theranos	Complement C3	Document Number: CL-RPT-145004 Revision: A
	Validation Document	Effective Date: 1/17/2014
Complement C3 Validation Report		

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1 ASSAY BACKGROUND

1.1 Complement proteins are acute phase reactants that bind to the surface of blood-borne bacteria, thereby allowing for complement mediated lysis or opsonization by macrophages. Complement levels rise rapidly during inflammation and infection, and can drop in the setting of autoimmune diseases, particularly those in which complement is deposited in tissues such as the lung or kidney (eg Goodpasture's disease, Wegener's Granulomatosis, acute glomerulonephritis, mebranoproliferative glomurelonephritis). Patients deficient in C3 protein are often more susceptible to infection with encapsulated bacteria such as Hemophilus Influenza and Neisseria Meningiditis. Complement C3 levels are often viewed in combination with C4 levels to provide a more focused clinical differential diagnosis. For instance in acute glomerulonephritis, C3 levels are decreased while C4 levels are normal while in Systemic Lupus Erythematosus, both C3 and C4 are decreased.

2 REGULATION AND GUIDANCE

2.1 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

Sample is reacted with a buffer containing antibody specific for human Complement C3 (β_1 C-globulin). The absorbance (340/694 nm) of the resulting turbid solution is proportional to the concentration of C3 in the sample. By constructing a standard curve from the absorbance of standards, C3 concentration of sample can be 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 = 73 mg/dL

5.1.2 Mid = 155 mg/dL

5.1.3 High = 235 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.

Table 1: Precision at 3 medical decision limits

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Precision

CLSI guideline EP05-A2 section 10.8

Level = L1

Number of observations	40
Number of runs	20
Number of days	10
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	70.46

	SD	95% CI	CV	Allowable Total SD
Repeatability	0.55	0.42 to 0.79	0.8%	-
Between-run	0.73		1.0%	-
Between-day	1.49		2.1%	-
Within-laboratory	1.75	1.26 to 2.84	2.5%	5.92

Imprecision is less than allowable total imprecision: 8.4%.

Level = L2

Number of observations	40
Number of runs	20
Number of days	10
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	154.74

	SD	95% CI	CV	Allowable Total SD
Repeatability	1.40	1.07 to 2.02	0.9%	-
Between-run	0.19		0.1%	-
Between-day	1.90		1.2%	-
Within-laboratory	2.37	1.76 to 3.60	1.5%	13.00

Imprecision is less than allowable total imprecision: 8.4%.

Level = L3

Number of observations	40
Number of runs	20
Number of days	10
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	242.43

	SD	95% CI	CV	Allowable Total SD
Repeatability	2.21	1.69 to 3.19	0.9%	-
Between-run	3.59		1.5%	-
Between-day	6.44		2.7%	-
Within-laboratory	7.69	5.57 to 12.45	3.2%	20.36

Imprecision is less than allowable total imprecision: 8.4%.

6.2 Acceptance criteria:

Total allowable error (TAE %) of 25%, was selected as the acceptance criteria for this assay following proficiency guidelines recommended by the American Proficiency Institute Peer Data for 2013 CHEMISTRY / IMMUNOLOGY / IMMUNOHEM -1ST EVENT. Allowable bias was calculated as the residual error budget after precision values (CV %) were subtracted from TAE (%).

Table II Total Allowable Error % (TAE%)

	Level 1	**Level 2**	**Level 3**
TAE%	25	25	25
CV (%)	2.5	1.5	3.2
Allowable Bias (%)	22.5	23.5	21.7
Bias (%)	3	3	3
Decision	Pass	Pass	Pass

7 BIAS ESTIMATION:Serum (Siemens) versus K2-EDTA (Theranos)

7.1 The Siemens-recommended tube type for C3 is Serum, however, the Theranos preferred tube type is EDTA-plasma. Since a potential exists for anticoagulant incompatibility, a study was performed to estimate bias between assay values obtained from EDTA-Plasma versus Serum.

7.2 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.

7.3 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.

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

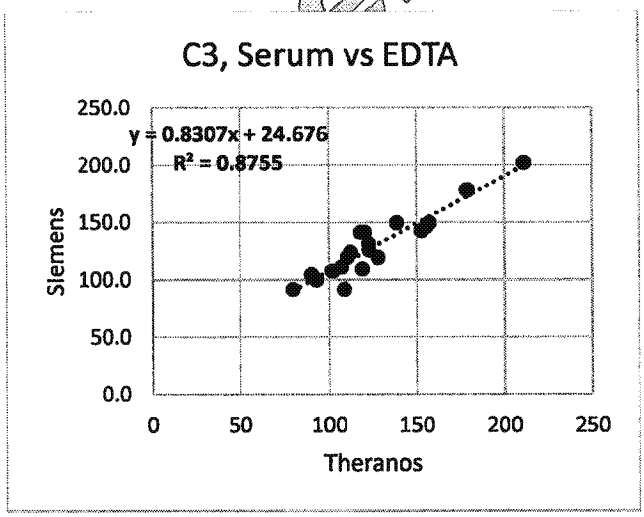

Figure I: Bias estimation, Siemens versus Theranos Methods

Sample #	Siemens	Serum	Theranos	EDTA	% difference	T-corr	%difference-corr
1	C3	155.80	T-C3	146.94	0.06	147.18	5.53
2	C3	**210.80**	T-C3	**202.43**	0.04	**213.98**	-1.51
3	C3	108.40	T-C3	91.85	0.17	**80.86**	25.40
4	C3	118.80	T-C3	109.49	0.08	102.10	14.06
5	C3	120.00	T-C3	141.45	-0.16	140.57	-17.14
6	C3	157.20	T-C3	149.66	0.05	150.46	4.29
7	C3	122.70	T-C3	126.37	-0.03	122.42	0.23
8	C3	107.00	T-C3	111.53	-0.04	104.56	2.28
9	C3	110.20	T-C3	120.07	-0.09	114.84	-4.21
10	C3	152.80	T-C3	143.07	0.07	142.52	6.73
11	C3	**178.60**	T-C3	**178.70**	0.00	**185.41**	-3.82
12	C3	90.20	T-C3	104.35	-0.15	95.91	-6.33
13	C3	93.00	T-C3	100.43	-0.08	91.19	1.94
14	C3	101.90	T-C3	107.96	-0.06	100.26	1.61
15	C3	112.00	T-C3	123.78	-0.10	119.30	-6.52
16	C3	122.60	T-C3	131.43	-0.07	128.51	-4.82
17	C3	**79.70**	T-C3	91.92	-0.14	80.95	-1.57
18	C3	138.90	T-C3	149.78	-0.08	150.60	-8.42
19	C3	127.90	T-C3	119.78	0.07	114.49	10.49
20	C3	117.90	T-C3	141.20	-0.18	140.27	-18.98
Average		126.32		129.61		126.32	-0.04

Table III: Bias estimation Theranos versus Siemens methods

8 CTN REFERENCE RANGE VERIFICATION

8.1 20 unique fingerstick samples collected in capillary tube and nanotainers (CTNs) were collected from healthy donors and assayed in duplicate 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 -24.676)/.8307). Corrected values are shown in the column labelled T-corr. The column labelled %difference-corr, indicates that the correction procedure was successful, because the average difference between predicate and Theranos assays is eliminated by this transformation.

8.2 17/20 (85%) of corrected CTN values fell within the predicate reference range (90-170 mg/dL), however, excluding Theranos values where the corresponding predicate value fell

out of the reference range (Table III, column 3, bold, 16/17 of corrected Theranos values fell within the reference range (CLSI guidance C28-A3c). Therefore the predicate reference range is verified for the Theranos method.

9 REFERENCES

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

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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.