

HDL-direct FL

HD F080 CH	4 x 20 ml
HD F245 CH	12 x 20 ml

SUMMARY OF TEST

Blood total cholesterol levels have long been known to be related to coronary heart disease (CHD). In recent years, in addition to total cholesterol, high density lipoprotein cholesterol (HDL-C) has become an important tool used to assess an individual risk of developing CHD since a strong negative relationship between HDL-C concentration and the incidence of CHD was reported. Thus, there has been substantial interest in HDL-C analysis. Selective chemical precipitation techniques are widely used for the determination of HDL-C such as heparin-manganese, dextran-magnesium, and phosphotungstate-magnesium. However, these techniques require physical separation via centrifugation, which is not suited to large scale lab use. The HDL-direct test eliminates the precipitation procedure by employing a specific antibody, and thus, can be applied on automatic analyzers.

PRINCIPLE OF THE METHOD

Anti human 3-lipoprotein antibody in reagent A binds to lipoproteins (LDL, VLDL, and chylomicrons) other than HDL. The antigen-antibody complexes formed block enzyme reactions when reagent B is added. Cholesterol esterase and cholesterol oxidase in reagent B react only with HDL-C. Hydrogen peroxide produced by the enzyme reactions with HDL-C yields a blue color complex upon oxidative condensation of F-DAOS [N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoroaniline, sodium salt] and 4-aminoantipyrine in the presence of peroxidase. By measuring the absorbance of the blue color complex produced, at the optimum wavelength of 593 nm, the HDL-C concentration in the sample can be calculated when compared with the absorbance of the HDL-C Calibrator.

KIT COMPONENTS

For in vitro diagnostic use only.

The components of the kit are stable until expiration date on the label.

Keep away from direct light sources.

Reagent A: F080 3 x 20 ml (liquid) blue cap
F245 9 x 20 ml (liquid) blue cap

Composition: Good's buffer pH 7.0 30 mmol/l, 4-aminoantipyrine 0.9 mmol/l, POD 2400 U/l, ascorbate oxidase 2700 U/l, and anti human-lipoprotein antibody, blend of 5-chloro-2-methyl-2-H-isothiazol-3-one [EC No 247-500-7] and 2-methyl-2-H-isothiazol-3-one [EC No 220-239-6] (3:1) in concentration 0,0015-0,06%

Reagent B: F080 1 x 20 ml (liquid) red cap
F245 3 x 20 ml (liquid) red cap

Composition: Good's buffer pH 7.0 30 mmol/l, cholesterol esterase 4000 U/l, cholesterol oxidase 20000 U/l, and F-DAOS 0.8 mmol/l.

Store all components at 2-8°C.

MATERIAL REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution. HDL/LDL-C calibrator (cod. HD CA12 CH)

REAGENT PREPARATION

Use separate reagents ready to use.

Stability: up to expiration date on labels at 2-8°C.

Stability since first opening of vials: 60 days at 2-8°C.

PRECAUTIONS

Labelling: Xi; R43 May cause sensitization by skin contact
S20 When using do not eat, drink or smoke
S24 Avoid contact with skin
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S36/37/39 Wear suitable protective clothing, gloves, and eye/face protection
S46 If swallowed, seek medical advice immediately and show this container or label
S56 Dispose of this material and its container at hazardous or special waste collection point.

SPECIMEN

Use serum as a specimen. It is recommended to measure HDL-C immediately after collection. Ascorbic acid, bilirubin, and hemoglobin do not have a significant effect on the measurement.

TEST PROCEDURE

Wavelength:	600 nm
Lightpath:	1 cm
Temperature:	37°C
dispense in cuvette reagent A:	360 µl
add sample:	4 µl
mix, incubate at 37°C for 5 minutes.	
dispense in cuvette reagent B:	120 µl
mix, incubate 5 minutes at 37°C. Read absorbances of calibrator (As) and samples (Ax) against reagent blank.	

RESULTS CALCULATION

serum/plasma sample:

HDL-C mg/dl = Ax/As x calibrator value

EXPECTED VALUES

Adult male: 35.3 - 79.5 mg/dl
Adult female: 42.0 - 88.0 mg/dl

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose a reliable and compatible human based control sera is needed.

If required, different calibrators are available:

- a multiparametric, human based calibrator :

AT 0030 CH AUTOCAL H 10 x 3 ml

- a specific calibrator for LDL-C and HDL-C:

HD CA12 CH HDL/LDL-C CALIBRATOR 4 x 3 ml

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity

the method is linear up to 220 mg/dl.

If the limit is exceeded, it is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)

the limit of detection is 1 mg/dl.

Interferences

no interference was observed by the presence of:

hemoglobin ≤ 500 mg/dl
bilirubin (free) ≤ 50 mg/dl
bilirubin(conjugated) ≤ 40 mg/dl
ascorbic acid ≤ 50 mg/dl

Precision

intra assay (n=10)	mean (mg/dl)	SD (mg/dl)	CV%
sample 1	32.1	0.18	0.55
sample 2	88.9	0.61	0.68

Comparison

a comparison between HDL-direct FL and other methods has shown the following results:

HDL-C Chema = x
HDL-C competitor = y
n = 50

$y = 0.96x + 2.5 \text{ mg/dl}$ $r^2 = 0.998$

WASTE DISPOSAL

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

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

REFERENCES








Rifai, N. and Warnick, G.R., Ed. Laboratory Measurement of Lipids, Lipoproteins and Apolipoproteins AACC Press. Washington, DC, USA, 1994.

Burtis, C.A. and Ashwood E.R., Ed. Tietz Textbook of Clinical Chemistry 2 Ed. Saunders, Philadelphia, 1994, Gordon, T., Castelli, W.P., Hjortland, M.C., et al., Am. J. Med. 62,707- 714, (1977).

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SYMBOLS

	for in vitro diagnostic use only
	lot of manufacturing
	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions