DIRECT HDL CHOLESTEROL

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QUANTITATIVE DETERMINATION OF DIRECT HDL CHOLESTEROL IVD

PRINCIPLE:

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 chromogenic coupler to develop color for the quantitative determination of HDL-C.

CLINICAL SIGNIFICANCE:

Lipoproteins serves to solubilize and transport cholesterol and other lipids in the bloodstream. Different lipoprotein classes have been shown to have various effects on the progression of coronary heart disease (CHD). High density lipoproteins are associated with decreased risk and are seen as a protective factor.

The measurement of HDL cholesterol (HDL-C) is a powerful predictor of CHD that provides the opportunity for early diagnosis and intervention to halt the progress of cardiovascular disease.

REAGENT COMPONENT AND CONCENTRATIONS:

Reagent 1: Good's Buffer 50 mmol/L, Cholesterol oxidase <1000 U/L Peroxidase <1300 ppg U/L, (DSBmT) <1 m/M, Preservative <0.06%

Reagent 2: Good's Buffer, Cholesterol esterase <1500 U/L, 4-AAP <1 mM, Detergent <2%, Preservative <0.06%

PRESENTATION:

Store all reagents at 2-8^{0}C		No. of Bottles		
		20ml/ 50 T	40ml/100 T	80ml/200T
1	1HDL Cholesterol	1	1	1
2	2 HDL Cholesterol	1	1	1
3	HDL/LDL Calibrator	1	1	1
4	Distilled Water	1	1	1

SAFETY PRECAUTIONS AND WARNINGS:

For I*n-Vitro* diagnostic use.

Performance cannot be guaranteed, if the reagents are used in other procedures or for other purposes.

WORKING REAGENT PREPARATION:

Reagents are ready to use as supplied.

HDL/LDL Calibrator Preparation & Stability:

Refer the calibrator insert before use.

REAGENT STORAGE AND STABILITY:

When stored at 2-8^{0}C reagent is stable until the expiration date stated on the bottle and kit box label.

SPECIMEN COLLECTION AND HANDLING:

Patients are not required to fast, prior to blood collection, Serum, EDTA-treated or heparinized plasma are the recommended specimen. If not analyzed promptly, specimens need to be stored for longer than 5 days, they may be stored frozen at -80^{0}C.

CE

PROCEDURE FOR SEMIAUTOMATED ANALYZERS.

REACTION PARAMETERS:

- Type of Reaction : Fixed Time
- Wavelength : 546 nm
- Flow cell temperature : 37^{0}C
- Delay Time : 300 Sec
- Interval Time : 300 Sec
- No. of Reading : 2
- Sample Volume : 10 µl
- Reagent Volume (R1+R2) : 750 + 250 µl
- Calibrator Concentration : As mentioned on vial
- Light Path : 1 cm
- Zero setting with : Distilled water

PROCEDURE:

1. Bring the Reagents and the photometer to 37^{0}C.
2. Pipette into a cuvette:
   Reagent 1 750 µL
   Serum/Calibrator 10 µL
3. Mix and incubate for 5 min at 37^{0}C then immediately add R2 250 µL. Read the absorbance (A1) after 5 seconds. After 5 minutes, read the absorbance (A2)

PROCEDURE FOR FULLY AUTO ANALYZER

REACTION PARAMETERS:

- Type of Reaction : Fixed Time
- Wavelength : 546 and 700nm
- Flow cell temperature : 37^{0}C
- Delay Time : 300 Sec
- Interval Time : 300 Sec
- No. of Reading : 2
- Sample Volume : 3 µl
- Reagent Volume (R1+R2) : 300 + 100 µl
- Calibrator Concentration : As mentioned on vial
- Light Path : 1 cm
- Zero setting with : Distilled water

Sample + Reagent 1 — 37^{0}C, 5 min — Measurement (Abs 1) difference between 700 nm & 546 nm)

Reagent 2 — 37^{0}C, 5 min — Measurement (Abs 2) difference between 700 nm & 546 nm)

CALCULATION:

Results are calculated automatically by the instrument as follows:

$$\text{HDL Chol. (mg/dl)} = \frac{\text{(A2-A1) of Unknown}}{\text{(A2-A1) of calibrator}} \times \text{Calibrator Value}$$

QUALITY CONTROL:

Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions, if controls do not meet the acceptable tolerance.

EXPECTED VALUES:

The expected values for serum HDL cholesterol are as follows 40-70 mg/dl (1.06-1.87 mmol/L)

Each laboratory must establish its own range of expected values. According to the NCEP, HDL values greater than or equal to 60 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.

PERFORMANCE CHARACTERISTICS:

Measuring range : From detection limit of 1.0 mg/dl to linearity limit of 150 mg/dl.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with Nacl 9 g/L and multiply the result by 2.

Precision:

	Intra –assay		
Mean (mg/dl)	32.6	47.3	68.5
SD	0.33	0.25	0.63
CV	0.65	0.78	0.65

Inter – assay		
32.9	46.9	69.1
0.91	0.97	1.05
1.25	1.11	1.33

INTERFERENCES:

No interference of Ascorbic Acid upto 100 mg/dl.

No interference of Bilirubin & Bilirubin Conjugate upto 40 mg/dl.

No interference of Hemoglobin upto 500 mg/dl.

LINEARITY:

The method is linear upto a concentration of 150 mg/dl (4.0 mmol/L). Specimens with HDL values above 150 mg/dL (4.0mmol/L) should be diluted with isotonic saline and reassayed. Multiply results by the dilution factor.

BIBLIOGRAPHY:

Matsuzaki Y., Kawaguchi E., Norita Y.etal Evaluation of Two Kinds of Reagents for Direct Determination of HDL-Cholesterol. J.Anal Bio Sc 1996; 19:419-427.

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