ENVOY® 500 HDL CHOLESTEROL REAGENT KIT

APPLICATION PARAMETERS

PRIMARY PARAMETERS

Code	HDL
Bar-Code	Active
Code for Bar-Code	310
Test Methodology	Selective
Method	Sample Blank (A)
Kind of Process	Linear
1st Filter	578
2nd Filter	700
Reaction direction	Increasing

REAGENTS

Number of reagents	2
Reagent 1 Volume µL	300
Concentrated	Inactive
Reagent 2 Volume µL	100
Concentrated	Inactive

SAMPLE

	Serum	Urine
Name	HDL Cholesterol	
Sample µL	4	N/A
Pre-Dilution 1:	1	N/A
Post-Dilution 1:	1	N/A

TIMES

Sample Starter	Inactive
Delay Time	0
Reading Time	60
Reagent 1 Incubation Time	300
Reagent 2 Incubation Time	240

CHECK PARAMETERS

Reagent Limit (mABS)	200
Curve Acceptance (%)	100

RE-RUN SERUM

Test Limit (Conc)		150
Low Test Limit (Conc)		1.1
Initial ABS (mABS)		N/A
Final ABS (mABS)		N/A
Max ABS Delta (mABS)		N/A
Prozone Check		Inactive
Normal Range	Min	Max
Man	[User defined]	
Woman	[User defined]	
Child	[User defined]	
Re-run hyperactive		Inactive
Re-run pathological		Inactive

RE-RUN URINE

Test Limit (Conc)		N/A
Low Test Limit (Conc)		N/A
Initial ABS (mABS)		N/A
Final ABS (mABS)		N/A
Max ABS Delta (mABS)		N/A
Prozone Check		Inactive
Normal Range	Min	Max
Man		N/A
Woman		N/A
Child		N/A
Re-run hyperactive		Inactive
Re-run pathological		Inactive

SECONDARY PARAMETERS

1st Unit Serum	mg/dL
2nd Unit Serum	Inactive
1st Unit Urine	N/A
2nd Unit Urine	Inactive
Dynamic Blank	Active
Needle washes	[From Settings Table]
Cuvette washes	[From Settings Table]
Special wash	[From Settings Table]
Instrumental Factor	1.000
Shift	0.000
Reagent Blank	Every Day
Decimals	0

STANDARD PARAMETERS

Factor	[Determined by calibration]
Minimum	270
Maximum	900
No. of Samples	1
Max Var. (%)	10
Timed re-run	Inactive
N. replicates	3
Reagents ABS	[Determined by Envoy]
Pos.	[From Settings Table]
Conc.	[From calibrator label]
ABS	[Determined by Envoy]
% last calibration	100

PROGRAMMING INSTRUCTIONS

Detailed instructions for programming reagent parameters are provided in the Envoy 500 Operator manual and Envoy 500 Settings Table.

If the Envoy 500 Chemistry System is not pre-programmed, a HDL cholesterol code must first be added before the parameters can be entered. On the menu bar, select «Test → Test Directory.» A new window will open up listing all the codes for the tests that are installed on the instrument. Click on the «New Code» button, type «HDL» into the Code field and select «Save.»

To program the application parameters, check the box next to the code for the HDL test, and select the «Parameters» button located at the bottom of the window. To program standard information, click the «Standards» button located at the bottom of the window.

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ENVOY® 500 HDL CHOLESTEROL REAGENT KIT

Product no. 55301

For *in vitro* diagnostic use

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

☛INTENDED USE

Envoy® 500 HDL Cholesterol Reagent is for the quantitative determination of high density lipoprotein (HDL) cholesterol in serum and plasma on Envoy 500 Series Analyzers.

SUMMARY

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.[1] 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.[2]

HDL cholesterol is measured using a two step process.

Step 1:
HDL, LDL, VLDL, Chylomicrons —(Accelerator + CO + POD + DSBmT)→ Non Reactive LDL, VLDL, Chylomicrons

Step 2:

HDL-Cholesterol —(HDL Specific Detergent)→ HDL Disrupted

HDL-Cholesterol + O_2 —(CO + CE)→ Cholest-4-ene-3-one + H_2O_2

2 H_2O_2 + 4-Aminoantipyrine + DSBmT —(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 solubilizing 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

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. 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

- This reagent 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 contaminations.
- Dispose of contents in accordance with all local, state and federal regulations.
- For more information, Safety Data Sheet (SDS) is available on request for professional user.

PREPARATION

Both HDL Cholesterol Reagent 1 and HDL Cholesterol Reagent 2 are ready for use as packaged.

STORAGE AND STABILITY

Store these reagents at 2 to 8 °C. Do not freeze. Unopened reagents are stable to the expiration dates on the bottle labels.

Open reagents are stable for 28 days onboard the Envoy 500 Chemistry System.

☛SPECIMENS

SERUM AND PLASMA 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.[3]
- 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.
- For best results, HDL should be analyzed on the day of collection. 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.[3]

COMPATIBLE ADDITIVES

Acceptable chemical preservatives are lithium and sodium heparin. Do not use anticoagulants containing citrate or any other chemical additives.

PROCEDURE

☛MATERIALS PROVIDED

The Envoy 500 HDL Cholesterol Reagent Kit includes the following components:

4 x 30.4 mL boats of Envoy 500 HDL Cholesterol Reagent 1
4 x 11.4 mL bottles of Envoy 500 HDL Cholesterol Reagent 2

MATERIALS REQUIRED BUT NOT PROVIDED

Envoy 500 HDL Calibrator (product no. 55119)
Envoy 500 Serum Controls (product no. 55131)
Normal saline (0.85% saline)

ASSAY PROCEDURE

Program the instrument using the application parameters and programming instructions provided at the end of this Instructions For Use.

REAGENT INSTALLATION AND USE

The Envoy 500 HDL Cholesterol Reagent is ready to use as packaged.

Snap the small reagent bottle onto the reagent boat if it has become dislodged during shipping. Mix the reagents by gently inverting the assembled boat several times.

Record the installation date on the label and insert the assembled boat into the designated position on the reagent tray.

Let the reagent equilibrate on the instrument for at least 30 minutes before use.

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

CALIBRATION

Calibrate the instrument after loading new reagent, 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 operator 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 at least daily. Control materials may be of human or animal origin, but should represent both clinically normal and elevated levels of high density lipoprotein cholesterol. Controls should also be assayed after maintaining the instrument, loading a new reagent, and calibrating the analyzer.

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.

☛LIMITATIONS / INTERFERING SUBSTANCES

- This method has not been certified by the Cholesterol Reference Method Laboratory Network.

- Do not report results outside of the usable range.

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

- Lipemia may interfere with this test.

- Effects of icterus, hemolysis, and lipemia are estimated through the assay of pools spiked with ditaurobilirubin, red blood cell hemolysate and Intralipid® 20% solution. The effect of ascorbic acid was also tested. Observed biases are shown below. Substances that affect results by more than both

3 mg/dL and 4% are reported as interfering substances in the Specimens section.

Effects of Common Substances on HDL Cholesterol Recoveries

Interferant	Concentration	Changes in Recoveries
Ascorbic Acid	3.0 mg/dL	-0.3 at 57 mg/dL†
Ditaurobilirubin	40 mg/dL*	-0.7 at 57 mg/dL‡
RBC hemolysate	200 mg/dL*	-0.8 at 56 mg/dL‡
Intralipid 20% solution	240 mg/dL*	-3.3 at 52 mg/dL
	400 mg/dL*	-5.4 at 52 mg/dL
	800 mg/dL*	-1.5 at 54 mg/dL
	2000 mg/dL*	+2.5 at 54 mg/dL

* Refers to bilirubin, hemoglobin, and/or triglyceride concentration
† Effect is not statistically significant at α = 0.05.
‡ The observed effect is less than 3 mg/dL. This substance is not reported as an interfering substance.

- 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 high density lipoprotein cholesterol results. For additional information, refer to *Effects of Drugs on Clinical Laboratory Tests*[7] and *Effects of Preanalytical Variables on Clinical Laboratory Tests.*[8]

PERFORMANCE CHARACTERISTICS

USABLE RANGE

The linear range of this assay is listed below. Specimens that exceed the upper limit of this range should be diluted with normal saline and reanalyzed. Multiply the results of diluted specimens by the appropriate dilution factors.

Conventional Units	SI Units
5 to 150 mg/dL	0.13 to 3.89 mmol/L

EXPECTED VALUES

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 [4]:

Risk Classification	Conventional Units	SI Units
High risk	< 40 mg/dL	< 1.03 mmol/L
Low risk	≥ 60 mg/dL	≥ 1.55 mmol/L

LIMIT OF DETECTION

The limit of detection (LoD) for HDL cholesterol is 0.46 mg/dL, which was determined based on the NCCLS protocol EP17-A[5] with proportions of false positives (α) less than 5% and false negatives (β) less than 5%. This LoD is based on 80 determinations, with 40 blank and 40 low level samples, and LoB = 0.29 mg/dL.

ANALYTICAL SENSITIVITY

An absorbance change of 0.003 A on the Envoy 500 Chemistry System corresponds to a change in HDL cholesterol concentration of approximately 1 mg/dL (0.03 mmol/L).

METHOD COMPARISON

One hundred and sixty serum and 152 plasma specimens were collected from individual adult patients and assayed for high density lipoprotein cholesterol using an Envoy 500 Chemistry System and another commercially available method. Results were compared by least squares and Passing - Bablok regression and the following statistics were obtained.

Serum/Plasma Comparison

n = 312 range = 5 to 158 mg/dL

Least Squares Regression

Envoy 500 = 0.7 mg/dL + 1.021 x Competitive Method

$s_{(y.x)}$ = 2.4 mg/dL r = 0.995

Passing - Bablok Regression

Envoy 500 = 0.7 mg/dL + 1.015 x Competitive Method

PRECISION

Two lipid controls were each assayed in triplicate twice per day over 8 days on an Envoy 500 Chemistry System. Estimates of within run and total imprecision are calculated analogous to the methods described in NCCLS publication EP3-T.[6]

Precision of HDL Cholesterol Recoveries in mg/dL

			Within Run		Total	
Sample	n	mean	1SD	%CV	1SD	%CV
Level 1	45	36.8	0.52	1.4%	0.72	2.0%
Level 2	48	71.1	0.68	1.0%	1.25	1.8%

☛REFERENCES

1. Badiman 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. Warnick G, et al., *National Cholesterol Education Program Recommendations for Measurement of High Density Lipoprotein Cholesterol; Executive Summary* Clin Chem, 41 10:1427 1995.
4. 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.
5. *Protocols for the Determination of Limits of Detection and Limits of Quantitation; Approved Guideline*. NCCLS Document EP17-A. NCCLS, Wayne PA, 2004.
6. *Tentative Guidelines for Manufacturers for Establishing Performance Claims for Clinical Chemical Methods, Replication Experiment* NCCLS Publication: Vol. 2 No. 20. Villanova, PA, 1982.
7. Young D S, *Effects of Drugs on Clinical Laboratory Tests: Fifth Edition* AACC Press: Washington, DC, 2000.
8. Young D S, *Effects of Preanalytical Variables on Clinical Laboratory Tests: Second Edition* AACC Press: Washington, DC, 1997.

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☛GLOSSARY OF SYMBOLS

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

☛: Modification from previous version

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