BILIRUBIN (Total & Direct)

(Modified Jendrassik & Grof's Method)

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INTENDED FOR USE:

The Bilirubin T&D is an *in vitro* assay for the quantitative determination of Bilirubin in serum, plasma.

CLINICAL SIGNIFICANCE:

Bilirubin is mainly formed from the hemo portion of aged or damaged RBC's. It then combines with albumin to form a complex, which is not water soluble. This is referred to as indirect or unconjugated bilirubin. In the liver this bilirubin complex is combined with glucuronic acid into a water soluble conjugate. This is referred to as conjugated or direct bilirubin. Elevated levels of bilirubin are found in liver (Hepatitis, Cirrhosis) excessive haemolysis/destruction of RBC (hemolytic jaundice) obstruction of the biliary tract (obstructive jaundice) and in drug induced reactions. The differentiation between the direct and indirect bilirubin is important in diagnosing the cause of hyper bilirubinemia.

PRINCIPLE:

Conjugated (Direct) bilirubin present in serum reacts with diazotized sulphanilic acid to yield azobilirubin which absorbs at 546 nm. Total bilirubin present in serum reacts with diazoated sulphanilic acid in presence of activator to yield azobilirubin, which absorbs at 546 nm.

Bilirubin + Diazotized + Sulphanilic acid ⟶ Azobilirubin Compound

REAGENT COMPOSITION:

R1 Total Bilirubin Reagent	
Sulphanilic acid	10 mmol/L
Hcl	40 mmol/L
Sodium benzoate	20 mmol/L
Caffeine	25 mmol/L
Surfactant, Preservative	qs
R2 Total Diazo Reagent	
Sodium Nitrate	1.5 mmol/L
R3 Direct Bilirubin Reagent	
Sulphanilic acid	10 mmol/L
Hcl	10 mmol/L
R4 Direct Diazo Reagent	
Sodium Nitrate	15 mmol/L

REAGENT STORAGE:

All reagents are stable at room temperature (15-30°C) till the expiry date mentioned on the label. Reagents are ready to use and should be protected from direct light.

SAMPLE COLLECTION:

Unhemolyzed Serum, Heparinized plasma or EDTA plasma. Bilirubin is reported to be stable in the sample for 4 days at 2-8°C protected from light as it is photosensitive. Lipaemic samples should be avoided.

PRECAUTIONS & WARNING:

Avoid pipette with mouth. The preparation, according to current regulation, is classified as not dangerous. The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation. Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes. The samples must be handle as potentially infected from HIV or Hepatitis.

SYSTEM PARAMETERS: (Total and Direct Bilirubin)

Reaction Type	:	End Point Method
Wave Length	:	546 nm
Optical path length	:	1 cm
Abs max	:	≤ 0.1
Flow cell Temperature	:	37^0C
Blank	:	Sample Blank
Incubation Time	:	10 min
T&D Reagent Volume	:	1000 uL
Diazo Reagent Volume	:	25 uL
Sample Volume	:	50 uL
Factor	:	26.31
Reaction Slope	:	Increasing
Low Normal	:	0
High Normal	:	1.0
Linearity	:	20
Units	:	mg/dL

Note: Young, et al., give an exhaustive list of drugs and other substances known to affect the circulating level of Bilirubin. In this assay, as in all laboratory procedures, materials which come in contact with specimens should be clean and free of contamination by heavy metals, detergents, and other chemicals. Direct sunlight may cause up to a 50 % decrease in Bilirubin level within 1 hour.

ASSAY PROCEDURE:

Total Bilirubin Estimation:

Pipette in to test tubes labeled Sample Blank (SB) and Sample Test (ST) as follows:

Addition Sequence	Sample Blank (SB)	Sample Test (ST)
R1 Total Bilirubin Reagent	1000 µL	1000 µL
R2 Total Diazo Reagent	…	25 µL
Mix well and proceed		
Sample	50 µL	50 µL

Mix well and incubate at room temperature for 10 minutes and read absorbance of Sample Blank (SB) Sample Test (ST) at 546 nm.

Direct Bilirubin Estimation:

Pipette in to test tubes labeled Sample Blank (SB) and Sample Test (ST) as follows:

Addition Sequence	Sample Blank (SB)	Sample Test (ST)
R3 Direct Bilirubin Reagent	1000 µL	1000 µL
R4 Direct Diazo Reagent	…	25 µL
Mix well and proceed		
Sample	50 µL	50 µL

Mix well and incubate at Room temperature for 10 minutes and read absorbance of Sample Blank (SB) and Sample Test (ST) at 546 nm.

CALCULATION:

Total Bilirubin in mg/dL = Abs ST - Abs SB X 26.31
Direct Bilirubin in mg/dL = Abs ST - Abs SB X 26.31

SENSITIVITY / LIMIT OF DETECTION:

The method will accurately measure Bilirubin (T&D) level is 0.03 mg/dL.

LINEARITY:

The procedure is linearity up to 20 mg/dL. If values exceed this limit dilute the sample with normal saline and repeat the assay. Calculate the value using the proper dilution factor.

QUALITY CONTROL:

To ensure adequate quality control, the use of commercial reference control serum is recommended with each assay batch. Use of quality control material checks both the instrument and reagent function

REFERENCE RANGE:

Total Bilirubin : Up to 1.0 mg/dL
Direct Bilirubin : Up to 0.3 mg/dL
Note: It is recommended that each laboratory establish its own normal range representing its patient population.

INTERFERENCE:

No interference was observed by up to hemoglobin 50 gm/L and triglycerides 2000 mg/dL.

PRECISION:

Precision studies were performed with two controls using NCCLS protocol EP5 –A. The results of the precision studies are shown below:

Total Bilirubin:

Sample	Within - Run		Between - Run		Total	
	Mean	CV%	Mean	CV%	Mean	CV%
Control-1	1.74	1.24	1.89	1.32	3.63	2.56
Control-2	4.22	1.95	4.35	2.25	8.57	4.25

Direct Bilirubin:

Sample	Within - Run		Between - Run		Total	
	Mean	CV%	Mean	CV%	Mean	CV%
Control-1	0.45	1.48	0.49	1.65	0.94	3.13
Control-2	1.45	1.97	1.55	1.98	3.43	3.95

REFERENCES:

1. Jendrassik, L., Grof, P., (1938) Biochem. 2,297: 81.
2. Sherlock S. (1951) p.204 in Liver Disease, Churchill, London.

SYMBOLS USED ON THE LABELS:

MONOZYME
Reagents & Equipments
MONOZYME LIFE SCIENCES
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