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ULTRA N-geneous® HDL Cholesterol Reagent

Catalog number HDCE-70-5955-03

INTENDED USE

For the quantitative determination of high-density lipoprotein cholesterol (HDL-C) 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 quantitation of HDL-C combines ultracentrifugation and chemical precipitation to separate HDL from other lipoproteins, followed by cholesterol measurement by Abell-Kendall analysis.[11] This method is too time consuming and labor intensive for use in routine analysis.[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 Ultra N-geneous® HDL Cholesterol assay is a homogeneous method for directly measuring HDL-C levels 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 chromagenic 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

HDL, LDL, VLDL, Chylomicron	Accelerator + CO / DSBmT + Peroxidase →	Non-reactive LDL, VLDL, Chylomicrons
HDL	HDL Specific Detergent →	HDL disrupted
HDL Cholesterol	Cholesterol esterase / Cholesterol oxidase →	Δ^4 Cholestenone + H_2O_2
H_2O_2 + DSBmT + 4-AAP	Peroxidase →	Color Development

REAGENTS

Composition of Reagents

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

Reagent Preparation

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

Storage and Stability

Store Ultra N-geneous® HDL 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.

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. Ultra N-geneous® HDL Cholesterol Reagent must be used with Ultra N-geneous® HDL Cholesterol Calibrator.

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.

Assay Procedure

Below is a general example of the Ultra N-geneous® HDL Cholesterol assay procedure for an automated analyzer.

Sample 3µL + Reagent 1 300µL → 37°C, 5 min → Measurement (absorbance difference between 700 nm and 600 nm) →

Reagent 2 100µL → 37°C, 5 min → Measurement (absorbance difference between 700 nm and 600 nm) → HDL-C result

Genzyme has validated applications for several automated analysers. This information is available through your distributor.

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Materials Provided

Description	Volume Per Bottle	Catalog number
Reagent 1	60 mL	HDCE-70-5955-03
Reagent 2	20 mL	

Materials Required but not Provided

1. Genzyme Ultra N-geneous® HDL Cholesterol Calibrator, Genzyme catalog number HCCE-70-5954.
2. HDL cholesterol control sera or quality control material (See "Quality Control Procedures").
3. Automated clinical chemistry analyzer capable of accommodating two-reagent assays.
4. HDL Linearity Verifier Kit – Catalog number 80-5943-00.

Calibration

The Genzyme Ultra N-geneous® HDL Cholesterol Calibrator is required for calibration. The value of the Ultra N-geneous® HDL Cholesterol Calibrator was assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL). Calibration materials have concentrations around the medical decision level. Refer to the Ultra N-geneous® HDL 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 Procedures

Reliability of test results should be routinely monitored with control sera or quality control materials that reasonably emulate performance on patient specimens.[11] Quality control materials are intended for use only as monitors of accuracy and precision. 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. 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-P for interference testing in clinical chemistry.[13]

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

Refer to the work of Young for a review of drug effects on serum HDL cholesterol levels.[13]

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. Endogeneous triglyceride levels gave acceptable performance up to 2000 mg/dL. Samples with triglyceride level >2000 mg/dL should not be diluted.

Expected Values

The expected values for serum HDL Cholesterol are as follows:[15]

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

Each laboratory must establish its own range of expected values.

According to the NCEP, HDL values greater than or equal to 40 mg/dL are considered desirable, and values greater than or equal to 60 mg/dL are considered to offer some protection against coronary heart disease. Values below 40 mg/dL are considered to be a significant independent risk factor for coronary heart disease.[10]

SPECIFIC PERFORMANCE CHARACTERISTICS

Accuracy

Accuracy of the Ultra N-geneous® HDL Cholesterol method was verified by comparison to the Designated Comparison Method (DCM) for HDL cholesterol[12] and a previous Liquid N-geneous® HDL Cholesterol Assay. Studies comparing the Ultra N-geneous® HDL Cholesterol Assay to the DCM produced the following results on the Hitachi 911 Analyzer:

Method	Ultra N-geneous® HDL Cholesterol	Designated Comparison Method (DCM)
n	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 Ultra N-geneous® HDL Cholesterol method to a previous Liquid N-geneous® HDL method produced the following results:

Method	Ultra N-geneous® HDL Cholesterol	Liquid N-geneous® HDL Cholesterol
n	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 Ultra N-geneous® HDL 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 (<40 mg/dL)	Mid (40 - 59 mg/dL)	High (≥60mg/dL)
n	20	20	20
Mean (mg/dL)	32.9	50.6	101.4
Std 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 Ultra N-geneous® HDL 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 (<40 mg/dL)	Mid (40 - 59 mg/dL)	High (≥60mg/dL)
n	40	40	40
Mean HDL-C (mg/dL)	32.8	50.0	100.1
Std Deviation (mg/dL)	0.4	0.7	1.1
Coefficient of Variation (%)	1.3	1.5	1.1

Total Error Determination

Total error [11,16,17] 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).[18] The % Bias of the Ultra N-geneous® HDL Cholesterol assay was calculated using the linear regression formula, derived from the comparison of the Ultra N-geneous® HDL Cholesterol method to the Designated Comparison Method for HDL Cholesterol shown above.[16, 17] The CV_T is calculated as $CV_T = (CV_B^2 + CV_W^2)^{1/2}$.[18] The results of the total error analysis for the Ultra N-geneous® HDL 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 Linerity Verifier. Linearity samples were prepared according to the package insert instructions. The Ultra N-geneous® HDL 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 Ultra N-geneous® HDL 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 Ultra N-geneous® Cholesterol = 1.01 RM – 2.48 mg/dL. Patient specimens with triglyceride levels up to 2000 mg/dL may be used.

Separate studies comparing the lyophilized N-geneous® HDL 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
N-geneous® Mean (mg/dL)	47	45	58
N-geneous® 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 N-geneous® 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.

Definitions for Symbols

CE — This product fulfills the requirements of the European Directive for In Vitro Diagnostic Medical Devices.

REF — Catalog number

IVD — For in vitro diagnostic use

2°C 8°C — Temperature Limitation

Manufactured by

LOT — Batch code

Use by

Consult instructions for use

N-geneous® is a trademark of Genzyme Corporation

These instructions for use apply to Ultra N-geneous® HDL Cholesterol Reagent kit with carton label version LC5955.01

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ULTRA N-geneous® HDL Cholesterin Reagenz

Katalognummer HDCE-70-5955-03

VERWENDUNGSZWECK

Zur quantitativen Bestimmung von Lipoprotein-Cholesterin hoher Dichte (HDL-C) in Humanserum oder Plasma.

EINLEITUNG

Plasmalipoproteine sind kugelförmige Teilchen, die Cholesterin, Triglyceride, Phospholipide und Proteine in unterschiedlichen Mengen enthalten. Phospholipid, freies Cholesterin und Protein stellen die Außenfläche des Lipoproteinteilchens dar, während der innere Kern hauptsächlich verestertes Cholesterin und Triglyceride enthält. Diese Teilchen dienen als Lösungsvermittler und transportieren Cholesterin und Triglyceride in den Blutkreislauf.

Die relativen Protein- und Lipidanteile bestimmen die Dichte dieser Lipoproteine und bieten eine Grundlage für ihre Klassifizierung.[1] Die Klassen sind: Chylomikron, Lipoprotein sehr niedriger Dichte (VLDL), Lipoprotein niedriger Dichte (LDL) und Lipoprotein hoher Dichte (HDL). Zahlreiche klinische Studien belegen, dass sich die verschiedenen Lipoprotein-Klassen sehr