HDL Cholesterol (High Density Lipoprotein)

Purpose

This procedure provides instructions for performing HDL CHOLESTEROL (High Density Lipoprotein). The HDLC method is an *in vitro* diagnostic test for the quantitative measurement of high density lipoprotein cholesterol (HDL-C) in human serum and plasma on the Dimension Vista® System.

Policy Statements

This procedure applies to all personnel who perform testing on the Dimension Vista® System, at Children's Hospitals and Clinics of Minnesota.

Principle

The HDLC assay measures 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 and cholesterol oxidase that react with HDL cholesterol. In the presence of oxygen, the HDL cholesterol is oxidized to Δ4-cholestenone and hydrogen peroxide. The generated hydrogen peroxide then reacts with 4-aminoantipyrine and sodium 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) endpoint technique. The color intensity of the dye is directly proportional to the serum HDL-C concentration.

The reference method for measuring HDL cholesterol utilizes ultracentrifugation and chemical precipitation to separate HDL from the other lipoproteins, followed by a determination of the cholesterol content using the Abell-Kendall method. Since this procedure is tedious and requires an ultracentrifuge, most laboratories now use methods that selectively measure HDL-C by chemical means.

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 blood stream.

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. The determination of serum HDL cholesterol level is a useful tool in identifying at-risk patients. The Adult Treatment Panel of the National Cholesterol Education Program (NCEP) recommends that all adults 20 years of age and over should have their total and HDL cholesterol levels measured at least every 5 years to screen for risk of coronary artery disease.

Total cholesterol and triglycerides are required as well for determination of lipid risk factors for coronary artery disease. HDLC is especially apt to be low in male subjects who are obese and sedentary, in those who smoke cigarettes, and in those who have diabetes mellitus. Uremia is also associated with lower HDLC. Exercise, appropriate diet, and moderate ethanol intake increase HDLC.

Those at least risk for coronary artery disease have low TC, low triglycerides, and high HDLC. Even in men with moderately low cholesterol, HDLC is protective against CAD. Women have higher serum HDLC than men. This may be due to hormonal factors.

Analyzer

PRIMARY METHOD: Siemens Dimension Vista 500, Siemens Healthcare Diagnostics Inc

SECONDARY (BACKUP) METHOD: Siemens Dimension Vista 500 (opposite campus)

Sunquest Test Codes

HDLC High Density Lipoprotein Cholesterol in plasma or serum

Specimen

Plasma (lithium heparin), or Serum. Refer to specimen collection procedures for collection of diagnostic blood specimens by venipuncture.

Patient Preparation:

- The patient should be on a stable diet for 3 weeks and should fast for 9 – 12 hours prior to collection of the specimen
- Venipuncture should occur prior to administration of Metamizole due to the potential for falsely depressed results[8]. Metamizole is an anti-pyretic banned in most countries

Sample Volume: 200 µL **Minimum volume:** 100 µL **Actual Test Volume**: 1.3 µL

Stability: RT / 8 hours, 2-8 °C / 7 days, -70 °C for up to three months.
Samples may be frozen once
Note: When using samples stored at 2 – 8 °C or -70 °C, increases or decreases of up to 10% may be observed in HDL-C concentrations.

Rejection criteria: Unlabelled specimens.

Preparation:

1. Whole blood specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis.
2. 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.
3. Specimens should be free of particulate matter.
4. Transfer serum or plasma to a properly labeled Siemens SSC nested on a bar-coded pilot tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.

Reagents

Product Description	***Product Code***	***Stability***
HDLC Flex® reagent cartridge All reagents are liquid and ready to use.	K3048A	**Store at:** 2 - 8 °C. **Unopened:** Refer to carton for expiration date. **On-board:** Sealed wells on the instrument are stable for 30 days. **Open well stability:** 3 days for wells 1– 12
LIPID CAL Liquid, ready for use	KC220A	**Store at:** -25 to -15 °C **Unopened:** Refer to carton for expiration date. **Unopened/Thawed:** 30 days at 2 – 8 °C **On-board:** Once the vial stopper is punctured, assigned values are stable for 7 days when stored on board the Dimension Vista® System. **Opened**: Once the cap is removed, assigned values are stable for 30 days when recapped and stored at 2-8 °C. Do not use on board the Vista.

Risk and Safety

- Contains sodium azide (< 0.1%) as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds. Dispose of properly by flushing with plenty of water.
- Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact or ingestion.
- Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

Calibration

Analytical Measuring Range	3 – 150 mg/dL
Reference Material:	LIPID CAL (KC220A)
Typical Calibration Levels:	Level 1 (Calibrator A): 0 mg/dL Level 2 (Calibrator B): 158 mg/dL
Calibration Scheme:	2 levels, n = 5
Calibration Frequency:	• Every 90 days for any one lot • For each new lot of Flex® reagent cartridges • After major maintenance or service, if indicated by quality control results • As indicated in laboratory quality control procedures
Analytical Measuring Range	• Cal Verification and AMR verification are performed at least once every six (6) months. • Touch Advanced → Calibrations → Calibrations by Lot, select method HDLC and “Order a Linearity Study” • See iGuide “Calibration by Lot” for more information.

Quality Control

Product: BioRad Mulitqual® 1 & 3 Control Levels, contained in Vista vials

Frequency: Two levels each day of use

Stability: Stable until the date on vial when stored at -20 to -50 °C protect from light. Thawed and unopened, 7 days at 2 -8 °C. 7 days on board the Vista, and 5 days opened and stored at 2 – 8 °C

Preparation: Allow the control to stand at 18 – 25 °C for 30 minutes until completely thawed. Gently mix the vials until homogeneous to dissolve any precipitate.

Sunquest Control names: Level 1 = C-MQ1, Level 2 = C-MQ3

Acceptable ranges:

- Ranges are current in Sunquest and the instrument. Refer to the Quality Control in Chemistry procedure for QC exception codes.
- If a control value is outside the confidence interval, the determination must be repeated. If the repeat determination confirms the deviation, a new reference curve should be established.
- Do not release patient results until the cause of deviation has been identified and corrected
- When a new lot of assayed control is received, validate the manufacturer’s insert range by running the new lot in parallel with the current lot, and confirming that the results obtained are within the stated range
- When a new lot of unassayed control is received, verify new ranges by running the new lot in parallel with the current lot 30 times, and calculate a new range using the method mean ± 3 SD. Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes.

Interferences

Hemolysis, Icterus & Lipemia (HIL) Index Values:

H	I	L
-	-	-

Non-Interfering Substances: Interference is less than 10% at an HDL concentration level of 40 mg/dL

- Hemoglobin: up to 1000 mg/dL
- Bilirubin: conjugated up to 60 mg/dL, and unconjugated: up to 80 mg/dL
- Lipemia (Intralipid®): up to 1000 mg/dL

Interfering Substances:
LDL-C was evaluated for cross reactivity in the presence of 42.6 mg/dL HDL-C. Crossreactivity was -1.5%

Refer to the Siemens DFU for a list of non-interfering substances.

Reference Range

Age	NCEP Guidelines	Range (mg/dL)
2 – 17 yrs	Normal	≥ 60
	Low	<40
	Borderline Low	40 – 60
≥ 18 yrs	Normal	40 – 60
	Low	< 40
	High	> 60

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Critical Values

None defined.

Limitations

The instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in HDLC results. Refer to your Dimension Vista® Operator's Guide for the meaning of report flags and comments. Any report containing flags and/or comments should be resolved prior to reporting.

Abnormal liver function may affect lipid metabolism; when present, HDL-C and LDL-C results are of limited diagnostic value. In some patients with abnormal liver function, the HDL-C result may significantly differ from the designated comparison method (DCM) result.

In very rare cases, presence of a gammopathy, in particular type IgM (Waldenstrom's macroglobulinemia), may cause unreliable results.

Dilutions

Maximum Dilution:	1:4
Surplus Rack:	Samples with results >150 mg/dL reflex to a 1:4 dilution.
Limited Rack:	Samples with results >150 mg/dL should be repeated as an Add-On Test with a 1:4 dilution.
Manual Dilution:	Do not manually dilute

Result Reporting

- Results between 3 - 150 mg/dL without error messages are released
- Results below 3 mg/dL: report as < 3 mg/dL instead of the numerical value.
- Results >150 mg/dL are reported as the numerical result following a maximum dilution of 1:4
- Results that exceed the assay range following the maximum dilution are reported as >600 mg/dL.

Append the following comment to patients 2-17 years
NHANES guidelines
Low: <40
Borderline low: 40-59
Normal: ≥ 60

Append the following comment to patients ≥18 years
National Cholesterol Education
Program (NCEP) guidelines:
≥18 y:
Low HDL: <40
Normal: 40-60
Desirable: >60

Specimen Storage

Promptly stopper tested specimen and store upright in specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer.

References

1. Vista HDLC Flex® reagent cartridge insert sheet PN 782048.001 Issue Date 2013-09-04 Rev. C
2. Vista Lipid Calibrator insert sheet, Siemens Healthcare Diagnostics, PN 751220.002 – US, issue date 02/2014, Rev C.
3. US Department of Health and Human Services, National Institutes of Health Publication # 01-3305, May 2001
4. US Department of Health and Human Services, Summary Report of the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents:
5. Mayo Medical Laboratories, online test catalogue, 1/06/2012
6. Jacobs & DeMott Laboratory Test Handbook, Lexi-Comp, Inc, Hudson, OH, 5th Edition, 2001, p.192, 212-218.
7. Biorad Multiqual Chemistry Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618
8. Medical Device Correction Notice DC-16-02.A.US – March 17, 2016

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.	Linda Lichty	2/21/2012	New test
2.	L. Lichty	12/17/2013	Siemens CLSI HDLC procedure for Vista, issue date 9/4/13, Rev C
3.	L. Lichty	6/15/2015	Updated for Vista
4.	L.Lichty	3/28/2016	Metamizole warning