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DIRECT HDL CHOLESTEROL REAGENT (MANUAL AND AUTOMATED)

INTENDED USE
For the quantitative determination of high-density lipoprotein (HDL) in human serum or plasma on automated analyzer. For *in vitro* diagnostic use only.

SUMMARY AND EXPLANATION OF THE TEST
Cholesterol is a fatty substance found in blood, bile and brain tissue. It serves as a precursor to bile acids, steroids and vitamin D. The concentration of total cholesterol in serum has been associated with metabolic, infectious and coronary heart diseases. In the plasma, cholesterol is transported by three lipoproteins: high-density lipoprotein (HDL-Cholesterol), low density lipoprotein (LDL-Cholesterol), and very low density lipoprotein (VLDL-Cholesterol).[1]

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 cardio protective mechanism. Low HDL-C levels have repeatedly been associated with an increased risk of coronary heart disease and coronary artery disease. Thus the determination of serum HDL cholesterol has been recognized as a useful tool in identifying high-risk patients.

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.[10] This method is considered too time consuming and labor intensive for use in routine analysis.[11] 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.[10] 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 form both long turnaround times and poor reproducibility.

PRINCIPLE
The Direct 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. The first reagent stabilizes LDL, VLDL, and chylomicrons. The second reagent contains PEG modified enzymes that selectively react with the cholesterol present in the HDL particles. Consequently, only the HDL cholesterol is subject to cholesterol measurement.

REAGENT COMPOSITION
1. Direct HDL Cholesterol Reagent 1:
   Magnesium chloride 100mM, Aminoantipyrene 1 mmol/L, buffer, pH 7.0±0.1, preservative.
2. Direct HDL Cholesterol Reagent 2:
   Peroxidase from Horseradish (POD) 4KU/L, Cholesterol Oxidase from Nocardia sp. (PEG-CO) 1 KU/L, Cholesterol Esterase from Pseudomonas (PEG-CE) 1KU/L, N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS) 0.3g/L, buffer, pH 7.0±0.1, surfactant, preservative.

WARNINGS AND PRECAUTIONS
1. For *in vitro* diagnostic use only.
2. Exercise the normal precautions required for the handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent.
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. Do not use the reagent after the expiration date printed on the kit.

STORAGE AND STABILITY
Store the reagent set at 2 – 8 °C. The reagent is stable until the expiration date indicated on the bottle label.

SPECIMEN COLLECTION AND STORAGE
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 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 1 month. For storage periods of 1 month to 2 years, samples should be preserved at -70°C.[10]

INTERFERENCES
All interference studies were conducted according to the procedures recommended in NCCLS guideline NO. EP7-P for interference testing in clinical chemistry.[12] Hemoglobin levels up to 100 mg/dl, Triglyceride levels up to 1800mg/dl and Bilirubin levels up to 20 mg/dl were found to exhibit negligible interference (<5%) on this method. 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.[13]

MATERIALS PROVIDED
1. Direct HDL Cholesterol Reagent 1 (Ready to use)
2. Direct HDL Cholesterol Reagent 2 (Ready to use)

MATERIALS REQUIRED BUT NOT PROVIDED
1. Direct HDL/LDL Cholesterol Calibrator (Cat. # H514-A)
2. Lipid Control Set
3. Automated chemistry analyzer or spectrophotometer capable of measuring absorbance at 700 and 600 nm.

MANUAL PROCEDURE
1. Label test tubes: blank, standard, control, patient, etc
2. Pipette 750 µl of Direct HDL Cholesterol Reagent 1 and place in 37°C water bath for 5 minutes.
3. Add 10 µl of sample to respective tubes and mix well.
4. Add 250 µl of Direct HDL Cholesterol Reagent 2 and incubate in 37°C water bath for 5 minutes.
5. Measure difference in absorbance at 700 and 600 nm.

AUTOMATED PROCEDURE
Note: All analyzer applications should be validated in accordance with NCEP and CLIA recommendations.[10]
1. Use 4 ul sample with 300 ul of Direct HDL Cholesterol Reagent 1.
2. Equilibrate to 37°C for 5 minutes.
3. Add 100 ul of Direct HDL Cholesterol Reagent 2.
4. Equilibrate to 37°C for 5 minutes.
5. Measurement (Absorb. Difference between 700nm & 600 nm)

CALCULATIONS
(A = Absorbance)

$$\frac{\Delta A \text{ (patient)}}{\Delta A \text{ (standard)}} \times \text{Concentration of calibrator (mg/dl)} = \text{Concentration of patient (mg/dl)}$$

Example:
Δ A (patient) = 0.40, Δ A (calibrator) = 0.32, Concentration of calibrator = 53 mg/dl.

$$\frac{0.40}{0.32} \times 53 = 66.3 \text{ mg/dl}$$

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

mg/dl × 0.02586 = mmol/L HDL cholesterol

LIMITATIONS

1. Anticoagulants containing citrate should not be used.
2. Protect the reagents from direct sunlight.
3. Store the reagents as per instructions.
4. Samples with values greater than 150 mg/dl must be diluted 1:1 with saline and re-assayed. Multiply the result by two.

CALIBRATION

The Direct HDL/LDL Cholesterol Calibrator is required for calibration. Calibrate with each bottle change or lot change or if control results are found to be out of range. The value of the Direct HDL/LDL calibrator was assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL). Refer to Direct HDL/LDL Cholesterol calibrator kit package insert for instructions. If control results are found to be out of range, the procedure should be recalibrated.

QUALITY CONTROL

Reliability of test results should be routinely monitored with control materials that reasonably emulate performance of patient specimens.[10] Quality control materials are intended for use only as monitors of accuracy and precision. Any HDL/LDL controls would be suitable for use with this assay. The National Cholesterol Education Program (NCEP) Lipid Standardization Panel (LSP) recommends two levels of controls, one in the normal range (35-65 mg/dl) and one near the concentrations for decision making (<35 mg/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 materials are intended for use only as monitors of accuracy and precision. Controls should be run with every working shift in which HDL-C assays are performed. It is recommended that each laboratory establish their frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

EXPECTED VALUES[2]

The expected values for serum HDL cholesterol are as follows: [14]

< 40 mg/dl Low

≥ 60 mg/dl High

It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

According to the NCEP, HDL values greater than or equal to 35 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 35 mg/dl are considered to be a significant independent risk factor for coronary heart disease.[9]

PERFORMANCE CHARACTERISTICS

Assay Range: 2 – 150 mg/dl

Accuracy: Accuracy of the Direct HDL Cholesterol Reagent method was verified by comparison to the Designated Comparison Method (ultracentrifugation, chemical precipitation and enzymatic cholesterol analysis which has been standardized to the Abell-Kendall method)[10] and another manufacturer's lyophilized Direct HDL Cholesterol method to the Designated Comparison Method produced the following results:

Studies comparing the Direct HDL Cholesterol method to Pointe Scientific AutoHDL Cholesterol Reagent Set on Hitachi 717 Analyzer produced the following results:

Method	Direct HDL Cholesterol	AutoHDL Cholesterol
N	52	52
Mean HDL Cholesterol	46	48.3
Range (mg/dl)	22.3-96.8	21.3-92.1
Regression Analysis	y=0.93x+0.73mg/dl	
Correlation Coefficient	r=0.916	

Precision: Within Day precision for the Direct HDL Cholesterol Reagent was determined on Hitachi 717 following a modification of NCCLS document EP5-T2.[15] Within Day precision studies produced the following results:

	Sample 1	Sample 2
N	25	25
Mean HDL Cholesterol	38.1	83.5
Standard Deviation (mg/dl)	1.54	1.94
Correlation of Variation (%)	4.0	2.3

Day to Day precision for the Direct HDL Cholesterol Reagent was also determined on Hitachi 717 following a modification of NCCLS document EP5-T2.[15] Day to Day precision studies produced the following results:

	Sample 1	Sample 2
N	25	25
Mean HDL Cholesterol	38.2	83.4
Standard Deviation (mg/dl)	1.74	2.2
Correlation of Variation (%)	4.5	2.6

Sensitivity: The analytical sensitivity of the Direct HDL Cholesterol Reagent was determined on Hitachi 717 as 2 mg/dl of HDL Cholesterol.

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H513: 03/2018

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