

BILIRUBIN T&D (EP)

(Mod.Jendrassik & Grof's Method)



INTRODUCTION

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.

METHOD 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 caffeine - benzoate accelerator. The intensity of the colour formed is directly proportional to the amount of bilirubin present in the sample.

TOTAL BILIRUBIN

Bilirubin + Diazotized Sulphanilic acid + Sodium Nitrite $\xrightarrow{\text{caffeine + benzoate}}$ Azobilirubin Compound

DIRECT BILIRUBIN

Bilirubin + Diazotized Sulphanilic acid + Sodium Nitrite $\xrightarrow{\hspace{2cm}}$ Azobilirubin Compound

Reagent Name	Pack Size 2x50ml	Pack Size 2x100ml
R1 Direct Reagent	1 x 50 ml	1 x 100 ml
R2 Direct Nitrite	1 ml	2 ml
R3 Total Reagent	1 x 50 ml	1 x 100 ml
R4 Total Nitrite	1 ml	2 ml

WORKING REAGENT PREPARATION AND STABILITY

The reagents are stable at R.T till expiry date printed on the package.

CONCENTRATIONS IN THE TEST

TOTAL BILIRUBIN DIRECT BILIRUBIN

Sulphanilic acid	- 32 mmol/l	Sulphanilic acid	- 32 mmol/l
Conc. HCL	- 165 mmol/L	Conc. HCL	- 165 mmol/L
Sodium Nitrite	- 290mmol/L	Sodium Nitrite	- 290mmol/L
Caffein	- 150 mmol/L		

WARNINGS AND NOTES

Product for in vitro diagnostic use only.

Reagents are ready to use. Do not pipette with mouth

ADDITIONAL EQUIPMENT

- Automatic analyzer or photometer able to read at 546 nm
- Thermostat at 25°C or 37°C
- General laboratory equipment

SPECIMEN

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

These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analyzers. Programme Sheets are available on request.

MANUAL PROCEDURE - DIRECT BILIRUBIN

Wavelength	546 nm
Temperature	25°C/37°C
Cuvette	1 cm

Pipette into the cuvette:

Reagent	Blank (B)	Test (T)
R1 Direct reagent	1000 µl	1000 µl
R2 Direct Nitrite	-	20 µl
Sample	50 µl	50 µl

Mix well and incubate at R.T. for exactly 5 min. Measure absorbance of the Test Samples (Abs.T) immediately against their respective Blanks within 8 mins.

MANUAL PROCEDURE - TOTAL BILIRUBIN

Wavelength	546 nm
Temperature	25°C/37°C
Cuvette	1 cm

Pipette into the cuvette:

Reagent	Blank (B)	Test (T)
R3 Total reagent	1000 µl	1000 µl
R4 Total Nitrite	-	20 µl
Sample	50 µl	50 µl

Mix well and incubate at R.T. for exactly 5 min. Measure absorbance of the Test Samples (Abs.T) immediately against their respective Blanks within 8 mins.

CALCULATION

Total Bilirubin in mg/dl = (Abs.T - Abs.B) X 30 (Factor)

Direct Bilirubin in mg/dl = (Abs.T - Abs.B) X 20 (Factor)

REFERENCE VALUES

Direct bilirubin	upto 0.3 mg/dl
Total bilirubin	upto 1.0 mg/dl

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

NOTE

1. In case of Cuvette Volume is more than 1.0 ml requisite volume of reagents and sample can be multiplied keeping reagent to sample ratio same.
2. Sequence of reagent addition should be followed strictly as per the procedure.

PERFORMANCE CHARACTERISTICS

Linearity: The procedure is linear up to 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.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

Tietz, N.W., textbook of clinical Chemistry. Gambino, S.R.,Michealson, M.Gambino. S.R.et al, Jama, W.B. Saunders Co. (1983).

SYSTEM PARAMETERS

	Direct	Total
Method	End Point	End Point
Wavelength	546 nm	546 nm
Zero Setting	Serum Blank	Serum Blank
Temperature Setting	30° C	30° C
Incubation Temperature	30° C	30° C
Incubation Time	5 mins	5 mins
Delay Time	----	----
Read Time	----	----
No. of Reading	----	----
Interval Time	----	----
Sample Volume	0.05 ml (50 ul)	0.05 ml (50 ul)
Reagent Volume	1.02 ml (1020 ul)	1.02 ml (1020 ul)
Standard Concentration	----	----
Units	mg/dl	mg/dl
Factor	20	30
Reaction Slope	Increasing	Increasing
Linearity	20 mg/dl	20 mg/dl



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