CDC Vitamin D Standardization-Certification Program (VDSCP)

Participation Evaluation Report

25-hydroxyvitamin D in serum (25OHD)

Lab ID: LXX **Cycle:** #
Data Collection Time Frame: Date

Mean Bias Evaluation Results

Acceptable bias criteria is ± 5.0%.[3]

Bias (%)					
Mean	**SD**	**95% Confidence Interval**		**Proportion of Samples Meeting Bias Criteria**	**Evaluation Result**
-3.4	4.2	-4.8	-2.1	55%	WITHIN RANGE

Mean Imprecision Evaluation Results

Acceptable imprecision criteria is ≤ 10.0%.[3]

Imprecision (%CV)				
Mean	**SD**	**10th and 90th Percentile**		**Evaluation Result**
3.8	1.4	1.8	6.0	WITHIN RANGE

Certification Status: CERTIFIED

Information planned to be published on the CDC Website:
Please review the information to ensure it is correct and is how you would like to be represented on the CDC website as a standardized laboratory.

Participant	Measurement Principle	Method Identifier	Measurement Range (nmol/L)	Proportion of Samples Meeting Bias Criteria	Participant contact information	Certification Date
				55%		

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DATA ANALYSIS FOR TOTAL 25(OH)D

Lab & Platform ID: LXX

The following report summarizes information on bias and imprecision of total 25-hydroxyvitamin D [25(OH)D] measurements performed as part of the VDSCP. Total 25-hydroxyvitamin D [25(OH)D] is defined as the sum of 25(OH)D2 and 25(OH)D3. The bias is the difference between the mean value reported for a sample by the participant and the reference value assigned to the sample.

The reference measurements were performed in Professor Dr. Linda Thienpont's Laboratory at the University of Ghent and at the Centers for Disease Control and Prevention by Mineva et al.[1,2] Reference values can be found in Appendix B of this report.

Method comparison and bias estimation were performed by the procedures described in CLSI document EP9-A2 "Method Comparison and Bias Estimation Using Patient Samples."[3] Linear regression analysis and difference plots were calculated using Analyze-It Software package (Version 2.21 Excel 12+). Findings on measurement bias and imprecision were compared against the suggested performance criteria for 25(OH)D of $\pm$5.0 % bias and $\leq$10.0 % for imprecision.[4]

The following pages include four graphs described in Section 4.2 of CLSI EP9-A2. Graphs B1 (sample mean) and B2 (individual values) are scatter plots with linear regression line and 95% prediction intervals. Graphs B3 (sample mean) and B4 (individual values) are bias plots showing the suggested bias criterion. Additional information on bias, imprecision and total error is provided, which is not part of the CLSI EP9-A2 protocol.

To be considered certified, a participant must meet criteria for bias and imprecision.

SUMMARY OF DATA POINTS RECEIVED BY PARTICIPANT for TOTAL 25(OH)D

Expected data points:	160
Sample Means:	40
Data points outside reportable range (3.03 - 225 nmol/L):	0
Data points not reported by participant:	0
Data points removed according to EP9-A2:	0

*Please refer to **Appendix A** for a summary of all reported data.*

References

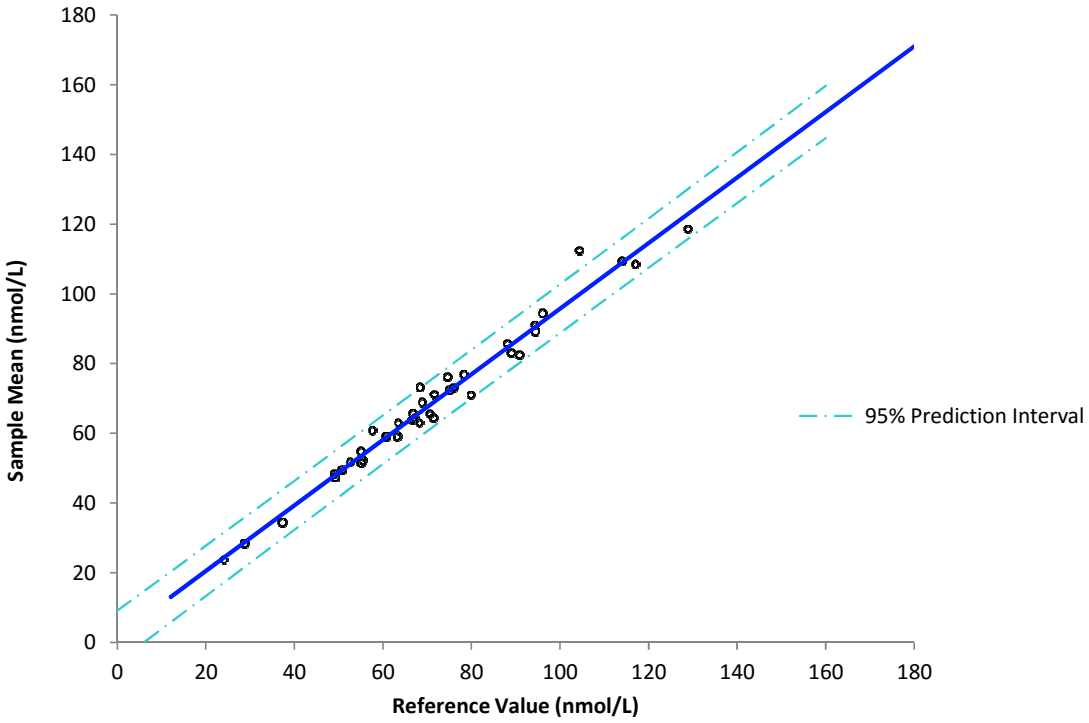
(1) Stepman HC, Vanderroost A, Van Uytfanghe K, Thienpont LM. Candidate Reference Measurement Procedures for Serum 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 by Using Isotope-Dilution Liquid Chromatography-Tandem Mass Spectrometry. Clin Chem. 2011; 57: 441-448.
(2) Mineva EM, Schleicher RL, Chaudhary-Webb M, Maw KL, Botelho JC, Vesper HW, Pfeiffer CM. A Candidate Reference Measurement Procedure for Quantifying Serum Concentrations of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 Using Isotope-Dilution Liquid Chromatography-Tandem Mass Spectrometry. Analytical and Bioanalytical Chemistry. 2015; 407(19): 5615-5624.
(3) Clinical Laboratory Standards Institute. Method Comparison and Bias Estimation Using Patient Samples (CLSI document EP9-A2). Wayne, PA: Clinical Laboratory Standards Institute. 2002.
(4) Stöckl D, Sluss PM, Thienpont LM. Clin Chim Acta 2009; 408: 8-13.
(5) Fraser CG. Biological variation: from principles to practice. Washington DC. AACC Press, 2001.
(6) Clinical Laboratory Standards Institute. CLSI document C37. Wayne, PA: Clinical Laboratory Standards Institute. 1999.

LINEAR REGRESSION USING MEAN OF REPORTED VALUES

B1 in Section 4.2 CLSI EP9-A2

n	40
R^2	0.9882

Term	Coefficient	95% CI		SE	p
Intercept	1.7011	-1.0610	4.4631	1.3644	0.2201
Slope	0.9403	0.9065	0.9741	0.0167	<0.0001

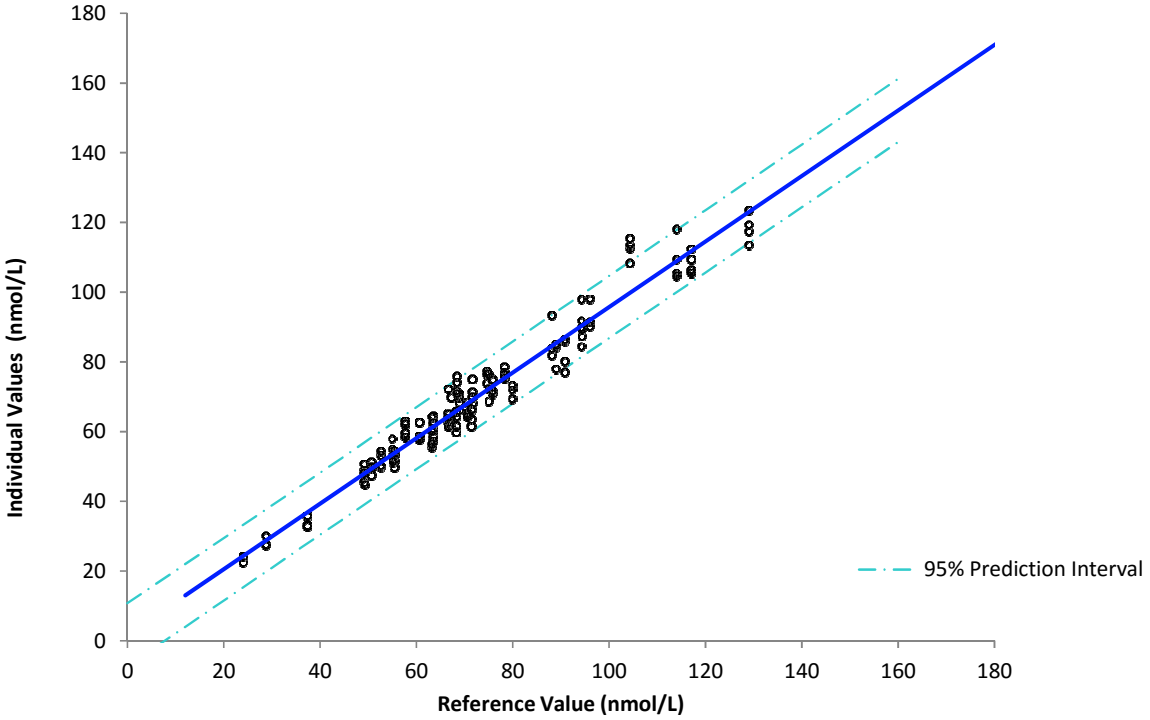


LINEAR REGRESSION USING INDIVIDUAL REPORTED VALUES

B2 in Section 4.2 CLSI EP9-A2

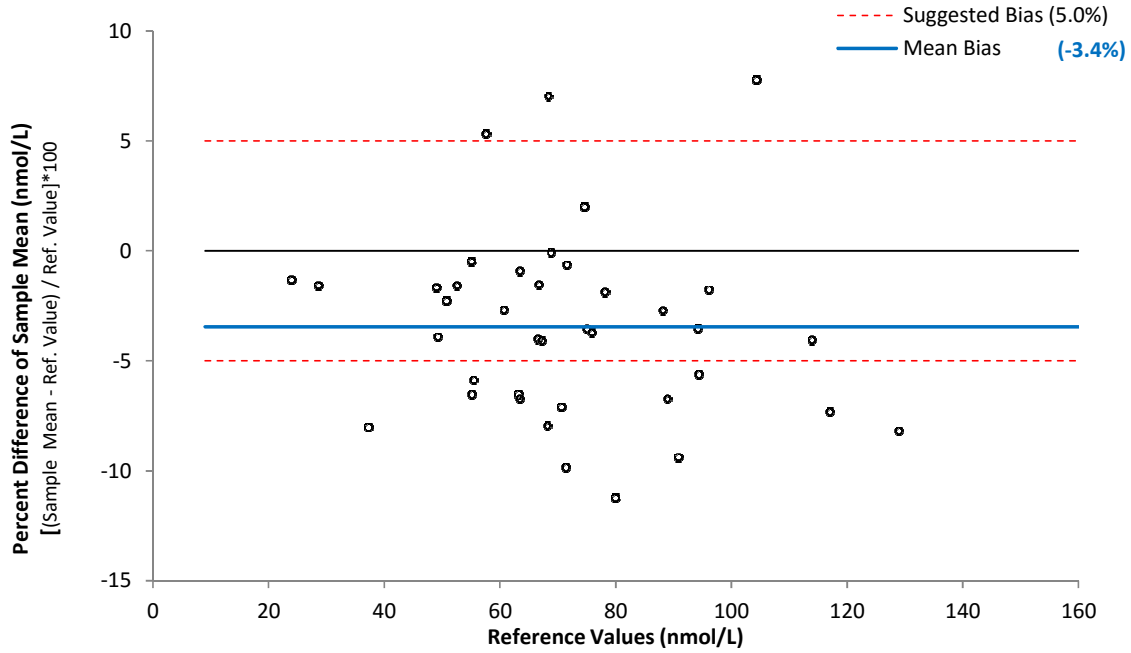
n	160
R^2	0.9786

Term	Coefficient	95% CI		SE	p
Intercept	1.7011	-0.0866	3.4887	0.9051	0.0620
Slope	0.9403	0.9184	0.9622	0.0111	<0.0001



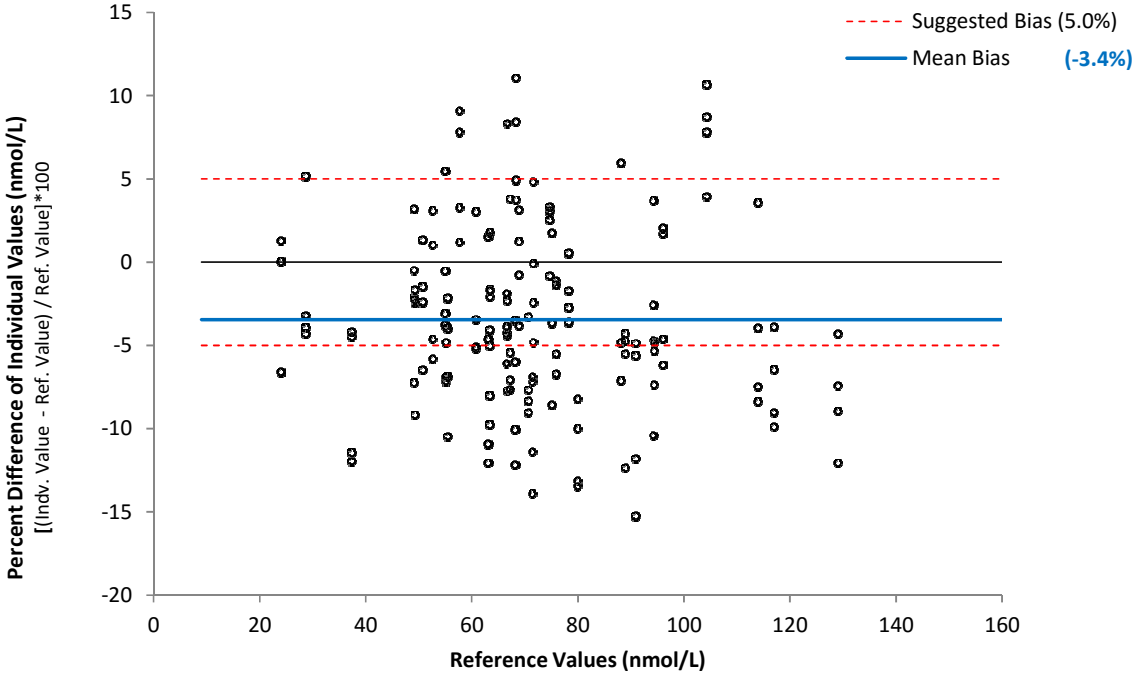
DIFFERENCE PLOT USING SAMPLE MEAN OF REPORTED VALUES

B3 in Section 4.2 CLSI EP9-A2



DIFFERENCE PLOT USING INDIVIDUAL REPORTED VALUES

B4 in Section 4.2 CLSI EP9-A2



BIAS EVALUATION OF MEAN REPORTED VALUES (N=40)

Acceptable bias criteria is ± 5.0%.[4]

Overall Mean Bias on Mean of Reported Values Per Sample Data

For bias assessments, the mean of replicate measurements (n=4) was calculated for each sample and compared to the reference value. The mean bias was calculated from the individual sample biases of all 40 samples to determine the overall bias.

Bias (%)						
Mean	**SD**	**95% Confidence Interval**		**Min**	**Max**	**Evaluation Result of Mean Bias**
-3.4	4.2	-4.8	-2.1	-11.2	7.8	WITHIN RANGE

The following evaluations are provided for information only.

Data Evaluation Using Subgroups on Mean Reported Values

(Section 6.3 in CLSI EP9)

			Bias (%)					
Subgroup	**Reference Value Conc. Range (nmol/L)**		**Mean**	**SD**	**95% Confidence Interval**		**Min**	**Max**
1 (n=13)	24.0	63.2	**-2.9**	3.5	-4.8	-0.9	-8.0	5.3
2 (n=13)	63.4	75.1	**-2.9**	4.6	-5.4	-0.3	-9.8	7.0
3 (n=14)	75.9	220	**-4.5**	4.5	-7.0	-2.1	-11.2	7.8

The following evaluations are provided for information only.

Mean of Reported Results WITHIN RANGE of Bias Criterion

Assessment was made using mean values reported from 4 replicate measurements

	n	**Reference Value Concentration Range (nmol/L)**		**Samples within suggested criterion (%)**
Group 1	13	24.0	63.2	62
Group 2	13	63.4	75.1	62
Group 3	14	75.9	220	43
OVERALL	**40**	**24.0**	**220**	**55**

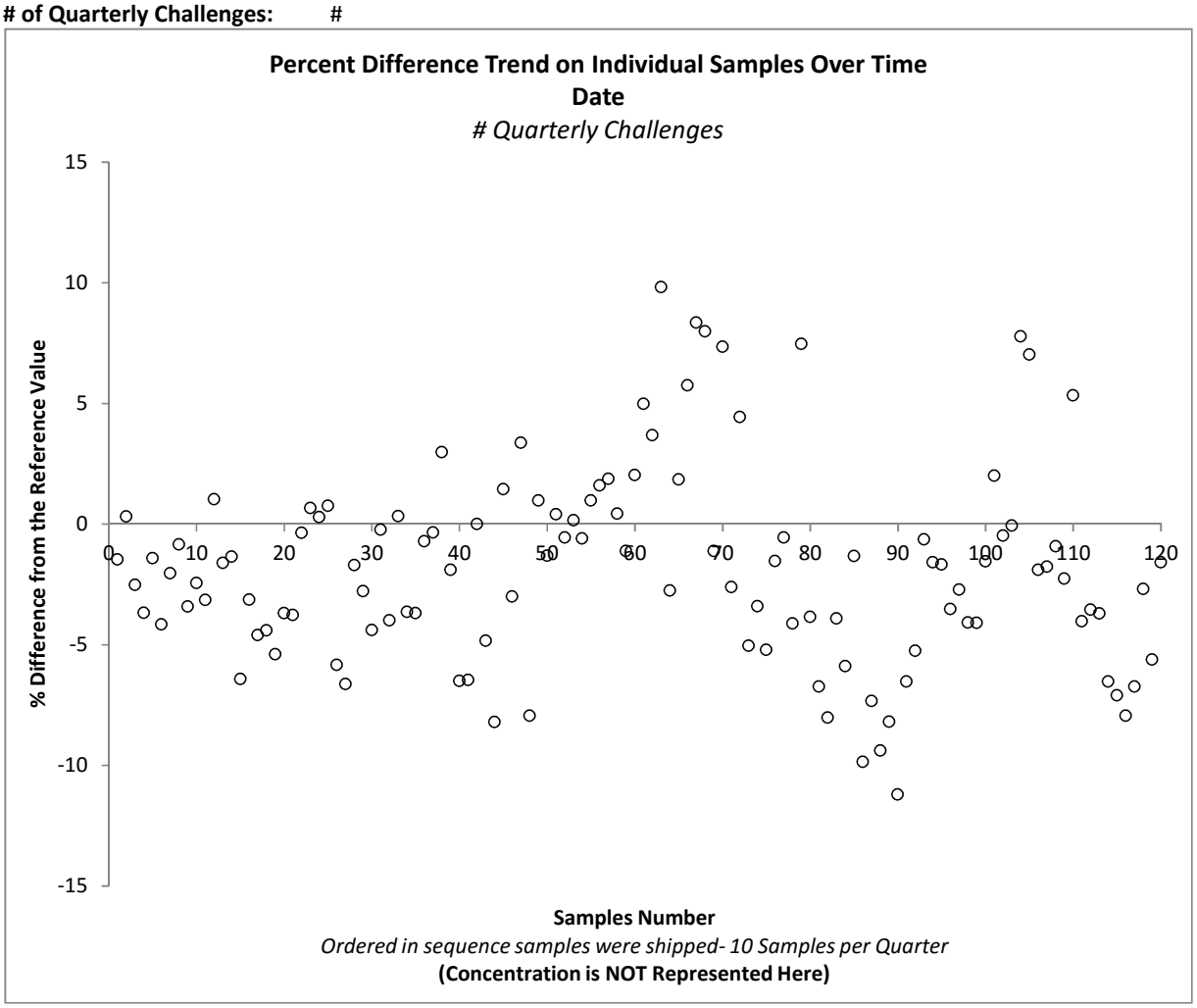
BIAS EVALUATION OF MEAN REPORTED VALUES (N=40)- CON'T

The following evaluations are provided for information only.

Percent Difference Trend on Individual Samples Over Time

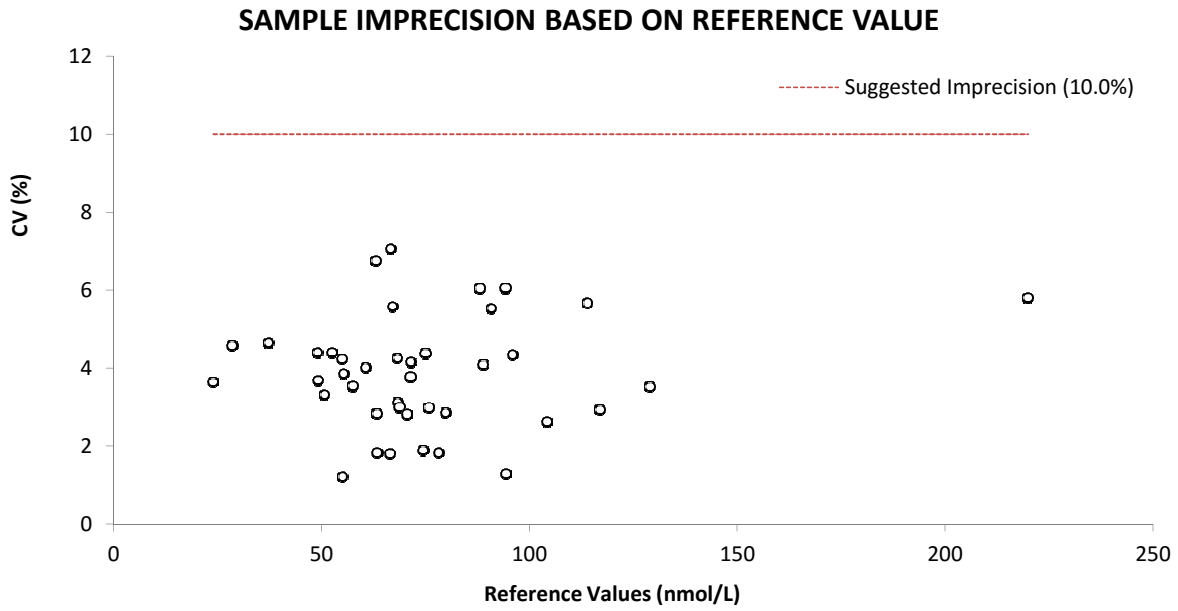
Dates: Date

# of Quarterly Challenges: #



IMPRECISION EVALUATION

Acceptable imprecision criteria is ≤ 10.0%.[4] (Not part of EP9-A2)



Overall Mean Imprecision of Reported Values

For imprecision assessments, the percent coefficient of variation (%CV) of the replicate measurements (n=4) was calculated for each sample. The mean imprecision was calculated from the %CV from all 40 samples to determine the overall imprecision.

Imprecision (%CV)						
Mean	**SD**	**10th and 90th Percentile**		**Min**	**Max**	**Evaluation Result of Mean Imprecision**
3.8	1.4	1.8	6.0	1.2	7.0	WITHIN RANGE

IMPRECISION EVALUATION (CON'T)

The following evaluations are provided for information only.

Acceptable imprecision is ≤ 10.0%.[4] (Not part of EP9-A2)

Data Evaluation Using Subgroups

			Imprecision (%CV)					
Subgroup	**Reference Value Conc. Range (nmol/L)**		**Mean**	**SD**	**10th - 90th Percentile**		**Min**	**Max**
1 (n=13)	24.0	63.2	**4.0**	1.2	2.0	5.9	1.2	6.7
2 (n=13)	63.4	75.1	**3.6**	1.5	1.8	6.5	1.8	7.0
3 (n=14)	75.9	220	**4.0**	1.6	1.5	6.0	1.3	6.0

Samples within Imprecision Criterion

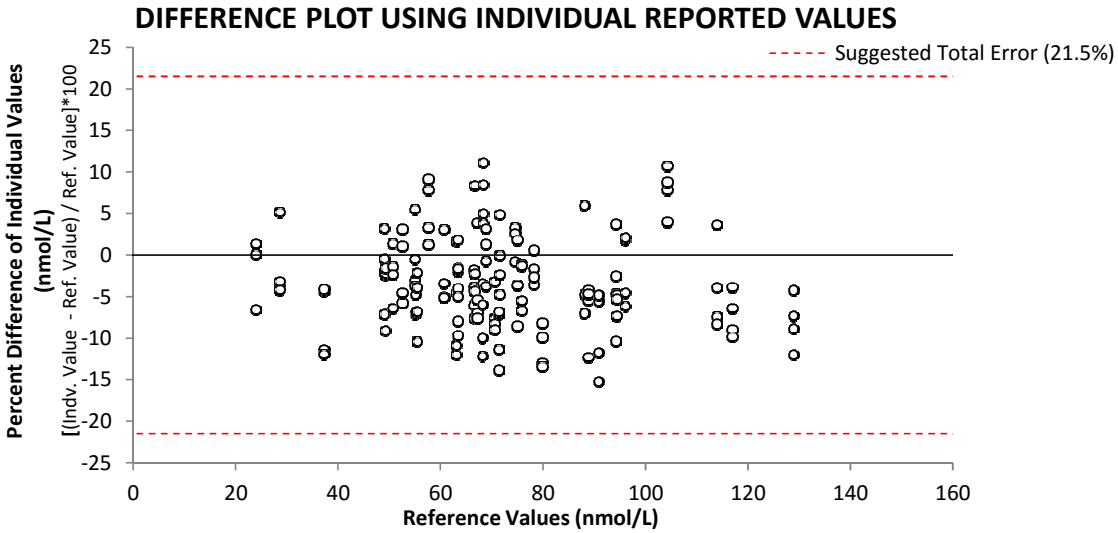
Assessment was made using individual values reported over 2 days (n=4) for each sample.

	n	**Reference Value Concentration Range (nmol/L)**		**Samples within suggested criterion (%)**
Group 1	13	24.0	63.2	100
Group 2	13	63.4	75.1	100
Group 3	14	75.9	220	100
OVERALL	**40**	**24.0**	**220**	**100**

TOTAL ERROR ASSESSMENT USING INDIVIDUAL REPORTED VALUES

The following evaluations are provided for information only.

The suggested total error was calculated[5] as ± 21.5% based on the suggested bias and imprecision of ± 5.0% and ≤ 10.0%, respectively. (Not part of EP9-A2)



The following evaluations are provided for information only.

Samples within Suggested Total Error Criterion

Assessment was made using the percent difference of individual values reported over 2 days.

	n	Reference Value Concentration Range (nmol/L)		Samples within suggested criterion (%)
Group 1	53	24.0	63.2	100
Group 2	53	63.4	75.1	100
Group 3	54	75.9	220	100
OVERALL	**160**	**24.0**	**220**	**100**

APPENDIX A: REPORTED VALUES

			Reported Results (nmol/L)				
Sample #	**Phase**	**Shipment Date**	**Day 1**		**Day 2**		**Overall Mean**
1	2	Date	57.7	58.7	57.6	62.6	**59.1**
2	2	Date	27.6	27.8	30.2	27.5	**28.2**
3	2	Date	68.2	71.6	75.1	69.9	**71.2**
4	2	Date	84.5	91.9	97.8	89.9	**91.0**
5	2	Date	117	113	119	123	**118**
6	2	Date	83.9	81.9	93.4	83.9	**85.7**
7	2	Date	53.2	49.6	50.2	54.2	**51.8**
8	2	Date	65.4	62.6	63.8	64.0	**63.9**
9	2	Date	76.9	78.7	75.4	76.1	**76.8**
10	2	Date	62.2	64.6	62.5	62.4	**62.9**
11	2	Date	24.4	22.5	24.1	24.1	**23.7**
12	2	Date	105	109	118	104	**109**
13	2	Date	97.8	98.1	91.7	90.2	**94.4**
14	2	Date	68.4	65.2	64.8	64.3	**65.7**
15	2	Date	80.2	77.0	85.8	86.5	**82.4**
16	2	Date	69.5	69.2	73.4	72.0	**71.0**
17	2	Date	35.7	33.1	35.8	32.9	**34.3**
18	2	Date	62.5	62.1	69.8	63.6	**64.5**
19	2	Date	106	105	109	112	**108**
20	2	Date	45.6	48.9	50.7	48.1	**48.3**
21	2	Date	48.1	44.8	48.2	48.5	**47.4**
22	2	Date	63.8	65.2	72.3	61.6	**65.7**
23	2	Date	71.8	76.0	74.2	71.0	**73.2**
24	2	Date	75.1	74.9	70.8	71.7	**73.1**
25	2	Date	89.9	87.5	89.8	89.4	**89.1**
26	2	Date	76.9	77.1	74.0	76.5	**76.1**
27	2	Date	50.0	51.4	49.5	47.4	**49.6**
28	2	Date	198	201	225	208	**208**
29	2	Date	65.9	64.2	59.9	61.4	**62.8**
30	2	Date	55.6	56.3	64.2	60.3	**59.1**
31	2	Date	58.4	57.3	60.9	60.3	**59.2**
32	2	Date	71.1	69.8	68.4	66.3	**68.9**
33	2	Date	53.4	58.1	54.8	53.0	**54.8**
34	2	Date	68.7	72.4	72.3	76.4	**72.4**
35	2	Date	51.7	49.7	53.3	54.3	**52.2**
36	2	Date	62.9	62.2	59.6	58.4	**60.7**
37	2	Date	52.5	51.2	51.2	51.3	**51.5**
38	2	Date	112	108	113	115	**112**
39	2	Date	63.3	61.5	66.3	66.5	**64.4**
40	2	Date	84.1	85.2	84.7	77.9	**83.0**

APPENDIX B: REFERENCE VALUES

- All materials are non-pooled human sera from single donors obtained following the CLSI C37-A protocol.[6]
- Concentrations were assigned by Professor Dr. Linda Thienpont at the University of Ghent by ID-LC-MS/MS and Mineva et al. at the Centers for Disease Control and Prevention by ID-LC-MS/MS.[1,2]
- $25(OH)D_3$ and $25(OH)D_2$ concentrations are reference values, but Epi-$25(OH)D_3$ concentrations should only be considered as target values.
- Only Total 25(OH)D will be used in Phase 2 Certification to evaluate platforms. Concentrations for $25(OH)D_3$, $25(OH)D_2$, and epi-$25(OH)D_3$ are provided

	<LOQ (1.50 nmol/L)
$25(OH)D_3$	25-hydroxyvitamin D3
$25(OH)D_2$	25-hydroxyvitamin D2
25(OH)D	Total 25-hydroxyvitamin D
Epi-$25(OH)D_3$	C-3 epimer 25-hydroxyvitamin D3

	Reference Values				Target Values	
					Epi-$25(OH)D_3$	
					nmol/L	*%CV*
PS01		1.4	<LOQ		5.02	4.0
PS02		2.9		4.5	<LOQ	
PS03		1.3	<LOQ		4.09	2.2
PS04		0.6	<LOQ		8.91	2.9
PS05		0.5	<LOQ		10.4	9.6
PS06		1.0	<LOQ		6.09	7.4
PS07		0.1	<LOQ		3.49	1.9
PS08		1.5	<LOQ		2.35	1.9
PS09		1.1		1.1	3.24	0.0
PS10		1.2		1.7	2.80	0.1
PS11		1.8	<LOQ		<LOQ	
PS12		0.7		7.0	10.5	1.5
PS13		0.3	<LOQ		6.59	0.1
PS14		1.8		2.1	4.24	3.6
PS15		1.3		0.5	7.37	4.3
PS16		1.5		2.8	4.53	6.6
PS17		1.5		1.3	1.98	9.6
PS18		1.8	<LOQ		9.72	12.6
PS19		1.3	<LOQ		9.93	7.9
PS20		0.9		1.3	3.18	2.3
PS21		0.5	<LOQ		2.42	5.5
PS22		1.1	<LOQ		3.77	15.1
PS23		1.6	<LOQ		3.39	0.0
PS24		1.7	<LOQ		3.37	3.8
PS25		0.3		3.2	4.87	12.5
PS26		2.0		0.4	2.92	0.1
PS27		2.3		2.3	1.77	0.1
PS28		0.7	<LOQ		48.2	5.9
PS29		1.0		6.4	3.54	11.0
PS30		1.8		2.9	3.38	3.0
PS31		2.0		2.6	4.90	5.3
PS32		1.9	<LOQ		4.49	0.1
PS33		0.9		2.4	3.05	0.6
PS34		1.4	<LOQ		4.73	16.0
PS35		0.3	<LOQ		3.05	17.3
PS36		1.9	<LOQ		2.00	0.1
PS37		1.0		2.6	2.23	14.2
PS38		0.8	<LOQ		6.71	0.0
PS39		1.1		1.8	4.68	3.6
PS40		0.6	<LOQ		5.22	8.2

(1) Stepman HC, Vanderroost A, Van Uytfanghe K, Thienpont LM. Candidate Reference Measurement Procedures for Serum 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 by Using Isotope-Dilution Liquid Chromatography-Tandem Mass Spectrometry. Clin Chem. 2011; 57: 441-448.

(2) Mineva EM, Schleicher RL, Chaudhary-Webb M, Maw KL, Botelho JC, Vesper HW, Pfeiffer CM. A Candidate Reference Measurement Procedure for Quantifying Serum Concentrations of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 Using Isotope-Dilution Liquid Chromatography-Tandem Mass Spectrometry. Analytical and Bioanalytical Chemistry. 2015; 407(19): 5615-5624.

(6) Clinical Laboratory Standards Institutes (CLSI C37-A). Wayne, PA: Clinical Laboratory Standards Institute. 1999.