DXC (TBIL) TOTAL BILIRUBIN

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PURPOSE

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

PRINCIPLE

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

BACKGROUND

Clinical Significance

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

Methodology

TBIL reagent is used to measure the total bilirubin concentration by a timed endpoint Diazo method. In the reaction, the bilirubin reacts with diazo reagent in the presence of caffeine, benzoate, and acetate as accelerators to form azobilirubin.
The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 35 parts reagent. The system monitors the change in absorbance at 520 nanometers. This change in absorbance is directly proportional to the concentration of TBIL in the sample and is used by the System to calculate and express TBIL 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 and 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	• 8 hours at 18-26°C • 48 hours at 2-8°C • After 48 hours, freeze at -15 to -20°C

Criteria 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 Total Bilirubin Reagent Cartridges (2 x 300 tests), 4427454 or (2 x 400 tests), kit # 476861
One Preparation Insert

Volume per Test	
Sample Volume	8 µL
Total Reagent Volume	280 µL
Cartridge Volumes	A 255 µL B 25 µL C – –

Reactive Ingredients	
Sodium Benzoate	347 mmol/L
Caffeine	173.9 mmol/L
Sulfanilic acid	27 mmol/L
HCl	50 mmol/L
Sodium Nitrite	0.36 mmol/L
Sodium Acetate	609 mmol/L

Also non-reactive chemicals necessary for optimal system performance.

Reagent Preparation

For P/N 442745 (300 tests): Quantitatively transfer 100 microliters (0.1 mL) of the contents from the smallest compartment (C) into the center compartment (B).

For P/N 476861 (400 tests): Quantitatively transfer 200 µL (0.2 mL) of the contents from the smallest compartment (C) into the center compartment (B).

Replace the cartridge caps and **gently** invert the cartridge several times to ensure adequate mixing. Thorough mixing is necessary for successful calibration.

Acceptable Reagent Performance

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

Reagent Storage and Stability

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

CALIBRATION

Calibrator Required

SYNCHRON® Systems Bilirubin Calibrator
Deionized water (low level calibrator)

Calibrator Preparation

No preparation is required.

Calibrator Storage and Stability

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

Calibrator Information

NOTE: Since Total Bilirubin is a calibrated chemistry and also requires "quantitative" reagent preparation it is important to follow proper reagent handling, preparation and storage procedures, especially when utilizing the within-lot calibration feature. Before reporting patient results on successive within-lot cartridges, always analyze and review calibration and quality control data.

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 TBIL 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

NOTE: When using the within-lot calibration feature it is highly recommended that recovery be confirmed on subsequent cartridge(s) from the same lot number by analyzing quality control material prior to analyzing or reporting any patient results.

1. If necessary prepare reagent as defined in the Reagent Preparation section of this chemistry information sheet and 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

1. 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)
Sodium Heparin	29 Units/mL	NSI[b]
Lithium Heparin	29 Units/mL	NSI
Ammonium Heparin	29 Units/mL	NSI

2. The following anticoagulants were found to be incompatible with this method:

Anticoagulant	Level Tested for In Vitro Interference	Average Plasma-Serum Bias (mg/dL)
Sodium Citrate	1.7 mg/mL	≤-0.8
Potassium Oxalate/ Sodium Fluoride	4.0 / 5.0 mg/mL	≤-0.4

PERFORMANCE CHARACTERISTICS

Reference Range

Age	Range	Critical High
0 – 23 hrs	0.0 – 4.0 mg/dL	7.1 mg/dL
23 – 28 hrs	0.0 – 5.0 mg/dL	8.1 mg/dL
28 – 32 hrs	0.0 – 5.6 mg/dL	9.1 mg/dL
32 – 36 hrs	0.0 – 6.3 mg/dL	10.1 mg/dL
36 – 40 hrs	0.0 – 7.0 mg/dL	11.1 mg/dL
40 – 47 hrs	0.0 – 7.8 mg/dL	12.1 mg/dL
47 – 54 hrs	0.0 – 8.5 mg/dL	13.1 mg/dL
54 – 60 hrs	0.0 – 9.0 mg/dL	14.1 mg/dL
60 – 71 hrs	0.0 – 9.5 mg/dL	15.1 mg/dL
71 – 90 hrs	0.0 – 11.2 mg/dL	16.1 mg/dL
90 – 108 hrs	0.0 – 12.0 mg/dL	17.1 mg/dL
108 – 119 hrs	0.0 – 13.0 mg/dL	17.1 mg/dL
119 – 132 hrs	0.0 – 13.2 mg/dL	17.1 mg/dL
132 – 143 hrs	0.0 – 13.2 mg/dL	17.1 mg/dL
143 hrs – 1 mon	0.0 – 11.6 mg/dL	12.1 mg/dL
1 mon – 18 yrs`	0.0 – 1.9 mg/dL	
18 yrs – 60 yrs	0.1 – 1.5 mg/dL	
60 yrs – 90 yrs	0.2 – 1.1 mg/dL	
>90 yrs	0.2 – 0.9 mg/dL	

For Critical Value reporting protocol, refer to FHS Critical Policy

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 – 30.0 mg/dL

Samples with concentrations outside the analytical range will be reported as "<0.1 mg/dL" ("<1.7 µmol/L"). Samples reported out as greater than the analytical range may be confirmed by diluting with human serum with a known bilirubin value and reanalyzing. The appropriate dilution factor should be applied to the reported result.

Reporting results outside of analytical range

Lower limit of detection	0.1 mg/dL	Result below 0.1, report as <0.1 mg/dL
Upper limit of detection	30.0 mg/dL	Results >30 should be diluted with azide free human serum albumin, reanalyzed and dilution factor applied. The maximum allowable dilution is x2. Results >60 are reported as >60 mg/dL.

Sensitivity

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

LIMITATIONS

None identified.

Interferences

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

Substance	Source	Maximum Level Tested	Observed Effect
Hemoglobin	RBC hemolysate	100 mg/dL INDEX of 3	≤+0.24 mg/dL
Lipemia	Intralipid[g]	200 mg/dL INDEX of 5 Airfuge recommended	≤-0.24 mg/dL
Azide	NA[h]	5 mg/dL	≤+0.24 mg/dL
Citrate	NA	900 mg/dL	≤±0.20 mg/dL
Oxalate	NA	1000 mg/dL	≤±0.20 mg/dL
Gentisic Acid	NA	5 mg/dL	≤+0.24 mg/dL
Acetoacetate	NA	0.2 mg/mL	≤+0.7 mg/dL
		1.08 mg/mL	≤+3.7 mg/dL

2. Lipemic samples >2+ should be ultra-centrifuged and the analysis performed on the infranate.

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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