

Bilirubin (Direct) Reagent

Catalog #: 43707

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

This reagent is intended for the *in vitro* quantitative determination of direct bilirubin in human serum.

CLINICAL SIGNIFICANCE

Red blood cells at the end of their circulating life are broken down in the reticulo-endothelial system, mainly the spleen. The resulting haem, once the iron is removed, is then converted to bilirubin. This process accounts for about 80 percent of the 300 mg of bilirubin formed daily. Other sources of bilirubin include the breakdown of myoglobin and cytochromes and the catabolism of immature red blood cells in the bone marrow. Once formed, bilirubin is transported to the liver bound to albumin as it is water insoluble. This fraction of bilirubin is referred to as indirect or unconjugated bilirubin. In the liver bilirubin is conjugated to glucuronic acid (mono and di glucuronides) to form conjugated bilirubin by the enzyme uridyl diphosphate glucuronyl transferase. Conjugated bilirubin or direct bilirubin is excreted via the biliary system into the intestine where it is metabolized by bacteria to a group of products known collectively as sterco-bilinogen. Elimination of is almost complete and plasma levels are normally negligible. Total Bilirubin is the sum of the unconjugated and conjugated fractions. Total bilirubin is elevated in conditions causing obstruction of the bile duct, hepatitis, cirrhosis, in hemolytic disorders and several inherited enzyme deficiencies. Indirect bilirubin is elevated by pre-hepatic causes such as hemolytic disorders or liver diseases resulting in impaired entry, transport or conjugation within the liver. Monitoring of bilirubin in the newborn, particularly if premature is of particular importance. Since the hepatic handling of bilirubin in such cases is often immature, jaundice due to a rise in unconjugated bilirubin is common. Unconjugated bilirubin if not bound to albumin is able to cross the blood brain barrier more easily, increasing the danger of cerebral damage.¹

METHODOLOGY

Most methods currently used for assaying bilirubin are based on the reaction between bilirubin and diazotised sulphanic acid solutions. In aqueous solution only the direct (conjugated) bilirubin will react in this manner. The SDI Direct Bilirubin reagent uses an acid diazo method. Conjugated bilirubin reacts with diazotised sulphanic acid to produce an acid azobilirubin, the absorbance of which is proportional to the concentration of direct bilirubin in the sample and can be measured at 550 nm. For bichromatic analyzers the blank reading should be taken at 660 nm.

REAGENT COMPOSITION

Active Ingredients	Concentration
HCl	103 mM
Sulphanilic acid	9.8 mM
Sodium Nitrite	145 mM

Precautions:

- This reagent is for *in vitro* diagnostic use only.
- DO NOT pipette by mouth. If spilt, thoroughly wash affected areas with water.

REAGENT PREPARATION

Test Reagent:

Add Sodium Nitrite Reagent to Direct Bilirubin Reagent in the ratio of 1:100. For example, to 10 mL of Direct Bilirubin Reagent add 0.1 mL of Sodium Nitrite (This is approximately equal to 3 drops but it is recommended that this be verified by the operator).

Blank Reagent:

Use Direct Bilirubin Reagent as supplied.

REAGENT STORAGE

Store reagent between 2-25°C. The reagent is stable until the expiry date stated on the bottle and kit box label.

Working Reagent:

Working reagent is stable for at least 21 days at 2-8°C.

REAGENT DETERIORATION:

Do not use the reagent if:

- Turbid
- Reagent absorbance > 0.1 AU at 550 nm.
- The reagent fails to meet stated parameters of performance.

SPECIMEN COLLECTION AND HANDLING

- The best specimen is unhemolyzed serum.
- Specimens should be protected from bright light as bilirubin is photolabile.
- Specimens may be stored refrigerated for 3 days.⁶

INTERFERENCE

Studies to determine the level of interference for hemoglobin, and lipemia were carried out, the following results were obtained:

Hemoglobin:

No significant interference (± 10%) from hemoglobin up to 100 mg/dL.

Lipemia:

No significant interference (± 10%) from lipemia up to 202 mg/dL measured as triglycerides.

Young DS⁵ has published a comprehensive list of drugs and substances which may interfere with the Direct Bilirubin assay.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- An SDI CA480 Clinical Chemistry System
- Deionized water and related equipment, e.g.: pipettes
- Analyzer specific consumables, e.g.: sample cups
- Control, and Calibrator materials such as those provided by SDI Biomed.

ASSAY PROCEDURE

It is recommended that a sample blank be performed on each sample. A sample blank can be obtained by simply using Direct Bilirubin Reagent as supplied *without* the addition of Sodium Nitrite.

Direct Bilirubin	System Parameters
TEMPERATURE:	37°C
WAVELENGTH:	550 nm
ASSAY TYPE:	Endpoint
DIRECTION:	Increase
SAMPLE / RGT RATIO:	1 : 20
e.g. Sample Vol.	0.05 mL (50µL)
Reagent Vol.	1.0 mL
INCUBATION TIME:	10 Min

Procedure Notes

Samples with values above 20 mg/dL should be diluted 1:1 with water, rerun, and results multiplied by 2.

Calculations

- Abs. = Absorbance

$$\frac{\text{Abs. Sample Test} - \text{Abs. Sample Blank}}{\text{Abs. Standard Test} - \text{Abs. Standard Blank}} \times$$

$$\text{Concentration of Standard} = \frac{\text{Direct Bilirubin}}{\text{(mg/dL)}}$$

Sample calculation:

Abs. Sample Test	= 0.350
Abs. Sample Blank	= 0.010
Abs. Standard Test	= 0.250
Abs. Standard Blank	= 0.010
Concentration of Standard	= 4.0 mg/dL

$$\frac{0.350 - 0.01}{0.250 - 0.01} \times 4 = \frac{0.34}{0.24} \times 4 = 5.7 \text{ mg/dL Direct Bilirubin}$$

NOTE: To obtain values in mmol/L, multiply mg/dL x 17.10 = mmol/L

CALIBRATION

An appropriate serum calibrator can be used.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known Direct Bilirubin values.

EXPECTED VALUES⁶

Adults and infants over 1 month old 0.0 - 0.2 mg/dL.
It is recommended each laboratory verify this range or derives a reference interval for the population that it serves.⁷

PERFORMANCE

Linearity:

When run as recommended the assay is linear from 0 to 20.0 mg/dL.

Method Comparison:

Studies performed between this procedure and a similar Direct Bilirubin Reagent yielded the following results:

Number of sample pairs:	57
Range of samples:	0.10 – 20.3 (mg/dL)
Correlation Coefficient:	0.999
Slope:	0.928
Intercept:	0.06 (mg/dL)

Precision:

Within Run	Level 1	Level 2	Level 3
n=40			
Mean (mg/dL)	0.19	1.67	4.05
S.D. (mg/dL)	0.02	0.04	0.07
C.V. (%)	11.8	2.1	1.7

Total

	Level 1	Level 2	Level 3
n=40 (10 days / 2 runs per day / 2 replicates per run)			
Mean (mg/dL)	0.19	1.67	4.05
S.D. (mg/dL)	0.03	0.08	0.19
C.V. (%)	16.2	4.6	4.7

Sensitivity:

A calibration factor of approximately 27.3 was obtained, which is equivalent to a sensitivity of 0.0366 D Abs per mg/dL.

REFERENCES

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