DIRECT BILIRUBIN

OSR61181	**4 x 45 mL**	**R1**
	4 x 9 mL	**R2**

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

Summary
The determination of direct bilirubin is helpful in the differentiation of hepatic disorders. The increase in total bilirubin associated with obstructive jaundice is primarily due to the direct (conjugated) fraction. Both direct and indirect bilirubin are increased in the serum with hepatitis[1].

Methodology
This Direct Bilirubin Reagent utilizes a variation of the classical method developed by Van den Bergh and Mueller[2]. 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 540/600 nm.

Bilirubin + 3,5-Dichlorophenyl Diazonium $(BF)_4$ ⟶ Azobilirubin

System Information
For AU400/400^e, AU600/640/640^e and AU2700/5400 Beckman Coulter Analyzers.

Reagents
Final concentration of reactive ingredients:

3,5-Dichlorophenyldiazonium tetrafluoroborate	0.08	mmol/L
Hydrochloric acid	100	mmol/L

Precautions
1. For *in vitro* diagnostic use.
2. Do not ingest, harmful if swallowed.

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 30 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 free from hemolysis is the recommended specimen. 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, direct bilirubin in serum is stable for up to 3 days at 2 – 8°C and for approximately three months when stored frozen at ≤ -70°C in the dark.[1]

Interfering Substances
Results of studies[3] show that the following substances interfere with this direct bilirubin assay.

The criteria for no significant interference is recovery within 12% of the initial value

Hemolysis:	Interference less than 12% up to 25 mg/dL hemoglobin or concentration change ≤ 0.1 mg/dL for samples containing < 0.8 mg/dL Direct Bilirubin
Lipemia:	Interference less than 12% up to 500 mg/dL Intralipid* or concentration change ≤ 0.1 mg/dL for samples containing < 0.8 mg/dL Direct Bilirubin

* Intralipid is a 20% IV fat emulsion used to emulate extremely turbid samples. Approximate triglyceride concentration is 1500 mg/dL.

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[4] for a compilation of reported interferences with this test.
In very rare cases gammopathy, especially monoclonal IgM (Waldeström's macroglobulinemia), may cause unreliable results. Interference from commonly used drugs was not evaluated with this assay.

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

Direct Bilirubin

Materials Provided
Direct Bilirubin Reagent
Materials Required But Not Provided
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 21 days. Calibration of this direct bilirubin procedure is accomplished by use of the Chemistry Calibrator (Cat # DR0070), which is traceable to a Master Calibrator.

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 0.05 up to 10 mg/dL. The limit of quantitation was determined to be less than 0.05 mg/dL using CLSI protocol EP17-A[5]. Linearity was tested over a range of 0.02 to 10 mg/dL with recovery within 10% or 0.1 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:[1] 0.0 – 0.2 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[7]
Estimates of precision, based on CLSI recommendations,[6] are consistent with typical performance. The within run precision is less than 7% CV or SD ≤ 0.07 and total precision is less than 8% CV or SD ≤ 0.21. Assays of control sera were performed and this data reduced following CLSI guidelines above.

N = 80	Repeatability (Within Run)		Within Laboratory (Total)	
Mean, mg/dL	SD	CV%	SD	CV%
0.14	0.00	3.18	0.01	5.29
2.17	0.01	0.58	0.07	3.38
5.41	0.04	0.67	0.19	3.46

Method Comparison[7]
Patient samples were used to compare this Direct Bilirubin Reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	AU640
X Method	Method 2
Slope	1.097
Intercept	-0.028
Correlation Coeff. (r)	0.9971
No. of Samples (n)	50
Range (mg/dL)	0.06 – 8.00

Sensitivity
Typical change in absorbance per minute for 1 mg/dL of Direct Bilirubin is 22.7 mAbsorbance.

References

1. Burtis, C.A. and Ashwood, E.R. (eds), Tietz Textbook of Clinical Chemistry, 3rd Edition, W.B. Saunders, 1999.
2. Van den Bergh, A. and Mueller, P., Biochem Z, 77: 90, 1916.
3. CLSI/NCCLS, Interference Testing in Clinical Chemistry, EP7-A2, 2005.
4. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
5. CLSI/NCCLS, Protocols for Determination of Limits of Detection and Limits of Quantitation; EP17-A, 2004.
6. CLSI/NCCLS, Evaluation of Precision Performance of Quantitative Measurement Methods, EP5-A2, 2004.
7. Data is on file for specific AU analyzers.

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