# BILIRUBIN TOTAL
Diazotized Sulphanilic Acid Method

**Quantitative determination of Total Bilirubin in human Serum / Plasma**
**Only for *In Vitro* Diagnostic use**

## ORDER INFORMATION

| REF | Cont. |
|---|---|
| BIT 100 | 2 X 50 mL |
| BIT 200 | 2 X 100 mL |
| BIT 300 | 3 X 100 mL |
| BIT 1000 | 2 X 500 mL |

## CLINICAL SIGNIFICANCE
Bilirubin is a breakdown product of hemoglobin, insoluble in water. It is transported from the spleen to the liver and excreted into bile. Hyperbilirubinemia results from the increase of bilirubin concentrations in plasma. Causes of hyperbilirubinemia:
Total bilirubin: Increase hemolysis, genetic errors, neonatal jaundice, ineffective erythrpoiesis, and drugs.

## METHOD
Photometric Test Method.

## PRINCIPLE
Total bilirubin in the sample reacts with diazotized sulfanilic acid forming a coloured complex that can be measured by spectrophotometry. Both Direct and indirect bilirubin couple with diazo in the presence of Accelerator. The terms “Direct” and “total” refer to the reaction characteristics of serum bilirubin in the absence or presence of solubilising (accelerating) reagents. The “Direct” and “indirect” bilirubin is only approximately equivalent to the conjugated and unconjugated fractions.

## REAGENT
Reagent 1 : Bilirubin Total Reagent.
Reagent 2 : Total Nitrite Reagent

## REAGENT PREPARATION
**Working Reagent**: Mix bilirubin total reagent and total nitrite reagent in the ratio of 1:50 i.e for making 5 ml working reagent add 100 µl of respective total nitrite reagent to 5.0 ml of bilirubin total reagent.

## REAGENT STORAGE AND STABILITY
When stored at RT reagent is stable until the expiration date stated on the bottle and kit box label
**Working Reagent**
This working reagent is stable for 7 days at 2-8ºC. The reagent may develop slight yellow colour upon storage but this does not affect the performance.

## WARNING AND PRECAUTIONS
- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.

## WASTE MANAGEMENT
Please refer to local legal requirements.

## MATERIALS REQUIRED BUT NOT PROVIDED
- NaCl solution 9 g/L
- General laboratory equipment

## SAMPLE COLLECTION AND PRESERVATION
**Serum or heparin plasma**
It is very important to store the sample protected from light!
Stability: 7 days at 2 – 8°C
6 months at –20°C in case of immediate freezing.
Freeze only once! Discard contaminated specimens!

## ASSAY PROCEDURE
**Operating Instructions**
- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned work load.
- Bring all reagents, standard and samples to room temperature 18 - 28ºC, prior to analysis.

| AUTOMATED PARAMETERS | |
|---|---|
| Wavelength I<br>Wavelength II | 546 nm<br>630 nm |
| Cuvette Light Path | 1 cm |
| Reaction Type | End Point |
| Reaction Temperature | 37 °C |
| Measurement | Against Reagent blank |
| Sample Volume | 50 µl |
| Working Reagent Volume | 1000 µl |
| Incubation | 5 mins. |
| Factor | 27.00 |
| Low Normal | 0.0 mg/dl |
| High Normal | 1.2 mg/dl |
| Linearity | 25 mg/dl |

## MANUAL ASSAY PROCEDURE

**Pipette into Test Tubes**

| | |
|---|---|
| Working reagent | 1000 µl |
| Sample | 50 µl |

- Mix & Incubate for 05 min. at 37 °C or 10 min. at R.T. Measure absorbance at 546/630 nm.

## SAMPLE DILUTIONS
- This method is linear upto a concentration of 25 mg/dL.
- Dilute samples above this concentration 1:1 with 0.9% saline
- Repeat assay. Multiply the result by 2.

## CALCULATION

Results are calculated, usually automatically by the instrument, as follows:

Total Bilirubin (mg/dl) = Abs of test X 27

## CLIBRATORS AND CONTROLS
For the calibration of automated photometric systems the commercially available suitable multi-calibrator is recommended.

It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.

Each laboratory should establish corrective action in case of deviations in control recovery.

## PERFORMANCE CHARACTERISTICS
## WITHIN RUN

| Sample | Mean Concentration | SD | CV % |
|---|---|---|---|
| Randox 2 | 1.84 | 0.01 | 0.32% |
| Randox 3 | 6.30 | 0.04 | 0.57% |

## RUN TO RUN

| Sample | Mean Concentration | SD | CV % |
|---|---|---|---|
| Randox 2 | 1.84 | 0.02 | 1.06% |

| | | | |
|---|---|---|---|
| Randox 3 | 6.26 | 0.04 | 0.69% |

## LINEARITY

The method is linear upto a concentration of 25mg/dL. Dilute samples above this concentration 1:1 with 0.9% saline solution and repeat assay. Multiply the result by 2.

**Limit of detection**: The limit of detection for Bilirubin direct is 0.2 mg/dL.

## METHOD COMPARISON

A comparison of Paramcare Bilirubin direct with a commercially available assay (x) using 20 samples gave following results: $R^2 = 0.9900$

## REFERENCE VALUES

| Adults | |
|---|---|
| Total Bilirubin | Up to 1.2 mg/dL = 3.4 mmol/L |

| Newborns (Total bilirubin) : | | |
|---|---|---|
| Age | premature | full-term |
| Up to 24 h | 1.0-8.0 mg/dL = 17-137mmol/L | 2.0-6.0mg/dL= 34-103 mmol/L |
| Up to 48 h | 6.0-12.0 mg/dL = 103-205mmol/L | 6.0-10 mg/dL = 103-171 mmol/L |
| 3-5 days | 10-14 mg/dL = 171-239 mmol/L | 4.0-8.0mg/dL= 68-137 mmol/L |

The reference values are to be considered as indicative only. Every laboratory should establish its own normal range.

## LIMITATION OF THE PROCEDURE

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

## INTERFERENCE

- Hemoglobin: No interference found upto 150 mg/dL.
- Lipemia will interfere.
- These characteristics have been obtained using an automatic analyzer. Results may vary if a different instrument or a manual procedure is used.

## BIBLIOGRAPHY

Pearlman FC and Lee RTY. Detection and measurement of bilirubin in serum, with use of surfactants as solubilizing agents.
Clin Chem 1974; 20: 447-453.

## GLOSSARY OF SYMBOL

| | |
|---|---|
|  | Consult Instruction for Use |
|  | Catalog Number |
|  | Store between |
|  | Manufacturer |
|  | Keep away from sunlight |



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