HDL-CHOLESTEROL

For discrete analyzers
Method: Immonosuppression
Product Code: 1418-0247
Packaging: 4 x 27 ml (R1) + 4 x 9 ml (R2)
Store at 2 – 8°C
For *in vitro* use

INTENDED USE
MEDICON Cholesterol is a reagent for the enzymatic determination of Cholesterol in human serum and plasma, with BECKMAN COULTER AU400/600/600-IVD/640/2700/5400 and other discrete analyzers. For *in vitro* diagnostic use only.

CLINICAL SIGNIFICANCE
HDL-Cholesterol plays a major role in the transportation of cholesterol from the points of storage to the tissues towards the liver, from where it's discarded as cholic acids. Reduced HDL levels suggest liver and intestinal failure—since HDL is produced there—as well as increased risk of atherosclerosis and cardiovascular disease.

METHOD PRINCIPLE
Cholesterol is determined according to the following reaction scheme:

LDL, VDL, chylomicrons $\xrightarrow[\text{antibody}]{\text{Anti-human-β-Lipoprotein}}$ antigen-antibody complex

HDL-cholesterol + H_2O + O_2 $\xrightarrow{\text{CHE, CHOD}}$ Cholist-4-en-3-one + Fatty acids + H_2O_2

H_2O_2 + 4-AA + F-DAOS $\xrightarrow{\text{POD}}$ Blue colouring + 2 H_2O

CHE: cholesterol esterase
CHOD: cholesterol oxidase
POD: peroxidase

Cholesterol esters are hydrolysed by cholesterol esterase and produce cholesterol. Cholesterol is oxidized by cholesterol oxidase producing Hydrogen Peroxide. Under the catalytic action of Peroxidase, Hydrogen Peroxide reacts with 4-Aminoantipyrine and Phenol and produces a red colored Quinoneimine dye. The increase of absorbance at 500 nm is proportional to the concentration of cholesterol in the sample.

METHOD LIMITATIONS
Refer to the book "Effects of Preanalytical Variables on Clinical Laboratory Tests" for possible interference of other pharmaceutical agents in this particular test. Interference of other agents is described in the "Clinical Guide to Laboratory Tests".
The reagent is designed for in vitro diagnostic use only. In vitro diagnostic reagents can be hazardous. They should be handled according to good laboratory practices and techniques. Avoid inhalation and contact with eyes and skin.

REAGENT COMPOSITION
For *in vitro* diagnostic use only.

Components	Final concentration
Anti-human-β-Lipoprotein antibody	
Cholesterol Esterase	0.8 IU/mL
Cholesterol Oxidase	4.4 IU/mL
Peroxidase	1.7 IU/mL
Ascorbate Oxidase	2.0 IU/mL
Good's buffer pH=7.0	30 mM
N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3.5-dimethoxy-4-fluoroaniline (F-DAOS) :	0.20 mM
4-aminoantipyrine	0.67 mM
Preservatives	

WARNINGS – PRECAUTIONS
- The reagent is designed for in vitro diagnostic use only. In vitro diagnostic reagents can be hazardous. They should be handled according to good laboratories practices and techniques. Avoid inhalation and contact with eyes, skin and mucous membranes
- Samples should be considered as potentially infectious. Handle according to the universal precautions and good laboratory practices.
- The reagent contains sodium azide (NaN_3 < 0.1%). Avoid swallowing and contact of the reagent with skin and mucous membrane.
- Dispose all waste according to national laws.
- MSDS is available by MEDICON upon request.

PREPARATION
Reagents are liquid, ready to use, and placed at their corresponding places on the analyzer. Vials bear barcode for recognition by BECKMAN COULTER AU Series analyzers.

REAGENT DETERIORATION
The reagent should not be used:
- When it does not exhibit the specified linearity or recovery of control values lies outside the acceptable range after recalibration.
- After prolonged exposure to sunlight or high temperatures.
- When reagent 1 (R1) has been accidentally frozen.

SHELF LIFE
Unopened, the reagent is stable up to the stated expiry date when stored at 2 – 8°C. Once opened, the reagent remains stable for 1 month if stored refrigerated on the instrument at 2 – 8°C.

SAMPLE
Use serum, EDTA (1 mg/dL) or heparinised-plasma as specimen. Patient must fast for 12 hours prior to sampling. When possible, separation of HDL must be done on the same day as the sampling. HDL is stable for 4 days at 4 – 6°C. Statistically but not clinically, there is a significant reduction of HDL when assay takes place after the sample has remained at –20°C for 7 – 14 days.

CALIBRATION
MEDICON HDL Calibrator (1578-0242) or the MEDICON MEDI-CAL Calibrator (1578-0891), or BECKMAN COULTER HDL Calibrator Cat. No. ODC0011 (MEDICON code: 4478-0242) can be used for calibration. Calibrate the assay every 1 month. Recalibrate following preventive maintenance or replacement of a critical part of the analyzer, when using a new reagent kit or a new reagent lot number, or when Quality Control results are out of range.

QUALITY CONTROL
MEDICON provides the following products for quality control: Clinical Chemistry Control levels 1 and 2 (1578-0901-12, 1578-0902-12), or the BECKMAN COULTER HDL/LDL-Cholesterol Control Serum ODC0005 (MEDICON code: 4478-0249), or the BECKMAN COULTER CONTROL SERUM ODC0003 and ODC0004 (MEDICON code: 4478-0941 and 4478-0942, respectively).
Control values and limits should fall within acceptable intervals adjusted to the requirements of each laboratory. Target values should be verified with the corresponding working protocol. Results outside the specified values even after recalibration could be due to reagent deterioration, instrument malfunction or error during test procedure.

MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT
Cholesterol calibrator
Quality control material
Automated biochemistry analyzer
Common laboratory equipment

REFERENCE INTERVALS
Increased risk factor: < 35 mg/dL
Negative risk factor: > 60 mg/dL
Each laboratory should determine its own expected values as dictated by good laboratory practice.

WASTE DISPOSAL
This product contains sodium azide (NaN_3), which forms sensitive explosive compounds with copper or lead. Flush waste pipes with water after the disposal of undiluted reagent in order to avoid azide build up in the drain pipes.

SPECIFIC PERFORMANCE CHARACTERISTICS
Linearity
The assay is linear within measuring range 0 – 180 mg/dL. When values exceed this range samples should be diluted accordingly.
Sensitivity
The lowest detectable level of Cholesterol is estimated at 0.00 mg/dL.
The lowest detection limit (LDL) is defined as the lowest concentration of analyte that is distinguishable from zero. A sample free of analyte is
assayed 20 times within the assay and the LDL is calculated as the absolute mean plus three standard deviations.
Precision

Level (mg/dL)	Within run %CV	Total %CV
34	1.06	2.11
136	0.88	1.66

Precision is estimated on two concentration levels of analyte according to NCCLS protocol EP-5T (20 consecutive days, 2 runs per day, 2 repeats per run).
Interference
Lipemic: insignificant up to 1000 mg/dL intralipid®
Hemoglobin: insignificant up to 500 mg/dL
Non Conj. Bilirubin: insignificant up to 20 mg/dL
Conj. Bilirubin: insignificant up to 20 mg/dL
Ascorbic acid: insignificant up to 3 mg/dL
Refer to Young[4] for further information on interfering substances
Method Comparison
A comparison was performed between this reagent and another commercially available product. The results were as follows on a BECKMAN COULTER AU series analyzer:
Y = 0.9858X + 1.0335 R=0.9982 N=44 Sample range: 15.80 – 99.40 mg/dL

BIBLIOGRAPHY

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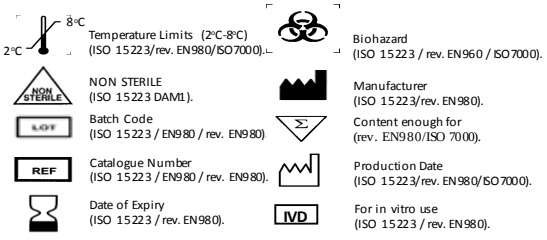
2. Burtis, CA and Ashwood, ER, ed. Tietz Textbook of Clinical Chemistry. 2nd. ed. Philadelphia: W.B. Saunders Company Ltd., 1994.
3. Jacobs, DJ, Demott, WR, Grady, HJ, Horvat, RJ, Huestis, DW and Kasten, BL, JR, eds. Laboratory Test Handbook. 4th. ed. Ohio, Hudson: Lexi-Comp Inc., 1996.
4. Young DS. Effects of Preanalytical Variables on Clinical Laboratory Tests. 2nd. ed. Washington: The American Association for Clinical Chemistry Press, 1997.
5. Warnick, G.R., et al, National Cholesterol Education Program, recommendations for Measurement of HDL-Cholesterol: Executive Summary Clin. Chem. 1995; 41: 1427-1433

SYMBOLS


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