AU

Instructions For Use

DBili
Direct Bilirubin

REF OSR6111 4 x 6 mL R1 (DBILC), 4 x 6 mL R1 (DBILB)
OSR6211 4 x 20 mL R1 (DBILC), 4 x 20 mL R1 (DBILB)
OSR6611 4 x 173 mL R1 (DBILC), 4 x 173 mL R1 (DBILB)

For *in vitro* diagnostic use only.

PRINCIPLE

INTENDED USE

Photometric colour test for the quantitative determination of direct bilirubin in human serum and plasma on Beckman Coulter analysers.

OSR6611 for use on the AU5800, AU2700 and AU5400 systems only.

SUMMARY AND EXPLANATION

Reference[1,2]

80 – 85% of bilirubin produced daily originates from haemoglobin released by the breakdown of senescent erythrocytes, the remaining 15 – 20% results from the breakdown of haem-containing proteins such as myoglobin, cytochromes, catalases and from bone marrow as a result of ineffective erythropoiesis.

Because of its poor solubility in water unconjugated bilirubin (indirect bilirubin) is transferred to the liver bound to albumin. Inside the hepatocytes it is rapidly conjugated with glucuronic acid to produce bilirubin mono- and diglucuronide (direct bilirubin) which are then excreted in bile together with all other normal biliary constituents.

Whereas prehepatic jaundice (e.g. haemolytic anemia and neonatal jaundice) is primarily associated with an increase in unconjugated bilirubin, the assessment of direct bilirubin is helpful in the determination of hepatic and post-hepatic jaundice. Diseases of hepatic origin with predominantly conjugated hyperbilirubinemia include acute and chronic viral hepatitis, liver cirrhosis and hepatocellular carcinoma. Diseases of post hepatic origin with predominantly conjugated hyperbilirubinemia include extrahepatic cholestasis and liver transplant rejection. Chronic congenital conjugated hyperbilirubinemias include Dubin-Johnson and Rotor syndrome. The differentiation between chronic congenital hyperbilirubinemias and acquired types of bilirubininemia is accomplished via the measurement of bilirubin fractions and the detection of normal liver enzyme activities.

METHODOLOGY

Reference[3]

A stabilised diazonium salt, 3,5 Dichlorophenyl diazonium tetrafluoroborate (DPD) couples directly with direct (conjugated) bilirubin in an acid medium to form azobilirubin. The absorbance at 570 nm is proportional to the direct bilirubin concentration in the sample.

CHEMICAL REACTION SCHEME

Bilirubin + DPD → Azobilirubin

SPECIMEN

TYPE OF SPECIMEN

Serum and heparinised plasma: stable for 3 days when protected from light and stored 15…25°C.[1]

Even slight haemolysis can cause a reduction in value and such samples should be avoided.

Lipemic samples should also be avoided.

REAGENTS

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.

Dispose of all waste material in accordance with local guidelines.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

3,5 Dichlorophenyl diazonium tetrafluoroborate	0.08 mmol/L

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

GHS HAZARD CLASSIFICATION

Direct Bilirubin Blank R1	DANGER	
		
	H314	Causes severe skin burns and eye damage.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
	P303+P361+P353	IF ON SKIN (or hair): Rinse skin with water.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310	Immediately call a POISON CENTER or doctor/physician.
		Hydrochloric Acid 2 - 5%
		Sulfuric Acid 20 - 50%
		Sulfosalicylic Acid, Dihydrate 0.5 - 1%
Direct Bilirubin Color R1	DANGER	
		
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		Sulfosalicylic Acid, Dihydrate 0.5 - 1%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

REAGENT PREPARATION

The reagents are ready for use and can be placed directly on board the instrument.

STORAGE AND STABILITY

The reagents are stable, protected from light, unopened, up to the stated expiry date when stored at 2…8°C. Once open, reagents stored on board the instrument are stable for 21 days.

CALIBRATION

CALIBRATOR REQUIRED

System Calibrator Cat. No. 66300.

The calibrator value is traceable to a Beckman Coulter Master Calibrator.

Recalibrate the assay every 21 days, or when the following occur:

Change in reagent lot or significant shift in control values;

Major preventative maintenance was performed on the analyser or a critical part was replaced.

QUALITY CONTROL

Controls Cat. No. ODC0003 and ODC0004 or other control materials with values determined by this Beckman Coulter system may be used.

Each laboratory should establish its own control frequency however good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration is performed.

The results obtained by any individual laboratory may vary from the given mean value. It is therefore recommended that each laboratory generates analyte specific control target values and intervals based on multiple runs according to their requirements. These target values should fall within the corresponding acceptable ranges given in the relevant product literature.

If any trends or sudden shifts in values are detected, review all operating parameters.

Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement. The paediatric application is suitable for use with small volume serum/plasma samples.

CALCULATIONS

The Beckman Coulter analysers automatically compute the direct bilirubin concentration of each sample.

REPORTING RESULTS

REFERENCE INTERVALS

Reference[1]	
Adults and Children	< 3.4 µmol/L (< 0.2 mg/dL)

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

PROCEDURAL NOTES

INTERFERENCES

Results of studies conducted to evaluate the susceptibility of the method to interference were as follows:

Lipemia: Interference less than 10% up to 300 mg/dL Intralipid

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

N-acetyl-p-benzoquinone imine (metabolite of Paracetamol) will generate erroneously low results in samples for patients that have taken an overdose of Paracetamol.

Refer to Young[4] for further information on interfering substances.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

LINEARITY

The test is linear within a concentration range of 0 – 171 µmol/L (0 – 10 mg/dL).

SENSITIVITY

The lowest detectable level on a DxC 700 AU analyser was estimated at 0.28 µmol/L.

The lowest detectable level represents the lowest measurable level of direct bilirubin that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

METHODS COMPARISON

Patient serum samples were used to compare this Direct Bilirubin (OSR6111) assay on the AU640 against another commercially available direct bilirubin assay. Results of linear regression analysis were as follows:

y = 0.789x – 0.7	r = 0.998	n = 99	Sample range = 0.2 – 151.5 µmol/L

PRECISION

Estimates of precision, based on CLSI recommendations[5], are consistent with typical performance. The within run precision is ≤5% CV or SD ≤ 2.6 µmol/L and total precision is ≤7.5% CV or SD ≤ 3.9 µmol/L. 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.

n = 80	Within-run		Total	
Mean µmol/L	**SD**	**CV%**	**SD**	**CV%**
13.9	0.32	2.30	0.46	3.34
22.6	1.02	4.53	1.15	5.08
106.6	4.07	3.82	4.55	4.27

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	Description
DBC1N	Direct Bilirubin Colour (Serum)
DBB1N	Direct Bilirubin Blank (Serum)
DBC1NP	Direct Bilirubin Colour (Serum Paediatric)
DBB1NP	Direct Bilirubin Blank (Serum Paediatric)

Setting Sheet Footnotes

‡ The above parameters must be entered twice using test names DBILC (Colour Rg) and DBILB (Blank Rg). Set the test as SAMPLE BLANK in the INTER RELATED TEST menu

User defined
† System Calibrator Cat. No.: 66300
* Values set for working in SI units (µmol/L). To work in mg/dL divide by 17.1

§ Set the factor range for the blank reagent at –99999 to 99999

ж Set the test as SAMPLE BLANK in the COMMON TEST PARAMETERS TEST NAME SAMPLE BLANK menu.

REVISION HISTORY

Added new languages

Preceding version revision history

Updated REAGENTS section

Revised Interferences section.

REFERENCES

1. Thomas L. Bilirubin. In: Thomas L, ed. Clinical laboratory diagnostics. Use and assessment of clinical laboratory results. Frankfurt/Main: TH-Books Verlagsgesellschaft, 1998:192-202.

2. Balistreri WF, Shaw LM. Liver Function. In: Tietz NW, ed. Fundamentals of clinical chemistry. Philadelphia:WB Saunders Company, 1987:733-737.

3. Hymans van den Bergh AA, Mueller P. Ueber eine direkte and indirekte diazoreaktion auf bilirubin. Biochem Z 1916, 77:90.

4. Young DS. Effects of Drugs on Clinical Laboratory Tests, AACC, 5th ed. AACC Press, 2000.

5. CLSI/NCCLS, Evaluation Protocol EP5-T2, 1992.

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