TRAINING UPDATE

Lab Location:	SGMC	**Date Distributed:**	7/20/2021
Department:	Core Lab	**Due Date:**	8/20/2021

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Amikacin Assay by Atellica CH Analyzer SGMC.C3048 v2

Description of change(s):

Section	Reason
10.6	Added steps to program dilution

This revised SOP will be implemented on July 28, 2021

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	**Amikacin Assay by Atellica CH Analyzer**
Prepared by	Ashkan Chini Date: 4/30/2021
Owner	Robert SanLuis Date: 4/30/2021

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

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1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Amikacin, Peak	Atellica CH Analyzer	AMIKP
Amikacin, Trough		AMIKT
Amikacin, Random		AMKR

Synonyms/Abbreviations
Amikin

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Emit® Amikacin 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.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	**Trough:** Collect immediately before dose (within 30 minutes) **Peak:** Collect at end of a 60 minute IV fusion, or 30 minutes after end of 30 minute infusion, or 60 minutes after IM dose
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Serum None
Collection Container	Serum: Red top tube, Serum separator tube (SST)
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 7 days
	Refrigerated: 7 days
	Frozen: 7 days
Timing Considerations	N/A
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.
Compromising Physical Characteristics	Gross hemolysis. Reject sample and request a recollection. Credit the test with the appropriate LIS English text code explanation of HMT (Specimen markedly hemolyzed)
Other Considerations	Allow Red Top or SST to clot completely prior to centrifugation.

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration.

4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Amikacin	Siemens, Syva® Emit® Amikacin Assay Cat. No. 10445383
EMPTY1 Reagent Pack	Siemens Atellica CH, Cat. No. 11538114
EMPTY2 Reagent Pack	Siemens Atellica CH, Cat. No. 11538115

4.2 Reagent Preparation and Storage

Reagent	• Enzyme Reagent A • Antibody/Substrate Reagent B • Buffer Concentrate
Container	Glass vial
Storage	Store at 2-8°C
Stability	• **Unopened:** stable until expiration date printed on each vial • Working Reagent A remains stable at 2 - 8C for 12 weeks • Working Reagent B remains stable at 2 - 8C for 12 weeks • Buffer Solution remains stable at 20 - 25C for 12 weeks • **Pre-filled Empty Reagent Pack:** Onboard per well is 30 days
Reagent A & B Reconstitution	Reconstitute both Enzyme Reagent A and Antibody/Substrate Reagent B using 6 mL of reagent grade water. • Gently swirl the vial to dissolve the contents. • Allow to equilibrate at 20 - 25C for the minimum of 2 hours.
Working Buffer Solution Preparation	Make a 1:15 dilution of Buffer Concentrate using reagent grade water as the diluent. • Mix 4 mL of Buffer Concentrate with 56 mL of reagent grade water; invert gently to ensure it is homogenous. • Prepare two different Buffer Solutions, one for Working Reagent A and one for Working Reagent B.
1:9 Working A & B Reagent Preparation	Note: Use sterile containers labeled with reagent name, lot number, and expiration date for the following steps: 1. Working Reagent A: Make a 1:9 dilution of reconstituted Enzyme Reagent A using Buffer Solution as the diluent. Invert both the reconstituted Enzyme Reagent A vial and Buffer Solution gently to ensure they are homogenous. Mix 6 mL of reconstituted Enzyme Reagent A with 48 mL of Buffer Solution; invert gently to ensure it is homogenous. 2. Working Reagent B: Make a 1:9 dilution of reconstituted Antibody/Substrate Reagent B using Buffer Solution as the diluent. Invert both the reconstituted Antibody/Substrate Reagent B vial and Buffer Solution gently to ensure they are homogenous. Mix 6 mL of reconstituted Antibody/Substrate Reagent B with 48 mL of Buffer Solution; invert gently to ensure it is homogenous. **Note:** After preparation, allow the working reagents A and B to equilibrate at 2–8C for one hour before proceeding to the next step. Transfer reagents into empty reagent packs according to the table below. Try to avoid bubbles as much as possible. Label the

	reagent packs with reagent name, lot number, date prepared, and expiration date.

Amikacin Reagent	Empty Reagent	Volume per Well	Tests per Well
Reagent A	EMPTY1, Well 1 (W1)	8.2 mL	75
Reagent B	EMPTY2, Well 1 (W1)	8.2 mL	75

4.3 Loading Pre-filled Empty Reagent Pack on Atellica CH

Note: Load one set of Empty Reagent Pack on board at a time. Since there is no way to differentiate between empty flexes on board, operator must load one set of Empty Reagent Pack and identify them before loading the next set.

Load one set of Empty Reagent Pack on board. On the Atellica CH screen the reagent picture will generate a red flag. Select the reagent picture highlighted in red to select the method and lot number.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Amikacin Calibrator	Siemens, Syva® Emit® Amikacin Assay, Cat. No. 10445383

Note: Reagents A and B and calibrators are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

5.2 Calibrator Preparation and Storage

Calibrator	Amikacin Calibrator
Preparation	Reconstitute each calibrator vial using 1 mL of reagent grade water, gently swirl the vial to dissolve the contents. Allow to equilibrate at 20-25C for the minimum of 2 hours. **Note:** Calibrator Level 0 is not required for calibration. It is used as a diluent for samples with values > 50 µg/mL. Refer to section 10.6.
Storage/Stability	• **Unopened vials:** stable until the expiration date on the vial • **Reconstituted:** stable at 2-8C for 12 weeks

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Amikacin Calibrator

Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in µg/mL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (19 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (19 days), for calibrated reagent packs on the system. • When indicated by quality control results. • After major maintenance or service. At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.
Calibration Scheme	See Package Insert for specific calibration scheme.
Procedure	Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions.

5.4 Tolerance Limits

IF……	THEN……
If result fall within assay-specific specification, and QC values are within acceptable limits,	proceed with analysis
If result falls outside assay-specific specification, or QC values are out of Acceptable limits,	troubleshoot the assay and/or instrument and repeat calibration

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
InteliQ Immunoassay Plus Controls, Levels 1, 2 & 3	Bio-Rad Laboratories Cat. No. 12009948, 12009949, 12009950

6.2 Control Preparation and Storage

Control	InteliQ Immunoassay Plus Controls
Preparation	Allow to thaw at room temperature (18-25C) for approximately 60 minutes or until completely thawed. Once thawed, gently invert the tube several times to ensure homogeneity.
Storage/Stability	**Frozen**: until the expiration date if unopened at -20 to -70C **Thawed and Unopened:** 30 days at 2-8C **Thawed and Onboard:** 14 days at 2-8C Note: Stability for PSA and Folate is shorter.

6.3 Frequency

Analyze all levels of QC material after every calibration and each day of testing (notated on the QC frequency sheets posted on the instruments).

Refer to the Atellica QC Schedule and the Siemens Atellica Quick Reference Guide.

6.4 Tolerance Limits and Criteria for Acceptable QC

Step	Action
1	Acceptable ranges for QC are programmed into the instrument's Quality Control software system and Unity Real Time, and may be posted near the instrument for use during computer downtime.
2	**Run Rejection Criteria** • Anytime the established parameters are exceeded (if one QC result exceeds 2 SD), the run is considered out of control (failed) and patient results must not be reported. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program. • Corrective action documentation must follow the Laboratory Quality Control Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.

- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Siemens Atellica CH Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

- System Fluids
- Assorted calibrated pipettes (MLA or equivalent) and disposable tips

8. PROCEDURE

Atellica CH Amikacin is required to perform this test.

Amikacin is performed on the Atellica CH Analyzer after the method is calibrated and Quality Controls are acceptable.

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Instrument Set-up Protocol
1.	Perform any required instrument maintenance.
2.	Ensure that the instrument has sufficient primary and ancillary reagents.
3.	Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate.
4.	Check calibration status and re-calibrate as needed.

8.2	Specimen Testing
1.	Centrifuge the specimens.
2.	Load the sample in the Atellica rack and place the rack into the Sample Handler to initiate testing. **NOTE: If not equipped with an in-line decapper unit, samples must be de-capped prior to loading on the Atellica system
3.	Refer to the general operating procedure for detailed steps.
4.	Follow protocol in section 10.6 "Repeat Criteria and Resulting" for samples with results above the analytical measurement range (AMR). Investigate any flagged results and repeat as necessary.
5.	Append the appropriate English text code qualifier messages to any samples requiring a comment regarding sample quality and/or any other pertinent factors.

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

The instrument automatically calculates the concentration of Amikacin in µg/mL.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results up to one decimal point.

10.3 Units of Measure

µg/mL

10.4 Clinically Reportable Range (CRR)

2.5 – 100.0 µg/mL

10.5 Review Patient Data

Each result is reviewed for error messages. Resolve any problems noted before issuing patient reports.

10.6 Repeat Criteria and Resulting

All repeats must replicate the original result within the total allowable error (TEa) of the assay. Refer to TEa listing for specific information.

Values that fall below or within the AMR or CRR may be reported without repeat. Values that exceed the upper ranges must be repeated.

IF the result is …	THEN…
< 2.5 µg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 2.5 µg/mL
≥ 50.0 µg/mL	**Manual Dilution:** Make a two-fold (1:2) dilution **Diluent:** Emit® Amikacin Calibrator 0 To program the dilution: • Go to **Patient Order** tab • Click **Create Patient Orders** tab • Enter accession number and press **Enter** • Select the assay (test) from the list displayed • The test will default to x1 (undiluted) – uncheck this • Enter the manual dilution factor 2 in the appropriate field • Press **Enter** and print barcode The barcode has the dilution factor embedded in it and the instrument will do the calculation automatically. No multiplication is required on the user end. Label tube with barcode and load.
> 100.0 µg/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 100.0 µg/mL -REP" Bring to the attention of Tech in Charge (TIC) or Group Lead to check for integrity issues prior to release of results.

Message	Code
Verified by repeat analysis	Append –REP to the result.

11. EXPECTED VALUES

11.1 Reference Ranges

Random: None established
Peak: 20.0 – 30.0 µg/mL
Trough: 4.0 – 8.0 µg/mL

11.2 Critical Values

Random: > 30.0 µg/mL
Peak: > 30.0 µg/mL
Trough: > 8.0 µg/mL

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Monitoring Amikacin concentrations in serum is the most effective means of ensuring adequate therapy. Amikacin concentration in serum correlates better with antibacterial activity than does dosage. A standard dose of amikacin does not always yield a predictable serum level because the drug's 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. Patients with impaired renal function, dialysis patients, burn patients, and neonatal or elderly patients should be monitored closely. Exposure to high concentrations for a prolonged period may cause renal impairment or ototoxicity.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/Modified
- **Validated Test Modifications:** Specimen stabilities have been modified from the package insert based on in-house stability studies performed at Quest Diagnostics. (per *Amikacin by Siemens Immunoassay on the Beckman Coulter/Olympus AU 400/600/5400/2700 Series,* SOP ID QDTX722)

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

2.5 – 50.0 µg/mL

14.2 Precision

Within Run	Number of Replicates	Mean µg/mL	Coefficient of Variation (%)
1	20	9.2	8.6
2	20	9.9	3

14.3 Interfering Substances

Patient samples containing kanamycin will cause significant elevation of amikacin results.

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. Atellica Solutio QC Schedule
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Retention of Records and Materials (Lab Policy)
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)
10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of Amikacin Reagent

17. REFERENCES

1. Quest Diagnostics SOP, Amikacin by Siemens Immunoassay on the Beckman Coulter/Olympus AU 400/600/5400/2700 Series, SOP ID QDTX722.
2. Package Insert, Syva® Emit® Amicakin Reagent, Siemens Healthcare Diagnostics Inc., 07/2019.
3. Package Insert, Syva® Emit® Amicakin Application Sheet, Siemens Healthcare Diagnostics Inc., 11/2020.
4. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
1	7/19/21	10.6	Added steps to program dilution	H Genser	R SanLuis

19. ADDENDA

None