NISTIR 8143

NIST/NIH Vitamin D Metabolites Quality Assurance Program Report of Participant Results: Summer 2015 Comparability Study (Exercise 11)

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National Institute of Standards and Technology
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ABSTRACT

The National Institute of Standards and Technology (NIST) has established a Vitamin D Metabolites Quality Assurance Program (VitDQAP) in collaboration with the National Institutes of Health (NIH) Office of Dietary Supplements. Participants in the eleventh exercise of this program, the Summer 2015 Comparability Study, were asked to use the methodology of their choice to measure concentrations of 25-hydroxyvitamin D in pooled human serum control and study materials distributed by NIST. The study materials consisted of VitDQAP-I and VitDQAP-II (materials designed for the VitDQAP). Standard Reference Material (SRM) 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum Level 1 was provided as a control material. Participants provided their data to NIST, where it was compiled and evaluated for trueness relative to the NIST value and concordance within the participant community. A report of results was provided to all participants of the study, and laboratories were identified by code numbers known only to them. The results from this eleventh study are reported along with a summary of the analytical methods used.

OVERVIEW OF THE SUMMER 2015 COMPARABILITY STUDY

For the Summer 2015 comparability study of the collaborative National Institute of Standards and Technology and National Institutes of Health (NIST/NIH) Vitamin D Metabolites Quality Assurance Program (VitDQAP), human serum control and study materials were distributed to participants for evaluation. Standard Reference Material (SRM) 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum Level 1 (SRM 968d L1) was provided as a control material for assay validation. For SRM 968d L1 (Control), the participants were provided the NIST target values within the data reporting sheet so that they could qualify their methods prior to analyzing the study samples. The study materials consisted of two vials, each containing a sample of pooled human serum. In this study, Vial A was VitDQAP-I, and Vial B was VitDQAP-II, both of which contain endogenous levels of the vitamin D metabolites. Participants were asked to determine 25-hydroxyvitamin D in each of the human serum control and study samples. Individual concentration values for 25-hydroxyvitamin D_3 ($25(OH)D_3$), 25-hydroxyvitamin D_2 ($25(OH)D_2$), and 3-epi-25-hydroxyvitamin D_3 (3-epi-$25(OH)D_3$) were requested along with a total concentration of 25-hydroxyvitamin D: $25(OH)D_{Total} = 25(OH)D_2 + 25(OH)D_3$.

There were 48 participants and 55 datasets (7 participants provided data from two methods) in the Summer 2015 comparability study. Seventeen (17) of the datasets originated from immunoassay (IA) techniques, including 11 from chemiluminescence immunoassay (CLIA), two from enzyme immunoassay (EIA), three from radioimmunoassay (RIA), and one from chemiluminescence enzyme immunoassay (CLEIA). **Appendix A-1** summarizes the IA methods used by the participants. Thirty-eight (38) of the datasets originated from liquid chromatographic (LC) methods; of those, 33 were from LC with tandem mass spectrometric detection (LC-MS/MS), one was from LC-MS, and four were from LC with ultraviolet absorbance detection (LC-UV). The LC-MS/MS and LC-MS methods are collectively referred to as $LC\text{-}MS^n$. A summary of the LC methods used by the participants may be found in **Appendices A-2** and **A-3**. Note: The methodological information provided on the data reporting sheet was used to update the list from previous comparability studies. For prior participants that did not provide method details for the Summer 2015 study, the information in the appendices were not edited and may not be current.

The raw data received from all participants are summarized in **Appendix B**. The IA methods do not distinguish between $25(OH)D_3$ and $25(OH)D_2$, and hence IA participants reported single values for $25(OH)D_{Total}$ in the control and study materials. The LC methods measure the vitamin D metabolites separately, and the majority of the LC participants reported values for $25(OH)D_3$ in addition to $25(OH)D_{Total}$. Thirteen LC participants reported non-zero results for $25(OH)D_2$ in at least one of the study materials, and 11 participants reported results for 3-epi-$25(OH)D_3$. One participant also reported values for 24(R), 25-dihydroxyvitamin D_3 (24(R),$25(OH)_2D_3$), which is not represented in **Appendix B**.

Appendix B also provides the summarized NIST results for each of the serum materials. A detailed description of the NIST method is provided in the next section of this report.

SUMMARY OF THE NIST METHOD USED TO EVALUATE THE CONTROL AND STUDY MATERIALS

NIST used isotope dilution LC-MS/MS (ID-LC-MS/MS) [1] to determine the vitamin D metabolites ($25(OH)D_3$, $25(OH)D_2$, and 3-epi-$25(OH)D_3$) in the control and study materials evaluated in this comparability study. The ID-LC-MS/MS approach is a reference measurement procedure (RMP) for $25(OH)D_3$ and $25(OH)D_2$ that is recognized by the Joint Committee for Traceability in Laboratory Medicine (JCTLM).

The NIST values for $25(OH)D_3$, $25(OH)D_2$, and 3-epi-$25(OH)D_3$ in VitDQAP-I (Vial A) and VitDQAP-II (Vial B) are reported with expanded uncertainties (U) that incorporate components for measurement variability and measurement uncertainty associated with the density of the materials and the purity of the reference standards. In addition, the measurements include an additional 1 % type B uncertainty for unknown systematic errors, which is consistent with the practice used at NIST for clinical measurements [1]. For SRM 968d L1 (Control), the NIST values for $25(OH)D_3$ and 3-epi-$25(OH)D_3$ are reported as described for VitDQAP-I (Vial A) and VitDQAP-II (Vial B), but the value for $25(OH)D_2$ was well below the limit of quantitation and was estimated to be 0.1 ng/mL based on one measurement.

The values for $25(OH)D_{Total}$ in VitDQAP-I (Vial A), VitDQAP-II (Vial B) and SRM 968d L1 (Control) are the sum of the individual values for $25(OH)D_3$ and $25(OH)D_2$, and the expanded uncertainty incorporates measurement uncertainties for the two analytes.

[1] Tai, S. S.-C.; Bedner, M.; Phinney, K.W.; *Anal. Chem.* **2010** *82*, 1942-1948.

SUMMER 2015 COMPARABILITY STUDY RESULTS AND DISCUSSION

Results for 25(OH)D_{Total}

A summary of the individual participant data for total 25-hydroxyvitamin D (25(OH)D_{Total}) in VitDQAP-I (Vial A), VitDQAP-II (Vial B), and SRM 968d L1 (Control) is provided in **Table 1**.

The community results are summarized at the bottom of **Table 1** for all reported methods, the IA methods only, the LC methods only, and the LC-MS^n methods only. The community results include the total number of quantitative values reported (N); the median value; the median absolute deviation from the median (MADe), a robust estimate of the standard deviation; and the percent coefficient of variation (CV %).

Table 1 also presents the NIST results for 25(OH)D_{Total} in the control and the two study materials.

Table 1. Summary of participant and NIST results for 25(OH)D_{Total} (ng/mL) in VitDQAP-I (Vial A), VitDQAP-II (Vial B), and SRM 968d L1 (Control).

Lab	Method	VitDQAP-I Vial A	VitDQAP-II Vial B	SRM 968d L1 Control
026	LC-MS/MS	31.9	38.6	12.4
030a	RIA	26.2	37.2	15.8
056a	LC-MS/MS	30.7	36.1	12.1
056b	LC-MS/MS	32.3	37.4	13.6
060	LC-MS/MS	31.5	35.8	13.7
110	LC-UV	19.5	31.3	12.6
116	LC-MS/MS	35.3	42.1	13.8
150	LC-MS/MS	26.8	32.6	10.2
180	RIA	27.7	32.1	13.9
187	LC-MS/MS	29.9	37.6	12.1
188	CLIA	31.1	36.8	11.9
189	LC-UV	33.5	38.4	11.0
194	LC-MS/MS	31.0	42.0	n/r
196	CLIA	32.1	38.6	14.4
197	LC-MS/MS	33.6	41.1	12.2
198a	LC-MS/MS	35.5	45.3	12.7
198c	CLIA	32.0	39.2	7.3
199	LC-MS/MS	30.9	38.4	12.6
204b	LC-MS/MS	33.6	39.1	12.2
209	LC-MS/MS	31.9	39.4	12.5
211	LC-MS/MS	32.7	39.0	11.3
212	LC-MS/MS	32.8	40.5	13.2
214b	CLIA	23.4	31.1	21.1
214c	LC-MS/MS	32.6	39.0	12.5
215	LC-MS/MS	30.8	36.0	12.0
216	LC-MS/MS	34.2	43.3	12.8
217	LC-MS/MS	28.4	38.4	15.7
218a	CLIA	30.2	45.7	13.5
218b	LC-MS/MS	29.2	42.6	12.3
220a	LC-MS/MS	34.0	41.5	12.6
221b	LC-UV	29.2	34.5	9.6
221c	LC-MS	28.4	36.1	11.5
225	LC-MS/MS	34.3	38.0	13.4
228a	LC-MS/MS	31.5	39.9	12.5
231b	CLIA	36.0	46.0	14.5
243a	LC-UV	34.4	34.4	12.2
243b	LC-MS/MS	34.5	34.5	12.2
244	LC-MS/MS	29.3	37.4	12.7
249	LC-MS/MS	32.8	39.0	12.8
251	LC-MS/MS	36.0	46.0	n/r
253	LC-MS/MS	37.1	44.0	14.3
255	LC-MS/MS	37.7	47.5	16.4
256	CLIA	27.0	30.9	16.0
258	CLIA	40.4	48.5	18.1
259	LC-MS/MS	30.2	33.0	14.0
261	CLIA	41.5	50.5	22.2
262	CLIA	29.0	38.4	17.7
267	CLEIA	29.9	36.7	12.4
268a	RIA	28.2	34.2	13.9
268b	EIA	46.7	58.7	28.4
270	LC-MS/MS	29.8	35.5	12.4
271	LC-MS/MS	23.2	36.3	13.0
272	LC-MS/MS	31.5	40.0	12.3
273	EIA	24.2	40.3	14.1
274	CLIA	31.5	48.4	18.5

n/r = not reported or not determined

		VitDQAP-I Vial A	VitDQAP-II Vial B	SRM 968d L1 Control
All methods	N	55	55	53
	Median	31.5	38.6	12.7
	MADe	3.4	3.9	1.2
	CV%	11	10	9.3
IA methods	N	17	17	17
	Median	30.2	38.6	14.5
	MADe	3.7	9.7	3.1
	CV%	12	25	21
LC methods	N	38	38	36
	Median	31.9	38.5	12.5
	MADe	3.0	3.6	0.6
	CV%	9.5	9.4	4.4
LC-MS^n	N	34	34	32
	Median	31.9	39.0	12.6
	MADe	2.7	3.9	0.6
	CV%	8.6	9.9	5.0
	NIST Value	32.0	37.5	12.5
	U	0.8	0.9	0.4

For all participant datasets, the single reported values for 25(OH)D_{Total} in VitDQAP-I (Vial A), VitDQAP-II (Vial B), and SRM 968d L1 (Control) are plotted in **Figure 1**, **Figure 2**, and **Figure 3**, respectively. The results from immunoassay methods are displayed with open dark blue circles (○), and the results from the LC-based methods are displayed with open light blue circles (○). The results from the individual methods were sorted separately, as indicated by the x-axis labels.

From the single reported values for all datasets for a given technique (IA or LC), the consensus median and the consensus expanded uncertainty (2 × MADe) were determined. For both of the major techniques (IA or LC) in each figure, the solid lines (——) and (——) represent the consensus median, and the dashed lines (- - - - -) and (- - - - -) represent the consensus expanded uncertainty interval (median ± 2 × MADe). The laboratories with results that fall between the two dashed lines are within the consensus range for their technique (IA or LC).

The red lines (——) in each figure (**Figures 1 – 3**) represent the NIST value and its associated uncertainty (i.e., value ± *U*). NIST has confidence that the "true" value for each material lies within this interval. When these lines are not within the consensus ranges for each technique (IA or LC), then there may be method bias.

Specific results for each of the three study materials are summarized below. Note that the assessment is based on the actual reported values, not the lines and symbols, which have been enlarged to show detail and the laboratory number.

VitDQAP-I (Vial A): **Figure 1**

- For the IA results, three reported values are outside of the consensus range (two CLIA, one EIA).
- For the LC results, two reported values are outside of the consensus range (one LC-MS^n, one LC-UV).
- The consensus median value for the IA results is lower than the NIST expanded uncertainty range (red lines).
- The consensus median value for the LC results is comparable to the NIST expanded uncertainty range (red lines).
- The NIST expanded uncertainty range (red lines) falls within the consensus range for both IA and LC.

VitDQAP-II (Vial B): **Figure 2**

- For the IA results, the data appear to be non-normally distributed, and the consensus variability is not well-described by the MADe estimation; however, one EIA result is outside the consensus range.
- For the LC results, two LC-MS^n values are outside the consensus range (both LC-MS^n).
- The consensus median values for both the IA and the LC results are comparable with the NIST expanded uncertainty range (red lines).

SRM 968d L1 (Control): **Figure 3**

- For the IA results, four reported values are outside of the consensus range (three CLIA, one EIA).
- For the LC results, eight reported values are outside of the consensus range (six LC-MS^n, two LC-UV).
- The consensus median value for the IA results is higher than the NIST expanded uncertainty range (red lines).
- The consensus median value for the LC results is comparable to the NIST expanded uncertainty range (red lines).
- The NIST expanded uncertainty range (red lines) falls within the consensus range for both IA and LC.

Figure 1. Participant and NIST results for 25(OH)D_{Total} in VitDQAP-I (Vial A) as determined by immunoassay (CLIA, EIA, RIA, and CLEIA) and LC (LC-MS^n and LC-UV) methods.

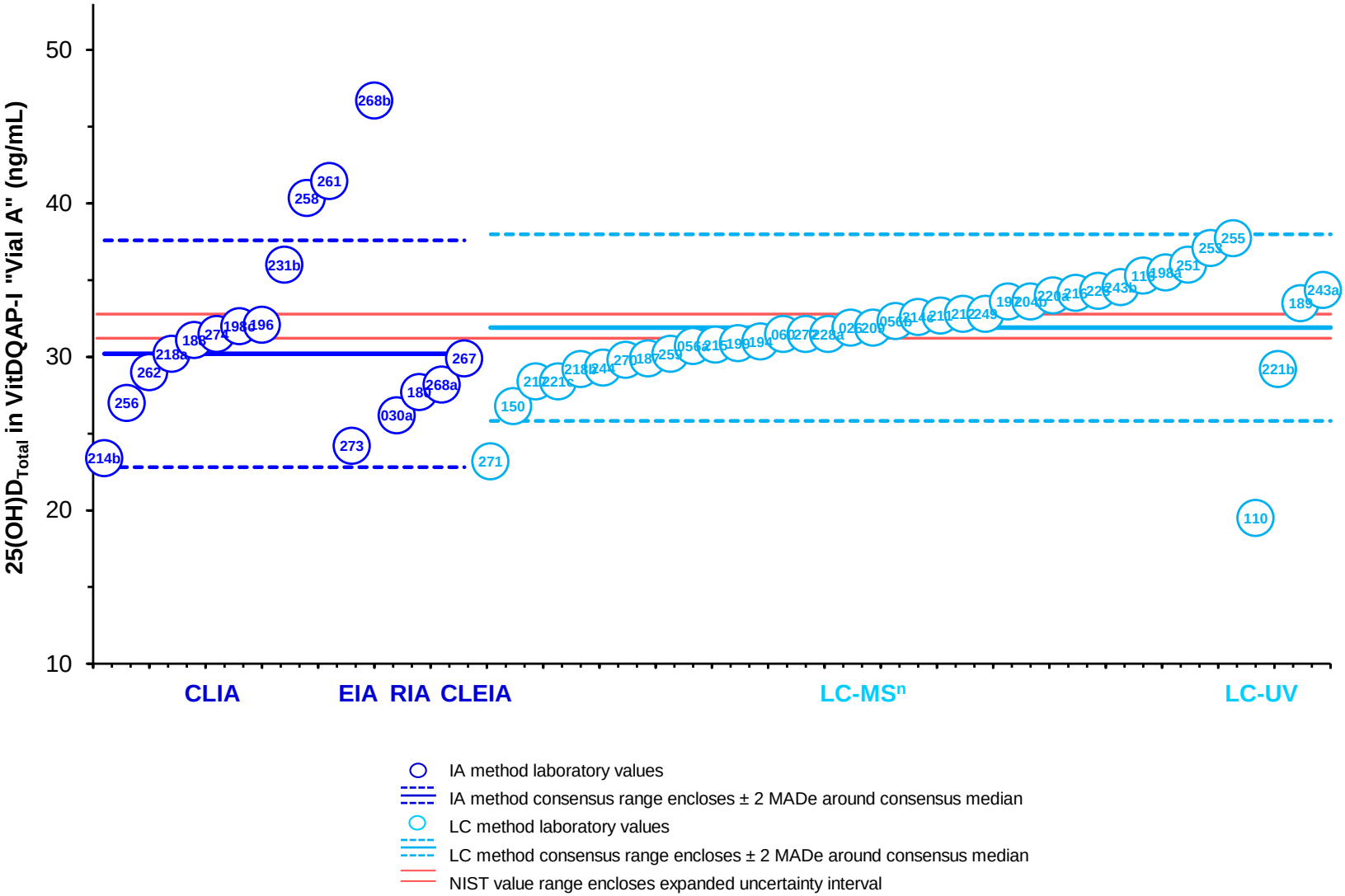


Figure 2. Participant and NIST results for 25(OH)D_{Total} in VitDQAP-II (Vial B) as determined by immunoassay (CLIA, EIA, RIA, and CLEIA) and LC (LC-MS^n and LC-UV) methods.

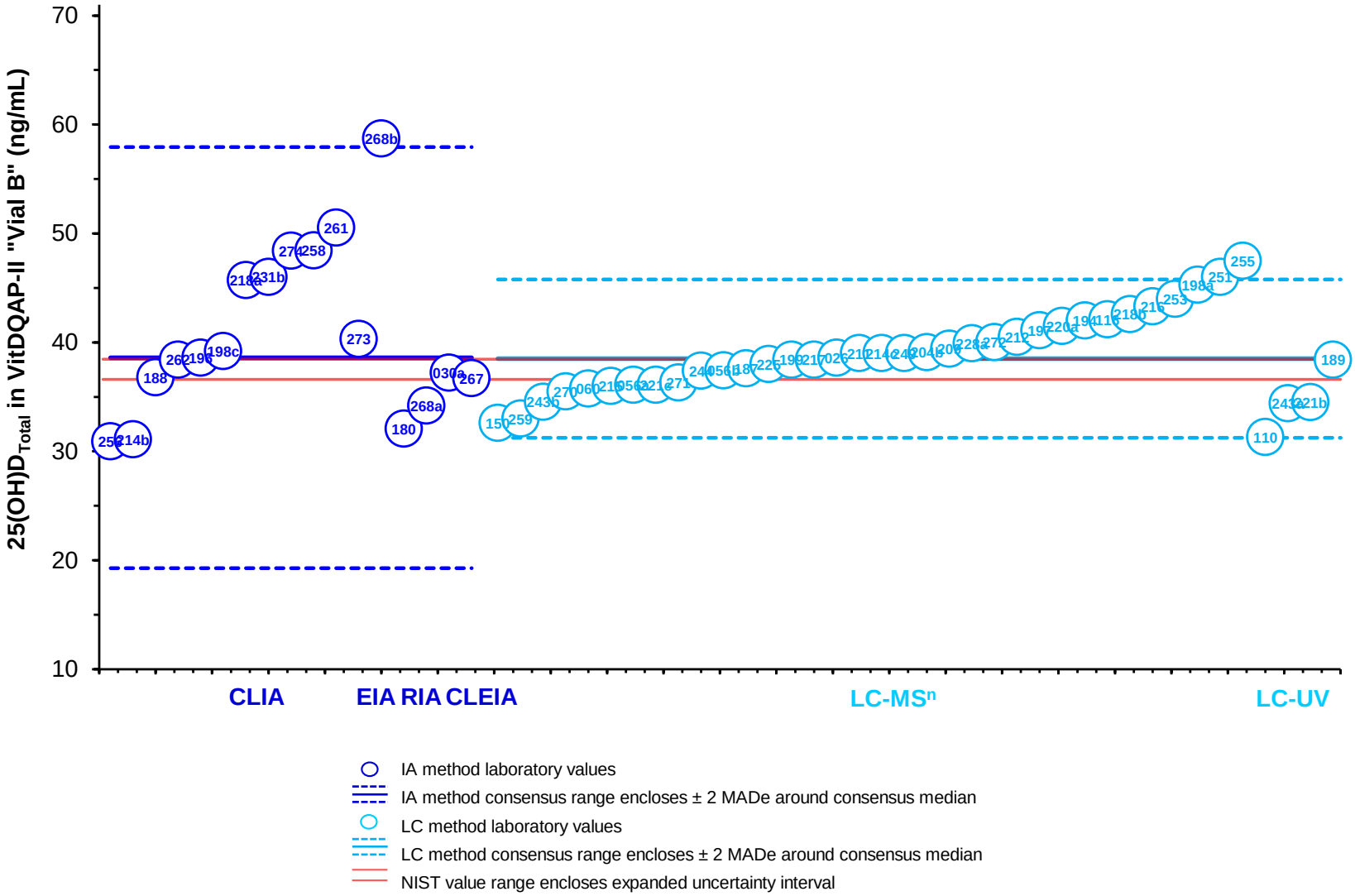


Figure 3. Participant and NIST results for 25(OH)D_{Total} in SRM 968d Level 1 (Control) as determined by immunoassay (CLIA, EIA, RIA, and CLEIA) and LC (LC-MS^n and LC-UV) methods.

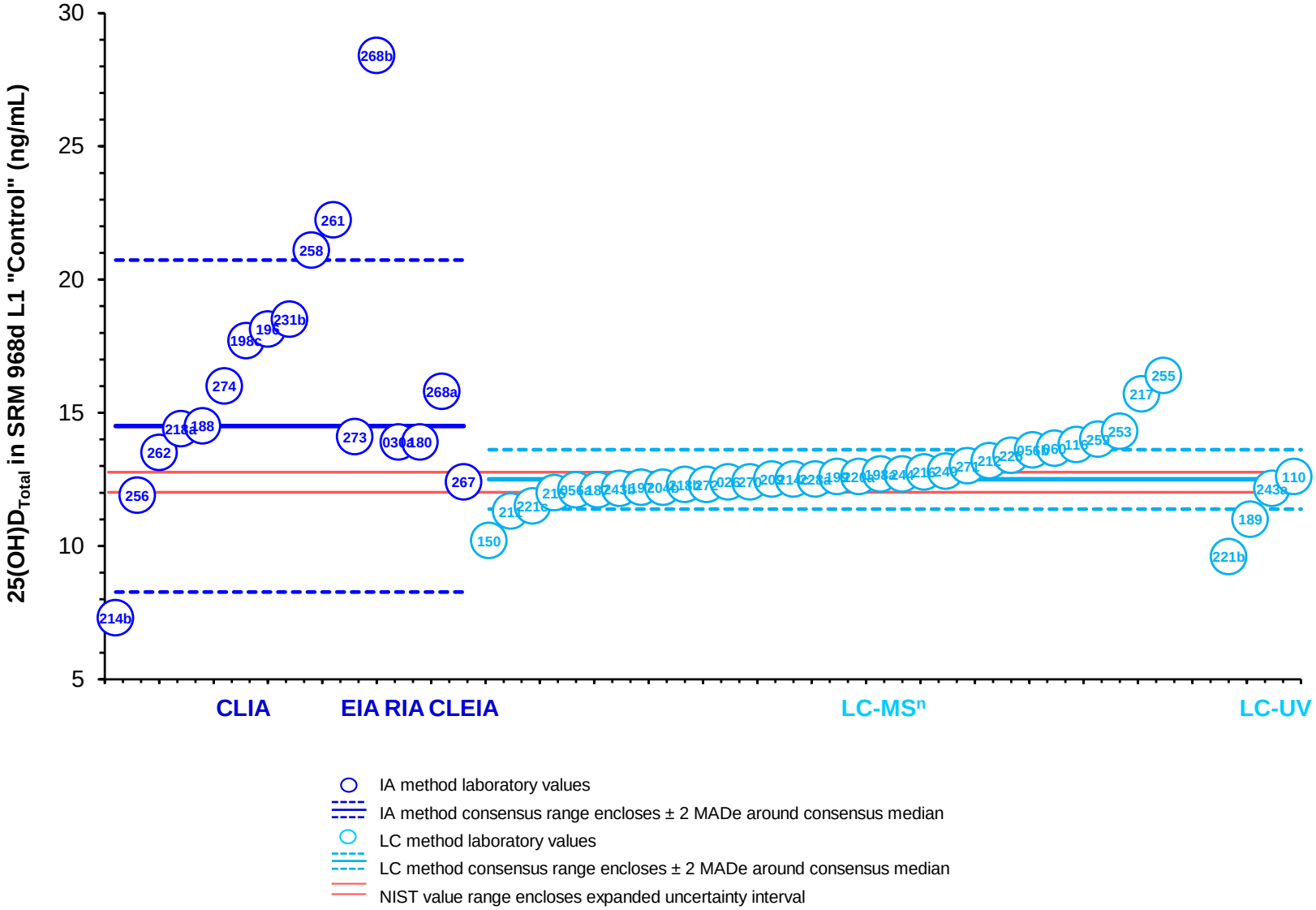


Figure 4 presents direct graphical comparisons of the 25(OH)D_{Total} results for a) VitDQAP-I (Vial A) and VitDQAP-II (Vial B), and b) VitDQAP-II (Vial B) and SRM 968d L1 (Control). In each plot, there are two blue consensus boxes, one for IA methods and one for LC methods (as indicated). Laboratory results that are within the consensus range for both study materials are within the blue consensus boxes. Conversely, laboratory results that fall outside of (or on the edge of) either of the consensus boxes are not included in the consensus ranges and are highlighted with their laboratory code numbers. In each plot, the NIST values for the materials are denoted with a red diamond symbol (◆), and the Youden line (y=x) centered on the NIST value is illustrated by a red line (——) across the magnitude of the y-axis and x-axis, respectively.

Specific results as assessed from the Youden comparison plots are summarized below.

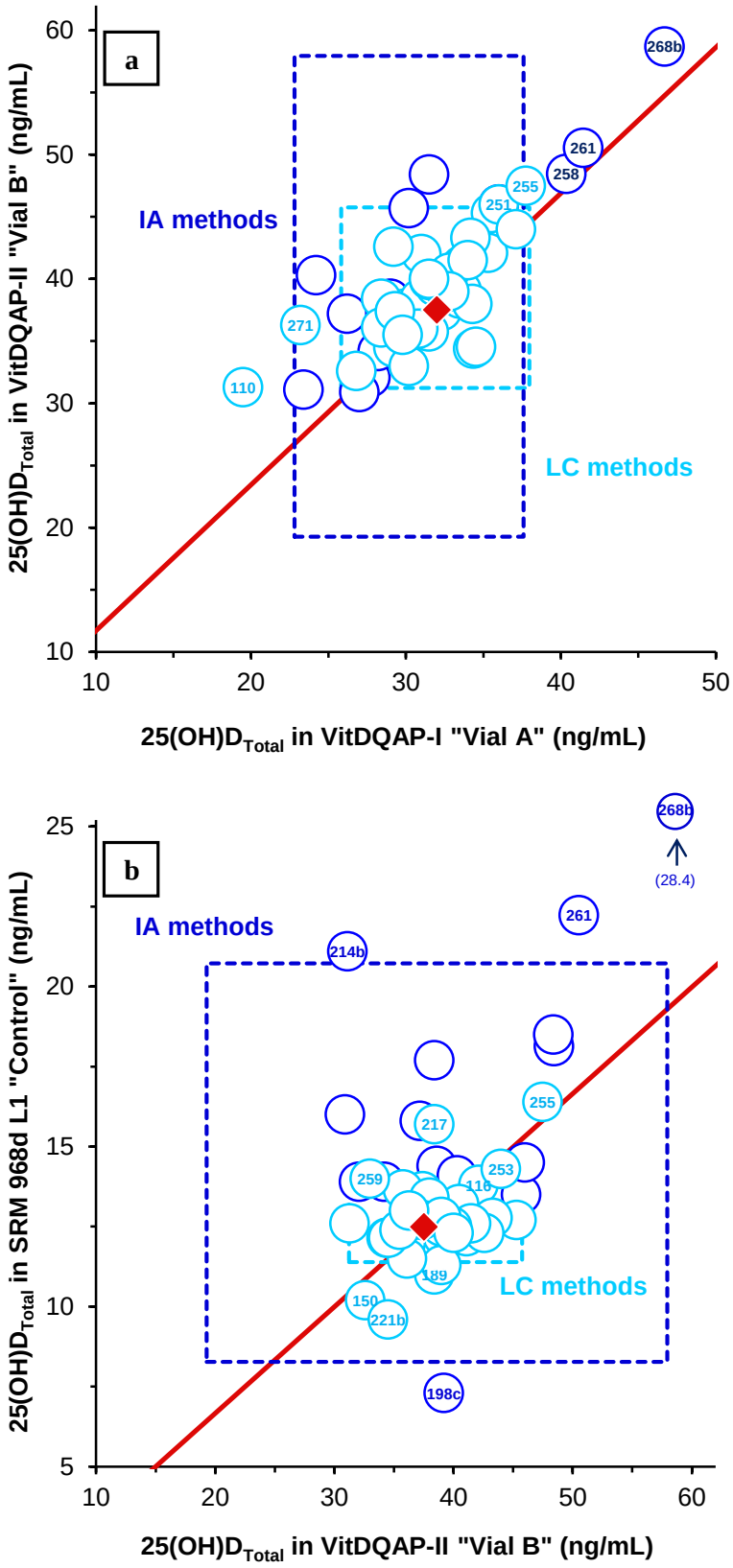
VitDQAP-I (Vial A) and VitDQAP-II (Vial B): **Figure 4 a**

- IA results that are not included in the consensus ranges include: 258, 261, and 268b.
- LC results that are not included in the consensus ranges include: 110, 251, 255 and 271.
- The Youden line runs through the center of the LC consensus box and near the center of the IA consensus box, illustrating that both the IA and LC results are in agreement with each other and with the NIST results for these materials.
- The linear trend (results closely aligned with the Youden line) indicates participant-specific analytical bias.

VitDQAP-II (Vial B) and SRM 968d L1 (Control): **Figure 4 b**

- The consensus box for the IA results is extremely large for these two materials, which hinders an assessment of the outliers; however, the IA results that are not included in the consensus ranges include numbers 198c, 214b, 261, and 268b.
- LC results that are not included in the consensus ranges include numbers 116, 150, 189, 217, 221b, 253, 255, and 259.
- The Youden line runs through the center of the LC consensus box and through the bottom of the IA consensus box, illustrating that the LC results are in better agreement with the NIST results than are the IA results for these materials.
- The lack of strong linear trend suggests either significant differences between SRM 968d L1 (Control) and VitDQAP-II (Vial B) (e.g., concentration difference) or the ‘attractor’ effect of participants knowing the correct value for the control.

Figure 4. Youden comparison plot of the results for 25(OH)D_{Total} in a) VitDQAP-I (Vial A) and VitDQAP-II (Vial B) and b) VitDQAP-II (Vial B) and SRM 968d L1 (Control) for all methods.



Discussion of Results for 25(OH)D_{Total}

In the Summer 2015 comparability study, both study materials VitDQAP-I (Vial A) and VitDQAP-II (Vial B) and SRM 968d L1 (Control) contain predominantly 25(OH)D_3 as the metabolite contributing to 25(OH)D_{Total}. The CV %'s of 11 %, 10 %, and 9.3 % (all methods) for VitDQAP-I (Vial A), VitDQAP-II (Vial B), and (Control), respectively, are consistent with participant performance for other materials containing predominantly 25(OH)D_3 that were evaluated in previous comparability studies of the VitDQAP.

The Summer 2015 exercise was the second to utilize study materials that were evaluated in previous comparability studies of the VitDQAP. VitDQAP-I (Vial A) was also evaluated in the Summer 2014 comparability study (Vial A), and VitDQAP-II (Vial B) was previously evaluated in Summer 2013 (Vial A). **Table 2** provides the program results for each of these two study materials for the labs participating in the current study. Using the results in **Table 2**, the participant performance for these materials over time can be assessed. When the summary statistics at the bottom of **Table 2** are compared, the median and CV % results are generally consistent across both comparability studies in which the materials were evaluated with the exception of the IA results for VitDQAP-II, which has a significantly higher CV % of 25 % in the current study, compared to 6.3 % in Summer 2013. The higher CV % in the present study is attributable to the non-normal distribution of the IA results, which led to an overestimation of the MADe and the resulting CV % (see **Figure 2**).

Figure 5 presents direct graphical comparisons of the 25(OH)D_{Total} results for 25(OH)D_{Total} in a) VitDQAP-I (Vial A) in the present study (Summer 2015) and in a previous study (Summer 2014) and b) VitDQAP-II (Vial B) in the present study (Summer 2015) and in a previous study (Summer 2013). The features of the plots are the same as described for **Figure 4**. The clustering of results around the NIST value in both **Figure 5 a** and **b** illustrates that there are not consistent within-laboratory biases for VitDQAP-I and VitDQAP-II over 1 and 2 years, respectively, and that the within-round variability is consistent with the over-time variability. While the vast majority of labs yield results that are within the consensus boxes for their techniques, the labs that fall outside are not in as good statistical control. For VitDQAP-I, these labs include 110, 188, 258, and 261 (**Figure 5a)**, and for VitDQAP-II, these labs include 030a, 110, 188, 198a and 255 (**Figure 5b)**.

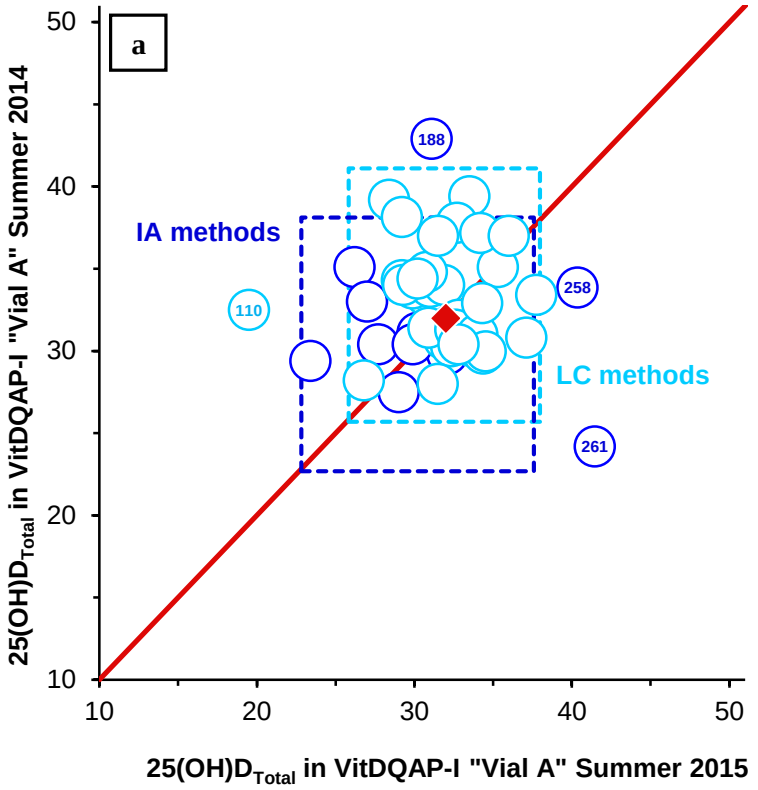
Table 2. Summary of participant data for 25(OH)D_{Total} (ng/mL) in VitDQAP-I in the current study (Vial A) and a prior study (Summer 2014) as well as for VitDQAP-II in the current study (Vial B) and a prior study (Summer 2013).

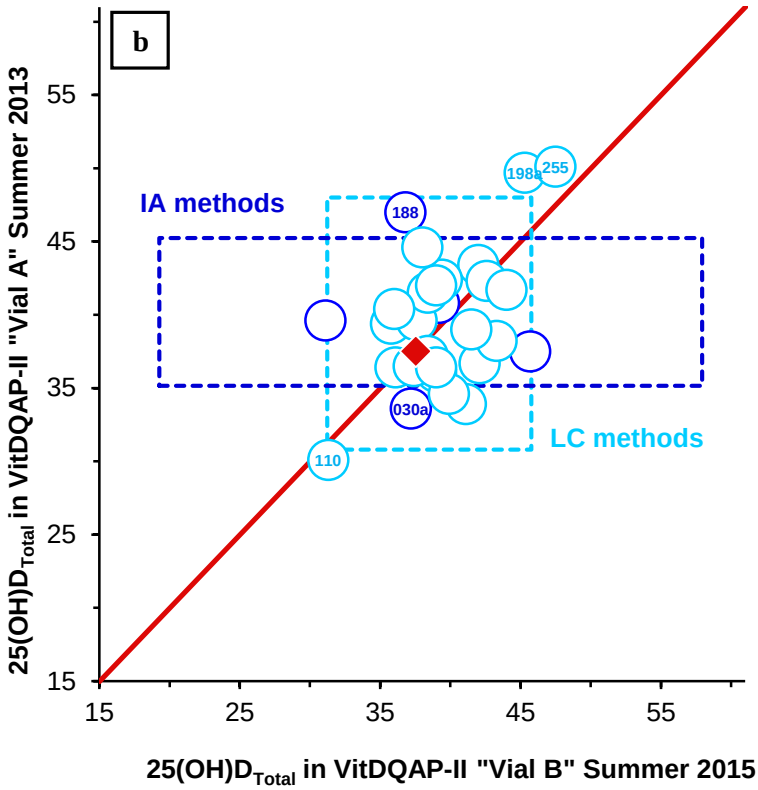
		VitDQAP-I		VitDQAP-II	
		Summer 2015	Summer 2014	Summer 2015	Summer 2013
Lab	Method	Vial A	Vial A	Vial B	Vial A
026	LC-MS/MS	31.9	30.7	38.6	X
030a	RIA	26.2	35.1	37.2	33.6
056a	LC-MS/MS	30.7	33.4	36.1	36.4
056b	LC-MS/MS	32.3	30.3	37.4	X
060	LC-MS/MS	31.5	28.0	35.8	39.4
110	LC-UV	19.5	32.5	31.3	30.1
116	LC-MS/MS	35.3	35.1	42.1	36.7
150	LC-MS/MS	26.8	28.2	32.6	X
180	RIA	27.7	30.4	32.1	X
187	LC-MS/MS	29.9	33.8	37.6	39.6
188	CLIA	31.1	42.9	36.8	47.0
189	LC-UV	33.5	39.4	38.4	X
194	LC-MS/MS	31.0	33.9	42.0	43.4
196	CLIA	32.1	29.8	38.6	40.9
197	LC-MS/MS	33.6	30.3	41.1	33.9
198a	LC-MS/MS	35.5	X	45.3	49.7
198c	CLIA	32.0	X	39.2	40.8
199	LC-MS/MS	30.9	31.4	38.4	41.5
204b	LC-MS/MS	33.6	30.8	39.1	X
209	LC-MS/MS	31.9	34.0	39.4	42.4
211	LC-MS/MS	32.7	37.8	39.0	42.0
212	LC-MS/MS	32.8	31.9	40.5	X
214b	CLIA	23.4	29.4	31.1	39.6
214c	LC-MS/MS	32.6	31.3	39.0	36.1
215	LC-MS/MS	30.8	34.8	36.0	40.4
216	LC-MS/MS	34.2	37.2	43.3	38.2
217	LC-MS/MS	28.4	39.2	38.4	37.2
218a	CLIA	30.2	31.1	45.7	37.5
218b	LC-MS/MS	29.2	38.1	42.6	42.3
220a	LC-MS/MS	34.0	31.0	41.5	39.0
221b	LC-UV	29.2	34.3	34.5	X
221c	LC-MS	28.4	X	36.1	X
225	LC-MS/MS	34.3	32.9	38.0	44.6
228a	LC-MS/MS	31.5	37.0	39.9	34.6
231b	CLIA	36.0	X	46.0	X
243a	LC-UV	34.4	29.8	34.4	X
243b	LC-MS/MS	34.5	30.0	34.5	X
244	LC-MS/MS	29.3	34.0	37.4	36.5
249	LC-MS/MS	32.8	30.4	39.0	36.4
251	LC-MS/MS	36.0	37.0	46.0	X
253	LC-MS/MS	37.1	30.8	44.0	41.7
255	LC-MS/MS	37.7	33.4	47.5	50.1
256	CLIA	27.0	33.0	30.9	X
258	CLIA	40.4	33.9	48.5	X
259	LC-MS/MS	30.2	34.4	33.0	X
261	CLIA	41.5	24.2	50.5	X
262	CLIA	29.0	27.5	38.4	X
267	CLEIA	29.9	30.4	36.7	X
268a	RIA	28.2	X	34.2	X
268b	EIA	46.7	X	58.7	X
270	LC-MS/MS	29.8	X	35.5	X
271	LC-MS/MS	23.2	X	36.3	X
272	LC-MS/MS	31.5	X	40.0	X
273	EIA	24.2	X	40.3	X
274	CLIA	31.5	X	48.4	X

X= did not participate in that study

		VitDQAP-I		VitDQAP-II	
		Summer 2015	Summer 2013	Summer 2015	Summer 2013
		Vial A	Vial A	Vial B	Vial A
All methods	N	55	44	55	29
	Median	31.5	32.7	38.6	39.6
	MADe	3.4	3.4	3.9	4.2
	CV%	11	10	10	10
IA methods	N	17	11	17	6
	Median	30.2	30.4	38.6	40.2
	MADe	3.7	3.9	9.7	2.5
	CV%	12	13	25	6.3
LC methods	N	38	33	38	23
	Median	31.9	33.4	38.5	39.4
	MADe	3.0	3.9	3.6	4.3
	CV%	9.5	12	9.4	11
LC-MS^n	N	34	29	34	22
	Median	31.9	33.4	39.0	39.5
	MADe	2.7	3.9	3.9	4.2
	CV%	8.6	12	9.9	11
	NIST Value	32.0	32.0	37.5	37.5
	U	0.8	0.8	0.9	0.9

Figure 5. Youden comparison plot of the results for 25(OH)D_{Total} (ng/mL) in a) VitDQAP-I (Vial A) in the present study (Summer 2015) and in a previous study (Summer 2014 – Vial A) and b) VitDQAP-II (Vial B) in the present study (Summer 2015) and in a previous study (Summer 2013 – Vial A).





LC Results for 3-Epi-25(OH)D_3

Of the two major techniques IA and LC, only the LC methods can independently measure the individual metabolites 25(OH)D_2, 25(OH)D_3 and 3-epi-25(OH)D_3. In the Summer 2015 comparability study of the VitDQAP, the study materials and the control contained negligible amounts of the 25(OH)D_2 metabolite, and hence the 25(OH)D_{Total} values reported in **Table 1** generally reflect the 25(OH)D_3 concentrations measured by the LC participants. However, both the VitDQAP-I (Vial A) and VitDQAP-II (Vial B) study materials contained measurable concentrations of the 3-epi-25(OH)D_3 metabolite, which does not contribute to the reported 25(OH)D_{Total} values.

Of the 38 LC participants in the Summer 2015 comparability study, 11 reported values for 3-epi-25(OH)D_3 in at least one of the materials. The study results and the NIST values for 3-epi-25(OH)D_3 are presented in **Table 3**. For each material, the median LC result agrees well with the NIST value.

Table 3. Summary of participant and NIST results for 3-epi-25(OH)D_3 in VitDQAP-I (Vial A), VitDQAP-II (Vial B), and SRM 968d L1 (Control).

		VitDQAP-I	VitDQAP-II	SRM 968d L1
Lab	Method	Vial A	Vial B	Control
026	LC-MS/MS	1.9	3.6	0.7
056a	LC-MS/MS	1.6	2.8	0.5
060	LC-MS/MS	1.8	3.2	0.7
150	LC-MS/MS	1.7	3.2	0.5
204b	LC-MS/MS	n/d	3.5	n/d
216	LC-MS/MS	1.4	3.0	0.7
228a	LC-MS/MS	1.8	2.7	0.7
243a	LC-UV	1.7	1.9	n/d
243b	LC-MS/MS	1.6	2.0	n/d
249	LC-MS/MS	1.6	3.2	0.8
272	LC-MS/MS	1.8	3.5	0.8
LC methods	N	10	11	8
	Median	1.7	3.2	0.67
	MADe	0.1	0.5	0.12
	CV%	7	17	19
LC-MS^n	N	9	10	8
	Median	1.7	3.2	0.67
	MADe	0.1	0.5	0.12
	CV%	7	16	19
	NIST Value	1.7	3.2	0.65
	U	0.1	0.1	0.03

n/d = not detected

Dihydroxyvitamin D_3 Metabolite

This is the second comparability study in which a participant reported results for at least one of the dihdroxy metabolites, 24(R),25$(OH)_2D_3$, in each of the study materials. The results provided by participant 60 for this metabolite include:

	24(R),25$(OH)_2D_3$ (ng/mL)
VitDQAP-I (Vial A)	2.44
VitDQAP-II (Vial B)	6.82
SRM 968d L1 (Control)	1.02

NIST has developed a candidate RMP for the determination of 24(R),25$(OH)_2D_3$ and has assigned reference values for this metabolite in SRM 972a. However, NIST is not providing values for 24(R),25$(OH)_2D_3$ for the VitDQAP study materials at this time.

Conclusions from the Summer 2015 Comparability Study of the VitDQAP

The Summer 2015 comparability study was the 11th exercise for the VitDQAP. Over 11 studies, the participant performance has been consistent for study materials that contain predominantly 25$(OH)D_3$; the CV has been in the range from 7 % to 19 %, and the median values (all methods) have been biased slightly high or were comparable to the NIST values. In the Summer 2015 comparability study, VitDQAP-I (Vial A), VitDQAP-II (Vial B), and SRM 968d L1 (Control) also contain predominantly 25$(OH)D_3$ and follow these longstanding trends. In addition, Summer 2015 represents the second study in which both VitDQAP-I (Vial A) and VitDQAP-II (Vial B) were evaluated in the VitDQAP. **Table 2** and **Figure 5** contains the program results for this material in both studies and demonstrates the consistency of the participant results for these study materials.

Appendix A-1. Summary of immunoassay methods as reported by the study participants.

Laboratory Number	*IA Method*	*Sample Preparation*	*Vendor/kit**
30a	RIA	Samples were extracted with acetonitrile	A
180	RIA	Samples were extracted with acetonitrile	A
188	CLIA	n/r	B
196	CLIA	No sample preparation required	C
198c	CLIA	n/r	n/r
214b	CLIA	n/r	C
218a	CLIA	Direct analysis	C
231b	CLIA	n/r	B
256	CLIA	n/r	C
258	CLIA	n/r	D
261	CLIA	No sample preparation required	D
262	CLIA	n/r	E
267	CLEIA	n/r	F
268a	RIA	n/r	G
268b	EIA	n/r	H
273	EIA	n/r	n/r
274	CLIA	n/r	D

n/r = not reported

*NIST cannot endorse or recommend commercial products, therefore individual vendors/kits are indicated with a unique letter but not identified

Appendix A-2. Summary of LC-MS^n methods as reported by the study participants.

Laboratory Number	*Internal Standard (IS)*	*Sample Preparation*	*Chromatographic Conditions*	*Detection: MRM ions*
26	25(OH)D_2-d_6 and 25(OH)D_3-d_6	Liquid-liquid extraction method	PFP column (100 mm × 3.2 mm); isocratic elution with 82 % methanol/18 % water; flow 0.4 mL/min	25(OH)D_3 401/365; 25(OH)D_2 413/355; 3-epi-25(OH)D_3 401/365
56a	25(OH)D_2-d_3; 25(OH)D_3-d_6; 3-epi-25(OH)D_3-d_3	Samples were extracted with hexane, evaporated, then reconstituted with 69 % methanol	PFP column (100 mm × 2.1 mm; 1.9 µm); isocratic elution; flow 0.4 mL/min	25(OH)D_3 383/365; 25(OH)D_3-d_6 389/371; 25(OH)D_2 395/377; 25(OH)D_2-d_3 398/380; 3-epi-25(OH)D_3 383/365; 3-epi-25(OH)D_3-d_3 386/368
56b	n/r	n/r	n/r	n/r
60	25(OH)D_3 -d_6 25(OH)D_3-d_3 24,25$(OH)_2D_3$-d_6	IS was added, and then samples were extracted with acetonitrile, evaporated, and reconstituted with 90 % methanol/10 % water	PFP column (100 mm × 3.0 mm; 2.6 µm); gradient with water, methanol and acetonitrile (0.05 % formic acid)	25(OH)D_3 383/211; 25(OH)D_3-d_6 389/211; 25(OH)D_2 413/355; 3-epi-25(OH)D_3 401/383
116	25(OH)D_3 -d_6	Serum proteins were precipitated with methanol	Online SPE; reversed-phase column; isocratic elution with 95 % methanol/5 % water; flow 0.6 mL/min	25(OH)D_3 383/211; 25(OH)D_3-d_6 389/211; 25(OH)D_2 395/269
150	25(OH)D_2-d_6 and 25(OH)D_3-d_3	Sample (200 µL) was mixed with IS solution, liquid-liquid extracted, centrifuged, supernatant evaporated, and reconstituted in mobile phase	PFP column (100 mm × 3.0 mm; 2.6 µm); isocratic separation with 74 % methanol/26 % water (2 mmol/L ammonium acetate, 0.1 % formic acid); flow 0.5 mL/min	25(OH)D_3 401/383, 401/365; 25(OH)D_2 413/395, 413/365
187	deuterated standards for 25(OH)D_2 and 25(OH)D_3	SPE	C18 column (50 mm × 2.1 mm; 3 µm); gradient with methanol and water	25(OH)D_2 413/395; 25(OH)D_3 401/383
194	25(OH)D_3 -d_6	Proteins precipitated with acetonitrile, top layer removed, evaporated, and reconstituted with methanol	C8 column (50 mm × 2 mm); isocratic elution with 70 % acetonitrile/ 30 % water; flow 0.7 mL/min	25(OH)D_2 395/119; 25(OH)D_3 383/211
197	25(OH)D_3 -d_6	Precipitating agent added (200 µL with 20 ng IS) to each serum sample (200 µL), calibrator and control sample followed by mixing, centrifugation, and analysis	C18 column (50 mm × 4.6 mm; 5 µm); column temp 45 °C; gradient with water and methanol; flow 1.0 mL/min	n/r
198a	25(OH)D_3 -d_6	Proteins precipitated with methanol, followed by $ZnSO_4$ addition, hexane extraction, centrifugation, evaporation under N_2, and reconstitution in methanol (0.1 % formic acid)	C18 column (50 mm x 2.1 mm; 3.5 µm); isocratic elution with 85 % methanol (0.1 % formic acid); flow 0.5 mL/min	25(OH)D_3 401/383, 401/365; 25(OH)D_2 413/395, 413/355; 25(OH)D_3-d_6 407/389, 407/371
199	proprietary	proprietary	proprietary	proprietary
204b	25(OH)D_2-d_3; 25(OH)D_3-d_6; 3-epi-25(OH)D_3-d_3	Protein crash with 73 % methanol followed by liquid-liquid extraction with hexane, centrifugation, evaporation, and reconstitution in mobile phase	PFP column (100 mm × 2.1 mm; 1.9 µm); column temperature 30 °C; isocratic elution with 73 % methanol/27 % water; flow 0.4 mL/min	APCI 25(OH)D_3 383/365, 383/257; 25(OH)D_2 395/377, 395/209; 3-epi-25(OH)D_3 383/365, 383/257

209	25(OH)D_3 -d_6	Proteins were precipitated with 5 % $ZnSO_4$ in methanol	C8 column (50 mm × 2 mm; 5 µm); gradient with water/methanol; flow 0.7 mL/min	APCI 25(OH)D_3 383/229,383/211; 25(OH)D_3-d_6 389/211; 25(OH)D_2 395/269, 395/119
211	25(OH)D_3-d_6	Proteins precipitated with acetonitrile containing IS followed by centrifugation	Turbulent flow column (32 mm x 4.6 mm; 3 µm)	25(OH)D_3 383/365 (quant), 383/257 (qual); 25(OH)D_2 395/209 (quant), 395/377 (qual)
212	25(OH)D_3-d_6	Serum (100 µL) proteins precipitated using 5 % methanol/95 % acetonitrile containing the IS (350 µL)	C8 column (50 mm × 2 mm; 3 µm); gradient of 60 % to 98 % acetonitrile (0.1 % formic acid)	25(OH)D_3 383/229, 383/211; 25(OH)D_2 395/269, 395/119
214c	25(OH)D_3-d_6	Samples were extracted with hexane, centrifuged, evaporated, and filtered	Column (50 mm × 2.1 mm); isocratic elution with 85 % methanol/ 15 % water/ 0.1 % formic acid; flow 0.3 mL/min	25(OH)D_3 401/383; 25(OH)D_3-d_6 407/389; 25(OH)D_2 413/395
215	25(OH)D_3-d_6	Protein precipitation with methanol/isopropanol and $ZnSO_4$; supernatant extracted using SPE	C18 column (50 mm × 2.1 mm; 2.6 µm) column; gradient with water (0.1 % formic acid, 5 mmol/L ammonium formate) and methanol (0.05 % formic acid)	ESI 25(OH)D_3 401/383; 25(OH)D_2 413/395; 25(OH)D_3-d_6 407/389
216	Derivatized deuterated standard	Samples extracted using liquid-liquid extraction then labeled with a derivatization reagent	Reversed-phase column (150 mm × 2.1 mm); gradient from 25 % water (0.05 % formic acid) to 50 % acetonitrile (0.05 % formic acid); flow 0.2 mL/min	n/r
217	25(OH)D_3-d_6	Protein precipitation with $ZnSO_4$ in methanol followed by SPE	C8 column (50 mm × 2.1 mm; 1.7 µm); gradient of 70 % to 98 % methanol (with 0.1 % formic acid); flow 0.4 mL/min	25(OH)D_3 401/159 (quant), 401/383 (qual); 25(OH)D_2 413/83 (quant), 413/395 (qual)
218b	From vendor-supplied vitamin D kit	Samples were extracted, separated, centrifuged, and evaporated.	C18 column (50 mm × 2.1 mm; 1.7 µm); isocratic elution with methanol and water; flow 0.45 mL/min	25(OH)D_3 298
220a	25(OH)D_2-d_3 and 25(OH)D_3-d_6	Protein crash with 90 % methanol/ 10 % $ZnSO_4$ and then acetonitrile/ 1 % formic acid; sample filtered; phospholipids removed with SPE	C18 column (20 mm × 2.1 mm; 2.7 µm); gradient with water and acetonitrile; flow 1 mL/min; column 40 °C	MRM with dehydrated precursor and product ions
221c	25(OH)D_2-d_6 and 25(OH)D_3-d_3	Protein crash with acetonitrile containing IS; SPE extraction; elution with methanol/acetonitrile solution; evaporation; reconstitution with acetonitrile	PFP column (50 mm × 3.0 mm; 2.7 µm); elution with methanol/water/formic acid; column 40 °C	LC-MS SIM 25(OH)D_3 383; 25(OH)D_2 395; 25(OH)D_3-d_6 389; 25(OH)D_2-d_6 401
225	25(OH)D_3-d_6	Liquid-liquid extraction	PFP column (100 mm × 2.1 mm); gradient with methanol/water	25(OH)D_3 401/107; 25(OH)D_2 413/83
228a	n/r	n/r	n/r	n/r
243b	25(OH)D_3-d_6	Samples (400 µL) were mixed with solution containing the IS (400 µL) and the mobile phase (500 µL); samples were centrifuged; supernatant was diluted; portion (50 µL) was injected	PFP column (150 mm × 2 mm); isocratic separation with 85 % methanol/15 % water; flow 0.3 mL/min	25(OH)D_3 383/257; 25(OH)D_2 395/269; 25(OH)D_3-d_6 389/263;
244	25(OH)D_3-d_6	Protein precipitation followed by filtration	CN column; mobile phase consisting of distilled water (formic acid) and methanol	25(OH)D_3 383/211; 25(OH)D_3-d_6 389/211; 25(OH)D_2 395/269

249	25(OH)D_2-d_3; 25(OH)D_3-d_6; 3-epi-25(OH)D_3-d_3	Serum was deproteinated with NaOH and 90 % acetonitrile/ 10 % methanol followed by SPE	PFP column (100 mm × 2.1 mm; 1.8 µm); gradient separation with water (2 mmol/L ammonium acetate) and methanol; flow 0.35 mL/min	25(OH)D_3 401/159; 25(OH)D_2 413/159
251	25(OH)D_2-d_3 and 25(OH)D_3-d_3	Protein precipitation followed by SPE	Phenyl column (50 mm × 2.1 mm; 1.7 µm); gradient with water and methanol (0.1 % formic acid, 2 mmol/L ammonium acetate); flow 0.45 mL/min	25(OH)D_3 401/159 (quant), 401/365 (qual); 25(OH)D_2 413/83 (quant), 413/355 (qual); 25(OH)D_3-d_3 404/162; 25(OH)D_2-d_3 416/358
253	25(OH)D_2-d_3 and 25(OH)D_3-d_3	The sample was extracted, centrifuged, and derivatized	C18 column (150 mm × 2.1 mm); gradient separation with water and methanol; flow 0.4 mL/min	25(OH)D_2 588; 25(OH)D_3 576
255	deuterium labeled compound	Samples were extracted and derivatized with 4-phenyl-1,2,4-triazoline-3,5-dione	Reversed-phase column (50 mm × 2.1 mm); gradient with methanol; flow 0.5 mL/min	25(OH)D_3 607/298; 25(OH)D_2 619/298
259	25(OH)D_3-d_6	Liquid-liquid extraction using hexane	C8 column; gradient with methanol/water/0.1 % formate; column temperature 40 °C	25(OH)D_3 401/355; 25(OH)D_2 413/355; 25(OH)D_3-d_6 407/371
270	25(OH)D_3-d_6	Samples were precipitated, centrifuged, evaporated, reconstituted, centrifuged, and upper layer injected	C18 column (300 mm × 4.6 mm; 3.5 µM); isocratic separation with 50 % water/ 50 % methanol; flow 1.0 mL/min	25(OH)D_3 401/383; 25(OH)D_2 413/395; 25(OH)D_3-d_6 407/389
271	25(OH)D_3-d_6	Protein precipitation	C8 column (3 µm); gradient with water/acetonitrile/0.1 % formic acid; flow 0.7 mL/min	25(OH)D_3 383/229; 25(OH)D_2 395/269
272	Isotopically labeled internal standards	Samples were precipitated and centrifuged before injection	Analytical column and trap column from a kit; separation using a binary gradient system and an additional isocratic pump	25(OH)D_3 383/365, 383/299; IS (1): 386/257, 386/232; 25(OH)D_2 395/269, 395/251; 3-epi-25(OH)D_3 383/257, 383/299; 3-epi-25(OH)D_2 395/269, 395/251; IS (2): 386/257, 386/232

C18 = octadecyl; C8 = octyl; PFP = pentafluorophenyl; SPE = solid phase extraction; CN = cyano;

MRM = multiple reaction monitoring; quant/qual = quantitative/qualitative ions; n/r = not reported;

APPI = atmospheric pressure photoionization; APCI = atmospheric pressure chemical ionization; ESI = electrospray ionization

Appendix A-3. Summary of LC-UV methods as reported by the study participants.

Laboratory Number	*Internal Standard (IS)*	*Sample Preparation*	*Chromatographic Conditions*	*Wavelength*
110	n/a	Samples (500 µL) were mixed with ethanol (500 µL), extracted twice with hexane/methylene chloride (5:1), evaporated, and reconstituted	C18 column (2.1 mm × 100 mm; 1.8 µm); gradient with acetonitrile/methanol (85:15) and isopropanol (100 %)	267 nm
189	unidentified	Protein precipitation followed by SPE	Reversed-phase column (150 mm × 4.6 mm); isocratic separation; flow 0.7 mL/min	265 nm
221b	laurophenone	Protein crash with acetonitrile solution containing IS, followed by SPE, elution with methanol/acetonitrile solution, evaporation, and reconstitution with acetonitrile	CN column (150 mm × 5 mm; 3.5 µm); elution with methanol/water/formic acid; column temperature 47 °C	275 nm
243a	dodecanophenone	Samples (400 µL) were mixed with solution containing the IS (400 µL), precipitation reagent was added (500 µL), and portion of upper layer (50 µL) was injected	C18 column (100 mm × 3 mm); isocratic elution with water and isobutanol; flow 1.2 mL/min; column temperature 25 °C	264 nm

C18 = octadecyl; SPE = solid phase extraction; CN = cyano; n/a = not applicable

Appendix B. Raw participant data and NIST results for $25(OH)D_2$, $25(OH)D_3$, $25(OH)D_{Total}$, and 3-epi-$25(OH)D_3$ in VitDQAP-I (Vial A), VitDQAP-II (Vial B), and SRM 968d L1 (Control).

		$25(OH)D_2$ (ng/mL)			$25(OH)D_3$ (ng/mL)			$25(OH)D_{Total}$ (ng/mL)			3-epi-$25(OH)D_3$ (ng/mL)		
		VitDQAP-I	VitDQAP-II	SRM 968d L1	VitDQAP-I	VitDQAP-II	SRM 968d L1	VitDQAP-I	VitDQAP-II	SRM 968d L1	VitDQAP-I	VitDQAP-II	SRM 968d L1
Lab	Method	Vial A	Vial B	Control	Vial A	Vial B	Control	Vial A	Vial B	Control	Vial A	Vial B	Control
026	LC-MS/MS	<1.0	<1.0	<1.0	31.9	38.6	12.4	31.9	38.6	12.4	1.9	3.6	0.7
030a	RIA	n/a	n/a	n/a	n/a	n/a	n/a	26.2	37.2	15.8	n/a	n/a	n/a
056a	LC-MS/MS	0.6	0.6	0.6	30.1	35.5	11.5	30.7	36.1	12.1	1.6	2.8	0.5
056b	LC-MS/MS	0.7	< 0.6	< 0.6	31.7	37.4	13.6	32.3	37.4	13.6	n/r	n/r	n/r
060	LC-MS/MS	0.7	0.4	0.2	30.8	35.4	13.5	31.5	35.8	13.7	1.8	3.2	0.7
110	LC-UV	n/r	n/r	n/r	n/r	n/r	n/r	19.5	31.3	12.6	n/r	n/r	n/r
116	LC-MS/MS	<3.3	<3.3	<3.3	35.3	42.1	13.8	35.3	42.1	13.8	<4	<4	<4
150	LC-MS/MS	0.7	0.4	0.0	26.1	32.2	10.2	26.8	32.6	10.2	1.7	3.2	0.5
180	RIA	n/a	n/a	n/a	n/a	n/a	n/a	27.7	32.1	13.9	n/a	n/a	n/a
187	LC-MS/MS	<1.5	<1.5	<1.5	29.9	37.6	12.1	29.9	37.6	12.1	n/r	n/r	n/r
188	CLIA	n/a	n/a	n/a	n/a	n/a	n/a	31.1	36.8	11.9	n/a	n/a	n/a
189	LC-UV	n/r	n/r	n/r	33.5	38.4	11.0	33.5	38.4	11.0	n/r	n/r	n/r
194	LC-MS/MS	<7	<7	n/r	31.0	42.0	n/r	31.0	42.0	n/r	n/r	n/r	n/r
196	CLIA	n/a	n/a	n/a	n/a	n/a	n/a	32.1	38.6	14.4	n/a	n/a	n/a
197	LC-MS/MS	<5	<5	0.0	33.6	41.1	12.2	33.6	41.1	12.2	n/r	n/r	n/r
198a	LC-MS/MS	<5	<5	<5	35.5	45.3	12.7	35.5	45.3	12.7	n/r	n/r	n/r
198c	CLIA	n/a	n/a	n/a	n/a	n/a	n/a	32.0	39.2	7.3	n/a	n/a	n/a
199	LC-MS/MS	<2.0	<2.0	<2.0	30.9	38.4	12.6	30.9	38.4	12.6	n/r	n/r	n/r
204b	LC-MS/MS	n/d	n/d	n/d	33.6	39.1	12.2	33.6	39.1	12.2	n/d	3.5	n/d
209	LC-MS/MS	<1.0	<1.0	<1.0	31.9	39.4	12.5	31.9	39.4	12.5	n/r	n/r	n/r
211	LC-MS/MS	0.0	0.0	0.0	32.7	39.0	11.3	32.7	39.0	11.3	n/r	n/r	n/r
212	LC-MS/MS	<2	<2	<2	32.8	40.5	13.2	32.8	40.5	13.2	n/r	n/r	n/r
214b	CLIA	n/a	n/a	n/a	n/a	n/a	n/a	23.4	31.1	21.1	n/a	n/a	n/a
214c	LC-MS/MS	1.0	0.4	0.2	31.6	38.6	12.3	32.6	39.0	12.5	n/r	n/r	n/r
215	LC-MS/MS	<2	<2	<2	30.8	36.0	12.0	30.8	36.0	12.0	n/r	n/r	n/r
216	LC-MS/MS	0.8	0.5	0.2	33.4	42.8	12.6	34.2	43.3	12.8	1.4	3.0	0.7
217	LC-MS/MS	n/d	n/d	1.3	28.4	38.4	14.4	28.4	38.4	15.7	n/r	n/r	n/r
218a	CLIA	n/a	n/a	n/a	n/a	n/a	n/a	30.2	45.7	13.5	n/a	n/a	n/a
218b	LC-MS/MS	0.0	0.0	0.0	29.2	42.6	12.3	29.2	42.6	12.3	n/r	n/r	n/r
220a	LC-MS/MS	<5	<5	<5	34.0	41.5	12.6	34.0	41.5	12.6	n/r	n/r	n/r
221b	LC-UV	0.0	0.0	0.0	29.2	34.5	9.6	29.2	34.5	9.6	n/r	n/r	n/r
221c	LC-MS	0.0	0.0	0.0	28.4	36.1	11.5	28.4	36.1	11.5	n/r	n/r	n/r
225	LC-MS/MS	<5	<5	<5	34.3	38.0	13.4	34.3	38.0	13.4	n/r	n/r	n/r
228a	LC-MS/MS	n/r	n/r	n/r	31.5	39.9	12.5	31.5	39.9	12.5	1.8	2.7	0.7
231b	CLIA	n/a	n/a	n/a	n/a	n/a	n/a	36.0	46.0	14.5	n/a	n/a	n/a
243a	LC-UV	0.9	0.3	n/d	33.4	34.1	12.2	34.4	34.4	12.2	1.7	1.9	n/d
243b	LC-MS/MS	0.9	0.5	n/d	33.6	34.1	12.2	34.5	34.5	12.2	1.6	2.0	n/d
244	LC-MS/MS	<5	<5	<5	29.3	37.4	12.7	29.3	37.4	12.7	n/r	n/r	n/r
249	LC-MS/MS	0.0	0.0	0.0	32.8	39.0	12.8	32.8	39.0	12.8	1.6	3.2	0.8
251	LC-MS/MS	<4	<4	n/r	36.0	46.0	n/r	36.0	46.0	n/r	n/r	n/r	n/r
253	LC-MS/MS	0.7	0.5	0.2	36.4	43.5	14.1	37.1	44.0	14.3	n/r	n/r	n/r
255	LC-MS/MS	0.4	0.2	0.0	37.3	47.3	16.4	37.7	47.5	16.4	n/r	n/r	n/r
256	CLIA	n/a	n/a	n/a	n/a	n/a	n/a	27.0	30.9	16.0	n/a	n/a	n/a
258	CLIA	n/a	n/a	n/a	n/a	n/a	n/a	40.4	48.5	18.1	n/a	n/a	n/a
259	LC-MS/MS	n/d	n/d	<2	30.2	33.0	14.0	30.2	33.0	14.0	n/r	n/r	n/r
261	CLIA	n/a	n/a	n/a	n/a	n/a	n/a	41.5	50.5	22.2	n/a	n/a	n/a
262	CLIA	n/a	n/a	n/a	n/a	n/a	n/a	29.0	38.4	17.7	n/a	n/a	n/a
267	CLEIA	n/a	n/a	n/a	n/a	n/a	n/a	29.9	36.7	12.4	n/a	n/a	n/a
268a	RIA	n/a	n/a	n/a	n/a	n/a	n/a	28.2	34.2	13.9	n/a	n/a	n/a
268b	EIA	n/a	n/a	n/a	n/a	n/a	n/a	46.7	58.7	28.4	n/a	n/a	n/a
270	LC-MS/MS	2.3	2.3	1.6	27.5	33.2	10.8	29.8	35.5	12.4	n/r	n/r	n/r
271	LC-MS/MS	<4	<4	<4	23.2	36.3	13.0	23.2	36.3	13.0	n/r	n/r	n/r
272	LC-MS/MS	0.7	0.4	0.0	30.8	39.6	12.3	31.5	40.0	12.3	1.8	3.5	0.8
273	EIA	n/a	n/a	n/a	n/a	n/a	n/a	24.2	40.3	14.1	n/a	n/a	n/a
274	CLIA	n/a	n/a	n/a	n/a	n/a	n/a	31.5	48.4	18.5	n/a	n/a	n/a

n/a = not applicable (for immunoassay methods); n/r = not reported or not determined; n/d = not detected; < X = less than a reported quantitation limit of X

	NIST Value	0.68	0.44	0.1*	31.3	37.1	12.4	32.0	37.5	12.5	1.7	3.2	0.65
	U	0.06	0.04	---	0.8	0.9	0.4	0.8	0.9	0.4	0.1	0.1	0.03

*estimated value (no uncertainty determined)