the operator.

Calibration

For calibration use the "Multicalibrator" I.S.E. S.r.l. code R0300000006.

Traceability:

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

Calibration Stability

For the instrumentation series Miura, the calibration is recommended to be done every 10 days.

I.S.E S.r.l.
Via Delle Driadi, 45 – 00133 Roma - Italy
Tel.+39 06+ 20610289; FAX +39 06 2018131
E-mail: info@logotech-ise.com
www.logotech-ise.com

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Method for automated instrumentation

Analyzer:	**I.S.E. Miura**		
Analyte Name :	**Direct Bilirubin**	Ref.:	A-R0100000201
Method Code:	**BD**		
Type:	**Differential – Smpl Blk**		
Unit:	**mg/dL**		
Filter F1 / F2	**546 nm**		
Blank in:	**Not Used**		

Step	Reaction volume	U.M.
Volume reagent R1:	**180**	µl
Volume reagent R2:	**45**	µl
Sample volume:	**23**	µl
First uncubation	**36**	Sec.
Final uncubation	**300**	Sec.

Reagents included in the kit
The reagents are described above.

Materials required but not supplied in the kit
Calibrators and controls.

NORMAL VALUES

Serum, plasma: < 0.2 mg/dL (3.4 µmol/L)

It is recommended that each laboratory establish its own expected range. For diagnostic purposes, results obtained should always be evaluated taking into consideration the patient's history and all other clinical findings.

ANALYTICAL CHARACTERISTICS/PERFORMANCE

Linearity
The method is linear from 0.15 up to 18 mg/dL (2.6 to 307.9 µmol/L) of Direct Bilirubin. In the case of higher concentrations, repeat the test on samples diluted in physiological saline and multiply the result by the dilution factor.

Detection limit
Determined according to SFBC protocol, the detection limit is equal to 0.02 mg/dL (0.3 µmol/L).

Interferences
According to SFBC recommendations, studies have been performed to determine the level of interference from different compounds:
Turbidity: no significant interference up to 500 mg/dL (5.65 mmol/L) triglyceride equivalent.
Haemoglobin: negative bias from 250 mg/dL (2.5g/L).
Ascorbic acid: positive bias from 2 mg/dL (114 µmol/L).

In very rare cases, monoclonal gammapathies (multiple myeloma), in particular IgM type (Waldenstrom's macroglobulinemia) can cause unreliable results.
Other compounds may interfere.

Precision of the method

Within run reproducibility on serum:

Mean	N	Low level	Medium level	High level
mg/dL	20	0.60	2.03	6.14
µmol/L	20	10.3	34.7	105.0
CV%	20	2.0	2.1	1.5

Between run reproducibility on serum:

Mean	N	Low level	Medium level	High level
mg/dL	84	0.65	2.52	6.91
µmol/L	88	11.1	43.1	118.2
CV%	87	3.5	3.6	2.8

Sensitivity
The average variation of the analytical signal is 66 mΔA per mg/dL of bilirubin (3.9 mΔA per µmol/L) for a light path of 1 cm.

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

The product is in conformity with D.L: 8 September 2000, no. 332 "Actuation of the directive 98/79/EC regarding in vitro medical diagnostic devices".

Correlation
A comparative study has been performed between this and another commercial method on 43 human serum samples. The sample concentrations range form 0.10 to 15.70 mg/dL (1.7 to 268.5 µmol/L).
The parameters of linear regression are as follows:
Correlation coefficient: (r) = 0.9958

Linear regression: y = 1.083 x -0.11 mg/dL (1.9 µmol/L)

Symbols used on labels and packaging

IVD = In vitro diagnostic medical device
REF = Catalogue Number
LOT = Lot Number
= Manufacturer
= Expiration date
= Temperature limitation
= Instruction for use

Reference.

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