SPECTRUM

The Creative Approach to Bioscience

Pediatric Total BILIRUBIN

REF: 221 001 (2 x 50 ml) 50 Test
REF: 221 002 (2 x 100 ml) 100 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 Diagnostics Total Bilirubin single reagent is intended for in-vitro quantitative, diagnostic determination of Total Bilirubin in human serum on both manual and automated 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

Modified Bergh & Muller method (Colorimetric, End point)

Assay Principle

The azobilirubin produced by the reaction between bilirubins and the diazonium salt of 3,5-dichlororphenyl diazonium tetrafluoroborate 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

Reagent (R)

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

Serum Blank Reagent (SB)

Buffered Saline

For further information, refer to the Pediatric Total 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

The reagents are supplied ready to use.
Stability:at 2 °C to 8 °C up to the expiration date labeled on the bottles.Once opened the reagent is stable foe 2 months at specified temperature.
Always keep bottles closed tightly and protect from light.

Specimen collection and preparation

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.

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.

Additional Material may be required:

- Bilirubin Calibrators
This reagent can be used manually (see methods below) and on most Autoanalyzers, Applications are available on request.

System Parameters

Wavelength	546 nm
Optical path	1 cm
Assay type	Colorimetric End-point
Direction	Increase
Temperature	25 ^{O}C or 37 ^{O}C
Zero adjustment	Reagent blank
Sensitivity	0.1 mg/dL (1.71 µmol/l)
Linearity	25 mg/dL (427.5 µmol/l)

Procedures

	Blank	Serum Blank	Sample
Serum blank reagent (SB)	.....	1 ml	.....
Reagent (R)	1 ml	.....	1 ml
Sample	.....	25 µl	25 µl

Mix well and let stand 5 minutes at 25 ^{O}C or 2 min at 37 ^{O}C , then read absorbance of sample blank and sample against reagent blank.

Calculation

$\Delta A = A_{sample} - A_{sample\ blank}$
Total Bilirubin Factor = 56
Serum Total Bilirubin conc. (mg/dL) = $\Delta A \times 56$
Conversion Factor = mg/dl x 17.1 = µmol/l

Performance Characteristics

Precision
Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	0.77	4.24
SD	0.014	0.155
CV%	1.82	3.57

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	0.87	4.62
SD	0.03	0.124
CV%	3.45	2.68

Quality Control

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

Methods Comparison

A comparison between Spectrum Diagnostics Pediatric Total Bilirubin reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.978 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 0.1 mg/dl (1.71 µmol/l)

Linearity

The reaction is linear up to Bilirubin concentration of 25 mg/dl (427.5 µmol/l)

Interfering substances

Haemolysis
Avoid haemolysis since it interferes with the test.

Lipemia
Lipemic specimens interfere with the test.

Expected Values

Total Bilirubin

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)

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

Analytical Range

0.1 - 25 mg/dl.

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.r P. Neue serumeisenbestimmung auf dem GSA II. Lab Med.1980;4:240-244.
5. Williams HL, Johnson DJ, Haut MJ. Simultaneous spectrophotometry of Fe2+ and Cu2+ in serum denatured with guanidine hydrochloride. Clin Chem.1977;23:237-240.
6. Makino et al.: Clinica Chimica Acta, 171 (1988), 19-28.

ORDERING INFORMATION	
CAT. NO.	**QUANTITY**
221 001	2 x 50 ml
221 002	2 x 100 ml

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