

HDL Reagent

Catalog #: 43715

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

This reagent is intended for the *in vitro* quantitative determination of High Density Lipoprotein (HDL) Cholesterol in human serum.

SUMMARY AND EXPLANATION

Lipoproteins are spherical-shaped particles that contain varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipids and proteins make up the outer surface of the lipoprotein particle, while the core contains mostly of cholesterol in the esterified form and triglycerides, the purpose of the lipoprotein particles is to transport cholesterol and triglyceride through the bloodstream.

The relative amounts of the protein and lipid constituents determine the density of the lipoprotein particles and provide a bases for their classification.¹ These classes are: chylomicron, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). There have been many clinical studies that have shown that these lipoprotein particles have very distinct and varied effects on the risk of coronary heart disease.²

The role of HDL particles in lipid metabolism is primarily the uptake and transport of cholesterol from peripheral tissue to the liver. This process is known as reverse cholesterol transport and has been proposed as a cardioprotective mechanism.³ Low HDL-C levels have repeatedly been associated with an increased risk of coronary heart disease and coronary artery disease.⁴ Thus, the determination of serum HDL cholesterol has been recognized as a useful tool in identifying high 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 cholesterol and HDL cholesterol levels measured at least every 5 years to screen for risk of coronary heart disease.

The CDC reference method for HDL cholesterol uses ultracentrifugation followed by chemical precipitation to separate HDL from other lipoproteins, followed by cholesterol measurement using a modified Abell-Kendall assay.⁴ This method is considered too time consuming and labor intensive for use in routine analysis. Historically, most laboratories have used one of several methods for the selective precipitation and removal of LDL and VLDL, followed by the enzymatic measurement of HDL-C in the supernatant fraction. Since almost all of these methods required manual separation steps, HDL cholesterol determinations could not be fully automated. Also the dilution of the sample resulted in an enzymatic determination of cholesterol with low sensitivity. As a result, the routine determination of HDL cholesterol has suffered from both long turnaround times and poor reproducibility.

METHODOLOGY^{2,3}

The SDI HDL Cholesterol Auto-Easy Reagent is an immunoinhibition reagent method which directly measures serum HDL-C levels without the need for any off-line pretreatment or centrifugation steps. The method is in a two reagent format.

The first reagent contains anti human b-lipoprotein antibody which bind to lipoproteins (LDL, VLDL and chylomicrons) other than HDL.

HDL Cholesterol

The second reagent contains enzymes which then selectively react with the cholesterol present in the HDL particles. Consequently only HDL cholesterol is subject to cholesterol measurement.

REAGENT COMPOSITION

Active Ingredients

Reagent 1
Good's Buffer
Peroxidase (Horseradish)
4-aminoantipyrine
Anti human b-lipoprotein antibody (sheep)
pH 7.0 ± 0.1

Reagent 2
Good's Buffer
Cholesterol oxidase (Nocardia)
Cholesterol esterase (Pseudomonas)
(N-ethyl-N-(2-hydroxy-3-sulfoethyl)-5-dimethoxy-4-fluoranyl) FDAO
pH 7.0 ± 0.1

Concentration

30 mM
2400 U/L
0.9 mM

30 mM
20,000 U/L
4,000 U/L
0.8 mM

Precautions and Warnings:

- For *in vitro* diagnostic use only. DO NOT pipette by mouth. Avoid contact with skin and eyes. If spilt, thoroughly, wash affected areas with water. For further information, consult the SDI HDL Immunoinhibition Reagent Material Safety Data Sheet.
- 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.
- Do not use the reagent after the expiration date printed on the kit.

REAGENT PREPARATION

Reagent 1: Supplied ready to use.
Reagent 2: Supplied ready to use.

REAGENT STORAGE

- Store the reagent at 2-8°C.
- The reagent is stable until the expiration date printed on the label when stored at 2-8°C.

REAGENT DETERIORATION:

DO NOT USE REAGENT IF:

- The reagent develops turbidity.
- The reagent fails to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING

- Serum: Use fresh, unhemolyzed serum.
- Samples are stable for 4 days refrigerated at 2-8°C, for 1 month at -20°C or for 2 years at -70°C.⁴

INTERFERENCES

Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out according to NCCLS No EP7-P.⁵ The following results were obtained:

Hemoglobin:

No significant interference from hemoglobin up to 500 mg/dL.

Bilirubin:

No significant interference from bilirubin up to 40 mg/dL.

Lipemia:

No significant interference from lipemia up to 1200 mg/dL measured as triglycerides.

A number of drugs and substances may affect the accuracy of this test. See Young, et al.⁶

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A SDI CA480 Clinical Chemistry System
- Deionized water and related equipment, e.g.: pipettes
- Analyzer specific consumables, e.g.: sample cups
- HDL Control and HDL Calibrator materials, such as those provided by SDI Biomed.

ASSAY PROCEDURE

HDL (Immunoinhibition)

Temperature 37°C
Primary Wavelength 600 nm
Secondary Wavelength 700 nm
Assay Type Endpoint
Direction Increase
Sample Volume 4 µL
Reagent 1 Volume 300 µL
Incubation Time 5 mins
Reagent 2 Volume 100 µL
Incubation Time 5 mins

Calculations

$$\frac{A(\text{patient})}{A(\text{standard})} \times \text{Concentration of standard (mg/dL)} = \text{HDL Cholesterol (mg/dL)}$$

Limitations:

- Unit Conversion: mg/dL x 0.02586 = mmol/L
- Protect reagents from direct sunlight and store as directed.
- Young, D.S.⁶ has published a comprehensive list of drugs and substances which may interfere with this assay.

CALIBRATION

The assay requires the use of SDI HDL/LDL Calibrator. The value of the SDI HDL/LDL Calibrator is assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL).

Refer to appropriate instrument operator manual for calibration interval.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known HDL Cholesterol values. The National Cholesterol Education Program (NCEP) in the USA has recommended that two levels of controls, one in the normal range (35 - 65 mg/dL) and one near the concentration for decision making (<35 mg/dL), be run in the same manner as patient samples. An acceptable range of HDL Cholesterol values should be established with this method by repeat analysis. Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control.

EXPECTED VALUES⁷

The expected value for serum HDL Cholesterol is as follows:
40 -60 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 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.

PERFORMANCE

Linearity:

When run as recommended the assay is linear from 1 to 180 mg/dL

Method Comparison:

Accuracy of the SDI HDL Immunoinhibition reagent method was verified by comparison to the instrument manufacturer's reagent.

Number of samples pairs:	55
Range of samples (mg/dL):	15 - 104
Correlation Coefficient:	0.997
Slope:	1.052
Intercept (mg/dL):	-2.0

Precision:

Within Run n=20	Level 1	Level 2	Level 3
Mean (mg/dL)	31.4	53.6	73.1
S.D. (mg/dL)	0.36	0.32	0.70
C.V. (%)	1.15	0.60	0.96
Total n=80 (20 days / 2 runs per day / 2 replicates per run)			
Mean (mg/dL)	23.2	68.9	112.2
S.D. (mg/dL)	0.56	1.67	2.38
C.V. (%)	2.41	2.42	2.12

REFERENCES

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- National Committee for Clinical Laboratory Standards, National Evaluation Protocols for Interference Testing, Evaluation Protocol Number 7, Vol. 4, No. 8, June 1984.
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