**Intended Use**
System reagent for the quantitative determination of Direct Bilirubin in human serum and plasma on Beckman Coulter AU analyzers.

*Direct Bilirubin reagent OSR6511 for use on AU2700/5400/680 system only.

**Summary**
Bilirubin is an end product of hemoglobin catabolism. It is conjugated with glucuronic acid in the liver, and the conjugated form is cleared from the circulation by excretion in the bile. Both conjugated (direct) and unconjugated (indirect) forms of bilirubin circulate loosely bound to albumin.

The assessment of direct bilirubin is helpful in the determination of hepatic disorders. The increase in the total bilirubin associated with obstructive jaundice is primarily due to the direct fraction. Both direct and indirect bilirubin are increased in the serum with hepatitis. In the newborn patient with hemolytic jaundice and neonatal jaundice, the increase in the total bilirubin is primarily due to the indirect bilirubin fraction. This jaundice may be caused by Rh, ABO, or other blood group incompatibilities, by hepatic immaturity, or by hereditary defects in bilirubin conjugation.

**Methodology**
This Direct Bilirubin Reagent utilizes a variation of the classical method developed by Van den Bergh and Mueller. Direct (conjugated) bilirubin couples directly with a diazonium salt of 3,5-dichloroaniline (DPD) in an acid medium to form azobilirubin.

The direct bilirubin in serum is directly proportional to the color development of azobilirubin which is measured bichromatically at 570/660 nm.

**System Information**
For AU400/400e/480, AU600/640/640e/680 and AU2700/5400/AU5800 Beckman Coulter Analyzers.

**Reagents**
Final concentration of reactive ingredients:
- 3,5-Dichlorophenyldiazonium tetrafluoroborate 0.09 mmol/L
- Hydrochloric acid 84 mmol/L
- Sulfuric acid 37 %

**Precautions**
1. For in vitro diagnostic use.
2. WARNING! CORROSIVE! Causes severe burns. Contains Sulfuric acid and Hydrochloric acid. Do not pipet by mouth. Avoid contact with eyes, skin or clothing. In case of contact, immediately flush affected areas with plenty of water for 15 minutes. Obtain medical attention immediately for eye contact or ingestion.

**Preparation of Reagents**
The Direct Bilirubin Reagents are ready for use. No preparation is required.

**Storage and Stability**
1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 - 8°C.
2. Opened reagents are stable for 21 days when stored in the refrigerated compartment of the analyzer.
3. Protect from light.

**Indications of Deterioration**
Visible signs of microbial growth, gross turbidity, precipitate, or change in color in the Direct Bilirubin reagent may indicate degradation and warrant discontinuance of use.

**Specimen Collection and Preparation**
Serum or heparinized plasma samples, free from hemolysis, are the recommended specimens. Protect specimen from light and assay as soon as possible.

**Sample Storage and Stability**
Exposure to direct sunlight can decrease bilirubin in samples by 50% within one hour. When well protected from light, bilirubin in serum is stable for up to 3 days at 2 - 8°C and for approximately three months when stored at ≤-20°C.

**Interfering Substances**
Results of studies show that the following substances interfere with this direct bilirubin assay.

The criteria for no significant interference is recovery within 10% of the initial value.

**Hemolysis:**
No significant Interference up to 10 mg/dL Hemolysate

**Lipemia:**
No significant Interference up to 300 mg/dL Intralipid*

* Intralipid, manufactured by KabiVitrum Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young for a compilation of reported interferences with this test.
Direct Bilirubin

In very rare cases gammopathy, especially monoclonal IgM (Waldeström’s macroglobulinemia), may cause unreliable results.

Procedure

A complete list of test parameters and operational procedure can be found in the User’s Guide appropriate to the analyzer.

Materials Provided

Direct Bilirubin Reagent

Materials Required But Not Provided

Chemistry Calibrator (Cat. No. DR0070)

Stability of Final Reaction Mixture

The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration

The frequency of calibration is every 7 days. Calibration of this direct bilirubin procedure is accomplished by use of the Chemistry Calibrator (Cat # DR0070), which is traceable to the National Institutes of Standards and Technology (NIST) Standard Reference Material (SRM) 916a.

Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Quality Control

During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration; with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User’s Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory’s standard procedure.

Results

Automatically printed out for each sample in mg/dL at 37°C. For SI units (µmol/L) the result must be multiplied by 17.1.

Dynamic Range

The Direct Bilirubin procedure is linear from 0 up to 10 mg/dL. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Expected Values

Adult: 0.03 - 0.18 mg/dL

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the Direct Bilirubin Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision

Estimates of precision, based on CLSI recommendations, are consistent with typical performance. The within run precision is ≤5% CV or SD ≤ 0.15 mg/dL and total precision is ≤7.6% CV or SD ≤ 0.23 mg/dL. Assays of control sera and plasma were performed and this data reduced following CLSI guidelines above.

The following data was obtained on an AU2700 using 3 plasma pools analysed over 20 days.

<table>
<thead>
<tr>
<th>N = 80</th>
<th>Within run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, mg/dL</td>
<td>SD</td>
<td>CV%</td>
</tr>
<tr>
<td>1.2</td>
<td>0.02</td>
<td>1.80</td>
</tr>
<tr>
<td>1.6</td>
<td>0.06</td>
<td>3.54</td>
</tr>
<tr>
<td>6.6</td>
<td>0.24</td>
<td>3.71</td>
</tr>
</tbody>
</table>

Method Comparison

Patient samples were used to compare this Direct Bilirubin Reagent. The table below demonstrates representative performance on the AU analyzers.

<table>
<thead>
<tr>
<th>Y Method</th>
<th>X Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>AU640</td>
<td>AU600</td>
</tr>
<tr>
<td>Slope</td>
<td>0.9996</td>
</tr>
<tr>
<td>Intercept</td>
<td>- 0.006</td>
</tr>
<tr>
<td>Correlation Coeff. (r)</td>
<td>0.9996</td>
</tr>
<tr>
<td>No. of Samples (n)</td>
<td>180</td>
</tr>
<tr>
<td>Range (mg/dL)</td>
<td>0.01-6.03</td>
</tr>
</tbody>
</table>

Sensitivity

Typical change in absorbance per minute for 1 mg/dL of Direct Bilirubin is 23 mAbsorbance.

References

5. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
7. Data is on file for specific AU analyzers.

Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA