I. Purpose

Instructions for the quantitative analysis of digoxin in human serum or plasma on the Beckman Coulter AU Clinical Chemistry analyzers.

A	Principle
	The enzyme in the Emit 2000 Digoxin Assay is manufactured using recombinant DNA technology. The assay is a homogeneous enzyme immunoassay technique used for the analysis of digoxin and its active metabolites in serum or plasma. The assay is based on competition between drug in the sample and drug labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme functions only with the recombinant variant of the bacterial (Leuconostoc mesenteroides) enzyme employed in the assay.

B	Clinical Indication
	Digoxin is a potent cardiac glycoside widely prescribed for the treatment of patients suffering from congestive heart failure, as well as some types of cardiac arrhythmia. Digoxin intoxication is a common and serious problem in the clinical setting. This is, in part, a result of the fact that cardiac glycosides have a low therapeutic ratio (a very small difference between therapeutic and tissue toxic levels). Coupled with the narrow therapeutic range is a marked patient variability in response to the same dosage of drug, often resulting in unpredictable serum drug levels. Intoxication symptoms are often indistinguishable from the original condition for which the drug was prescribed. It may not be immediately apparent whether the patient has been under or overdosed. Monitoring serum digoxin levels combined with other clinical data can provide the physician with useful information to aid in adjusting patient dosage, achieving optimal therapeutic effect while avoiding useless subtherapeutic or harmful toxic dosage levels.

II. Scope

All Testing Personnel, specifically Clinical Laboratory Scientists (CLS).

III. Safety Precautions

Testing personnel must take normal infectious disease precautions, including but not limited to PPE.

IV. Specimen

All blood should be handled as though potentially infectious. Follow laboratory bloodborne pathogen policy and guidelines when handling body fluid specimens.

A	**Specimen Requirements**
	1. Type: a. Serum (Plain Red top only) i. Separate serum/plasma from cells as soon as possible if testing is delayed. ii. Use of tubes with separator gel (SST or PST) should be avoided due to possible absorption of the drug by the gel.
	2. Sample Collection Time a. For reliable interpretation of results, collect samples either after the drug's distribution phase or immediately before the next oral dose (at least 6 hours after administration). b. Samples drawn before the drug has completed its distribution phase will not accurately reflect the level of drug in the myocardium. These samples cannot be used to evaluate cardiac response, as serum levels do not represent tissue levels until at least 6 hours after oral dose or 4 hours after an intravenous dose. c. To evaluate maintenance doses, collect samples when digoxin levels are at steady state—the time to reach steady state is normally from three to five elimination half-lives but may be prolonged in patients with impaired renal function.
	3. Volume: a. Minimum - 0.5 mL b. Sample Size (dead space excluded) – 17 uL
	4. Stability: a. Room temperature (15-25°C): 8 hours b. Refrigerated (2 - 8°C): 7 days c. Frozen (<-20°C): 6 months i. Repeated freeze-thaw cycles should be avoided. For transporting, maintain the sample temperature at 2-8°C.
	5. Unacceptable specimen: a. Whole blood b. Tubes with separator gel (SST or PST) i. In the event that SST/PST is received and redraw is not possible due to timed collection, append Result Comment **TDMSST** "Specimen received in inappropriate draw tube. Results may be affected and should be interpreted with caution." Refer to reporting section.
	6. Special Handling: a. Samples that contain particulate matter, fibrous material, gel-like masses, appear unusual, or were frozen: i. If sample is frozen, thaw at room temperature (15-25°C). ii. Vigorously mix sample in a vortex for at least 30 seconds. iii. Centrifuge sample at > 2000 rpm for 15 minutes. iv. Collect a specimen from the middle portion of the sample. Avoid collecting lipids from the top portion or particulate matter from the bottom portion.

V. Equipment Calibration and Maintenance

A	**Calibration**
	1. Perform a multi-point calibration (5AB) using a water blank and the Emit 2000 Digoxin Calibrators.
	2. Frequency: a. Every 7 days b. Each new set of reagent (recommended, but not required) c. When reagent lot changes d. When QC has shifted e. After major preventive maintenance, or replacement of a critical part
	3. Calibrator: Emit 2000 Digoxin Calibrators(ng/mL) : 0, 0.5, 1.0, 2.0, 3.0, 5.0
	4. Preparation: a. Calibrators are packaged in a ready to use liquid form and may be used directly from the refrigerator. b. Record open dates and initials
	5. Storage and Stability a. Store at 2-8°C, upright, and with caps tightly closed when not in use. b. Unopened and opened calibrators are stable until the expiration date printed on the label if stored as directed. c. Do not freeze the calibrators or expose them to temperatures above 32°C.
B	**Maintenance**
	Refer to SFOFCD-0408. Refer to SFOWI-1268. Refer to AU680 Chemistry Analyzer User's Guide-Chapter (8) -Maintenance

VI. Supplies

All reagents must be dated upon receipt and upon installation. The "on-board" expiration date must also be indicated on installed reagents.

A **Reagent**

1. Preparation
   a. Reagents 1 and 2 are provided as a matched set. Do not interchange with components of kits with different lot numbers.

Reagent	Ingredient	Concentration	Preparation
R1 (Antibody/ Substrate)	Non-sterile rabbit antibodies reactive to digoxin G6P NAD Acidic amphoteric dipeptide buffer Bovine serum albumin	N/A	Ready for use
R2 (Enzyme)	Digoxin labeled with recombinant G6PDH HEPES/Tris buffer Bovine serum albumin	N/A	Ready for use

2. Storage & Stability
   a. Opened bottle expiration date is monitored by the analyzer.
   b. Do not use the reagent kit or calibrators after the expiration date.
   c. Do not freeze reagents or expose them to temperatures above 32°C.

	Storage	Expiration Date
Unopened	2 - 8°C	Stable until expiration date on label
Opened	In refrigerated compartment of the analyzer	90 days Record open date & initials on bottle.

3. Indications of Deterioration:
   a. Discoloration, especially yellowing, of the reagent, visible signs of microbial growth, turbidity or precipitation in reagent may indicate degradation and warrant discontinuance of use.

4. Precautions:
   a. Reagents contain sodium azide preservatives. Flush with plenty of water when discarding reagents.
   b. Reagents and calibrators contain a preservative that may cause sensitivity on contact with skin.

VII. Quality Control

A	QC Material & Stability
	Refer to SFOFCD-0407

B	**Frequency**
	1. Two levels of QC every 24 hours 2. Each new reagent bottle (even if same Lot #) 3. Each new reagent lot 4. After every calibration 5. After each shipment of the same Lot # 6. After specific maintenance or troubleshooting as detailed in the operators manual or after service/repair.
C	**Acceptability Criteria**
	1. Refer to SFOWI-0218

VIII. Procedure

A	**Sample Analysis**
	1. Refer to SFOWI-1268 (AU680 General Operating Procedures)
B	**Dilutions**
	<table><tr><th>On-Board Auto Dilution</th><th>Maximum Dilution</th><th>Diluent</th></tr><tr><td>X1</td><td>X5</td><td>Calibrator 0</td></tr></table>
	1. Auto dilution: N/A
	2. Manual dilution: a. If the result exceeds the AMR, manually dilute the sample with Calibrator 0 using a higher dilution factor than the on-board dilution (x1). Refer to SFOFCD-0412 for manual dilution instructions. b. Make two independent dilutions, e.g., x2, and x5 c. Place the manually diluted samples on the Red Rack. d. Manually order sample I.D., the test, sample type, and enter the dilution factor in the "Sample Dilution Rate" field to re-run the assay. e. Ensure dilution factor is included as part of the sample identity [e.g., last 5 digits of Acc# x2, last 5 digits of Acc# x5] f. The system will automatically multiply the results with dilution factors. g. Review the dilution results for any flags and LIH index. h. Confirm diluted results are within AMR. No "G" or "F" flag. i. When maximum dilution is reached, it is acceptable to have a "F" flag for extremely high sample. In this case, the result is reported as > upper limit of the reportable range. j. Confirm the final results from the two dilutions agree within 10%. k. Report results from the lower dilution. Do not average the two diluted results. l. Consult supervisor if the two dilutions do not agree. m. Manually enter final result into Millennium. Results from manual dilutions do not upload to LIS and therefore manual entry into Millennium is needed.

C	**Repeats**
	1. Follow laboratory repeat policy
	2. Review instrument printouts for result reasonableness, questionable results are repeated.
	3. Review instrument printouts for LIH indices and any flags. a. If lipemic index = 4+,5+ or ABN, airfuge the sample and repeat Digoxin assay.

IX. Limitations, Reportable Range, Calculations, Reference Range, Interpretation and Result Reporting

A	**Limitations**
	1. No clinical significant interference has been found in samples spiked with: a. Bilirubin: up to 30 mg/dL Bilirubin b. Hemolysis: up to 800 mg/dL Hemolysate c. Human gamma globulins: 80 mg/mL.
	2. Endogenous, digoxin-like immunoreactive factors (DLIF) have been detected in the serum and plasma of neonates, pregnant women, and patients in renal and hepatic failure. Several studies have established that these factors can cause falsely elevated digoxin measurements when assayed by commercially available immunoassays. a. In rare instances, individuals have antibodies that interfere with the assay by depressing its enzymatic rate. This rate depression may cause low test results. b. Fab fragments of antidigoxin antibodies, found in the serum and plasma of individuals being treated for digoxin intoxication, have the potential to interfere with any immunoassay in which they are not separated from digoxin before testing.
	3. Sensitivity: a. 0.2 ng/mL b. This represents the lowest measurable concentration of Digoxin that can be distinguished from 0 ng/mL with 95% confidence.

4. Specificity:
    a. The compounds listed in the following table do not interfere with the Emit 2000 Digoxin Assay when tested in the presence 1.0 ug/mL digoxin. Levels tested were at or above maximum physiological or pharmacological concentrations

Therapeutic Drugs	
Compound	**Conc. Tested (g/mL)**
Furosemide	50
Hydrochlorothiazide	100
Lidocaine	100
Phenytoin	100
Procainamide	100
Propranolol	100
Quinidine	100
Secobarbital	100
Spironolactone	10
Cholesterol	10
Cortisol	10
Cortisone	10

Endogenous Substances and Synthetic Hormones	**Concentration Tested (µg/mL)**
Estriol	10
Prednisolone	10
Prednisone	10
Progesterone	10
Testosterone	5

B **AMR & Reportable Range**

AMR
0.2 - 5.0 ng/mL

Reportable Range
0.2 - 25.0 ng/mL
Results outside the linear limits are reported as such.

C **Calculations**

All calculations are automatically performed by the Beckman Coulter AU680 Analyzer and RILIS.

D **Reference Range**

Therapeutic range 0.0 - 2.0 ng/mL

Therapeutic Drug Monitoring: The therapeutic drug dose and date/time and TDM specimen collection date/time are documented in the electronic medical record, KP-Health Connect, for the treating clinician's/pharmacist's assessment.

E	**Critical Values**
	≥ 2.5 ng/mL
F	**Early Notification Values**
	None
G	**Result Reporting & Interpretation**
	1. Confirm ALL flags and indices are properly addressed before reporting any result.
	2. Report Digoxin results in ng/mL and to one decimal place.
	3. Review the instrument printout for result reasonableness, LIH indices and any flags.
	4. If any index shows ABN, visually check the sample appearance to confirm the index ABN is correct. Extreme lipemia, hemolysis, or icterus can show all indices as "ABN". a. Lipemia and hemolysis can cause unpredictable increase or decrease in Digoxin. b. Refer to SFOFCD-0411 "AU680 Comment Codes for Reporting Interference due to Lipemia, Icterus, and Hemolysis"
	5. If testing was performed on SST/PST, append **TDMSST** as Result Comment.

X. Corrective Action

A	**QC Out of Acceptable Range**
	1. Review data and LJ charts. If an out of control value appears to be random error, repeat control on new QC aliquot.
	2. Refer to SFOWI-0218 and SFOSOP-0288 for additional QC troubleshooting steps.
	3. Do not use the instrument for patient tests until issues are resolved. Beckman Coulter Tech Support is available at 1-800-854-3633.
B	**Instrument Warning or Error Flag Displayed**
	1. See Reference Manual for troubleshooting steps.
	2. Do not use the instrument for patient tests until issues are resolved. Beckman Coulter Tech Support is available at 1-800-854-3633.

XI. Associated Documents and Records

SFOFCD-0407
SFOFCD-0408
SFOFCD-0411
SFOFCD-0412
SFOWI-0218
SFOWI-1268
SFOSOP-0288

XII. References

1. RWLQCWI-2082 rev.4 AU 680 Digoxin
2. RLWI-2692 rev.2 MWS Digoxin on Architect i2000
3. Beckman Coulter Instructions for Use: Digoxin, CLSIOSR4H229.02 March 2012
4. Digoxin Package Insert dated September 2011