TOTAL BILIRUBIN

OSR6112	**4 x 15 mL**	**R1 Color Reagent (TBILC)**
	4 x 15 mL	**R1 Blank Reagent (TBILB)**
OSR6212	**4 x 40 mL**	**R1 Color Reagent (TBILC)**
	4 x 40 mL	**R1 Blank Reagent (TBILB)**
OSR6512*	**4 x 107 mL**	**R1 Color Reagent (TBILC)**
	4 x 107 mL	**R1 Blank Reagent (TBILB)**

Intended Use
System reagent for the quantitative determination of Total Bilirubin in human serum and plasma on Beckman Coulter AU analyzers.
*Total Bilirubin reagent OSR6512 for use on the AU2700/5400/680 system only.

Summary
Measurement 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, hematologic, and metabolic disorders, including hepatitis and gall bladder block.

Methodology
Total bilirubin in serum is composed of direct (conjugated) bilirubin and indirect (unconjugated) bilirubin. Since the introduction of the diazo method for bilirubin determination by Ehrlich in 1883,[1] several modifications have been proposed to enhance the reaction. The primary reason for these modifications is that while direct bilirubin couples directly with the diazotized compound to yield a colored product, the indirect portion of bilirubin requires a solubilizing agent, such as a surfactant.

This Total Bilirubin Reagent is a variation of the classical method. A stabilized diazonium salt, 3,5-dichlorophenyldiazonium tetrafluoroborate (DPD), reacts with bilirubin to form azobilirubin which absorbs at 570/660 nm. Caffeine and a surfactant are used as reaction accelerators.

The absorbance at 570/660 nm is proportional to the bilirubin concentration in the sample. A separate serum blank is performed to eliminate endogenous serum[2] interferences.

$$\text{Bilirubin} + \text{DPD} \xrightarrow[\text{surfactant}]{\text{caffeine}} \text{Azobilirubin}$$

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

Reagents
Final concentration of reactive ingredients:

Caffeine	2.1	mmol/L
3,5-Dichlorophenyldiazonium Tetrafluoroborate	0.31	mmol/L

Also contains preservative and surfactant.

Precautions
1. For *in vitro* diagnostic use.
2. Do not ingest. Toxicity has not been established.
3. Do not pipette by mouth. Avoid contact with skin, eyes 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
For OSR6112, OSR6212 and OSR6512, the Total 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 90 days when stored in the refrigerated compartment of the analyzers.
3. Protect from Light.

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

Specimen Collection and Preparation
Serum, heparinized or EDTA plasma samples, are the recommended specimens. Samples should be protected from light.

Sample Storage and Stability
It has been reported that 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 3 days when stored at 2 - 8°C, or three months when stored at ≤ -20°C.[2]

Interfering Substances
Results of studies[3] show that the following substances interfere with this Total Bilirubin procedure.

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

Hemolysis: No significant interference up to 500 mg/dL Hemolysate
Lipemia: No significant Interference up to 500 mg/dL Intralipid*

* Intralipid, manufactured by KabiVitrium 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[4] for a compilation of reported interferences with this test.

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

Total Bilirubin

Procedure

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

Materials Provided

Total 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 30 days. Calibration of this Total Bilirubin procedure is accomplished by use of the Chemistry Calibrator (Cat # DR0070), which is traceable to the National Institute 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 Total Bilirubin procedure is linear from 0 up to 30 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[5]

Adults[5]: 0.3 - 1.0 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 Total 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 3% CV or SD ≤ 0.07 and total precision is less than 5% CV or SD ≤ 0.10. Assays of control sera were carried out and data reduced following CLSI guidelines above:

N = 60	Within run		Total	
Mean, mg/dL	SD	CV%	SD	CV%
0.85	0.01	1.24	0.02	2.65
2.11	0.01	0.61	0.04	2.01
4.78	0.03	0.53	0.16	3.31
9.82	0.03	0.33	0.21	2.13

Method Comparison[7]

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

Y Method	AU640
X Method	Method 2
Slope	1.046
Intercept	- 0.065
Correlation Coeff. (r)	1.000
No. of Samples (n)	100
Range (mg/dL)	0.08 – 24.90

Sensitivity

Typical change in absorbance for 1 mg/dL of total bilirubin is 88 mAbsorbance.

References

1. Ehrlich, P., Charite-Annalen, 8: 140, 1883.
2. Tietz, N.W.(ed), Fundamentals of Clinical Chemistry, 3rd Edition, W.B., Saunders, 1987.
3. CLSI/NCCLS, Interference Testing in Clinical Chemistry, EP7-P, 1986.
4. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
5. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
6. CLSI/NCCLS Evaluation of Precision Performance EP5-A Vol.19 No. 2 1999.
7. Data is on file for specific AU analyzers.

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