

# HDL Cholesterol (Direct)

## 1.0 INTENDED USE

This reagent is intended for the quantitative determination of high-density lipoprotein cholesterol (HDL-C) in human serum or plasma.

## 2.0 BACKGROUND

### 2.1 METHOD AND HISTORY

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 (11.1). These 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 (11.2).

The principle role of HDL in lipid metabolism is the uptake and transport of cholesterol from peripheral tissue to the liver through a process known as reverse cholesterol transport (a proposed cardioprotective mechanism) (11.3). Low HDL-C levels are associated with an increased risk of coronary heart disease and coronary artery disease (11.4-11.9). 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 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 coronary heart disease risk (11.9).

The CDC reference method for the quantitation of HDL-C combines ultracentrifugation and chemical precipitation to separate HDL from other lipoproteins, followed by cholesterol measurement using the Abell-Kendall assay (11.10). This method is too time consuming and labor intensive for use in routine analysis (11.11). Therefore, most laboratories utilized one of several methods for selective precipitation and removal of LDL and VLDL, followed by the enzymatic measurement of HDL-C in the supernatant fraction (11.10). 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.

### 2.2 TEST PRINCIPLE

This HDL Cholesterol assay is a homogeneous method for directly measuring serum HDL-C levels without the need for any off-line pretreatment or centrifugation steps.

The method is in a two-reagent format (illustrated below). The first reagent contains a mixture of polymers and polyanions that bind to the surface of LD, VLDL, and chylomicrons. These complexed lipoproteins are stabilized even in the presence of detergent, which is added as part of the second reagent, together with the remaining components of a cholesterol reagent. HDL particles on the other hand are not stabilized by the polymers and polyanions and become solubilized by the detergent. Consequently, only the HDL Cholesterol is subject to cholesterol measurement.

## 3.0 SPECIMEN COLLECTION AND STORAGE

### 3.1 SPECIMEN 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) (11.10).

Plasma: Specimens may be collected in EDTA or heparin. Centrifuge and remove the plasma as soon as possible after collection (within 3 hours) (11.10).

Samples containing triglyceride levels of > 2,871 mg/dl should be diluted with one part sample to one part physiological saline before assaying. Multiply the result by two.

### 3.2 SPECIMEN STORAGE

If not analyzed promptly, specimens may 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 -20°C for up to one month. For storage periods of 1 month to 2 years, samples should be preserved at -70°C (11.10).

## 4.0 MATERIALS

(1 x 30 ml, 1 x 10 ml)  
(2 x 60 ml, 2 x 20 ml)

The reagents necessary for the determination of HDL Cholesterol are included in the kit.

### 4.1 COMPOSITION OF REAGENTS

Reagent 1:  $\alpha$ -cyclodextrin 0.5 mM, dextran sulfate 0.5 g/l, magnesium chloride 2.0 mM, HSDA 0.3 g/l, buffer, pH 7.0  $\pm$  0.1, preservative. aPolyanion Neutralizes ionic charges on the surface of LDL thereby strengthening the binding with the polymer.

Reagent 2: POD > 15,000 U/L, PEG-CO > 5,000 U/L, PEG-CE > 800 U/L, 4-aminoantipyrene 0.5 g/l, buffer, pH 7.0  $\pm$  0.1, surfactant, preservative.

HSDA = sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline.

PEG-CO=Cholesterol Oxidase from Nocardia sp.

PEG-CE=Cholesterol Esterase from Pseudomonas

POD=Peroxidase from Horseradish

### 4.2 WARNINGS AND PRECAUTIONS

4.2.1 For In Vitro diagnostic use.

4.2.2 Do not pipette by mouth.

4.2.3 All specimens used in this 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.

4.2.4 Do not use the reagent after the expiration date printed on the kit.

### 4.3 WORKING REAGENT PREPARATION

Reagent 1: Reagent 1 is ready to use.

Reagent 2: Reagent 2 is ready to use.

### 4.4 REAGENT STORAGE AND STABILITY

All unopened reagents are stable until the expiration date on the kit label when stored at 2-8°C.

### 4.5 ADDITIONAL MATERIALS REQUIRED

4.5.1 HDL Cholesterol (Direct) Calibrator.

4.5.2 Tri-level Lipid Control,

4.5.3 4.5.3 Automated clinical chemistry analyzer capable of accommodating two-reagent assays.

## 5.0 PROCEDURE

Below is a general example of the HDL test procedure for an automated analyzer. All analyzer applications should be validated in accordance with NCEP and CLIA recommendations (11.10). For assistance with applications on automated analyzers, please contact Biotron Diagnostics technical service department at 1-800-595 8766.

Sample + Reagent 1	37°C	Reagent 2	37°C	Measurement	
4µl	300µl	5 min	100µl	5 min	(absorbance difference between 700nm & 600nm)
HDL-C Result					

## 6.0 CALIBRATION

The value of the HDL 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 HDL Cholesterol Calibrator kit package insert for instructions.

## 7.0 INTERPRETATION OF RESULTS

### 7.1 EXPECTED VALUES

The range of expected values is (11.14):

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

Each laboratory must establish its own range of expected values.

According to the NCEP, HDL values greater than or equal to 35mg/dl are considered desirable, and values greater than or equal to 60mg/dl are considered to offer some protection against coronary heart disease. Values below 35mg/dl are considered to be a significant independent risk factor for coronary heart disease (11.9).

### 7.2 SI UNITS

To convert from conventional units to S.I. units, multiply the conventional units by 0.02586.

mg/dl X 0.02586=mmol/L HDL-Cholesterol

### 7.3 LIMITATIONS

- 7.3.1 Anticoagulants containing citrate should not be used.
- 7.3.2 Protect the reagents from direct sunlight.
- 7.3.3 Store the reagents as per instructions.
- 7.4.3 Samples with values greater than 150 mg/dl must be diluted 1:1 with saline and reassyed. Multiply the result by 2.

### 8.0 INTERFERING SUBSTANCES

All interference studies were conducted according to NCCLS guideline No. EP7-P for interference testing in clinical chemistry (11.12).

Hemoglobin levels up to 100 mg/dl and bilirubin levels up to 20 mg/dl were found to exhibit negligible interference (<5%); interference was observed at 400 mg/dl.

Samples with levels of interfering substances higher than the upper limits should be diluted with physiological saline before assaying. Refer to the work of Young for a review of drug effects on serum HDL Cholesterol levels (11.13).

### 9.0 QUALITY CONTROL

Reliability of test results should be routinely monitored with control sera or quality-control materials that reasonable emulate performance on patient specimens (11.10). 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 (35-65mg/dl) and one near the concentrations for decision making (<35mg/dl). An acceptable range of HDL Cholesterol values should be established for the controls by repeat analysis. The recovery of control values within the appropriate range should be the criteria used in evaluation of future assay performance.

Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

### 10.0 SPECIFIC PERFORMANCE CHARACTERISTICS

#### 10.1 ACCURACY

Accuracy of the direct HDL Cholesterol Reagent method was verified by comparison to the designated comparative method (ultracentrifugation, chemical precipitation and Abell-Kendall cholesterol analysis) (11.10) and the Biotron lyophilized direct HDL method. Studies comparing the HDL Cholesterol method to the designated comparative method produced the following results:

<u>Method</u>	<u>Direct HDL Cholesterol</u>	<u>Designated Comparative Method</u>
n	30	30
Mean (mg/dl)	55	55
Range (mg/dl)	26-94	26-94
Standard Deviation (mg/dl)	18	19
Regression Analysis	y=0.93x - 3.85	
Correlation Coefficient	r=0.989	

  

<u>Method</u>	<u>Direct HDL Cholesterol</u>	<u>Lyophilized Direct HDL Method</u>
n	95	95
Mean (mg/dl)	59	59
Range (mg/dl)	27-109	24-118
Standard Deviation (mg/dl)	20	21
Regression Analysis	y=0.91x + 6.08	
Correlation Coefficient	r=0.979	

#### 10.2 PRECISION

Within-day precision for the direct HDL Cholesterol Reagent was determined following a modification of NCCLS document EP5-T2 (11.15.) Within-day precision studies produced the following results:

	<u>Low</u>	<u>Medium</u>	<u>High</u>
n	20	20	20
Mean (mg/dl)	38	68	85
Standard Deviation (mg/dl)	0.9	1.0	1.2
Coefficient of Variation (%)	2.4	1.5	1.4

Day-to-day precision was determined following a modification of NCCLS document EP5-T2 (11.15.) Day-to-day precision studies produced the following results:

	<u>Low</u>	<u>Medium</u>	<u>High</u>
n	20	20	20
Mean (mg/dl)	37	66	84
Standard Deviation (mg/dl)	0.8	1.5	1.6
Coefficient of Variation (%)	2.2	2.3	1.9

### 11.0 REFERENCES

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