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Prealbumin Immunoassay Validation Report

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TABLE OF CONTENT

1 ASSAY BACKGROUND 3
2 REGULATION AND GUIDANCE 3

3 PRINCPLE OF THE ASSAY 3

4 CALIBRATION 4
5 QUALITY CONTROL 5
6 PRECISION 5-8
7 BIAS ESTIMATION SIEMENS VS THERANOS 9-10
8 REFERENCE RANGE VERIFICATION 10

9 REFERENCES 12

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Prealbumin Immunoassay Validation Report

1 ASSAY BACKGROUND

Prealbumin (transthyretin or thyroxine-binding protein) is a 55KDa protein, synthesized in the liver, and is involved in thyroid hormone (T3 and T4) as well as Vitamin A transport. Prealbumin complexes with retinol binding protein to bind Vitamin A, and is capable of binding T3 or T4 at a different site. Prealbumin has a shorter half-life than albumin or transferrin and therefore may be a more sensitive marker for protein-calorie malnutrition or liver disease. Prealbumin is also a negative acute-phase reactant with levels falling rapidly during periods of infection or inflammation. During concomitant malnutrition and inflammation, prealbumin levels may be extremely low. The Kidney Diseases Outcome Initiative (KDOQI) has set a goal for prealbumin >30 mg/dL in patients undergoing dialysis. Prealbumin can also be elevated with elevated corticosteroids, or with non-steroidal anti-inflammatory use.

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

A sample is incubated with assay buffer. The antibody reagent, which is specific for human prealbumin, is then added. The resulting formation of antibody-antigen complex results in an increase in turbidity. The absorbance of the resulting turbid solution is proportional to the concentration of prealbumin in the sample.

Samples were diluted 1:3.125 in saline prior to the assay.

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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.

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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 = 31 mg/dL

5.1.2 Mid = 23.1 mg/dL

5.1.3 Low = 12.3 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.

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Figure 1: Level 1 Precision

Precision (CLSI EP05-A2)

Prealbumin (PREA) (mg/dL) using ADVIA at Theranos

Establish the precision of a measurement procedure (EP05-A2)

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	11.56

	SD	95% CI	CV	Allowable Total SD
Repeatability	0.39	0.30 to 0.57	3.4%	-
Between-run	0.09		0.8%	-
Between-day	0.18		1.5%	-
Within-laboratory	0.44	0.36 to 0.58	3.8%	1.16

Imprecision is less than allowable total imprecision: 10%.

Figure 2: Level 2 Precision

Prealbumin Immunoassay Validation Report

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	22.85

	SD	95% CI	CV	Allowable Total SD
Repeatability	0.36	0.28 to 0.53	1.6%	-
Between-run	0.00		0.0%	-
Between-day	0.22		1.0%	-
Within-laboratory	0.43	0.34 to 0.57	1.9%	2.28

Imprecision is less than allowable total imprecision: 10%.

Figure 3: Level 3 Precision

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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	31.62

	SD	95% CI	CV	Allowable Total SD
Repeatability	0.23	0.18 to 0.34	0.7%	-
Between-run	0.49		1.5%	-
Between-day	0.66		2.1%	-
Within-laboratory	0.85	0.63 to 1.35	2.7%	3.16

Imprecision is less than allowable total imprecision: 10%.

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

7.1 The Siemens-recommended tube type for Prealbumin 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.

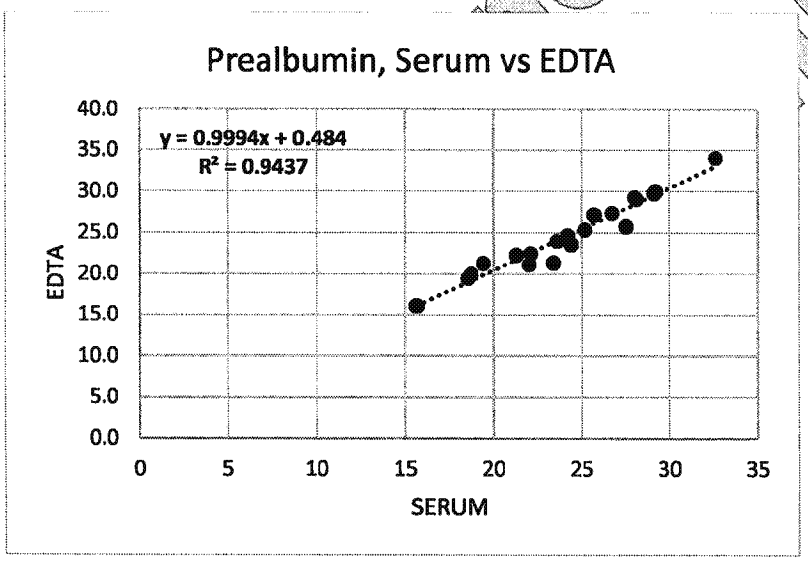

Figure I

Sample #	Siemens	Serum	Theranos	EDTA	%Difference
1	PREALB	23.4	T-PREAL	21.4	-8.5
2	PREALB	27.5	T-PREAL	25.8	-6.2
3	PREALB	32.6	T-PREAL	34.1	4.6
4	PREALB	22.0	T-PREAL	21.2	-3.6

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5	PREALB	23.6	T-PREAL	24.0	1.7
6	PREALB	24.2	T-PREAL	24.7	2.1
7	PREALB	24.4	T-PREAL	23.6	-3.3
8	PREALB	18.5	T-PREAL	19.5	5.4
9	PREALB	22.1	T-PREAL	22.5	1.8
10	PREALB	29.2	T-PREAL	30.0	2.7
11	PREALB	19.4	T-PREAL	21.3	9.8
12	PREALB	21.3	T-PREAL	22.3	4.7
13	PREALB	25.7	T-PREAL	27.2	5.8
14	PREALB	26.7	T-PREAL	27.4	2.6
15	PREALB	28.1	T-PREAL	29.1	3.6
16	PREALB	15.6	T-PREAL	16.1	3.2
17	PREALB	28.0	T-PREAL	29.3	4.6
18	PREALB	29.1	T-PREAL	29.8	2.4
19	PREALB	18.7	T-PREAL	20.0	7.0
20	PREALB	25.2	T-PREAL	25.4	0.8
Average					2.1

Table III: Bias estimation, Serum (Siemens) versus EDTA (Theranos)

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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-4.84/0.9994), and are shown in table IV.

8.2 **20/20 (100%) of corrected CTN values fell within the predicate reference range (10-40 mg/dL) (CLSI guidance C28-A3c).** Therefore the predicate reference range is verified.

Sample #	Siemens	EDTA
1	PREALB	20.9
2	PREALB	25.3
3	PREALB	33.6
4	PREALB	20.7
5	PREALB	23.5
6	PREALB	24.2
7	PREALB	23.1
8	PREALB	19.0
9	PREALB	22.0
10	PREALB	29.5
11	PREALB	20.8
12	PREALB	21.8
13	PREALB	26.7
14	PREALB	26.9
15	PREALB	28.6
16	PREALB	15.6
17	PREALB	28.8
18	PREALB	29.3
19	PREALB	19.5
20	PREALB	24.9

Table IV: Reference range verification: Corrected Theranos (CTN) values

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9 REFERENCES

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