HDL Ultra Cholesterol Reagent

INTENDED USE

For the quantitative measurement of high-density lipoprotein cholesterol (HDL-C) concentration in human serum or plasma.

SUMMARY

Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipid, free cholesterol and protein constitute the outer surface of the lipoprotein particle, while the inner core contains mostly esterified cholesterol and triglyceride. These particles serve to solubilize and transport cholesterol and triglyceride in the bloodstream.

The relative proportions of protein and lipid determine the density of these lipoproteins and provide a basis on which to begin their classification.[1] The classes are: chylomicron, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Numerous clinical studies have shown that the different lipoprotein classes have very distinct and varied effects on coronary heart disease risk.[2]

The principle role of HDL in lipid metabolism is the uptake and transport of cholesterol from peripheral tissues to the liver through a process known as reverse cholesterol transport (a proposed cardioprotective mechanism).[3] Low HDL-C levels are strongly associated with an increased risk of coronary heart disease and coronary artery disease.[4-9] Hence, the determination of serum HDL-C is a useful tool in identifying high-risk patients. The Adult Treatment Panel of the National Cholesterol Education Program (NCEP) recommends that in all adults 20 years of age and over, a fasting lipoprotein profile (total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride) should be obtained once every five years to screen for coronary heart disease risk.[10]

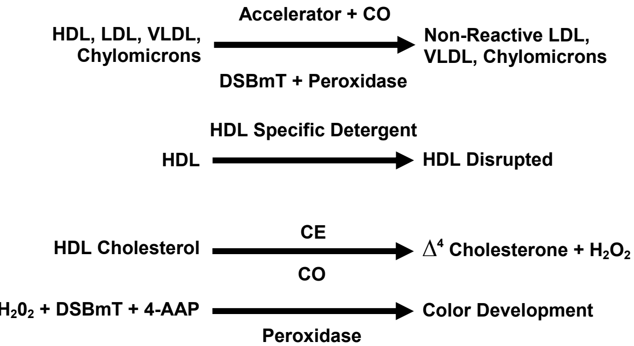
The reference method for the quantification of HDL-C combines ultracentrifugation and chemical precipitation to separate HDL from other lipoproteins, followed by cholesterol measurement by the Abell-Kendall method.[11] The first routine methods widely utilized by laboratories involved selective precipitation and removal of LDL and VLDL, followed by the enzymatic measurement of HDL-C in the supernatant fraction.[11] Since these methods require off-line pretreatment and separation steps the assay procedures cannot be fully automated. As a result, routine determination of HDL-C has suffered from long handling times and poor reproducibility.

PRINCIPLE

The HDL Ultra Cholesterol assay is a homogeneous method for directly measuring HDL-C concentrations in serum or plasma without the need for any off-line pretreatment or centrifugation steps.

The method is in a two reagent format and depends on the properties of a unique detergent, as illustrated. This method is based on accelerating the reaction of cholesterol oxidase (CO) with non-HDL unesterified cholesterol and dissolving HDL selectively using a specific detergent. In the first reagent, non-HDL unesterified cholesterol is subject to an enzyme reaction and the peroxide generated is consumed by a peroxidase reaction with DSBmT yielding a colorless product. The second reagent consists of a detergent capable of solubilizing HDL specifically, cholesterol esterase (CE) and chromogenic coupler to develop color for the quantitative determination of HDL-C. This may be referred to as the Accelerator Selective Detergent methodology.

Accelerator Selective Detergent Methodology



REAGENTS

Composition of Reagents:

Component	Ingredients	Concentration
Reagent 1	Buffer	
	Cholesterol oxidase (Fr: E. Coli)	<1000 U/L
	Peroxidase (Fr: Horseradish)	<1300 ppg U/L
	N,N-bis(4-sulphobutyl)-m-toluidine-disodium(DSBmT)	<1 mM
	Accelerator	<1 mM
	Preservative	<0.06%
	Ascorbic Oxidase (Fr: Curcubita sp.)	<3000 U/L
Reagent 2	Buffer	
	Cholesterol esterase (Fr: Pseudomonas sp.)	<1500 U/L
	4-Aminoantipyrine (4-AAP)	<1mM
	Detergent	<2%
	Preservative	

Precautions and Warnings

1. For *In Vitro* Diagnostic Use.
2. Do not pipette by mouth.
3. All specimens used in the test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing.
4. Do not use the reagents after the expiration date printed on the reagent label.
5. HDL Ultra Cholesterol Reagent must be used with HDL Ultra Cholesterol Calibrator.

Reagent Preparation
Reagent 1: Ready to use as packaged.
Reagent 2: Ready to use as packaged.

Storage and Stability
Store HDL Ultra Cholesterol reagents at 2-8°C.

Unopened reagents are stable until the expiration date on the reagent bottle label.

Reagent 1 is stable open on the analyzer for 4 weeks at 2-8°C.

Reagent 2 is stable open on the analyzer for 4 weeks at 2-8°C.

DO NOT FREEZE

Indications of Deterioration
Inability to recover control values.
Presence of turbidity.

SPECIMEN COLLECTION AND PREPARATION

Serum, EDTA-treated or heparinized plasma drawn from the patient after a 12 – 14 hour fast are the required specimens.

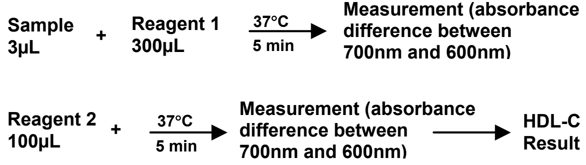
Serum: Collect whole blood by venipuncture and allow to clot. Centrifuge and remove the serum as soon as possible after collection (within 3 hours).[11]

Plasma: Specimens may be collected in EDTA or lithium or sodium heparin. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours).[11]

Serum or plasma should not remain at 15-30°C longer than 14 hours. If assays are not completed within 14 hours, serum or plasma should be stored at 2-8°C for up to 1 week. If specimens need to be stored for more than 1 week, they may be preserved at less than -70°C for up to 3 months. Samples may be frozen once. Refer to NCCLS Document H18-A for further instructions on specimen collection, handling, and storage.

PROCEDURE

Assay
Below is a general example of the HDL Ultra Cholesterol assay procedure for an automated analyzer.



For assistance with applications on automated analyzers, please contact Genzyme Technical Marketing at 800-332-1042.

Materials Provided
Both the HDL Ultra Cholesterol Reagent 1 and Reagent 2 are required for the measurement of HDL cholesterol.

Description	Configuration	Catalog Number
HDL Ultra Cholesterol Reagent	R1 1 x 60 mL R2 1 x 20 mL	6121
HDL Ultra Cholesterol Reagent	R1 1 x 250 mL R2 1 x 80 mL	6122
HDL Ultra Cholesterol Reagent 2	R2 1 x 20 mL	80-7433-00

Materials Required but not Provided

Description	Configuration	Catalog Number
HDL Ultra Cholesterol Calibrator	3 x 1 mL	6272-3

1. HDL cholesterol control sera or quality control material (See “Quality Control Procedures”).
2. Automated clinical chemistry analyzer capable of accommodating two-reagent assays.
3. Class A volumetric pipettes.
4. Distilled, deionized, Type II water or equivalent.

Calibration
The HDL Ultra Cholesterol Calibrator is required for calibration. The value of the HDL Ultra Cholesterol Calibrator was assigned by procedures traceable to the CDC HDL cholesterol reference method.[20, 21] Calibration materials have concentrations at approximately the medical decision level. Refer to the HDL Ultra Cholesterol Calibrator kit package insert for instructions. Refer to the instrument operator’s manual for analyzer specific procedures and for guidance in determining calibration frequency.

Quality Control values should be within the expected range.

Quality Control
Reliability of test results should be routinely monitored with control sera or quality control materials that reasonably emulate performance on patient specimens.[11] The National Cholesterol Education Program (NCEP) Lipid Standardization Panel (LSP) recommends two levels of controls, one in the normal range (40-65 mg/dL) and one near the concentrations for decision making (<40 mg/dL). An acceptable range of HDL cholesterol values should be established by each laboratory. If control values are not within the expected range, confirm that procedures were performed correctly and follow normal troubleshooting measures. If assistance is required call Genzyme Technical Marketing at 800-332-1042.

Quality control requirements should be established in accordance with local, state, and/or federal regulations or accreditation requirements.

RESULTS

To convert from conventional units to S.I. units, multiply the conventional units by 0.0259

mg/dL x 0.0259 = mmol/L HDL-Cholesterol

Interfering Substances

All interference studies were conducted according to a modified NCCLS guideline No. EP7 for interference testing in clinical chemistry.[13]

Substances Tested	Concentration with no significant (±10%) interference
Bilirubin Conjugated	60 mg/dL
Bilirubin Total	60 mg/dL
Hemoglobin	1000 mg/dL
Ascorbic Acid	100 mg/dL
Lipemia using Intralipid®	1800 mg/dL
Gamma-globulins	5000 mg/dL

Refer to the work of Young for a review of drug effects on serum HDL cholesterol levels.[14]

Limitations

1. Anticoagulants containing citrate should not be used.
2. Protect the reagents from direct sunlight.
3. Store the reagents at 2-8°C. Do not freeze the reagents.
4. The NCEP recommends that dietary and/or drug treatment not be based on a single HDL cholesterol result.
5. Lipemia: no interference from Intralipid® up to 1800 mg/dL.
6. Endogenous triglyceride levels gave acceptable performance up to 2000 mg/dL. Samples with triglyceride level >2000 mg/dL should not be diluted.
7. Samples from patients of cirrhotic liver have been reported to give HDL results lower than reported from reference method.[15]

Expected Values

The following NCEP cutpoints for patient classification are used to assess the risk and management of coronary heart disease.[10, 16]

Males: 30 - 70 mg/dL
Females: 30 - 85 mg/dL

SPECIFIC PERFORMANCE CHARACTERISTICS

Accuracy

Accuracy of the HDL Ultra Cholesterol method was verified by comparison to the Designated Comparison Method (DCM) for HDL cholesterol[12] and the previous HDL Ultra Cholesterol Assay.

Studies comparing the HDL Ultra Cholesterol Assay to the DCM produced the following results on the Hitachi 911 Analyzer:

Method	HDL Ultra Cholesterol	Designated Comparison Method (DCM)
n	52	52
Mean (mg/dL)	58.3	56.3
Range (mg/dL)	33.6-133.0	32.0-133.0
Regression Analysis	Ultra = 0.99(DCM) + 2.81 mg/dL	
Correlation Coefficient	0.996	

Studies comparing the HDL Ultra Cholesterol method to the previous HDL Direct Liquid Select Cholesterol method produced the following results:

Method	HDL Ultra Cholesterol	Previous HDL Direct Liquid Select Cholesterol
n	101	101
Mean (mg/dL)	56.4	54.2
Range (mg/dL)	33.6-133.0	31.5-132.8
Regression Analysis	Ultra = 0.98 (Liquid) + 3.42 mg/dL	
Correlation Coefficient	0.996	

Precision

Within-run precision for the HDL Ultra Cholesterol method was determined using three levels of frozen pooled human serum. Each run consisted of twenty replicate samples. Within-run precision studies produced the following results on the Hitachi 911 Analyzer:

Serum Pool	LOW	MID	HIGH
n	20	20	20
Mean (mg/dL)	32.9	50.06	101.4
Standard Deviation (mg/dL)	0.3	0.2	0.7
Coefficient of Variation (%)	0.8	0.5	0.7

Between-run precision was determined using three levels of frozen pooled human serum. The HDL Ultra Cholesterol assay was run in duplicate and analyzed twice per day over 10 days. Between-run precision studies produced the following results:

Serum Pool	LOW	MID	HIGH
n	40	40	40
Mean HDL Cholesterol(mg/dL)	32.8	50.0	100.1
Standard Deviation (mg/dL)	0.4	0.7	1.1
Coefficient of Variation (%)	1.3	1.5	1.1

Total Error Determination

Total error[11,17,18] is a measure of the overall analytical performance of an assay, and combines both accuracy and precision. Total error is equal to the % Bias + 1.96 x the Total C.V. (CV_T).[19] The % Bias of the HDL Ultra Cholesterol assay was calculated using the linear regression formula, derived from the comparison of the HDL Ultra Cholesterol method to the Designated Comparison Method for HDL cholesterol shown above.[17,18] The CV is calculated as $CV_T = (CV_B^2 + CV_W^2)^{1/2}$.[19] The results of the total error analysis for the HDL Ultra Cholesterol assay on the Hitachi 911 Analyzer at low, medium and high HDL Cholesterol levels using samples with triglycerides <400 mg/dL are shown below.

HDL Cholesterol Concentration	% Bias	Total CV	Total Error
30 mg/dL	8.05%	1.53%	11.05%
50 mg/dL	4.31%	1.58%	7.40%
80 mg/dL	2.21%	1.29%	4.73%

Linearity (Dilution and Recovery)

Linearity studies were conducted using the Genzyme HDL Cholesterol Linearity Verifier. Linearity samples were prepared according to the package insert instructions. The HDL Ultra Cholesterol reagent was found to be linear from 2.5 mg/dL to 200 mg/dL with a deviation from the linear line of less than or equal to 4 mg/dL or 5%. Patient samples with HDL cholesterol levels exceeding 200 mg/dL should be diluted with physiological saline before assaying. Multiply the result

obtained from the manual dilution by the appropriate dilution factor.

Other Performance Studies

In a study comparing the HDL Ultra Cholesterol method to the Reference Method (RM) for HDL cholesterol (ultracentrifugation, chemical precipitation and Abell-Kendall cholesterol analysis)[11] 41 patient specimens with elevated triglyceride values (triglyceride levels greater than the 95th percentile) were analyzed. The correlation coefficient for this study was r = 0.968 and the regression equation was HDL Ultra Cholesterol = 1.01 RM – 2.48 mg/dL. Patient specimens with triglyceride levels up to 2,000 mg/dL may be used.

Separate studies comparing the lyophilized HDL Ultra Cholesterol assay to the phosphotungstic acid (PTA) precipitation method at three Physician's Office Laboratories (POL) produced the following results:

POL Current Method	POL Site 1	POL Site 2	POL Site 3
n	40	42	40
HDL Ultra Mean (mg/dL)	47	45	58
HDL Ultra Range (mg/dL)	24.4-89.7	28.3-94.9	25.8-97.1
Slope	0.88	1.05	0.77
Intercept (mg/dL)	2.90	-1.32	11.10
Correlation Coefficient	0.97	0.99	0.98

Within-run precision at the three POL sites was determined using three levels of frozen pooled human serum. Each run consisted of twenty replicate samples. Within-run precision studies at the three POL sites produced the following results:

Serum Pool	LOW <35 mg/dL	MID 35-60 mg/dL	HIGH >60 mg/dL
POL Site 1	n=20	n=20	n=20
Mean (mg/dL)	19.5	44.1	70.8
S.D. (mg/dL)	0.6	1.8	1.1
C.V. (%)	2.9	4.0	1.5
POL Site 2	n=20	n=20	n=20
Mean (mg/dL)	29.5	49.3	71.5
S.D. (mg/dL)	1.8	2.4	3.6
C.V. (%)	6.2	4.8	5.1
POL Site 3	n=20	n=20	n=20
Mean (mg/dL)	33.3	45.6	76.8
S.D. (mg/dL)	0.3	0.4	0.5
C.V. (%)	1.0	0.8	0.7

Note: Each site received a unique set of three serum pools.

Between-run precision was determined at three POL sites using three levels of frozen pooled human serum. The HDL Cholesterol assay was run in duplicate over multiple days. Between-run precision studies produced the following results:

Serum Pool	LOW <35 mg/dL	MID 35-60 mg/dL	HIGH >60 mg/dL
POL Site 1	n=16	n=16	n=16
Mean (mg/dL)	19.0	41.2	65.3
S.D. (mg/dL)	1.3	1.8	3.4
C.V. (%)	6.9	4.5	5.2
POL Site 2	n=20	n=20	n=20
Mean (mg/dL)	25.7	46.4	68.2
S.D. (mg/dL)	1.4	1.6	2.4
C.V. (%)	5.3	3.4	3.5
POL Site 3	n=40	n=40	n=40
Mean (mg/dL)	33.6	45.7	76.2
S.D. (mg/dL)	0.8	0.9	1.5
C.V. (%)	2.4	2.0	2.0

Note: Each site received a unique set of three serum pools.

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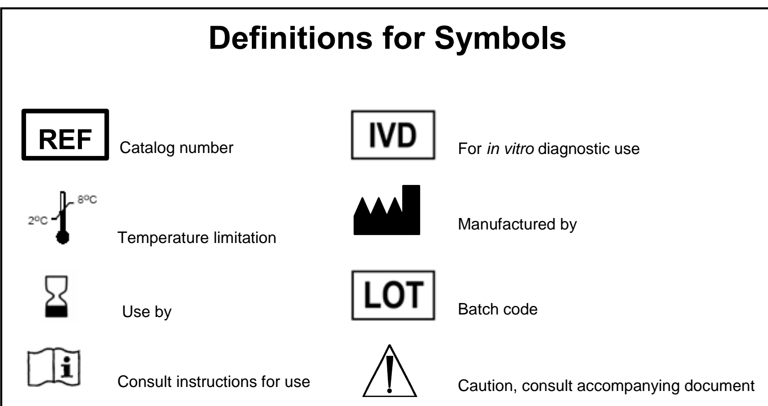

Manufactured by:

The Americas
31 New York Avenue
Framingham, MA 01701-9322
USA

Phone: 800-999-6578
Fax : 610-594-8585
Email: usdiagcustomerservice@genzyme.com

www.genzymediagnostics.com

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