

BILIRUBIN KIT

(Mod. Jendrassik & Grof's method)

For the determination of Direct & Total Bilirubin in serum.
(For Invitro Diagnostic Use Only)

Summary

Bilirubin is mainly formed from the heme 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 diseases (Hepatitis, cirrhosis), excessive hemolysis / 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 hyperbilirubinemia.

Principle

Bilirubin reacts with diazotized sulphanilic acid to form a coloured azobilirubin compound. The unconjugated bilirubin couples with the sulphanilic acid in the presence of a caffeine-benzoate accelerator. The intensity of the colour formed is directly proportional to the amount of bilirubin present in the sample.

Bilirubin + Diazotized Sulphanilic acid \longrightarrow Azobilirubin Compound

Normal reference values

Serum (Direct) : upto 0.2 mg/dl
(Total) : upto 1.0 mg/dl

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	35 Tests	75 Tests	2 x 250 ml
L1 : Direct Bilirubin Reagent	75 ml	150 ml	250 ml
L2 : Direct Nitrite Reagent	4 ml	4 ml	8 ml
L1 : Total Bilirubin Reagent	75 ml	150 ml	250 ml
L2 : Total Nitrite Reagent	4 ml	4 ml	8 ml
S : Artificial Standard (10 mg/dl)	10 ml	10 ml	-

Storage / Stability

All reagents are stable at R.T. till the expiry mentioned on the label.

Reagent Preparation

Reagents are ready to use. Do not pipette with mouth.

Sample material

Serum. Bilirubin is reported to be stable in the sample for 4 days at 2-8°C protected from light as it is photosensitive.

Procedure

Wavelength / filter : 546 nm / Yellow - Green
Temperature : R.T.
Light path : 1 cm

Direct Bilirubin Assay

Pipette into clean dry test tubes labelled as Blank (B), and Test (T) :

Addition Sequence	B (ml)	T (ml)
Direct Bilirubin Reagent (L1)	1.0	1.0
Direct Nitrite Reagent (L2)	-	0.05
Sample	0.1	0.1

Mix well and incubate at R.T. for exactly 5 min. Measure the absorbance of the Test Samples (Abs.T) immediately against their respective Blanks.

Total Bilirubin Assay

Pipette into clean dry test tubes labelled as Blank (B), and Test (T) :

Addition Sequence	B (ml)	T (ml)
Total Bilirubin Reagent (L1)	1.0	1.0
Total Nitrite Reagent (L2)	-	0.05
Sample	0.1	0.1

Mix well and incubate at R.T. for 10 min. Measure the absorbance of the Test Samples (Abs.T) immediately against their respective Blanks.

Calculations

Total or Direct Bilirubin in mg/dl = Abs.T X 13

Linearity

This procedure is linear upto 20 mg/dl. If values exceed this limit, dilute the sample with distilled water and repeat the assay. Calculate the value using the proper dilution factor.

Note

In case the exact wavelength is not available the artificial standard (S) may be used. Measure the absorbance of the artificial standard against distilled water with the appropriate filter and keep the same for future calculations by dividing the Abs.T with the Abs. of the Std. x 10. Discard the Artificial Standard after use.

In case of neonates where the sample quantity is a limitation, and the samples have high bilirubin (above 3 mg/dl), only 0.05 ml / 0.02 ml of the sample may be used for bilirubin estimation. The calculation factor in this case would be 24.9 / 60.5 respectively instead of 13. In case of using the standard the value of the same would be 19.1 / 46.5 mg/dl respectively instead of 10 mg/dl.

References

Jendrassik, L., Grof, P., (1938) Biochem. 2,297 : 81

System Parameters

Reaction : End Point
Wavelength : 546 nm
Zero Setting : Sample Blank
Incub. Temp. : R.T.
Incub. Time : 5 min. / 10 min.
Delay Time : ---
Read Time : ---
No. of read. : ---
Interval : ---
Sample Vol. : 0.10 ml
Reagent Vol. : 1.05 ml
Standard : ---
Factor : 13
React. Slope : Increasing
Linearity : 20 mg/dl
Units : mg/dl

Note for Colorimeters**Calculations For Colorimeters**

Total or Direct Bilirubin in mg/dl = $\frac{\text{Abs. T} - \text{Abs. B}}{\text{Abs. Std. against DW}} \times 10$



Gitanjali, Dr. Antonio Do Rego Bagh,
Bambolim Complex P.O. GOA - 403 202, INDIA

BIL(J&G):02(P)