I. Purpose

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

A	Principle
	The Emit tox™ Salicylic Acid Assay is a homogeneous enzyme immunoassay technique used for the quantitative analysis of salicylic acid in human serum or plasma. This 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 can be 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
	Acetylsalicylic acid (aspirin) and other salicylates are rapidly metabolized to salicylic acid after ingestion. Aspirin is widely used for its analgesic, antipyretic, and anti-inflammatory properties. It is found in a number of over-the-counter and prescription medications, and as such can be inadvertently overdosed by adults or can cause accidental poisoning in children. Chronic salicylate poisoning (salicylism) occurs most commonly in seniors who regularly take large doses of aspirin, often for osteoarthritis and then gradually increase their doses or develop renal insufficiency. Signs and symptoms of salicylism include fever, vomiting, and tachypnea. Acute overdosage can cause nausea, vomiting, dehydration, hyperpnea, oliguria, and tinnitus. Severe poisoning can cause coma, convulsions, severe hyperpnea, and metabolic acidosis. In suspected acute overdose cases, determination of salicylic acid concentrations in serum helps determine the severity of toxicity and the steps toward detoxification. In children, accidental poisoning or serious intoxication occurs frequently and is sometimes fatal. Pediatric patients who are dehydrated are especially susceptible to salicylate intoxication. Additionally, in children with varicella infections or influenza-like illnesses, testing for serum salicylates may either implicate or rule out Reyes syndrome. For patients on chronic aspirin therapy, monitoring salicylic acid concentrations in serum, along with careful clinical assessment, is the most effective means of ensuring adequate therapy. Therapeutic ranges for salicylic acid are frequently very close to the levels associated with toxic manifestations. Salicylate concentrations in the blood generally correlate with both adverse and therapeutic effects.

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. Serum levels drawn less than 6 hours after a toxic dose can be used to confirm overdose. To use the Done nomogram to predict the severity of the toxic reaction, sample must be drawn at least 6 hours after ingestion of the toxic dose. Repeat testing within 2 - 3 hours is recommended to ensure that absorption is complete and to determine the effectiveness of the therapeutic intervention. b. After therapeutic doses of salicylates, peak levels are reached at 2 hours.
	3. Volume: a. Minimum - 0.5 mL b. Sample Size (dead space excluded) – 3 uL
	4. Stability: a. Refrigerated (2 - 8°C): 7 days b. Frozen (<-20°C): 6 months
	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 Salicylic Acid 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™ Salicylic Acid Calibrators (mg/dL) :0, 5, 10, 20, 40, 80.
	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. The Emit tox™ Salicylic Acid Calibrators are light sensitive. i. Store calibrators at 2-8°C, upright, in their original brown containers 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 salicyclic acid G6P NAD Preservatives, including 0.1% sodium azide and stabilizers	N/A	Ready for use
R2 (Enzyme)	Salicylic acid labeled bacterial 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	42 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. 0.2 mg/dL
   b. This represents the lowest concentration of Salicylic Acid that can be distinguished from 0 mg/dL with 95% confidence.

3. Specificity:
   a. The compounds listed in the following table do not interfere with the Emit tox™ Salicylic Acid Assay when tested in the presence of 30 mg/dL salicylic acid. Levels tested were at or above maximum physiological or pharmacological concentrations.

Compounds that Do Not Interfere	
Acetaminophen	Gentistic Acid
Acetylsalicylic Acid	Ibuprofren
p-Aminosalicylic Acid	Indomethacin
Benzoic Acid	Methyl Salicylate
Caffeine	Naproxen
Codeine	Salicylamide
Diflunisal	Salicylsalicylic Acid
2,3 Dihydroxybenzoic Acid	Salicyluric Acid
Fenoprofen	

B **AMR & Reportable Range**

AMR
2.5 - 80 mg/dL

Reportable Range
3 - 800 mg/dL
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 - 30 mg/dL

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 mg/dL

F **Early Notification Values**

None

G	**Result Reporting & Interpretation**
	1. Confirm ALL flags and indices are properly addressed before reporting any result.
	2. Report Salicylic Acid 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-2087 rev.4 AU 680 TDM Salicylates
2. Beckman Coulter Instructions for Use: tox Salicylic Acid, CLSIOSR7S229.02 March 2012
3. Salicylates Package Insert dated May 2012