I. Purpose

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

A	Principle
	The Emit tox™ Acetaminophen Assay is a homogeneous enzyme immunoassay technique used for the quantitative analysis of acetaminophen in human serum or plasma. In this assay, serum or plasma is mixed with reagent 1, which contains antibodies to acetaminophen and the coenzyme nicotinamide adenine dinucleotide (NAD). Subsequently reagent 2, containing acetaminophen labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) is added. This assay is based on competition between drug in the sample and drug labeled with the enzyme 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 NAD to NADH, resulting in an absorbance change that can be measured spectrophoto-metrically. Endogenous serum G6PDH does not interfere because the coenzyme functions only with the bacterial (Leuconostoc mesenteroides) enzyme employed in the assay.

B	Clinical Indication
	Acetaminophen is a widely used analgesic and antipyretic found in a number of over-the-counter and prescription products. When consumed in overdose quantities, acetaminophen may cause severe liver and kidney damage, or death. Patients may have few or no symptoms early after acute overdose of acetaminophen. The only reliable early diagnostic indicator is provided by a quantitative measurement of the serum drug level. Clinical evidence of liver and kidney damage is usually delayed for 24 hours or more after ingestion. This is well past the time when a prophylactic antidote, acetylcysteine, can be effectively administered. Acetylcysteine is highly effective in preventing liver damage, especially if administered within 8 to 10 hours after overdose, and improves survival in patients with hepatic failure when initiated 12 to 16 hours after overdose. Measurement of serum acetaminophen may also be used to estimate the drug elimination half-life. Serum half-life is recommended when the time of the ingestion is not known. Acetaminophen half-life is used to judge toxicity and may be a better predictor of hepatotoxicity than a single serum measurement.

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 at least 4 hours after drug ingestion to ensure the plasma or serum concentrations have peaked. b. Ingestion of massive quantities of acetaminophen or of a modified-release preparation may result in delayed peak serum acetaminophen levels. In such cases, repeated serum concentrations should be obtained. c. If the time of ingestion is not known, the acetaminophen half-life, an indicator of potential hepatotoxicity, may be estimated by drawing 2 or more blood samples at intervals of 2 to 3 hours.
	3. Volume: a. Minimum - 0.5 mL b. Sample Size (dead space excluded) – 3 uL
	4. Stability: a. Refrigerated (2 - 8°C): 14 days b. Frozen (<-20°C): 45 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 tox Acetaminophen 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 tox™ Acetaminophen Calibrators (ug/mL): 0, 10, 25, 50, 100, 200.
	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 sheep antibodies reactive to acetaminophen G6P NAD Non-sterile bovine serum albumin	N/A	Ready for use
R2 (Enzyme)	Acetaminophen labeled with bacterial G6PDH 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	28 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.

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>Deionized 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. 0.12 ug/mL b. This represents the lowest concentration of acetaminophen 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 tox™ Acetaminophen Assay when tested in the presence 50 ug/mL acetaminophen. Levels tested were at or above maximum physiological or pharmacological concentrations.

	<table><tr><th colspan="2">Compounds that Do Not Interfere</th></tr><tr><td>Acetaminophen cysteine</td><td>Cysteine</td></tr><tr><td>Acetaminophen glucuronide</td><td>Diazepam</td></tr><tr><td>Acetaminophen mercapturate</td><td>Methionine</td></tr><tr><td>Acetaminophen sulfate</td><td>Phenacetin</td></tr><tr><td>Acetylcysteine</td><td>Phenylephrine hydrochloride</td></tr><tr><td>Amitriptyline</td><td>Propoxyphene</td></tr><tr><td>Caffeine</td><td>Salicylic acid</td></tr><tr><td>Codeine</td><td>Secobarbital</td></tr></table>
B	**AMR & Reportable Range**
	AMR 10 - 200 ug/mL
	Reportable Range 10 - 2000 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 - 30 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**
	≥31 ug/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 Acetaminophen 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.
	6. Interpretive Comment: a. NCAL interpretive comment is automatically attached to each Acetaminophen result reported: "Acetaminophen levels must be interpreted in relation to the time since ingestion, the formulation (immediate or extended release), and acute vs chronic ingestion. Consult appropriate nomograms or the California Poison Control System."

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-2080 rev.3 AU 680 TDM Acetaminophen
2. Beckman Coulter Instructions for Use: tox Acetaminophen, CLSIOSR7A229.02 March 2012
3. Package Insert, May 2012