TRAINING UPDATE

Lab Location:	SGMC & WOMC	**Date Distributed:**	2/18/2021
Department:	Core Lab	**Due Date:**	3/18/2021

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

High Sensitivity Troponin I by Dimension Vista® System SGMC.C2007 v2

Description of change(s):

Note: The information added about the calibration process is already in practice.

Section	Reason
5.3	Added lot specific correlation factors

This revised SOP was implemented on February 18, 2021

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

Technical SOP

Title	**High Sensitivity Troponin I by Dimension Vista® System**
Prepared by	Ashkan Chini Date: 11/5/2020
Owner	Robert SanLuis Date: 11/5/2020

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	Dimension Vista® System	TROPI1

Synonyms/Abbreviations
Troponin, Tropi, TNIH

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Dimension Vista TNIH assay is a homogeneous, sandwich chemiluminescent immunoassay based on LOCI technology. The LOCI reagents include two synthetic bead reagents and two biotinylated anti-cardiac troponin I monoclonal antibody fragments. The first bead reagent (Sensibeads) is coated with streptavidin and contains photosensitizer dye. The second bead reagent (Chemibeads) is coated with a third anti-cardiac troponin I monoclonal antibody and contains chemiluminescent dye. Sample is incubated with Chemibeads and biotinylated antibodies to form bead-cardiac troponin I-biotinylated antibody sandwiches. Sensibeads are added and bind to the biotin to form bead-pair immunocomplexes. Illumination of the complex at 680 nm generates singlet oxygen from Sensibeads which diffuses into the Chemibeads, triggering a chemiluminescent reaction. The resulting signal is measured at 612 nm and is a direct function of the cardiac troponin I concentration in the sample.

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
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	Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours 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.

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	Siemens, Flex® reagent cartridge, Cat. No. K6427
CTNI Sample Diluent	Siemens Healthcare Diagnostics, Cat. No. KD692

4.2 Reagent Preparation and Storage

Reagent	**High Sensitivity Troponin I**
Container	Reagent cartridge
Storage	Store at 2-8°C
Stability	• Stable until expiration date stamped on reagent cartridges. • Sealed wells on the instrument are stable for 30 days. • Open wells: 7 days for wells 1 - 12
Preparation	All reagents are liquid and ready to use.

Reagent	**CTNI Sample Diluent**
Container	Reagent bottle
Storage	Store at 2-8°C
Stability	• **Unopened:** stable until expiration date stamped on bottle • **Opened:** o Stable for 30 days when recapped and stored at 2-8°C. **Do not use this vial on board the instrument.** o Once the stopper is punctured, stable for 30 days on board the instrument.
Preparation	CTNI SDIL is ready for use. No preparation is required.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
TNIH CAL	Siemens Dimension Vista®, Cat. No. KC627

5.2 Calibrator Preparation and Storage

Calibrator	High Sensitivity Troponin I Calibrator (TNIH CAL)
Preparation	Before use, thaw at room temperature (18–30°C) for one hour (no more than two hours), swirl and invert gently to mix.
Storage/Stability	• Store at -15°C to -25°C • **Unopened Frozen:** stable until expiration date on the box. • **Unopened Thawed:** 3 days when stored at 2-8°C • **Opened Calibrator:** o Once the stopper is punctured, stable for 3 days when stored on board Dimension Vista System. o Once cap is removed, stable for 3 days when recapped immediately after use and stored at 2-8°C. **Do not use this vial on board the instrument.**

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	TNIH CAL
Assay Range	See section 14.1
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in pg/mL. **Note**: Each reagent carton contains an alert card with lot specific correlation factors. These factors must be entered into the Vista system prior to processing QC and patient samples. To apply the correlation factors correctly: • Only one lot of High Sensitivity Troponin I reagents can be in use at any given time. • Auto-calibration of new lots must be de-selected in the Calibration Triggers on Method Configuration Screen.
Additional information	• The new lot of TNIH reagent is calibrated on one analyzer. QC is performed at the time of the calibration. Coefficient values are not changed at this time. Correlation / patient sample comparison is performed between the new lot and the old lot. • Once all the data is collected for the new lot studies (calibration, QC and correlation), the new lot of TNIH is removed off of the instrument. • When the old lot is used up and the new lot is loaded, the coefficients are changed at this time.
Frequency	• Every new reagent cartridge lot. • Every 30 days for any one lot • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	5 levels, n = 5

5.4 Calibration Procedure

Auto Calibration:

1. Place the required calibrator vials in a carrier. Make sure the barcode labels are entirely visible through the slots.
2. Place the carrier in the loading area.
3. Position the carrier with the labels facing away from the user.
4. Press the **Load** button.
5. Automatic calibration requires that calibrators be on the instrument. As the time for processing approaches, the instrument reviews onboard inventory for the appropriate calibrators.

Manual Calibration:

1. Verify that calibrators and reagents are in inventory on the instrument.
2. Press **System > Method Summary > Calibration**.

3. Select a method from the sidebar menu. Press the **Order Calibration** button on the screen.
4. Verify that the information on the screen is correct. Verify that the calibrator lot is correct using the drop-down menu.
   a. When calibrating using Vials press **OK**.
   b. When calibrating using Cups, check the Use Cups box. This displays the rack and cup position fields. For additional cups use the positions in ascending order. Be sure to use the number of calibration levels and cups as specified in the method IFU. Scan the rack barcode and place calibrator cups in an adapter in position 1 on a rack. Press **OK** and load the rack on the instrument.
5. The status field in the calibration screen changes sequentially to Awaiting Scheduling, Preparing Calibrators and Processing.

5.5 Tolerance Limits

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

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Liquichek™ Cardiac Markers Plus Control LT Levels 1B, 2 and 3	Bio-Rad Laboratories Cat # 27105, 147 and 148

6.2 Control Preparation and Storage

Control	Liquichek Cardiac Markers Plus Control LT, Level 1B, 2 and 3
Preparation	Allow the frozen control to thaw at room temperature (18-25°C) for approximately 30 minutes or until completely thawed. Swirl the contents gently to ensure homogeneity. (Do not use a mechanical mixer). Immediately load the vial on the analyzer. After each use, promptly replace the stopper and return to 2-8°C storage.
Storage/Stability	**Frozen**: stable until the expiration date at -20 to -70° C. **Thawed and Opened**: Once the stopper is punctured, stable for 5 days when stored at 2-8°C. Once thawed, do not re-freeze

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 Dimension Vista® QC Schedule in the Laboratory policy Quality Control Program and in the Dimension Vista® 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

Dimension Vista® System

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 product
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

TNIH Flex® reagent cartridge Cat. No. K6427 is required to perform this test.

High Sensitivity Troponin I is performed on the Dimension Vista® System after the method is calibrated (see Reference Material in Calibration section) 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	Sample Processing
1.	A sample rack holding tubes or cups is placed on the rack input lane.
2.	The sample shuttle moves the rack to the barcode reader which identifies the rack and samples to the system.
3.	The rack moves into the sample server and to the rack positioner.
4.	At the same time, aliquot plates move from the aliquot loader into position.
5.	The aliquot probe aspirates the sample from the tubes or cups and dispenses it into the wells of the aliquot plates.
6.	After each aspirate-dispense action, the probe is thoroughly rinsed inside and out to prevent sample carryover.
7.	When sample aspiration is completed, the sample server moves the rack back to the sample shuttle, where it is placed on the output lane and can be removed by the operator.

8.2	Specimen Testing
1.	For QC placement and frequency, refer to the Dimension Vista® QC Schedule in the Laboratory QC Program.
2.	Follow the instructions, outlined in the Dimension Vista® Operator's Manual
3.	The instrument reporting system contains error messages to warn the user of specific malfunctions. Results followed by such error messages should be held for follow-up. Refer to the Dimension Vista® system manual "Error messages" section for troubleshooting.
4.	Follow protocol in Section 10.6 "Repeat criteria and resulting" for samples with results above or below the Analytical Measurement Range (AMR). Investigate any failed delta result and repeat, if 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.

Test Conditions	
Sample Volume:	10 µL
Chemibead Reagent Volume:	20 µL
Biotinylated Antibody Volume:	20 µL
Sensibead Reagent Volume:	20 µL
Assay Buffer Volume:	100 µL
Reaction Time:	10 minutes
Test Temperature:	37° C
Wavelength:	680 and 612 nm
Type of measurement:	Chemiluminescence

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 result as whole number.

10.3 Units of Measure

pg/mL

10.4 Clinically Reportable Range (CRR)

4 – 125,000 pg/mL

10.5 Review Patient Data

Each result is reviewed for error messages. Refer to the Dimension Vista system manual "Error messages" section for troubleshooting. 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…
< 4 pg/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 4 pg/mL
≥ 25,000 pg/mL	**On Board Automated Dilution:** Results ≥ 25,000.0 pg/mL will automatically have repeat testing performed into the instrument using dilution factor of 5. No multiplication is necessary.

IF the result is …	THEN…
> 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. Refer to your Dimension Vista Operator's Guide.

The expected maximum observed standard deviations for repeatability using n = 5 replicates at the following Cardiac Troponin I concentrations are:

TNIH Concentration	Acceptable S.D. Maximum
50.0 pg/mL	4.8 pg/mL
500.0 pg/mL	31.4 pg/mL

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

4 – 25,000 pg/mL

Note: The lower limit in the manufacturer's package insert is 3 pg/mL. The laboratory has chosen to use a value of 4 to standardize with testing performed on the EXL analyzers at GEC.

14.2 Precision

Material	Mean pg/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Plasma 1	12.4	0.69	0.71
Plasma 2	50.7	0.98	1.37
Plasma 3	76.4	1.44	1.77

14.3 Interfering Substances

- Patient samples may contain cardiac troponin-specific autoantibodies that could react in immunoassays to give depressed results.
- Specimens that contain biotin at a concentration of 300 ng/mL demonstrate a less than 10% change in results. Biotin concentrations greater than this may lead to falsely depressed results for patient samples. Testing specimens from renal dysfunction patients taking biotin may lead to false negative results. Therefore, do not use this device in patients with renal impairment (eGFR<60), unless it is confirmed that the patient is not taking biotin. Patients taking more than 20 mg/day of biotin may have falsely negative results, and should not use this test.
- Dextran 40 at 60 g/L increases the troponin result in plasma at 35.1 pg/mL and 1337.4 pg/mL by 22% and 4% respectively.
- Protein Gamma Globulin at 6 g/dL increases the troponin result in plasma samples at approximately 40 pg/mL and 1000 pg/mL of troponin.

HIL Interference:
The TNIH assay was evaluated for interference according to CLSI EP07-A2.36 Bias is the difference in the results between the control sample (without the interferent) and the test sample (contains the interferent) expressed in percent. Lithium heparin plasma test sample ranges were: 40 ± 20 pg/mL and 1350 ± 650 pg/mL. Bias exceeding 10% is considered interference.

Substance tested	Substance Concentration	Bias %
Hemoglobin (hemolysate)	400 mg/dL	<10
Bilirubin (unconjugated)	40 mg/dL	<10
Bilirubin (conjugated)	30 mg/dL	<10
Lipemia Intralipid	3000 mg/dL	<10

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

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

TNIH Flex® Reagent Cartridge may cause an allergic skin reaction.
Contains: 5-chloro-2-methyl-3(2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone.
Wear protective gloves/protective clothing/eye protection/face protection.

IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention.

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

16. RELATED DOCUMENTS

1. Dimension Vista® Clinical Chemistry System Operator's Manual
2. Dimension Vista® Calibration/Verification Procedure
3. Dimension Vista® Cal Accept Guidelines
4. Dimension Vista® Calibration summary
5. Dimension Vista® Sample Processing, Startup and Maintenance procedure
6. Laboratory Quality Control Program
7. QC Schedule for Siemens Dimension Vista®
8. Laboratory Safety Manual
9. Safety Data Sheets (SDS)
10. Dimension Vista® Limits Chart (AG.F200)
11. Quest Diagnostics Records Management Procedure
12. Dimension Vista® System Error Messages Chart
13. Centrifuge Use, Maintenance and Function Checks (Lab policy)
14. Specimen Acceptability Requirements (Lab policy)
15. Repeat Testing Requirement (Lab policy)
16. Critical Values (Lab policy)
17. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
18. Current package insert TNIH Flex® Reagent Cartridge K6427

17. REFERENCES

1. Package Insert, TNIH Flex® Reagent Cartridge K6427, Siemens Healthcare Diagnostics Inc., 6/11/2019.
2. Package Insert, TNIH CAL, Siemens Healthcare Diagnostics Inc., 06/2020.
3. Package Insert, Liquichek Cardiac Markers Plus Control LT, Levels 1, 2, 3,1A, 1B and 1C; Bio-Rad Laboratories, 06/2020.
4. Package Insert, CTNI Sample Diluent, Siemens Healthcare Diagnostics Inc., 04/2019.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
1	2/9/21	5.3	Added lot specific correlation factors	L Barrett	R SanLuis

19. ADDENDA

None