TRAINING UPDATE

Lab Location:	SGMC & WAH	**Date Distributed:**	3/19/2016
Department:	Core	**Due Date:**	4/12/2016
		Implementation:	**4/13/2016**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Cholesterol, Total by Dimension Vista® System SGAH.C78, WAH.C74 v3

Cholesterol, High Density Lipoprotein (HDLC) by Dimension Vista® System SGAH.C104, WAH.C100 v2

Triglycerides by Dimension Vista® System SGAH.C111, WAH.C107 v1

Description of change(s):

All 3 SOPS – main change is to section 3

Section	Reason
3.2	Specify anticoagulant, **add instructions for lipemia**
6.4, 6.6	Replace LIS with Unity Real Time
11.1	Add ranges for calculated values
11.3	Add report comment for lipid panel

CHOL

1	Add battery code
9	Correct to state performed by LIS

HDL

4.2	Add hazard/chemical information
5.2	Update stability

TRIG

1	Remove outdated battery codes
4.2	Add hazard/chemical information
5.2	Update stability

These revised SOPS will be implemented on April 13, 2016

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

Approved draft for training (version 3)

Technical SOP

Title	**Cholesterol, Total by Dimension Vista® System**	
Prepared by	Ashkan Chini	Date: 6/22/2012
Owner	Robert SanLuis	Date: 10/21/2013

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

Review		
Print Name	**Signature**	**Date**

Form revised 2/02/2007

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1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Cholesterol, Total	Dimension Vista® System	CHOL

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

Department
Chemistry

Form revised 2/02/2007

2. ANALYTICAL PRINCIPLE

Cholesterol esterase (CE) catalyzes the hydrolysis of cholesterol esters to produce free cholesterol which, along with preexisting free cholesterol, is oxidized in a reaction catalyzed by cholesterol oxidase (CO) to form cholest-4-ene-3-one and hydrogen peroxide. In the presence of horseradish peroxidase (HPO), the hydrogen peroxide thus formed is used to oxidize N, N-diethylaniline- HCl/4-aminoantipyrine (DEA-HCl/AAP) to produce a chromophore that absorbs at 540 nm. The absorbance due to oxidized DEA-HCl/AAP is directly proportional to the total cholesterol concentration and is measured using a polychromatic (540, 452, 700 nm) endpoint technique.

$$\text{Cholesterol esters} \xrightarrow{\text{CE}} \text{Cholesterol} + \text{Fatty Acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CO}} \text{Cholest-4-ene-3-one} + H_2O_2$$

$$2\,H_2O_2 + \text{DEA} - \text{HCl/AAP} \xrightarrow{\text{HPO}} 4\,H_2O + \text{Oxidized DEA} - \text{HCl/AAP}$$

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: 5 - 7 days

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Criteria	
	Frozen: 3 months
	Instrument on board aliquot stability 2 hours
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	**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.

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Cholesterol	Siemens, Flex® reagent cartridge, Cat. No. K1027

4.2 Reagent Preparation and Storage

NOTES: Each container must be labeled with (1) substance name, (2) lot number, (3) expiration date, (4) any special storage instructions; check for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration. Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Reagent	**Cholesterol**
Container	Reagent cartridge
Storage	Store at 2-8° C
Stability	• Reagent is stable until expiration date stamped on the reagent cartridges. • Sealed wells on the instrument are stable for 30 days. • Once wells 1 - 12 have been entered by the instrument, they are stable for 7 days.
Preparation	Hydrating, mixing and diluting are automatically performed by the instrument.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
CHEM 1 CAL	Siemens Dimension Vista®, Cat. No. KC110B

5.2 Calibrator Preparation and Storage

NOTE: Date and initial all calibrators upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) any special storage instructions; check for visible signs of degradation. **When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration.**

Calibrator	CHEM 1 CAL
Preparation	Allow CHEM 1 Calibrator to thaw and equilibrate to room temperature (22 – 28° C) for 1 hour. Before use, **gently** invert the calibrator vials at least 10 times to ensure that the contents are thoroughly mixed. **Do not vortex.**
Storage/Stability	• Store at -25 to - 15° C • **Unopened calibrator** is stable until expiration date stamped on the box. • **Opened Calibrator:** once the stopper of the vial is punctured, assigned values are stable for 7 days when stored on board the Dimension Vista System.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	CHEM 1 CAL

Assay Range	50 – 600 mg/dL
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in mg/dL
Frequency	• Every new reagent cartridge lot. • Every 90 days for any one lot • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	2 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™ Unassayed Chemistry Control Levels 1 and 2	Bio-Rad Laboratories Cat. No. 691 and 692

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation. A barcode label is produced and placed on the vial.

Control	Liquichek Unassayed Chemistry Controls, Level 1 and 2
Preparation	Allow the frozen control to stand at room temperature (18-25°C) until completely thawed. Swirl the contents gently to ensure homogeneity. (Do not use a mechanical mixer) Use immediately. After each use, promptly replace the stopper and return to 2-8°C storage.
Storage/Stability	Once the control is thawed, all analytes will be stable for 15 days at 2-8°C. Unthawed controls are stable until the expiration date at -20 to -70°C.

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

Step	Action
1	Acceptable ranges for QC are programmed into the instrument's Quality Control software system and Unity Real Time, and may be posted near the instrument for use during computer downtime.
2	**Run Rejection Criteria** • Anytime the established parameters are exceeded (if one QC result exceeds 2 SD), the run is considered out of control (failed) and patient results must not be reported.

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

6.5 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.

6.6 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.

6.7 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 -20 to -70°C.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

CHOL Flex® reagent cartridge Cat. No. K1027 is required to perform this test.

Cholesterol 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.

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.

8.1	Sample Processing
1.	A sample rack holding tubes or cups is placed on the rack input lane.

8.1	Sample Processing
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 10.5 "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:	1.25 µL
Reagent 1 Volume:	36.5 µL
Reagent 2 Volume:	10.8 µL
Reaction Time:	5.6 minutes
Test Temperature:	37° C
Wavelength:	540, 542 & 700 nm
Type of measurement:	Polychromatic endpoint

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

VLDL = Triglycerides / 5

Total Cholesterol / HDL ratio = TC / HDL

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)

50 – 3,000 mg/dL

10.5 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 within the AMR or CRR may be reported without repeat. Values that fall outside these ranges must be repeated.

IF the result is …	THEN…
< 50 mg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 50 mg/dL
≥ 600 mg/dL	**On Board Automated Dilution:** Results ≥ 600 mg/dL will automatically have repeat testing performed into the instrument using dilution factor of 4. No multiplication is necessary.

> 2,400 mg/dL	**Manual Dilution:** Using the primary tube, make the smallest dilution possible to bring the raw data within the AMR. Maximum allowable dilution: x 5 **DILUENT**: WATER Enter dilution factor as a whole number. Re-assay. Readout is corrected for dilution.
> 3,000 mg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 3,000 mg/dL-REP" Bring to the attention of your supervisor prior to releasing result.

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 Report 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. Refer to your Dimension Vista Operator's Guide.

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

CHOL Concentration	Acceptable S.D. Maximum
180 mg/dL	17 mg/dL
450 mg/dL	30 mg/dL

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

50 – 600 mg/dL

14.2 Precision

Material	Mean mg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Multiqual Control			
Level 1	176	4 (2)	5 (3)
Level 2	278	4 (2)	7 (2)

14.3 Interfering Substances

Potassium Oxalate/Sodium Fluoride can decrease cholesterol results an average of 12% and should not be used.

HIL Interference:
The CHOL method was evaluated for interference according to CLSI/NCCLS EP7-A2. Bias, defined as the difference between the control sample (does not contain interferent) and the test sample (contains interferent), is shown in the table below. Bias exceeding 10% is considered "interference".

Substance tested	Substance Concentration	CHOL mg/dL	Bias %
Hemoglobin (hemolysate)	1000 mg/dL	202	<10
Bilirubin (unconjugated)	5 mg/dL 10 mg/dL 20 mg/dL 40 mg/dL	202	<10 -11 -13 -26
Bilirubin (conjugated)	5 mg/dL 10 mg/dL 20 mg/dL 40 mg/dL	202	<10 -12 -13 -32
Lipemia Intralipid®	1000 mg/dL 3000 mg/dL	202	<10 -11.3

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

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. Material Safety Data Sheets (MSDS)
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 Functions Checks (Lab policy)
14. Hemolysis, Icteria and Lipemia Interference (Lab policy)
15. Repeat Testing Requirement (Lab policy)
16. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
17. Current package insert CHOL Flex® Reagent Cartridge K1027

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, CHOL Flex® Reagent Cartridge K1027, Siemens Healthcare Diagnostics Inc., 07/28/2014.
3. Package Insert, CHEM I CAL, Siemens Healthcare Diagnostics Inc., 03/2015.
4. Package Insert, Unassayed Liquichek Chemistry Controls, Bio-Rad Laboratories, 08/2014.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
000	10/21/13		Update owner	L Barrett	R SanLuis
000	10/21/13	10, 14	Change values to whole numbers	L Barrett	R SanLuis
000	10/21/13	11.1	Change adult range	L Barrett	R SanLuis
000	10/21/13	16	Update titles	L Barrett	R SanLuis
000	10/21/13	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L Barrett	R SanLuis
1	6/12/14	5.2	Updated open calibrator stability	A Chini	R SanLuis
2	2/17/16	1	Add battery code	A Chini	R SanLuis
2	2/17/16	3.2	Specify anticoagulant, add instructions for lipemia	L Barrett A Chini	R SanLuis
2	2/17/16	6.4, 6.6	Replace LIS with Unity Real Time	L Barrett	R SanLuis
2	2/17/16	9	Correct to state performed by LIS	A Chini	R SanLuis
2	2/17/16	11.1	Add ranges for calculated values	A Chini	R SanLuis
2	2/17/16	11.3	Add report comment for lipid panel	A Chini	R SanLuis
2	2/17/16	17	Update package insert information	A Chini	R SanLuis

19. ADDENDA

None

Approved draft for training (version 2)

Technical SOP

Title	**Cholesterol, High Density Lipoprotein (HDLC) by Dimension Vista® System**
Prepared by	Ashkan Chini Date: 6/25/2012
Owner	Robert SanLuis Date: 2/4/2015

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

Review		
Print Name	**Signature**	**Date**

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1. TEST INFORMATION

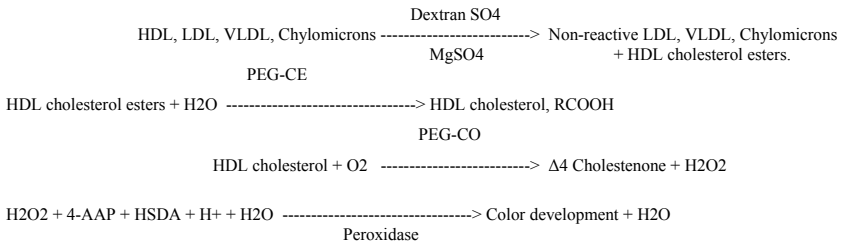
Assay	Method/Instrument	Local Code
High Density Lipoprotein Cholesterol	Dimension Vista® System	HDL

Synonyms/Abbreviations
HDL, HDLC; Included in Batteries/Packages: LIPD

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The HDLC assay measures serum HDL cholesterol levels directly without the need for sample pretreatment or specialized centrifugation steps, using a two reagent format. In the first reaction, chylomicrons, VLDL and LDL form water soluble complexes with dextran sulfate in the presence of magnesium sulfate. These complexes are resistant to the polyethylene glycol (PEG)-modified cholesterol esterase (CE) and cholesterol oxidase (CO) that react with HDL cholesterol. In the second reaction, in the presence of oxygen, the HDL cholesterol is oxidized to Δ-4-cholestenone and hydrogen peroxide. The generated hydrogen peroxide then reacts with 4-aminoantipyrine (4-AAP) and N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HSDA) in the presence of peroxidase to form a colored dye that is measured using a bichromatic (600/700 nm) technique. The color intensity of the dye is directly proportional to the serum HDL-C concentration.



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	Plasma (Lithium Heparin)
-Other Acceptable	Serum
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)
Volume - Optimum	1.0 mL
- Minimum	0.5 mL
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 8 hours
	Refrigerated: 7 days
	Frozen: 3 months
	Instrument on board aliquot stability: 2 hours
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	**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.

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
HDLC	Siemens, Flex® reagent cartridge, Cat. No. K3048A

Quest Diagnostics
Site: SGAH & WAH
Title: **Cholesterol, High Density Lipoprotein (HDLC) by Dimension Vista® System**

Form revised 2/02/2007

4.2 Reagent Preparation and Storage

NOTES: Each container must be labeled with (1) substance name, (2) lot number, (3) expiration date, (4) any special storage instructions; check for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration. Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds.
Wear protective clothing, gloves and eye/face protection.

Reagent	**High Density Lipoprotein Cholesterol**
Container	Reagent cartridge
Storage	Store at 2-8° C
Stability	• Reagent is stable until expiration date stamped on the reagent cartridges. • Sealed wells on the instrument are stable for 30 days. • Once wells 1 - 12 have been entered by the instrument, they are stable for 3 days.
Preparation	All reagents are liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
LIPID CAL	Siemens Dimension Vista®, Cat. No. KC220A

5.2 Calibrator Preparation and Storage

NOTE: Date and initial all calibrators upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) any special storage instructions; check for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration.

Calibrator	LIPID CAL
Preparation	Allow LIPID calibrator to thaw and equilibrate to room temperature (22 – 28° C) for 1 hour. Before use, **gently** invert the calibrator vials at least 10 times to ensure that the contents are thoroughly mixed. **Do not vortex.**

Storage/Stability	• Store at -25 to -15°C • **Unopened frozen calibrator** is stable until expiration date stamped on the box. • **Opened Calibrator:** once the stopper of the vial is punctured, assigned values are stable for 7 days when stored on board the Dimension Vista System.

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	LIPID CAL
Assay Range	3 – 150 mg/dL
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in mg/dL
Frequency	• Every new reagent cartridge lot. • Every 90 days for any one lot • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	2 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™ Unassayed Chemistry Control Levels 1 and 2	Bio-Rad Laboratories Cat. No. 691 and 692

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation. A barcode label is produced and placed on the vial.

Control	Liquichek Unassayed Chemistry Controls, Level 1 and 2
Preparation	Allow the frozen control to stand at room temperature (18-25°C) until completely thawed. Swirl the contents gently to ensure homogeneity. (Do not use a mechanical mixer) Use immediately. After each use, promptly replace the stopper and return to 2-8°C storage.
Storage/Stability	Once the control is thawed, all analytes will be stable for 15 days at 2-8°C. Unthawed controls are stable until the expiration date at -20 to -70°C.

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

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 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.

6.6 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.

6.7 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.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

HDLC Flex® reagent cartridge Cat. No. K3048A is required to perform this test.

High Density Lipoprotein Cholesterol 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.

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.

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 10.5 "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:	1.3 µL
Reagent 1 Volume:	135 µL
Reagent 2 Volume:	44.4 µL
Reaction Time:	8.9 minutes
Test Temperature:	37° C
Wavelength:	600 & 700 nm
Type of measurement:	Bichromatic endpoint

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

VLDL = Triglycerides (Trig) ÷ 5

Total Cholesterol/HDL Ratio = TC ÷ HDL

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)

3 – 600 mg/dL

10.5 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 within the AMR or CRR may be reported without repeat. Values that fall outside these ranges must be repeated.

IF the result is …	THEN…
< 3 mg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 3 mg/dL
≥ 150 mg/dL	**On Board Automated Dilution:** Results ≥ 150 mg/dL will automatically have repeat testing performed into the instrument using dilution factor of 4. No multiplication is necessary.
> 600 mg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 600 mg/dL-REP" Bring to the attention of your supervisor prior to releasing result.

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 Report 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. Refer to your Dimension Vista Operator's Guide.

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

HDLC Concentration	Acceptable S.D. Maximum
35 mg/dL	1.5 mg/dL
70 mg/dL	2.2 mg/dL

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

3 – 150 mg/dL

14.2 Precision

Material	Mean mg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Multiqual Unassayed Control			
Level 1	26	0.6 (2.3)	0.7 (2.7)
Level 2	47	0.8 (1.6)	1.1 (2.3)
Level 3	67	1.3 (1.9)	1.4 (2.1)
Serum Pool	41	0.7 (1.8)	0.9 (2.3)

14.3 Interfering Substances

HIL Interference:
The HDLC method was evaluated for interference according to CLSI/NCCLS EP7-A2. Bias, defined as the difference between the control sample (does not contain interferent) and the test sample (contains interferent), is shown in the table below. Bias exceeding 10% is considered "interference".

Substance tested	Substance Concentration	HDLC mg/dL	Bias %
Hemoglobin (hemolysate)	1000 mg/gL	40	<10
Bilirubin (unconjugated)	80 mg/dL	40	<10
Bilirubin (conjugated)	60 mg/dL	40	<10
Lipemia Intralipid®	1000 mg/dL	40	<10

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

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. Material Safety Data Sheets (MSDS)
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. Hemolysis, Icteria and Lipemia Interference (Lab policy)
15. Repeat Testing Requirement (Lab policy)
16. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
17. Current package insert HDLC Flex® Reagent Cartridge K3048

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, HDLC Flex® Reagent Cartridge K3048A, Siemens Healthcare Diagnostics Inc., 03/27/2015.
3. Package Insert, LIPID CAL, Siemens Healthcare Diagnostics Inc., 12/2014.
4. Package Insert, Unassayed Liquichek Chemistry Controls, Bio-Rad Laboratories, 08/2014.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
000	2/4/15		Update owner	L Barrett	R SanLuis
000	2/4/15	5.2	Change in frozen storage temperature	L Barrett	R SanLuis
000	2/4/15	7.2	Change freezer requirements	L Barrett	R SanLuis
000	2/4/15	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13	L Barrett	R SanLuis
1	2/17/16	3.2	Specify anticoagulant, add instructions for lipemia	L Barrett A Chini	R SanLuis
1	2/17/16	4.2	Add hazard/chemical information	A Chini	R SanLuis
1	2/17/16	5.2	Update stability	A Chini	R SanLuis
1	2/17/16	6.4, 6.6	Replace LIS with Unity Real Time	L Barrett	R SanLuis
1	2/17/16	11.1	Add ranges for calculated values	A Chini	R SanLuis
1	2/17/16	11.3	Add report comment for lipid panel	A Chini	R SanLuis
1	2/17/16	17	Update package insert information	A Chini	R SanLuis

19. ADDENDA

None

Approved draft for training (version 1)

Technical SOP

Title	**Triglycerides by Dimension Vista® System**		
Prepared by	Ashkan Chini	Date:	6/25/2012
Owner	Robert SanLuis, ~~Jean Buss~~	Date:	2/17/2016

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

Review		
Print Name	**Signature**	**Date**

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1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Triglycerides	Dimension Vista® System	TRIG

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

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The triglycerides method is based on an enzymatic procedure in which combinations of enzymes are employed for the measurement of serum or plasma triglycerides. The sample is incubated with lipoprotein lipase (LPL) enzyme reagent that converts triglycerides into free glycerol and fatty acids. Glycerol kinase (GK) catalyzes the phosphorylation of glycerol by adenosine-5- triphosphate (ATP) to glycerol-3-phosphate. Glycerol-3-phosphate-oxidase (GPO) oxidizes glycerol-3-phosphate to dihydroxyacetone phosphate and hydrogen peroxide (H2O2). The catalytic action of peroxidase (POD) forms quinoneimine from H2O2, aminoantipyrine and 4-chlorophenol. The change in absorbance due to the formation of quinoneimine is directly proportional to the total amount of glycerol and its precursors in the sample and is measured using a bichromatic (510, 700 nm) endpoint technique.

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + Fatty Acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-Phosphate + ADP}$$

$$\text{Glycerol-3-Phosphate + } O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate + } H_2O_2$$

$$2H_2O_2 + \text{Aminoantipyrine + 4-Chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine + HCL + } 4H_2O$$

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	Fasting specimen 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	None
Other	Avoid blood collection tubes containing glycerol lubricated stoppers which will falsely elevate results.

3.2 Specimen Type & Handling

Criteria	
Type -Preferred	Plasma (Lithium Heparin)
-Other Acceptable	Serum
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)
Volume - Optimum	1.0 mL
- Minimum	0.5 mL
Transport Container and Temperature	Collection container or Plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 8 hours Refrigerated: 7 days Frozen: 3 months Instrument on board aliquot stability 2 hours
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	**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.

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Triglycerides	Siemens, Flex® reagent cartridge, Cat. No. K2069

Quest Diagnostics
Site: SGAH & WAH
Title: **Triglycerides by Dimension Vista® System**

Form revised 2/02/2007

4.2 Reagent Preparation and Storage

NOTES: Each container must be labeled with (1) substance name, (2) lot number, (3) expiration date, (4) any special storage instructions; check for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration. Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds.
Wear protective clothing, gloves and eye/face protection.

Reagent	**Triglycerides**
Container	Reagent cartridge
Storage	Store at 2-8° C
Stability	• Reagent is stable until expiration date stamped on the reagent cartridges. • Sealed wells on the instrument are stable for 30 days. • Once wells 1 - 12 have been entered by the instrument, they are stable for 7 days.
Preparation	All reagents are liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	**Supplier and Catalog Number**
CHEM 2 CAL	Siemens Dimension Vista®, Cat. No. KC120

5.2 Calibrator Preparation and Storage

NOTE: Date and initial all calibrators upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) any special storage instructions; check for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration.

Calibrator	CHEM 2 CAL
Preparation	CHEM 2 CAL is ready for use.

Storage/Stability	• Store at 2-8° C • **Unopened calibrator** is stable until expiration date stamped on the box. • **Opened Calibrator:** once the stopper of the vial is punctured, assigned values are stable for 24 hours when stored on board the Dimension Vista System.

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	CHEM 2 CAL
Assay Range	2 – 1000 mg/dL
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in mg/dL
Frequency	• Every new reagent cartridge lot. • Every 90 days for any one lot • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	2 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™ Unassayed Chemistry Control Levels 1 and 2	Bio-Rad Laboratories Cat. No. 691 and 692

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation. A barcode label is produced and placed on the vial.

Control	Liquichek Unassayed Chemistry Controls, Level 1 and 2
Preparation	Allow the frozen control to stand at room temperature (18-25°C) until completely thawed. Swirl the contents gently to ensure homogeneity. (Do not use a mechanical mixer) Use immediately. After each use, promptly replace the stopper and return to 2-8°C storage.
Storage/Stability	Once the control is thawed, triglycerides will be stable for 6 days at 2-8°C. Unthawed controls are stable until the expiration date at -20 to -70°C.

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

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 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.

6.6 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.

6.7 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 -20 to -70°C.
- Centrifuge

7.3 Supplies

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

8. PROCEDURE

TRIG Flex® reagent cartridge Cat. No. K2069 is required to perform this test.

Triglycerides 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.

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.

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 10.5 "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:	1.6 µL
Reagent Volume:	55 µL
Reaction Time:	5.6 minutes
Test Temperature:	37° C
Wavelength:	510 & 700 nm
Type of measurement:	Bichromatic endpoint

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)

2 – 5000 mg/dL

10.5 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 within the AMR or CRR may be reported without repeat. Values that fall outside these ranges must be repeated.

IF the result is …	THEN…
< 2 mg/dL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 2 mg/dL
≥ 1000 mg/dL	**On Board Automated Dilution:** Results ≥ 1000 mg/dL will automatically have repeat testing performed into the instrument using dilution factor of 4. No multiplication is necessary.
> 4,000 mg/dL	**Manual Dilution**: Using the primary tube, make the smallest dilution possible to bring the raw data within the AMR. Maximum allowable dilution: x 5 **DILUENT**: Water Enter dilution factor as a whole number. Re-assay. Readout is corrected for dilution.
> 5,000 mg/dL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 5,000 mg/dL-REP" Bring to the attention of your supervisor prior to releasing result.

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. Refer to your Dimension Vista Operator's Guide.

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

TRIG Concentration	Acceptable S.D. Maximum
70 mg/dL	9 mg/dL
375 mg/dL	26 mg/dL

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

2 – 1000 mg/dL

14.2 Precision

Material	Mean mg/dL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Multiqual Control			
Level 1	68	2 (3)	3 (4)
Level 2	384	6 (2)	9 (2)

14.3 Interfering Substances

Ascorbic acid at a concentration of 5 mg/dL decreases triglycerides results by 11.8% at triglyceride concentration of 180 mg/dL.

HIL Interference:
The TRIG method was evaluated for interference according to CLSI/NCCLS EP7-A2. Bias, defined as the difference between the control sample (does not contain interferent) and the test sample (contains interferent), is shown in the table below. Bias exceeding 10% is considered "interference".

Substance tested	Substance Concentration	TRIG mg/dL	Bias %
Hemoglobin (hemolysate)	1000 mg/dL	180	<10
Bilirubin (unconjugated)	5 mg/dL	180	<10
	10 mg/dL		11
	20 mg/dL		20
	60 mg/dL		24
Bilirubin (conjugated)	60 mg/dL	180	<10

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

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. Material Safety Data Sheets (MSDS)
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 Functions Checks (Lab policy)
14. Hemolysis, Icteria and Lipemia Interference (Lab policy)
15. Repeat Testing Requirement (Lab policy)
16. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
17. Current package insert TRIG Flex® Reagent Cartridge K2069

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, TRIG Flex® Reagent Cartridge K2069, Siemens Healthcare Diagnostics Inc., 08/20/2013.
3. Package Insert, CHEM 2 CAL, Siemens Healthcare Diagnostics Inc., 03/18/2015.
4. Package Insert, Unassayed Liquichek Chemistry Controls, Bio-Rad Laboratories, 08/2014.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
000	2/17/16		Update owner	L Barrett	R SanLuis
000	2/17/16	1	Remove outdated battery codes	L Barrett	R SanLuis
000	2/17/16	3.2	Specify anticoagulant, add instructions for lipemia	L Barrett A Chini	R SanLuis
000	2/17/16	4.2	Add hazard/chemical information	A Chini	R SanLuis
000	2/17/16	5.2	Update stability	A Chini	R SanLuis
000	2/17/16	6.4, 6.6	Replace LIS with Unity Real Time	L Barrett	R SanLuis
000	2/17/16	11.3	Add report comment for lipid panel	A Chini	R SanLuis
000	2/17/16	16	Update titles	L Barrett	R SanLuis
000	2/17/16	17	Update package insert information	A Chini	R SanLuis
000	2/17/16	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.	L Barrett	R SanLuis

Quest Diagnostics
Site: SGAH & WAH
Title: **Triglycerides by Dimension Vista® System**

19. ADDENDA

None

Form revised 2/02/2007