HDL Cholesterol. Direct. Enzymatic colorimetric Ref.:HDL-010
Quantitative Determination of HDL Cholesterol
Only for *in vitro* use in clinical laboratory
Store at 2-8ºC 60+20 mL

HDL CHOLESTEROL

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PRINCIPLE OF THE METHOD
Directly determination of serum HDLc (high-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation of the sample.
The method depends on the properties of a detergent which solubilizes only the HDL so that the HDL-c is released to react with the cholesterol esterase, cholesterol oxidase and chromogens to give colour. The non HDL lipoproteins LDL, VLDL and chylomicrons are inhibited from reacting with the enzymes due to absorption of the detergents on their surfaces.
The intensity of the color formed is proportional to the HDLc concentration in the sample.

CLINICAL SIGNIFICANCE
HDL particles serve to transport lipoproteins in the blood-stream.
HDL is known as "good cholesterol" because high levels are thought to lower the risk of heart disease and coronary artery disease.
A low HDL cholesterol levels, is considered a greater heart disease risk[1,5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1	GOOD pH 7.0	
	Cholesterol oxidase	< 1000 U/L
	Peroxidase	<1300 U/L
	DSBmT	< 1 mM
R 2	GOOD pH 7.0	
	Cholesterol esterase	< 1500 U/L
	4 – Aminoantipyrine (4-AP)	< 1 mM
	Detergent	< 2%
	Ascorbic oxidase	< 3000 U/L
HDLc/ LDLc CAL	Calibrator. Lyophilized human serum.	

PRECAUTIONS
HDLc/ LDLc CAL
Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

PREPARATION
- **R 1 and R 2**: Are ready to use.
- **HDLc/ LDLc CAL:** Dissolve the contents with 1 mL of distilled water. Cap vial and mix gently to dissolve contents.

STORAGE AND STABILITY
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not freeze the reagents.
- **R 1 and R 2**: Once opened is stable 8 weeks at 2-8ºC.
- **HDLc/ LDLc CAL:** Once reconstitute 1 week at 2-8ºC or 5 weeks at –20ºC.

Do not use reagents over the expiration date.

Signs of reagent deterioration:
- Presence of particles and turbidity.

ADDITIONAL EQUIPMENT
- Spectrophotometer or colorimeter measuring at 600 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES
Serum or heparinized plasma, free of hemolysis[1]: Anticoagulants containing citrate should not be use.
Removed from the blood clot as soon as possible
Stability of the sample: 7 days at 2-8ºC .

PROCEDURE
1. Assay conditions:
   Wavelength: . . . . . 600 -700 nm
   Cuvette: . . . . . 1 cm light path
   Temperature 37ºC
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Calibrator	Sample
R 1 (µL)	300	300	300
Calibrator (µL)	--	3	--
Sample (µL)	--	--	3

4.Mix and incubate for 5 min at 37ºC.
5. Read the absorbance (A_1) of the samples and calibrator.
6. Add:

	Blank	Calibrator	Sample
R 2 (µL)	100	100	100

7. Mix and incubate for 5 min. at 37ºC.
8. Read the absorbance (A_2) of the samples and calibrator, against the Blank.
9. Calculate the increase of the absorbance $\Delta A = A_2 - A_1$.

CALCULATIONS

$$\frac{(\Delta A)\text{Sample}}{(\Delta A)\text{Calibrator}} \times \text{Calibrator conc.} = \text{mg/dL of HDL-c in the sample}$$

Conversion factor: mg/dL x 0.0259= mmol/L.

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 tolerances.

REFERENCE VALUES[2]

	Men	Women
Low risk	> 50 mg/dL	> 60 mg/dL
Normal risk	35 – 50 mg/dL	45 – 60 mg/dL
High risk	< 35 mg/dL	< 45 mg/dL

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
Measuring range: From detection limit of 2.5 mg/dL to linearity limit of 200 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			Inter-assay		
Mean (mg/dL)	32.9	50.6	101.4	32.8	50.0	100.1
SD	0.3	0.2	0.7	0.4	0.7	1.1
CV	0.8	0.5	0.7	1.3	1.5	1.1

Sensitivity: 1 mg / dL = 0.0016 A.
Accuracy: Results obtained using BSM reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.996.
Regression equation: y= 0.98 + 3.42 mg/dL.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES
No interferences were observed to bilirubin T. and D. up to 60 mg/dL, hemoglobin up to 1000 mg/dL or lipemia up to 1800 mg/dL.
A list of drugs and other interfering substances with HDL cholesterol determination has been reported by Young et. al[3,4].

NOTES
BSM has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

BIBLIOGRAPHY

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PCT/JP97/04442