DXI & ACCESS (DIG) DIGOXIN

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PURPOSE

To provide instruction on how to perform Digoxin testing on the DXI & Access instruments.

PRINCIPLE

The Digoxin reagent, when used in conjunction with the Beckman Access or DXI Systems and Access Calibrators, is intended for quantitative determination of Digoxin concentration in human serum or plasma.

BACKGROUND

Clinical Significance

Digoxin is a potent, cardiac glycoside isolated from the leaves of Digitalis lanta. This glycoside is a widely prescribed drug used in the treatment of congestive heart failure, atrial fibrillation, atrial flutter, supraventricular tachycardia, and other cardiac disorders. Treatment with digoxin results in more effective cardiac contractility, decrease in conduction velocity, and a slowing of the heart rate.

The therapeutic range for digoxin is narrow. Desired effects become exaggerated with administration of an excess of digoxin leading to conditions difficult to distinguish from the original cardiac symptoms. Coupled with the narrow therapeutic to toxic range is a considerable variability in patient response to the same dose. In different patients identical doses of digoxin result in different serum levels mainly due to individual variation in myocardial sensitivity, absorption, diet, and excretion of the drug. The variability in bioavailability of different drug formulations and interactions with other drugs, particularly drugs affecting the electrolyte balance such as diuretics, also affect individual patient responses. Measuring a serum sample drawn 6–8 hours after the administration of digoxin allows for equilibration of serum and tissue digoxin levels. Monitoring digoxin levels can assist the physician in adjusting digoxin dosage.

Methodology

The Access Digoxin assay is a competitive binding immunoenzymatic assay. A sample is added to the reaction vessel with anti-digoxin antibody, digoxin-alkaline phosphatase conjugate, and paramagnetic particles coated with goat anti-rabbit capture antibody. Digoxin in the sample competes with the digoxin-alkaline phosphatase conjugate for binding sites on a limited amount of specific anti-digoxin antibody. Resulting antigen: antibody complexes bind to the capture antibody on the solid-phase. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of digoxin in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

RELATED DOCUMENTS

R-PO-CH-0810	Quality Control Program General Laboratory
R-PO-CH-0809	Quality Control Westgard Rules Statistics

R-PR-AD-0540	Specimen Rejection/Cancellation Protocol
J-F-CH-0824	DXI & Access Controls
J-F-CH-0825	DXI Calibrators
M-F-CH-0820	Chemistry Controls
M-F-CH-0826	Chemistry Calibrators
J-F-CH-2000	Access 2 and DXI 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 are the preferred specimens.

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.

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

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

1. R1: Access Digoxin Reagent Pack
   Cat. No. 33710: 100 determinations, 2 packs, 50 tests/pack.

   Provided ready to use. Store upright and refrigerate at 2 to 10°C. Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument. Stable until the expiration date stated on the label when stored at 2 to 10°C. Stable at 2 to 10°C for 14 days after initial use. Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range. If the reagent pack is damaged (i.e., broken elastomer), discard the pack. All antisera are polyclonal unless otherwise indicated.

Reactive Ingredients	
Paramagnetic particles coated with goat anti-rabbit IgG suspended in TRIS buffered saline, with surfactant, bovine serum albumin (BSA) matrix, < 0.1% sodium azide, and 0.0125% Cosmocil** CQ.	R1a
Digoxin-alkaline phosphatase (bovine) conjugate in TRIS buffered saline, with surfactant, BSA matrix, < 0.1% sodium azide, and 0.0125% Cosmocil CQ.	R1b
Rabbit antibody to digoxin in TRIS buffered saline, with surfactant, BSA matrix, rabbit IgG, < 0.1% sodium azide, and 0.0125% Cosmocil CQ.	R1c

2. Access Digoxin Calibrators
Cat. No. 33715: S0-S5, 4.0 mL/vial

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e. assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Provided ready to use. Store upright and refrigerate at 2 to 10°C. Mix contents by gently inverting before use. Avoid bubble formation. Stable until the expiration date stated on the label when stored at 2 to 10°C. Signs of possible deterioration are control values out of range. Refer to calibration card and or vial labels for exact concentrations.

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

S0: Human serum, < 0.1% sodium azide, and 0.025% Cosmocil** CQ. Contains 0.0 ng/mL (nmol/L) digoxin.

S1–S5: Digoxin in human serum at levels of approximately 0.5, 1.0, 2.0, 4.0 and 6.0 ng/mL (0.6, 1.3, 2.6, 5.1 and 7.7 nmol/L), respectively, with < 0.1% sodium azide, and 0.025% Cosmocil CQ.

Calibration Card: 1

3. Access Substrate
Cat. No. 81906: 4 x 130 mL

Provided ready to use. Refer to the following chart for storage conditions and stability. An increase in substrate background measurements may indicate instability.

Condition	Storage	Stability
Unopened	2 to 8°C	Until expiration date stated on the label
Equilibration prior to use (unopened)	15 to 30°C (room temperature)	Minimum 18 hours Maximum 14 days
In use (opened)	External fluids tray substrate position	Maximum 14 days

R2 Substrate: Lumi-Phos 530 (buffered solution containing dioxetane Lumigen* PPD, fluorescer, and surfactant).

Refer to the appropriate system manuals and/or Help system for detailed instructions.

4. **Access®, Access 2, SYNCHRON LX®i:**
   Access Wash Buffer II, Cat. No. A16792
   UniCel® DxI:
   Unicel DxI: Wash Buffer II, Cat. No. A16793

   Provided ready to use. Stable until the expiration date stated on the label when stored at room temperature (15 to 30°C). An increase in substrate background measurements or increased relative light units for the zero calibrators in “sandwich”-type assays may indicate instability.

   R3 Wash Buffer: TRIS buffered saline, surfactant, < 0.1 sodium azide, and 0.1% ProClin*** 300.

   Refer to the appropriate system manuals and/or Help system for detailed instructions.

5. Quality Control (QC) materials: commercial control material.

6. Access Immunoassay System and supplies

7. Warnings and Precautions
   - For *in vitro* diagnostic use.
   - Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
   - Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.
   - Each serum/plasma pool used in the preparation of this product has been tested and found negative for the presence of fibrinogen.
   - Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.
   - Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.
   - The Material Safety Data Sheet (MSDS) is available upon request.

CALIBRATION

An active calibration curve is required for all tests. For the Access Digoxin assay, calibration is required every 28 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

The Access Digoxin Calibrators are provided at six levels - zero and approximately 0.5, 1.0, 2.0, 4.0, and 6.0 ng/mL - prepared gravimetrically from crystalline digoxin and human serum. Assay calibration data are valid up to 28 days.

Calibrators run in duplicate.

QUALITY CONTROL

See Related Documents J-F-CH0824 DXI & Access Controls and M-F-CH0820 Chemistry Controls

STEPS

1. Instrument Operation: Refer to the appropriate system manuals and/or Help system for preparation and operation.

2. Assay Procedure: Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

RESULTS

Patient test results are determined automatically by the system software using a weighted four parameter logistic curve (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

PERFORMANCE CHARACTERISTICS

Reference range

Therapeutic	0.5 – 2.0 ng/mL
Possible toxic effects	> 2.0 ng/mL
Critical	> 3.0 ng/mL

For Critical Value reporting protocol, refer to Policy

Analytic Range

Type	Conventional Units
Serum or Plasma	0.2-6.0 ng/mL

Reporting results outside the analytical range

Lower Limit of range	0.2 ng/mL	Results below 0.2 should be reported as <0.2 ng/mL
Upper limit of range	6.0 ng/mL	Results >6.0 should be diluted with Digoxin Zero (S0) Calibrator, reanalyzed and dilution factor applied. The maximum allowable dilution is X2. Results >12.0 should be reported as >12.0 ng/mL.

LIMITATIONS

Substance	Level Tested	Observed Effect
Hemoglobin	1000 mg/dL INDEX of 10	No Significant Interference
Bilirubin	10 mg/Dl INDEX of 7	No Significant Interference
Lipemia (Triglycerides)	1800 mg/Dl INDEX of 10	No Significant Interference

1. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.(8, 9)

2. Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

3. The Access Digoxin results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.

4. Samples containing up to 10 mg/dL (171 µmol/L) bilirubin, lipemic samples containing the equivalent of 1800 mg/dL (20.32 mmol/L) triglycerides, and hemolyzed samples containing up to 1000 mg/dL (10 g/L) hemoglobin do not affect the concentration of digoxin assayed. In addition, 3 g/dL (30 g/L) human albumin added to the endogenous albumin in the sample does not affect the concentration of digoxin assayed.

5. The following table describes the cross-reactivity of the assay with substances that are similar in structure to digoxin.

Substance	Analyte Added (ng/mL)	Cross-Reactivity (%)
Digoxin	1	103
Digitoxin	10	2.902
Digitoxigenin	10	1.926
Ouabain	1000	0.0202
Prednisone	1000	0.0008
Spironolactone	1000	0.0053
Digoxigenin	1.0	68.38
Aldosterone	100	0.0290
Cortisol	1000	0.0001
Furosemide	1000	0.0001
Progesterone	1000	0.0023
Testosterone	1000	0.0007

6. The lowest detectable level of digoxin distinguishable from zero (Access Digoxin Calibrator S0) with 95% confidence is 0.20 ng/mL (0.3 nmol/L).

7. **NOTE**: Digoxin metabolites, cardiac glycosides, and some synthetic steroid-like medications can interfere in the assay of digoxin due to cross-reactivity with the digoxin-specific antibody. In addition, Valdes reports that the presence of endogenous digoxin-Like Immunoreactive Factor (DLIF) in some samples may interfere in the assay due to cross-reactivity with the digoxin-specific antibody. Apparently toxic digoxin levels can occur in patients receiving both digoxin and diuretics due to electrolyte imbalances, not assay cross-reactivity.

PROCEDURAL NOTES

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.

2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.

3. Use fifty-five (55) µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.

4. The system default unit of measure for sample results is ng/mL. To change sample reporting units to the International System of Units (SI units), nmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply ng/mL by multiplication factor 1.281.

5. Due to interferences which may occur with *in vitro* diagnostic assays, replicate testing may be indicated to ensure the integrity of analytical results.

REFERENCES

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DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
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