HDL-DIRECT

(Selective Detergent)

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Ref. CC3-HDL.13M, 20ml/50Test
CC3-HDL.13MU, 40ml/100Test
CC3-HDL.13MV, 80ml/200Test

INTENDED USE

Quantitative Determination of HDL Cholesterol (Direct).

DIAGNOSTIC 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.

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-Cholesterol.

PRESENTATION

All reagents to be stored at 2-8^{0}C	No. of Bottles 20ml/50 T	40ml/100 T	80ml/200 T
• 1 HDL Direct	1	1	1
• 2 HDL Direct	1	1	1
• HDL/LDL Calibrator	1	1	1
• Distilled Water	1	1	1

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• Good's Buffer	50 mmol/L
• Cholesterol oxidase	<1000 U/L
• Peroxidase	<1300 ppg U/L
• (DSBmT)	<1 m/M
• Preservative	<0.06%
• Cholesterol esterase	<1500 U/L
• 4-AAP	<1 Mm
• Detergent	<2%
• Preservative	<0.06%
• HDLc/LDLc CAL	Standard, Lyophilized human serum

PRECAUTIONS

For I*n-Vitro* diagnostic use.

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

PREPARATION OF WORKING REAGENT

Direct HDL Cholesterol reagents are ready to use.

HDL/LDL Calibrator Preparation & Stability

Refer the calibrator insert before use.

REAGENT STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8^{0}C and contamination are prevented during their use.

R1 and R2: Once opened is stable for 30 days at 2-8^{0}C.

SPECIMEN COLLECTION

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.

PROCEDURE FOR SEMI AUTOMATED 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

TEST PROCEDURE

Pipette into Test Tube	Calibrator	Sample
Reagent 1 (µL)	750	750
Calibrator (µL)	10	-
Sample (µL)	-	10

Mix and Incubate for 5 min at 37^{0}C than add,

Reagent 2 (µL)	250	250

Mix and read absorbance (A1) after 5 second. Incubate for 5 min at 37^{0}C. 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

Pipette into Test Tube	Calibrator	Sample
Reagent 1 (µL)	300	300
Calibrator (µL)	3	-
Sample (µL)	-	3

Mix and Incubate for 5 min at 37^{0}C and measurement (Abs 1) difference between 700 nm& 546 nm than add,

Reagent 2 (µL)	100	100

Mix and Incubate for 5 min 37^{0}C and measurement (Abs 2) difference between 700 nm & 546 nm.

TEST RESULTS

Results are calculated automatically by the instrument as follows,

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

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.

LIMITATIONS FOR INTERFERENCE

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.

NORMAL VALUES

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.

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.

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

REFERENCES

1. 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.