

Stanbio Direct Bilirubin LiquiColor® (DCA) Procedure No. 0235

Indication: For the quantitative colorimetric determination of Direct Bilirubin in Serum and Plasma.

Summary and Principle^{1,2}

The Stanbio Direct Bilirubin LiquiColor® test is a device intended to measure the levels of bilirubin (direct) in serum and plasma. Measurements of the levels of bilirubin, an organic compound formed during the normal and abnormal destruction of red blood cells, is used in the diagnosis and treatment of liver, hemolytic, hematological, and metabolic disorders, including hepatitis and gall bladder block.

Bilirubin is a breakdown product of hemoglobin. Free, unconjugated bilirubin is extremely apolar and nearly insoluble in water, thus forming a complex with albumin for the transport in the blood from the spleen to the liver. In the liver, bilirubin is conjugated with glucuronic acid and the resulting water soluble bilirubin glucuronides are excreted via the bile ducts.

Hyperbilirubinemia can be caused by increased bilirubin production due to hemolysis (pre-hepatic jaundice), by parenchymal damages of the liver (intra-hepatic jaundice). A chronic congenital (predominantly unconjugated) hyperbilirubinemia called Gilbert's syndrome is quite frequent in the population. High levels of total bilirubin are observed in 60-70% of neonates due to an increased postpartal breakdown of erythrocytes and because of delayed function of enzymes for bilirubin degradation. Common bilirubin methods detect either total bilirubin or direct bilirubin. Determinations of direct bilirubin measure mainly conjugated, water soluble bilirubin. Unconjugated bilirubin can therefore be estimated as the difference between total bilirubin and direct bilirubin.

The Stanbio method for quantifying direct bilirubin relies on the reaction that direct bilirubin in presence of diazotized 2,4-dichloroaniline forms a red colored azocompound in acidic solution.

Reagents

Direct Bilirubin Buffer (R1), Cat. No. 0236

EDTA-Na₂, 0.07 mmol/L
NaCl, 6.6 gm/L
Sulfamic acid, 70 mmol/L

Direct Bilirubin Color Reagent (R2), Cat. No. 0237

2,4-Dichlorophenyl-diazonium salt, 0.09 mmol/L
HCl, 130 mmol/L
EDTA-Na₂, 0.02 mmol/L

Precautions: For In Vitro Diagnostic Use.

Reagent Preparation: The reagents are supplied ready-to-use.

Reagent Storage and Stability: The reagents are stable when stored at 2 -8°C until the expiration date on their respective labels. Do not freeze the reagents. Reagent 2 (R2) must be protected from light.

Materials Required But Not Provided

Spectrophotometer capable of absorbance readings at 550 (546-500) nm. Accurate pipetting devices, Cuvettes, Vortex mixer, Interval timer Ser-T-Cal® Multicalibrator, Cat. No. 0550-605

Specimen Collection and Preparation^{3,4}

1. Serum, or plasma collected by any type of heparin. Sample should be free of hemolysis.

2. Samples must be protected from both sunlight and white artificial light, as bilirubin is highly photolabile.

Sample Stability: Bilirubin is stable in serum or plasma 4-7 days at 2-8°C and for 3 months when frozen (-20°C). Freeze only once.

Interfering Substances: No interference was observed by ascorbic acid up to 30 mg/dL, hemoglobin up to 50 mg/dL and lipemia up to 2000 mg/dL triglycerides. An extensive list of drugs or other agents interfering with bilirubin methodologies has been reported by Young et al⁵.

Automated Analyzer

Parameters should be employed in programming automated analyzers for Direct Bilirubin. Consult your instrument manual for programming instructions. For a specific instrument application contact Stanbio's Customer Service Department. Only analyzers employing a 2 reagent delivery system can utilize this methodology.

Manual Procedure

1. Pipet into cuvettes labeled Reagent Blank (RB), K (Calibrator), and S (Specimen) the following volumes (mL).

	RB	K	S
Bilirubin Buffer (R1)	1.0	1.0	1.0
Calibrator (K)	-	0.100	-
Sample	-	-	0.100
Water	0.100	-	-

2. Mix, incubate for 3 - 5 minutes @ 25°C/37°C and read the absorbance (A1).

3. Add 0.25 mL of Bilirubin Color Reagent (R2), mix and incubate for exactly 5 minutes @ 37 °C, or 10 minutes @ 25 °C, then read the absorbance (A2).

Results

$$\Delta A = [(A2 - A1) \text{ sample or calibrator}] - [(A2 - A1) \text{ blank}]$$

Values are derived from the following calculation:

$$\text{Direct Bilirubin (mg/dL)} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Cal (K)}} \times \text{Conc. Cal. (mg/dL)}$$

Quality Control

1) Stanbio Laboratory recommends the use of Ser-T-Fy® I (Normal) and Ser-T-Fy® II (Abnormal) Controls (reorder numbers G427-86 and G428-86, respectively). See the instructions for use for preparation, use and control ranges.

2) Two levels of controls are run each day the instrument is run prior to reporting patient results. Controls are also run when a new lot number of reagent is loaded or recalibration of the test is performed.

3) Refer to the instructions for use for the established ranges. Recovered control values outside of the established ranges are considered out of control and requires the following corrective action.

a) Repeat the same control but do not report patient results unless the repeated control results are within the established limits.

b) If the repeated control results are still outside of established limits, prepare a fresh bottle of control and repeat the test. If the results are within range, report patient results.

c) If the results from the fresh controls are still out of range, recalibrate the analyzer and repeat the controls. If the controls are within range, repeat test with patient specimens.

d) If after recalibrating on the existing reagent the control results are still out of range, recalibrate on fresh reagent and repeat the controls. If the control results are in range, repeat test with patient specimens.

e) If none of the preceding produces acceptable QC results, contact Stanbio Laboratory Technical Service Department (800-531-5535).

4) The mean value and expected range found on the instructions for use are derived from interlaboratory data. The expected range includes instrument, reagent, and laboratory variations. This laboratory's mean of several determinations may not duplicate the mean value found in the instructions for use, but should fall within the expected range.

5) Each laboratory should establish its own mean and precision parameters.

Expected Values⁶

Adult/elderly/child: 0.1 - 0.3 mg/dL

Performance Characteristics⁷

Date obtained using the EPOS 5060 analyzer

Precision:

Intra-assay Precision n = 20	Mean	SD	CV
Sample Number	mg/dL	mg/dL	%
1	0.36	0.01	3.12
2	0.76	0.01	1.46
3	2.07	0.03	1.30

Intra-assay Precision n = 20	Mean	SD	CV
Sample Number	mg/dL	mg/dL	%
1	0.35	0.01	3.34
2	0.75	0.01	1.00
3	2.13	0.02	0.71

Correlation: Determination of bilirubin by the procedure described (y) and by a another commercially available test (x) using 85 samples gave the following results: $y = 0.95x - 0.04$; $r = 0.995$.

Correlation of Serum vs. Plasma: Determination of bilirubin by the procedure described by (y) serum and by x (plasma) using 22 samples gave the following results: $y = 1.0118x - 0.0078$; $r = 0.9999$.

Sensitivity: The procedure showed a sensitivity of 0.1 mg/dL per 0.001 absorbance units.

Linearity: Linear from 0.1 to 10 mg/dL.

References

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4. Routh JI. In Fundamentals of Clinical Chemistry. NW Tietz, Ed Saunders, Philadelphia, 1976, pp. 1035-1043.
5. Young DS et al. Clin Chem 21:22, 1980.
6. Pagana, KD, and TJ Pagana, Mosby's Diagnostic and Laboratory Test Reference, Mosby, St. Louis, 1995, pp. 108.
7. Stanbio Laboratory data.

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