Bilirubin (Diazo with Sulphanilic Acid method)

In-vitro Diagnostic reagent kit for quantitative determination of Bilirubin in serum/plasma sample on Photometric System.

Reagent

Reagent T1 : Total Bilirubin Reagent 1
Reagent T2: Total Bilirubin Reagent 2
Reagent D1: Direct Bilirubin Reagent 1
Reagent D2: Direct Bilirubin Reagent 2

Principle

Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. In the presence of accelerator (cetrimide), conjugated and unconjugated bilirubin reacts with diazotized sulfanilic acid to form azobilirubin (Bilirubin Total). In the absence of accelerator only conjugated bilirubin reacts (Bilirubin Direct). The increase of absorbance at 546 nm is proportional to bilirubin concentration.

Summary

Approximately 80-85% of the bilirubin produced is derived from the heme moiety of the hemoglobin 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 Roter syndrome) and obstruction to the flow of bile (most often produced by gallstones or by tumours) induce an important elevation of conjugated bilirubin and in a minor extent an increase of unconjugated bilirubin (conjugated hyperbilirubinemia).

Reagents Storage Instructions and Stability

Reagents up to the end of the indicated month of expiry, if stored at 2°–30°C, protected from light and contamination is avoided.
Do not freeze the reagents!

Composition and Concentrations

Sulphanilic acid 7 g/L, Dimethyl Sulphoxide 0.5 mL/L, Conc. HCl 10 mL/L, Sodium Nitrite 7.0 g/L.

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Reagents are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin, plasma or EDTA plasma separate at the latest 1h after blood collection from cellular contents.
Stability in plasma/Serum
10 days at 2° –8°C
30 days at –20°C

Assay Procedure

Wavelength	546 nm (540-560 nm)
Optical path	10 mm
Temperature	R.T°C
Measurement	Against sample blank

Total Bilirubin

	Sample Blank (A1)	Sample Test (A2)
Reagent T1	1000 µL	1000 µL
Reagent T2	---	20 µL
Sample	50 µL	50 µL
Mix, Incubate for 5 min. at R.T°C. Read absorbance against sample blank.		

Direct Bilirubin

	Sample Blank (A1)	Sample Test (A2)
Reagent D1	1000 µL	1000 µL
Reagent D2	---	20 µL
Sample	50 µL	50 µL
Mix, Incubate for 5 min. at R.T°C. Read absorbance against sample blank.		

Calculation

Take ΔA (sample) and multiply by the corresponding factor from below:
ΔA (sample) = Sample Test (A2) – Sample Blank (A1)
Bilirubin (Total & Direct) mg/dL = ΔA (sample) x factor (21)

Quality Controls

For internal quality control any normal and abnormal controls should be assayed with each batch of samples.
Each laboratory should establish corrective action in case of deviations in control recovery.

Warning and Precautions

1. Keep out of reach of children. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
2. Take off immediately all contaminated clothing.
3. Wear suitable gloves and eye/face protection.
4. Always use safety pipettes to pull the reagents into a pipette.
5. Reagents may contain some non-reactive and preservative components. It is suggested to handle carefully, avoid direct contact with skin and do not swallow.
6. For professional use only!

Performance Characteristics

Measuring range
The test has been developed to determine bilirubin within a measuring range from 0.25 – 30 mg/dL. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result is multiplied by 2.

Linearity/Limit of maximum Detection

Linearity of detection is 30 mg/dL.

Sensitivity/Limit of Detection

The lower limit of detection is 0.25 mg/dL.

Specificity/Interferences

No interference was observed by triglycerides up to 800 mg/dL.

Precision

Total Bilirubin

Intra-assay n=20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.93	0.03	3.01
Sample 2	1.65	0.03	2.06
Sample 3	4.27	0.05	1.18

Inter-assay n=20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.92	0.03	3.65
Sample 2	1.53	0.05	3.07
Sample 3	3.98	0.08	2.05

Direct Bilirubin

Intra-assay n=20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.31	0.01	3.70
Sample 2	0.83	0.03	4.02
Sample 3	2.00	0.03	1.62

Inter-assay n=20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.32	0.01	3.19
Sample 2	0.87	0.03	3.07
Sample 3	2.12	0.03	1.63

Method Comparison

A comparison of Precision Biomed Bilirubin (y) with a commercially available test (x) using 15 samples gave following results:
Total Bilirubin: $y = 0.973x - 0.025$; $r^2 = 0.995$
Direct Bilirubin: $y = 0.988x + 0.024$; $r^2 = 0.982$

Reference Range

Total Bilirubin		mg/dL
Children	>1 month	0.2-1.0
Adults		0.1-1.2

Direct Bilirubin	mg/dL
Adults and children	≤0.2

Note: It is recommended that each laboratory should establish its own reference range based on the patient population.

Quick Reference

Parameter	Total Bilirubin		Direct Bilirubin	
	Sample blank	Sample	Sample blank	Sample
Mode	End Point		End Point	
Wavelength	546 nm		546 nm	
Path length	10mm		10mm	
Reagent T1	1000 µL	1000 µL	--	--
Reagent T2	---	20 µL	----	----
Reagent D1	---	----	1000µL	1000 µL
Reagent D2	---	----	----	20 µL
Sample	50 µL	50 µL	50 µL	50 µL
Incubation	5 min.		5 min.	
Temperature	R.T°C		R.T°C	
Factor	21		21	
Linearity	30 mg/dL		30 mg/dL	
Sensitivity	0.5 mg/dL		0.02 mg/dL	
Normal range				
Children	>1 month	0.2-1.0 mg/dL	--	<0.2 mg/dL
Adults	--	0.1-1.2 mg/dL	--	<0.2 mg/dL

Pack Size

Cat No.	Configuration	Pack
BIL00200	Reagent T1-100 ML Reagent T2- 5 ML Reagent D1- 100 ML Reagent D2- 5 ML	200ML
BIL01000	Reagent T1-500 ML Reagent T2- 10 ML Reagent D1- 500 ML Reagent D2- 10 ML	1000ML

Literature

1. Tietz, N.W., Clinical guide to laboratory tests. 3rd Ed., (W.B. Saunders eds. Philadelphia USA), (1995), 90. Vassault, A., et al., Protocole de validation de techniques.
2. Thomas L ed. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft, 1998: p. 192 - 202.
3. Tolman KG, Rej R. Liver function. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1125-77.
4. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 18-9.
5. Rand RN, di Pasqua A. A new diazo method for the determination of bilirubin. Clin Chem 1962;6:570-8.
6. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.

Version : BIL/00

IVD In Vitro Diagnostic Use | See Pack Insert For Procedure | Single Use only | CE
Temperature Limit | Manufacturer's Address | Manufacturing Date | Expiry Date | LOT Lot Number

Manufactured in India by :
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