

BILIRUBIN T&D

(Mod. Jendrassik & Grof's method)



DIATEK

Diagnostic reagent for quantitative in vitro determination of Total & Direct Bilirubin in serum on photometric systems.

Presentation

Pack Size	Direct Bilirubin Reagent (R1)	Direct Bilirubin Nitrite Reagent (R2)	Total Bilirubin Reagent (R3)	Total Bilirubin Nitrite Reagent (R4)
2x100ml	1x100ml	1x5ml	1x100ml	1x5ml

Summary

Bilirubin is a breakdown product of hemoglobin. Free, unconjugated bilirubin is extremely apolar and nearly insoluble in water, thus forming a complex with albumin for the transport in the blood from the spleen to the liver. In the liver, bilirubin is conjugated with glucuronic acid and the resulting water soluble bilirubin glucuronic acid is excreted via the bile ducts.

Hyperbilirubinemia can be caused by increased bilirubin production due to hemolysis (Pre-hepatic jaundice), by parenchymal damages of the liver (intra-hepatic jaundice) or by occlusion of bile ducts (post-hepatic jaundice). A chronic congenital (predominantly unconjugated) hyperbilirubinemia called Gilbert's syndrome is quite frequent in the population. High levels of total bilirubin are observed in 60-70% of neonates due to an increased postpartal breakdown of erythrocytes and because of delayed function of enzymes for bilirubin degradation. Common bilirubin methods detect either total bilirubin or direct bilirubin. Determinations of direct bilirubin measure mainly conjugated, water soluble bilirubin. Unconjugated bilirubin can therefore be estimated as the difference between total bilirubin & direct bilirubin.

Method

Colorimetric test, Modified Jendrassik & Grof's method

Principle

Bilirubin + Diazotized Sulphanilic acid \rightarrow Azobilirubin Compound

Reagents

Components and concentrations

	Total Bilirubin	Direct Bilirubin
Sulphanilic acid	10 mmol/L	10 mmol/L
Conc. HCL	40 mmol/L	40 mmol/L
Sodium Nitrite	15 mmol/L	1.5 mmol/L
Caffeine	25 mmol/L	-
Sodium Benzoate	20 mmol/L	-

Storage instructions & reagent stability

The reagents are stable upto the end of the indicated month of expiry, if stored at room temperature. Do not freeze the reagents.

Waste Management

Please refer to local legal requirements.

Reagent Preparation

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

Specimen

Serum.

It is very important to store the sample protected from light, as it is photosensitive.

Stability:	1 day	at	15-25°C
	4 days	at	2-8°C
	3 months	at	-20°C freeze only once

discard contaminated specimens

Assay Procedure

Wavelength	:	546nm
Optical path	:	1 cm
Temperature	:	20-25 °C/ 37 °C
Measurement	:	Against sample blank

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Direct Bilirubin

	Sample Blank (A1 _{SB})	Sample (A1 _S)
Direct Bilirubin Reagent (R1)	1000µl	1000µl
Direct Bilirubin Nitrite Reagent (R2)	-	25µl
Sample	50µl	50µl

Mix well and incubate at R.T for exactly 10 min. or at 37°C for 5 min. Measure the absorbance of test samples immediately against their respective blanks

$$\Delta A_D = (A1_S) - (A1_{SB})$$

Total Bilirubin

	Sample Blank (A2 _{SB})	Sample (A2 _S)
Total Bilirubin Reagent (R3)	1000µl	1000µl
Total Bilirubin Nitrite Reagent (R4)	-	25µl
Sample	50µl	50µl

Mix well and incubate at R.T for exactly 10 min. or at 37°C for 5 min. Measure the absorbance of test samples immediately against their respective blanks

$$\Delta A_T = (A2_S) - (A2_{SB})$$

Calculation

Direct Bilirubin in mg/dl = $\Delta A_D \times \text{Factor}$

Total Bilirubin in mg/dl = $\Delta A_T \times \text{Factor}$

Factor = 26.30

Linearity

This procedure is linear upto 25 mg/dl. If values exceed this limit, dilute the sample with normal saline and repeat the assay. Calculate the value using the dilution factor.

Quality Control

To ensure adequate quality control each run should include assayed normal & abnormal controls.

Reference range

Direct Bilirubin : upto 0.2 mg/dl

Total Bilirubin : upto 1.0 mg/dl

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

References

1. Jendrassik L., Grof P., Biochem 2., 297,81 (1938)
2. Tietz N.W., Fundamentals of clinical chemistry (1976), p 1037



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