I. Purpose

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

A	**Principle**
	The Emit® 2000 Gentamicin Plus 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 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**
	Gentamicin is an aminoglycoside antibiotic that acts by inhibiting bacterial protein synthesis. It is used to treat serious infections due to many aerobic and anaerobic types of bacteria. Monitoring gentamicin concentrations in serum, along with careful clinical assessment, is the most effective means of ensuring adequate therapy for the following reasons: 1. Gentamicin concentration in serum correlates better with antibacterial activity than does dosage. 2. A standard dose of gentamicin does not always yield a predictable serum level because drug concentration also depends on the patient's volume of distribution and on drug elimination. These factors are influenced by the mode of administration, the volume of extracellular fluid, renal function, and physiological changes during therapy. 3. Gentamicin has a narrow range of safe and effective serum 4. concentrations. 5. Exposure to high concentrations for a prolonged period may cause renal impairment or ototoxicity. 6. Patients with impaired renal function should be monitored closely while on gentamicin therapy because nephrotoxicity caused by gentamicin may be difficult to distinguish from the symptoms of underlying renal disease. In addition, patients with compromised renal function eliminate gentamicin more slowly than patients with normal renal function.

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. Collect a trough sample just before the next scheduled dose. When adjusting dosage, measure peak and trough levels during the same dosing interval.
	3. Volume: a. Minimum - 0.5 mL b. Sample Size (dead space excluded) – 2 uL
	4. Stability: a. Refrigerated (2 - 8°C): 6 weeks 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. Test immediately or store sample frozen: i. High concentrations of beta-lactam antibiotics (penicillins and cephalosporins) inactivate gentamicin in vivo and in vitro. ii. Analyze specimens containing beta-lactam antibiotic and gentamicin immediately upon receipt, or store them frozen to prevent in vitro inactivation and low quantitation. b. 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 Gentamicin 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 Gentamicin Calibrators (ug/mL) :0, 0.6, 2.0, 4.0, 6.0, 10.
	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 To Avoid Bacterial Growth or Water contamination: - Change diluent daily. - Thoroughly clean bottle weekly. To Avoid Contamination of Probes: - Ensure correct concentration of wash solution in the bottles. - Thoroughly clean wash solution bottles weekly.

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 gentamicin G6P NAD	N/A	Ready for use
	R2 (Enzyme)	Gentamicin labeled with G6PDH Tris buffer	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	49 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>X3</td><td>X3</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. Samples that contain gentamicin in combination with netilmicin or sisomicin cannot be reliably quantitated by this assay.
	3. High concentrations of beta-lactam antibiotics (penicillins and cephalosporins) inactivate gentamicin in vivo and in vitro.
	4. Sensitivity: a. 0.25 ug/mL b. This represents the lowest concentration of Gentamicin that can be distinguished from 0 ug/mL with 95% confidence.

5. Specificity:
    a. Netilmicin and sisomicin, aminoglycosides structurally related to gentamicin, cross-react significantly with this assay.
    b. Aminoglycosides are not generally co-administered in clinical practice, but more than one aminoglycoside may be present when switching from one treatment to another.
    c. Samples that contain gentamicin in combination with netilmicin or sisomicin cannot be reliably quantitated by this assay.
    d. The compounds listed in the following table do not interfere with the Emit 2000 Gentamicin Plus Assay when tested in the presence of 4.0 g/mL gentamicin. Levels tested were at or above maximum physiological or pharmacological concentrations.

Compound	Concentration Tested (g/mL)
Amikacin	500
Carbenicillin	500
Cephalothin	500
Chloramphenicol	500
Clindamycin	500
Erythromycin	500
Penicillin G	500*
Sulfamethoxazole	600
Tetracycline	100
Tobramycin	50
Trimethoprim	25

B **AMR & Reportable Range**

AMR
0.3 - 10.0 ug/mL

Reportable Range (Linear Limits in LIS)
0.3 - 30.0 ug/mL
Results outside the linear limits are reported as “<0.3 ug/mL”, or “>30.0 ug/mL”

C **Calculations**

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

D **Reference Range**

Peak: 5.0 - 10.0 ug/mL
Random: Not Established
Trough: 0.0 - 1.9 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**
	None
F	**Early Notification Values**
	Trough: ≥2.1 ug/mL
G	**Result Reporting & Interpretation**
	1. Confirm ALL flags and indices are properly addressed before reporting any result.
	2. Report Gentamicin results in ug/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. 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. RWLQC-2083 rev.4 AU 680 Gentamicin
2. Beckman Coulter Instructions for Use: Gentamicin Plus, CLSIOSR4T229.02 March 2012