Bilirubin Total & Direct
Jendrassik modified method
Serum/Plasma

INTENDED USE

For the quantitative estimation of Bilirubin Total & Direct in human serum and plasma.

INTRODUCTION

Bilirubin is the orange-yellow pigment derived from senescent red blood cells. It is extracted and biotransformed in the liver and excreted in bile and urine.

MODIFIED JENDRASSIK - GROF METHOD

Since the development of the original procedure by Jendrassik-Grof, a number of modifications have been made to speed up the reaction and reduce interference.

The Jendrassik-Grof method is slightly more complex but has the following advantages over the Evelyn-Malloy Method.

- It is insensitive to sample pH changes.
- It is insensitive to a 50-fold variation in protein concentration of the sample.
- It has adequate optical sensitivity even for low bilirubin concentrations.
- It has minimal turbidity and a relatively constant serum blank.
- It is not affected by haemoglobin up to 750 mg/dl.

TEST PRINCIPLE

Both conjugated and unconjugated bilirubin react with diazotized sulphanilic acid. Reaction with unconjugated bilirubin is hastened by the use of caffeine sodium benzoate as an accelerator for total bilirubin estimation. The pink colour developed is measured at 540 nm and the intensity of colour formed is directly proportional to the bilirubin concentration in the sample.

KIT CONTENTS

- **Reagent 1 : Nitrite (T&D Reagent)**
- **Reagent 2 : Sulphanilic Acid (T&D Reagent)**
- **Reagent 3 : Accelerator (Total Reagent)**
- **Reagent 4: Saline (Direct Reagent)**
- **Product Insert**

PREPARATION OF WORKING SOLUTION

All reagents are ready to use.

STORAGE AND STABILITY

Bilirubin T&D Reagents are stable till the expiry date mentioned on the labels when stored at 2 - 8°C.

SPECIMEN COLLECTION AND STORAGE

Unhemolysed serum or heparinised plasma. Samples should be used on the same day. If necessary, the samples may be preserved in a refrigerator at 2 - 8°C for 24 hours.

PRECAUTIONS

1. Storage conditions as mentioned on the kit Should be adhered.
2. Do not freeze or expose the Reagents to higher temperature as it may affect the performance of the kit.
3. Before conducting the assy bring all the reagents to room temperature.
4. After use store the kit contents immediately as 2 - 8°C.
5. Avoid contamination of the Reagents during the assay process.
6. Use clean glassware free from dust or debris.
7. Bilirubin levels may be reduced if the samples are exposed to light.

NOTE

Please note that Reagent 3 may develop needle shaped crystals at low temperatures, which should be dissolved by warming at 37°C before use.

PROCEDURE (Automated)

Refer to specific instrument application instructions.

TEST PROCEDURE (Manual)

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

	Total Bilirubin		Direct Bilirubin	
Pipette into Test Tube	Blank	Test	Blank	Test
Reagent-1	-	50 µl	-	50 µl
Reagent-2	100 µl	100 µl	100 µl	100 µl
Sample	-	100 µl	-	100 µl
Reagent-3	1000 µl	1000 µl	-	-
Reagent-4	-	-	1000 µl	1000 µl
	Mix and incubate at 37°C for 5 minutes.		Mix and incubate at 37°C for 5 minutes.	
Read the absorbance of Total Bilirubin and Direct Bilirubin of Test absorbance against reagent blank absorbance at Bichromatic mode (Main wavelength) 535 nm (530-570 nm) or Green filter and (Reference wavelength) 635 nm (600-650 nm) or Red filter.				

CALCULATION

Total Bilirubin Conc. in mg/dl =
Abs. of Test - Abs. of Blank x Factor (15)

Direct Bilirubin Conc. in mg/dl =
Abs. of Test - Abs. of Blank x Factor (11)

Expected value for Bilirubin*

Conjugated (Direct)	Upto 0.25 mg/dl
Unconjugated	0.2 - 0.8 mg/dl
Total	Upto 1.0 mg/dl

*It is recommended that each laboratory should establish its own normal range.

Expected value for Infant Total Bilirubin*

Infants	Premature	Full-Term
24 h	1-6 mg/dl	2-6 mg/dl
48 h	6-10 mg/dl	6-10 mg/dl
3-5 days	10-14 mg/dl	4-8 mg/dl

*It is recommended that each laboratory should establish its own normal range.

PERFORMANCE

1. **Linearity :** 20 mg/dl
2. **Comparison**
   Comparative studies were conducted on random samples using Bilirubin T&D and a reference method. The resultant coefficient of correlation was found to be 0.999 and the corresponding regression equation was y = 1.009x + 0.188.
3. **Precision**
   For **Precision**, within run and between run studies were carried out using samples having low abnormal and high abnormal values (1-10mg/dl). The maximum coefficient of variation obtained was 4.3%.
4. **Specificity**
   Bilirubin T&D method is relatively free of interference from commonly occurring substances in the blood.

AUTOMATED APPLICATIONS

Bilirubin T & D Reagents can be used with most of the semi-automated analysers. Input parameters for semi auto/auto analysers are given as follows :

Input Parameters	Total Bilirubin	Direct Bilirubin
Type of reaction	End Point	End Point
Mode	Bichromatic	Bichromatic
Main Wavelength	535 (530-570 nm) or Green filter	535 (530-570 nm) or Green filter
Reference Wavelength	635 (600-650 nm) or Red filter	635 (600-650 nm) or Red filter
Incubation Time	5 minutes	5 minutes
Incubation Temperature	37°C	37°C
Sample Volume	100 µl	100 µl
Blank with	Reagent	Reagent
Factor	15	11
Upper Normal Value	Upto 1.0 mg/dl	0.25 mg/dl
Linearity	20 mg/dl	20 mg/dl
Units	mg/dl	mg/dl

CLINICAL SIGNIFICANCE

Defects in bilirubin metabolism resulting in jaundice can occur at each step in the metabolic pathway. The disorders are usually classified as (1) inherited disorders of bilirubin metabolism and (2) jaundice of the newborn. All of these disorders are characterised by predominant elevations in either conjugated or unconjugated bilirubin in the absence of other abnormal liver tests.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of control sera (normal and abnormal) with known Direct and Total Bilirubin concentrations.

REFERENCES

- Jendrassik, Land Grof, S. (1938) A short text book of Clinical Pathology, 3rd edition, Reprinted in 1979.
- Tietz text book of Clinical Chemistry, Chapter 33, 3rd edition, 2000.

Manufactured by:
Vanguard Diagnostics Private Limited
C-123, Phase-I, Okhla Industrial Area
New Delhi-110 020, INDIA.

Tel: 011-4172 7222,
Email:info@vanguarddiagnostics.com
Website : www.vanguarddiagnostics.com

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