BILIRUBIN

Modified Jendrassik & Grof`s Method

Intended Use:
Bilirubin (Total & Direct) test reagent/kit is a medical device intended for the estimation of Bilirubin (Total & Direct) in serum or plasma.

Clinical Significance
Hyperbilirubinemia (an abnormal elevation of bilirubin, whether conjugated or unconjugated) in plasma is an indication of a disturbance in bilirubin metabolism. This condition is caused either by an overproduction of bilirubin or by an impairment in the metabolic pathway. The increase in bilirubin production is usually caused by a rapid destruction of erythrocytes, resulting from blood diseases such as haemolilytic anemia. In newborns the increase in bilirubin may be caused by Rh, ABO, or other blood group incompatibility, by sepsis, hepatic immaturity, or by a variety of hereditary defects in bilirubin conjugation.
An impairment in the bilirubin metabolism is caused either by an enzyme deficiency or by a physical obstruction in bilirubin flow such as biliary (bile duct) obstruction. The hyperbilirubinemia leads to *kernicterus* (deposition of unconjugated bilirubin in brain and nerve cells) or *jaundice* (discoloration of mucus membranes, sclera and skin caused by the deposition of bilirubin pigment).

Principle
Bilirubin + Diazotized Sulphanilic acid ⟶ Azobilirubin Compound

Reagent Composition

R1 Direct Reagent	Sulphnilic Acid- 10 mmol/L Hydrochloric Acid – 40 mmol/L
R2 Direct Nitrite	Sodium Nitrite – 15 mmol/L
RI Total Reagent	Caffiene – 25mmol/L
RII Total Nitrite	Sodium Nitrite – 50 mmol/L

Working Reagent Preparation
Reagent is ready to use.

Stability and Storage
Store at Room Temp or below 30ºC.
All the kit contents are stable until the expiry date stated on the label. Do not use reagents over the expiration date. Store the vials tightly closed protected from light and prevent contaminations during the use.

Materials required
- Photometer or spectrophotometer with a thermostat cell compartment set at 25/30/37ºC, capable of reading at 546 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length
- Pipettes to measure reagent and samples.

Sample and Stability

Serum or plasma
Fresh hemolysis-free serum.
Store in the dark until use.
Samples can be frozen at –15ºC or below in which case bilirubin is stable for 2 months.

Know Interfering Substances
- Lipemia (intralipid < 5 g/L) does not interfere.
- Direct Bilirubin. (Hemoglobin 2 g/L) may affect the results.
- Total Bilirubin. (Hemoglobin 16 g/L) does not interfere.
- Other drugs and substances may interfere[3].
- Lipemic samples interfere with the assay. The interference can be corrected by preparing a sample blank before applying the general formula of calculation.

ASSAY PROCEDURE
DIRECT BILIRUBIN ASSAY
Pipette into clean dry test tubes labeled as Blank (B) and Test (T):

Addition Sequence	B_D	T
R1 Direct Reagent	1000µl	1000µl
R2 Direct Nitrite Reagent	-	25 µl
Mix well and proceed		
Sample	50 µl	50 µl

Mix well and incubate at R.T(<30°C) for exactly 10 min or 5 min at 37^{0}C. Measure the absorbance of the Test samples (Abs.T) immediately against their respective Blanks.

TOTAL BILIRUBIN ASSAY
Pipette into clean dry test tubes labeled as Blank (B), and Test (T):

Addition Sequence	B_T	T
RI Total Reagent	1000µl	1000µl
RII Total Nitrite Reagent	-	25 µl
Mix well and proceed		
Sample	50 µl	50 µl

Mix well and incubate at R.T(<30°C) for exactly 10 min or 5 min at 37^{0}C. Measure the absorbance of the Test samples (Abs.T) immediately against their respective Blanks.

Calculations:
Direct Bilirubin in mg/dl = (Abs.T – Abs.BD)x 26.30

Total Bilirubin in mg/dl = (Abs.T – Abs.BT) x 26.30

Quality Control
To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.
If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable results.

Linearity:
This procedure is linear up to 25 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.

Reference Values

Serum (Direct)	:	up to 0.2 mg/dl
(Total)	:	up to 1.0 mg/dl

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

General System Parameters

	Direct Bilirubin	**Total Bilirubin**
Mode	Differential Mode	Differential Mode
Reaction	Increasing	Increasing
Wavelength	546 nm	546 nm
Blank with	Sample	Sample
Sample Volume	50 µl	50 µl
Reagent Volume	R1-1000µl+R2-25µl	RI-1000µl+RII-25µl
Factor	26.3	26.3
Incub. Temp.	37^0C	37^0C
Incub. Time	5 min.	5 min.
Delay Time	5 sec	5 sec
Normal Range	Up to 0.2	Up to 1.0
Linearity	Up to 25	Up to 25
Unit	mg/dl	mg/dl

Notes

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.

Reference

1. Jendrassik L and Grf.P. (1938) BIOCHEM 2;297.81

For *in vitro* Diagnostic use only.