SPECTRUM

The Creative Approach to Bioscience

Bilirubin (TOTAL AND DIRECT)
Jendrassik Grof

REF: 222 001 (255ml)	100 Test	**REF:** 222 002 (750ml)	300 Test
R1 Sulphanilic Acid	1 x 45 ml	R1 Sulphanilic Acid	2 x 65 ml
R2 Nitrite	1 x 10 ml	R2 Nitrite	2 x 15 ml
R3 Caffeine	1 x 100 ml	R3 Caffeine	3 x 100 ml
R4 Tartarate	1 x 100 ml	R4 Tartarate	3 x 100 ml

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. 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

Colorimetric Diazo method.

Assay Principle

The total bilirubin concentration is determined in presence of caffeine by the reaction with diazotized sulphanilic acid to produce an intensely colored diazo dye (560-600 nm). The intensity of color of this dye formed is proportional to the concentration of total bilirubin.
Direct bilirubin is determined in absence of caffeine by the direct reaction with diazotized sulphanilic acid to form red-colored azobilirubin, the color intensity of which measured at 546 nm is proportional to the concentration of the direct bilirubin in the sample.

$$\text{Sulfanilic acid} + NaNO_2 \xrightarrow{HCL} \text{Diazotized sulfanilic acid}$$

$$\text{Bilirubin} + \text{Diazotized sulfanilic acid} \xrightarrow{pH\ 1.4} \text{Azobilirubin}$$

Reagents

Reagent 1 (R1)
Sulfanilic acid 31.0 mmol/l
HCL 0.20 N

Reagent 2 (R2)
Sodium nitrite 28.0 mmol/l

Reagent 3 (R3)
Caffeine 0.28 mol/l
Sodium benzoate 0.55 mol/l

Reagent 4 (R4)
Tartarate 0.99 mol/l
Sodium hydroxide 2.0 N

Reagent 4 contains caustic material.
Corrosive (C)
R35 Causes severe burns.
R41 Risk of serious damage to eyes.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 After contact with skin, wash immediately with plenty of soap and water.
For further information, refer to the Bilirubin reagent material safety data sheet.

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.

Reagent Preparation, Storage and Stability

Spectrum bilirubin reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when stored at room temperature.The opened vials are 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 °C	4 – 8 °C	20 – 25 °C
Total	6 months	7 days	1 day
Direct	6 months	7 days	2 days

Procedure

Total Bilirubin

	Sample blank	Sample
Reagent 1	200 µl	200 µl
Reagent 2	-----	1 drop
Reagent 3	1.0 ml	1.0 ml
Sample	200 µl	200 µl
Mix and incubate for 10 minutes at 20 – 25 °C. then add;		
Reagent 4	1.0 ml	1.0 ml

Mix and incubate for 5 minutes at 20 – 25 °C. Measure absorbance of sample (A_{sample}) against sample blank at 578 nm(560 - 600 nm) The color intensity is stable for 30 minutes.

Direct Bilirubin

	Sample blank	Sample
Reagent 1	200 µl	200 µl
Reagent 2	-----	1 drop
Saline 0.9% NaCl	2.0 ml	2.0 ml
Sample	200 µl	200 µl

Mix and incubate for exactly 5 minutes at 20 – 25 °C. Measure absorbance of sample (A_{sample}) against sample blank at 546 nm (530 - 560 nm).

Calculation

Total bilirubin (mg/dl) = $A_{Sample} \times 10.8$

Direct bilirubin (mg/dl) = $A_{Sample} \times 14.4$

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 both total and direct bilirubin.

Linearity

The reaction is linear up to a total bilirubin concentration of 30 mg/dL (513 µmol/L) and a direct bilirubin concentration of 10 mg/dL (171 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	< 0.2-1.0 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)

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 – 30 mg/dL (1.7 – 513 µmol/L)
Direct bilirubin : 0.1 – 10 mg/dL (1.7 – 171 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.

ORDERING INFORMATION	
CATALOG NO.	QUANTITY
222 001 222 002	100 test 300 test

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