BECKMAN COULTER

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SYNCHRON CX® System(s) Chemistry Information Sheet

DBIL
Direct Bilirubin

REF 439715 (200 tests/cartridge)
REF 476856 (300 tests/cartridge)

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

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

CLINICAL SIGNIFICANCE

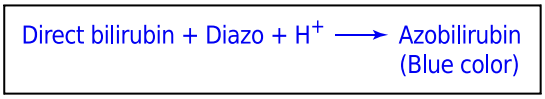
Direct bilirubin measurements are used in the diagnosis and treatment of liver, hemolytic, hematological, and metabolic disorders, including hepatitis and gall bladder block.

METHODOLOGY

DBIL reagent is used to measure DBIL concentration by a timed endpoint diazo method.[1,2] In the reaction, DBIL combines with diazo to form azobilirubin.

The SYNCHRON CX® 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.

CHEMICAL REACTION SCHEME



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SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.[3] 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.[4]
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.[4]
3. Bilirubin is photosensitive. Protect samples from light.

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

SAMPLE VOLUME

A filled 0.5 mL sample cup is the optimum volume. For optimum volume in primary tube samples, refer to Primary Sample Tube Chart Template (P/N 248511) for minimum volume requirements.

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 Direct Bilirubin Reagent Cartridges (2 x 200 tests) or (2 x 300 tests)

VOLUMES PER TEST

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

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

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

Also non-reactive chemicals necessary for optimal system performance.

⚠ CAUTION

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

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

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

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 your facility's acceptance criteria.

REAGENT STORAGE AND STABILITY

DBIL reagent, when stored unopened at room temperature, will obtain the shelf-life indicated on the cartridge label.[5] Once opened, the reagent 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: 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

If unopened, the SYNCHRON® Systems Bilirubin Calibrator may be stored at -15°C to -20°C until the expiration date printed on the calibrator ampule. 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

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 or with certain parts replacements or maintenance procedures, as defined in the SYNCHRON CX *Operating Instructions* manual. This assay has within-lot calibration available. Refer to Section 6 of the SYNCHRON CX *Operating Instructions* manual for information on this feature.
3. For detailed calibration instructions refer to Section 6 of the SYNCHRON CX *Operating Instructions* 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. The explanation of these error codes can be found in Appendix G of Section 10 in the SYNCHRON CX *Operating Instructions* manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

At least two levels of control material, normal and abnormal, 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 SYNCHRON CX® System(s) *Operations Manual*. More frequent use of controls or the use of additional controls is left to the discretion of the user based on work load and work flow.

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)

1. If necessary, load the reagent onto the system as directed in Section 6 of the SYNCHRON CX *Operating Instructions* manual.

2. After reagent load is completed, calibration may be required. Refer to Section 6 of the SYNCHRON CX *Operating Instructions* manual for details of the calibration procedure.
3. Program samples and controls for analysis as directed in Section 6 of the SYNCHRON CX *Operating Instructions* manual.
4. After loading samples and controls onto the system, follow the protocols for system operation as directed in Section 6 of the SYNCHRON CX *Operating Instructions* manual.

CALCULATIONS

The system performs all calculations internally to produce the final reported result. SYNCHRON CX4/5 Systems do not calculate the final result for sample dilutions made by the operator. In these cases, the result produced by the instrument must be multiplied by the dilution factor before reporting the final result. SYNCHRON CX4CE/5CE/7 Systems (including the CX DELTA and CX PRO Systems) 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

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	0.0 – 0.2 mg/dL	0.0 – 3.4 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

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

ANTICOAGULANT	LEVEL TESTED FOR IN VITRO INTERFERENCE	AVERAGE PLASMA-SERUM BIAS (mg/dL) @ +37°C
Ammonium Heparin	29 Units/mL	NSI[a]
Lithium Heparin	29 Units/mL	NSI
Sodium Heparin	29 Units/mL	NSI
Sodium Citrate	6.6 mg/mL	NSI
Potassium Oxalate/Sodium Fluoride	4.0 / 5.0 mg/mL	NSI

a NSI = No Significant Interference (within ±0.3 mg/dL or 10%).

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:

Table 4.0 Interferences

SUBSTANCE	SOURCE	LEVEL TESTED	OBSERVED EFFECT ON ANALYTE[a]
Bilirubin	Bovine (unconjugated)	30 mg/dL	NSI[b]
Lipemia	Intralipid[c]	(4+) 500 mg/dL	-0.25 @ 0.5 mg/dL
Ascorbic Acid	NA[d]	3.0 mg/dL	NSI
Sodium Azide	NA	5.0 mg/dL	NSI

a Plus (+) or minus (-) signs in this column signify positive or negative interference.
b NSI = No Significant Interference (within ±0.30 mg/dL or 10%).
c Intralipid is a registered trademark of KabiVitrum, Inc., Clayton, NC 27250.
d NA = Not applicable.

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.

Table 5.0 Hemoglobin Interferences

SUBSTANCE	SOURCE	LEVEL TESTED	MAXIMUM OBSERVED EFFECT ON ANALYTE[a]
Hemoglobin	RBC hemolysate	(4+) 400 mg/dL	≤+0.3 @ 0.5 mg/dL
		(2+) 200 mg/dL	≤-0.3 @ 3.1 mg/dL
		(2+) 200 mg/dL	≤-0.9 @ 8.3 mg/dL

a Plus (+) or minus (-) signs in this column signify positive or negative interference.

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

PERFORMANCE CHARACTERISTICS

ANALYTIC RANGE

The SYNCHRON CX® 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 – 10 mg/dL	1.7 – 171 µmol/L

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.

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 7.0 Reportable Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS

EQUIVALENCY

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

Serum or plasma:

Y (SYNCHRON CX Systems)[a]	= 1.084X - 0.02
N	= 100
MEAN (SYNCHRON CX Systems)[a]	= 2.35
RANGE OF Y	= 0 - 11
MEAN (SYNCHRON AS®)	= 2.17
RANGE OF X	= 0 - 10
CORRELATION COEFFICIENT (r)	= 0.9859

a Data shown was collected using the SYNCHRON CX4/CX5 Systems. Equivalency between SYNCHRON CX Systems has been established by Deming regression analysis to SYNCHRON CX4/CX5 Systems.

Refer to References (14) for guidelines on performing equivalency testing.

PRECISION

A properly operating SYNCHRON CX® System(s) should exhibit precision values less than or equal to the maximum performance limits in the table below. Maximum performance limits were derived by an examination of the precision of various methods, proficiency test summaries, and literature sources.

Table 8.0 Maximum Performance Limits

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	3.0	52.0	5.0
Total	Serum/Plasma	0.23	3.9	3.0	52.0	7.5

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 the SYNCHRON CX® System(s) evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.[15] 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 Means & Point Estimates	
					SD	% CV
Within-run	Control Level 1	1	80	0.85	0.03	3.53
	Control Level 2	1	80	4.29	0.04	0.93
	Control Level 3	1	80	5.47	0.05	0.91
Total	Control Level 1	2	160	0.85	0.03	3.53
	Control Level 2	2	160	4.29	0.05	1.17
	Control Level 3	2	160	5.47	0.07	1.28

a The serum/plasma point estimate is based on the pooled data from 2 systems, each run for 20 days, 2 runs per day, 2 observations per run on instruments operated and maintained according to the manufacturer's instructions.

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on the SYNCHRON CX® System(s) and are not intended to represent the performance specifications for this reagent.

ADDITIONAL INFORMATION

For more detailed information on SYNCHRON CX Systems, refer to the appropriate SYNCHRON CX manual.

SHIPPING DAMAGE

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

REFERENCES

1. Malloy, H. T., Evelyn, K. A., *J. Biol. Chem.*, 119 481 (1937).
2. Winkleman, J., Cannon, D. C., Jacobs, S. L., *Clinical Chemistry: Principles and Technics*, 1061, Harper and Row, Hagerstown, MD (1974).
3. Tietz, N. W., "Specimen Collection and Processing; Sources of Biological Variation", *Textbook of Clinical Chemistry*, 2nd Edition, W. B. Saunders, Philadelphia, PA (1994).
4. National Committee for Clinical Laboratory Standards, *Procedures for the Handling and Processing of Blood Specimens*, Approved Guideline, NCCLS publication H18-A, Villanova, PA (1990).
5. "USP XXII, NF XVII", United States Pharmacopeial Convention, Inc., Rockville, MD (1990).
6. CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, U.S. Government Printing Office, Washington, D.C. (1984).
7. Tietz, N. W., *Clinical Guide to Laboratory Tests*, 2nd Edition, W. B. Saunders, Philadelphia, PA (1990).
8. National Committee for Clinical Laboratory Standards, *How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory*, Approved Guideline, NCCLS publication C28-A, Villanova, PA (1994).
9. Tietz, N. W., ed., *Fundamentals of Clinical Chemistry*, 3rd Edition, W. B. Saunders, Philadelphia, PA (1987).
10. Henry, J. B., *Clinical Diagnosis and Management by Laboratory Methods*, 18th Edition, W. B. Saunders Company, Philadelphia, PA (1991).
11. Young, D. S., *Effects of Drugs on Clinical Laboratory Tests*, 3rd Edition, AACC Press, Washington, D.C. (1990).
12. Friedman, R. B., Young, D. S., *Effects of Disease on Clinical Laboratory Tests*, 2nd Edition, AACC Press, Washington, D.C. (1989).
13. Young, D. S., *Effects of Preanalytical Variables on Clinical Laboratory Tests*, AACC Press, Washington, D.C. (1993).
14. National Committee for Clinical Laboratory Standards, *Method Comparison and Bias Estimation Using Patient Samples*, Tentative Guideline, NCCLS publication EP9-T, Villanova, PA (1993).
15. National Committee for Clinical Laboratory Standards, *Precision Performance of Clinical Chemistry Devices*, Tentative Guideline, 2nd Edition, NCCLS publication EP5-T2, Villanova, PA (1992).

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