theranos	Alpha-1 Acid Glycoprotein	Document Number: CL-RPT-145016 Revision: A
	Validation Document	Effective Date: 1/17/2014
Alpha-1 acid glycoprotein Validation Report		

Author(s):

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

Reviewer(s)

Signature: Sharada Sivaraman	Date: 3/19/14
Name: Sharada Sivaraman, PhD	Title: Immunoassay Team Leader

Signature: [signature]	Date: 3/19/2014
Name: Daniel Young, Ph.D.	Title: Vice President

Approver(s):

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

[signature] 9/19/15

Sunil S. Dhawan M.D.

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1 ASSAY BACKGROUND

Measurements of a1-acid glycoprotein (orosomucoid) may aid in the diagnosis of collagen (connective tissue) disorders, tuberculosis, infection, extensive malignancy and diabetes.

a1-Acid glycoprotein is a sensitive acute-phase protein. a1-Acid glycoprotein concentration changes in response to trauma. a1-Acid glycoprotein is rich in carbohydrate content and synthesized in the liver. Its concentration in blood is increased during acute or chronic inflammation (for example, Crohn's disease, systemic lupus erythematosus, and rheumatoid arthritis), hemolysis, cancer, wound healing, and pneumonia.

Decreased levels of a1-acid glycoprotein are associated with pregnancy, estrogen therapy, severe hepatic damage, and nephrotic syndromes.

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

In the ADVIA Chemistry α_1-Acid Glycoprotein (AAG) assay, sample is diluted and reacts with a buffer that contains an antibody specific for α_1-acid glycoprotein. The formation of the antibody-antigen complex during the reaction results in an increase in turbidity, the extent of which is measured as the amount of light absorbed at 340 and 694 nm. The α_1-acid glycoprotein concentration in the sample is determined by constructing a standard curve from the absorbance of standards.

Plasma samples were diluted 1:9.15 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 1:9.15 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 = 42.5 mg/dL

5.1.2 Mid= 71 mg/dL

5.1.3 High = 98.2 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 as shown in section 5. The following data describes 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	39.09		
	SD	95% CI	CV
Repeatability	1.55	1.15 to 2.36	4.0%
Between-run	0.00		0.0%
Between-day	0.00		0.0%
Within-laboratory	1.55	1.23 to 2.09	4.0%

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	75.66		
	SD	95% CI	CV
Repeatability	1.26	0.94 to 1.92	1.7%
Between-run	1.95		2.6%
Between-day	0.82		1.1%
Within-laboratory	2.46	1.87 to 3.60	3.3%

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	105.68		
	SD	95% CI	CV
Repeatability	1.87	1.39 to 2.84	1.8%
Between-run	2.36		2.2%
Between-day	0.28		0.3%
Within-laboratory	3.02	2.33 to 4.29	2.9%

6.2 Acceptance criteria:

Total allowable error (TAE %) of 16%, was selected as the acceptance criteria for this assay. These goals have been provided by Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, Minchinela J, Perich C, Simon M. "Current databases on biologic variation: pros, cons and progress." Scand J Clin Lab Invest 1999;59:491-500.

Table II Total Allowable Error (%)

	Level 1	Level 2	Level 3
TAE%	16	16	16
CV (%)	4	3.3	2.9
Allowable Bias (%)	12	12.7	14.1
Bias (%)	1.9	1.9	1.9
Decision	Pass	Pass	Pass

7 BIAS ESTIMATION: COMPARISON OF PREDICATE WITH THERANOS METHODS

7.1 Twenty (20) venous samples were run using the predicate Siemens protocol without dilution, and in parallel on the Theranos assay with pre-dilution. Results were plotted in a scatter diagram, and a simple linear regression was performed (Figure I). Raw data as well as the scatter-plot summarizing the results are shown in Table III.

7.2 Mean bias comparing methods was calculated as follows: %Bias=[(Theranos-Siemens)/Siemens]*100 and results are shown in the column labelled "% difference", and indicated in section 6.2

7.3 Mean bias is less than the allowable bias therefore the acceptance criteria PASS.

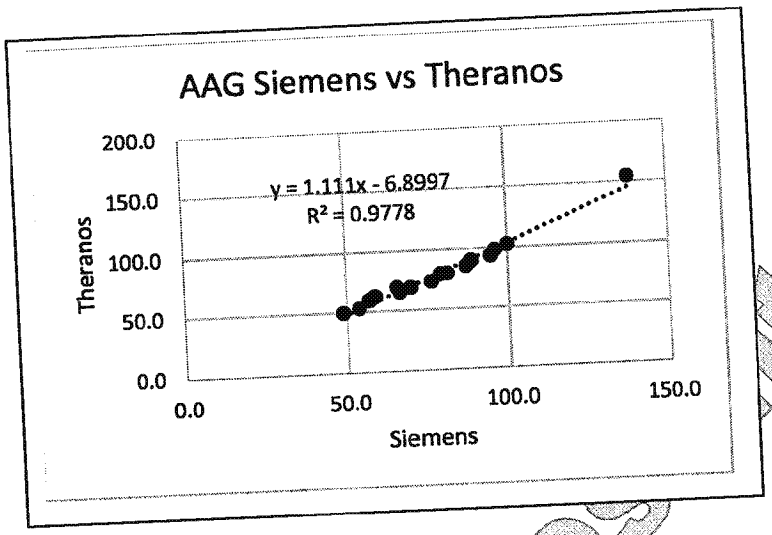

Figure 1: Table III Bias estimation, comparison of Theranos with Siemens methods

Sample #	Siemens	EDTA	Theranos	EDTA	% difference	T-corr
1	AAG	137.6	T-AAG	155.0	-12%	145.72
2	AAG	58.8	T-AAG	62.9	-7%	62.83
3	AAG	70.0	T-AAG	69.7	0%	68.95
4	AAG	65.6	T-AAG	70.0	-6%	69.22
5	AAG	57.0	T-AAG	59.8	-5%	60.04
6	AAG	100.0	T-AAG	102.9	-3%	98.83
7	AAG	78.9	T-AAG	79.8	-1%	78.04
8	AAG	**53.9**	T-AAG	53.6	1%	**54.46**
9	AAG	70.0	T-AAG	69.4	1%	68.68
10	AAG	66.5	T-AAG	65.6	1%	65.26
11	AAG	94.7	T-AAG	93.8	1%	90.64
12	AAG	79.1	T-AAG	80.2	-1%	78.40
13	AAG	87.2	T-AAG	85.9	2%	83.53
14	AAG	96.1	T-AAG	98.5	-2%	94.87
15	AAG	76.2	T-AAG	73.9	3%	72.73
16	AAG	**48.9**	T-AAG	50.0	-2%	**51.21**
17	AAG	81.3	T-AAG	80.4	1%	78.58

18	AAG	88.5	T-AAG	89.5	-1%	86.77
19	AAG	88.9	T-AAG	90.2	-1%	87.40
20	AAG	57.2	T-AAG	60.0	-5%	60.22

Table III Bias estimation, comparison of Theranos with Siemens methods

8 CTN REFERENCE RANGE VERIFICATION

8.1 20 unique capillary CTN samples were collected from healthy donors and assayed in singlicate using the Theranos methods, as shown in Table III. Resulting values were corrected to match more closely with the predicate using the regression equation as follows: Corrected value=(CTN value -6.8997)/0.9778, and are shown in the column labelled T-corr.

8.2 Values excluded from analysis, based on the fact that the matching venous sample value was also out of reference range, are shown bold.

8.3 2 out of 18 values were outside the reference range for both predicate (Siemens) and Theranos methods. Of the remaining 18 usable values, 18/18 were within the manufacturer recommended reference range (58-155 mg/dL) (CLSI document C28A3).

8.4 The predicate reference range is therefore verified.

9 REFERENCES

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

9.2 DeSilva B, Smith W, Weiner R, et al. Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. Pharmaceutical Res. 2003; 20:1885-1900.

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

9.5 StatisPro (version 1.13.00). Clinical and Laboratory and Standards Institute, Wayne, PA. 07/14/2011.

9.6 Dexter-Immunoassay (version1.0), Theranos, Inc., 2009.

9.7 EP10-A3, Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline—Third Edition, 2006, Clinical and Laboratory Standards Institute, Wayne, PA.

9.8 EP15-A2, User Verification of Performance for Precision and Trueness; Approved Guideline—Second Edition. 2005, Clinical and Laboratory Standards Institute, Wayne, PA.

9.9 EP09-A2-IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Second Edition (Interim Revision), 2010, Clinical and Laboratory Standards Institute, Wayne, PA.

9.10 EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition, 2004, Clinical and Laboratory Standards Institute, Wayne, PA.

9.11 EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, 2003, Clinical and Laboratory Standards Institute, Wayne, PA.

9.12 EP7-A2, Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition, 2004, Clinical and Laboratory Standards Institute, Wayne, PA.

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