

Exp#6


Bilirubin

Total and Direct bilirubin

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Definition

Bilirubin: is the yellow breakdown product of normal haemoglobin & excreted in bile and urine

(DIRECT (Conjugated	(INDIRECT(Unconjugated
<p>bound to albumin and sent to the liver. In the liver it is conjugated with glucuronic acid</p> <p>soluble in water.</p>	<p>Erythrocytes  Hb</p> <p>The heme is then turned into unconjugated bilirubin in the reticulo-endothelial cells of the spleen</p> <p>.not soluble in water</p>

Clinical Significance

.An increase in bilirubin conc. Is called jaundice-

High level of direct type means that the bile is not being properly excreted

.High levels of indirect mean more Hb is being damaged-

**Hyberbilirubinemia, Elevation of total bilirubin*
:occur due to**

Over production of bilirubin-

Excessive hemolysis of RBC-

Liver diseases e.g. hepatitis & cirrhosis-

Obstruction of biliary tract e.g. gall stones-

Both conjugated (direct) and unconjugated-
(indirect) bilirubin are increased in hepatitis

The relative proportion of the conjugated fraction-
increases with progression of the disease until
eventually the liver loses its ability to carry out the
conjugation reaction

Principle

Direct Bilirubin:

Bilirubin in the serum is coupled with diazotized sulfanilic acid to form azobilirubin (purple color).

The intensity of absorption of purple color that is formed is proportional to the bilirubin concentration in the serum.

This is the calculation of Direct Bilirubin

Total Bilirubin:

Add methanol to accelerates the reaction of unconjugated bilirubin in the serum, and a value of total bilirubin is obtained after letting the sample stand for 5 minutes.

***This is the calculation of Total Bilirubin**

The total bilirubin value represents the sum of the bilirubin glucuronide (direct) and the unconjugated (indirect) bilirubin.

Specimen

serum

While processing samples, protect from direct light as loss of bilirubin may occur.

Procedure

:Total Bilirubin-1

Tubes	Reagent blank	Test blank	Test	CAL
DW	μl 100	--	--	--
Test	--	μl 100	μl 100	--
CAL	--	--	--	μl 100
RT	--	ml 1	--	--
WR	1ml	--	1ml	1ml

.Mix and let stand for 2 min. at RT

Wavelength= 540 nm

Read A. of test blank against DW

Read A. of samples against reagent blank

Direct Bilirubin -2

Tubes	Reagent blank	Test blank	Test	CAL
DW	μ 100	--	--	--
Test	--	μ 100	μ 100	--
CAL	--	--	--	μ 100
RD	--	ml 1	--	--
WR	ml 1	--	1ml	ml 1

.Mix and stand for 5 min. at 37 C

Wavelength= 540 nm

read A of test blank against DW-1

read A of samples against reagent blank-2

Calculation

:Same calculation for total and direct

$$\frac{\text{Abs. test} - \text{Abs. test blank}}{\text{Abs. CAL}} \times \text{Conc. Of CAL} = \text{mg/dl}$$

Normal Value

:Adult

Total up to 1.0 mg/dl

Direct up to 0.2 mg/dl
