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.C3010	Cholesterol, HDL (D-HDL) by Atellica CH Analyzer
SGMC.C3008	Cholesterol, Total (Chol-2) by Atellica CH Analyzer
SGMC.C3023	Lactate Dehydrogenase (LDLP) by Atellica CH Analyzer
SGMC.C3025	Triglycerides (Trig) by Atellica CH Analyzer

Description of change(s):

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

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

These SOPs were implemented on May 19, 2021

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

Technical SOP

Title	**Cholesterol, HDL (D-HDL) by Atellica CH 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
Cholesterol, HDL	Atellica CH Analyzer	HDL

Synonyms/Abbreviations
HDL, HLDC, Included in Batteries/Packages: LIPD

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica CH Direct HDL Cholesterol (D‑HDL) assay measures HDL cholesterol in serum and plasma without prior separation, based on procedures developed by Izawa, Okada, and Matsui. Cholesterol from non-HDL particles is released and eliminated in the first step of the reaction. Cholesterol in HDL particles is released in the second step by the detergent in D-HDL Reagent 2, and the HDL cholesterol is measured by a Trinder reaction.
The assay consists of 2 distinct reaction steps:

1. Elimination of chylomicrons, VLDL cholesterol, and LDL cholesterol by cholesterol esterase and cholesterol oxidase. Peroxide produced by the oxidase is removed by catalase.
2. Specific measurement of HDL cholesterol after release of HDL cholesterol by surfactant in D‑HDL Reagent 2. Catalase from step 1 is inhibited by sodium azide in D‑HDL Reagent 2. The intensity of the quinoneimine dye produced in the Trinder reaction is directly proportional to the cholesterol concentration when measured at 596/694 nm.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	Blood should be collected after a 12 hour period of fasting by normal procedures.
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: To be determined
	Refrigerated: 7 days
	Frozen: 3 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	**Lipemic Samples**: Ultra-centrifugation removes lipemia. Thus, if lipid testing (CHOL, TRIG, HDL, or LDL) is requested, testing for lipids must be performed prior to ultra-centrifugation. **Note**: Saved aliquot must be clearly marked as ultra-centrifuged. 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
Direct HDL Cholesterol (D-HDL)	Siemens, Atellica CH, Cat. No. 11097630

4.2 Reagent Preparation and Storage

Reagent	**Direct HDL Cholesterol (D-HDL)**
Storage	• Store at 2-8°C • Protect from light sources
Stability	Reagents are stable onboard the system for 89 days.
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
HDL/LDL Cholesterol Calibrator (HDL/LDL CAL)	Siemens Atellica CH, Cat. No. 11099402

5.2 Calibrator Preparation and Storage

Calibrator	HDL/LDL Cholesterol Calibrator (HDL/LDL CAL)
Preparation	1. Open the vial carefully. 2. Add 1.0 mL of reagent grade water into the vial using a calibrated pipette. Replace stopper. 3. Let the vials stand for 30 minutes at room temperature to allow the lyophilized material to completely dissolve. 4. Gently swirl and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** stable for 3 days.

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	HDL/LDL Cholesterol Calibrator (HDL/LDL CAL)

Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in mg/dL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (60 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (30 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**: 7 days at 2-8C for HDL Chol **Thawed and Opened**: 7 days at 2-8C for HDL Chol 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. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program. • Corrective action documentation must follow the Laboratory Quality Control Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.

- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

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

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Siemens Atellica CH Analyzer

7.2 Equipment

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

7.3 Supplies

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

8. PROCEDURE

Atellica CH Direct HDL Cholesterol (D-HDL) is required to perform this test.

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

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

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

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

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

9. CALCULATIONS

Total Cholesterol (TC) = High density lipoprotein (HDL) + Low density lipoprotein (LDL) + Very low density lipoprotein (VLDL) or TC = HDL + LDL + VLDL

The following calculations are performed by the LIS (Sunquest) when a Lipid Panel is performed:

Calculated LDL = TC – HDL – VLDL

Notes:

If the calculated LDL is a negative number, the LIS will automatically report it as: Unable to calculate, recommend direct measurement of LDL.

If any of the variables in the calculation is a non-numeric result, the LIS will automatically report the calculated LDL as ‘Unable to calculate’

VLDL = Triglycerides (Trig) ÷ 5

Note: If the triglyceride result is non-numeric, the LIS will automatically report VLDL as ‘Not calculated’

Total Cholesterol/HDL Ratio = TC ÷ HDL

Note: If either the total cholesterol or HDL result is non-numeric, the LIS will automatically report the ratio as 'Unable to calculate'

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

mg/dL

10.4 Clinically Reportable Range (CRR)

20 – 258 mg/dL

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…
< 20 mg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 20 mg/dL
≥ 129 mg/dL	**On Board Automated Dilution:** Results ≥ 129 mg/dL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 258 mg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 258 mg/dL -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 (>19 years):	>39 mg/dL	>39 mg/dL
Pediatric:		
15 – 19 years	36-76	31-65
5 – 14 years	37-75	38-76

Calculated LDL: < 130 mg/dL
VLDL: 8 – 32 mg/dL
Chol / HDL Ratio: < 5.0

11.2 Critical Values

None established

11.3 Standard Required Messages

The following comment is automatically added to the report by the LIS when a lipid panel is ordered:

Lipid Interpretation

RISK OF CORONARY HEART DISEASE		
TOTAL CHOL. / HDL-CHOL. RATIO		
	MEN	WOMEN
½ average risk	3.4	3.4
average risk	5.0	4.4
2 times average risk	9.6	7.1
3 times average risk	23.4	11.0

12. CLINICAL SIGNIFICANCE

Measurements of HDL-C are used as an aid in the diagnosis of lipid disorders (such as diabetes mellitus), various liver and renal diseases, and in the assessment of risk for atherosclerosis and cardiovascular disease.
Plasma lipoproteins are spherical particles of varying composition. The outer surface of these particles is made up of phospholipids, free cholesterol and protein; the inner core contains mostly esterified cholesterol and triglyceride. Lipoproteins function to solubilize and transport cholesterol and triglycerides in the bloodstream.
Four types of lipoproteins are recognized clinically based on the relative proportions of their lipid and protein content: chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). The primary function of HDL is to transport cholesterol from peripheral tissues to the liver where it is metabolized. This process, known as reverse cholesterol transport, has been proposed to be a cardiovascular protective mechanism. Patients with low levels of HDL cholesterol are generally considered to be at increased risk for coronary artery disease.

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)

20 – 129 mg/dL

14.2 Precision

Material	Mean mg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Plasma	37.5	0.68	2.0
Serum QC	64.6	0.39	1.6
Serum	101.5	0.71	0.7

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	mg/dL	Bias %
Hemoglobin	500 mg/dL	36.1	7
Bilirubin (unconjugated)	30 mg/dL	35.6	-1
Bilirubin (conjugated)	30 mg/dL	33.1	6
Lipemia Intralipid®	1000 mg/dL	34.5	1

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) < limit of detection (LoD) and LoD ≤ 5.0 mg/dL, and a limit of quantitation (LoQ) ≤ 20 mg/dL. The LoD corresponds to the lowest concentration of HDL cholesterol that can be detected with a probability of 95%. The LoD for the Atellica CH D‑HDL assay is 2.1 mg/dL, and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 0.5 mg/dL. The LoQ corresponds to the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable error ≤ 20 %CV. The LoQ of the Atellica CH D‑HDL assay is 2.7 mg/dL.

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

HDL/LDL CAL contains 2-methyl-2H-isothiazol-3-one hydrochloride. May produce an allergic reaction.

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)
10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of Direct HDL Cholesterol 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, Direct HDL Cholesterol Reagent, Siemens Healthcare Diagnostics Inc., 08/2020.
3. Package Insert, HDL/LDL Cholesterol Calibrator (HDL/LDL 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	**Cholesterol, Total (Chol-2) by Atellica CH 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
Cholesterol, Total	Atellica CH Analyzer	CHOL

Synonyms/Abbreviations
CHOL, Included in Batteries/Packages: LIPD

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The cholesterol esters are hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. The cholesterol is converted to cholest-4-en-3-one by cholesterol oxidase in the presence of oxygen to form hydrogen peroxide. A colored complex is formed from hydrogen peroxide, 4-aminoantipyrine and phenol under the catalytic influence of peroxidase. The absorbance of the complex is measured as an endpoint reaction at 505/694 nm.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

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

3.2 Specimen Type & Handling

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

Criteria	
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 8 hours
	Refrigerated: 2 days
	Frozen: Not established
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	**Lipemic Samples:** Ultra-centrifugation removes lipemia. Thus, if lipid testing (CHOL, TRIG, HDL, or LDL) is requested, testing for lipids must be performed prior to ultra-centrifugation. **Note:** Saved aliquot must be clearly marked as ultra-centrifuged. 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
Cholesterol-2 (Chol-2)	Siemens, Atellica CH, Cat. No. 11097609

4.2 Reagent Preparation and Storage

Reagent	**Cholesterol-2 (Chol-2)**
Storage	• Store at 2-8°C • Protect the product from light sources
Stability	Onboard per well: 26 days
Preparation	Reagent is liquid and ready to use. **Note:** The Chol-2 reagent should be kept refrigerated at 2–8°C when not in use. The detergent in this reagent may appear cloudy if maintained at room temperature. If the reagent appears cloudy, restore it to a temperature of 2–8°C, and then mix the reagent by gently inverting the sealed reagent pack until the detergent precipitate dissolves. Once the reagent appears clear again, return the pack to cold storage in a refrigerator. Reagent performance is not affected once reagent cloudiness dissipates

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

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

5.2 Calibrator Preparation and Storage

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

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Chemistry Calibrator (CHEM CAL)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in mg/dL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (50 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (7 days), for calibrated reagent packs on the system. • When indicated by quality control results. • After major maintenance or service. At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.
Calibration Scheme	See Package Insert for specific calibration scheme.
Procedure	Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions.

5.4 Tolerance Limits

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

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
InteliQ 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 CHOL **Thawed and Opened**: 14 days at 2-8C for CHOL 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. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program. • Corrective action documentation must follow the Laboratory Quality Control Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

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

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Siemens Atellica CH Analyzer

7.2 Equipment

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

7.3 Supplies

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

8. PROCEDURE

Atellica CH Cholesterol-2 (Chol-2) is required to perform this test.

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

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

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

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

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

9. CALCULATIONS

Total Cholesterol (TC) = High Density Lipoprotein (HDL) + Low Density Lipoprotein (LDL) + Very Low Density Lipoprotein (VLDL)

The following calculations are performed by the LIS (Sunquest) when a Lipid Panel is performed:

TC = HDL + LDL + VLDL

Calculated LDL = TC – HDL – VLDL

Notes:

If the calculated LDL is a negative number, the LIS will automatically report it as: “Unable to calculate, recommend direct measurement of LDL”

If any of the variables in the calculation is a non-numeric result, the LIS will automatically report the calculated LDL as "Unable to calculate".

VLDL = Triglycerides (Trig) ÷ 5

Note: If the triglyceride result is non-numeric, the LIS will automatically report VLDL as "Not calculated"

Total Cholesterol / HDL ratio = TC / HDL

Note: If either the total cholesterol or HDL result is non-numeric, the LIS will automatically report the ratio as "Unable to calculate"

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

mg/dL

10.4 Clinically Reportable Range (CRR)

25 – 1,236 mg/dL

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…
< 25 mg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 25 mg/dL

IF the result is …	THEN…
≥ 618 mg/dL	**On Board Automated Dilution:** Results ≥ 618 mg/dL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 1,236 mg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 1236 mg/dL -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):	< 200 mg/dL	< 200 mg/dL
Pediatric:		
16 – 18 years	101 - 200	105 - 200
14 – 15 years	125 - 200	101 - 200
12 – 13 years	120 - 200	122 - 200
10 – 11 years	122 - 200	120 - 200
7 – 9 years	107 - 200	107 - 200
4 – 6 years	103 - 184	103 - 184
1 – 3 years	37 - 178	37 - 178
7 – 11 months	68 - 200	83 - 200
2 – 6 months	59 - 200	53 - 194
0 – 1 month	56 - 195	37 - 174

Calculated LDL: < 130 mg/dL
VLDL: 8 – 32 mg/dL
Chol / HDL Ratio: < 5.0

11.2 Critical Values

None established

11.3 Standard Required Messages

The following comment is automatically added to the report by the LIS when a lipid panel is ordered:

Lipid Interpretation

RISK OF CORONARY HEART DISEASE TOTAL CHOL. / HDL-CHOL. RATIO	MEN	WOMEN
½ average risk	3.4	3.4
average risk	5.0	4.4
2 times average risk	9.6	7.1
3 times average risk	23.4	11.0

12. CLINICAL SIGNIFICANCE

Lipids and lipoproteins in circulation have been strongly associated with coronary heart disease (CHD), associated lipid metabolism disorders, and atherosclerosis, a cause of CHD.

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)

25 – 618 mg/dL

14.2 Precision

Material	Mean mg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum Pool	115	0.88	1.49
Serum QC	170	0.99	1.65
Serum QC	276	1.26	2.12
Plasma Pool	584	3.1	5.5

14.3 Interfering Substances

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

Substance tested	Substance Concentration	mg/dL	Bias %
Hemoglobin (hemolysate)	500 mg/dL	162	6
Bilirubin (unconjugated)	20 mg/dL	166	9
Bilirubin (conjugated)	60 mg/dL	126	8
Lipemia Intralipid®	1000 mg/dL	152	2

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

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

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. QC Schedule for Siemens Atellica Solution
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Quest Diagnostics Records Management Procedure
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)
10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of Cholesterol-2 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, Cholesterol-2 Reagent, Siemens Healthcare Diagnostics Inc., 07/2019.
3. Package Insert, Chemistry Calibrator (CHEM CAL), Siemens Healthcare Diagnostics Inc., 04/2020.
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	**Triglycerides (Trig) by Atellica CH Analyzer**
Prepared by	Ashkan Chini Date: 4/27/2021
Owner	Robert SanLuis Date: 4/27/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Triglycerides	Atellica CH Analyzer	TRIG

Synonyms/Abbreviations
TGL, TRIG, Included in Batteries/Packages: LPNL

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The triglycerides are converted to glycerol and free fatty acids by lipase. The glycerol is then converted to glycerol-3-phosphate by glycerol kinase followed by its conversion by glycerol-3-phosphate-oxidase to hydrogen peroxide. A colored complex is formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase. The absorbance of the complex is measured as an endpoint reaction at 505/694 nm.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	Fasting specimens preferred. Patient should be fasting for 12 hours before collection.
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: To be determined
	Refrigerated: 7 days
	Frozen: 3 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	**Lipemic Samples:** Ultra-centrifugation removes lipemia. Thus, if lipid testing (CHOL, TRIG, HDL, or LDL) is requested, testing for lipids must be performed prior to ultra-centrifugation. **Note:** Saved aliquot must be clearly marked at ultra-centrifuged. 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
Triglycerides (Trig)	Siemens, Atellica CH, Cat. No. 11097591

4.2 Reagent Preparation and Storage

Reagent	**Triglycerides (Trig)**
Storage	• Store at 2-8°C • Protect from light sources
Stability	Onboard per well: 30 days
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

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

5.2 Calibrator Preparation and Storage

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

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	Chemistry Calibrator (CHEM CAL)
Assay Range	See Package Insert for specific assay ranges.
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in mg/dL
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (60 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (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	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**: 7 days at 2-8C for Trig **Thawed and Opened**: 7 days at 2-8C for Trig 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. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program. • Corrective action documentation must follow the Laboratory Quality Control Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples.

Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.

- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Siemens Atellica CH Analyzer

7.2 Equipment

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

7.3 Supplies

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

8. PROCEDURE

Atellica CH Triglycerides (Trig) is required to perform this test.

Triglycerides 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.

8.1	Instrument Set-up Protocol
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 Triglycerides in mg/dL.

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

mg/dL

10.4 Clinically Reportable Range (CRR)

10 – 1100 mg/dL

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…
< 10 mg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 10 mg/dL
≥ 550 mg/dL	**On Board Automated Dilution:** Results ≥ 550 mg/dL will automatically have repeat testing performed into the instrument using dilution factor of 2. No multiplication is necessary.
> 1100 mg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 1100 mg/dL -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):	0 – 149 mg/dL	0 – 149 mg/dL
Pediatric:		
16 – 18 years	35 - 134	32 - 134
14 – 15 years	36 - 129	32 - 158
12 – 13 years	35 - 124	22 - 138
10 – 11 years	37 - 134	22 - 131
7 – 9 years	26 - 123	26 - 123
4 – 6 years	30 - 110	30 - 110
1 – 3 years	25 - 119	25 - 119
1 – 11 months	34 - 340	42 - 279
8 – 30 days	33 - 270	37 - 279
0 – 7 days	26 - 159	19 - 174

11.2 Critical Values

None established

11.3 Standard Required Messages

The following comment is automatically added to the report by the LIS when a lipid panel is ordered:

Lipid Interpretation

RISK OF CORONARY HEART DISEASE		
TOTAL CHOL. / HDL-CHOL. RATIO		
	MEN	WOMEN
½ average risk	3.4	3.4
average risk	5.0	4.4
2 times average risk	9.6	7.1
3 times average risk	23.4	11.0

12. CLINICAL SIGNIFICANCE

Triglycerides are water-insoluble lipids consisting of three fatty acids linked to one glycerol molecule. Triglycerides are transported in the blood as core constituents of all lipoproteins, but the greatest concentration of these molecules is carried in the triglycerides-rich chylomicrons and very low density lipoproteins (VLDL). Through the action of lipases and bile acids, triglycerides are hydrolyzed into glycerol and fatty acids which are absorbed by adipose tissue for storage or by other tissues requiring a source of energy. A peak concentration of chylomicron-associated triglycerides occurs within 3–6 hours after ingestion of a fat-rich meal; however, the rate of absorption of fats is highly variable, depending on the individual and dietary composition of the fat. After absorption, triglycerides are resynthesized in the epithelial cells and combined with cholesterol and a number of apolipoproteins to form chylomicrons.
Triglycerides measurements obtained are used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, other diseases involving lipid metabolism, or various endocrine disorders.

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)

10 – 550 mg/dL

14.2 Precision

Material	Mean mg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum	106	0.8	2.6
Serum QC	228	1.1	2.9
Plasma	424	2	4.1

14.3 Interfering Substances

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

Substance tested	Substance Concentration	mg/dL	Bias %
Hemoglobin	500 mg/dL	159	8
Bilirubin (unconjugated)	5 mg/dL	143	8
Bilirubin (conjugated)	15 mg/dL	147	-8

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) ≤ limit of detection (LoD) and LoD ≤ 8 mg/dL, and a limit of quantitation (LoQ) ≤ 10 mg/dL. The LoD corresponds to the lowest concentration of triglycerides that can be detected with a probability of 95%. The LoD for the Atellica CH Trig assay is 4 mg/dL, and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 3 mg/dL. The LoQ corresponds to the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable error ≤ 25%. The LoQ of the Atellica CH Trig assay is 10 mg/dL, and was determined using multiple patient samples that were assayed using 3 reagent lots, over a period of 3 days, using total analytical error definition of bias + 2SD.

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 Triglycerides 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, Triglycerides Reagent, Siemens Healthcare Diagnostics Inc., 06/2020.
3. Package Insert, Chemistry Calibrator (CHEM CAL), Siemens Healthcare Diagnostics Inc., 04/2020.
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	**Lactate Dehydrogenase (LDLP) by Atellica CH Analyzer**
Prepared by	Ashkan Chini Date: 4/27/2021
Owner	Robert SanLuis Date: 4/27/2021

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Lactate Dehydrogenase, Serum / Plasma	Atellica CH Analyzer	LDH
Lactate Dehydrogenase, Body Fluid		FLD

Synonyms/Abbreviations
LD, LDH, LDI

Department
Chemistry

2. ANALYTICAL PRINCIPLE

Lactate dehydrogenase (LD) catalyzes the conversion of L-lactate to pyruvate in the presence of nicotinamide adenine dinucleotide (NAD). The enzymatic activity of LD is proportional to the rate of production of NADH (reduced NAD). The amount of NADH produced is determined by measuring the increase in absorbance at 340/410 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, plasma and body fluid 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**	Serum, Body Fluid Plasma (Lithium Heparin) Serum
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST) Body Fluid: Sterile/Clean container or tube

Criteria		
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL	
Transport Container and Temperature	Collection container or Plastic vial at room temperature	
Stability & Storage Requirements	Room Temperature:	Plasma/serum - 7 days Body fluid – to be determined
	Refrigerated:	Plasma/serum - 4 days Body fluid – to be determined
	Frozen:	Plasma/serum - 42 days
Timing Considerations	To avoid falsely elevated results due to high red blood cell LD levels, separate specimens from the clot as soon as possible.	
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. Carefully evaluate plasma data because of the possible effects of sample handling on LD levels. Elevations in plasma LD levels can occur as a result of the release of LD from red blood cells or platelets. For this reason, serum is the preferred sample. 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
Lactate Dehydrogenase L-P (LDLP)	Siemens, Atellica CH, Cat. No. 11097594

4.2 Reagent Preparation and Storage

Reagent	**Lactate Dehydrogenase L-P (LDLP)**
Storage	Store at 2-8°C
Stability	Onboard per well: 28 days
Preparation	Reagent is liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
ENZ 1 Calibrator (ENZ 1 CAL)	Siemens Atellica CH, Cat. No. 11099317

5.2 Calibrator Preparation and Storage

Calibrator	ENZ 1 Calibrator (ENZ 1 CAL)
Preparation	Calibrators are liquid and ready to use.
Storage/Stability	• Store at 2-8°C • Protect from heat and light sources. • **Unopened:** stable until expiration date stamped on box • **Opened:** remains stable for 30 days when recapped immediately after use.

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	ENZ 1 Calibrator (ENZ 1 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 (60 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	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 LDH **Thawed and Opened**: 14 days at 2-8C for LDH 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. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program. • Corrective action documentation must follow the Laboratory Quality Control Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples.

Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.

- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Siemens Atellica CH Analyzer

7.2 Equipment

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

7.3 Supplies

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

8. PROCEDURE

Atellica CH Lactate Dehydrogenase L-P (LDLP) is required to perform this test.

LDLP 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.

8.1	Instrument Set-up Protocol
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 Lactate Dehydrogenase 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)

14 – 4500 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…
< 14 U/L	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 14 U/L
≥ 750 U/L	**On Board Automated Dilution:** Results ≥ 750 U/L will automatically have repeat testing performed into the instrument using dilution factor of 6. No multiplication is necessary.
> 4500 U/L	If the recommended dilution does not give results within the clinically reportable range, report as: "> 4500 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

Serum / Plasma:
Male: 85 – 227 U/L
Female: 81 – 234 U/L

Body Fluid: Reference ranges have not been established for this sample type

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Lactate dehydrogenase (LD) is present in the cytoplasm of all cells in the body. The concentration of LD in tissues is several hundred-fold higher than in serum or plasma and even a small amount of tissue damage can lead to an elevation in LD activity. This makes LD especially useful in the diagnosis and monitoring of disease states where tissue turnover is accelerated such as the liver, cardiac muscle, skeletal muscle, kidneys, and erythrocytes.

LD is elevated in myocardial or pulmonary infarction, leukemias, hemolytic anemias, non-viral hepatitis, sickle cell disease, lymphoma, renal infarction, acute pancreatitis and any condition that results in the leaking of cytoplasm. It is moderately elevated in cirrhosis, obstructive jaundice, renal disease, skeletal muscle diseases, neoplastic diseases and congestive heart failure. LD is markedly elevated in megaloblastic and pernicious anemia, metastatic carcinoma, viral hepatitis, shock, hypoxia and extreme hyperthermia.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared for serum / plasma
- **FDA Status:** FDA Modified for body fluid
- **Validated Test Modifications:** Testing validated for body (serous) fluid specimens

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)

14 – 750 U/L

14.2 Precision

Material	Mean U/L	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum Pool	189	1.7	0.9
QC	403	2.7	0.7
Plasma Pool	557	1.5	0.3

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 %
Bilirubin (conjugated)	30 mg/dL	121	-3
Bilirubin (unconjugated)	30 mg/dL	120	1
Lipemia Intralipid®	650 mg/dL	122	1

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) < limit of detection (LoD) and LoD ≤ 20 U/L, and a limit of quantitation (LoQ) ≤ 25 U/L. The LoD corresponds to the lowest concentration of lactate dehydrogenase that can be detected with a probability of 95%. The LoD for the Atellica CH LDLP assay is 3 U/L, and was determined using 120 determinations, with 60 blank and 60 low level replicates, and a LoB of 2 U/L. The LoQ corresponds to the lowest amount of analyte in a sample that

can be accurately quantitated with a total allowable error ≤ 15 U/L. The LoQ of the Atellica CH LDLP assay is 11 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 LDLP reagent may cause an allergic skin reaction. Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Wash contaminated clothing before reuse.
Contains: 2-chloracetamide (R2)

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 Lactate Dehydrogenase L-P Reagent

17. REFERENCES

1. Package Insert, Lactate Dehydrogenase L-P Reagent, Siemens Healthcare Diagnostics Inc., 11/2019.
2. Package Insert, ENZ 1 Calibrator (ENZ 1 CAL), Siemens Healthcare Diagnostics Inc., 07/2019.
3. Package Insert, InteliQ Assayed Multiqual Controls, Bio-Rad Laboratories, 07/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

None