Bilirubin

Modified Jendrassik & Grof's Method

Code : 10004 (3x100 ml)

(For the Analyser/Colorimetric estimation of total and direct Bilirubin in Serum / Plasma)
In VITRO USE Only.

SUMMARY & EXPLANATION OF TEST:

Bilirubin is formed from hemoglobin in the reticuloendothelial system and transported to liver as Bilirubin-Albumin complex. In liver, a major part of the Bilirubin is converted to Bilirubin diglucuronide (also called conjugated Bilirubin/direct Bilirubin), which is water soluble. Unconjugated Bilirubin (also called free Bilirubin/indirect Bilirubin) is not water soluble. The abnormal retention of Bilirubin usually results in Jaundice, a condition that is characterized by increased Bilirubin in blood and deposition of a brownish Yellow pigment in the skin, sclera, and mucous membrane. Bilirubin estimation is based on its coupling with diazotized sulfanilic acid to form a pink colored pigment in an acidic medium. A major limitation in the common methods of Bilirubin estimation is that diazotization reaction of unconjugated bilirubin is very slow.

Bilirubin Kit is based on the modified Jendrassik & Grof's method in which caffeine is used as an activator. Hence, the time required for the color development of indirect Bilirubin is reduced to only 5 minutes. This Bilirubin Kit is rapid, simple and highly reliable. The standard used is an artificial standard calibrated with native Bilirubin as per the recommendations of the College of American Pathologist and also the American Association of Clinical Chemists.

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 absence of an activator, while for total Bilirubin (Direct & Indirect) the diazotization is carried out in the presence of an activator.

REAGENTS:

1.	Diazo A	2x100 ml
2.	Diazo B	1x10 ml
3.	Activator	1x100 ml
4.	Artificial Standard (10mg %)	2x10 ml

The reagents are ready to use and usable upto the expiration date when stored at room temperature. Artificial standard which is provided separately should be stored at 2-8^{o}C.

SAMPLE :

Serum or Plasma

1. Lipemic samples should be avoided. Fasting samples are preferred.
2. Hemolysis should be avoided, because hemoglobin produces falsely low values with diazo methods.
3. Both conjugated and unconjugated Bilirubin are photo-Oxidized on exposure to white or ultraviolet light. Specimens should be protected from direct exposure to either artificial or sunlight as soon as they are drawn.
4. Bilirubin in serum is stable for 3 days at 2-8^{o}C in the dark.

EXPECTED RANGE:

Direct Bilirubin : 0.0 - 0.2 mg%
Total Bilirubin : 0.2 - 1.0 mg%

LINEARITY:

This method is linear upto 20 mg%. Samples exceeding 20 mg% should be diluted and reassayed. The result has to be multiplied by the dilution factor.

INSTRUCTIONS:

1. Strictly adhere to the pipetting sequence mentioned in the procedure. Color will not develop in case of change in pipetting sequence.
2. Artificial standard should be used initially when the first test is performed with the kit, and the same absorbance can be used for subsequent assay calculations.
3. Do not reuse the artificial standard.

DIRECTIONS FOR USE ON ANALYSERS:

Reaction Type	:	End point with standard/factor
Wave Length	:	540 nm (green filter)
Incubation Temp	:	Room Temperature (dark)
Incubation Time	:	5 min. (total) 1 min. (direct)
Standard	:	10 mg%
Light Path	:	1 cm
Linearity	:	20 mg%
Unit	:	mg%

PROCEDURE:

Take, 4 clean dry test tubes labelled as T_1 & T_2 (for total Bilirubin) and D_1&D_2 (for direct Bilirubin). Add the reagents as shown below.

For Colorimeter use

	T_1	T_2	D_1	D_2
Diazo A(1)	1.0ml	1.0ml	1.0ml	1.0ml
Diazo B(2)	0.1ml	--	0.1ml	--
Activator (3)	1.0ml	1.0ml	--	--
Distilled Water	2.5ml	2.6ml	3.5ml	3.6ml
Serum/Plasma	0.2ml	0.2ml	0.2ml	0.2ml

For Analyser use

	T_1	T_2	D_1	D_2
Diazo A (1)	0.25ml	0.25ml	0.25ml	0.25ml
Diazo B (2)	0.025ml	--	0.025ml	--
Mix thoroughly then proceed				
Activator (3)	0.25ml	0.25ml	--	--
Distilled Water	0.5ml	0.5ml	0.75ml	0.75ml
Serum/Plasma	0.05ml	0.05ml	0.05ml	0.05ml

Mix well and read the absorbance of D_1&D_2 exactly after one minute on Photocolorimeter at 540nm against distilled water (Hg546nm). Mix well and keep the tubes T_1&T_2 in dark at room temperature for 5 minutes, then read absorbance of T_1 & T_2 and of Artificial Standard (4) at 540nm or using green filter, within 30 minutes.

CALCULATIONS :

With Artificial Standard

a) Total Bilirubin in mg % : $\frac{\text{A of } T_1 - T_2}{\text{A of Standard}} \times 10 \text{ (Std.Conc)}$

b) Direct Bilirubin in mg % : $\frac{\text{A of } D_1 - D_2}{\text{A of Standard}} \times 10 \text{ (Std.Conc)}$

★ Standard factor for Analysers 27.77

NOTES:

★ Due to variations in inter - laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology.

★ Final diagnosis should be based on a co-relation of test results with other clinical observations / Diagnostic tools.

BIBLIOGRAPHY:

1. Jendrassik, L & Grof, P. (1938) Biochem.Z.297:81:
2. Powell,W. (1994) Am. J. Clin Path, 8:55

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