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.C3043	Myoglobin (MYO) by Atellica IM Analyzer
SGMC.C3036	B-Type Natriuretic Peptide (BNP) by Atellica IM Analyzer
SGMC.C3039	High-Sensitivity Troponin I (TnIH) by Atellica IM Analyzer
SGMC.C3041	Creatine Kinase (CK-L) by Atellica CH Analyzer
SGMC.C3055	Creatine Kinase MB (CKMB) by Atellica IM 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 May 19, 2021

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

Technical SOP

Title	**Myoglobin (MYO) by Atellica IM Analyzer**
Prepared by	Ashkan Chini Date: 4/21/2021
Owner	Robert SanLuis Date: 4/21/2021

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
Myoglobin	Atellica IM Analyzer	MYOGL

Synonyms/Abbreviations
Myoglobin Quant, MYO

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica IM MYO assay is a 2-site sandwich immunoassay using direct chemiluminescent technology, which uses constant amounts of 2 antibodies. The first antibody, in the Lite Reagent, is a goat polyclonal anti-myoglobin antibody labeled with acridinium ester. The second antibody, in the Solid Phase, is a mouse monoclonal anti‑myoglobin antibody, which is covalently coupled to paramagnetic particles. A direct relationship exists between the amount of myoglobin present in the patient sample and the amount of relative light units (RLUs) detected by the system.

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	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: Not specified, to be determined
	Frozen: Not specified
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
Myoglobin (MYO)	Siemens, Atellica IM, Cat. No. 10995649
Atellica IM Multi-Diluent 10	Siemens, Atellica IM, Cat. No. 10995640

4.2 Reagent Preparation and Storage

Reagent	**Myoglobin (MYO)**
Storage	• Store at 2-8°C • Store in upright position. Protect from heat and light source.
Stability	Reagents are stable onboard the system for 28 days.
Preparation	Reagent is liquid and ready to use. Before loading primary reagent packs onto the system, mix them by hand and visually inspect the bottom of the reagent pack to ensure that all particles are re-suspended.

Reagent	**Atellica IM Multi-Diluent 10**
Storage	• Store at 2-8°C • Store in upright position.
Stability	Remains stable onboard for 28 days
Preparation	Reagent is liquid and ready to use. Before loading primary reagent packs onto the system, mix them by hand and visually inspect the bottom of the reagent pack to ensure that all particles are re-suspended.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	**Supplier and Catalog Number**
Calibrator U (CAL U)	Siemens Atellica IM, Cat. No. 10995519

5.2 Calibrator Preparation and Storage

Calibrator	Calibrator U (CAL U)
Preparation	1. Add 2.0 mL of reagent grade water into each vial using a calibrated pipette. Replace cap. 2. Let the vials stand for 15–20 minutes at room temperature to allow the lyophilized material to dissolve. 3. Gently mix and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C in an upright position. • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** remains stable for 4 hours at room temperature and 24 hours at 2-8°C.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Calibrator U (CAL U)
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 (33 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (14 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	To load a new lot of reagent on IM Module: Note: Calibrate the new lot of reagent as soon as it is loaded on the instrument. If the reagent sits un-calibrated for a short period of time (24 hours) then it will not be eligible for a "Lot Calibration" and another new reagent will need to be loaded onboard. 1. From the home page of the IM Module Screen (small screen attached on the IM Module) make sure the analyzer status is in Standby. 2. On the IM Module Screen select Reagent Loader. Make sure the Reagent Drawer status is unlocked. Open the reagent drawer, load the reagent and then close it. Once the reagent is scanned, the IM Module Screen will populate message "Missing TDef for lot" next to the reagent. The Reagent Drawer status remains unlocked. 3. Both Reagent Master Curve and Calibrator Package Insert need to be scanned using the Atellica Solution's main monitor. To differentiate between the two: • Reagent Master Curve has MC TDEF printed right below the assay name. • Calibrator Package Insert has CAL printed right above the assay name. 4. To scan the Reagent Master Curve, go to Set up – Test Definition – IM Test Definition. Scan the barcode.

	5. To scan the Calibrator Package Insert, go to Calibration – Calibrator Definition. Scan the barcode. 6. Re-open the Reagent Drawer and close it. This time its status should change to locked, meaning the reagent is going to be loaded onboard ready for calibration.

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 Cardiac Markers Plus Control LT, Levels 1B, 2, & 3	Bio-Rad Laboratories Cat. No. 12009959, 12009956, 12009957

6.2 Control Preparation and Storage

Control	InteliQ Cardiac Markers Plus Control LT, Levels 1B, 2, & 3
Preparation	Allow to thaw at room temperature (18-25C) for approximately 40 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 Onboard:** 10 days at 2-8C for myoglobin Note: Stability for troponin and BNP 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 IM 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 IM Myoglobin (MYO) is required to perform this test.

Myoglobin is performed on the Atellica IM 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 Myoglobin 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 as a whole number.

10.3 Units of Measure

ng/mL

10.4 Clinically Reportable Range (CRR)

3 – 100,000 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…
< 3 ng/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 3 ng/mL
≥ 1000 ng/mL	**On Board Automated Dilution:** Results ≥ 1000 ng/mL will automatically have repeat testing performed into the instrument using dilution factor of 100. No multiplication is necessary.
> 100,000 ng/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 100,000 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

10 – 92 ng/mL

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Myoglobin is a heme protein found in both cardiac and skeletal muscle cells and is released in the serum when damage occurs to these cells. In the absence of skeletal muscle trauma or other factors associated with non-cardiac related increase in circulating myoglobin, myoglobin levels have been used as an early marker for detection of myocardial infraction (MI). Following myocardial necrosis associated with MI, myoglobin is one of the first markers to rise above normal levels, increasing measurably above baseline within 1 – 3 hours post infract, peaking at 6 – 12 hours and returning to baseline within 24 – 36 hours. Reports suggest the measurement of myoglobin as an aid in risk stratification of chest pain patients and as an aid in the diagnosis of myocardial infraction. Negative predictive values for myocardial infraction of up to 100% have been reported at certain periods after the onset of symptoms.

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 – 1000 ng/mL

14.2 Precision

Material	Mean ng/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum A	18.03	0.31	N/A
Serum B	832.09	17.92	2.2
Control 1	52.29	1.02	N/A
Control 2	155.95	3.07	2.0
Control 3	361.51	7.6	2.1

14.3 Interfering Substances

Hemolysis, Icterus, Lipemia (HIL), and other interferences

Specimens that are …	Demonstrate ≤ 5% change in results up to …
Hemolyzed	1000 mg/dL of hemoglobin
Icteric	40 mg/dL of conjugated bilirubin 40 mg/dL of unconjugated bilirubin
Lipemic	1000 mg/dL of triglycerides
Proteinemic	12.5 g/dL of protein

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have an analytical sensitivity of ≤ 3.00 ng/mL, a limit of blank ≤ 3.00 ng/mL, and a limit of detection (LoD) ≤ 6.00 ng/mL. The analytical sensitivity for the Atellica IM MYO assay is 2.53 ng/mL. The LoB corresponds to the highest measurement result that is likely to be observed for a blank sample. The LoB of the Atellica IM MYO assay is 2.56 ng/mL. The LoD corresponds to the lowest concentration of myoglobin that can be detected with a probability of 95%. The LoD for the Atellica IM MYO assay is 3.57 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 Myoglobin Reagent

17. REFERENCES

1. Package Insert, Myoglobin Reagent, Siemens Healthcare Diagnostics Inc., 07/2019.
2. Package Insert, Calibrator U (CAL U), Siemens Healthcare Diagnostics Inc., 08/2019.
3. Package Insert, InteliQ Cardiac Markers Plus Control LT, Bio-Rad Laboratories, 9/2020
4. Package Insert, Atellica IM Multi-Diluent 10, Siemens Healthcare Diagnostics Inc., 7/2019

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**B-Type Natriuretic Peptide (BNP) by Atellica IM Analyzer**
Prepared by	Ashkan Chini Date: 4/21/2021
Owner	Robert SanLuis Date: 4/21/2021

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
B-type Natriuretic Peptide	Atellica IM Analyzer	BNPT

Synonyms/Abbreviations
BNP

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica IM BNP assay is a fully automated 2‑site sandwich immunoassay using direct chemiluminescent technology which uses constant amounts of 2 monoclonal antibodies. The first antibody, in the Lite Reagent, is an acridinium-ester-labeled mouse monoclonal anti‑human BNP F(ab)2 fragment specific to the ring structure of BNP. The second antibody, in the Solid Phase, is a biotinylated mouse monoclonal anti-human antibody specific to the C‑terminal portion of BNP, which is coupled to streptavidin magnetic particles. A direct relationship exists between the amount of BNP present in the patient sample and the amount of relative light units (RLUs) detected by the system.

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 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	Plasma (K2 EDTA)
Collection Container	Lavender Top Tube
Volume - Optimum **- Minimum**	Full Tube 1.0 mL

Criteria	
Transport Container and Temperature	Collection container or Plastic vial at room temperature, **as BNP is unstable in glass containers.**
Stability & Storage Requirements	Room Temperature: Not recommended
	Refrigerated: 24
	Frozen: 9 months (separated plasma)
Timing Considerations	If plasma is not tested within 24 hours, then centrifuge the sample and store separated plasma at -20C or colder. Mix thoroughly after thawing and store at 2-8C until testing. Test within 8 hours.
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	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
B-Type Natriuretic Peptide (BNP)	Siemens, Atellica IM, Cat. No. 10995471

4.2 Reagent Preparation and Storage

Reagent	B-Type Natriuretic Peptide (BNP)
Storage	• Store at 2-8°C • Store in an upright position. • Protect from heat and light.

Stability	Reagents are stable onboard the system for 42 days.
Preparation	Reagent is liquid and ready to use. Before loading primary reagent packs onto the system, mix them by hand and visually inspect the bottom of the reagent pack to ensure that all particles are re-suspended.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
B-Type Natriuretic Peptide Calibrator (BNP CAL)	Siemens Atellica IM, Cat. No. 10995473

5.2 Calibrator Preparation and Storage

Calibrator	B-Type Natriuretic Peptide Calibrator (BNP CAL)
Preparation	1. Add 2.0 mL of reagent grade water into each vial using a calibrated pipette. Replace cap. 2. Let the vials stand for 15–20 minutes at room temperature to allow the lyophilized material to dissolve. 3. Gently mix and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C in an upright position. • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** stable for 8 hours at room temperature, 5 days refrigerated, 60 days frozen.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	B-Type Natriuretic Peptide Calibrator (BNP CAL)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in pg/mL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (61 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (42 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	To load a new lot of reagent on IM Module: Note: Calibrate the new lot of reagent as soon as it is loaded on the instrument. If the reagent sits un-calibrated for a short period of time (24 hours) then it will not be eligible for a "Lot Calibration" and another new reagent will need to be loaded onboard. 1. From the home page of the IM Module Screen (small screen attached on the IM Module) make sure the analyzer status is in Standby. 2. On the IM Module Screen select Reagent Loader. Make sure the Reagent Drawer status is unlocked. Open the reagent drawer, load the reagent and then close it. Once the reagent is scanned, the IM Module Screen will populate message "Missing TDef for lot" next to the reagent. The Reagent Drawer status remains unlocked. 3. Both Reagent Master Curve and Calibrator Package Insert need to be scanned using the Atellica Solution's main monitor. To differentiate between the two: • Reagent Master Curve has MC TDEF printed right below the assay name. • Calibrator Package Insert has CAL printed right above the assay name. 4. To scan the Reagent Master Curve, go to Set up – Test Definition – IM Test Definition. Scan the barcode. 5. To scan the Calibrator Package Insert, go to Calibration – Calibrator Definition. Scan the barcode. 6. Re-open the Reagent Drawer and close it. This time its status should change to locked, meaning the reagent is going to be loaded onboard ready for calibration.
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 Cardiac Markers Plus Control LT, Levels 1B, 2, & 3	Bio-Rad Laboratories Cat. No. 12009959, 12009956, 12009957

6.2 Control Preparation and Storage

Control	InteliQ Cardiac Markers Plus Control LT, Levels 1B, 2, & 3
Preparation	Allow to thaw at room temperature (18-25C) for approximately 40 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 Onboard:** 5 days at 2-8C for BNP

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

Step	Action
	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 IM 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 IM B-Type Natriuretic Peptide (BNP) is required to perform this test.

BNP is performed on the Atellica IM 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 B-Type Natriuretic Peptide (BNP) in pg/mL

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results as a whole number.

10.3 Units of Measure

pg/mL

10.4 Clinically Reportable Range (CRR)

2 – 5000 pg/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 pg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 2 pg/mL
> 5000 pg/mL	Report as: "> 5000 pg/mL"

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

11. EXPECTED VALUES

11.1 Reference Ranges

0 – 100 pg/mL

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

This assay is indicated for the measurement of plasma BNP as an aid in the diagnosis and assessment of the severity of heart failure. In patients with acute coronary syndromes (ACS), this test, in conjunction with other known risk factors, can also be used to predict survival as well as to predict the likelihood of future heart failure. Heart failure is an important clinical syndrome which compromises left ventricular systolic or diastolic function or a combination of both. Heart failure occurs when the heart us unable to pump blood at a rate sufficient for metabolic requirements. Its most common causes are coronary artery disease, hypertension, valvular heart diseases and cardiomyopathies. Accurate and early diagnosis is important since effective therapeutic interventions are available, which improve both morbidity and mortality. Based on clinical signs and symptoms, the severity of heart failure is classified into four classes of increasing disease progression according to the New York Heart Association classification.

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 – 5000 pg/mL

14.2 Precision

Material	Mean pg/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Plasma A	9.2	0.3	3.7
Plasma B	12.2	0.3	2.6
Plasma C	36.2	0.7	1.9
Plasma D	654.6	12	1.8
Plasma E	1523.7	19.50	1.3
Plasma F	4325.2	64.2	1.5

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.

Specimens that are …	Demonstrate ≤ 5% change in results up to …
Icteric	25 mg/dL of unconjugated bilirubin
Lipemic	800 mg/dL of triglycerides 1000 mg/dL of cholesterol
Uremic	200 mg/dL of urea 2.5 mg/dL of creatinine

Specimens that are …	Demonstrate ≤ 7% change in results up to …
Icteric	25 mg/dL of conjugated bilirubin
Proteinemic	5.3 g/dL of human IgG

Specimens that are …	Demonstrate ≤ 10% change in results up to …
Hemolyzed	100 mg/dL of hemoglobin
Biotin	38 ng/mL of biotin

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have an analytical sensitivity < 2.0 pg/mL, functional sensitivity < 4.0 pg/mL, a limit of blank (LoB) < 2.0 pg/mL and limit of detection (LoD) < 4.0 pg/mL The analytical sensitivity for the Atellica IM BNP assay is 1.4 pg/mL. The LoB of the Atellica IM BNP assay is 1.1 pg/mL. The LoD for the Atellica IM BNP assay is 2.4 pg/mL. The functional sensitivity of the Atellica IM BNP assay is < 1.5 pg/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 B-Type Natriuretic Peptide (BNP) Reagent

17. REFERENCES

1. Package Insert, BNP Reagent, Siemens Healthcare Diagnostics Inc., 11/2020
2. Package Insert, BNP CAL, Siemens Healthcare Diagnostics Inc., 08/2019
3. Package Insert, InteliQ Cardiac Markers Plus Control LT, Bio-Rad Laboratories, 9/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**High-Sensitivity Troponin I (TnIH) by Atellica IM Analyzer**
Prepared by	Ashkan Chini Date: 4/21/2021
Owner	Robert SanLuis Date: 4/21/2021

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
High Sensitivity Troponin I	Atellica IM Analyzer	TROPI1

Synonyms/Abbreviations
Troponin, Tropi, TNIH

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica IM TnIH is a 3-site sandwich immunoassay using direct chemiluminescent technology. The Solid Phase reagent consists of magnetic latex particles conjugated with streptavidin with 2 bound biotinylated capture monoclonal antibodies, each recognizing a unique cTnI epitope. The Lite Reagent comprises a conjugate with an architecture consisting of a proprietary acridinium ester and a recombinant anti-human cTnI sheep Fab covalently attached to bovine serum albumin (BSA) for chemiluminescent detection. A direct relationship exists between the amount of troponin I present in the patient sample and the amount of relative light units (RLUs) detected by the system.

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	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: 24 hours
	Frozen: 40 days
Timing Considerations	For serum specimens, complete clot formation should take place before centrifugation. Serum should be physically separated from cells as soon as possible from the time of collection.
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. For plasma specimens, avoid transferring white blood cells or platelets from the layer located just above the red blood cells. 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
High-Sensitivity Troponin I (TnIH)	Siemens, Atellica IM, Cat. No. 10997840
APW3	Siemens, Atellica IM, Cat. No. 10998580
Multi-Diluent 11	Siemens, Atellica IM, Cat. No. 10995642

4.2 Reagent Preparation and Storage

Reagent	**High-Sensitivity Troponin I (TnIH)**
Storage	• Store at 2-8°C • Store in an upright position. • Protect from heat and light sources.
Stability	Reagents are stable onboard the system for 28 days.
Preparation	Reagent is liquid and ready to use. Before loading primary reagent packs onto the system, mix them by hand and visually inspect the bottom of the reagent pack to ensure that all particles are re-suspended.

Reagent	**APW3**
Storage	• Store at 2-8°C • Store in an upright position.
Stability	It is stable onboard the system for 28 days.
Preparation	It is liquid and ready to use.

Reagent	**Multi-Diluent 11**
Storage	• Store at 2-8°C • Store in an upright position.
Stability	It is stable onboard the system for 28 days.
Preparation	It is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
TnIH CAL	Siemens Atellica IM, Cat. No. 10997840

5.2 Calibrator Preparation and Storage

Calibrator	TnIH CAL
Preparation	**Low Calibrator:** TnIH CAL L is liquid and ready to use. Gently mix and invert the vials to ensure homogeneity of the material. **High Calibrator:** 1. Add 1.00 mL of reagent grade water into the vial using calibrated pipette. Replace cap. 2. Let the vial stand for 15–20 minutes at room temperature to allow the lyophilized material to dissolve. 3. Gently mix and invert the vial to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C in an upright position. • **Unopened:** stable until expiration date stamped on the box. • **Low Calibrator, Opened:** stable for 4 hours at 2-8°C or 30 days at ≤ -20°C. • **High Calibrator, Reconstituted:** stable for 4 hours at 2-8°C or 30 days at ≤ -20°C.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	TnIH CAL
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in pg/mL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (47 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (31 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	Use the lot specific master curve and test definition sheet provided with the reagents. Calibrators in the kit must only be used with reagents from that assay kit lot. Do not use calibrators from another kit.

	Generate lot specific barcode labels to use with calibrator samples.
	To load a new lot of reagent on IM Module: Note: Calibrate the new lot of reagent as soon as it is loaded on the instrument. If the reagent sits un-calibrated for a short period of time (24 hours) then it will not be eligible for a "Lot Calibration" and another new reagent will need to be loaded onboard. 1. From the home page of the IM Module Screen (small screen attached on the IM Module) make sure the analyzer status is in Standby. 2. On the IM Module Screen select Reagent Loader. Make sure the Reagent Drawer status is unlocked. Open the reagent drawer, load the reagent and then close it. Once the reagent is scanned, the IM Module Screen will populate message "Missing TDef for lot" next to the reagent. The Reagent Drawer status remains unlocked. 3. Both Reagent Master Curve and Calibrator Package Insert need to be scanned using the Atellica Solution's main monitor. To differentiate between the two: • Reagent Master Curve has MC TDEF printed right below the assay name. • Calibrator Package Insert has CAL printed right above the assay name. 4. To scan the Reagent Master Curve, go to Set up – Test Definition – IM Test Definition. Scan the barcode. 5. To scan the Calibrator Package Insert, go to Calibration – Calibrator Definition. Scan the barcode. 6. Re-open the Reagent Drawer and close it. This time its status should change to locked, meaning the reagent is going to be loaded onboard ready for calibration.
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 Cardiac Markers Plus Control LT, Levels 1B, 2, & 3	Bio-Rad Laboratories Cat. No. 12009959, 12009956, 12009957

6.2 Control Preparation and Storage

Control	InteliQ Cardiac Markers Plus Control LT, Levels 1B, 2, & 3
Preparation	Allow to thaw at room temperature (18-25C) for approximately 40 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 Onboard:** 5 days at 2-8C for troponin

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

Step	Action
	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 IM 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 IM High-Sensitivity Troponin I (TnIH) is required to perform this test.

TnIH is performed on the Atellica IM 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 High-Sensitivity Troponin I in pg/mL.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results as a whole number

10.3 Units of Measure

pg/mL

10.4 Clinically Reportable Range (CRR)

3 – 125,000 pg/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 pg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 3 pg/mL
≥ 25,000 pg/mL	**On Board Automated Dilution:** Results ≥ 25,000 pg/mL will automatically have repeat testing performed into the instrument using dilution factor of 5. No multiplication is necessary.
> 125,000 pg/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 125,000 pg/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

< 83 pg/mL

11.2 Critical Values

Initial (first) critical value: > 100 pg/mL

Treatment of **subsequent critical values** is based on delta criteria:

Prior Critical Value	Delta Threshold	Example
101 - 500 pg/mL	Value doubles	Prior value of 101, next value must be 202 or greater
501 – 1,000 pg/mL	Increase of 250	Prior value of 600, next value must be 850 or greater
1,001 pg/mL or more	Increase of 1,000	Prior value of 2,000, next value must be 3,000 or greater

If the subsequent critical value does NOT qualify to be called, document this by appending the code **TROPC** to the result. This code translates to "Laboratory value indicates a critical value previously reported."

Notes:

- Data Innovations (DI) will flag results that meet delta criteria to be called (Error code contains 'CALL' and the Error name contains 'CALL TROP').
- When DI is down, ALL critical troponin values must be called.

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Troponin is the contractile regulatory protein complex of striated muscle. It is found periodically along the thin filament of the myofibrils, in conjunction with the protein tropomyosin. The troponin complex consists of three distinct polypeptide components: troponin-C (the calcium binding element), troponin-I (the actinomyosin ATPase inhibitory element), and troponin-T (the tropomyosin binding element). The complex serves to regulate the calcium dependent interaction of myosin and actin and thus plays an integral role in muscle contraction. Troponin-I exists in three distinct molecular forms which correspond to

specific isotypes found in fast-twitch skeletal muscle, slow-twitch skeletal muscle, and heart, respectively.

Several reports in the literature have indicated that cardiac troponin-I is released into blood within hours of the onset of symptoms of myocardial infarction and that it remains elevated for several days post-infarction. The cumulative data from these reports indicate that troponin-I levels become abnormal 4–8 hours following onset of chest pain, peak at 12–16 hours, and remain elevated for 5–9 days following an infarction.

Measurement of cardiac troponin-I levels provide sensitive and specific determination of myocardial injury over a wide diagnostic window. Elevations in cardiac troponin-I levels have been observed across a spectrum of acute coronary syndromes including Q-wave MI, non-Q-wave MI and unstable angina. A significantly higher incidence of mortality has been observed in patients with non-Q-wave MI and unstable angina who have detectable levels of cardiac troponin-I. This suggests that cardiac troponin-I provides a means for risk stratification of these individuals.

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 – 25,000 pg/mL
Note: manufacture insert lists lower limit as 2.50, rounded to whole number to match our facility's reporting practice.

14.2 Precision

Material	Mean pg/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum 1	12.72	0.55	4.3
Serum 2	127.93	2.3	1.8
Serum 3	1334.97	22.28	1.7
Serum 4	13815.89	192.05	1.4
Plasma 1	12.03	0.49	4.1
Plasma 2	131.21	2.23	1.7
Plasma 3	1363.38	27.11	2.0
Plasma 4	12862.97	212.91	1.7

14.3 Interfering Substances

- The use of a single sample type (either lithium plasma or serum) is recommended for troponin analysis when collecting serial samples from the same patient.
- Heterophilic antibodies and rheumatoid factor in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
- Samples from patients receiving preparations of mouse monoclonal antibodies for therapy or diagnosis may contain Human Anti-Mouse Antibodies (HAMA). Such samples may show either falsely elevated or falsely depressed values when tested with this method.
- Specimens from some individuals with pathologically high gamma globulin levels may demonstrate depressed troponin values. Additional information may be required for diagnosis.
- An unknown interference was observed in analytical spiking and dilution studies casing negative bias that may affect interpretation of patient results. The unknown interference may be due to the presence of troponin autoantibodies, which have been reported in up to 10% of patients with or without AMI and up to 20% of patients positive for rheumatoid factor. If the cTnI result is below the 99th percentile at the 1st blood draw, at least 2 additional blood samples should be drawn before results are interpreted as negative for AMI.

Hemolysis, Icterus, Lipemia (HIL), and other interferences:

Specimens that are …	Demonstrate ≤ 10% change in results up to …
Hemolyzed	500 mg/dL of hemoglobin
Icteric	40 mg/dL of conjugated bilirubin 60 mg/dL of unconjugated bilirubin
Lipemic	2000 mg/dL of triglycerides

Specimens that contain …	Demonstrate ≤ 10% change in results up to …
Biotin	3500 ng/mL
Cholesterol	500 mg/dL
Protein Albumin	6g/dL
Protein Gamma Globulin	2.5 g/dL
Total Protein	12 g/dL

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability

The assay is designed to have a limit of detection (LoD) ≤ 1.6 pg/mL, and a limit of quantitation (LoQ) ≤ 3.0 pg/mL. The LoB of the Atellica IM TnIH assay is 0.50 pg/mL. The LoD corresponds to the lowest concentration of cTnI that can be detected with a probability of 95%. The LoD for the Atellica IM TnIH assay is 1.60 pg/mL.

The LoQ of the Atellica IM TnIH assay is 2.50 pg/mL. Report results below the LoQ as < 2.50 pg/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 High-Sensitivity Troponin I Reagent

17. REFERENCES

1. Package Insert, TnIH Reagent, Siemens Healthcare Diagnostics Inc., 04/2019.
2. Package Insert, InteliQ Cardiac Markers Plus Control LT, Bio-Rad Laboratories, 9/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None

Technical SOP

Title	**Creatine Kinase (CK-L) by Atellica CH Analyzer**
Prepared by	Ashkan Chini Date: 4/25/2021
Owner	Robert SanLuis Date: 4/25/2021

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
Creatine Kinase	Atellica CH Analyzer	CPK

Synonyms/Abbreviations
CK, CPK, CKI

Department
Chemistry

2. ANALYTICAL PRINCIPLE

Creatine Kinase reacts with creatine phosphate and adenosine diphosphate (ADP) to form adenosine triphosphate (ATP), which is coupled to the hexokinase-G6PD (glucose-6-phosphate dehydrogenase) reaction, generating NADPH (reduced nicotinamide adenine dinucleotide phosphate). The concentration of NADPH is measured by the increase in absorbance at 340/596 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)
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: 4 hours
	Refrigerated: 5 days
	Frozen: 2 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
Creatine Kinase (CK-L)	Siemens, Atellica CH, Cat. No. 11097640

4.2 Reagent Preparation and Storage

Reagent	**Creatine Kinase (CK-L)**
Storage	Store at 2-8°C
Stability	Reagents are stable onboard the system for 29 days.
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
ENZ 3 Calibrator (ENZ 3 CAL)	Siemens Atellica CH, Cat. No. 11099319

5.2 Calibrator Preparation and Storage

Calibrator	ENZ 3 Calibrator (ENZ 3 CAL)
Preparation	1. Thaw the calibrator at room temperature for 30–45 minutes. **Note:** Do not thaw in a water bath or in water above 25°C. 2. Gently invert the calibrator vial at least 10 times to ensure that the contents are thoroughly mixed. **Do not vortex.**
Storage/Stability	• Store at -15 to -25°C • **Unopened:** stable until expiration date stamped on the box. • **Opened:** 30 days when recapped immediately after use and stored at 2-8°C.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	ENZ 3 Calibrator (ENZ 3 CAL)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in U/L
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (202 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (21 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 CK **Thawed and Opened**: 7 days at 2-8C for CK 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 -15 to -25°C for calibrator.
- Freezer capable of sustaining range not to exceed -20 to -70°C for QC.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

Atellica CH Creatine Kinase (CK-L) is required to perform this test.

Creatine Kinase 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

8.2	Specimen Testing
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 Creatine Kinase in U/L.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results as a whole number.

10.3 Units of Measure

U/L

10.4 Clinically Reportable Range (CRR)

15 – 65,000 U/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…
< 15 U/L	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 15 U/L
≥ 1300 U/L	**On Board Automated Dilution:** Results ≥ 1300 U/L will automatically have repeat testing performed into the instrument using dilution factor of 50. No multiplication is necessary.
> 65,000 U/L	If the recommended dilution does not give results within the clinically reportable range, report as: "> 65,000 U/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

Age	Female	Male
Adult (>18 years):	21 – 215 U/L	32 – 232 U/L
Pediatric:		
15 – 18 years	28 - 142	34 - 147
11 – 14 years	31 - 172	31 - 152
2 – 10 years	25 - 177	31 - 152
13 months – 23 months	25 - 177	28 - 162
3 – 12 months	27 - 242	25 - 172
0– 90 days	43 - 474	29 - 303

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Measurements of creatine kinase are used in the diagnosis and treatment of myocardial infraction and muscle diseases. Creatine kinase may also be elevated following muscle injury or strenuous exercise.

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)

15 – 1300 U/L

14.2 Precision

Material	Mean U/L	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum Pool 1	962	4.0	0.4
Serum Pool 2	1152	3.4	0.3
Plasma Pool	198	1.5	0.8
QC 1	84	1.3	1.6
QC 2	258	3.1	1.2
QC 3	640	2.4	0.4

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	U/L	Bias %
Hemoglobin	125 mg/dL	100	10
Bilirubin (conjugated)	60 mg/dL	85	0
Bilirubin (unconjugated)	60 mg/dL	87	-3
Lipemia Intralipid®	1000 mg/dL	97	4

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) ≤ 10 U/L, a limit of detection (LoD) ≤ 15 U/L, and a limit of quantitation (LoQ) ≤ 25 U/L. The LoD corresponds to the lowest concentration of creatine kinase that can be detected with a probability of 95%. The LoD for the Atellica CH CK_L assay is 6 U/L, and was determined using 225 determinations, with 75 blank and 75 low level replicates, and a LoB of 1 U/L. The LoQ corresponds to the lowest amount of analyte in a sample at which the total error is ≤ 10 U/L. The LoQ of the Atellica CH CK-L assay is 6 U/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 CK_L reagent may damage the unborn child. Do not handle until all safety precautions have been read and understood. Wear protective gloves/protective clothing/eye protection/face protection. Use personal protective equipment as required. IF exposed or concerned: Get medical advice/attention.
Contains: Imidazole; ADVIA Chemistry CK-L Reagent 1

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 Creatine Kinase Reagent

17. REFERENCES

1. Ghoshal, Amit K. and Soldin, Steven J., Evaluation of the Dade Behring Dimension® RxL: Integrated chemistry system-pediatric reference ranges. Clinica Chimica Acta 2003; 331:144
2. Package Insert, Creatine Kinase Reagent, Siemens Healthcare Diagnostics Inc., 02/2018.
3. Package Insert, ENZ 3 Calibrator (ENZ 3 CAL), Siemens Healthcare Diagnostics Inc., 07/2019.
4. 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	**Creatine Kinase MB (CKMB) by Atellica IM Analyzer**
Prepared by	Ashkan Chini Date: 4/21/2021
Owner	Robert SanLuis Date: 4/21/2021

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
Creatine Kinase MB	Atellica IM Analyzer	CKMB

Synonyms/Abbreviations
MMB, CKMB

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica IM CKMB assay is a 2-site sandwich immunoassay using direct chemiluminescent technology, which uses constant amounts of 2 antibodies. The first antibody, in the Lite Reagent, is a mouse monoclonal anti‑CK‑MB antibody labeled with acridinium ester. The second antibody, in the Solid Phase, is a mouse monoclonal anti‑CK‑BB antibody, which is covalently coupled to paramagnetic particles. A direct relationship exists between the amount of CK‑MB present in the patient sample and the amount of relative light units (RLUs) detected by the system.

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	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: 4 hours
	Refrigerated: 48 hours
	Frozen: Not specified
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
Creatine Kinase MB (CKMB)	Siemens, Atellica IM, Cat. No. 10995530
CKMB DIL	Siemens, Atellica IM, Cat. No. 10995533

4.2 Reagent Preparation and Storage

Reagent	**Creatine Kinase MB (CKMB)**
Storage	• Store at 2-8°C • Store in an upright position. Protect from heat and light sources.
Stability	Reagents are stable onboard the system for 28 days.
Preparation	Reagent is liquid and ready to use. Before loading primary reagent packs onto the system, mix them by hand and visually inspect the bottom of the reagent pack to ensure that all particles are re-suspended.

Reagent	**CKMB DIL**
Storage	• Store at 2-8°C • Store in an upright position.
Stability	It remains stable onboard the system for 28 days.
Preparation	It is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Creatine Kinase MB Calibrator (CKMB CAL)	Siemens Atellica IM, Cat. No. 10995532

5.2 Calibrator Preparation and Storage

Calibrator	Creatine Kinase MB Calibrator (CKMB CAL)
Preparation	1. Add 2.0 mL of reagent grade water that is 20–25°C into each vial using a calibrated pipette. Replace cap. 2. Gently mix the vials for 30 minutes on a mechanical mixing device (a rocker or rotator, for example) at room temperature to allow the lyophilized material to dissolve. Alternatively: Manually mix by inverting 10 times every 10 minutes for a period of 30 minutes, or until reconstitution is complete. 3. Gently mix and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C in an upright position. • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** 4 hours at room temperature or 14 days at 2-8°C.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Creatine Kinase MB Calibrator (CKMB 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 (66 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (28 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	To load a new lot of reagent on IM Module: Note: Calibrate the new lot of reagent as soon as it is loaded on the instrument. If the reagent sits un-calibrated for a short period of time (24 hours) then it will not be eligible for a "Lot Calibration" and another new reagent will need to be loaded onboard. 1. From the home page of the IM Module Screen (small screen attached on the IM Module) make sure the analyzer status is in Standby. 2. On the IM Module Screen select Reagent Loader. Make sure the Reagent Drawer status is unlocked. Open the reagent drawer, load the reagent and then close it. Once the reagent is scanned, the IM Module Screen will populate message "Missing TDef for lot" next to the reagent. The Reagent Drawer status remains unlocked. 3. Both Reagent Master Curve and Calibrator Package Insert need to be scanned using the Atellica Solution's main monitor. To differentiate between the two: • Reagent Master Curve has MC TDEF printed right below the assay name. • Calibrator Package Insert has CAL printed right above the assay name. 4. To scan the Reagent Master Curve, go to Set up – Test Definition – IM Test Definition. Scan the barcode.

	5. To scan the Calibrator Package Insert, go to Calibration – Calibrator Definition. Scan the barcode. 6. Re-open the Reagent Drawer and close it. This time its status should change to locked, meaning the reagent is going to be loaded onboard ready for calibration.
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 Cardiac Markers Plus Control LT, Levels 1B, 2, & 3	Bio-Rad Laboratories Cat. No. 12009959, 12009956, 12009957

6.2 Control Preparation and Storage

Control	InteliQ Cardiac Markers Plus Control LT, Levels 1B, 2, & 3
Preparation	Allow to thaw at room temperature (18-25C) for approximately 40 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 Onboard:** 20 days at 2-8C for CKMB. Stability for other assays 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 IM 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 IM Creatine Kinase MB (CKMB) is required to perform this test.

CKMB is performed on the Atellica IM 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.

8.1	Instrument Set-up Protocol
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 Creatine Kinase MB (CKMB) 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 one decimal point.

10.3 Units of Measure

ng/mL

10.4 Clinically Reportable Range (CRR)

0.2 – 3,000.0 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…
< 0.2 ng/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.2 ng/mL
≥ 300.0 ng/mL	**On Board Automated Dilution:** Results ≥ 300.0 ng/mL will automatically have repeat testing performed into the instrument using dilution factor of 10. No multiplication is necessary.
> 3,000.0 ng/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 3000.0 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

0.0 – 3.6 ng/mL

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

The Creatine Kinase MB (CKMB) is found primarily in cardiac tissue, with substantially lower concentration also seen in skeletal muscle. The quantitation of CKMB is routinely ordered as part of the cardiac panel and is useful in the diagnosis of acute myocardial

infraction (AMI). Typically, in cases of uncomplicated AMI, serial determinations show a pattern wherein CKMB levels become elevated within 4 – 8 hours after onset of pain, peak between 12 – 24 hours and then drop to normal by 48 hours. CKMB concentrations have also been used to assess the extent of AMI and subsequent re-infraction.

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)

0.2 – 300.0 ng/mL
Note: Lower level changed to one decimal point to match reporting practice.

14.2 Precision

Material	Mean ng/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum A	90.4	1.08	1.2
Serum B	195.9	2.29	1.2
Control 1	2.2	0.08	3.8
Control 2	12.5	0.14	1.1
Control 3	63.3	0.62	1.0

14.3 Interfering Substances

Hemolysis, Icterus, Lipemia (HIL), and other interferences

Specimens that are …	Have an insignificant effect on the assay up to …
Hemolyzed	150 mg/dL of hemoglobin
Icteric	40 mg/dL of bilirubin
Lipemic	1000 mg/dL of triglycerides

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have an analytical sensitivity of ≤ 0.18 ng/mL, a limit of blank (LoB) ≤ 0.18 ng/mL, and a limit of detection (LoD) ≤ 0.36 ng/mL.
The analytical sensitivity for the Atellica IM CKMB assay is 0.08 ng/mL. The LoB corresponds to the highest measurement result that is likely to be observed for a

blank sample. The LoB of the Atellica IM CKMB assay is 0.13 ng/mL. The LoD corresponds to the lowest concentration of CK‑MB that can be detected with a probability of 95%. The LoD for the Atellica IM CKMB assay is 0.31 ng/mL, and was determined using 162 determinations, with 72 blank and 90 low-level replicates, and an LoB of 0.13 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 Creatine Kinase MB Reagent

17. REFERENCES

1. Package Insert, CKMB Reagent, Siemens Healthcare Diagnostics Inc., 07/2019
2. Package Insert, Creatine Kinase MB Calibrator (CKMB CAL), Siemens Healthcare Diagnostics Inc., 08/2019
3. Package Insert, InteliQ Cardiac Markers Plus Control LT, Bio-Rad Laboratories, 9/2020
4. Package Insert, CKMB DIL, Siemens Healthcare Diagnostics Inc., 07/2019

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None