theranos	Immunoglobulin M	Document Number: CL-RPT-145012 Revision: A
	Validation Document	Effective Date: 1/17/2014
Immunoglobulin M (IgM) Assay Validation Report		

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1 ASSAY BACKGROUND

Immunoglobulins are a group of related proteins responsible for removal of bacteria, viruses and other pathogenic organisms from the body. Five major immunoglobulins exist varying in structure and function: IgA, IgG, IgM, IgE and IgD. In serum, IgA, IgG and IgM are most common. Immunoglobulin constitutes approximately 20% of total serum protein. IgM accounts for approximately 6% of all immunoglobulins in serum. IgM is particularly effective in eliminating gram negative bacteria, and fixes complement more effectively than IgG. IgM does not cross the placenta because it exists in pentamers connected by J-chain protein.

IgM deficiency can be seen in the context of primary immunodeficiency, as well as chronic lymphocytic leukemia (CLL), while increased IgM serum protein is seen in rheumatoid arthritis, chronic infection, and Waldrenstrom's macroglobulinemia (Lymphoplasmacytic Lympoma).

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 ADVIA Chemistry IGM_2 assay is a PEG-enhanced immunoturbidimetric method. When serum or plasma containing human IgM is mixed with the IGM_2 reagents, agglutination takes place that results in an increase in turbidity. This turbidity is measured at 340 and 694 nm. By constructing a standard curve from the absorbencies of standards, concentrations of IgM 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 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 = 64.7 mg/dL

5.1.2 Mid = 149 mg/dL

5.1.3 Low = 220 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 listed in section 5.

Table I: Precision at 3 decision levels

Precision

CLSI guideline EP05-A2 section 10.8

Level = Level 1

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	65.74

	SD	95% CI	CV	Allowable Total SD
Repeatability	1.54	1.18 to 2.23	2.3%	-
Between-run	1.58		2.4%	-
Between-day	4.92		7.5%	-
Within-laboratory	5.39	3.83 to 9.11	8.2%	19.72

Imprecision is less than allowable total imprecision: 30%.

Level = Level 2

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	155.66

	SD	95% CI	CV	Allowable Total SD
Repeatability	2.34	1.79 to 3.38	1.5%	-
Between-run	3.88		2.5%	-
Between-day	10.85		7.0%	-
Within-laboratory	11.76	8.29 to 20.16	7.6%	46.70

Imprecision is less than allowable total imprecision: 30%.

Level = Level 3

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	232.56

	SD	95% CI	CV	Allowable Total SD
Repeatability	6.85	5.24 to 9.89	2.9%	-
Between-run	3.77		1.6%	-
Between-day	15.69		6.7%	-
Within-laboratory	17.53	12.57 to 28.94	7.5%	69.77

Imprecision is less than allowable total imprecision: 30%.

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	64.7	65.7	101.5%
2	149	155.7	104.5%
3	220	232.6	105.5%

6.3 Acceptance criteria:

Total allowable error (TAE %) of 30%, 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. SD as well as total error guidelines as stated in 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 (%). Values in brackets indicate the closest/corresponding API levels obtained from 2013 proficiency data.

Table II

	Level 1	Level 2	Level 3
TAE% (3XCV %)	30	30	30
CV (%)	8.2	7.6	7.5
Allowable Bias (%)	21.8	22.4	22.5
Bias (%) see section 7.1	10.2	4.4	5.3

7 BIAS ESTIMATION: THERANOS METHOD

7.1 Bias estimation was conducted by comparing the average value of 4 replicate Theranos measurements to the assigned QC level controls, and calculating percent recovery (Nominal value-Theranos value/Assigned Value)*100, and percent bias (100%-percent recovery), and included in total error calculations as shown in Section 6.1.2.

Level 1 (66 mg/dL), Average Theranos value: 72.7 mg/dL, Percent Bias; 10.2%
Level 2 (156 mg/dL), Average Theranos value: 162.8 mg/dL, Percent Bias: :4.4%
Level 3 (233 mg/dL), Average Theranos value: 245.3 mg/dL. Percent Bias: 5.3%

The total bias was less than the allowable bias based on the total allowable error and precision estimates.

8 CAPILLARY TUBE AND NANOTAINER (CTN) 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. The 2-4 fingerstick samples were pooled and tested in replicates of 2. Figure 2 shows the values obtained in this study, and the relationship between datasets is described using simple linear regression.

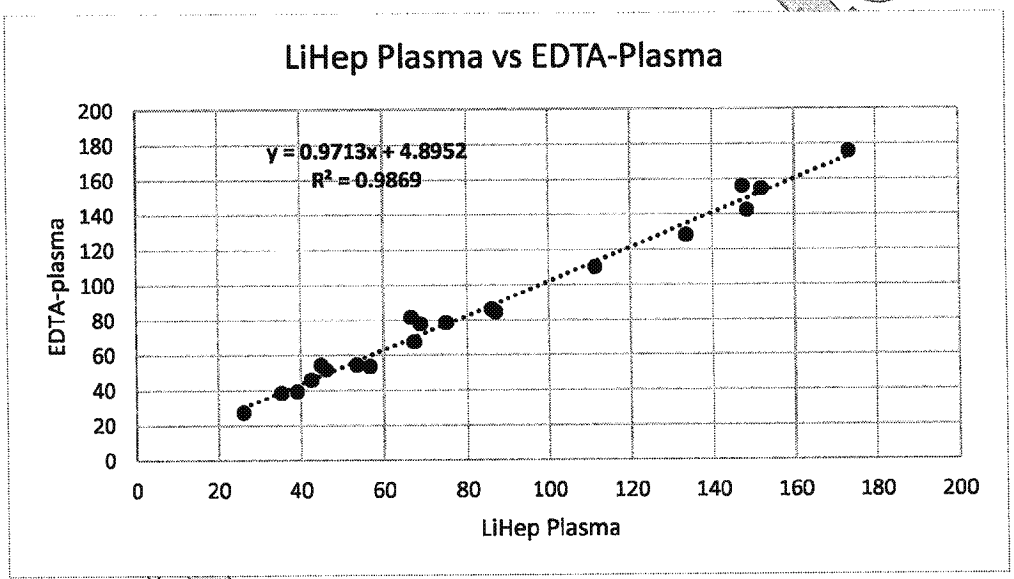

Figure 2:Bias estimation, LiHep Plasma versus EDTA-Plasma

The raw data used to establish this Scatterplot is shown in Table III below:

IgM	EDTA plasma						LiHep Plasma				
Donor	Rep-1	Rep-2	Average	STDEV	CV	CV%	Rep-1	Rep-2	Average	STDEV	%CV
1	65.7	69.8	67.75	2.9	0.043	4.3	69.7	64.9	67.3	3.39	5.04
2	154.8	157.1	155.95	1.6	0.010	1.0	146.4	148.4	147.4	1.41	0.96
3	156.5	152.5	154.5	2.8	0.018	1.8	153.6	150.6	152.1	2.12	1.39
4	76.3	79.9	78.1	2.5	0.033	3.3	68.5	69.2	68.85	0.49	0.72
5	54.5	54.3	54.4	0.1	0.003	0.3	43.4	46.3	44.85	2.05	4.57
6	129.4	127.2	128.3	1.6	0.012	1.2	134.6	132.6	133.6	1.41	1.06
7	26.6	28.3	**27.45**	1.2	0.044	4.4	26.7	25.3	**26**	0.99	3.81
8	52.8	50.7	51.75	1.5	0.029	2.9	47.9	44.2	**46.05**	2.62	5.68
9	38.8	40.3	**39.55**	1.1	0.027	2.7	38.9	39.1	**39**	0.14	0.36
10	82.7	80.8	81.75	1.3	0.016	1.6	65.4	67.9	66.65	1.77	2.65
11	45.4	46.7	**46.05**	0.9	0.020	2.0	44.6	40.4	**42.5**	2.97	6.99
12	109	111.2	110.1	1.6	0.014	1.4	108.6	114	111.3	3.82	3.43
13	83.1	86.4	84.75	2.3	0.028	2.8	84.3	89.6	86.95	3.75	4.31
14	171.1	181	176.05	7.0	0.040	4.0	171.5	175.1	173.3	2.55	1.47
15	84.6	88.3	86.45	2.6	0.030	3.0	85.2	86.8	86	1.13	1.32
16	52.7	54.8	53.75	1.5	0.028	2.8	57	56.2	56.6	0.57	1.00
17	77	80.5	78.75	2.5	0.031	3.1	74.3	75.7	75	0.99	1.32
18	51.2	58.1	54.65	4.9	0.089	8.9	52.3	54.6	53.45	1.63	3.04
19	38.1	39	**38.55**	0.6	0.017	1.7	33.5	36.8	**35.15**	2.33	6.64
20	140.8	143.8	142.3	2.1	0.015	1.5	148.3	148.8	148.55	0.35	0.24

Table III: Bias estimation, LiHep Plasma versus EDTA-Plasma

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

8.3 Acceptance Criteria were as follows:

8.3.1 All samples must have %CV within 20%

8.3.2 This criterion is satisfied.

8.3.2 Scatter plots should have a slope of 1 +/- 0.3 and R^2 greater than 0.8

8.3.2.1 This criterion is satisfied.

9 REFERENCE RANGE VERIFICATION

20 unique EDTA-CTN samples were collected and assayed in duplicate using the Theranos methods, as described in Section 8, and the average value was calculated. Of the 20 values obtained, 4 were excluded because the matching predicate value was also out of range. 16 out of the remaining 16 Theranos values (100%) were within the manufacturer's stated reference range for expected values (50-300 mg/dL), The predicate RR (50-300mg/dL) is therefore verified (CLSI guidance C28-A3c).

10 REFERENCES

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

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