BECKMAN COULTER™ CE

SYNCHRON® System(s) Chemistry Information Sheet

DIGN Digoxin

REF 650182

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

DIGN reagent, when used in conjunction with UniCel® DxC 600/800 System(s) and SYNCHRON® Systems Drug Calibrator 2 set, is intended for quantitative determination of total digoxin concentration in human serum or plasma.

CLINICAL SIGNIFICANCE

Digoxin is administered for the treatment of congestive heart failure and certain types of cardiac arrhythmias. It is monitored for possible toxicity and as a guide for acute and maintenance therapy.

METHODOLOGY

DIGN reagent is used to measure the digoxin concentration by a particle enhanced turbidimetric inhibition immunoassay method.[1] Particle-bound drug (PBD) binds to digoxin specific antibody (Ab) resulting in the formation of insoluble aggregates causing increased turbidity. Non-particle-bound digoxin in the patient sample competes with the PBD for the antibody binding sites, inhibiting the formation of insoluble aggregates. The rate and amount of particle aggregate is inversely proportional to the concentration of digoxin in the sample.

SYNCHRON® System(s) automatically proportion the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 15 parts reagent. The system monitors the aggregate formation by measuring the change in absorbance at 560 nanometers. This change in absorbance is inversely proportional to the concentration of DIGN in the sample and is used by the System to calculate and express the DIGN concentration. This calculation is based upon a multi-point calibration curve.

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SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.[2] Freshly drawn serum or plasma are the specimens of choice. Acceptable anticoagulants are listed in 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.[3]
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]

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 DIGN Reagent Cartridges (2 x 100 tests)

VOLUMES PER TEST

Sample Volume	15 µL
Total Reagent Volume	225 µL
Cartridge Volumes	
A	185 µL
B	15 µL
C	25 µL

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

Digoxin Particle Reagent	3.7 mL
Monoclonal anti-Digoxin Antibodies (mouse)	3.3 mL
Digoxin Reagent Buffer	28.0 mL

Also non-reactive chemicals necessary for optimal system performance.

⚠ CAUTION

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

⚠ CAUTION

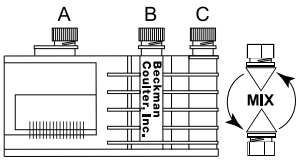
Sodium azide preservative may form explosive compounds in metal drain lines. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).

> ⚠ 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.[4]**

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

SYNCHRON® Systems Drug Calibrator 2 set
At least two levels of control material
Saline

REAGENT PREPARATION



1. Gently invert the cartridge several times prior to loading onto the system.
2. Check for bubbles or foam in compartments; break any bubbles.

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

Digoxin reagent and its components, when stored unopened at +2°C to +8°C, will remain stable until the expiration date printed on the kit label. Once opened, the reagent is stable for 30 days at +2°C to +8°C unless the expiration date is exceeded. DO NOT FREEZE. Do not expose reagent to temperatures above +35°C or to direct sunlight.

Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON® Systems Drug Calibrator 2 set

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

SYNCHRON® Systems Drug Calibrator 2 set is stable until the expiration date printed on the calibrator bottle if stored capped in the original container at +2°C to +8°C.

> ⚠ 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.[4]

Calibrator storage location:

CALIBRATION INFORMATION

1. The system must have a valid calibration factor in memory before control or patient samples can be run.
2. Under typical operating conditions the DIGN 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)

1. If necessary, 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

Therapeutic DIGN concentrations vary significantly, depending upon the individual. The lower limit for one patient may be ineffective in another, while the upper limit may prove toxic in a third. The physician should determine the appropriate reference interval for each patient. The reference intervals listed below were taken from the literature.[5]

Table 2.0 Reference intervals

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Therapeutic	Serum/Plasma (Congestive heart failure)	0.8 – 1.5 ng/mL	1.0 – 1.9 nmol/L
	Serum/Plasma (Arrhythmias)	1.5 – 2.0 ng/mL	1.9 – 2.6 nmol/L
Toxic	Serum or Plasma (Adult)	> 2.5 ng/mL	> 3.2 nmol/L
	Serum or Plasma (Child)	> 3.0 ng/mL	> 3.8 nmol/L

INTERVALS	SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Therapeutic			
Toxic			

Refer to References (6, 7, 8) for guidelines on establishing laboratory-specific reference intervals.

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

ANTICOAGULANT TEST RESULTS

The following anticoagulants were assessed by Deming regression analysis with a minimum of 50 paired digoxin free serum and plasma samples to which purified digoxin was added. Values of serum (X) ranging from 0.4 ng/mL to 4.5 ng/mL were compared with the values for plasma (Y) yielding the following results.

Table 3.0 Anticoagulant Test Results

ANTICOAGULANT	LEVEL OF ANTICOAGULANT TESTED	DEMING REGRESSION ANALYSIS
Lithium Heparin	14 Units/mL	Y = 1.004X - 0.08; r = 0.996
Sodium Heparin	14 Units/mL	Y = 0.994X - 0.06; r = 0.994

LIMITATIONS

None identified

INTERFERENCES

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

Table 4.0 Interferences

SUBSTANCE	SOURCE	LEVEL TESTED	OBSERVED EFFECT
Hemoglobin	RBC hemolysate	500 mg/dL	NSI[a]
Bilirubin	Porcine	30 mg/dL	NSI
Rheumatoid Factor[b]	Human	300 IU/mL	NSI
Lipemia	Human	4+	NSI
Paraprotein	Human	500 mg/dL	NSI

a NSI = No Significant Interference (for digoxin value ≤ 2.0 ng/mL interference is ≤ 0.20 ng/mL, > 2.0 ng/mL interference is < 10%).
b Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.

2. Digoxin-like immunoreactive factors (DLIF) or substances (DLIS) have been identified in blood from patients in renal failure, liver failure, newborns, and pregnant women in the third trimester. Studies have established that the

presence of DLIF or DLIS in a sample can result in a false elevation of digoxin when assayed by commercially available immunoassays.[9,10]

3. In very rare cases, patient samples may contain a particle agglutinating protein which may produce low results with this assay. If this is suspected please follow directions for RESULTS SUPPRESSED OIR LO in the PERFORMANCE CHARACTERISTICS, ANALYTICAL RANGE section.
4. Use of the SYNCHRON Systems Digoxin Assay is not recommended for the monitoring of digoxin levels in patients undergoing Digoxin Immune FAb (i.e. DIGIBIND) treatment.*
5. Specimens containing particulate matter should be clarified by centrifugation.
6. Refer to References (11,12,13) for other interferences caused by drugs, disease and preanalytical variables.
7. For assays employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample. Human anti-mouse antibodies may be present in samples from patients who have received immunotherapy or diagnostic procedures utilizing monoclonal antibodies or in individuals who have been regularly exposed to animals.[14,15] Additionally, other heterophile antibodies, such as human anti-goat antibodies may be present in patient samples. Interpretation of results should be done in the context of the overall clinical presentation of the patient, including symptoms, clinical history, data from additional tests and other appropriate information.

SPECIFICITY

Specificity of the Digoxin Assay:

Cross reactivity is expressed as a ratio of the recovered digoxin concentration to the concentration of the cross-reactant.

$$\frac{[\text{Digoxin}]}{[\text{Cross-reactant}]} \times 100\% = \%\ \text{Cross-reactivity}$$

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The following compounds have been tested for cross-reactivity in the Digoxin assay:

Table 5.0 Cross Reactivity[a]

COMPOUND	CROSS-REACTANT CONCENTRATION (ng/mL)	% CROSS-REACTIVITY
Amiodarone	100,000	0.000
Canrenone	100,000	0.003
Cholesterol	10,000	-0.001
Dehydroisoandrosterone (DHEA)	10,000	0.000
Dehydroisoandrosterone sulfate (DHEAS)	10,000	0.000
Deslanoside	2.27	55.5
Digitonin	10,000	0.005
Digitoxigenin	420	1.02
Digitoxigenin-bis-digitoxoside	2.5	97.6
Digitoxin	10	35.2
Digitoxose	10,000	0.002
Digoxigenin	33	6.82
Digoxigenin-bis-digitoxoside	1.75	85.1
Digoxigenin-mono-digitoxoside	1.9	99.0
Dihydrodigoxin	115	2.63

Table 5.0 Cross Reactivity, Continued

COMPOUND	CROSS-REACTANT CONCENTRATION (ng/mL)	% CROSS-REACTIVITY
Diltiazem	10,000	-0.002
DMSO	100,000	0.000
β-Estradiol	10,000	0.001
Estriol	10,000	-0.002
Furosemide	50,000	0.000
Gitoxin	180	1.81
11-α-hydroxyprogesterone	10,000	0.001
17-α-hydroxyprogesterone	10,000	0.000
11-β-hydroxyprogesterone	10,000	0.005
6-hydroxy-7-thiomethylspironolactone	100,000	0.000
Hydrocortisone (cortisol or 17-hydroxycorticosterone)	10,000	0.001
Lanatoside C	1.55	53.6
Lidocaine	100,000	0.000
β-Methydigoxin	2.27	59.9
Ouabain	860	0.356
Phenytoin	100,000	0.001
Prednisolone	10,000	-0.001
Prednisone	10,000	-0.001
Procainamide	75,000	-0.001
Progesterone	10,000	0.003
Propranolol	100,000	0.000
Proscillaridin A	340	0.985
Quinidine	100,000	0.000
Spironolactone	100,000	0.000
Testerone	10,000	0.002
7-Thiomethylspirolactone	100,000	0.000
7-Thiospirolactone	100,000	0.000

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.

PERFORMANCE CHARACTERISTICS

ANALYTIC RANGE

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

Table 6.0 Analytical Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Serum or Plasma	0.2 – 4.5 ng/mL	0.256 – 5.76 nmol/L

Samples with concentrations outside of the analytical range will be reported as "<0.2 ng/mL" ("<0.256 nmol/L") or ">4.5 ng/mL" (">5.76 nmol/L").

Samples reported out as greater than the analytical range may be confirmed by diluting with saline and reanalyzing. The appropriate dilution factor should be applied to the reported result.

Samples reported out as less than the analytical range should be confirmed by diluting one part of the original patient sample with one part of Level 4 of SYNCHRON® Systems Drug Calibrator 2 set. The assayed result of this dilution, when multiplied by 2, should approximate the original value of the known sample to confirm the low patient result. The confirmed result should be reported out as "<0.2 ng/mL" ("<0.256 nmol/L"). If the assayed result of the first dilution, when multiplied by 2, does not approximate the original result of the known sample, do not report result; assay by another method.

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 DIGN determination is 0.2 ng/mL (0.256 nmol/L).

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of samples obtained from patients on digoxin therapy (N=93, in the range of 0.2 ng/mL to 3.0 ng/mL), and sera with purified digoxin added, (N=19, in the range of 2.2 ng/mL to 4.0 ng/mL), to an accepted clinical method.

Serum

Y (SYNCHRON LX Systems)	= 1.054X - 0.05
N	= 112
MEAN (SYNCHRON LX Systems)	= 1.55
MEAN (TDx)[a]	= 1.52
CORRELATION COEFFICIENT (r)	= 0.990

a TDx is a registered trademark of Abbott Laboratories.

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
		ng/mL	nmol/L	ng/mL	nmol/L	
Within-run	Serum	0.10	0.128	2.0	2.56	5.0
Total	Serum	0.15	0.192	2.0	2.56	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 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 (ng/mL)	EP5-T2 Calculated Point Estimates	
						SD	%CV
Within-run	Serum	Control 1	1	80	0.80	0.05	6.06
	Serum	Control 2	1	80	1.78	0.03	1.78
	Serum	Control 3	1	80	2.49	0.07	2.62
Total	Serum	Control 1	1	80	0.80	0.09	11.25
	Serum	Control 2	1	80	1.78	0.11	6.30
	Serum	Control 3	1	80	2.49	0.12	4.64

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.

SHIPPING DAMAGE

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

REVISION HISTORY

Revision AF
Revised Quality Control section and updated European Hazard Classification.

Revision AG
Updated corporate address; 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
Added new language requirement: Czech, and Korean.

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

Added Beckman Coulter trademark statement and disclaimer.

FOOTNOTES

* DIGIBIND is a registered trademark of Burroughs-Wellcome Company.

REFERENCES

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17. 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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