Spectrum For Diagnostic Industries

Bilirubin
Jendrassik Grof

IVD

Cat. No.	Pack size
104 05 125	(500 ml) 200 tests

Intended Use

Bilirubin reagent is intended for the in-vitro quantitative and diagnostic determination of bilirubin in human serum or plasma on both automated and manual systems.

Introduction

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.

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 intenisity of which measured at 546 nm is proportional to the concentration of the direct bilirubin in the sample.

Direct bilirubin is determined as follows:

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

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

Reagents

Reagent 1
Sulfanilic acid 31.0 mmol/L
HCL 0.20 N

Reagent 2
Sodium nitrite 28.0 mmol/L

Reagent 3
Caffeine 0.28 mol/L
Sodium benzoate 0.55 mol/L

Reagent 4
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.

Reagents preparation, storage and stability

Bilirubin reagents are supplied ready-to-use and stable up till the expiration date labeled on the bottles when stored at room temperature.Once opened, the open vial is stable for 6 months at the specified temperature if contamination is avoided.

Deterioration

Do not use the bilirubin reagents if precipitate forms.Failure to recover the control values within assigned range may indicate 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

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} x 10.8

Direct bilirubin (mg/dL) = A_{Sample} x 14.4

Quality control

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

Interference

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

1- Adults and infants >1 month	< 0.2-1.0 mg/dL	(3.4-17 µmol/L)
2- Newborns premature (3-5 d)	10-14 mg/dL	(171-239 µmol/L)
3- Newborns:		
a) 3-5 d	4.0 - 8.0 mg/dL	(68-137 µmol/L)
b) <48 h	6.0 - 10.0 mg/dL	(103-171 µmol/L)
c) <24 h	2.0 - 6.0 mg/dL	(34-103 µmol/L)
Direct Bilirubin	0 – 0.3 mg/dL	(0 – 51 µmol/L)

Performance characteristics

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

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

Sensitivity

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).

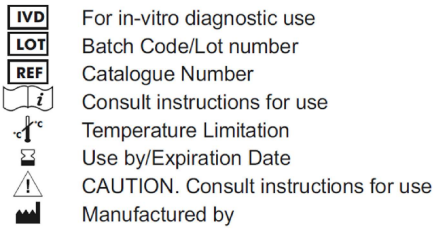
Waste Disposal

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.Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimetric method. J Biol Chem.
2.Balistreri WF, Shaw LM. Liver function. In: Tietz NW, ed. Fundamentals of clinical chemistry.3 rd ed. Philadelphia:WB Saunders.

SYMBOLS IN PRODUCT LABELLING



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