LIQUIZYME

DIRECT BILIRUBIN

(DMSO Method)

BEACON

Code	Product Name	Pack Size
LS055A	Liquizyme Direct Bilirubin	240 ml
LS055B	Liquizyme Direct Bilirubin	1200 ml

Intended Use

Diagnostic reagent for quantitative *in vitro* determination of Bilirubin in human serum.

Clinical Significance

Bilirubin is a breakdown product of haemoglobin. Bilirubin formed in the reticulo endothelial system is transported bound by albumin to the liver. This bilirubin is water insoluble and is known as indirect or unconjugated bilirubin. In the liver, bilirubin is conjugated to glucuronic acid to form direct bilirubin. Conjugated bilirubin is excreted via the biliary system into the intestine. Here it is metabolised by bacteria to urobilinogen & stercobilinogen.

Principle

In the determination of Bilirubin Total, Bilirubin is coupled with diazotized sulfanilic acid in the presence of ethylene glycol and dimethylsulfoxide as solvents to produce an intensely colored diazo dye. The intensity of colour of this solution is proportional to the concentration of the bilirubin total in the sample.

Reaction

Direct Bilirubin

Bilirubin + Sulphanilic acid + Sodium Nitrite ⟶ Azobilirubin

Contents:

Reagent 1 : Direct Bilirubin R eagent

Buffer : < 15 mmol/l
Sulphanilic Acid : >20 mmol/l

Reagent 2 : Direct Nitrite Reagent

Sodium Nitrite : < 10 mmol/l

Reagent 3: Bilirubin Artificial Standard : 10 mg/dl

Ready to use

Unit Conversion

mg/dl x 16.95 = µmol/l

Normal Value :

Serum Direct Bilirubin : upto 0.3 mg/dl

Each Laboratory should establish it's own normal range representing its patient population.

Reagent Preparation

Reagents are liquid, ready to use.

Stability and storage

The unopened reagents are stable till the expiry date stated on the bottle and label when stored at room temperature.

Direct Bilirubin

Performance Data

Data contained within this section is representative of performance on Beacon system. Data obtained in your laboratory may differ from these values.

Limit of quantification : 0.0052 mg
Linearity : 20 mg/dl
Measuring range : 0.0052 – 20 mg/dl

Intra-assay precision Within run (n=20)	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample 1	0.251	0.01	3.59
Sample 2	1.15	0.01	0.47

Inter-assay precision Run to run (n=20)	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample 1	1.16	0.01	1.02

Comparison

A comparison between Beacon Direct Bilirubin(y) and a commercially available test (x) using 20 samples gave following results :

y = 0.993 x + 0.011 mg/dl
r = 0.999

Linearity:

This procedure is linear upto 20 mg/dl. If the values exceed this limit, dilute the sample with normal saline (NaCl 0.9%) and repeat the assay. Multiply result by dilution factor.

Interferences

Following substances do not interfere:
haemoglobin up to 7.5 g/l, triglycerides up to 1500 mg/dl.

Warning And Precautions

For in vitro diagnostic use. To be handled by entitled and professionally educated person.

Procedure:

Pipette into clean dry test tubes labeled as Blank (B), and Test (T) :

Direct Bilirubin

Addition Sequence	(B)	(T)
Direct Bilirubin Reagent	1000 µl	1000 µl
Direct Bilirubin Activator	-	20 µl
Serum	50 µl	50 µl

Mix well and incubate at 37°C for exactly 5 minutes. Measure the absorbance of the Test Samples (Abs. T) immediately against their respective Blanks.

Calculation

With factor:

Direct Bilirubin = OD of test - OD of sample blank x Factor (20)

With artificial standard:

$$\text{Bilirubin Concentration (mg/dl)} = \frac{\text{OD test - OD of sample Blank}}{\text{OD of standard}} \times 10$$

Assay Parameters For Photometers

Mode	End Point
Wavelength	546
Sample Volume (µl)	50
Direct Bilirubin Reagent (µl)	1000
Direct Bilirubin Activator (µl)	20
Incubation Time	5 min
Reaction temperature (°C)	37
Linearity Low (mg/dl)	0.0052
Linearity High (mg/dl)	20
Blank with	Serum
Standard Concentration	10 mg/dl
Unit	mg/dl

References

1. Cornall, A. G., Bardawill, C. J., David, M. M.: J. Biol. Chem. 177, 751, 1949.
2. Doumas, B. T., Bayse, D. D. a kol.: Clin. Chem. 27, 1642, 1981.
3. Chromý, V., Fischer, J.: Clin. Chem. 23, 754, 1977.
4. Chromý, V., Fischer, J., Vozníèek, J.: Z. Med. Labor. Diagn. 21, 333, 1980.
5. Tietz Textbook of Clinical Chemistry and Molecular diagnostics. Burtis, C.A.
6. Ashwood, E.R., Bruns, D.E.; 5th edition, WB Saunders.

ymbols Used On Labels

S

REF	Catalogue Number		Manufacturer
	See Instruction for Use	LOT	Lot Number
CONT	Content		Storage Temperature
	Expiry Date	IVD	In Vitro Diagnostics

BEA/24/BDD/LS/IFU Ver- 01
23/07/2024

BSCIC JAS-ANZ ISO 9001 : 2015 BSCIC ISO 13485:2016