Direct HDL-CHOLESTEROL

INTENDED USE

Vitro D-HDL reagent is intended for the in vitro quantitative determination of High-Density Lipoproteins (HDL) in serum on manual systems.

METHOD

Direct Enzymatic colorimetric, Liquid

CLINICAL SIGNIFICANCE

Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipids, free cholesterol, and protein constitute the outer surface of the lipoprotein particle, while the inner core contains mostly esterified cholesterol and triglycerides. The particles serve to solubilize and transport cholesterol and triglycerides in the blood stream[2]. The relative proportions of protein and lipid determine the density of these lipoproteins, and provide a basis on which to begin their classification. These classes are: chylomicron, very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high-density lipoprotein (HDL). Numerous clinical studies have shown that the different lipoprotein classes have very distinct and varied effects on coronary heart disease risk[3]. The principle role of HDL in lipid metabolism is the uptake and transport of the cholesterol from peripheral tissues to the liver through a process known as reverse cholesterol transport (a proposed cardio protective mechanism)[4]. Low HDL-cholesterol levels are associated with an increased risk of coronary heart disease and coronary artery disease. Hence the determination of serum HDL-cholesterol is a useful tool in identifying high-risk patients[5].

ASSAY PRINCIPLE

The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents.LDL, VLDL, and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol Oxidase (CHOD) and cholesterol esterase (CHER). The enzymes selectively react with HDL to produce H_2O_2 which is detected through a Tinder reaction.

EXPECTED VALUES

Males [6]	
Good prognosis	> 50 mg/dl (>1.4 mmol/L)
Standard risk	35 – 50 mg/dl (0.9 -1.4 mmol/L)
Risk indicator	< 35 mg/dl (<0.9 mmol/l)
Females [6]	
Good prognosis	> 60 mg/dl (>1.7 mmol/L)
Standard risk	45 – 65 mg/dl (1.2 -1.7 mmol/L)
Risk indicator	< 45 mg/dl (<1.2 mmol/l)

REAGENTS

	MES buffer (pH 6.5)
	TODB N, N-Bis (4-sulfobutyl)-3- methylaniline)
	Polyvinyl sulfonic acid
R_1	Polyethyleneglycol-methyl ester
	MgCl2
	Detergent
	EDTA
R_2	MES buffer (pH 6.5)
	Cholesterol esterase
	Cholesterol Oxidase
	Peroxidase
	4-aminoantipyrine
	detergent.

- **Reagent Preparation & Stability**

All reagents are stable up to the expiry date given on label when stored at 2-8 °C protected from light.

SPECIMEN

- Use fresh non-hemolyzed serum.
- EDTA and heparinized plasma are the only accepted anticoagulants.
- A specimen drawn from a fasting patient is preferred, although non-fasting specimens are considered acceptable[8].

Specimen Preparation & Stability

- Serum separated from the blood clot within 4 hours at room temperature.
- Store plasma at 4°C prior to analysis. EDTA plasma has the advantage that lipoproteins have enhanced stability during storage at 4°C.
- Specimens should preferably be analyzed on the day of collection.
- HDL-cholesterol is stable in specimen for 4 days at 4 °C or 7-14 days at -20°C. HDL-cholesterol decreased significantly, but the decrease is not clinically relevant[9].

PROCEDURE

Wavelength	580 - 600 nm
Cuvette	1 cm light path
Temperature	37 °C
Zero adjustment	against Reagent Blank

	Blank	Calibrator	Specimen
R1	300 µl	300 µl	300 µl
Calibrator	……	4 µl	……
Specimen	……	……	4 µl
Mix and Incubate for 5 min at 37°C. Then add :			
R2	100 µl	100 µl	100 µl

Mix, incubate for 5 minutes at 37°C. Measure the absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.
The color is stable for 60 minutes.

CALCULATION

Calculate the HDL-cholesterol concentration by using the following formulae:

HDL-Cholesterol Concentration=

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Calibrator}} \times \text{Calibrator value}$$

- **Unit conversion**

mg/dl x 0.0259 = mmol/l

PERFORMANCE CHARACTERISTICS

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run		Between Day	
Control	Level I	Level II	Level I	Level II
Number of samples	20	20	20	20
Mean (mg/dl)	28	76.1	27.5	75.3
SD (mg/dl)	0.25	0.81	1.26	2.04
CV (%)	0.89	1.06	4.60	2.71

Method Comparison

Comparison studies were carried out using another similar commercially available HDL reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression.

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EC REP

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MDSS GmbH, Burckhardtstr. 1
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The results obtained using 50 samples were the following:

Correlation coefficient: 0.938

Regression equation: y= 0.9825x + 1.41606

Linearity

When run as recommended, the assay is linear up to 180 mg/dl.

Specimens with values above 180.0 mg/dl should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

Sensitivity

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a pathlength of 1 cm.

When run as recommended the sensitivity of this assay is 1.0 mg/dl

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established HDL-cholesterol values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

No Interferences were observed with

ascorbic acid up to 100 mg/dL

haemoglobin up to 750 mg/dL

bilirubin up to 23.4 mg/dL.

A list of drugs and other interfering substances with HDL- Cholesterol determination has been reported by Young et al

WARNING & PRECAUTION

- Vitro D-HDL reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Don't use D-HDL reagent if it has become cloudy.
- Specimen with more than 1700 mg/dl triglycerides should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

BIBLIOGRAPHY

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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
15801	40 ml
15802	80 ml

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ISO 13485:2016 CE

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