BILIRUBIN (TOTAL)
DC Method

DIATEK

CLINICAL SIGNIFICANCE :

Bilirubin, a product of red blood cell destruction, is a bile pigment normally found in the blood. The average life expectancy of red blood cells is 120 days. Approximately 6 gm of hemoglobin is released per day due to their disintegration. Reticuloendothelial cells from the spleen, liver, and bone marrow phagocytize aged red cells and convert the released hemoglobin to bilirubin. Serum albumin links to bilirubin and transports it to the liver where it is metabolized. Elevated serum bilirubin can indicate impairment of liver excretory function, excessive hemolysis, or biliary tract obstruction. Hyperbilirubinemia can also be associated with obstructive jaundice, hemolytic and hepatic jaundice, infectious hepatitis, and pernicious anemia.

TEST PRINCIPLE :

Photometric test with stabilized 2,4- Dichlorophenyldiazonium salt ("DC") :
Bilirubin forms a red diazo dye with DC-derivative in acidic solution. Bound bilirubin is released by detergents.

REAGENTS COMPOSITION :

R1: Phosphate-Buffer 40 mmol/L, NaCl 9 g/L Detergent, Stabilisors

R2: 2,4-Dichlorophenyldiazoniumsalt 0.09 mmol/L, HCl 30 mmol/L , Detergent, Stabilisors

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8 ºC, and contaminations prevented during their use. Do not use reagents over the expiration date. Once opened the reagent is stable for 1 months at 2 - 8ºC.

KIT CONTENTS :

Pack size : (LW120ml) CODE No. BILTLW01

Reagent 1: 5 x 20 ml Reagent 2 : 2 x 10 ml

Pack size : (480ml) CODE No. BILT02

Reagent 1: 4 x100 ml Reagent 2 : 2 x 40 ml

Pack size : (60ml) CODE No. BILT01

Reagent 1: 1 x 50 ml Reagent 2 : 1 x 10 ml

SAMPLES :

Serum or heparinised plasma
(Bilirubin is very light sensitive, therefore carefully protect from light)

CALIBRATORS & CONTROLS:

For the calibration of automated analyzers Greiner Multicalibrator Unical is recommended, for quality control use Greiner GmbH normal and abnormal control, Unitrol I and Unitrol II.

INTERFERENCES

The following analyze were tested up to the levels indicated and found not to interfere with
- Ascorbic Acid: up to 30 mg/dL
- Hemoglobin: no interferences up to 50 mg/dL
- Triglycerides: no interference up to 1000 mg/dL

PROCEDURE : Before the assay begins, bring all the reagents to room temperature.

Total Bilirubin

Wave length : 546 nm (540-560)
Temperature : 37ºC
Cuvette : 1 cm light path
Read against Reagent Blank (RB)

	Reagent Blank	Sample/Calibrator
Sample/Calibrator		20 µl
Reagent 1	1000 µl	1000 µl
Mix incubate for 3 - 5 min read Absorbance A1 and then add		
Reagent 2	200 µl	200 µl
Mix incubate for exactly 5 min and read A2		

$\Delta A = [(A2-A1)\ Sample / Calibrator\ ] - [(A2-A1)RB]$

CALCULATION :

With Calibrator

$$\text{Bilirubin [mg/dl]} = \frac{\Delta A \text{ sample}}{\Delta A \text{ Calibrator}} \times C \text{ [mg/dl]}$$

C = Concentration of Calibrator

REFERENCE RANGE :

Adult : upto 1.2 mg/dL

The above reference range is guideline and all the laboratories must establish their own normal reference range. Final diagnosis should be made with correlation of clinical factors.

PRECAUTIONS :

1. Do not pipette by mouth therefore avoid contact of the reagent with skin. Do not swallow, avoid contact to skin. In case of skin contact wash off with with plenty of water.

2. Use clean glassware and microtips while pipetting

3. Avoid contamination of the reagent during the assay process.

4. Before the assay begins, bring all the reagents to room temperature.

5. Do not freeze or expose the reagents to high temperature and protect from direct sunlight as it will affect the performance of the kit.

6. Programmes for specific autoanalysers are available on request.

8. For accuracy of results, the assay procedure, reagent preparation and storage has to be meticulously followed.

9. As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

Performance Data (37°C) :

Precision

Within - run reproducibility
N=20

	Mean mg/dl	SD mg/dl	CV %
Sample 1	0.89	0.03	3.05
Sample 2	1.02	0.02	2.32
Sample 3	4.83	0.05	0.95

Between - run reproducibility
N=20

	Mean mg/dl	SD mg/dl	CV %
Sample 1	0.87	0.02	2.74
Sample 2	1.15	0.04	3.49
Sample 3	4.65	0.13	2.86

LINEARITY AND DETECTION LIMIT :

The linearity limit of the standard serum procedure is up to an total Bilirubin activity of 30 mg/dl and detection limit 0.1mg/dl.
At higher concentration activities the sample has to be diluted 1 + 4 with normal saline, the result multiplied by 5.

BIBLIOGRAPHY

1. Thomas L ed. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft, 1998.p. 192-202.

2. Tolman KG, Rej R. Liver function. In: Burtis CA,Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1125-77.

3. Rand RN, di Pasqua A. A new diazo method for thedetermination of bilirubin. Clin Chem 1962;6:570-8.

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