

REF: BIL099100 100 Test REF: BIL099260 60 Test
 R1 Direct Bilirubin 1 x 100 ml R1 Direct Bilirubin 2 x 30 ml
 R2 Total Bilirubin 1 x 100 ml R2 Total Bilirubin 2x 30 ml
 R3 Nitrite 1 x 15ml R3 Nitrite 1x 5ml

Intended Use

BioMed Diagnostics bilirubin reagent is intended for the in-vitro

Quantitative, diagnostic determination of bilirubin in human serum on both automated and manual systems.

Background

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

DMSO. Colorimetric method.

Assay Principle

Bilirubin is converted to colored diazotized sulfanilic acid and Measured photometrically. Of the two fractions presents in serum, bilirubin glucuromide and free bilirubin loosely bound to albumin. Only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulfoxide (DMSO) to react (bilirubin indirect). In the determination of indirect bilirubin the direct is also determined, the results correspond to total bilirubin.

Reagents

Reagent 1 (R1) D- Bilirubin 30 mmol/l
 Sulfanilic acid 150 mmol/l
 HCL
 Reagent 2 (R2) T- Bilirubin 30 mmol/l
 Sulfanilic acid 150 mmol/l
 HCL 7 mol/l
 Dimethylsulfoxide(DMSO)
 Reagent 3 (R3) 29 mmol/l
 Sodium Nitrite

Precautions and Warnings

R1/R2/RT: Corrosive (C)
 R35 Causes severe burns.
 S28 After contact with skin, wash immediately with plenty of soap and water.

Do not ingest or inhale. 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.

Reagent Preparation, Storage and Stability

BioMed bilirubin reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when stored at 2 - 8 oC

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Use by/Expiration Date
IVD	For in-vitro diagnostic use		CAUTION. Consult instructions for use
LOT	Batch Code/Lot number		Manufactured by
REF	Catalogue Number		(C) - Corrosive
	Consult instructions for use		
	Temperature Limitation		

Deterioration

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

Specimen Collection and Preservation

Avoid 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.

Stability:

	-20 oC	4 – 8 oC	20 – 25 oC
Total	6 months	7 days	1 day
Direct	6 months	7 days	2 days

Procedure

Direct Bilirubin

	Sample blank	Sample
Reagent 1 (D)	1ml	1 ml
Reagent 3	-----	50 µ
Sample	50 µ	50 µ

Mix and incubate for 5 minutes at 20 – 25 oC. Measure absorbance of sample (Asample) against sample blank at 546 nm(530 - 580 nm)

Total Bilirubin

	Sample blank	Sample
Reagent 2 (T)	1 ml	1ml
Reagent 3	-----	50 µ
Sample	50 µ	50 µ

Mix and incubate for exactly 5 minutes at 20 – 25 oC. Measure absorbance of sample (Asample) against sample blank at 546 nm (530 - 580 nm).

Calculation

{ (A)Sample - (A) Sample blank } x Factor* = mg/dl

*Theoretical Factor

Direct bilirubin = 14

Total bilirubin = 19.1

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

Note

For bilirubin determination in newborns, pipette 50 µ of sample. Multiply the result by 2.

Quality Control

Normal & abnormal commercial control serum of known concentrations Should be analyzed with each run.

Performance Characteristics Precision

Within run (Repeatability)

	Total		Direct	
	Level 1	Level 2	Level 1	Level 2
n	20	20	20	20
Mean (mg/dL)	0.79	4.37	0.299	0.77
SD	0.016	0.18	0.016	0.057
CV%	2.13	4.12	5.41	7.4

Run to run (Reproducibility)

	Total		Direct	
	Level 1	Level 2	Level 1	Level 2
n	20	20	20	20
Mean (mg/dL)	0.82	4.52	0.32	0.82
SD	0.02	0.27	0.023	0.062
CV%	2.24	4.21	5.57	8.1

Methods Comparison

A comparison between BioMed Diagnostics Bilirubin and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.975 was obtained.

Sensitivity

When run as recommended, the sensitivity of this assay is 0.1 mg/dL (1.7 µmol/L) for total and 0.04 mg/dL (0.68 µmol/L) for direct bilirubin.

Linearity

The reaction is linear up to a total bilirubin concentration of 18 mg/dL (308 µmol/L) and a direct bilirubin concentration of 18 mg/dL (308 µmol/L). Specimens showing higher concentration should be diluted 1+4 with physiological saline and repeat the assay (result*5).

Interfering substances Serum, plasma

Haemolysis

Avoid haemolysis since it interferes with the test.

Lipemia

Lipemic specimens interfere with the test.

Drugs

Theophyllin and propranolol may cause artificially low total bilirubin levels.

Expected Values

Total Bilirubin

Adults and infants >1 month < 0.2-1.1 mg/dL (3.4-17 µmol/L)
Newborns premature (3-5 d) 10-14 mg/dL (171-239 µmol/L)

Newborns:
(3-5 d) 4.0 - 8.0 mg/dL (68-137 µmol/L)
(<48 h) 6.0 - 10.0 mg/dL (103-171 µmol/L)
(<24 h) 2.0-6.0 mg/dL (34-103 µmol/L)

Direct Bilirubin 0 - 0.3 mg/dL (0 - 51 µmol/L)

BioMed Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Analytical Range

Total bilirubin : 0.1 - 18 mg/dL (1.7 - 308 µmol/L)
Direct bilirubin : 0.04 - 18 mg/dL (0.68 - 308 µmol/L)

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

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

ORDERING INFORMATION

CATALOG NO.	QUANTITY
BIL099160	60 test
BIL099250	150 test



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