TOTAL/DIRECT BILIRUBIN

(MODIFIED JENDRASSIC & GROUP OF METHODS)

PRINCIPLE

Bilirubin reacts with diazotized sulfanilic acid in acidic medium to form Azobilirubin, a pink colored complex whose absorbance is proportional to bilirubin concentration. Direct bilirubin being water soluble is allowed to react with diazotized sulfanilic acid in the presence of activator. Total Bilirubin (Direct & Indirect) the diazotization is carried out in the presence of an activator.

CLINICAL SIGNIFICANCE

Total & Direct Brilirubin estimation is important for diagnosis, differentiation and follow up of Jaundice

Hemolytic jaundice : Increased hemolysis results in elevation of Free/Indirect Bilirubin

Obstructive Jaundice : The direct bilirubin increases due to regulation of bile into hepatic circulation due to blockage of bile passage.
Hepatic Jaundice : Increase of both direct, indirect bilirubin is estimated to assess the extent of liver damage and subsequent progress or regress.

	Hemolytic	Hepatic	Obstructive
Direct Bilirubin	Normal	Increased	Increased
Indirect Bilirubin	Increased	Increased	Normal

SPECIMEN COLLECTION & STORAGE

Fasting Serum or Plasma sample is preferred.
Lipemic samples to be avoided.

Hemolysis should be avoided as hemoglobin produces falsely low values with diazo methods.

Both conjugated & Unconjugated bilirubin are photo oxidized on exposure to white/UV light.

Specimens should be protected from direct exposure to either artificial or sunlight as soon as they are drawn

PRECAUTIONS/NOTES

* Bilirubin Kit is for In-vitro diagnostic use only

* Strictly adhere to the pipetting sequence mentioned in the procedure

* Color will not develop in case of change pipetting sequence

KIT CONTENTS & STORAGE

Direct Bilirubin Reagent	1 x 100ml
Total Bilirubin Reagent	1 x 100ml
Total Diazo	1 x 3ml
Direct Diazo	1 x 3ml

REAGENT PREPARATION

All reagents are ready to use

SYSTEM PARAMETERS

TOTAL		DIRECT	
Reaction Type	End Point	Reaction Type	End Point
Wave Length	546nm	Wave Length	546nm
Incubation time	5 min dark	Incubation time	5min in dark
Factor	26.31	Factor	26.31
Blank	Sample Blank	Blank	Sample Blank
Linearity	25	Linearity	25
Unit	mg/dl	Unit	mg/dl
Temp	37^{o}	Temp	37^{o}

PROCEDURE

Take 4 clean test tube labeled as T1, T2 & D1,D2 add the reagents as shown below

TOTAL/DIRECT BILIRUBIN

(MODIFIED JENDRASSIC & GROUP OF METHODS)

Total Bilirubin	T1	T2	Direct Bilirubin	D1	D2
Total Bilirubin Reagent	1ml	1ml	Direct Bilirubin Reagent	1ml	1ml
Total Diazo	25 ml	…	Direct Diazo	25 µl	...
Serum/Plasma	50 µl	50 µl	Serum/Plasma	50 µl	50µl

Mix well and proceed

Mix well and keep at room temp for 5min then read the

absorbance of T1 & T2 at 546nm.

Mix well and keep at room temp for 5min then read the absorbance of D1 & D2 at 546nm.

CALCULATIONS

With Factor

Total Bilirubin in mg/dl = Abs of T1-T2 x 26.31

DirectBilirubin in mg/dl = Abs of D1-D2 x 26.31

LINEARITY

This method is linear up to 25 mg/dl. For sample values exceeding the linearity limit, dilute the sample suitably with normal saline and repeat the assay, Apply proper dilution factor while calculation. In its own normal range.

NORMAL RANGE

Total Bilirubin : upto 1.0mg/dl

Direct Bilirubin : upto 0.3mg/dl

Due to variations in inter-laboratory assay conditions , instruments and demography , it is recommended that each laboratory should establish.

REFERENCES

1. Carl A, Burtis and Edward R. Ashwood Liver Function in Tietz Fundamentals of Clinical chemistry .
W.B. Saunders and company Philadephia, P.A. (1996) 539 568.

2. Jendrassik , L&GROF , P,(1938) Biochem.Z.297:81

3. Recommendation on auniform Bilirubin standard, Standard methods of clinical chemistry (1965) Academic press, Vol5 P55

4. Powell. W.(1994) Am j. Clin Path 8.55