

# BILIRUBIN

Direct  
DCA Method

## INTENDED USE

Vitro bilirubin reagent is intended for the in vitro quantitative determination of direct bilirubin in serum or plasma on manual system.



## METHOD

DCA Method.

## BACKGROUND

Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from other-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. This process accounts for about 80% of bilirubin formed daily. Other sources of bilirubin include the breakdown of myoglobin and cytochromes and the catabolism of immature red cells in the bone marrow. In the liver, bilirubin is conjugated with glucuronic acid for solubilization to form conjugated or direct bilirubin for subsequent transport through the bile duct into the digestive tract where it is metabolized by bacteria to a group of products collectively known as stercobilinogen. Total bilirubin is the sum of the conjugated and unconjugated fractions. Pre-hepatic diseases or conditions such as hemolytic disease or liver diseases resulting in impaired entry, transport or conjugation within the liver cause elevation of unconjugated (indirect) bilirubin. Monitoring of bilirubin in newborns, particularly if premature, has special importance since the hepatic handling of bilirubin is immature leading to elevated unconjugated bilirubin. If not bound to albumin, unconjugated bilirubin is able to cross the blood brain barrier more easily, increasing the risk of cerebral damage. Total bilirubin is elevated in conditions causing obstruction of the bile duct, hepatitis, cirrhosis, in hemolytic disorders and several inherited enzyme deficiencies. There is information indicating elevated levels of direct bilirubin in patients with liver or biliary tract disease, even though, total bilirubin levels are normal. Therefore, the greatest diagnostic value of direct bilirubin assays stem from their ability to indicate occult liver disease<sup>4</sup>.

## ASSAY PRINCIPLE

Bilirubin present in the sample reacts with diazotized Dichloroaniline to form a coloured azocomplex whose intensity at 546 nm (540-560 nm) is directly proportional to the analyte concentration.

## EXPECTED VALUES

Direct Bilirubin	
Adults and infants	Up to 0.2 mg/dl Up to 3.4 µmol/l

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the bilirubin results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.

## REAGENTS

- R<sub>1</sub> Direct Reagent
- R<sub>2</sub> Direct Sodium nitrite

### • Reagent Preparation & Stability

All reagents are stable up to the expiry date given on label when stored at room temperature.

## SPECIMEN

Serum.

For direct bilirubin plasma specimen may be used, but the only accepted anticoagulants are heparin and oxalate.

### • Specimen Preparation & Stability

The specimen of choice is serum. Specimens should be assayed promptly after collection, since direct bilirubin is reportedly unstable<sup>5</sup>. If testing is delayed, specimens should be protected from exposure to light. Bilirubin remains stable in serum samples for 2 days at room temperature, 4 days at 4°C, or 3 months at -20°C, if care is taken to prevent exposure to light<sup>6</sup>.

## PROCEDURE

Wavelength	546 nm
Cuvette	1 cm light path
Temperature	20-25 °C
Zero adjustment	against specimen blank
Specimen	Serum

### Method 1

	Specimen blank	Specimen
R <sub>1</sub>	1000 µl	1000 µl
R <sub>2</sub>	.....	One drop
Specimen	100 µl	100 µl
Mix well, let stand 1 minutes at 37°C ( Maximum 2 minutes)		

### Method 2: Icteric or pediatric specimen

	Specimen blank	Specimen
R <sub>1</sub>	1000 µl	1000 µl
R <sub>2</sub>	.....	One drop
Specimen	20 µl	20 µl
Mix well, let stand 1 minutes at 37°C ( Maximum 2 minutes)		

Measure the absorbance of specimen (A<sub>specimen</sub>) against specimen blank.

**After 2 minutes indirect bilirubin reacts slowly with diazotized dichloroanilin and leads to over-estimated values.**

## CALCULATION

Calculate the bilirubin concentration by using the following formulae:

For method 1

$$\text{Direct Bilirubin Concentration} = \text{Specimen absorbance} \times 15.2 = \text{mg/dl}$$

For method 1

$$\text{Direct Bilirubin Concentration} = \text{Specimen absorbance} \times 71.2 = \text{mg/dl}$$

## Unit conversion

$$\text{mg/dl} \times 17.1 = \mu\text{mol/dl}$$

## QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established bilirubin values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

## INTERFERING SUBSTANCES

### Anticoagulants:

The only accepted anticoagulants are heparin and oxalate for direct bilirubin.

### • Drugs:

Young<sup>7</sup> in 1990 has published a comprehensive list of drugs and substances, which may interfere with this assay.

### • Hemolysis:

Avoid hemolyzed specimens. Even slight hemolysis interferes with the test. Haemoglobin interference is dependent on both analyte and hemoglobin concentration.

The interference becomes decreasingly significant with increasing concentration of total bilirubin.

### • Lipemia:

Avoid lipemic specimens. Even slight lipemia interferes with the test.



### WARNING & PRECAUTION

- Vitro bilirubin reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- All specimens must be protected from light. Serum bilirubin will decrease 50% in one hour if kept at room temperature and at direct sunlight.
- Don't use the reagent if it is turbid.

### PERFORMANCE CHARACTERISTICS

#### Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

Within Run		
Direct Bilirubin		
Number of samples	40	40
Mean (mg/dl)	0.78	2.28
SD (mg/dl)	0.015	0.01
CV (%)	1.31	0.65

Between Day		
Direct Bilirubin		
Control	Level I	Level II
Number of samples	40	40
Mean (mg/dl)	0.8	2.18
SD (mg/dl)	0.01	0.03
CV (%)	1.63	1.53

#### Method Comparison

Comparison studies were carried out using a similar commercially available Total Bilirubin reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs 929  
Range of sample results 0.05-26 mg/dl  
Mean of reference results 1.2 mg/dl  
Mean of Total Bilirubin results 1.2 mg/dl  
Slope 1.10  
Intercept 0.12 mg/dl  
Correlation coefficient 1.00

#### Sensitivity

The sensitivity is defined as the change of analytical response ( $\Delta A/\text{min}$ ) per unit change in analyte concentration at a pathlength of 1 cm.

When run as recommended the sensitivity of this assay is 0.01 mg/dl (0.17 mol/l).

### LINEARITY

When run as recommended, the assay is linear

up to 20 mg/dl (0.342 mmol/l) for method 1









up to 100 mg/dl (1.71 mmol/l) for method 2

If result exceeds 20 mg/dl (0.342 mmol/l) perform use method number 2.

### BIBLIOGRAPHY

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4. **Gambino, SR et al., (1967):** JAMA 201: 1047.
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7. **Young, DS (1990):** Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990: 3: 6-12.

### SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
	Batch code (Lot #)
	Catalog number
	Temperature limitation
	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

### ORDERING INFORMATION

REF	SIZE
1041	1 x 100 ml
1042	2 x 100 ml

Manufactured in Egypt by:  
Vitro Scient  
[www.vitrosient.com](http://www.vitrosient.com)

Technical Support:  
+202 26439699  
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+202 26439698  
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