Meril
Diagnostics

IVD For *in vitro* diagnostic use
Read this pack insert thoroughly before use

HDL Direct Reagent
CliniQuant – FSR

REF	Pack Size	R1 HDL Direct Reagent	R2 HDL Direct Reagent	R3 HDL Direct Calibrator	R4 HDL Direct Lipid Control
HDLFSR-01	2 x 24 /2 x 8ml	2 x 24ml	2 x 8ml	1 x 1ml	1 x 1ml
HDLFSR-02	4 x 30/4x 10ml	4 x 30ml	4 x 10ml	1 x 1ml	1 x 1ml

INTENDED USE

This reagent is intended for quantitative determination of high density lipoprotein cholesterol (HDL-C) concentration in human serum or plasma.

CLINICAL SIGNIFICANCE

High Density Lipoprotein (HDL) Cholesterol consists of a number of heterogeneous particles varying in size and content of lipid and apolipoproteins. HDL plays important role in cholesterol efflux, reducing stored cholesterol and returning cholesterol from the periphery to the liver for removal as bile acid, also serves as scavenger of lipid and apolipoprotein during normal catabolism. HDL cholesterol values are 1/5th of the total cholesterol values. There is inverse relationship between HDL Cholesterol and coronary heart diseases.

PRINCIPLE OF THE METHOD

The HDL Chloesterol test is a two reagent homogenous system for the selective measurement of serum or plasma HDL-Cholesterol in the presence of other lipoprotein particles. The assay is comprised of two distinct phases. In phase one, free cholesterol in non-HDL-lipoproteins is solubilized and consumed by cholesterol oxidase, peroxidase, and TOOS to generate a colorless end product. In phase two a unique detergent selectively solubilizes HDL-lipoproteins. The HDL cholesterol is released for reaction with cholesterol esterase, cholesterol oxidase and a chromogen system to yield a blue color complex which can be measured bichromatically at 546/670nm. The resulting increase in absorbance is directly proportional to the HDL-C concentration in the sample

$$\text{HDL, LDL, VLDL, Chylomicron} \xrightarrow[\text{TOOS + Peroxidase}]{\text{Accelerator + CO}} \text{Colourless End Product}$$

$$\text{HDL} \xrightarrow{\text{HDL Specific Detergent}} \text{HDL disrupted}$$

$$\text{HDL Cholesterol} \xrightarrow[\text{Cholesterol oxidase}]{\text{Cholesterol esterase}} \text{4-Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{TOOS} + \text{4AAP} \xrightarrow{\text{Peroxidase}} \text{Color Development}$$

KIT COMPONENTS

Composition :

R1 - HDL Direct Reagent : Good's Buffer 30 mmol/l, 4-AAP 0.9 mmol/l, POD 2400 U/l, Ascorbate Oxidase 2700 U/l

R2 - HDL Direct Reagent : Goods buffer 30 mmol/l, CHE 4000 U/l, CO 20000 U/l, DAOS 0.8 mmol/l

R3 - HDL Direct Calibrator : Concentration is lot specific, see vial label

R4 - HDL Direct Lipid Control : Concentration range is lot specific, see pack insert/instructions for use.

MATERIALS REQUIRED BUT NOT PROVIDED

Laboratory instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi automated, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/ rack, heating bath, saline.

REAGENT PREPARATION, STORAGE & STABILITY

Reagent 1 and Reagent 2 are ready to use.

Calibrator and Lipid Control should be reconstituted by adding distilled or deionized water mentioned on the vial label. Close the vial and let stand for 5 minutes. Dissolve the contents of the vial by swirling gently avoiding formation of foam. Do not shake.

Stability: unopened reagents, calibrator and lipid control are stable up to expiration date on labels at 2-8 °C. Do not freeze the reagents.

Once opened reagents are stable up to 4 weeks at 2-8 °C.

Once reconstituted, calibrator and lipid control are stable for 2 weeks at 2-8 °C. Reconstituted calibrator and lipid control may be aliquoted and stored for one month at -20 °C.

REAGENT DETERIORATION

Do not use reagents after the expiration date printed on the reagent label. Avoid thawing and freezing of aliquoted calibrator and lipid control.

WARNINGS AND PRECAUTIONS

1. Reagent may contain some non-reactive and preservative components. It is recommended to handle carefully by entitled and professionally educated person, avoiding contact with skin and ingestion. Do not pipette by mouth.

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2. Specimens should be considered infectious and handled appropriately.

3. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Use unhemolysed serum or EDTA plasma. Avoid use of citrate or heparin as anticoagulant. Serum / plasma are stable for 7 days at 2-8 °C or one month at -20°C when frozen once.

Programme Parameter for MERILYZER CliniQuant

Reading Mode	End Point
Calibrator Conc.	(mg/dl) See vial label
Filter - 1 (nm)	546
Filter - 2 (nm)	670
Temperature	37 °C
Volume (µl)	400
Delay Time (Sec)	5
Unit	mg/dl
Reaction Direction	Increase
Reference Low: (Men/Women)	30 / 45
Reference High: (Men/Women)	50/ 60
Linearity	100

TEST PROCEDURE

Dispense	Blank	Calibrator	Sample
Reagent 1	360 µl	360 µl	360 µl
Calibrator	-	5 µl	-
Sample	-	-	5 µl
Mix, incubate for 5 min at 37°C.			
Reagent 2	120 µl	120 µl	120 µl
Mix, incubate for 5 min at 37°C. Read absorbance of calibrator (Ac) & sample (Ax) against reagent blank at 546/670 nm.			

RESULT CALCULATION

Serum/plasma:

HDL Cholesterol mg/dl = Ax/Ac x Concentration of Calibrator

SI conversion factor: 1 mg/dl x 0.0259 = 1 mmol/l

EXPECTED VALUES	Male	Female
Low Risk	> 50 mg/dl	> 60 mg/dl
Moderate Risk	35 - 50 mg/dl	45 - 60 mg/dl
High Risk	< 30 mg/dl	< 45 mg/dl

It is recommended that each laboratory verifies this range or derives reference interval for the population it serves.

QUALITY CONTROL AND CALIBRATION

It is recommended to perform internal quality control with assayed lipid control provided in the kit, to confirm the validity of the test and assure the accuracy of patient result.

Using the Calibrator included, calibrate the assay:

a. When using a new reagent or lot

b. When QC values are out of range

PERFORMANCE CHARACTERISTICS

1. Linearity

The linearity is up to 100 mg/dl.

2. Sensitivity/ Limit of detection (LOD)

The limit of detection is 1.2 mg/dl.

The limit of quantification is 3.5 mg/dl.

3. Interferences

No interference has been observed for the following

Hemoglobin up to 1000 mg/dl; Bilirubin up to 40 mg/dl

Triglyceride up to 1800 mg/dl; Ascorbic acid up to 100 mg/dl

4. Precision

Intra-assay/ Within run precision

n = 20	Mean (mg/dl)	SD (mg/dl)	CV (%)
sample 1	28.39	0.45	1.5
sample 2	56.70	0.42	0.74

Inter-assay/ Run to run precision

n = 20	Mean (mg/dl)	SD (mg/dl)	CV (%)
sample 1	28.40	0.27	0.94
sample 2	59.37	0.67	1.12

5. Methods Comparison

Comparison was done between CliniQuant FSR- HDL Direct Reagent (y) and reference HDL Direct Reagent (x).

N = 77 $y = 0.9546x + 2.12$ $r^2 = 0.9753$

LIMITATIONS

Samples with values above 100 mg/dl should be diluted with D.I water 1:1, re-run and multiply results by 2.

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for correct waste disposal.

REFERENCES

1. Burtis, C.A., Ashwood, E.R., editors. Tietz Textbook of Clinical Chemistry. 2nd ed. Philadelphia, W.B. Saunders Company, 1994, p. 1024 – 1030

2. Data on file: Meril Diagnostics.

IFU/HDLFSR01/00

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Symbols used on Meril Diagnostics labels:

REF	Catalogue No.		Attention See Instruction for Use
LOT	Batch No.	IVD	*In vitro* Diagnostics
	Expiry Date		Consult Instruction for Use
	Manufacturer		Storage Temperature
	Keep Dry		Keep Away from Sunlight
	Manufacturing Date		Do not use if package is damaged
EC REP	Authorized European Representative in the European Community		