# Bilirubin Total

## (Jendrassik and Grof Method)

**Intended Use**

Bilirubin Total is a reagent kit used for the determination of Total Bilirubin based on Modified Jendrassik and Grof method.

**Principle**

Bilirubin reacts with diazotized Sulphanilic acid to produce azobilirubin (Pink colour). The pink colour thus produced is proportional to Bilirubin concentration measured at 546 nm 630/546/670 nm).

Bilirubin + Sulphanilic acid + Sodium nitrite $\xrightarrow{\text{Activator}}$ Azobilirubin

**Reagents provided**

1. Bilirubin Total Reagent (R1) - Ready to use
2. Bilirubin Total Reagent (R2) - Ready to use

**Reagent storage and stability**

The reagent kit should be stored at Room Temperature and is stable till the expiry date indicated on the label.

**Specimen collection and preservation**

Blood should be collected in a clean dry container. Although serum is preferred, plasma can also be used as sample. Following anticoagulants can be used for plasma separation:

- EDTA 2 mg/ml of blood
- HEPARIN 200 IU/ml of blood

Bilirubin is light sensitive. Avoid exposure of serum or plasma to direct light. Bilirubin in serum and plasma is stable for a day at 2° - 8°C or one month at -20°C. Samples should be brought to room temperature before use.

**Assay guidelines for Analyzers**

| | |
|---|---|
| Reaction Type | End Point |
| Reaction slope | Increasing |
| Incubation time | 5 min. at 37°C |
| Wave length | 546nm / 630nm |
| Zero Setting | Distilled Water |
| Sample Volume | 50 µl (0.05 ml) |
| Reagent Volume R1 | 800 µl (0.8 ml) |
| Reagent Volume R2 | 200 µl (0.2 ml) |
| Factor | 24 |
| Low normal | 0 mg/dl |
| High normal | 1 mg/dl |
| Linearity | Up to 30 mg/dl |

**Assay guidelines for Manual Procedure**

**Note**: The reagents R1, R2 and sample require proper mixing

| **Reagents** | **Test** |
|---|---|
| Reagent R1 | 800 µl (0.8 ml) |
| Reagent R2 | 200 µl(0.2 ml) |
| Serum / Plasma | 0.05 ml |

1) Mix thoroughly and incubate at 37°C for 5 minutes.
2) The absorbance of the test against distilled water at 546/630nm.

**Calculation**

Con. in sample mg/dl = Abs. ofTest x24

**Normal range**

Total Bilirubin : Up to 1 mg/dl

**Note**: Expected range varies from population to population and each laboratory should establish the normal range for its own population.

**Limitations**

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Dilute the specimen if the Bilirubin value is above 30 mg/dl. Suitable dilution can be done with normal saline. In such case the results obtained should be multiplied by dilution factor to obtain correct Bilirubin value.

**Quality Control**

It is recommended that each batch should include a normal and an abnormal commercial reference control serum or a known patient serum. Use of quality control serum, checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, and cleanliness of glassware, accuracy of pipetting and serum exposure to light.

**References**


1. Jendrassik, L., et al.(Biochem. 2. 297, 81 (1938)
2. Practical Clinical Biochem. Vol 1, 5th edition, H. Varley, page 1012, (1980).


**Symbols**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| IVD | In Vitro Diagnostics. | | Caution. | | Keep away from sun light. | | Date of Manufacture. |
| LOT | Batch No. | | Read Instructions. | | Fragile. | | Product Expiry Date. |
| CONT | Content. | | Storage Temperature. | | Keep Dry. | | Manufactured By. |
| REF | Catalogue No. | | | | | | |

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