theranos	Anti-Streptolysin O (ASO) Validation Report	Document Number: CL-RPT-145002 Revision: A
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Antistreptolysin O (ASO) Assay Validation Report		

Author(s):

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:	Date: 3/18/2014
Name: Daniel Young, Ph.D.	Title: Vice President

Approver(s):

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

9/19/14

Sunil S. Dhawan M.D.

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1 ASSAY BACKGROUND

During acute infection with group-A hemolytic Streptococci, antibodies to bacterial exotoxins are produced, that react with streptolysin O coated latex beads. ASO reactivity is also seen in rheumatic fever (valvular heart disease), and streptococcal glomerulonephritis. ASO can be used as an adjunct to the rapid strep test in the acute care setting.

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 those infections promoted by acute streptococcal infection, antibodies to the exotoxin of streptococcus are usually produced. By reacting suspended uniform latex particles coated with streptolysin-O with serum containing antibodies, an increase in turbidity occurs. By comparing with a standard, a quantitative value for the concentration of anti-streptolysin-O present in serum is obtained.

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 High = 85 IU/mL

5.1.2 Mid = 156 IU/mL

5.1.3 Low = 223 IU/mL

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 (High = 223 IU/mL, Mid = 156 IU/mL, Low = 85 IU/mL). The following data describes the results obtained:

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	81.99

	SD	95% CI	CV	Allowable Total SD
Repeatability	1.89	1.45 to 2.73	2.3%	-
Between-run	0.00		0.0%	-
Between-day	1.66		2.0%	-
Within-laboratory	2.52	1.96 to 3.53	3.1%	20.50

Imprecision is less than allowable total imprecision: 25%.

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	153.11

	SD	95% CI	CV	Allowable Total SD
Repeatability	2.12	1.62 to 3.06	1.4%	-
Between-run	2.81		1.8%	-
Between-day	1.41		0.9%	-
Within-laboratory	3.79	2.97 to 5.24	2.5%	38.28

Imprecision is less than allowable total imprecision: 25%.

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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	223.15

	SD	95% CI	CV	Allowable Total SD
Repeatability	2.80	2.14 to 4.04	1.3%	-
Between-run	3.33		1.5%	-
Between-day	3.74		1.7%	-
Within-laboratory	5.73	4.35 to 8.40	2.6%	55.79

Imprecision is less than allowable total imprecision: 25%.

6.1.2 The mean recovery was as follows:

Control#	Assigned (mg/dL)	Theranos (mg/dL)	% Recovery
1	85	82	96.4%
2	156	153	98%
3	223	223	100%

6.2 Acceptance criteria:

Total allowable error (TAE %) of 25%, 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 (82)	**Level 2** (153)	**Level 3** (223)
TAE%	25	25	25
CV (%)	3.1	2.5	2.6
Allowable Bias (%)	21.9	22.5	22.4
Bias (%) (Table III)	9	9	9

7 METHOD COMPARISON, THERANOS VERSUS PREDICATE

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 allowable bias therefore, the acceptance criteria PASS.

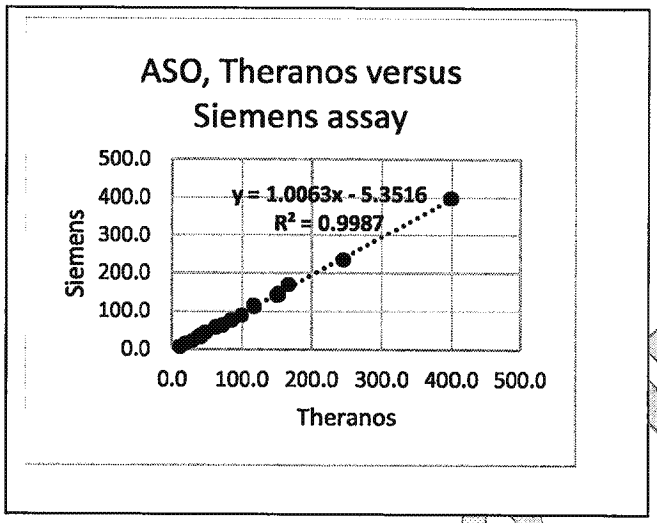

Figure 1: Bias estimation, Theranos versus Siemens assay

Sample #	Siemens	EDTA	Theranos	EDTA	% difference	T-corr	%difference
1	ASO_2	**398.0**	T-ASO	**398.0**	0%	**396.0**	0.00%
2	ASO_2	**244.4**	T-ASO	**235.5**	4%	234.6	3.64%
3	ASO_2	166.0	T-ASO	170.2	-2%	169.7	-2.53%
4	ASO_2	151.9	T-ASO	143.5	6%	143.1	5.53%
5	ASO_2	151.9	T-ASO	146.4	4%	146.0	3.62%
6	ASO_2	149.3	T-ASO	142.6	5%	142.2	4.49%
7	ASO_2	117.3	T-ASO	112.1	5%	111.9	4.43%
8	ASO_2	117.0	T-ASO	113.8	3%	113.6	2.74%
9	ASO_2	116.6	T-ASO	115.6	1%	115.4	0.86%
10	ASO_2	99.3	T-ASO	90.5	9%	90.5	8.86%
11	ASO_2	84.3	T-ASO	76.5	10%	76.5	9.25%
12	ASO_2	71.9	T-ASO	64.0	12%	64.1	10.99%
13	ASO_2	62.7	T-ASO	58.9	6%	59.1	6.06%
14	ASO_2	47.9	T-ASO	44.6	7%	44.8	6.89%
15	ASO_2	41.1	T-ASO	33.0	22%	33.3	19.71%
16	ASO_2	40.4	T-ASO	37.0	9%	37.3	8.42%
17	ASO_2	35.4	T-ASO	30.7	14%	31.0	13.28%

18	ASO_2	28.8	T-ASO	23.0	22%	23.4	20.14%
19	ASO_2	18.3	T-ASO	16.4	11%	16.8	10.38%
20	ASO_2	11.1	T-ASO	7.9	34%	8.4	28.83%
Average		107.7		103.0	9.0%	102.9	8.28%

Table III Bias estimation, Theranos versus Siemens assay

8 CTN REFERENCE RANGE VERIFICATION

8.1 20 unique capillary tube and nanotainer (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 +5.3516)/1.0063. The corrected values are shown in table III, under T-corr.

8.2 19/20 (95%) of corrected CTN values fell within the predicate reference range (<240 IU/mL), therefore the reference range is verified (CLSI C28-A3).

9 REFERENCES

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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.

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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.

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