# BILIRUBIN TOTAL & DIRECT
## (JENDRASSIK - GROF)

### INTENDED USE:

Reagent kit for the quantitative determination of Total and Direct Bilirubin in serum. JENDRASSIK-GROF method.

### CLINICAL SIGNIFICANCE:

Approximately 80-85% of the bilirubin produced is derived from the heme moiety of the hemoglobin released from aging erythrocytes in the reticuloendothelial cells. Bilirubin bound to albumin is transported into the liver where it is rapidly conjugated with glucuronide to increase its solubility. Then it is excreted into biliary canaliculi and hydrolyzed in the gastrointestinal tract. Unconjugated bilirubin serum concentration increases in case of overproduction of bilirubin (acute or chronic haemolytic anemias) and in case of disorders of bilirubin metabolism and transport defects (impaired uptake by liver cells: Gilbert's syndrome; defects in the conjugation reaction: Crigler-Najjar syndrome). Reduced excretion (hepatocellular damage hepatitis, cirrhosis...; Dubin- Johnson and Rotor syndrome) and obstruction to the flow of bile (most often produced by gallstones or by tumors) induce an important elevation of conjugated bilirubin and in a minor extent an increase of unconjugated bilirubin (conjugated hyperbilirubinemia).

### PRINCPLE:

Sulfanilic acid reacts with sodium nitrite to form diazotized Sulfanilic acid. In the presence of an accelerator (cetrimide), conjugated and unconjugated bilirubin reacts with diazotized Sulfanilic acid to form azobilirubin (Bilirubin Total). In the absence of accelerator, only conjugated bilirubin reacts (Bilirubin Direct). The increase of absorbance at 546 nm is proportional to bilirubin concentration.

### REAGENT COMPOSITION:

Reagent T1: Total Bilirubin reagent 1
Reagent T2: Total Bilirubin reagent 2
Reagent D1: Direct Bilirubin reagent 1
Reagent D2: Direct Bilirubin reagent 2

### MATERIALS REQUIRED BUT NOT PROVIDED:

- Clean & Dry Glassware.
- Micropipettes & Tips
-Colorimeter or Bio-Chemistry Analyzer.

### SAMPLES:

Serum free of hemolysis. Heparinized plasma.
Care must be taken to fill heparinized tubes according to the manufacturer's instructions. An insufficient filling may lead to erroneous results. Protect the samples from light before and during the analysis.

### STABILITY OF REAGENT:

When stored tightly closed at room temperature protected from light and contaminations prevented during their use; reagents are stable up to the expiry date stated on the label.

### WORKING REAGENT:

The Reagent is ready for use.

### GENERAL SYSTEM PARAMETERS:

| | |
|---|---|
| Reaction type | End Point (Increasing) |
| Primary Wave Length | 546 nm |
| Secondary Wave Length | 630 nm |
| Light Path | 1 Cm |
| Reaction Temperature | 37°C. |
| Blank / Zero Setting | Sample Blank |
| Reagent Volume | 1.02 ml |
| Sample Volume | 50 µl |
| Incubation Time | 5 Minute |
| Total Bilirubin Factor | 28 |
| Direct Bilirubin Factor | 14 |
| Linearity | 20 mg/dl |

### ASSAY PROCEDURE:

**Total Bilirubin:**

| | Serum Blank | Test |
|---|---|---|
| Reagent T1 | 1ml | 1ml |
| Reagent T2 | | 20 µl |
| Sample | 50 µl | 50 µl |

**Direct Bilirubin:**

| | Serum Blank | Test |
|---|---|---|
| Reagent D1 | 1ml | 1ml |
| Reagent D2 | | 20 µl |
| Sample | 50 µl | 50 µl |

Mix and read the absorbance of the tests against their respective sample blanks after a 5-minute incubation at 37°C.

### CALCULATIONS:

Total Bilirubin (mg/dl) = (Abs. Test-Abs. Sample Blank) X 28
Direct Bilirubin (mg/dl) = (Abs. Test-Abs. Sample Blank) X 14

### LINEARITY:

Reagent is Linear up to 20 mg/dl.

Dilute the sample appropriately and re-assay if the Total or Direct Bilirubin concentration exceeds 20 mg/dl. Multiply the result with dilution factor.

### REFERENCE NORMAL VALUE:

Total Bilirubin: (Adults and children over 10 days) 0.3-1.2 mg/dl
Direct Bilirubin: <0.2 mg/dl

### QUALITY CONTROL:

For accuracy it is necessary to run known controls with every assay.

### BIBLIOGRAPHY

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