BioMed- HDL - Cholesterol

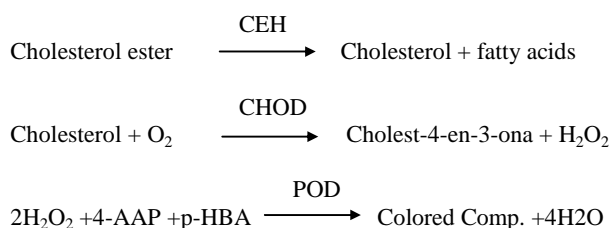## Precept. Reagent

REF: HDL114100 (2x50 ml)

INTENDED FOR USE:

For the quantitative determination of HDL cholesterol in serum and plasma

PRINCIPLE :

HDL-Cholesterol is obtained trough selective precipitation of LDL and VLDL lipoproteins, thus HDL lipoproteins remain in solution. HDL-Cholesterol in supernatant is treated as a sample for cholesterol assay according to the following reaction:

$$Cholesterol\ ester \xrightarrow{CEH} Cholesterol + fatty\ acids$$

$$Cholesterol + O_2 \xrightarrow{CHOD} Cholest\text{-}4\text{-}en\text{-}3\text{-}ona + H_2O_2$$

$$2H_2O_2 + 4\text{-}AAP + p\text{-}HBA \xrightarrow{POD} Colored\ Comp. + 4H_2O$$

Formed color is measured at 546 nm and is proportional to HDL-Cholesterol concentration in sample when used as directed.

SPECIMEN COLLECTION:

Fresh (or just defrosted) not hemolized serum or plasma (EDTA Na_2 , Na Heparin) .
Centrifuge and collect serum as soon as possible .
HDL_C in serum or plasma is stable up to 7 days at +2-8°C ,1 month at20^{o}C and 2 years at –70°C
Shake and bring the samples at room temperature (+15-25°C.) before using .

REAGENTS COMPOSITON :

The reagents set are stored at ambient temperature. Storage must not exceed expiration date on box label.

Precipitating Reagent	
Phosphotungstic acid Magnesium Chloride	0.55 mM 25 mM
HDL Cholerterol Standard	50 mg/dl

PACKAGE : Collection & Storage .

Store at +2-8°C.
Stable until the expiration date reported upon the package.
After the unsealing and the taking of the reagent , it is advised to close up the bottle immediately in order to avoid evaporation , direct light exposure and bacterial contamination .

PRECAUTION & WARNING:

Avoid pipetting by mouth .
The preparation , according to current regulation . is classified as not dangerous.
The total concentration of non active components (preservatives , detergents ,stabilizers) is below the minimum required for citation .
Anyway handle with care , avoid ingestion , avoid contact with eyes , skin and mucous membranes . The samples must be handle as potentially infected from HIV or Hepatitis .

REQUIRED MATERIALS NOT PROVIDED :

General Laboratory Equipment and instrumentations .

PROCEDURE :

This methodology describes the manual procedure to use the kit .
For automated procedure, ask for specific application .

Precipitation

Specimen	200 µl
Precipitant	500 µl

Mix and allow standing for 10 minutes at room temperature. Centrifuge for 10 minutes at 4000 rpm, or 2 minutes 12000rpm. Separate of the clear supernatant within two hours and determine the cholesterol content by the CHOD-PAP method. The supernatant may be stored up to five days at 2-8°C

Wavelength	546 nm
Optical path	1 cm
Incubation temperature	20, 25 or 37°C
Zero adjustment	Reagent blank

	BLANK	STANDARD	SAMPLE
BioMed Cholesterol Reagent	1000 µL	1000 µL	1000 µL
Distilled Water	10 µL		
Standard (R1)		10 µL	
Sample supernatant			100 µL

CALCULATION :

1. HDL Cholesterol

$$mg/dl = 50 \times \frac{(A)\ Sample}{(A)\ Standard}$$

2. LDL Cholesterol

$$LDL\ Cholesterol\ (mg/dl) = Total\ Cholesterol - \frac{Triglycerides}{5} - HDL\ Cholesterol$$

EXPECTED VALUES :

HDL:

Women	30-85 mg/dL
Men	30-70 mg/dL
LDL: Adults	66-178 mg/dL

The above mentioned values are to be considered as a reference.

It is strongly recommended that each laboratory establish its own normal range according to its geographic area , according to IFCC protocol .

Since CHOL   HDL has an elevated protective action against the risk of arising cardiovascular diseases , the following reference values can be used :

Protective Action	Men	Women
High	> 55mg/dL	> 65 mg/dL
None	35-55 mg/dL	45-65 mg/dL
Poor	< 35 mg/dL	< 45 mg/dL

WASTE DISPOSAL :

The disposal of the product must be in accordance with local regulation concerning waste disposal

QUALITY CONTROL :

It is recommended to execute the quality control at every kit utilization to verify that values are within the reference range indicated by the methodology.

PERFORMANCE :

MEASURE INTERVAL L INEARITY :	2-200 mg/dl
LOWEST MEASURABLE LIMIT :	2 mg/dl
SENSITIVITY :	1mg/dl= ΔA

PRECISSION WITH SERIES : n=20

LOW LEVEL	M = 30.2 mg/dl	C.V = 1.50%
MEDIUM LEVEL	M = 42.7 mg/dl	C. V = 1.18%
HIGH LEVEL	M = 75.6 mg/dl	C.V = 1.04%

PRECISSION AMONG SERIES : n=20

LOW LEVEL	M = 29.4 mg/dl	C.V = 2.68%
MEDIUM LEVEL	M = 41.9 mg/dl	C.V = 1.89%
HIGH LEVEL	M = 73.2 mg/dl	C.V = 3.22%
INTER, ANALIZED	30-72 mg/dl	
CORRELATION	r = 0.999	n=50
LIN. REGRESSION	y= 1.01 ×- 3.39	n=50

INTERFERENCE:

Interferences are negligible up to :			
Bilirubin	20 mg/dl		
Hemoglobin g/dl	0.4	Glucose	500 mg/dl
Ascorbic Acid mg/dl	40	Triglycerids	2000 mg/dl

METHOD LIMITATIONS:

If Triglycrides levels are higher than 2000 mg/dl , repeat the measure on a sample diluted 1:2 with physiological solution e multiply the results $\times 2$.
Do not use Anticoagulants containing citrate .
For through evaluation of the interfering substances ,consult : Young , D. S ,et al , AACC Press , Washington DC , , 3-104 (1990)

REFERENCES:

1. Tietz, N.W. (ed) Fundamentals of Clinical Chemistry W.B.Saunders Co., Philadelphia, 1976.
2. Watson, D., Clin. Chem. Acta 5 (637), 1960.
3.Trinder, P., Ann Clin. Biochem. 6 (24), 1969.
4. Castelli, W.P., et al., Circ. 55 (767) 1977.

📖	Consult Instructions for Use
⚠	Caution, Consult accompanying
IVD	In Vitro Diagnostic Medical
🌡	Temperature Limitation
🏭	Manufacturer
EC REP	Authorized Representative in the European Community
REF	Catalogue Number
LOT	Batch Code
⧖	Use by

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