I. Purpose

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

A	Principle
	The Emit® 2000 Phenytoin Assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in biological fluids. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) 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 bacterial (Leuconostoc mesenteroides) enzyme employed in the assay.

B	Clinical Indication
	Anticonvulsant drugs are those used to treat epilepsy as well as seizure disorders that are secondary to other diseases. The most common anticonvulsants are Phenytoin and Phenobarbital for major motor seizures. Phenytoin is used in the treatment of primary or secondary generalized tonic-clonic seizures (whole brain seizures), simple partial (focal cortical seizures), or complex partial seizures (temporal lobe seizures), and epilepsy. Monitoring serum phenytoin concentrations, along with careful clinical assessment, is the most effective means of improving seizure control, reducing the risk of toxicity; and minimizing the need for additional anticonvulsant medication for the following reasons: 1. Serum phenytoin concentrations correlate better with pharmacological activity than does dosage because of individual differences in absorption, metabolism, disease states, concomitant medication, and compliance. Serum concentration monitoring helps physicians individualize dosage regimens. 2. The hepatic enzyme system for metabolizing phenytoin can become saturated within the drug's therapeutic range. When this occurs, small dosage alterations can lead to unexpected drug accumulation and clinical toxicity. 3. Phenytoin is safe and effective only in a narrow range of serum concentrations.

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. Draw a sample within two to four hours after an intravenous loading dose and, at steady-state, collect a specimen representing the trough level just before the next scheduled dose.
	3. Volume: a. Minimum - 0.5 mL b. Sample Size (dead space excluded) – 2 uL
	4. Stability: a. Refrigerated (2 - 8°C): 30 days b. Frozen (<-20°C): 90 days
	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 Phenytoin Calibrators.

	2. Frequency: a. Every 10 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 Phenytoin Calibrators (ug/mL) :0, 2.5, 5.0, 10, 20, 40.
	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)	Mouse monoclonal antibodies reactive to phenytoin G6P NAD Preservatives, including 0.1% sodium azide and stabilizers	N/A	Ready for use
R2 (Enzyme)	Phenytoin labeled with G6PDH Tris buffer Preservatives, including 0.1% sodium azide and stabilizers	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	63 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>X10</td><td>X10</td><td>DI Water</td></tr></table>
	NOTE: On-board dilution = Maximum dilution
	1. Auto dilution: a. When results exceed the assay's AMR, an on-board auto-dilution is performed. Results are automatically multiplied by the instrument.
	2. Manual dilution: N/A
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.

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. Lipemia: up to 750 mg/dL Triglycerides
	2. Sensitivity: a. 0.5 ug/mL b. This represents the lowest concentration of phenytoin that can be distinguished from 0 ug/mL with 95% confidence.

3. Specificity:
    a. The compounds listed in the following table do not interfere with the Emit 2000 Phenytoin Assay when tested in the presence 10 ug/mL phenytoin. Levels tested were at or above maximum physiological or pharmacological concentrations.

Compounds that Do Not Interfere	
Amitriptyline	5-(p-Hydroxyphenyl)-5-phenylhydantoin glucuronide
Amobarbital	Imipramine
Carbamazepine	Mephenytoin
Carbamazepine-10,11-epoxide	Mephobarbital
Chlordiazepoxide	Methsuximide
Chlorpromazine	Pentobarbital
Clorazepate	Phenobarbital
Diazepam	Phensuximide
Ethosuximide	2-Phenyl-2-ethyl-malondiamide (PEMA)
Ethotoin	Primidone
5-Ethyl-5-phenylhydantoin	Promethazine
Glutethimide	Secobarbital
5-(p-Hydroxyphenyl)-5-phenylhydantoin	Valproic Acid

B **AMR & Reportable Range**

AMR
2.5 - 40 ug/mL

Reportable Range
3 - 400 ug/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: 10 - 20 ug/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**

≥41 ug/mL

F **Early Notification Values**

≥30 ug/mL

G	**Result Reporting & Interpretation**
	1. Confirm ALL flags and indices are properly addressed before reporting any result.
	2. Report Phenytoin results in ug/mL and in whole number
	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. 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-2086 rev.2 AU 680 TDM Phenytoin
2. Beckman Coulter Instructions for Use: Phenytoin, CLSIOSR4A229.02 March 2012