theranos	Beta-2 Microglobulin	Document Number: CL-RPT-145003 Revision: A
	Validation Document	Effective Date: 1/17/2014
Beta-2 Microgloblin Validation Report		

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1 ASSAY BACKGROUND

β 2-Microglobulin has a molecular weight of 11,800 Da and occurs on all nucleated cells as a component of the HLA complex. It is constantly released into the blood in small quantities. β 2-Microglobulin is freely filtered in the kidneys where it is reabsorbed and degraded in the renal tubules. Therefore, the serum levels found in healthy individuals remain at consistently low levels.

A rise of serum concentration occurs as a result of a higher release of β 2-microglobulin due to increased activity of the immune system, such as in infections or rheumatic diseases, cell death, or diminished elimination due to renal damage. The serum concentration of β 2-microglobulin is thus a sensitive marker for the glomerular filtration capacity of the kidneys.
Because the serum concentration of β 2-microglobulin can be elevated in a variety of disease states, its diagnostic application should always follow a clear clinical question and rule out the presence of other relevant diseases. Elevated concentrations of β 2-microglobulin in serum or plasma are also found in patients with multiple myeloma and chronic lymphatic leukemia. In situations with increased cell proliferation, its specificity can be enhanced by determining the β 2-microglobulin to cystatin C ratio.

2 REGULATION AND GUIDANCE

2.1 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 the β2-Microglobulin (B2M) assay, sample is diluted and reacted with a buffer that contains latex particles coated with antibody specific for β2-microglobulin. The formation of the antibody-antigen complex during the reaction results in an increase in turbidity, the extent of which is measured as the amount of light absorbed at 545 nm. The B2M concentration in a sample is determined by constructing a standard curve from the absorbance of a reagent blank and a single-level calibrator.

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 Low = 0.735 mg/L

5.1.2 Mid = 1.52 mg/L

5.1.3 High = 2.68 mg/L

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:

Table 1: Precision at 3 medical decision limits

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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	1.493

	SD	95% CI	CV	Allowable Total SD
Repeatability	0.022	0.017 to 0.032	1.5%	-
Between-run	0.019		1.3%	-
Between-day	0.012		0.8%	-
Within-laboratory	0.032	0.025 to 0.043	2.1%	0.149

Imprecision is less than allowable total imprecision: 10%.

Precision (CLSI EP05-A2)

Beta-2 Microglobulin (mg/dL) by ADVIA-diluted using Theranos 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	0.697

	SD	95% CI	CV	Allowable Total SD
Repeatability	0.019	0.014 to 0.027	2.7%	-
Between-run	0.000		0.0%	-
Between-day	0.012		1.7%	-
Within-laboratory	0.022	0.018 to 0.030	3.2%	0.070

Imprecision is less than allowable total imprecision: 10%.

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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	1.493

	SD	95% CI	CV	Allowable Total SD
Repeatability	0.022	0.017 to 0.032	1.5%	-
Between-run	0.019		1.3%	-
Between-day	0.012		0.8%	-
Within-laboratory	0.032	0.025 to 0.043	2.1%	0.149

Imprecision is less than allowable total imprecision: 10%.

Precision

CLSI guideline EP05-A2 section 10.8

Level = 3

Number of observations	40
Number of runs	20
Number of days	20
Runs per day	1
Replicates per run	2

CLSI guideline EP05-A2 section 10.4 recommends a minimum of 30 runs, with 2 replicates per run; or 20 runs, with 3 or more replicates per run.

Mean	2.658

	SD	95% CI	CV	Allowable Total SD
Repeatability	0.024	0.018 to 0.035	0.9%	-
Between-day	0.048		1.8%	-
Within-laboratory	0.054	0.042 to 0.075	2.0%	0.266

Imprecision is less than allowable total imprecision: 10%.

6.2 The mean recovery of controls 1,2 and 3 versus the assigned values was as follows:

Control#	Assigned (mg/L)	Theranos (mg/L)	% Recovery
1	0.735	0.697	94.8%
2	1.52	1.493	98.2%
3	2.68	2.658	99%

6.3 Acceptance criteria:

Total allowable error (TAE %) of 28%, 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, sample TM-02 (~3.6 mg/L) using a cutoff of +/- 2SD. Allowable bias was calculated as the residual error budget after precision values (CV %) were subtracted from TAE (%).

Table II Total Allowable Error % (TAE%)

	Level 1	Level 2	Level 3
TAE%	28	28	28

CV (%)	3.2	2.1	2.0
Allowable Bias (%)	24.8	25.9	26.0
Bias (%)	2	2	2
Decision	Pass	Pass	Pass

7 BIAS ESTIMATION: COMPARISON OF PREDICATE WITH THERANOS METHODS

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

Mean bias is less than the allowable bias therefore the acceptance criteria PASS

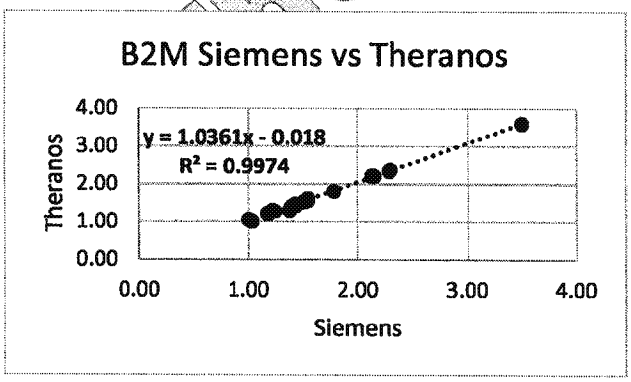

Figure 1 Bias Estimation, Predicate versus Theranos Assay

Sample #	Siemens	EDTA	Theranos	EDTA2	% difference
1	B2M	3.50	T-B2M	3.60	-3%
2	B2M	1.00	T-B2M	1.06	-6%
3	B2M	1.02	T-B2M	1.03	-1%
4	B2M	1.03	T-B2M	1.02	1%
5	B2M	1.18	T-B2M	1.22	-3%
6	B2M	1.18	T-B2M	1.21	-3%
7	B2M	1.22	T-B2M	1.30	-6%
8	B2M	1.23	T-B2M	1.28	-4%
9	B2M	1.37	T-B2M	1.31	4%
10	B2M	1.38	T-B2M	1.39	-1%
11	B2M	1.42	T-B2M	1.46	-3%
12	B2M	1.42	T-B2M	1.42	0%
13	B2M	1.50	T-B2M	1.53	-2%
14	B2M	1.54	T-B2M	1.56	-1%
15	B2M	1.54	T-B2M	1.61	-4%
16	B2M	1.78	T-B2M	1.81	-2%
17	B2M	2.13	T-B2M	2.22	-4%
18	B2M	2.15	T-B2M	2.22	-3%
19	B2M	2.29	T-B2M	2.35	-3%
20	B2M	2.30	T-B2M	2.38	-3%
Average					-2%

Table III: Method comparison ,predicate versus p-Assay (Theranos)

8 CTN REFERENCE RANGE VERIFICATION

8.1 20 unique fingerstick samples collected in capillary tube and nanotainers (CTNs) 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 +0.018)/1.0361

8.2 19/20 (95%) of corrected CTN values fell within the predicate reference range (1.0-2.4 mg/L) therefore the reference range is verified. (CLSI guidance C28-A3c). Raw values before and after correction are shown below; results which fall outside the RR are shown in red.

Sample #	EDTA
1	3.60
2	1.06
3	1.03
4	1.02
5	1.22
6	1.21
7	1.30
8	1.28
9	1.31
10	1.39
11	1.46
12	1.42
13	1.53
14	1.56
15	1.61
16	1.81
17	2.22
18	2.22
19	2.35
20	2.38

9 REFERENCES

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