TRAINING UPDATE

Lab Location:	SGMC	**Date Distributed:**	5/26/2021
Department:	Core Lab	**Due Date:**	6/26/2021

DESCRIPTION OF PROCEDURES

Name of procedure:

SOP #	Title
SGMC.C3048	Amikacin Assay by Atellica CH Analyzer
SGMC.C3007	Carbamazepine (Carb) by Atellica CH Analyzer
SGMC.C3011	Digoxin (Dgn) by Atellica CH Analyzer
SGMC.C3013	Gentamicin (Gent) by Atellica CH Analyzer
SGMC.C3024	Lithium (Li) by Atellica CH Analyzer
SGMC.C3018	Phenobarbital (Phnb) by Atellica CH Analyzer
SGMC.C3067	Phenytoin (Phny) by Atellica CH Analyzer
SGMC.C3015	Theophylline (Theo) by Atellica CH Analyzer
SGMC.C3014	Tobramycin (Tob) by Atellica CH Analyzer
SGMC.C3016	Valproic Acid (VPA) by Atellica CH Analyzer
SGMC.C3017	Vancomycin (Vanc) by Atellica CH Analyzer

Description of change(s):

These are the new assay SOPs for the Atellica Solution analyzers. Core technical staff must review and be familiar with -

- Specimen requirements
- Reagent, calibrator & QC stability and storage
- Ranges and dilutions

These SOPs were implemented on May19, 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.		

TABLE OF CONTENTS

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 Siemens Atellica QC Schedule and in 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:** Using the primary tube, make the smallest dilution possible to bring the raw data within the AMR. Maximum allowable dilution: x 2 **Diluent:** Emit® Amikacin Calibrator 0. Enter dilution factor as a whole number.
> 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. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
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

19. ADDENDA

None

Technical SOP

Title	**Carbamazepine (Carb) by Atellica CH Analyzer**
Prepared by	Ashkan Chini Date: 4/27/2021
Owner	Robert SanLuis Date: 4/27/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Carbamazepine	Atellica CH Analyzer	CRBM

Synonyms/Abbreviations
Tegretol

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The methodology for Carb involves a homogeneous particle-enhanced turbidimetric inhibition immunoassay (PETINIA) technique which uses a synthetic particle-carbamazepine conjugate (PR) and carbamazepine-specific, monoclonal antibody (Ab). Carbamazepine present in the sample competes with the particles for the antibody, thereby decreasing the rate of aggregation. Hence, the rate of aggregation is inversely proportional to the concentration of carbamazepine in the sample. The rate of aggregation is measured using bichromatic turbidimetric readings at 545/694 nm.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Plasma (Lithium Heparin) Serum
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)

Criteria	
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: 8 hours
	Refrigerated: 2 days
	Frozen: 30 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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Carbamazepine (Carb)	Siemens, Atellica CH, Cat. No. 11097515

4.2 Reagent Preparation and Storage

Reagent	Carbamazepine (Carb)
Storage	Store at 2-8°C
Stability	Reagents are stable onboard the system for 30 days per pack.
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
DRUG CAL II	Siemens Atellica CH, Cat. No. 11099405

5.2 Calibrator Preparation and Storage

Calibrator	DRUG CAL II
Preparation	Calibrators are ready to use. Allow to equilibrate to room temperature and mix thoroughly before use.
Storage/Stability	• Store at 2-8°C • **Unopened:** stable until expiration date stamped on the box. • **Opened:** remains stable for 30 days when recapped immediately after use.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	DRUG CAL II
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 (30 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (7 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 Siemens 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.

Step	Action
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 Carbamazepine (Carb) is required to perform this test.

Carbamazepine 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.

8.2	Specimen Testing
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 Carbamazepine 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)

0.4 – 40.0 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…
< 0.4 µg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.4 µg/mL
≥ 20.0 µg/mL	**On Board Automated Dilution:** Results ≥ 20.0 µg/mL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 40.0 µg/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 40.0 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

4.0 – 12.0 µg/mL

11.2 Critical Values

> 14.9 µg/mL

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Carbamazepine is a useful drug in the control of certain types of epilepsy. It is chemically different from other anti-convulsant agents and may achieve seizure control where other drugs have failed. Absorption from the intestine and metabolism by the liver is highly variable. Assay of plasma drug levels is useful in establishing maintenance dosage, determining compliance, and in evaluating possible toxic side effects. Carbamazepine is metabolized in the liver to form carbamazepine-10,11 epoxide which also has anti-convulsant action. The ratio of carbamazepine to epoxycarbamazepine in a patient on long term therapy is about seven to one, indicating that most of the plasma drug is the parent compound. Both carbamazepine and its metabolite are polar and not excreted in substantial quantities in either urine or bile. Further metabolism to dihydrodihydroxycarbamazepine occurs, and some of the drug is excreted in this form. The remainder of the excreted metabolic products have not

been identified. The biologic half-life appears to be highly variable from one individual to another and even within one individual. A low plasma carbamazepine value would exclude toxicity, while a high value might help to pinpoint the cause.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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)

0.4 – 20.0 µg/mL

14.2 Precision

Material	Mean µg/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum Pool A	3.2	0.05	1.5
Plasma Pool B	6.4	0.06	1.0
Plasma Pool	16.5	0.13	0.8

14.3 Interfering Substances

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	µg/mL	Bias %
Hemoglobin	500 mg/dL	3.0	1
Bilirubin (conjugated)	20 mg/dL	2.9	0
Bilirubin (unconjugated)	20 mg/dL	2.9	0
Lipemia Intralipid®	800 mg/dL	2.9	8

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) < 0.8 µg/mL and limit of detection LoD) ≤ 0.8 µg/mL. The LoD corresponds to the lowest concentration of carbamazepine that can be detected with a probability of 95%. The LoD for the Atellica CH Carb assay is 0.4 µg/mL, and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 0.3 µg/mL.

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.

Atellica Carb reagent may cause an allergic skin reaction. Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Wash contaminated clothing before reuse.
Contains: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2Hisothiazol-3-one (3:1) (R1, R2, and R3)

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
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 Carbamazepine Reagent

17. REFERENCES

1. Package Insert, Carbamazepine Reagent, Siemens Healthcare Diagnostics Inc., 07/2019.
2. Package Insert, DRUG CAL II, Siemens Healthcare Diagnostics Inc., 07/2019.
3. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Digoxin (Dgn) by Atellica CH Analyzer**
Prepared by	Ashkan Chini Date: 4/27/2021
Owner	Robert SanLuis Date: 4/27/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Digoxin	Atellica CH Analyzer	DIG

Synonyms/Abbreviations
Lanoxin

Department
Chemistry

2. ANALYTICAL PRINCIPLE

When digoxin is present in the sample, it competes with the digoxin-latex complex for the anti-digoxin antibody, thereby inhibiting the formation of the agglutination complex. The rate of agglutination is inversely proportional to the concentration of digoxin in the sample. By monitoring the change in scattered light as a change in absorbance at 694 nm, a concentration curve is obtained. The actual change in absorbance at 694 nm is inversely proportional to the concentration of digoxin in the sample.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Collect at any time requested by a physician, or at least 6-8 hours after last dose, regardless of route of administration (optimally 12-24 hours after a dose)
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Plasma (Lithium Heparin) Serum
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL

Criteria	
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 8 hours
	Refrigerated: 7 days
	Frozen: 6 months
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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Digoxin (Dgn)	Siemens, Atellica CH, Cat. No. 11097526

4.2 Reagent Preparation and Storage

Reagent	Digoxin (Dgn)
Storage	Store at 2-8°C
Stability	Onboard per well: 30 days
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
TDM Calibrator (TDM CAL)	Siemens Atellica CH, Cat. No. 11099439

5.2 Calibrator Preparation and Storage

Calibrator	TDM Calibrator (TDM CAL)
Preparation	1. Open each vial carefully. 2. Add 3.0 mL of reagent grade water into each vial using a calibrated pipette. Replace stopper. 3. Let the vials stand for 30 minutes at room temperature to allow the lyophilized material to dissolve. 4. Prior to use, to ensure homogeneity and to avoid foam formation, mix the contents by gently inverting the vials.
Storage/Stability	• Store at 2-8°C • Protect from heat and light sources. • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** remains stable for 7 days.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	TDM Calibrator (TDM CAL)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in ng/mL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (60 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (7 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 Siemens 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.

Step	Action
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 Digoxin (Dgn) is required to perform this test.

Digoxin 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.

8.2	Specimen Testing
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 Digoxin in ng/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 two decimal points.

10.3 Units of Measure

ng/mL

10.4 Clinically Reportable Range (CRR)

0.14 – 10.00 ng/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…
< 0.14 ng/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.14 ng/mL
≥ 5.00 ng/mL	**On Board Automated Dilution:** Results ≥ 5.00 ng/mL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 10.00 ng/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 10.00 ng/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

0.80 – 1.99 ng/mL

11.2 Critical Values

> 1.99 ng/mL

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Digoxin, a cardiac glycoside, is used as an antiarrhythmic agent, both alone and in conjunction with other drugs. Absorption from the gastrointestinal tract is variable: 60-80% of the administered dose is absorbed. Digoxin is excreted by the kidney almost entirely unchanged. Therefore, the patient's renal function is an important consideration in determining dosage. In persons with normal kidney function the half-life is about 1.5 days. The most serious complications of digoxin toxicity are ventricular arrhythmias: ventricular tachycardia and ventricular fibrillation.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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)

0.14 – 5.00 ng/mL

14.2 Precision

Material	Mean ng/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
QC	0.48	0.022	4.6
QC	1.51	0.036	2.4
Serum	2.68	0.086	3.2
Plasma	4.20	0.077	1.8

14.3 Interfering Substances

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	ng/mL	Bias %
Hemoglobin	500 mg/dL	0.79	8
Bilirubin (conjugated)	60 mg/dL	0.82	-6
Bilirubin (unconjugated)	60 mg/dL	0.73	7
Lipemia Intralipid®	399 mg/dL	0.77	9
Lipemia (triglycerides)	250 mg/dL	0.74	7

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) ≤ limit of detection (LoD) and LoD ≤ 0.14 ng/mL. The LoD corresponds to the lowest concentration of digoxin that can be detected with a probability of 95%. The LoD for the Atellica CH Dgn assay is 0.14 ng/mL, and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 0.08 ng/mL.

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. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
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 Digoxin Reagent

17. REFERENCES

1. Package Insert, Digoxin Reagent, Siemens Healthcare Diagnostics Inc., 11/2019.
2. Package Insert, TDM Calibrator (TDM CAL), Siemens Healthcare Diagnostics Inc., 07/2019.
3. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Gentamicin (Gent) by Atellica CH Analyzer**	
Prepared by	Ashkan Chini	Date: 4/27/2021
Owner	Robert SanLuis	Date: 4/27/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Gentamicin, Trough	Atellica CH Analyzer	GENTT
Gentamicin, Peak		GENTP
Gentamicin, Random		GENR

Synonyms/Abbreviations
GENT, GENTA

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The methodology for Atellica CH Gent involves a homogeneous particle-enhanced turbidimetric inhibition immunoassay (PETINIA) technique which uses a synthetic particlegentamicin conjugate (PR) and gentamicin-specific monoclonal antibody (Ab). Gentamicin present in the sample competes with the particles for the antibody, thereby decreasing the rate of aggregation. Hence, the rate of aggregation is inversely proportional to the concentration of gentamicin in the sample. The rate of aggregation is measured using bichromatic turbidimetric readings at 545/694 nm.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	**Trough**: Collect immediately prior to the next dose (within 30 minutes). Verify with the nurse in charge of the patient that the next dose has not yet been given. **Peak**: Collect thirty (30) minutes after completion of infusion or ninety (90) minutes after injection. An additional collection label CRN will print with each orderable. The tube type translation is “SEE RN”. It is solely used as a reminder for phlebotomy to first check with Nurse prior to collection.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Plasma (Lithium Heparin) Serum
Collection Container	Plasma: Mint green top tube (PST) 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: 8 hours
	Refrigerated: 2 days
	Frozen: 30 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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Gentamicin (Gent)	Siemens, Atellica CH, Cat. No. 11097516

4.2 Reagent Preparation and Storage

Reagent	**Gentamicin (Gent)**
Storage	Store at 2-8°C
Stability	Reagents are stable onboard the system for 30 days per pack.
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
DRUG CAL II	Siemens Atellica CH, Cat. No. 11099405

5.2 Calibrator Preparation and Storage

Calibrator	DRUG CAL II
Preparation	Calibrators are ready to use. Allow to equilibrate to room temperature and mix thoroughly before use.
Storage/Stability	• Store at 2-8°C • **Unopened:** stable until expiration date stamped on the box. • **Opened:** remains stable for 30 days when recapped immediately after use.

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	DRUG CAL II
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 (30 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (7 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 Siemens 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.

Step	Action
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 Gentamicin (Gent) is required to perform this test.

Gentamicin 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.

8.2	Specimen Testing
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 Gentamicin 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)

0.5 – 24.0 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…
< 0.5 µg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.5 µg/mL
≥ 12.0 µg/mL	**On Board Automated Dilution:** Results ≥ 12.0 µg/mL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 24.0 µg/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 24.0 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

Gentamicin Random None established
Gentamicin Peak 4.0 – 8.0 µg/mL
Gentamicin Trough 0.3 – 1.9 µg/mL

11.2 Critical Values

Gentamicin Random: > 11.9 µg/mL
Gentamicin Peak > 11.9 µg/mL
Gentamicin Trough > 1.9 µg/mL

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Gentamicin is an antibiotic effective against gram negative aerobic bacteria. It has a wide spectrum of antibiotic activity and relatively low toxicity. Gentamicin is a naturally occurring antibiotic produced by the organism Micromonospora purpurea. Gentamicin is administered either intramuscularly or intravenously. Peak concentrations are reached 60 minutes after intramuscular injection and after completion of intravenous injection.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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)

0.5 – 12.0 µg/mL

14.2 Precision

Material	Mean µg/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum Pool	1.5	0.08	5.2
Plasma Pool	4.2	0.07	1.7
Control 1	7.8	0.13	1.7

14.3 Interfering Substances

Aminoglycosides structurally similar to gentamicin (e.g. netilimicin, sagamicin, and sisomicin) may significantly cross-react with this assay.

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	µg/mL	Bias %
Hemoglobin	500 mg/dL	4.1	1
Bilirubin (conjugated)	20 mg/dL	4.1	2
Bilirubin (unconjugated)	20 mg/dL	4.1	-1
Lipemia Intralipid®	1000 mg/dL	4.2	4

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) < limit of detection (LoD) and LoD ≤ 0.5 µg/mL. The LoD corresponds to the lowest concentration of Gentamicin that can be detected with a probability of 95%. The LoD for the Atellica CH Gent assay is 0.4 µg/mL, and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 0.3 µg/mL.

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.

Atellica GENT reagent may cause an allergic skin reaction. Wear protective gloves/ protective clothing /eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Wash contaminated clothing before reuse.
Contains: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2Hisothiazol-3-one (3:1) (P1 and P2)

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
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 Gentamicin Reagent

17. REFERENCES

1. Package Insert, Gentamicin Reagent, Siemens Healthcare Diagnostics Inc., 07/2019.
2. Package Insert, DRUG CAL II, Siemens Healthcare Diagnostics Inc., 07/2019.
3. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Lithium (Li) by Atellica CH Analyzer**
Prepared by	Ashkan Chini Date: 4/28/2021
Owner	Robert SanLuis Date: 4/28/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Lithium	Atellica CH Analyzer	LI

Synonyms/Abbreviations
LI

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica CH Li assay is a colorimetric endpoint chemistry. The concentration of lithium in the sample is proportional to the increase in absorbance, which is due to formation of a lithium complex. The reaction absorbance is measured at 505/694 nm.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Collect specimen 8 to 12 hours after evening dose and before morning dose. Random samples are acceptable.
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: 24 hours
	Refrigerated: 7 days
	Frozen: 180 days

Criteria	
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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Lithium (Li)	Siemens, Atellica CH, Cat. No. 11097535

4.2 Reagent Preparation and Storage

Reagent	**Lithium (Li)**
Storage	Store at 2-8°C
Stability	Onboard per well: 13 days
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Chemistry Calibrator (CHEM CAL)	Siemens Atellica , Cat. No. 11099411

5.2 Calibrator Preparation and Storage

Calibrator	Chemistry Calibrator (CHEM CAL)
Preparation	1. Shake to break up lyophilized cake. 2. Open each vial carefully. 3. Using a calibrated pipette, add exactly 3.0 mL of reagent grade water into the vial. Replace the stopper. 4. Manually mix by inverting 10 times every 10 minutes for a period of 30 minutes, or until reconstitution is complete. 5. Prior to use, mix by inversion at least 5 times to ensure homogeneity. 6. Refrigerate any unused material. Prior to reuse, mix contents thoroughly.
Storage/Stability	• Protect from heat and light sources. • Store at 2-8°C • **Unopened:** stable until expiration date stamped on box. • **Reconstituted:** remains stable for 48 hours

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Chemistry Calibrator (CHEM CAL)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in mmol/L
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (63 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (4 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 Assayed Multiqual Control Levels 1 & 3	Bio-Rad Laboratories Cat. No. 12008256, 12008258

6.2 Control Preparation and Storage

Control	InteliQ Assayed Multiqual Control Levels 1 & 3
Preparation	Allow to stand at room temperature (18-25C) until completely thawed but not more than one (1) hour. Once thawed, gently invert 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 for Lithium **Thawed and Opened**: 14 days at 2-8C for Lithium Note: stability varies by assay

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 Siemens 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.

Step	Action
	• 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 Lithium (Li) is required to perform this test.

Lithium 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.

8.2	Specimen Testing
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 Lithium in mmol/L.

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 two decimal points.

10.3 Units of Measure

mmol/L

10.4 Clinically Reportable Range (CRR)

0.10 – 6.00 mmol/L

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…
< 0.10 mmol/L	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.10 mmol/L

IF the result is …	THEN…
≥ 3.00 mmol/L	**On Board Automated Dilution:** Results ≥ 3.00 mmol/L will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 6.00 mmol/L	If the recommended dilution does not give results within the clinically reportable range, report as: "> 6.00 mmol/L -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

0.60 – 1.20 mmol/L

11.2 Critical Values

> 2.10 mmol/L

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Lithium is used primarily to treat the manic phase of affective disorders, mania, and manic-depressive illness. The precise mechanism of action of lithium as a mood-stabilizing agent is not known. Lithium is administered in capsule, syrup, or tablet form as salts of either carbonate or citrate. It is readily absorbed from the gastrointestinal tract and does not bind appreciably to plasma proteins. Peak plasma concentrations are reached 2 to 4 hours after oral administrations. Approximately 95% of a single dose of lithium is excreted in the urine within 6 to 12 hours, with the remainder being slowly excreted over the next 10 to 14 days. Lithium concentrations are monitored to ensure patient compliance and prevent toxicity. Because there is a narrow therapeutic range of about 0.60 – 1.20 mmol/L, with significant risk of toxicity occurring above 1.5 mmol/L, determination of lithium concentration is crucial in the management of patients on lithium therapy. Since plasma values vary relative to time of last dose, a standardized 12-hour post-dose serum lithium concentration has been recommended to assess adequate therapy.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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)

0.10 – 3.00 mmol/L

14.2 Precision

Material	Mean mmol/L	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum	0.91	0.011	1.2
Serum QC	1.99	0.012	0.6
Plasma	2.76	0.015	0.5

14.3 Interfering Substances

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	mmol/L	Bias %
Hemoglobin	750 mg/dL	1.01	7
Bilirubin (conjugated)	40 mg/dL	0.97	-8
Bilirubin (unconjugated)	40 mg/dL	1.02	-8
Lipemia Intralipid®	500 mg/dL	1.04	-4

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) < limit of detection (LoD) and LoD ≤ 0.10 mmol/L. The LoD for the Atellica CH Li assay is 0.07 mmol/L, and a LoB of 0.05 mmol/L.

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.

Atellica Li reagent may be corrosive to metals. Causes severe skin burns and eye damage. Suspected of damaging the unborn child. Obtain special instructions before use. Do not

handle until all safety precautions have been read and understood. Wear protective gloves/protective clothing/eye protection/face protection. IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Immediately call a POISON CENTER or doctor/physician. IF SWALLOWED: rinse mouth. Do NOT induce vomiting. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF exposed or concerned: Get medical advice/attention. Absorb spillage to prevent material damage.
Contains: Potassium hydroxide; 2-(2-Methoxyethoxy) ethanol

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
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 Lithium Reagent

17. REFERENCES

1. Package Insert, Lithium Reagent, Siemens Healthcare Diagnostics Inc., 10/2020.
2. Package Insert, Chemistry Calibrator (CHEM CAL), Siemens Healthcare Diagnostics Inc., 04/2020.
3. Package Insert, InteliQ Assayed Multiqual Controls, Bio-Rad Laboratories, 07/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Phenobarbital (Phnb) by Atellica CH Analyzer**
Prepared by	Ashkan Chini Date: 4/27/2021
Owner	Robert SanLuis Date: 4/27/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Phenobarbital	Atellica CH Analyzer	PHENB

Synonyms/Abbreviations
PHNO, Phnb

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The phenobarbital assay is a homogeneous particle-enhanced turbidimetric inhibition immunoassay (PETINIA) technique which uses a synthetic particle-phenobarbital reagent (PR) and phenobarbital-specific monoclonal antibody (Ab). Phenobarbital present in the sample competes with the particles for the antibody, thereby decreasing the rate of aggregation. Hence, the rate of aggregation is inversely proportional to the concentration of phenobarbital in the sample. The rate of aggregation is measured using bichromatic turbidimetric readings at 545/694 nm.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method. **Trough**: collect just before the next dose.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred	Plasma (Lithium Heparin)
-Other Acceptable	Serum

Criteria	
Collection Container	Plasma: Mint green top tube (PST) 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: 8 hours
	Refrigerated: 2 days
	Frozen: 30 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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Phenobarbital (Phnb)	Siemens, Atellica CH, Cat. No. 11097514

4.2 Reagent Preparation and Storage

Reagent	Phenobarbital (Phnb)
Storage	Store at 2-8°C

Stability	Reagents are stable onboard the system for 30 days per pack.
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
DRUG CAL	Siemens Atellica CH, Cat. No. 11099336

5.2 Calibrator Preparation and Storage

Calibrator	DRUG CAL
Preparation	Allow to equilibrate to room temperature and mix thoroughly before use.
Storage/Stability	• Store at 2-8°C • **Unopened:** stable until expiration date stamped on the box. • **Opened:** 90 days when recapped immediately after use.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	DRUG CAL
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 (30 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (7 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.

5.5 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 Siemens 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.

Step	Action
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 Phenobarbital (Phnb) is required to perform this test.

Phenobarbital 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.

8.2	Specimen Testing
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 Phenobarbital 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)

3.0 – 160.0 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…
< 3.0 µg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 3.0 µg/mL
≥ 80.0 µg/mL	**On Board Automated Dilution:** Results ≥ 80.0 µg/mL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 160.0 µg/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 160.0 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

15.0 – 40.0 µg/mL

11.2 Critical Values

> 49.9 µg/mL

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

PHNO test results are used in monitoring levels of phenobarbital, an anti-epileptic drug and sedative-hypnotic drug, to ensure appropriate therapy and in the diagnosis and treatment of Phenobarbital overdose. Phenobarbital is particularly useful in grand mal and focal seizures and in seizures due to withdrawal of alcohol or barbiturates. Petit mal and psychomotor seizures do not respond to this drug and may be worsened.
Because of considerable inter-individual variation and the limited capacity for the liver to metabolize phenobarbital, blood concentrations should be monitored to obtain maximal anti-seizure effect. Once metabolism is saturated, small dosage changes may result in disproportionate changes in blood concentration and can cause wide variations in dosage requirements among patients.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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)

3.0 – 80.0 µg/mL

14.2 Precision

Material	Mean µg/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum Pool	14.1	0.20	1.4
Plasma Pool	32.0	0.38	1.2
Serum Pool	47.0	0.46	1.0
Serum Pool	67.6	0.61	0.9

14.3 Interfering Substances

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	µg/mL	Bias %
Hemoglobin	500 mg/dL	5.2	3
Bilirubin (conjugated)	80 mg/dL	5.0	6
Bilirubin (unconjugated)	80 mg/dL	5.6	2
Lipemia Intralipid®	200 mg/dL	4.7	10

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) < limit of detection (LoD) and LoD ≤ 3.0 µg/mL. The LoD corresponds to the lowest concentration of phenobarbital that can be detected with a probability of 95%. The LoD for the Atellica CH Phnb assay is 2.0 µg/mL, and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 1.5 µg/mL.

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.

Atellica Phnb reagent may cause an allergic skin reaction. Wear protective gloves/protective clothing/eye protection/face protection. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Wash contaminated clothing before reuse.

Contains: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (R1, R2, and R3)

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
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 Phenobarbital Reagent

17. REFERENCES

1. Package Insert, Phenobarbital Reagent, Siemens Healthcare Diagnostics Inc., 11/2019.
2. Package Insert, DRUG CAL, Siemens Healthcare Diagnostics Inc., 07/2019.
3. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Phenytoin (Phny) by Atellica CH Analyzer**
Prepared by	Ashkan Chini Date: 4/27/2021
Owner	Robert SanLuis Date: 4/27/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Phenytoin	Atellica CH Analyzer	PTN

Synonyms/Abbreviations
Dilantin, Phny

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica CH Phenytoin assay is a homogeneous particle enhanced turbidimetric inhibition immunoassay (PETINIA) technique which uses a synthetic particle-phenytoin reagent (PR) and phenytoin-specific monoclonal antibody (AB). Phenytoin present in the sample competes with the particles for the antibody, thereby decreasing the rate of aggregation. Hence, the rate of aggregation is inversely proportional to the concentration of phenytoin in the sample. The rate of aggregation is measured using bichromatic turbidimetric readings at 545 and 694 nm.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	**Peak**: Two hours after completion of IV loading does and eight hours after oral loading dose. **Trough**: Immediately before dose (within thirty minutes)
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Plasma (Lithium Heparin) Serum
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)

Criteria	
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: 24 hours
	Refrigerated: 2 days
	Frozen: 5 months
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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Phenytoin (Phny)	Siemens, Atellica CH, Cat. No. 11097510

4.2 Reagent Preparation and Storage

Reagent	**Phenytoin (Phny)**
Storage	Store at 2-8°C
Stability	Reagents are stable onboard the system for 30 days per pack.
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
DRUG CAL	Siemens Atellica CH, Cat. No. 11099336

5.2 Calibrator Preparation and Storage

Calibrator	DRUG CAL
Preparation	Allow to equilibrate to room temperature and mix thoroughly before use.
Storage/Stability	• Store at 2-8°C • **Unopened:** stable until expiration date stamped on the box. • **Opened:** 90 days when recapped immediately after use.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	DRUG CAL
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 (28 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (7 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 Siemens 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.

Step	Action
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 Phenytoin (Phny) is required to perform this test.

Phenytoin 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.

8.2	Specimen Testing
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 Phenytoin 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)

2.0 – 80.0 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…
< 2.0 µg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 2.0 µg/mL
≥ 40.0 µg/mL	**Manual Dilution:** Make a two-fold (1:2) dilution **Diluent**: level 1 calibrator as diluent 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
> 80.0 µg/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 80.0 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

10.0 – 20.0 µg/mL

11.2 Critical Values

> 29.9 µg/mL

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Phenytoin is widely used and effective for all types of seizure disorders except, absence seizures. It is of value in the treatment of elementary partial (focal) or complex partial epilepsy (psychomotor, temporal lobe) seizures, but ineffective in petit mal epilepsy.

Occasionally, it is used in treatment of cardiac arrhythmias. Because of considerable inter-individual variation and the limited capacity for the liver to metabolize phenytoin, blood concentrations should be monitored to obtain maximal anti-seizure effect. Once metabolism is saturated, small dosage changes may result in disproportionate changes in blood concentration and can cause wide variations in dosage requirements among patients.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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)

2.0 – 40.0 µg/mL

14.2 Precision

Material	Mean µg/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum Pool	8.2	0.18	2.2
QC	19.7	0.27	1.4
Plasma Pool	35.6	0.38	1.1

14.3 Interfering Substances

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	µg/mL	Bias %
Hemoglobin	300 mg/dL	2.9	9
Bilirubin (conjugated)	20 mg/dL	2.9	4
Bilirubin (unconjugated)	20 mg/dL	3.1	3
Lipemia Intralipid®	250 mg/dL	3.0	7

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) < limit of detection (LoD) and LoD ≤ 1.0 µg/mL. The LoD corresponds to the lowest concentration of phenytoin (dilantin, diphenylhydantoin) that can be detected with a probability of 95%. The

LoD for the Atellica CH Phny assay is 0.8 µg/mL, and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 0.4 µg/mL.

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.

Atellica Phny reagent may cause an allergic skin reaction. Wear protective gloves/protective clothing/eye protection/face protection. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Wash contaminated clothing before reuse.
Contains: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (R1, R2, and R3)

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
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 Phenytoin Reagent

17. REFERENCES

1. Package Insert, Phenytoin Reagent, Siemens Healthcare Diagnostics Inc., 07/2019.
2. Package Insert, DRUG CAL, Siemens Healthcare Diagnostics Inc., 07/2019.
3. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Theophylline (Theo) by Atellica CH Analyzer**
Prepared by	Ashkan Chini Date: 4/27/2021
Owner	Robert SanLuis Date: 4/27/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Theophylline	Atellica CH Analyzer	THEO

Synonyms/Abbreviations
Aminophylline, THEO

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica CH Theo assay is a homogeneous particle-enhanced turbidimetric inhibition immunoassay (PETINIA) technique which uses a synthetic particle-theophylline conjugate (PR) and theophylline-specific monoclonal antibody (Ab). Theophylline present in the sample competes with the particles for the antibody, thereby decreasing the rate of aggregation. Hence, the rate of aggregation is inversely proportional to the concentration of theophylline in the sample. The rate of aggregation is measured using a turbidimetric rate at 545/694 nm.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	After IV infusion loading dose and anytime during infusion **Trough:** Immediately before dose (within thirty minutes). **Peak:** Two hours after immediate release oral dose and four hours after sustained release oral dose.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Plasma (Lithium Heparin) Serum
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)

Criteria	
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: 8 hours
	Refrigerated: 7 days
	Frozen: 90 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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Theophylline (Theo)	Siemens, Atellica CH, Cat. No. 11097513

4.2 Reagent Preparation and Storage

Reagent	**Theophylline (Theo)**
Storage	Store at 2-8°C
Stability	Reagents are stable onboard they system for 30 days per pack.
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
DRUG CAL	Siemens Atellica CH, Cat. No. 11099336

5.2 Calibrator Preparation and Storage

Calibrator	DRUG CAL
Preparation	Allow to equilibrate to room temperature and mix thoroughly before use.
Storage/Stability	• Store at 2-8°C • **Unopened:** stable until expiration date stamped on the box. • **Opened:** 90 days when recapped immediately after use.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	DRUG CAL
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 (30 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (7 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 Siemens 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.

Step	Action
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 Theophylline (Theo) is required to perform this test.

Theophylline 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.

8.2	Specimen Testing
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 Theophylline 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)

2.0 – 80.0 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…
< 2.0 µg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 2.0 µg/mL
≥ 40.0 µg/mL	**On Board Automated Dilution:** Results ≥ 40.0 µg/mL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 80.0 µg/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 80.0 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

10.0 – 20.0 µg/mL

11.2 Critical Values

> 19.9 µg/mL

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Theophylline measurements may be used in the diagnosis and treatment of theophylline overdose, and in therapeutic drug monitoring. Theophylline is a methylated xanthine, 1,3-dimethylxanthine. It is structurally related to purines and uric acid, as well as to xanthine itself. The most commonly used compound is aminophylline, the double salt of theophylline and ethylenediamine. About 10% is excreted unchanged in the urine and the remaining 90% of the drug is converted to other compounds before it is eliminated from the body. The biologic half-life of theophylline varies from about 3.5 hours in young children to 8 – 9 hours in most adults. It is substantially prolonged in the presence of liver disease and/or cardiac decompensation.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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)

2.0 – 40.0 µg/mL

14.2 Precision

Material	Mean µg/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum Pool	7.0	0.1	1.4
Control 1	14.8	0.1	0.9
Plasma Pool	27.4	0.4	1.5

14.3 Interfering Substances

A theophylline metabolite, 1,3-dimethyl uric acid, is usually undetectable in samples from patients receiving theophylline. However, it can reach detectable levels in uremic patients. Theophylline values will be increased by 1.0 µg/mL in the presence of 10 µg/mL of 1,3-dimethyl uric acid.

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	µg/mL	Bias %
Hemoglobin	1000 mg/dL	6.0	-3
Bilirubin (conjugated)	80 mg/dL	5.8	4
Bilirubin (unconjugated)	80 mg/dL	5.8	4
Lipemia Intralipid®	1000 mg/dL	5.8	10

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) < limit of detection (LoD) and LoD ≤ 2.0 µg/mL. The LoD corresponds to the lowest concentration of theophylline that can be detected with a probability of 95%. The LoD for the Atellica CH Theo assay is 0.4 µg/mL, and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 0.3 µg/mL.

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.

Atellica Theo reagent is harmful to aquatic life with long lasting effects.
Contains: Particle Reagent / Buffer - 2-methyl-2H-isothiazol-3-one
Contains: 2-methyl-2H-isothiazol-3-one. May produce an allergic reaction.

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
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 Theophylline Reagent

17. REFERENCES

1. Package Insert, Theophylline Reagent, Siemens Healthcare Diagnostics Inc., 11/2019.
2. Package Insert, DRUG CAL, Siemens Healthcare Diagnostics Inc., 07/2019.
3. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Tobramycin (Tob) by Atellica CH Analyzer**
Prepared by	Ashkan Chini Date: 4/27/2021
Owner	Robert SanLuis Date: 4/27/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Tobramycin, Peak	Atellica CH Analyzer	TOBRP
Tobramycin, Trough		TOBRT
Tobramycin, Random		TOBR

Synonyms/Abbreviations
Tobramycin

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica CH Tob assay involves a homogeneous particle-enhanced turbidimetric inhibition immunoassay (PETINIA) technique which uses a synthetic particle-tobramycin conjugate (PR) and tobramycin-specific monoclonal antibody (Ab). Tobramycin present in the sample competes with the particles for the antibody, thereby decreasing the rate of aggregation. Hence, the rate of aggregation is inversely proportional to the concentration of tobramycin in the sample. The rate of aggregation is measured using bichromatic turbidimetric readings at 545/694 nm.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	**Trough**: Collect immediately prior to the next dose (within 30 minutes). Verify with the nurse in charge of the patient that the next dose has not yet been given. **Peak**: Collect thirty (30) minutes after completion of infusion or ninety (90) minutes after injection. An additional collection label CRN will print with each orderable. The tube type translation is “SEE RN”. It is solely used as a reminder for phlebotomy to first check with Nurse prior to collection.
Special Collection Procedures	N/A

Component	Special Notations
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Plasma (Lithium Heparin) Serum
Collection Container	Plasma: Mint green top tube (PST) 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: 8 hours
	Refrigerated: 72 hours
	Frozen: 30 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. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

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
Tobramycin (Tob)	Siemens, Atellica CH, Cat. No. 11097517

4.2 Reagent Preparation and Storage

Reagent	**Tobramycin (Tob)**
Storage	Store at 2-8°C
Stability	Reagents are stable onboard the system for 30 days per pack.
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
DRUG CAL II	Siemens Atellica CH, Cat. No. 11099405

5.2 Calibrator Preparation and Storage

Calibrator	DRUG CAL II
Preparation	Calibrators are ready to use. Allow to equilibrate to room temperature and mix thoroughly before use.
Storage/Stability	• Store at 2-8°C • **Unopened:** stable until expiration date stamped on the box. • **Opened:** remains stable for 30 days when recapped immediately after use.

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	DRUG CAL II
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 (30 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (7 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 Siemens 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 Tobramycin (Tob) is required to perform this test.

Tobramycin 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 Tobramycin 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)

0.3 – 24.0 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…
< 0.3 µg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.3 µg/mL
≥ 12.0 µg/mL	**On Board Automated Dilution:** Results ≥ 12.0 µg/mL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 24.0 µg/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 24.0 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

Tobramycin Random: None established
Tobramycin Peak: 4.0 – 8.0 µg/mL
Tobramycin Trough: 0.5 – 2.0 µg/mL

11.2 Critical Values

Tobramycin Random: > 12.0 µg/mL
Tobramycin Peak: > 12.0 µg/mL
Tobramycin Trough: > 2.1 µg/mL

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Tobramycin is an antibiotic effective against gram-negative aerobic bacteria. It has a wide spectrum of antibiotic activity and relatively low toxicity. Tobramycin is a naturally occurring antibiotic produced by the organism Streptomyces tenebarius. It has been used alone or in combination with other antibiotics in the treatment of serious gram-negative infections. Tobramycin is more effective against Pseudomonas species. Tobramycin is administered either intramuscularly or intravenously. Peak concentrations are reached 60 minutes after intramuscular injection and after completion of intravenous injection.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

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)

0.3 – 12.0 µg/mL

14.2 Precision

Material	Mean µg/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum	1.3	0.07	5
Serum	2.8	0.09	3.2
QC	5.3	0.07	1.4
Plasma	9.8	0.11	1.2

14.3 Interfering Substances

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	µg/mL	Bias %
Hemoglobin	500 mg/dL	4.4	< 1
Bilirubin (conjugated)	20 mg/dL	4.3	2
Bilirubin (unconjugated)	20 mg/dL	4.3	< 1
Lipemia Intralipid®	800 mg/dL	4.3	2

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) ≤ limit of detection (LoD) and LoD ≤ 0.3 µg/mL. The LoD corresponds to the lowest concentration of tobramycin that can be detected with a probability of 95%. The LoD for the Atellica CH Tob assay is value 0.2 µg/mL, and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 0.1 µg/mL.

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.

Atellica Tob reagent may cause an allergic skin reaction. Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Wash contaminated clothing before reuse.
Contains: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2Hisothiazol-3-one (3:1) (R1, R2, and R3)

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
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 Tobramycin Reagent

17. REFERENCES

1. Package Insert, Tobramycin Reagent, Siemens Healthcare Diagnostics Inc., 11/2019.
2. Package Insert, DRUG CAL II, Siemens Healthcare Diagnostics Inc., 07/2019.
3. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None