SPECTRUM

The Creative Approach to Bioscience

Bilirubin Total/Direct DMSO

REF: 225 001 **60 Test**
REF: 225 002 **120 Test**

SYMBOLS IN PRODUCT LABELLING

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

Intended Use

Spectrum 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 ranges 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. Diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize , 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 present 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

Sulfanilic acid	30 mmol/l
HCL	150 mmol/l

Reagent 2 (R2) T- Bilirubin

Sulfanilic acid	30 mmol/l
HCL	150 mmol/l
Dimethylsulfoxide(DMSO)	7 mol/l

Reagent 3 (R3)

Sodium Nitrite	29 mmol/l

Precautions and Warnings

S28 After contact with skin, wash immediately with plenty of soap and water.
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.

Reagent Preparation, Storage and Stability

Spectrum bilirubin reagents are supplied ready-to-use and stable up to the expiry date stated on the label when stored at 2 - 8 ^{o}C.The opened vial is stable for 6 months at the specified temperature if contamination is avoided.

Deterioration

Do not use the Spectrum 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 ^{o}C	4 – 8 ^{o}C	20 – 25 ^{o}C
Total	6 months	7 days	1 day
Direct	6 months	7 days	2 days

Procedure

Direct Bilirubin

	Sample blank	Sample
Reagent 1 (D)	1.5 ml	1.5 ml
Reagent 3	-----	50 µl
Sample	100 µl	100 µl

Mix and incubate for 5 minutes at 20 – 25 ^{o}C. Measure absorbance of sample (A_{sample}) against sample blank at 546 nm(530 - 580 nm)

Total Bilirubin

	Sample blank	Sample
Reagent 2 (T)	1.5 ml	1.5 ml
Reagent 3	-----	50 µl
Sample	100 µl	100 µl

Mix and incubate for exactly 5 minutes at 20 – 25 ^{o}C. Measure absorbance of sample (A_{sample}) against sample blank at 546 nm (530 - 580 nm).

Calculation

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

*Theoritical 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 µl of sample. Multiply the result by 2.

Quality Control

Normal and 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%	2.03	4.12	5.35	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	0.17	0.023	0.062
CV%	2.44	3.76	7.19	7.56

Methods Comparison

A comparison between Spectrum Bilirubin and a commercial reagent of the same methodology was performed on 200 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

Haemolysis
Avoid haemolysis since it interferes with the test.

Lipemia
Lipemic specimens interfere with the test.

Drugs
Theophylline 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 – 5.1 µmol/L)

Spectrum 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

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.

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