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

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

**Summary and Principle:**

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 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 postpartum 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-dichloranilin forms a red colored azo compound in acidic solution.

**Reagents**

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

EDTA-Na2, 0.07 mmol/L
NaCl, 6.6 g/mL
Sulfuric 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® Multicrater, Cat. No. 0550-605

**Specimen Collection and Preparation**

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 photosensitive.

**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 for 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 al.

**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).

<table>
<thead>
<tr>
<th></th>
<th>RB</th>
<th>K</th>
<th>S</th>
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<tbody>
<tr>
<td>Bilirubin Buffer (R1)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>Calibrator (K)</td>
<td>-</td>
<td>0.100</td>
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</tr>
<tr>
<td>Sample</td>
<td>-</td>
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<td>Water</td>
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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**

\[
\Delta A = [(A2 - A1) sample or calibrator] - [(A2 - A1) blank]
\]

Values are derived from the following calculation:

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

**Quality Control**

1) Stanbio Laboratories 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, 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. All controls should be run when a new lot number of reagent is loaded or recalibration of the test is performed.

**Expected Values**

Adult/child: 0.1 - 0.3 mg/dL

**Performance Characteristics**

- Date obtained using the EPOS 5060 analyzer

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<th>Precision:</th>
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<tbody>
<tr>
<td>intra-assay Precision n = 20</td>
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<tr>
<td>Sample Number</td>
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<tr>
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**Correlation of Serum vs. Plasma:**

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

**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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http://www.stanbio.com

For interlaboratory testing contact Stanbio Laboratory Technical Service Department (800-531-5553).