DXC (DBIL) DIRECT BILIRUBIN

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PURPOSE

To provide instructions for the quantitative determination of direct bilirubin on the DXC 600/800.

PRINCIPLE

DBIL reagent, when used in conjunction with UniCel® DxC 600/800 System(s) and SYNCHRON® Systems Bilirubin Calibrator, is intended for quantitative determination of direct (conjugated) bilirubin concentration in human serum or plasma.

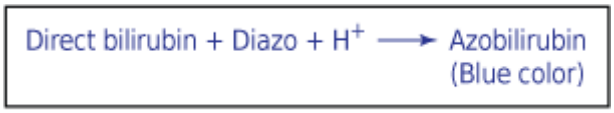
BACKGROUND

Clinical Significance

Direct bilirubin measurements are used in the diagnosis and treatment of liver, hemolytic, hematological, and metabolic disorders, including hepatitis and gall bladder block.

Methodology

DBIL reagent is used to measure DBIL concentration by a timed endpoint diazo method. In the reaction, DBIL combines with diazo to form azobilirubin.
The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used is one part sample to 32 parts reagent. The system monitors the change in absorbance at 560 nanometers. This change in absorbance is directly proportional to the concentration of DBIL in the sample and is used by the System to calculate and express the DBIL concentration.



RELATED DOCUMENTS

R-PO-CH0810	Quality Control Program General Laboratory
R-PO-CH0809	Quality Control Westgard Rules Statistics
R-PR-AD0540	Specimen Rejection/Cancellation Protocol
J-F-CH0820	DXC 800 Controls
M-F-CH0820	Chemistry Controls
J-F-CH0826	DXC 800 Calibrators
M-F-CH0826	Chemistry Calibrators
M-F-CH1940	DXC 600 (AMR) Analytical Measurement Range
J-F-CH1940	DXC 800 (AMR) Analytical Measurement Range

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum or plasma is the preferred specimen. Acceptable anticoagulants are listed in the PROCEDURAL NOTES section of this chemistry information sheet. Whole blood or urine are not recommended for use as a sample.

Specimen Storage and Stability

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

3. Bilirubin is photosensitive. Protect samples from light.

Sample Type	Volume	Sample Stability
Plasma/Serum	0.5mL	• Separate serum from cells within 2 hours • Room Temp 8 hours • Refrigerated 48 hours • After 48 hours freeze at -15 to -20°C • Protect from light

Critieria for Unacceptable Specimens

See Specimen Rejection/Cancellation Protocol

Sample Volume

A filled 0.5 mL sample cup is the optimum volume. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the Primary Tube Sample Template for your system.

REAGENTS

Contents

Each kit contains the following items:
Two Direct Bilirubin Reagent Cartridges (2 x 200 tests) Kit reorder #439715

Volume per Test	
Sample Volume	10 µL
Total Reagent Volume	320 µL
Cartridge Volumes	A 310 µL B 10 µL C – –

Reactive Ingredients	
Sulfanilic acid	27 mmol/L
HCl	51 mmol/L
Sodium nitrite	0.12 mmol/L

Also non-reactive chemicals necessary for optimal system performance.

Reagent Preparation

No preparation is required.

Acceptable Reagent Performance

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within acceptance criteria.

Reagent Storage and Stability

DBIL reagent, when stored unopened at room temperature, will obtain the shelf-life indicated on the cartridge label. Once opened, the reagent is stable for 30 days at +2°C to +8°C. Do not use beyond manufacturer's expiration date. DO NOT FREEZE.

CALIBRATION

Calibrator Required

SYNCHRON Systems Bilirubin Calibrator: The SYNCHRON Systems Bilirubin calibrator matrix is derived from stabilized human defibrinated serum. The assigned value of this calibrator can be traced directly to NIST Standard 916.
Deionized water (low level calibrator)

Calibrator Preparation

No preparation is required.

Calibrator Storage and Stability

SYNCHRON® Systems Bilirubin Calibrator is stable until the expiration date printed on the calibrator ampule when stored unopened at -15°C to -20°C. Opened calibrators that are resealed are stable for 24 hours at +2°C to +8°C. Do not use beyond the manufacturer's expiration date.

Calibrator Information

1. The system must have a valid calibration curve in memory before control or patient samples can be run.

2. Under typical operating conditions the DBIL reagent cartridge must be calibrated every 14 days and also with certain parts replacements or maintenance procedures, as defined in the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual. This assay has within-lot calibration available. Refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual for information on this feature.

3. For detailed calibration instructions, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

Traceability

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

See Related Documents J-F-CH0820 DXC 800 Controls & M-F-CH0820 Chemistry Controls

STEPS

1. If necessary, load the reagent onto the system.

2. After reagent load is completed, calibration may be required.

3. Program controls for analysis.

4. After loading controls onto the system, follow the protocols for system operation. To load samples manually refer to the FHS DXC Series Manual Sample Programming procedure. .For detailed testing procedures, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

CALCULATIONS

SYNCHRON® System(s) perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

ANTICOAGULANT TEST RESULTS

If plasma is the sample of choice, the following anticoagulants were found to be compatible with this method based on a study of 20 healthy volunteers:

Anticoagulant	Level Tested for In Vitro Interference	Average Plasma-Serum Bias (mg/dL)
Ammonium Heparin	29 Units/mL	NSI within ± 0.30 mg/dL or 10%
Lithium Heparin	29 Units/mL	NSI within ± 0.30 mg/dL or 10%
Sodium Heparin	29 Units/mL	NSI within ± 0.30 mg/dL or 10%
Sodium Citrate	6.6 mg/mL	NSI within ± 0.30 mg/dL or 10%
Potassium Oxalate/ Sodium Fluoride	4.0 / 5.0 mg/mL	NSI within ± 0.30 mg/dL or 10%

Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.

PERFORMANCE CHARACTERISTICS

Reference Range

Age	Range
0-2 Weeks	0.0-2.0 mg/dL
2-weeks-150 Years	0.0-0.4 mg/dL

Analytic Range

The SYNCHRON® System(s) method for the determination of this analyte provides the following analytical ranges:

Sample Type	Conventional Units
Serum or Plasma	0.1 – 10.0 mg/dL

The low end of the analytical range represents the minimum level of detection. Samples with concentrations exceeding the high end of the analytical range should be diluted with human serum albumin (azide free) and reanalyzed.

Reporting results outside of analytical range

Lower limit of detection	0.1 mg/dL	Results below 0.1; Report as <0.1mg/dL
Upper limit of detection	10.0 mg/dL	Results >10 should be diluted with human serum albumin (azide free), reanalyzed and dilution factor applies. The maximum allowable dilution is X2. Results >10.0 are reported as >20 mg/dL.

Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for DBIL determination is 0.1 mg/dL.

LIMITATIONS

1. The SYNCHRON Systems Direct Bilirubin method is an improved method which shows enhanced specificity for some conjugated fractions. However, no diazo direct bilirubin is able to estimate completely all possible conjugated bilirubin fractions.

2. Because the source of bilirubin in the SYNCHRON Systems Bilirubin Calibrator is not of human origin, the setpoint is value assigned to obtain good agreement on human samples with referee methods. This is implemented by including a factor in the database which is applied to the reported result. Therefore, the calibrator will not recover its setpoint value when run as sample.

3. Beckman Coulter has not validated this method for neonatal direct bilirubin values. Each laboratory should establish a reference range for neonatal values.

Interferences

1. The following substances were tested for interference with this methodology:

Substance	Source	Level Tested	Observed Effect
Bilirubin	Bovine (unconjugated)	30 mg/dL INDEX of 20	No Significant Interference within ± 0.30 mg/dL or 10%

Substance	Source	Level Tested	Observed Effect
Lipemia	Intralipid[g]	500 mg/dL INDEX of 10	-0.25 @ 0.5 mg/dL
Ascorbic Acid	NA[h]	3.0 mg/dL	No Significant Interference within ± 0.30 mg/dL or 10%
Sodium Azide	NA	5.0 mg/dL	No Significant Interference within ± 0.30 mg/dL or 10%
Hemoglobin	RBC hemolysate	400 mg/dL	≤ +0.3 @ 0.5 mg/dL
		200 mg/dL INDEX of 4	≤ -0.3 @ 3.1 mg/dL
		200 mg/dL	≤ -0.9 @ 8.3 mg/dL

Plus (+) or minus (-) signs signify positive or negative interference.

2. Interference from hemoglobin is a combination of chemical and spectral interference. For elevated direct bilirubin values, hemoglobin interference will change from a positive to a negative bias.

3. Refer to References (11,12,13) for other interferences caused by drugs, disease and preanalytical variables.

ADDITIONAL INFORMATION

For more detailed information on UniCel DxC Systems, refer to the appropriate system manual.

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DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
New format, added index level to interfering substances, and maximum allowable dilution			
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