BECKMAN COULTER

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SYNCHRON System(s)

Chemistry Information Sheet

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TBIL
Total Bilirubin

REF 442745 (300 tests/cartridge)
476861 (400 tests/cartridge)

For *In Vitro* Diagnostic Use

Rx Only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

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.

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.[1,2,3] 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.

CHEMICAL REACTION SCHEME



E015257L.EPS

SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.[4] Freshly drawn serum or plasma are the preferred specimens. 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.[5]
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.[5]
3. Bilirubin is photosensitive. Protect samples from light.

Additional specimen storage and stability conditions as designated by this laboratory:

SAMPLE VOLUME

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

Criteria for sample rejection as designated by this laboratory:

PATIENT PREPARATION

Special instructions for patient preparation as designated by this laboratory:

SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:

REAGENTS

CONTENTS

Each kit contains the following items:

Two Total Bilirubin Reagent Cartridges (2 x 300 tests) or (2 x 400 tests)

VOLUMES PER TEST

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

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS	
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.

Avoid skin contact with reagent. Use water to wash reagent from skin.

GHS HAZARD CLASSIFICATION

Total Bilirubin Reagent (Compartment A)	WARNING	
		
	H319	Causes serious eye irritation.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P337+P313	If eye irritation persists: Get medical advice/attention.
		Sodium Acetate 1 - 10%
		Sodium Benzoate 1 - 10%
Total Bilirubin Reagent (Compartment B)	DANGER	
		
		
	H314	Causes severe skin burns and eye damage.
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	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.
	P362+P364	Take off contaminated clothing and wash it before use.
		Hydrochloric Acid 0.1 - 1%
		Sulphanilic Acid 0.1 - 1%
Total Bilirubin Reagent (Compartment C)	WARNING	
		
	H302	Harmful if swallowed.
	H401	Toxic to aquatic life
	P264	Wash hands thoroughly after handling.
	P273	Avoid release to the environment.

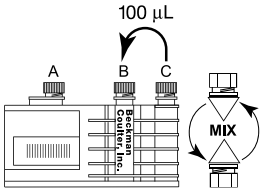
P301+P312	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. Sodium Nitrite 1 - 10%

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

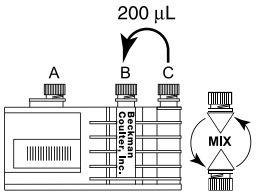
MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

SYNCHRON Systems Bilirubin Calibrator
Deionized water (low level calibrator)
At least two levels of control material
Human serum albumin (azide free)

REAGENT PREPARATION



For P/N 442745 (2 x 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 (2 x 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 unless the expiration date is exceeded. DO NOT FREEZE.

Reagent storage location:

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 unless the expiration date is exceeded.

⚠ CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.[6]

Calibrator storage location:

CALIBRATION INFORMATION

NOTICE

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

At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

Table 1.0 Quality Control Material

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

NOTICE

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 samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operations.

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

CALCULATIONS

The SYNCHRON System(s) performs 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.

REPORTING RESULTS

Equivalency between the SYNCHRON LX and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

REFERENCE INTERVALS

Each laboratory should establish its own reference intervals based upon its patient population. The reference intervals listed below were taken from literature.[7]

Table 2.0 Reference intervals

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Literature	Serum or Plasma	0.3 – 1.2 mg/dL	5.1 – 20.5 µmol/L

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Laboratory			

Refer to References (8,9,10) for guidelines on establishing laboratory-specific reference intervals.

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

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:

Table 3.0 Acceptable Anticoagulants[a]

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

a Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.
b NSI = No Significant Interference (within ±0.3 mg/dL or 6%).

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

Table 4.0 Incompatible Anticoagulants[a]

ANTICOAGULANT	LEVEL TESTED FOR IN VITRO INTERFERENCE	PLASMA-SERUM BIAS (mg/dL)[b]
Sodium Citrate	1.7 mg/mL	≤-0.8
Potassium Oxalate/Sodium Fluoride	4.0 / 5.0 mg/mL	≤-0.4

a Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.
b Bias is based on worst case instead of average. Plus (+) or minus (-) signs in this column signify positive or negative bias.

LIMITATIONS

Sample discoloration can interfere with photometric tests. The users of these test systems should evaluate the sample quality and identify potentially interfering substances in these samples. This evaluation is normally done by visual assessment.[11]

INTERFERENCES

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

Table 5.0 Interferences

SUBSTANCE	SOURCE	MAXIMUM LEVEL TESTED	OBSERVED EFFECT[a]
Hemoglobin	RBC hemolysate[b]	100 mg/dL	≤ +0.63 mg/dL
Lipemia	Intralipid[c]	200 mg/dL	≤-0.24 mg/dL
Azide[d]	NA[e]	5 mg/dL	≤+0.24 mg/dL
Citrate[d]	NA	900 mg/dL	≤±0.20 mg/dL

Table 5.0 Interferences, Continued

SUBSTANCE	SOURCE	MAXIMUM LEVEL TESTED	OBSERVED EFFECT[a]
Oxalate[d]	NA	1000 mg/dL	≤±0.20 mg/dL
Gentisic Acid[d]	NA	5 mg/dL	≤+0.24 mg/dL
Acetoacetate[d]	NA	0.2 mg/mL	≤+0.7 mg/dL
		1.08 mg/mL	≤+3.7 mg/dL

a Plus (+) or minus (-) signs in this column signify positive or negative interference.
b Hemolysis may cause falsely elevated results.
c Registered trademarks are the property of their respective owners.
d Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.
e NA = Not applicable.

2. Lipemic samples >2+ should be ultra-centrifuged and the analysis performed on the infranate.
3. The Naproxen metabolite, O-desmethylnaproxen, has demonstrated a positive interference with the Jendrassik-Grof method for total Bilirubin measurement.[12]
4. *N-acetyl-p-benzoquinone imine* (NAPQI), a metabolite of acetaminophen (paracetamol), may generate erroneously low results in samples for patients that have taken toxic doses of acetaminophen (paracetamol).
5. Refer to References (13,14,15) for other interferences caused by drugs, disease and preanalytical variables.

PERFORMANCE CHARACTERISTICS

ANALYTIC RANGE

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

Table 6.0 Analytical Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Serum or Plasma	0.1 – 30.0 mg/dL	1.7 – 513.0 µmol/L

Samples with concentrations outside the analytical range will be reported as "<0.1 mg/dL" ("<1.7 µmol/L") or ">30.0 mg/dL" (">513.0 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.

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 7.0 Reportable Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS

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 (1.7 µmol/L).

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods.

Serum or plasma (in the range of 0.2 to 28.2 mg/dL):

Y (SYNCHRON LX Systems)	= 0.96X + 0.31
N	= 79
MEAN (SYNCHRON LX Systems)	= 6.75
MEAN (SYNCHRON CX7 DELTA)	= 6.69
CORRELATION COEFFICIENT (r)	= 0.9997

Refer to References (16) for guidelines on performing equivalency testing.

PRECISION

A properly operating SYNCHRON System(s) should exhibit precision values less than or equal to the following:

Table 8.0 Precision Values

TYPE OF PRECISION	SAMPLE TYPE	1 SD		CHANGEOVER VALUE[a]		% CV
		mg/dL	µmol/L	mg/dL	µmol/L	
Within-run	Serum/Plasma	0.15	2.6	5.0	86.7	3.0
Total	Serum/Plasma	0.22	3.8	5.0	86.7	4.5

a When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

Comparative performance data for a SYNCHRON LX System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.[17] Each laboratory should characterize their own instrument performance for comparison purposes.

Table 9.0 NCCLS EP5-T2 Precision Estimate Method

TYPE OF IMPRECISION	SAMPLE TYPE		No. Systems	No. Data Points[a]	Test Mean Value (mg/dL)	EP5-T2 Calculated Point Estimates	
						SD	%CV
Within-run	Serum	Control 1	1	80	1.7	0.1	6.1
	Serum	Control 2	1	80	5.9	0.1	1.7
	Serum	Control 3	1	80	8.9	0.1	1.2
	Serum	Control 4	1	80	17.5	0.2	1.1

Table 9.0 NCCLS EP5-T2 Precision Estimate Method, Continued

TYPE OF IMPRECISION	SAMPLE TYPE		No. Systems	No. Data Points[a]	Test Mean Value (mg/dL)	EP5-T2 Calculated Point Estimates	
						SD	%CV
Total	Serum	Control 1	1	80	1.7	0.1	6.1
	Serum	Control 2	1	80	5.9	0.1	1.9
	Serum	Control 3	1	80	8.9	0.1	1.3
	Serum	Control 4	1	80	17.5	0.2	1.23

a The point estimate is based on the pooled data from one system, run for twenty days, two runs per day, two observations per run on an instrument operated and maintained according to the manufacturer's instructions.

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on a SYNCHRON LX System and are not intended to represent the performance specifications for this reagent.

ADDITIONAL INFORMATION

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

Beckman Coulter, the stylized logo, and the Beckman Coulter product and service marks mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries.

May be covered by one or more pat. - see www.beckmancoulter.com/patents.

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REVISION HISTORY

Revision AF

Revised Interferences section.

Revision AG

Updated corporate address; updated OSHA precaution, removed insert reference from content description, and removed EDTA as an Acceptable Anticoagulant claim.

Revision AH

Added Reagent Preparation visual aid to the Reagent Preparation section.

Revision AJ

Added Revision History

Revision AK

Revised Interferences section.

Revision AL

Added new language requirement: Czech, and Korean.

Revision AM

Removed references to CX and LX systems as they are discontinued effective 12/2013.

Added Beckman Coulter trademark statement and disclaimer.

Revision AN

Added GHS Classification information

Revision AP

Added new language requirement: Romanian

Revision AR

Added limitation statement and reference.

Revision AT

Updates to comply with requirements per Beckman Coulter Global Labeling Policy.

Revision AU

Additional changes to comply with requirements per Beckman Coulter Global Labeling Policy.

Revision AV

New statement (item #4) added under INTERFERENCES section.

Additional changes to comply with requirements per Beckman Coulter Global Labeling Policy.

Revision AW

Added new language requirement: Bulgarian, Serbian, and Vietnamese.

SYMBOLS KEY

Table 10.0

REF	Catalogue Number	IVD	In Vitro Diagnostic
CONTENTS	Contents		Temperature limit
	Manufacturer		Expiration Date
LOT	Batch code	SDS	Safety Data Sheet
CE	CE Mark		Consult Instructions for Use
EC REP	Authorized Representative in the European Community		Date of Manufacture
	Do not reuse	DANGER	DANGER
	Do Not Freeze		
Made in USA of US and Foreign Components		Made in USA of US and Foreign Components	

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