QUANTON BIOLIFE SCIENCES
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BILIRUBIN TOTAL
Diazotized Sulphanilic Acid Method

QBL/PDS/BIT_035

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 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: **Direct Reagent** – Mix Direct reagent and Direct nitrite in the ratio of 1:50 i.e for making 5 ml working reagent add 100 µl of respective nitrite reagent to 5.0 ml of Direct reagent.

REAGENT STORAGE AND STABILITY
When stored at 15-30ºC 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 Wavelength II	546 nm 630 nm
Cuvette Light Path	1 cm
Reaction Type	End Point
Reaction Temperature	37°C
Measurement	Against Reagent blank
Sample Volume	50 µl
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:

Direct 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	
Direct	Up to 0.5 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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