

# BILIRUBIN T&D

## (Modified Jendrasik & Grof)

### INTENDED USE

Bioline Bilirubin T&D is used for the quantitative determination of Total and Direct bilirubin in human serum.

### CLINICAL SIGNIFICANCE

Bilirubin is a metabolite of the heme portion of heme proteins, mainly hemoglobin. Normally it is excreted into the intestine and bile from the liver. The site of the catabolism of hemoglobin is the reticuloendothelial system (RES). Bilirubin is then released into the bloodstream where it binds tightly to albumin and is transported to the liver. Upon uptake by the liver, bilirubin is conjugated with glucuronic acid to form bilirubin mono and diglucuronide that are water soluble metabolites. The metabolites will react with aqueous diazo reagent and are commonly referred to as "direct bilirubin".

Elevation of total serum bilirubin may occur due to excessive hemolysis or destruction of the red blood cells e.g. hemolytic disease of the newborn, liver diseases e.g. Hepatitis and cirrhosis obstruction of the biliary tract e.g., gallstones. There is information in the literature indicating elevated levels of direct bilirubin in patients with liver or biliary tract diseases: even though, total bilirubin levels are normal. Therefore, the greatest diagnostic value of direct bilirubin assays stem from their ability to indicate occult liver disease.

### METHOD AND PRINCIPLE

Most chemical methods for the determination of total bilirubin are based on the reaction between diazotized sulfanilic acid and bilirubin to produce azobilirubin, which absorbs maximally at 546 nm. Such tests are often run in the presence and absence of an organic solvent e.g., methanol to distinguish free bilirubin from conjugated bilirubin on a differential solubility basis.

The intensity of the color produced is directly proportional to the amount of direct bilirubin concentration present in the sample.

### REAGENT COMPOSITION

Bilirubin Direct Reagent: Sulfanilic Acid 32mM, HCL 165 mM

Bilirubin Total Reagent: Sulfanilic Acid 32mM.

Hydrochloric Acid 165mM. Detergent 1%

Bilirubin Nitrite Reagent: Sodium Nitrite 60mM.

### WARNING AND PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. Specimens should be considered infectious and handled appropriately.
3. Do not pipette reagents by mouth. Avoid contact reagent with eyes, skin and clothing. Do not ingest. Wash hands after use.

### REAGENT PREPARATION

Direct Bilirubin working reagent: Add 0.010 ml (10 µl) of sodium nitrite reagent (T&D R2) per 1.0ml of Bilirubin Direct reagent (DR1) and mix well. **Improper mixing will lead to low results.**

Total Bilirubin working reagent: Add 0.020 ml (20 µl) of sodium nitrite reagent (T&D R2) per 1.0ml of Bilirubin Total reagent (TR1) and mix reagent. **Improper mixing will lead to low results.**

### REAGENT STORAGE AND STABILITY

1. All reagents are stored at room temperature (15 - 30°C).
2. Combined working reagent can be stored for up to eight (8) hours when kept in an amber bottle at room temperature.
3. Do not freeze reagents.
4. Avoid exposure to direct sunlight.

### REAGENT DETERIORATION

The reagent should be discarded if:

Sodium Nitrite reagent has a yellow discoloration.

Working reagent fails to achieve assigned assay values of fresh control sera.

### SPECIMEN COLLECTION AND STORAGE

1. Hemolysis interferes with the test, i.e. Hemolyzed samples should be avoided since they may give falsely low values.
2. All specimens for this assay must be carefully protected from light.
3. Bilirubin in serum is stable for 4-7 days when stored in the dark at 2-8°C

### INTERFERENCES.

1. Young et al. give an exhaustive list of drugs and other substances known to affect the circulating level of bilirubin.
2. In this assay, as in all laboratory procedures, materials, which come in contact with specimens, should be clean and free of contamination by heavy metals, detergents, and other chemicals.

### ASSAY PROCEDURE FOR SEMIAUTO ANALYZER.

Wavelength :546/670 nm or 546/630nm

Temperature :37°C

#### PROCEDURE FOR TOTAL BILIRUBIN

	Sample
<b>BILIRUBIN TOTAL (TR1)</b>	1000µL
<b>T&amp;D R2</b>	20µL
<b>SAMPLE</b>	50µL

#### PROCEDURE FOR DIRECT BILIRUBIN

	Sample
<b>BILIRUBIN DIRECT (DR1)</b>	1000µL
<b>T&amp;D R2</b>	10µL
<b>SAMPLE</b>	50µL

Mix thoroughly and incubate at 37°C for 5 minutes. The final color produced is stable for 60 minutes.

For pediatric samples with bilirubin over 3.0 mg/dl, use 0.025 µL of sample and then multiply the result by 2.

### CALCULATIONS

Direct Bilirubin (mg/dL)

546/670 = Abs.of Test x 12

546/630 = Abs.of Test x 17

Total Bilirubin (mg/dL)

546/670 = Abs.of Test x 21

546/630 = Abs.of Test x 24

### LIMITATIONS

1. Sera with values above 20 mg/dl must be diluted 1:1 with isotonic saline, reassayed and the final answer multiplied by two.
2. Serum hemoglobin levels of up to 1.0 g/dl do not interfere with results.

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### QUALITY CONTROL

Normal and abnormal control sera of known concentrations of Direct and Total Bilirubin should be analyzed routinely with each group of unknown specimens.

### EXPECTED VALUES

Direct Bilirubin: Infant (after one month) and adults: 0.0-0.5 mg/dl. It is strongly recommended that each laboratory establish its own normal range.

Total Bilirubin: Infants (after one month) and adults 0.2-1.2 mg/dl.

### PERFORMANCE CHARACTERISTICS

- Linearity:** 20mg/dl
- Sensitivity:** Based on an instrument resolution of 0.001 absorbance, the present procedure has a sensitivity of 0.02 mg/dl.
- Comparison:** A comparison study between the present method with an available commercial product using the same identical method on forty (40) fresh serum samples and two commercial serum controls, ranging from 0.10 mg/dl to 0.67 mg/dl yielded a coefficient of 0.98 and a regression equation of  $Y = 0.99X + 0.01$ .
- Precision studies:**

**Run-to-run:** Two commercial control sera were assayed for a period of 30 days and the following day-to-day precision was obtained.

N=22	Level 1	Level 2
Mean (mg/dL)	0.68	6.41
SD	0.05	0.63
CV%	7.3	9.7

**Within Run:** Two commercial control sera were assayed 20 times and the following within run precision was obtained.

N=22	Level 1	Level 2
Mean (mg/dL)	0.67	6.28
SD	0.02	0.15
CV%	2.9	2.3

### General Technical Parameters.

Mode	End Point Bichromatic
Primary Wavelength (Filter)	546 nm
Secondary Wavelength/Bichromatic	670/630 nm
Reaction Direction	Increasing
Sample Vol.	50µL
Reagent Vol. (Total)	1020µL
Reagent Vol. (Direct)	1010µL
Incubation Time	5 minutes
Calibration Method	Factor
Factor Total	546/670 nm- 21 546/630 nm- 24
Factor Direct	546/670 nm- 12 546/630 nm- 17
Linearity (TotalBil./DirectBil.)	20mg/dL
Decimal Places	2
Temp.	37°C
Unit	mg/dL
Ref.Low Adult & Children over 10day (Total)	0.2-1.2mg/dl.
Ref.High Adult & Children over 10day (Direct)	0.0-0.5mg/dl

### REFERENCES

- Tietz, N.W.:Fundamentals of Clinical Chemistry. W.B.YSaundersCo., Philadelphia, p. 1028(1976).
- Walters, M. Gerand, H., Microchem J. 15; 231 (1970).Michaelson, M.:Sand., J., J. Clin. Lab. Invest. (Suppl. 49) 13. 1(1961).
- Young,D.S.,et.al.:Clin.Chem.21, 10(1975). Gambino,S.R., et.al.: Bilirubin Assay Revised),Commission on Continuing Education.Am. Soc. of Clin. Path.,Chicago(1968).