EON 100 HDL CHOLESTEROL REAGENT

REF **77301** ☛*In vitro* diagnostic reagent, for professional use only

☛**CAUTION: Federal Law restricts this device to sale by or on the order of a licensed healthcare practitioner (Rx ONLY).**

INTENDED USE

EON 100 HDL CHOLESTEROL Reagent is for the quantitative determination of high density lipoprotein (HDL) in serum and plasma using the EON 100 Analyzer.

CLINICAL SIGNIFICANCE [1,2]

The principle role of high density lipoproteins (HDL) in lipid metabolism is the uptake and transport of cholesterol from the peripheral tissues to the liver through a process known as reverse cholesterol transport, which is a proposed cardio-protective mechanism. Low HDL-cholesterol levels are associated with an increased risk of coronary heart disease and coronary artery disease. Consequently the determination of serum HDL-cholesterol is a useful tool for identifying high risk patients. HDL-cholesterol results may also be indicative of various lipid disorders such as diabetes mellitus and other liver and renal diseases.

METHODOLOGY

HDL cholesterol is measured using a two step process.

Step 1:

HDL, LDL, VLDL, Chylomicrons $\xrightarrow{\text{Accelerator + CO + POD + DSBmT}}$ Non Reactive LDL, VLDL, Chylomicrons

Step 2:

HDL-Cholesterol $\xrightarrow{\text{HDL Specific Detergent}}$ HDL Disrupted

HDL-Cholesterol + O_2 $\xrightarrow{\text{CO + CE}}$ Cholest-4-ene-3-one + H_2O_2

2 H_2O_2 + 4-Aminoantipyrine + DSBmT $\xrightarrow{\text{POD}}$ Colored End Product

Non HDL-esterified and free cholesterol are consumed by cholesterol oxidase, peroxidase and DSBmT in step 1 yielding colorless products. HDL cholesterol is unaffected. Reagent 2 contains cholesterol esterase, a chromogenic coupler, and a detergent capable of selectively solubili zing the HDL cholesterol. In step 2, this reagent is added and the HDL cholesterol reacts to produce a chromogen that absorbs at 578 nm. The change in absorbance is proportional to the concentration of HDL cholesterol in the sample.
This reaction scheme may be referred to as the Accelerator Selective Detergent methodology.

REAGENTS

COMPOSITION

EON 100 HDL CHOLESTEROL Reagent 1 contains < 1,000 U/L Cholesterol oxidase (E. coli), < 1,300 ppg U/L Peroxidase (horseradish), < 1 mmol/L Disodium N, N-bis (4-sulfobutyl)-*m*-toluidine, < 1 mmol/L Accelerator, < 0.06 % Preservative, < 3,000 U/L Ascorbate oxidase (Curcubita), buffer, and other ingredients.

EON 100 HDL CHOLESTEROL Reagent 2 contains < 1,500 U/L Cholesterol esterase (Pseudomonas sp.), < 1 mmol/L 4-aminoantipyrine, < 2% Detergent, < 0.06% Preservative, buffer, and other ingredients.

☛WARNINGS AND PRECAUTIONS[3]

- The reagent kit is for professional *in vitro* diagnostic use only.
- Take normal precautions and adhere to good laboratory practice.
- Use clean or single use laboratory equipment only to avoid contamination.
- For more information, Safety Data Sheet (SDS) is available on request for the professional user.
- Handle and dispose of all human source materials as though capable of transmitting infectious agents using the universal precautions recommended by the Centers for Disease Control and Prevention (CDC). Do not pipette by mouth; do not eat, drink, smoke or apply cosmetics in areas where specimens are handled. Clean up spills immediately with a 0.5% sodium hypochlorite solution.
- Dispose of in accordance wih all local, state and federal regulations.

PREPARATION

EON 100 HDL CHOLESTEROL Reagent 1 and Reagent 2 are ready for use on the EON 100 Analyzer as packaged.

STORAGE AND STABILITY

Store the reagents at 2 to 8 °C and protected from light. Do not freeze. Unopened reagents are stable to the expiration dates on the bottle labels. The reagents are stable for 14 days onboard the EON100 Analyzer.

☛SPECIMENS[4]

COLLECTION AND STORAGE

- Fasting serum is the preferred specimen. Fasting heparinized plasma is also acceptable. Do not analyze whole blood. Collect specimens by venipuncture according to accepted clinical protocol. Patients should maintain their usual diet for at least two weeks before blood collection. Blood should be drawn after a 12 hour fast and after the subject has been sitting quietly for at least 5 minutes. Separate the serum or plasma sample from the cells within three hours of collection. For best results, HDL should be analyzed on the day of collection.
- Venipuncture should be performed prior to the administration of drugs. Of particular note, venipuncture performed during an acetaminophen overdose situation, when N-acetyl-p-benzoquinone imine (NAPQI) an atypical metabolic breakdown product of acetaminophen, may be present, may lead to erroneously low HDL Cholesterol results. Venipuncture performed during or immediately after administration of N-acetylcysteine (NAC), a drug used to treat acetaminophen overdose, or Metamizole may lead to erroneously low HDL cholesterol results.
- HDL cholesterol in serum and plasma is stable for up to two days at 2 to 8 °C, one month at -20 °C, or 2 years at -70 °C. Once thawed, the specimen may not be refrozen.

PROCEDURE

MATERIALS PROVIDED

The Eon100 HDL CHOLESTEROL Reagent Kit includes the following components:

4 x 11 mL bottles of EON 100 HDL CHOLESTEROL Reagent 1
4 x 3.7 mL bottles of EON 100 HDL CHOLESTEROL Reagent 2

MATERIALS REQUIRED BUT NOT PROVIDED

- EON HDL SERUM CALIBRATOR (REF. 77119 or formely sold under REF. 1419)
- EON SERUM CONTROL (REF. 77131 or formely sold under REF. 1421).

REAGENT INSTALLATION AND USE

Refer to the User Manual for additional information on installing reagents, programming the analyzer and running samples, calibrators and controls.

The EON 100 HDL CHOLESTEROL Reagent kit is ready to use as packaged.

Do not remove the caps from the bottles until you are ready to install the reagent on the analyzer. Before installing, mix the reagents by gently inverting the reagent bottles several times. Record the installation date on the label and insert the unit into the designated position on the reagent tray. Let the reagent equilibrate on the instrument for at least 30 minutes before use.

.../...

ELITech Clinical Systems SAS
Zone Industrielle
61500 SEES
France

For Technical questions, Please call or contact
(855) 354-8324 - www.elitechgroup.com
27 Wellington Road
Lincoln, Rhode Island 02865 - U.S.A.

(03/2016)
FTEON100-HDLL-v3

CALIBRATION

Calibrate the instrument after loading a new reagent lot, after maintenance and whenever quality control results fall outside established limits.

Under typical use conditions, calibration factors for this test are valid for 7 days. Refer to the User Manual for calibration procedures.

QUALITY CONTROL

Quality control requirements should be established in accordance with local, state and/or federal regulations or accreditation requirements.

Assay at least two levels of serum control daily. Serum Controls for EON 100 level 1 and level 2 may be used. Controls should also be assayed after calibrating, maintaining the instrument and after loading a new reagent unit.

Controls may be assayed more frequently based on laboratory workflow and the discretion of the user.

CALCULATIONS

All calculations are performed by the instrument.

To calculate the result in SI units (mmol/L), multiply the result in conventional units (mg/dL) by 0.0259.

PERFORMANCE CHARACTERISTICS

MEASURING RANGE

The measuring range of the assay defines the acceptable upper and lower limits of the range within which values may be reported. It is comprised of the Limit of Detection (lower limit) and the highest measured point that will be achieved throughout the lifespan of the reagent (upper limit).

Range	Conventional Units	SI Units
Serum/Plasma	3 to 150 mg/dL	0.08 to 3.9 mmol/L

LIMIT OF BLANK (LOB) AND LIMIT OF DETECTION (LOD)

Determined according to CLSI[5] EP17-A protocol

LoB = 0.7 mg/dL (0.02 mmol/L)

LoD = 3 mg/dL (0.08 mmol/L)

☛EXPECTED VALUES [6]

The NCEP (American National Cholesterol Education Program) has established the following classification for HDL cholesterol levels according to the risk of developing coronary heart disease:

Risk Classification	Level (mg/dL)	Level (mmol/L)
High risk	< 40	< 1.03
Low risk	≥ 60	≥ 1.55

Note : The quoted range should serve as a guide only. It is recommended that each laboratory verifies this range or establishes a reference interval for the intented population.

PRECISION

Determined according to CLSI[7] EP5-A2 protocol.Two levels of serum were analyzed in duplicate, two times per day, over a period of ten days (20 runs).

Precision of HDL Cholesterol in mg/dL

			Within Run		Total	
Sample	n	mean	1SD	%CV	SD	%CV
Level 1	40	63.8	1.9	3.0%	5.1	8.1%
Level 2	40	35.1	1.9	5.4%	2.8	8.0%

METHOD COMPARISON

A comparison of EON 100 HDL CHOLESTEROL assay (y) with a commercially available method (x) was performed based on CLSI EP9-A2[8] guidelines. A total of 42 human serum samples were assayed in the range from 12 to 136 mg/dL (0.31 – 3.54 mmol/L).

The following statistics were obtained:

Linear Regression	Deming Regression
y = 1.036x - 3.496 mg/dL	y = 1.040x - 3.768 mg/dL
R = 0.9969	R = 0.9969

☛LIMITATIONS / INTERFERING SUBSTANCES

- Do not report results outside of the usable range.

- Studies to determine the level of interference from biological compounds that may be normally present in serum or plasma were carried out on the EON 100.

No significant interference (± 10%) in HDL cholesterol recovery was observed in the presence of:

Lipemia (Intralipid® measured as Triglycerides):	No significant interference up to 2000 mg/dL
Bilirubin:	No significant interference up to 60 mg/dL (1026 µmol/L).
Hemoglobin:	No significant interference up to 1000 mg/dL (10 g/L).
Ascorbic Acid:	No significant interference up to 20 mg/dL (1.14 mmol/L).

- Results may be falsely low when the sample is taken while levels of NAC, NAPQI (a metabolite of acetaminophen (paracetamol)) or Metamizole are significant.

- Many other substances can affect HDL cholesterol results. For additional information, refer to Effects of Drugs on Clinical Laboratory Tests[9] and Effects of Preanalytical Variables on Clinical Laboratory Tests[10].

- The results of this assay should only be interpreted in conjunction with other diagnostic test results, clinical findings and the patient's medical history.

REFERENCES

1. Badimon J J, et al. *Regression of Atherosclerotic Lesions by High Density Lipoprotein Plasma Fraction in the Cholesterol-Fed Rabbit.* Journal of Clinical Investigation 1990 85:1234-41.
2. Burtis C A, Ashwood E R, Eds. *Tietz Textbook of Clinical Chemistry, Third Edition* W.B. Saunders Company: Philadelphia, PA, 1999.
3. *U. S. Department of Health and Human Services: Recommendation for Prevention of HIV Transmission in Health Care Settings.* MMW Report, Aug 21, 1987, Vol. 36, No. 2S.
4. Warnick G, et al., *National Cholesterol Education Program Recommendations for Measurement of High Density Lipoprotein Cholesterol; Executive Summary* Clin Chem, 41 10:1427 1995.
5. *Protocols for Determination of Limits of Detection and Limits of Quantification; Approved Guideline*. CLSI (NCCLS) document EP17-A (2004), **24** (34).
6. National Institutes of Health, National Cholesterol Education Program. *Detection Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), Final Report.* NIH Publication No. 02-5215, September 2002.
7. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline*—Second Edition. CLSI (NCCLS) document EP5-A2 (2004), **24** (25)
8. *Method Comparison and Bias estimation Using Patient Samples; Approved Guideline*—Second Edition. CLSI (NCCLS) document EP9-A2 (2002), **22** (19).
9. Young DS. *Effects of drugs on clinical laboratory tests. 5th Ed. Washington DC: AACC press; 2000.*
10. Young DS. *Effects of preanalytical variables on clinical laboratory tests. 3rd Ed. Washington DC: AACC press; 2007.*

GLOSSARY OF SYMBOLS

CONT	Contents		Manufacturer	REF	Catalog No.
LOT	Batch Code		See instruction for use		Use by
OPENED	Date opened	IVD	*In vitro* diagnostic device		Temperature Limitation
STAB DAYS	Number of days onboard stability	R1	Reagent 1	R2	Reagent 2

☛ : Modification from previous version

ELITech Clinical Systems SAS
Zone Industrielle
61500 SEES
France

For Technical questions, Please call or contact
(855) 354-8324 - www.elitechgroup.com
27 Wellington Road
Lincoln, Rhode Island 02865 - U.S.A.

(03/2016)
FTEON100-HDLL-v3