HDL-direct FL

HD F080 CH	4 x 20 ml
HD F245 CH	12 x 20 ml
HD F400 CH	4 x 100 ml

INTENDED USE

Reagent for quantitative in vitro determination of HDL-cholesterol in biological fluids.

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.

PRINCIPLE OF THE METHOD

Anti human β-lipoprotein antibody in reagent R1 binds to lipoproteins (LDL, VLDL, and chylomicrons) other than HDL. The antigen-antibody complexes formed block enzyme reactions when reagent R2 is added. Cholesterol esterase and cholesterol oxidase in reagent R2 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.

HDL-C R1	**F080**	**3 x 20 ml (liquid) blue cap**
	F245	**9 x 20 ml (liquid) blue cap**
	F400	**3 x 100 ml (liquid) blue cap**

Composition: Good's buffer pH 7.0 30 mmol/l, 4-amino-antipyrine 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%.

HDL-C R2	**F080**	**1 x 20 ml (liquid) red cap**
	F245	**3 x 20 ml (liquid) red cap**
	F400	**1 x 100 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 micro-pipettes. Glass or high quality polystyrene cuvettes. Saline solution.

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

HDL-C R1: Warning. May cause an allergic skin reaction (H317). Avoid breathing vapours (P261). IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water (P303+P361+P353). If skin irritation or rash occurs: Get medical advice (P333+P313).

HDL-C R2: It is not classified as hazardous.

N-acetylcysteine (NAC), metamizole and acetaminophen may cause interference in the Trinder reaction.[1,2]
To avoid interference, the blood withdrawal should be performed before drug administration.

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

Wavelenght:	600 nm
Ligthpath:	1 cm
Temperature:	37°C

dispense in cuvette reagent R1:	360 µl
add sample:	4 µl
mix, incubate at 37°C for 5 minutes.	
dispense in cuvette reagent R2:	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 the following human based control sera are available:
QUANTINORM CHEMA
with normal or close to normal control values
QUANTIPATH CHEMA
with pathological control values.
If required, a multiparametric, human based calibrator is available:
AUTOCAL H

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

$y = 0.96x + 2.5$ mg/dl $r^2 = 0.998$

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

1) N-acetylcysteine interference of Trinder-based assays. Genzen JR, Hunsaker JJ, Nelson LS, Faine BA, Krasowski MD. Clin Biochem. 2016 Jan;49(1-2):100-4
2) Drug interference in Trinder reaction. Wiewiorka O, Čermáková Z, Dastych M. Euromedlab 2017. ISSN 1437-4431
3) Rifai, N., Warnick, G.R. Ed. Laboratory Measurement of Lipids, Lipoproteins and Apolipoproteins AACC Press. Washington, DC, USA, 1994
4) Burtis, C. A and Ashwood, E. R., Ed. Tietz Textbook of Clinical Chemistry, 2nd Ed., Saunders, Philadelphia, 1994.
5) Gordon, T., Castelli, W.P., Hjortland, M.C., et al., Am. J. Med 62,707 - 714, (1977)

MANUFACTURER

Chema Diagnostica
Via Campania 2/4
60030 Monsano (AN) - ITALY - EU
phone +39 0731 605064
fax +39 0731 605672
e-mail: mail@chema.com
website: http://www.chema.com

SYMBOLS

IVD	*in vitro* diagnostic medical device
LOT	batch code
REF	catalogue number
	temperature limit
	use by date
	caution
	consult instructions for use

CE