

N-geneous® LDL-ST CHOLESTEROL REAGENT

INTENDED USE

For the direct, quantitative measurement of low-density lipoprotein cholesterol (LDL-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 These classes are: chylomicrons, 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.²⁻⁴ The studies all point to LDL cholesterol as the key factor in the pathogenesis of atherosclerosis and coronary artery disease (CAD)²⁻⁸, while HDL cholesterol has been observed to have a protective effect. Even within the normal range of total cholesterol concentrations, an increase in LDL cholesterol can occur with an associated increased risk for CAD.⁴

PRINCIPLE

The N-geneous® LDL-ST-C assay is a homogeneous method for directly measuring LDL-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. This detergent (Reagent 1) solubilizes only the non-LDL lipoprotein particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. A second detergent (Reagent 2) solubilizes the remaining LDL particles and a chromogenic coupler allows for color formation. The enzyme reaction with LDL-C in the presence of the coupler produces color that is proportional to the amount of LDL cholesterol present in the sample.

REAGENTS

Composition

Component	Ingredients	Concentration
Reagent 1	Buffer	
	Detergent 1	<1.0%
	Cholesterol esterase (Pseudomonas sp.)	<2500 U/L
	Cholesterol oxidase (Cellulomonas sp.)	<1500 U/L
	Peroxidase (Horseradish)	<1300 ppg U/L
	4-Aminoantipyrine	<0.1%
Reagent 2	Ascorbic oxidase (Curcubita sp.)	<3000 U/L
	Preservative	
	Buffer	
	Detergent 2	<1.0%
	N,N-bis(4-sulfobutyl)-m-toluidine, disodium (DSBmT)	<1.0 mM
Preservative		

Precautions and Warnings

1. For In Vitro diagnostic use.
2. Do not pipette by mouth.
3. Do not use the LDL-ST reagents after the expiration date printed on the label.
4. For use on Olympus Analyzers.

Preparation

N-geneous® LDL-ST Cholesterol Reagent 1 and Reagent 2: The reagents are supplied ready for use.

Storage and Stability

Unopened Reagents are stable until the expiration date shown on the label when stored at 2-8°C.

N-geneous® LDL-ST Cholesterol Reagent 1 and Reagent 2: Once opened, is stable up to 4 weeks at 2-8°C on the Olympus AU600 analyzer.

N-geneous® LDL-ST Cholesterol Reagent 1 and Reagent 2: Once opened, on board stability is 4 weeks at 2-8°C on the Olympus AU600 analyzer.

DO NOT FREEZE.

Indications of Deterioration

Inability to recover control values.
Presence of turbidity.

SPECIMEN COLLECTION AND PREPARATION

Patients are not required to fast prior to blood collection. Serum, EDTA-treated or heparinized plasma are the recommended 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).¹⁰

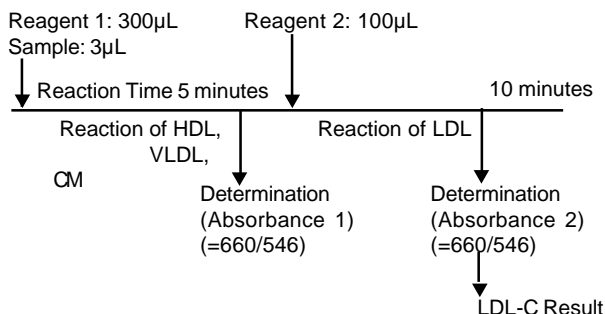
Plasma: Specimens may be collected in EDTA or heparin. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours).¹⁰

If not analyzed promptly, specimens may be stored at 2-8°C for up to 5 days. If specimens need to be stored for longer than 5 days, they may be stored frozen at -80°C. Samples may be frozen once. Refer to NCCLS Document H18-A for further instructions on specimen collection, handlight and storage.⁹

PROCEDURE

Assay

Below is a general example of the N-geneous® LDL-ST test procedure for a two reagent automated analyzer. All analyzer applications should be validated in accordance with NCEP and CLIA recommendations.^{10, 11} For assistance with applications on automated analyzers, please contact Genzyme Technical Marketing at 1-800-332-1042.



Materials Provided

Any of the following items may be included in the package you receive:

Description	Configuration	Catalog Number
N-geneous® LDL-ST Cholesterol Reagent 1	30 mL	80-5727-02
N-geneous® LDL-ST Cholesterol Reagent 2	10 mL	80-5729-02
N-geneous® LDL-ST Cholesterol Reagent 1	250 mL	80-5665-01
N-geneous® LDL-ST Cholesterol Reagent 2	80 mL	80-5714-00

Materials Required but not Provided

Description	Configuration	Catalog Number
Calibrator	3 x 1 mL	80-5666-02
LDL Linearity Verifier Kit	Low 1 x 3mL High 1 x 3 mL	80-5953-00

1. Class A volumetric pipettes.
2. Distilled, deionized, Type II water or equivalent.
3. Analyzer capable of running two reagent chemistries.

Calibration

The Genzyme N-geneous® LDL-ST Cholesterol Calibrator kit, is required for calibration. Other commercially available

LDL calibrators have not been tested with this assay and cannot be supported by Genzyme. Refer to the N-geneous® LDL-ST Cholesterol Calibrator kit package insert for a description of assignment procedures traceable to the National Reference System for cholesterol (NRS/CHOL).¹⁵ Refer to instrument operator's manual for analyzer specific calibration 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 quality control materials or serum pools that reasonably represent performance with patient specimens.¹² Controls or serum pools should be run with each assay to ensure that the reagents are functioning properly and that correct procedures have been followed. An acceptable range for each lot of control material should be established by the laboratory. If control values are not within the expected range, confirm procedures were performed correctly and follow normal troubleshooting measures. If assistance is required, call Genzyme Technical Marketing at 1-800-332-1042.

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 0.02586.¹²

$$\text{mg/dL} \times 0.02586 = \text{mmol/L LDL-cholesterol}$$

Limitations/Interfering Substances

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

Substance Tested

Concentration with no significant ($\pm 10\%$) interference

Bilirubin (conjugated)	20 mg/dL
Bilirubin (unconjugated)	20 mg/dL
Hemoglobin	500 mg/dL
Ascorbic Acid	50 mg/dL

1. Refer to the work of Young et al¹⁴ for a review of the effects of drugs on clinical laboratory tests.
2. Protect the reagent from direct sunlight.
3. Anticoagulants containing citrate should not be used.
4. Patient samples may only be frozen once.
5. Do not freeze reagents.
6. Samples with triglyceride values up to 1,183 mg/dL did not interfere with the results of the N-geneous® LDL-ST assay. Samples with triglyceride levels >1,183 mg/dL should not be diluted.

Expected Values

The following NCEP cutpoints for patient classification are used to assess the risk of coronary heart disease.¹⁰

LDL Cholesterol	Classification
<130 mg/dL (<3.36 mmol/L)	Desirable
130 - 159 mg/dL (3.36 - 4.11 mmol/L)	Borderline High Risk
≥160 mg/dL (>4.14 mmol/L)	High Risk

SPECIFIC PERFORMANCE CHARACTERISTICS

Accuracy

Accuracy of the N-geneous® LDL-ST Cholesterol method was verified by comparison to the Reference Method (Ultracentrifugation and cholesterol analysis) giving the following results on the Olympus AU600 Analyzer:

Method	N-geneous® LDL-ST Cholesterol	Reference Method
n	62	62
Mean (mg/dL)	119.6	119.0
Standard Deviation (mg/dL)	28.9	26.0
Regression Analysis	y=1.08x-8.7 mg/dL	
Correlation Coefficient	r=0.969	

Studies comparing the N-geneous® LDL-ST Cholesterol method to the N-geneous® LDL Cholesterol method produced the following results on the Olympus AU600 Analyzer:

Method	N-geneous® LDL-ST Cholesterol	Comparison Method
n	77	77
Mean (mg/dL)	121.6	121.3
Standard Deviation (mg/dL)	30.7	29.9
Regression Analysis	y=1.02x-1.7 mg/dL	
Correlation Coefficient	r=0.990	

Precision

Within-run precision of the N-geneous® LDL-ST Cholesterol test was determined using two levels of frozen pooled human serum. Each run consisted of 20 replicate samples. Within-run precision studies produced the following results on the Olympus AU600 Analyzer:

Serum Pool	Low	High
n	20	20
Mean (mg/dL)	93.8	233.5
Standard Deviation (mg/dL)	1.3	1.9
Coefficient of Variation (%)	1.4	0.8

Between-run precision was determined using two levels of frozen pooled human serum. The N-geneous® LDL-ST Cholesterol assay was run once per day in quadruplicate over 5 days. Between-run precision studies produced the following results on the Olympus AU600 Analyzer:

Serum Pool	Low	High
n	20	20
Mean (mg/dL)	86.9	188.4
Standard Deviation (mg/dL)	1.8	5.0
Coefficient of Variation (%)	2.1	2.7

Limit of Detection

The limit of detection of the N-geneous® LDL-ST assay, quantified 2 SDs plus the mean of twenty replicate measurements of saline, is 0.278 mg/dL on the Olympus AU600 analyzer.

Linearity

The N-geneous® LDL-ST Cholesterol method is linear from 6.6 mg/dL to 992 mg/dL. Both native and constructed specimens were used. The linearity range was established on the Olympus AU600 Analyzer.

REFERENCES

- Gotto, AM, Lipoprotein Metabolism and the Etiology of Hyperlipidemia, Hospital Practice, 23: Suppl. 1, 4 (1998).
- Crouse, JR, et al., Studies of low density lipoprotein molecular weight in human beings with coronary artery disease, J. Lipid Res., 26; 566 (1985).
- Badimon, JJ, et al., Regression of Atherosclerotic Lesions by High Density Lipoprotein Plasma Fraction in the Cholesterol-fed Rabbit, Journal of Clinical Investigation, 85:1234 (1990).
- Castelli, WP, et al., HDL Cholesterol and Other Lipids in the coronary Heart Disease, Circulation, 55:767 (1977).
- Barr, DP, et al., Protein-lipid Relationships in Human Plasma, Am. J. Med., 11:480 (1951).
- Gordon, T., et al., High Density Lipoprotein as a Protective Factor Against Coronary Heart Disease, Am. J. Med., 62:707 (1977).
- William, P., Robinson, D., Baily, A., High density lipoprotein and coronary risk factor, Lancet, 1:72 (1979).
- Kannel, WB, Castekku, WP, Gordon, T., Cholesterol in the prediction of atherosclerotic disease; New perspective factor against coronary heart disease, Am. J. Med., 62:707 (1977).
- National Committee for Clinical Laboratory Standards, Procedures for the Handling and Processing of Blood Specimens, Approved Guideline NCCLS Document H18-A, Number 12, Vol 10, (1990).
- Bachorik PS et al, National Cholesterol Education Program Recommendations for Measurement of Low-Density Lipoprotein Cholesterol; Executive Summary, Clinical Chemistry, Vol. 41, No. 10:1414, (1995).
- National Committee for Clinical Laboratory Standards, Statistical Quality Control for Quantitative Measurements; Principles and Definitions; Approved Guideline Second Ed, C24-A2, Vol. 19 No.5, Replaces C24-A, Vol. 11 No. 6, February 1999.
- Tietz, NW, Clinical Guide to Laboratory Tests, WB Saunders Co., Philadelphia, PA, 256 (1986).
- National Committee for Clinical Laboratory Standards, National Evaluation Protocols for Interference Testing, Evaluation Protocol, Proposed Guideline NCCLS Document EP7-P Number 7, Vol.6, No. 13, August, 1986.

14. Young, DS, Effects of drugs on Clinical Laboratory Tests, 3rd Ed., AACC Press, Washington, DC, 3-104 (1990).
15. National Reference System for Cholesterol. CRMLN LDL Cholesterol Protocol, May 2004.

Manufactured by:



Genzyme Corporation

One Kendall Square
Cambridge, MA
02139-1562
USA

Tel: 1-800-332-1042

Fax: 1-617-252-7759

www.genzymediagnosics.com

Genzyme Diagnostics

50 Gibson Drive
Kings Hill, West Malling
KENT ME19 4AF
United Kingdom

Tel: (+44) (0)1732-220022

Fax: (+44) (0)1732-220024

80-5665-20-02

4/05

N-geneous® is a registered trademark of Genzyme Corporation.