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Apolipoprotein B (ApoB) Assay Validation Report		

Author(s):

Signature:	Date: 2/19/2014
Name: Adam Rosendorff, MD	Title: Laboratory Director

Reviewer(s)

Signature: Sharada Sivaraman	Date: 3/31/14
Name: Sharada Sivaraman, PhD	Title: Immunoassay Team Leader

Signature:	Date: 3/25/2014
Name: Daniel Young, Ph.D.	Title: Vice President

Approver(s):

Signature:	Date: 2/19/2014
Name: Adam Rosendorff, M.D	Title: Laboratory Director

9/19/13

Sunil S. Dhawan M.D.

theranos	(Assay) Report	Document Number: Revision: A
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Apolipoprotein B immunoassay Validation Report on the ADVIA 1800 system		

TABLE OF CONTENT

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1 ASSAY BACKGROUND

Apolipoprotein B (APO B) is a distinct, lipid-binding protein that is the major protein moiety of all lipoproteins other than high-density lipoprotein (HDL). APO B is essential to the formation and release of lipoproteins into the plasma and interacts with the LDL receptors of peripheral cells thereby functioning in the recognition of cellular receptors for the catabolism of LDL.

Studies show a correlation between the development of coronary atherosclerosis and abnormal concentrations of lipoproteins. In atherosclerosis, the inner layer of the arterial wall thickens due to the accumulation of cellular material and deposits of several substances, particularly lipids. Because apolipoproteins are unique markers for the identification and differentiation of lipoproteins, they are the most probable determinants of the structural integrity and functional specificity of lipoproteins.

Measurement of APO B in serum of patients with significant arteriosclerotic alterations shows APO B levels significantly higher than a normal patient population. Abnormal APO B values also occur with dyslipoproteinemias. Adult-onset diabetes, and liver diseases including acute hepatitis and fatty liver result in elevated APO B values. Numerous studies suggest that serum Apolipoprotein A-1 (APO A-1) and APO B levels indicate the severity and extent of coronary artery stenosis better than serum total cholesterol and triglyceride In addition, studies indicate that patients with atherosclerosis are better distinguished from patients without the disease by the finding of increased plasma APO B levels than by the findings of decreased HDL-C and increased LDL-C levels. Measuring both APO A-1 and APO B and expressing the data as a ratio has been shown to be more effective than APO A-1 or APO B alone.

2 REGULATION AND GUIDANCE

The qualification/validation of the ELISA assays on the Theranos device will be in accordance with C.F.R. Ch IV, § 493.1253 "Standard: Establishment and verification of performance specifications" and outlined in CLSI guideline C28A3.

3 PRINCIPLE OF THE PROCEDURE

The APO B method is a polyethylene glycol (PEG) enhanced immunoturbidimetric assay. A sample containing human apolipoprotein B and specific antiserum form an

insoluble complex that is measured turbidimetrically at 340/694 nm. By constructing a

standard curve from the absorbances of standards, the concentration of apolipoprotein B is determined.

Plasma samples were diluted 1:3.125 fold in saline prior to analysis.

4 CALIBRATION

4.1 In 42 CFR Part 493.1255, it is required to perform calibration procedures with at least the frequency recommended by the manufacturer, or using criteria specified by the laboratory, or when calibration verification fails to meet acceptable limits.

4.1.1 The term "calibration verification," as used in CLIA, includes:

4.1.1.1 Confirming that a calibration meets the method manufacturer's specifications

4.1.1.2 Verifying that the calibration is suitable for the entire measuring interval (or "reportable range," which is the CLIA term)

4.2 Calibrators were diluted 1:3.125 and verified on the ADVIA system

4.2.1 This dilution factor is within the acceptable limits of the ADVIA internal calibration test.

4.3 For the purposes of this Validation Plan, calibration was carried out with every new lot of reagents.

4.3.1 Each level was tested in replicates of 3 and the average was used to create a standard curve for testing.

4.3.2 The calibration was verified using quality controls.

5 QUALITY CONTROL

5.1 Two to four level quality control samples, as appropriate to the assay, were analyzed with each calibration and before each test during the validation.

5.1.1 Low = 41.2 mg/dL

5.1.2 Mid= 98.8 mg/dL

5.1.3 High = 148 mg/dL

5.2 The QC levels are not included when generating the calibration curve.

6 PRECISION

6.1 Precision was evaluated according to CLSI standard EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods. A total of 20 runs were performed over 10 days with 2 runs per day and 2 replicates per run for a total of 40 data points. The following tables indicate the between-run, between-day and within-laboratory precision at 3 levels (Low = 45mg/dL, Mid= 102.6 mg/dL and High=152.2 mg/dL) The following data shows the results obtained.

Table I: Precision at 3 decision levels.

Precision

CLSI guideline EP05-A2 section 10.8

Level = L1

Number of observations	32
Number of runs	16
Number of days	8
Runs per day	2
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 40 runs, with 2 replicates per run.

Mean	45.4

	SD	95% CI	CV
Repeatability	0.7	0.5 to 1.1	1.6%
Between-run	0.5		1.2%
Between-day	0.6		1.3%
Within-laboratory	1.1	0.8 to 1.5	2.3%

Level = L2

Number of observations	32
Number of runs	16
Number of days	8
Runs per day	2
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 40 runs, with 2 replicates per run.

Mean	102.6

	SD	95% CI	CV
Repeatability	1.6	1.2 to 2.4	1.5%
Between-run	0.0		0.0%
Between-day	1.1		1.0%
Within-laboratory	1.9	1.5 to 2.7	1.9%

Level = L3

Number of observations	32
Number of runs	16
Number of days	8
Runs per day	2
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 40 runs, with 2 replicates per run.

Mean	152.2

	SD	95% CI	CV
Repeatability	1.7	1.3 to 2.6	1.1%
Between-run	0.9		0.6%
Between-day	1.0		0.7%
Within-laboratory	2.2	1.7 to 3.1	1.4%

6.2 The mean recovery of controls 1,2 and 3 versus the assigned values was as follows:

Control#	Assigned (mg/dL)	Theranos (mg/dL)	% Recovery
1	41.2	45.4	110%
2	98.8	102.6	104%
3	148	152.2	103%

6.3 Acceptance criteria:

Total allowable error (TAE %) of 27%, was selected as the acceptance criteria for this assay following CLIA proficiency guidelines as printed in the Federal Register February 28, 1992;57(40):7002-186.

	Level 1	**Level 2**	**Level 3**
TAE%	27	27	27
CV (%)	2.3	1.9	1.4
Allowable Bias (%)	24.7	25.1	25.6
Estimated bias (Sec. 6.2)	10%	4%	3%

Table II Total allowable error % (TAE%)

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7 BIAS ESTIMATION: Theranos versus Siemens methods

7.1 Mean bias between Theranos and Siemens methods was estimated based on % recovery of controls 1,2 and 3 (section 6.2), and, in each case, mean bias was less than allowable bias. Therefore bias criteria are satisfied.

8 CAPILLARY TUBE AND NANOTAINER (CTN) TO VENOUS BLOOD COMPARISON

8.1 Because the ADVIA system allows users to extend the reportable range, a pre-dilution of sample is valid, and the volume of sample obtained from a fingerstick in sufficient for testing on the system. To verify the comparability of fingerstick blood to venous blood, 20 unique patients donated 2 venous tubes of blood and 2-4 fingerstick samples in EDTA. Each sample of venous blood was tested and the 2 results were used as replicate tests. Fingerstick samples

were pooled and tested in replicates of 2. All samples should be within the reference range, and were also subject to the reference range criteria.

8.2 Calculated concentrations are based on the mean of 2 replicate tests.

8.3 Acceptance Criteria:

8.3.1 All samples must have %CV within 20%

8.3.2 Scatter plots should have a slope of 1 +/- 0.15 and R^2 greater than 0.9

8.3.3 Regression equation for CTN versus matched venous samples is shown below and passes acceptance criteria.

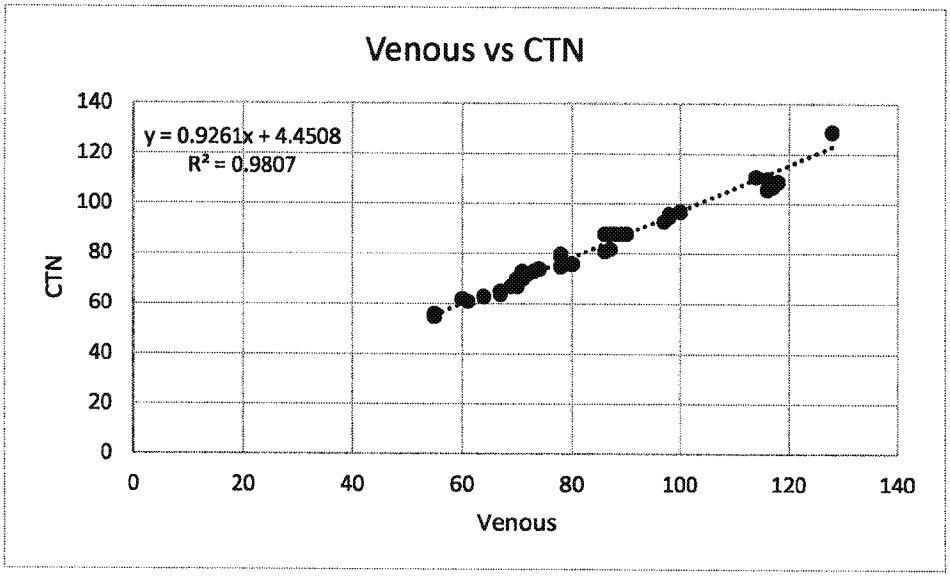

Figure 1: Capillary tube and nanotainer (CTN) comparison with vacutainer

9 REFERENCE RANGE VERIFICATION

9.1 20 unique venous and matched capillary tube and nanotainer (CTN) samples were collected and assayed in duplicate using the predicate and Theranos methods, and the average value was calculated. All samples were collected in EDTA. Values obtained from venous blood that fell out of the Siemens published reference range, as well as the corresponding values from matched CTN samples, were excluded from analysis. Of the 20 values obtained, all 20

were included for analysis, and all 20 (100%) fell within the both Siemens published reference ranges, 46-174 mg/dL (M) or 46-142 mg/dL (F).

9.2 The RR is therefore verified (CLSI guidance C28-A3c).

9.3 The raw data for this study is shown below:

Donor	Rep	EDTA Plasma	Average
1	2	123	122
	2	121	
2	1	120	119.5
	2	119	
3	1	118	118
11	1	118	
	2	117	116
20	1	115	
	2	97	96.5
18	1	96	
	2	96	96
19	1	96	
4	1	92	92
	2	92	
	2	89	88.5
9	1	88	
15	1	80	80
	2	80	
	2	75	74
12	1	73	
	2	73	72.5
16	1	72	
	2	72	71
17	1	70	
7	1	69	69
	2	69	
	2	68	68
10	1	68	

	1	66	67.5
	2	69	
5	1	65	64
	2	63	
8	1	61	61
	2	61	
13	1	60	59
	2	58	
6	2	58	57.5
	1	57	
14	1	56	51.5
	1	47	

Table III CTN values for reference range verification.

10 REFERENCES

10.1 Code of Federal Regulations, Title 42, Chapter IV, Subchapter G, Part 493, Subpart K, Sections 493.1217, 493.1253, and 493.1255.

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10.4 R (version 2.13.1). The R Foundation for Statistical Computing, 07/08/2011.

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