Spectrum For Diagnostic Industries

Total Bilirubin

IVD

Cat. No.	Pack size
107 02 030 107 05 030	(2 x 30 ml) 60 tests (5 x 30 ml 150 tests

Intended Use

Total Bilirubin reagent is intended for the in-vitro quantitative and diagnostic determination of bilirubin in human serum or plasma.

Introduction

The average level of the bilirubin produced in humans from different sources range between 250 to 300 mg/day, of which 85 % is derived from the heme moiety of the haemoglobin released from senescent erythrocytes that are destroyed in the reticuloendothelial system . The remaining 15 % is produced from erythrocytes destroyed in the bone marrow and from catabolism of other heme containing proteins such as cytochromes and myoglobin .
After it is produced in the peripheral tissues , bilirubin is transported to the liver in association with albumin . In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract. Disease or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

Method

MODIFIED BERGH & MÜLLER METHOD (Colorimetric, End Point)

Principle

The azobilirubin produced by the reaction between bilirubins and the diazonium salt of 3,5 dichlorophenyl tetraflouroborate salt shows maximum absorption at 540 nm. The intensity of the colour produced is proportional to the quantity of bilirubin which has reacted. In the presence of caffeine and surfactants as accelerators conjugated and free bilirubin participate in the reaction in the same way, so that the level of total bilirubin is determined.

Reagents

3,5-dichlorophenyl tetrafluoroborate	0.2 mmol/l
Caffeine	50 mmol/l
Surfactants and stabilizers	< 3%

Reagents preparation, storage and stability

Total Bilirubin Reagent is supplied ready to use.

Stability: at +2°C to +8°C up to the expiration date Once opened, the opened vial for reagent is stable for 2 months at the specified temperature.

Deterioration

Do not use the bilirubin reagents if precipitate forms Failure to recover control values within the assigned range may be an indication of reagent deterioration .

Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Specimen collection and preservation

Avoide exposure of the specimen to light. If plasma is used, only heparin and oxalate plasma are suitable. Other anticoagulants
should not be used. The average half-life of total bilirubin and direct bilirubin in serum is 17 days and few hours respectively

Procedure

Wavelength	546 nm
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample : Reagent Ratio	1 : 20
Temperature	37 ^{o}C or 20 – 25 ^{o}C
Incubation time	5 minutes at 20 – 25 ^{o}C
Zero adjustment	Reagent Blank
Reagent Blank Limits	Low 0.00 AU High 0.15 AU
Sensitivity	0.1 mg/dL
Linearity	25 mg/dL

Procedure 1 (with factor)

	Blank	sample blank	sample
Samlpe	.....	50 µl	50 µl
Reagent	1 ml	.....	1 ml
N.Saline	.....	1 ml	.....

Mix and incubate for 5 minutes at 15 -25^{o}C or 3 minutes at 37 ^{o}C. Measure absorbance of sample (A_{sample}) and Sample blank ($A_{sample\ blank}$) against reagent blank.

Calculation

ΔA Sample = $A_{sample} - A_{sample\ blank}$

Total Bilirubin Factor = 28

Serum Total Bilirubin Conc (mg/dl) = ΔA Sample x 28

Conversion Factor = mg/dl x 17.1 = µmol/l

Procedure 2 (with Bilirubin Calibrator)

	Blank	Calibrator	sample blank	sample
Samlpe	.....	.....	50 µl	50 µl
Reagent	1 ml	1 ml	.....	1 ml
Calibrator	.....	50 µl	.....	.....
N.Saline	.....	.....	1 ml	.....

Mix and incubate for 5 minutes at 15 -25^{o}Cor 3 minutes At 37 ^{o}C. Measure absorbance of sample (A_{sample}) Calibrator (A cal)and Sample blank ($A_{sample\ blank}$) against reagent blank.

Calculation

ΔA Sample = $A_{sample} - A_{sample\ blank}$

$$\text{T.Bilirubin concentration (mg/dl)} = \frac{(\Delta A \text{ Sample})}{(A_{cal.})} \times \text{conc. of cal}$$

Bilirubin calibrator is not included in the kit. Any commercial Bilirubin calibrator is required for the test (procedure 2)

Important Note :

For severely haemolyzed or lipemic sera, serum correction is required by performing serum blank. Use normal saline as serum blank reagent.

Quality control

Normal and abnormal control serum of known concentration should be analyzed with each run.

Interference

Hemolysis: Elevated levels of haemoglobin may interfere. Lipemia (Intralipid): Elevated levels of triglycerides may interfere.

Expected Values

Serum: 0.1 to 1.2 mg/dl .

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.

Performance Characteristics

Method Comparison

A comparison of the BIL-T (y) with a commercial obtainable assay (x) gave the following result:

y = 0.999 x + 0.250; r = 0.997

Precision

Within run (Repeatiblity)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	0.79	4.37
SD	0.016	0.18
CV%	2.13	4.12

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	0.82	4.52
SD	0.02	0.27
CV%	2.24	4.21

Sensitivity

The sensitivity of the reagent is 0.1 mg/dl. The lower detection limit represents the lowest measurable Bilirubin concentration that can be distinguished from zero.

Linearity

The reaction is linear up to a total bilirubin concentration of 25 mg/dl. Specimens showing higher concentration should be diluted 1+4 with physiological saline and repeat the assay (result × 5)

Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1. Balistreri WF, Shaw LM. Liver function. In: Tietz NW, ed. Fundamentals of clinical chemistry.3 rd ed. Philadelphia:WB Saunders: 1987:729-761.
2. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimetric method. J Biol Chem.1937:119:481-490.
3. Tietz NW, ed. Clinical guide to laboratory tests. 3rd ed.Philadephia: WB saunders; 1995:268-273.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use
LOT	Batch Code/Lot number
REF	Catalogue Number
	Consult instructions for use
	Temperature Limitation
	Use by/Expiration Date
	CAUTION. Consult instructions for use
	Manufactured by

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