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Immunoglobulin A (IgA) Validation Report

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1 ASSAY BACKGROUND

Immunoglobulin A (IgA) constitutes 10% of antibodies in the serum, and consists of two types: IgA1 and IgA2. Most (>90%) of the IgA that circulates is IgA1. IgA constitutes the first line of defense against pathogenic bacteria.

Selective immunoglobulin A (IgA) deficiency (SIGAD) is a relatively mild genetic immunodeficiency. People with this deficiency lack immunoglobulin A (IgA), a type of antibody that protects against infections of the mucous membranes lining the mouth, airways, and digestive tract. It is defined as an undetectable serum IgA level in the presence of normal serum levels of IgG and IgM. It is the most common of the primary antibody deficiencies.The incidence of this disorder is 1:300, and affects females more often than males. Affected persons are most often asymptomatic but may show an increased susceptibility to digestive, respiratory and urogenital infections due to the relative deficiency of protective antibodies in these sites.

IgA deficiency may manifest as part of rarer diseases in which B-cells fail to develop or mature such as Bruton's X-linked immunodeficiency and Common Variable Immunodeficiency, and occasionally in the setting of B-cell leukemias. Increased IgA levels can be seen in liver cirrhosis, IgA myeloma and rheumatoid arthritis.

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

Principles of the Procedure

The IGA_2 method is a PEG-enhanced immunoturbidimetric method. Sample containing human IgA 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 standard curve from the absorbances of standards, the concentration of IgA is determined.

Modification of the procedure was as follows:

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 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 High = 133 mg/dL

5.1.2 Mid = 292 mg/dL

5.1.3 Low = 430 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 as indicated in section 5.

6.2 Precision was evaluated according to CLSI standard EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods.

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	134.71

	SD	95% CI	CV	Allowable Total SD
Repeatability	2.89	2.21 to 4.18	2.1%	-
Between-run	3.41		2.5%	-
Between-day	4.26		3.2%	-
Within-laboratory	6.17	4.65 to 9.17	4.6%	26.94

Imprecision is less than allowable total imprecision: 20%.

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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	298.62

	SD	95% CI	CV	Allowable Total SD
Repeatability	5.89	4.51 to 8.51	2.0%	-
Between-run	5.00		1.7%	-
Between-day	8.88		3.0%	-
Within-laboratory	11.78	8.80 to 17.81	3.9%	59.72

Imprecision is less than allowable total imprecision: 20%.

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	445.88

	SD	95% CI	CV	Allowable Total SD
Repeatability	10.97	8.39 to 15.84	2.5%	-
Between-run	9.78		2.2%	-
Between-day	19.31		4.3%	-
Within-laboratory	24.26	17.90 to 37.66	5.4%	89.18

Imprecision is less than allowable total imprecision: 20%.

6.2.1 Acceptance criteria:

Total allowable error (TAE %) of 20%, 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

	Level 1 135 mg/dL	**Level 2** 296 mg/dL	**Level 3** 441 mg/dL
TAE%	20	20	20
CV (%)	4.6	3.9	5.4
Allowable Bias (%)	15.4	16.1	14.6
%Bias	6	6	6
Decision	Pass	Pass	Pass

7 BIAS ESTIMATION: Lithium-Heparin versus K2-EDTA

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

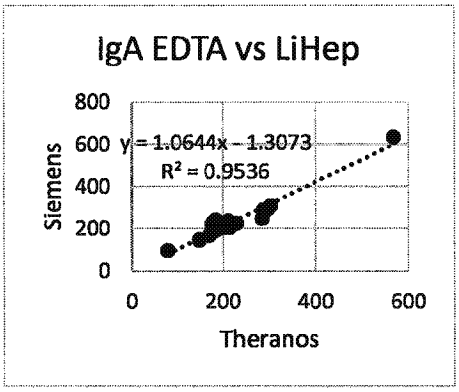

Fig. 1 Bias estimation, Siemens versus Theranos methods.

Sample #	Siemens	Li-Hep	Theranos	EDTA	% difference	T-Corr	% difference-corr
7	IGA_2	184.4	T-IgA	242.1	-27%	228.68	24.01
17	IGA_2	286.4	T-IgA	291.4	-2%	275.00	-3.98
18	IGA_2	294.5	T-IgA	291.8	1%	275.37	-6.49
5	IGA_2	177.5	T-IgA	184.9	-4%	174.94	-1.44
3	IGA_2	170.1	T-IgA	169.6	0%	160.57	-5.60
11	IGA_2	208	T-IgA	219	-5%	206.98	-0.49
15	IGA_2	228.6	T-IgA	225.3	1%	212.90	-6.87
10	IGA_2	206.1	T-IgA	229.6	-11%	216.94	5.26
4	IGA_2	174.5	T-IgA	223.8	-25%	211.49	21.20
16	IGA_2	283.4	T-IgA	249.7	13%	235.82	-16.79
12	IGA_2	210.7	T-IgA	237.6	-12%	224.45	6.53
14	IGA_2	215.3	T-IgA	219.4	-2%	207.35	-3.69
1	IGA_2	78	T-IgA	98.2	-23%	93.49	19.85
6	IGA_2	183.8	T-IgA	201.2	-9%	190.25	3.51
9	IGA_2	199.9	T-IgA	204.2	-2%	193.07	-3.42
20	IGA_2	**567.6**	T-IgA	635.8	-11%	**598.56**	5.45
2	IGA_2	147.1	T-IgA	148.6	-1%	140.84	-4.26
19	IGA_2	302.1	T-IgA	309.4	-2%	291.91	-3.37

13	IGA_2	214.3	T-IgA	205.8	4%	194.58	-9.20
8	IGA_2	185.8	T-IgA	195.4	-5%	184.81	-0.54
Average		225.905		239.14	-0.06098191	225.89938	0.00

Table III Bias estimation, Siemens versus Theranos methods.

8 CTN REFERENCE RANGE VERIFICATION

8.1 20 unique fingerstick samples collected in capillary tube and nonotainers (CTNs) from healthy donors were 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 +1.3073)/1.0644, and are shown in Table III under the column labelled T-Corr.

8.2 19/20 (95%) of corrected CTN values fell within the predicate reference range (40-350 mg/dL) (CLSI guidance C28-A3c).. Excluding Theranos values where the corresponding predicate value fell out of the reference range, 19/19 (100%) of corrected Theranos values fell within the reference range. Therefore the predicate reference range is verified for the Theranos method.

9 REFERENCES

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