theranos	Immunoglobulin G Validation Report	Document Number: CL-RPT-145011 Revision: A
	Validation Document	Effective Date: 1/17/2014
Immunoglobulin G (IgG) Assay Validation Report		

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1 ASSAY BACKGROUND

Immunoglobulin G (IgG) is the largest antibody component of protein in blood. Four distinct subtypes exist: (IgG1, IgG 2, IgG3, and IgG4). They differ according to their structure, ability to fix complement, ability to transport across the placenta and half-life. Polyclonal spikes in IgG are seen in infectious diseases and inflammation, while increased monoclonal IgG is seen in multiple myeloma or monoclonal gammopathy of undetermined significance (MGUS). Patients with frequent infections should be evaluated for IgG deficiency. More rarely, IgG deficiency can be seen in the context of primary immunodeficiency 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 ASSAY

Principles of the Procedure

The IGG_2 method is a PEG-enhanced immunoturbidimetric method. Sample containing human IgG is suitably diluted and then reacted with specific antiserum to form a precipitate that can be measured turbidimetrically at 340/694 nm. By constructing a calibration curve from the absorbances of calibrators, the concentration of IgG is determined.

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 fold 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 = 968 mg/dL

5.1.2 Mid = 1725 mg/dL

5.1.3 High = 2387 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.

6.1.1 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 indicated in section 5.

Table I: Precision at 3 decision levels

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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	960.9

	SD	95% CI	CV	Allowable Total SD
Repeatability	15.2	11.6 to 21.9	1.6%	-
Between-run	18.6		1.9%	-
Between-day	52.2		5.4%	-
Within-laboratory	57.5	40.8 to 97.2	6.0%	192.2

Imprecision is less than allowable total imprecision: 20%.

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	1740.6

	SD	95% CI	CV	Allowable Total SD
Repeatability	27.1	20.8 to 39.2	1.6%	-
Between-run	22.4		1.3%	-
Between-day	102.8		5.9%	-
Within-laboratory	108.7	76.3 to 188.5	6.2%	348.1

Imprecision is less than allowable total imprecision: 20%.

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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	2439.2

	SD	95% CI	CV	Allowable Total SD
Repeatability	53.6	41.0 to 77.5	2.2%	-
Between-run	47.1		1.9%	-
Between-day	158.2		6.5%	-
Within-laboratory	173.6	123.4 to 292.4	7.1%	487.8

Imprecision is less than allowable total imprecision: 20%.

6.2 Acceptance criteria:

Total allowable error (TAE %) of 25% , was selected as the acceptance criteria for this assay following CLIA proficiency and 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 (%). Values in brackets indicate the closest/corresponding API levels obtained from 2013 proficiency data.

Table II

	Level 1	Level 2	Level 3
TAE%	25	25	25
CV (%)	6	6.2	7.1
Allowable Bias (%)	19	18.8	17.9
Mean Bias	1	1	1
Decision	Pass	Pass	Pass

7 BIAS ESTIMATION:, Lithium-Heparin (Siemens) versus K2-EDTA (Theranos)

7.1 The Siemens-recommended tube type for IgG is Lithium-Heparin 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 Lithium-Heparin plasma.

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 6.1.2.

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

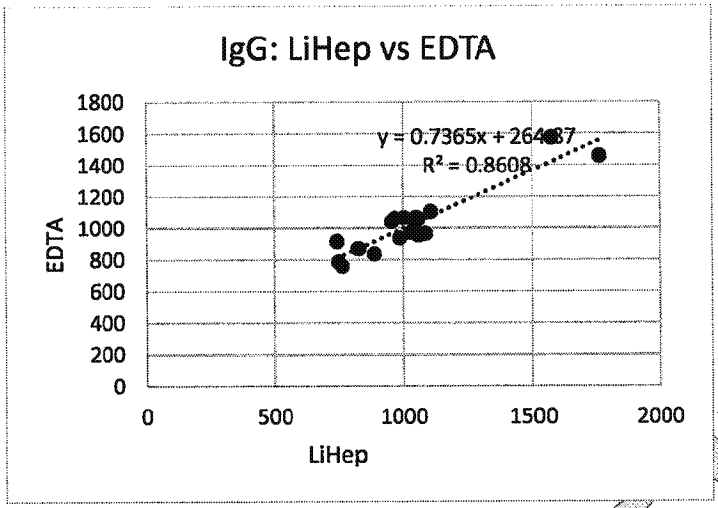

Fig 1

Sample #	Siemens	Li-Hep	Theranos	EDTA	% difference
7	IGG_2	660	T-IgG	808	-20%
17	IGG_2	741	T-IgG	920	-22%
18	IGG_2	748	T-IgG	788	-5%
5	IGG_2	764	T-IgG	763	0%
3	IGG_2	826	T-IgG	873	-6%
11	IGG_2	886	T-IgG	840	5%
15	IGG_2	954	T-IgG	1044	-9%
10	IGG_2	966	T-IgG	1064	-10%
4	IGG_2	986	T-IgG	942	5%
16	IGG_2	1004	T-IgG	1067	-6%
12	IGG_2	1030	T-IgG	975	5%
14	IGG_2	1043	T-IgG	992	5%
1	IGG_2	1050	T-IgG	1069	-2%
6	IGG_2	1053	T-IgG	1057	0%
9	IGG_2	1056	T-IgG	1064	-1%
20	IGG_2	1058	T-IgG	958	10%
2	IGG_2	1085	T-IgG	968	11%
19	IGG_2	1105	T-IgG	1108	0%
13	IGG_2	1571	T-IgG	1578	0%
8	IGG_2	1761	T-IgG	1461	19%
Average					**-1%**

Table III

8 CTN REFERENCE RANGE VERIFICATION

8.1 20 unique capillary tube and nanotainer (CTN) samples 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 -264.87)/.7365)

8.2 18/20 (90%) of corrected CTN values fell within the predicate reference range (650-1500 mg/dL) (modified cutoff of 90%, from CLSI guidance C28-A3c). Values falling outside of the reference range are indicated in red.

8.3 Therefore the reference range is verified.

8.4 Raw values before and after correction are shown below:

Sample #	Theranos	EDTA
V 7	T-IgG	727.4
17	T-IgG	882.8
18	T-IgG	699.6
5	T-IgG	664.9
3	T-IgG	817.6
11	T-IgG	771.8
15	T-IgG	1054.9
10	T-IgG	1082.7
4	T-IgG	913.4
16	T-IgG	1086.9
12	T-IgG	959.2
14	T-IgG	982.8
1	T-IgG	1089.6
6	T-IgG	1073.0
9	T-IgG	1082.7
20	T-IgG	935.6
2	T-IgG	949.5
19	T-IgG	1143.8
13	T-IgG	1796.1
8	T-IgG	1633.7

Table IV

9 REFERENCES

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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.

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