

# **Bilirubin (DIRECT)** Colorimetric

REF: 224 001 60 Test R1 Direct Bilirubin 2 x 90 ml R2 Nitrite 1 x 7

#### **Intended Use**

Spectrum 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 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 dámage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

## Method

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).

## Reagents

Reagent 1 (R1) D- Bilirubin Sulfanilic acid

30 mmol/l

Reagent 2 (R2) Sodium Nitrite 29 mmol/l

# **Precautions and Warnings**

**S28** After contact with skin, wash immediately with plenty of soap

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 2 - 8  $^{\circ}$  C. The opened vial is stable for 6 months at the specified temperature if contamination is avoided.

#### SYMBOLS IN PRODUCT LABELLING

ECREP Authorised Representative 

Use by/Expiration Date For in-vitro diagnostic use LOT Batch Code/Lot number Catalogue Number Consult instructions for use (Xi) - Irritant Temperature Limitation

CAUTION. Consult instructions for use

Manufactured by

## 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 direct bilirubin in serum is few hours

#### Stability:

	-20 °C	4 – 8 °C	20 – 25 <sup>o</sup> C
Direct	6 months	7 days	2 days

## **Procedure**

#### **Direct Bilirubin**

	Sample blank	Sample	
Reagent 1 (D)	1.5 ml	1.5 ml	
Reagent 2		50 μΙ	
Sample	100 μΙ	100 μΙ	

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

## Calculation

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

\*Theoritical Factor

Direct bilirubin = 14

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

# Performance Characteristics Precision

Within run (Repeatablity)

	Direct	
	Level 1	Level 2
n	20	20
Mean (mg/dL)	0.299	0.77
SD	0.016	0.057
CV%	5.41	7.4

Run to run (Reproducibility)

	Direct	
	Level 1	Level 2
n	20	20
Mean (mg/dL)	0.32	0.82
SD	0.023	0.062
CV%	5.57	8.1

## **Methods Comparison**

A comparison between Spectrum 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  $\mu$ mol/L) for total and 0.04 mg/dL (0.68  $\mu$ mol/L) for direct bilirubin.

## Linearity

The reaction is linear up to a direct bilirubin concentration of 18 mg/dL(308  $\mu$ mol/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

Theophylline and propranolol may cause artificially low total bilirubin levels.

## **Expected Values**

**Direct Bilirubin** 0 - 0.3 mg/dL  $(0 - 51 \mu\text{mol/L})$ 

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**

Direct bilirubin : 0.04 - 18 mg/dL (  $0.68 - 308 \mu \text{mol/L}$ )

## **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.

\$57: use appropriate container to avoid environmental contamination.
\$61: 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.3 rd 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.
- 3. Tietz NW, ed. Clinical guide to laboratory tests. 3rd ed.Philadephia: WB saunders; 1995:268-273.

ORDERING INFORMATION		
CATALOG NO.	QUANTITY	
224 001	60 test	



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