theranos	LDT Validation Report	Theranos HDL Assay	Rev:
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Description	Validation Report for Modified Siemens Assay of High Density Lipoprotein (HDL) in Lithium Heparin Plasma		
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Validation of Modified Siemens High Density Lipoprotein (HDL) Assay

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High Density Lipoprotein Plasma Assay

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I. Overview

High density lipoprotein (HDL) is the smallest of the lipoprotein particles and comprises a complex family of lipoprotein particles that exist in a constant state of dynamic flux as the particles interact with other HDL particles and with low density lipoprotein (LDL) particles and very low density lipoprotein (VLDL) particles. HDL has the largest proportion of protein relative to lipid, compared to other lipoproteins (>50% protein). Total cholesterol levels have long been known to be related to coronary heart disease. HDL cholesterol is also an important tool used to assess an individual's risk of developing coronary heart disease, since a strong negative relationship between HDL cholesterol concentration and the incidence of coronary heart disease has been reported. In some individuals, exercise increases the HDL cholesterol level; those with more physical activity tend to have higher HDL cholesterol values.

II. Method Principle

The method consists of 2 distinct reaction steps:

1. Elimination of chylomicrons, VLDL-Cholesterol, and LDL-Cholesterol by cholesterol esterase and cholesterol oxidase.

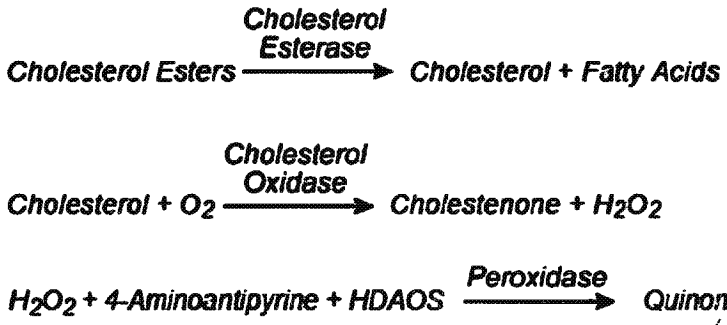
   The peroxides produced by the oxidase is removed by catalase.

$$\text{Cholesterol Esters} \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol + Fatty Acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholestenone} + H_2O_2$$

2. Specific measurement of HDL-Cholesterol after release of HDL-Cholesterol by surfactant in Reagent 2.

   Catalase from step 1 is inhibited by sodium azide in reagent 2. The intensity of the quinoneimine dye produced in the Trinder reaction is directly proporational to the HDL cholesterol concentration when measured at 596/694 nm.



Where HDAOS = N-(2-hydroxy-2-sulfopropyl)-3,5-dimethoxyaniline.

III. Definitions and Abbreviations

The following definitions and abbreviations are used in this document and related documents and attachments:

a. **Accuracy:** Accuracy is defined by CLSI as the closeness of agreement between a test result and an accepted reference value. Method accuracy is used in a different sense by the American Association of Pharmaceutical Scientists where it is expressed as percent relative error (%RE). Trueness, a related CLSI term, is the closeness of agreement between the average of a number of replicate measured quantity values and a reference quantity value.

b. **Analyte:** Component represented in the name of a measurable quantity. The closely related term measurand is defined as the particular quantity subject to measurement.

c. **Analytical sensitivity:** There are several alternative uses of this term. Most commonly, and for the purposes of this Validation Plan, it is used interchangeably with limit of detection. It is also used to describe the ability of an analytical method to assess small variations of the concentration of an analyte, such as the slope of the calibration curve (IUPAC).

d. **Analytical specificity:** Ability of a test or procedure to correctly identify or quantify an entity, including in the presence of interfering substance(s) or phenomena.

e. **Calibration:** Set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards. Under CLIA, calibration refers to the process of testing and adjusting an instrument, kit, or test system, to provide a known relationship between the measurement response and the value of the substance being measured by the test procedure (42 CFR 493.1217).

f. **Calibrator:** A substance, material, or article intended to be used to establish the measurement relationships of a diagnostic medical device.

g. **CLIA:** Clinical Laboratory Improvement Amendments of 1988, Congressional legislation that defined and requires specific quality assurance practices in clinical laboratories.

h. **CLSI:** Clinical and Laboratory Standards Institute.

i. **Coefficient of Variation:** The ratio of the standard deviation to the average, often multiplied by 100 and expressed as a percentage, abbreviated as %CV.

j. **Colorimetry:** A technique used to determine the concentration of colored compound(s) in solution.

k. **Interfering substance:** A substance or quantity thereof that is not the measurand but that affects the result of the measurement.

l. **IUPAC:** International Union of Pure and Applied Chemistry

m. **LDT:** Laboratory –developed Test.

n. **Linearity:** Linearity is the ability of a quantitative analytical method to provide results that are directly proportional to the concentrations of an analyte in test samples, within a given measuring interval. It is an important parameter to confirm when evaluating an analytical method because it verifies correct interpolation of results between points.

o. **LMR:** Lower end of the measuring range is the lowest level at which defined conditions, including all stated characteristic of the method, are met.

p. **LoB:** Limit of Blank is the highest value in a series of results on a sample that contains no analyte.

q. **LoD:** Limit of Detection is the lowest amount of analyte in a sample that can be detected with stated probability, although perhaps not quantified as an exact value.

r. **LoQ:** When used without a prefix, the Limit of Quantitation is the lowest actual concentration at which an analyte is reliably detected and at which uncertainty of the test result is less than or equal to the goal set by the manufacturer or laboratory. The term may also be used with prefixes L for lower (LLOQ) and U for upper (ULOQ), respectively. Note: LoB < LoD ≤ LoQ.

s. **Matrix:** All components of a material system, except the analyte. A specimen matrix is the biological milieu in which an analyte exists (e.g., plasma, serum, urine, or other body fluids).

t. **Measuring Interval (reportable range; analytical measurement range or AMR):** A measuring interval consists of all numeric values between the lower and upper numeric values for which a method can produce quantitative results suitable for clinical use. Where applicable, a linearity study is frequently used to establish or verify the measuring interval that can be reported for a measurement method. Alternatively, the lower limit of the measuring interval may be assigned as the LoQ (LLOQ).

u. **Precision:** Precision is the closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. It is usually expressed numerically in terms of standard deviation (SD) or percent Coefficient of Variation (%CV).

v. **Reference interval:** The interval between and including two reference limits. It is common practice to define a reference limit so a stated fraction of the reference values is less than or equal, or greater than or equal, to the respective upper or lower limit.

w. **SOP:** Standard Operating Procedure.

x. **Spectrophotometry:** The quantitative measurement of the transmission (or reflection) properties of a material as a function of wavelength.

y. **Testing System:** The entirety of the testing process, including instrument, sample, reagents, supplies, and procedures. Personnel are sometimes included in the definition.

IV. *Pre-clinical Validation*

a. Analytical Measurement Range

i. Limits of Blank, Detection and Quantitation

The limits of blank, detection, and quantitation were determined to be 0.16 mg/dL, 1.38 mg/dL, and 12.29 mg/dL (106% recovery), respectively.

Limit of blank

CLSI guideline EP17-A section 4.3.1

Level	Number of samples	N	Mean	SD
Blank	1	20	0.09	0.04

Alpha	5%
Parametric LoB	0.16

Limit of detection

CLSI guideline EP17-A section 4.3.2

Level	Number of samples	N	Pooled SD
Low	1	20	0.73

Beta	5%
Parametric LoD	1.38

Limit of quantitation

CLSI guideline EP17-A section 5.1

Level	Number of samples	N
Low	1	20

Bias	0.69
Pooled imprecision	0.73
95% total error	2.11
Allowable error	-
LoQ	1.38

Level	Sample	n	Assigned value	Mean	Median	SD	CV
Blank	1	20	0	0.09	0.10	0.04	49.7%
Low	1	20	11.6	12.29	12.45	0.73	5.9%

ii. Linearity

The Analytical Measurement Range (AMR) including linear measurement interval has been determined by Siemens. Refer to the **Analytical Range** section of the manufacturer product information insert for additional details.

b. Analytical Specificity

The analytical specificity for this assay was determined by observing the effect of bilirubin (10 mg/dL) on the recovery of HDL cholesterol (60.6 mg/dL) in a spiked plasma sample. No significant interference (NSI) was determined if the mean analyte concentration of an interferent-spiked sample reported within 10% of the mean analyte concentration of an un-spiked sample. Recovery of HDL cholesterol in the presence of bilirubin was 99% (see table below).

Analyte: HDL Cholesterol (mg/dL)	Interferent: Bilirubin (mg/dL)	% Recovery
60.6	10	99*

* NSI observed at interferent level tested.

c. Precision

Level = L1

Number of observations	80
Number of runs	40
Number of days	20
Runs per day	2
Replicates per run	2
Mean	24.43

	SD	95% CI	CV
Repeatability	0.52	0.43 to 0.67	2.1%
Between-run	0.13		0.5%
Between-day	0.23		0.9%
Within-laboratory	0.58	0.50 to 0.70	2.4%

Level = L2

Number of observations	80
Number of runs	40
Number of days	20
Runs per day	2
Replicates per run	2
Mean	48.18

	SD	95% CI	CV
Repeatability	2.81	2.31 to 3.60	5.8%
Between-run	0.89		1.8%
Between-day	0.00		0.0%
Within-laboratory	2.95	2.55 to 3.50	6.1%

Level = L3

Number of observations	78
Number of runs	39
Number of runs excluded	1
Number of days	20
% of days with 1 run	5%
Runs per day	2
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 40 runs

Mean	69.29

	SD	95% CI	CV
Repeatability	1.19	0.97 to 1.52	1.7%
Between-run	0.31		0.5%
Between-day	1.81		2.6%
Within-laboratory	2.18	1.75 to 2.90	3.1%

V. *Clinical Validation*

a. Method Comparison with Predicate (Accuracy/Comparability)

To test the accuracy of the assay on the Theranos System, 41 unique patient samples were screened on the predicate method (Siemens, Advia) and on the Theranos method. Using the predicate method twenty eight (28) values were below the high decision level of 60 mg/dL and thirteen (13) were above. Based on the results of the data examination, either a simple linear regression or alternative procedures were used to estimate expected (average) bias and the confidence interval of expected bias at the desired medical decision level(s) as per CLSI guidance EP09-A2. StatisPro was used for bias calculations. These estimates were compared with internal criteria to judge the acceptability of the Theranos method. Each sample was run in duplicate on the predicate, and the average used for comparison to the Theranos method. Some samples were stored before analysis on both methods. If the confidence interval for

the predicted bias includes the defined acceptable bias or if the acceptable bias is greater than the higher limit of the confidence interval of the predicted bias, then the data do not show that the bias of the Theranos method is different from the acceptable bias or there is a high probability (97%) that the predicated bias is acceptable, respectively. The acceptable bias at each medical decision level was determined based on the total allowable error (TEa) minus the measured precision at the level closest to that decision level. Total allowable error (TEa) was taken from American Proficiency Institute (API) peer proficiency testing criteria or CLIA proficiency testing criteria for acceptable analytical performance, as printed in the Federal Register February 28, 1992;57(40):7002-186, when available. The TEa for HDL-Cholesterol is 30%. The table below shows the allowable bias and precision at 2 levels (values shown in parentheses) and the corresponding closest medical desirable limits.

Table 1. Allowable Bias and Precision at the Medical Decision Levels

Medical Desirable Levels (mg/dL)	**>45.0 males (48.2)**	**>55.0 females (69.3)**
Precision (%)	**0.0**	**2.6**
Allowable Bias (%)	**30.0**	**27.4**

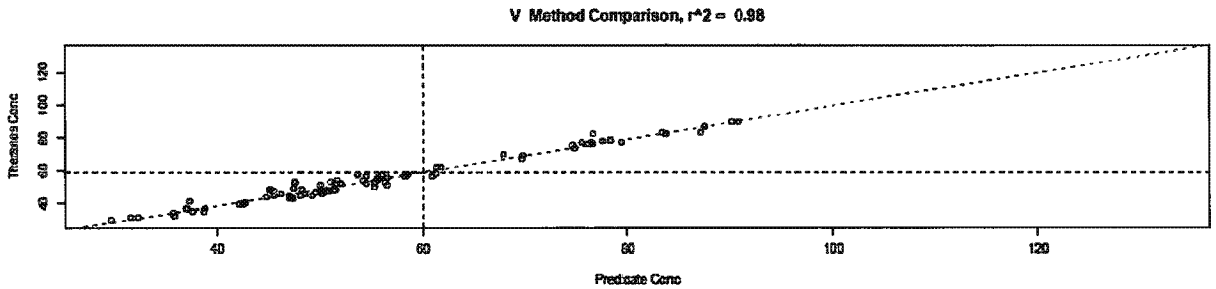


Figure 1. Graph showing Theranos method versus Predicate Method (Siemens Advia).

Simple linear regression was used to establish a slope, intercept and an r2. The slope, intercept and clinical correlation were determined to be 1.02, -2.03 and 0.98 respectively.

Comparability

CLSI guideline EP09-A2-IR section 7

Level ID	Value	Difference	SE	95% CI	Allowable difference
	30.000000	-1.5020116	0.57193680	-2.6608656 to -0.343157	5.0400000
	45.000000	-1.2557261	0.35210087	-1.9691502 to -0.542302	7.5600000
	90.000000	-0.5168697	0.70734586	-1.9500886 to 0.9163491	15.1200000

Difference is less than allowable bias: 16.8%.

The difference between the two methods is not greater than the allowable difference.

b. Transference and Verification of Reference Interval (Venous)

Reference interval for this analyte has been replaced by decision limits therefore verifying reference ranges is not required for High Density Lipoprotein Cholesterol. Eighteen (18) new normal venous samples were tested, 17 (94.4%) reported values above the high decision limit of 60 mg/dL and 1 (5.6%) reported a value below.

c. Verification of Reference Interval with Finger Stick Samples

Verifying finger stick sample reference ranges not required for High Density Lipoprotein Cholesterol. Eight (8) new venous matched finger sticks samples were also tested, all 8 (100%) reported values above the high decision limit of 60 mg/dL.

VI. Stability

a. Reagents

On-board Reagent Stability

System	Stability
ADVIA 1650/1800	14 days
ADVIA 2400	14 days

For all systems, unopened reagents are stable until the expiration date printed on the product label when stored at 2° - 8°C protected from light. Do not freeze reagents.

For complete details, refer to the Methods Introduction section of the system-specific Operator's Guide.

b. Sample

Plasma samples for HDL analysis are stable for 2 weeks at 2-8 °C, or at least 90 days at -20 °C.

c. Calibrators

The Siemens HDL/LDL Cholesterol Calibrator should be stored at 2-8 °C. Unopened the calibrator is stable until the expiration date on the vial label. After reconstitution the calibrator is stable for 3 days.

REVISION HISTORY

Revision Level	Effective Date	Initiator	ECO Number
A	11/06/2013	A. Rosendorff	CL ECO-00117

Section Number	Description and Justification of Changes
All	Initial Release