Assessment of Serum Total 25-Hydroxyvitamin D Assays for Vitamin D External Quality Assessment Scheme (DEQAS) Materials Distributed at Ambient and Frozen Conditions

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Abstract

The Vitamin D External Quality Assessment Scheme (DEQAS) distributes human serum samples four times per year to over 1000 participants worldwide for the determination of total serum 25-hydroxyvitamin D [25(OH)D)]. These samples are stored at -40 °C prior to distribution and the participants are instructed to store the samples frozen at -20 °C or lower after receipt; however, the samples are shipped to participants at ambient conditions (i.e., no temperature control). To address the question of whether shipment at ambient conditions is sufficient for reliable performance of various 25(OH)D assays, the equivalence of DEQAS human serum samples shipped under frozen and ambient conditions was assessed. As part of a Vitamin D Standardization Program (VDSP) commutability study, two sets of the same nine DEQAS samples were shipped to participants at ambient temperature and frozen on dry ice. Twenty-eight laboratories participated in this study and provided 34 sets of results for the measurement of 25(OH)D using 20 ligand binding assays and 14 liquid chromatography – tandem mass spectrometry (LC-MS/MS) methods. Equivalence of the assay response for the frozen vs. ambient DEQAS samples for each assay was evaluated using multilevel modeling, paired *t*-tests including a False Discovery Rate (FDR) approach, and ordinary least squares linear regression analysis of frozen versus ambient results. Using the paired *t*-test and confirmed by FDR testing, differences in the results for the ambient and frozen samples were found to be statistically significant at $p < 0.05$ for four assays (DiaSorin, DIAsource, Siemens, and SNIBE prototype). For all 14 LC-MS/MS assays, the differences in the results for the ambient- and frozen-shipped samples were not found to be significant at $p < 0.05$ indicating that these analytes were stable during shipment at ambient conditions. Even though assay results have been shown to vary considerably among different 25(OH)D assays in other studies, the results of this study also indicate that sample handling/transport conditions may influence 25(OH)D assay response for several assays.

Keywords: 25-hydroxyvitamin D_3, 25-hydroxyvitamin D_2, total 25-hydroxyvitamin D, liquid chromatography – tandem mass spectrometry (LC-MS/MS), Vitamin D External Quality Assessment Scheme (DEQAS), ligand binding assay

Introduction

The Vitamin D External Quality Assessment Scheme (DEQAS) has operated since 1989 [1] with the goal of ensuring the quality of analytical measurements for the determination of total serum 25-hydroxyvitamin D [25(OH)D], the primary marker for vitamin D status, which is defined as the sum of 25-hydroxyvitamin D_2 [25(OH)D_2] and 25-hydroxyvitamin D_3 [25(OH)D_3]. DEQAS distributes four sets of five human serum samples annually to over 1000 participating laboratories worldwide [1]. Participants report results based on a single analysis of each sample. Initially, individual participant results in DEQAS were compared to laboratory consensus results using an All-Laboratory Trimmed Mean (ALTM). Since 2013, however, DEQAS has been an accuracy-based program with participant results compared to target values established using reference measurement procedures (RMPs) at the U.S. National Institute of Standards and Technology (NIST) [2, 3], and since 2018 at the U.S. Centers for Disease Control and Prevention (CDC) [4]. DEQAS samples are stored at -40 °C and are distributed worldwide at ambient temperatures with participants instructed to store the samples frozen at -20 °C or lower after receiving them [1] .

Since 2012 the U.S. College of American Pathologists (CAP) has coordinated an accuracy-based vitamin D (ABVD) program for the determination of 25(OH)D, and all test samples are distributed within the United States frozen on dry ice and internationally on cold packs [5-7]. The question has been raised whether the shipment of serum samples at ambient temperature is sufficient for reliable measurements for 25(OH)D assays. The Vitamin D Standardization Program (VDSP) [8], which was established in 2010 by the Office of Dietary Supplements at the National Institutes of Health (NIH-ODS) to assist in the standardization of 25(OH)D measurements worldwide, conducted a multi-laboratory study in 2016, in conjunction with NIST [9], to assess the commutability of Standard Reference Materials (SRMs) and proficiency testing (PT)/external quality assessment (EQA) samples from CAP and DEQAS [10]. As part of this 2016 VDSP commutability study [10], two sets of the same nine DEQAS samples were shipped to participants, one set at ambient temperature and one set frozen on dry ice to address the question of the potential influence of shipping conditions on assay performance. Twenty-eight laboratories participating in this study provided results for the analysis of these DEQAS samples for the determination of 25(OH)D using 20 ligand binding assays and 14 LC-MS/MS methods. The results of this study to assess and compare the performance of 25(OH)D assays on samples distributed at ambient or frozen temperatures are reported in this paper. The complete

results from the commutability study [10] and from a comprehensive interlaboratory assessment of assay performance, including variability, bias, and the impact of metabolite content are reported elsewhere [11-13].

Materials and Methods

Measurands

The measurand for this study was serum total 25(OH)D (in nmol/L), which is defined as the sum of the concentrations of 25(OH)D_2 and 25(OH)D_3, without the inclusion of the concentration of 3-epi-25(OH)D_3.

Commutability Study 2 - Coordination and Responsibilities

The study of ambient versus frozen sample distribution was part of a larger VDSP study to assess commutability of SRMs and PT/EQA samples [10], which was co-designed and coordinated by NIST and NIH-ODS through the VDSP. NIST was responsible for analysis of the nine DEQAS samples to assign target values for 25(OH)D_2 and 25(OH)D_3 as part of the commutability study. NIH-ODS and VDSP LLC were responsible for conducting the data analyses.

SRM/PT/EQA Test Samples Evaluated

The DEQAS samples were from serum pools previously distributed as part of DEQAS quarterly exercises. DEQAS samples 1 through 9 were the following samples from DEQAS exercises conducted in April 2015 and July 2015: (1) 472, (2) 473, (3) 474, (4), 475 (5) 479, (6), 476, (7) 478, (8) 477, (9) 480 as described previously [10]. The DEQAS samples were assigned target values for serum total 25(OH)D using the NIST RMPs [3, 14] as described below.

Study design

Each participant received 18 DEQAS samples (each containing 0.6 mL of serum). Nine DEQAS samples were shipped from NIST in the U.S. to the participants by courier frozen on dry ice and nine samples were shipped separately from DEQAS in the U.K. by post at ambient temperature. Therefore, participants were not blinded to the identity of these samples within the complete set of SRM/PT/EQA samples. Participants were instructed to immediately upon receipt store all 18 DEQAS samples at -60 °C or lower until the time of analysis. Therefore, all samples were stored at frozen temperatures prior

to distribution and by the receiving laboratories except for the shipment phase when one set denoted as "ambient" was at ambient conditions for 1 to 2 weeks during shipment. The frozen samples were distributed from NIST to the laboratories in the U.S. on September 21, 2016 and to laboratories outside the U.S. on September 26, 2016. The DEQAS samples shipped at ambient temperature were sent from Imperial College Healthcare in the U.K. to laboratories on September 19, 2016. The laboratories analyzed the samples in October and November 2016 with the exception of two laboratories that analyzed the samples in January 2017 and one laboratory that reported results in April 2017.

Participants were to analyze the DEQAS samples in duplicate on one day as part of the protocol for VDSP Commutability Study 2 [10]. Briefly, duplicate measurements (i.e., sample preparations) were to be made from a single vial. Participants were requested to intersperse the 18 DEQAS samples among the 50 single-donor samples with the other SRMs and CAP ABVD samples in a prescribed assay run order. Participants were requested to use their routine laboratory procedures with normal internal quality control procedures and to provide results for serum total 25(OH)D in nmol/L with three significant figures in a reporting template provided by NIST.

Participants and assays used in this study

A total of 28 laboratories using 34 assays including 20 immunoassay-based assays (12 unique assays) and 14 LC-MS/MS assays provided results for the study. NIST also provided results based on ID LC-MS/MS RMPs [3]. The laboratories, assay manufacturers, and assay name/model reported in this study are listed in Table 1. Several laboratories used the same assay which provided two or more data sets including: Abbott (4), bioMérieux (2), DiaSorin (2), ImmunoDiagnostic Systems IDS-iSYS (2), Roche (2), Siemens (2), and SNIBE (2), where the number in parentheses is the number of participants using the assay. To avoid repetition of the assay name/model, the assay will be referred to by the manufacturer's name.

Data analysis

An ANOVA approach was used to test whether there were statistically significant differences between the LC-MS/MS assays and the ligand binding assays for the two sets of samples shipped either frozen or at ambient conditions. Three-level multi-level modeling results [15, 16] with random intercepts were used to assess differences in the frozen vs. ambient samples for results from all 34 laboratories (608 observations) using the following levels: shipping conditions (ambient vs. frozen), (2), sample number

(1 through 9), and individual laboratories (34). A multi-level modeling approach accounted for the within-laboratory and within-sample correlations. We tested whether or not mean level differences between shipping conditions, i.e., frozen vs. ambient, were dependent on the type of assay used, i.e., immunoassay vs LC-MS/MS, by adding an interaction term (shipping conditions x assay type) to the multi-level regression model. Because of concern for effects from multiple comparisons, the False Discovery Rate (FDR) approach, developed by Benjamini and Hochberg [17], was used to control the proportion of false-positive (statistically significant) results [15, 16]. Additional assessments were used to complement these results including comparison of analyte concentrations from RMPs and linear regression analysis of frozen versus ambient results. All calculations were performed using Stata 16 (StataCorp, LLC, College Station TX, US) and were confirmed using Analyze-It (Analyze-It Software, Leeds, UK), a statistical analysis add-in for Microsoft Excel.

Results and discussion

NIST assignment of target values and laboratory results

The DEQAS samples were assigned target values for 25(OH)D_2 and 25(OH)D_3 using RMPs [3] as reported in Camara et al. [10], and a summary of results is provided in Table 2. Results for the determination of 3-epi-25(OH)D_3 and 24R,25$(OH)_2D_3$ in these DEQAS samples are reported in Camara et al. [10]. The distribution of 25(OH)D_2 and 25(OH)D_3 in the DEQAS samples is shown graphically, arranged as increasing concentration of 25(OH)D, in Fig. S1 in Supplementary Materials. The results for the DEQAS samples for each of the 34 participating laboratories are summarized in Tables S1 through S5 for the ligand binding assays and in Tables S6 through S9 for the LC-MS/MS assays including the NIST RMPs.

Assessment of equivalence of DEQAS samples shipped frozen versus ambient temperature

The three-level multi-level modeling indicated that there was a statistically significant relationship between assay type and shipping condition, i.e., the difference between the ambient and frozen samples was significant for the ligand binding assays (difference = 1.29 nmol/L) but was not significant for the LC-MS/MS assays (difference = 0.06 nmol/L). As shown in Table 3, the means for all 20 ligand binding assays were 74.19 nmol/L (frozen) and 72.34 nmol/L (ambient), and the means of the 14 LC-MS/MS assays were 70.80 nmol/L (frozen) and 70.74 nmol/L (ambient).

The equivalence of the results from each laboratory (assay) was then assessed using a paired *t*-test at $p < 0.05$ and the results are summarized in Table 3. For 8 of the 20 ligand binding assays evaluated, the differences between the results for the ambient and frozen samples were determined to be significant at $p < 0.05$. These eight laboratory results represented five distinct assays, i.e., DiaSorin (Labs 9 and 24), DIAsource (Lab 2), Roche (Lab 19), Siemens (Labs 30 and 40), and SNIBE (Labs 5 and 31). For the DiaSorin, Siemens, and SNIBE prototype assays, two sets of results from different laboratories were available for each assay, and the differences between the ambient and frozen samples were found to be significant for both assay results. However, for the Roche assay reported by two laboratories, only one set of results was determined to have significant differences. At the $p < 0.10$ level, differences were found to be significant for two additional ligand binding assays (IDS-EIA and IDS-iSYS). Therefore, 7 of the 11 unique ligand binding assays evaluated in this study demonstrated statistically significant differences in response at the $p < 0.10$ level for the ambient and frozen samples. As shown in Table 3, for the seven ligand binding assays with differences in response for frozen vs ambient samples (and all ligand binding assays with differences in means > 1.0 nmol/L, the difference is positive (i.e., frozen mean concentration is greater than ambient mean concentration) except for the Siemens assay and the results for IDS-iSYS-1 (Lab 39). For the IDS-iSYS assay, two different kits were used in the study (denoted as IDS-iSYS-1 and IDS-iSYS-2) and results for the two kits differed significantly in the broader interlaboratory evaluation of bias [12]. At $p < 0.05$, there were no statistically significant differences between the ambient and frozen samples for all LC-MS/MS assays; at $p < 0.10$, the difference for one LC-MS/MS assay (Lab 33) was found to be statistically significant.

The results of the FDR testing are summarized in Table 4 and indicated that five ligand binding assays provide significant differences between the frozen and ambient DEQAS samples including SNIBE prototype (Labs 31 and 5), DiaSorin (Lab 9), Siemens (Lab 30), and DIAsource (Lab 2). These results confirm the statistically significant difference in response for the ambient- versus frozen-shipped samples for these four assays.

As shown in Table 2, the differences in concentrations of $25(OH)D_2$, $25(OH)D_3$, and total serum 25(OH)D determined using the LC-MS/MS RMPs in the frozen versus ambient samples were small with absolute differences ranging from 0.7% to 2.2% (mean 1.3%) compared with the %CVs for four replicate analyses ranging from 0.3% to 2.8% (mean 1.1%). A plot of the mean of the frozen samples versus the mean of ambient samples is shown in Fig. 1 for the ligand binding assays and the LC-MS/MS assays. For the LC-MS/MS assays, the slope is 0.988 whereas the slope for the ligand binding assays

is only 0.606 with considerable spread on the results (R^2 = 0.626) compared to the LC-MS/MS (R^2 = 0.983). Plots of the results for the ambient versus the frozen samples are shown in Fig. 2 using the NIST LC-MS/MS RMPs for 25(OH)D_3, 25(OH)D_2, and 24,25$(OH)_2D_3$ and a similar NIST ID-LC-MS/MS method for 3-epi-25(OH)D_3. Using a paired *t*-test, there were no significant differences for these four vitamin D metabolites in the DEQAS sample pairs (see Table S10). Percent differences between the means for the frozen and ambient samples were 0.34% 0.58%, 0.90%, and 1.5% for 25(OH)D_3, 25(OH)D_2, 3-epi-25(OH)D_3, and 24,25$(OH)_2D_3$, respectively. Comparison of the NIST LC-MS/MS results for the measured concentrations of four vitamin D metabolites in the frozen versus the ambient samples (Fig. 2) indicates that the metabolites concentrations are equivalent demonstrating stability during shipment at ambient conditions.

The response of individual assays was evaluated in a similar manner using plots of the mean results (two replicates) for the frozen sample versus the ambient sample for the nine DEQAS samples as shown in Figs. 3 and 4 for selected LC-MS/MS assays and ligand binding assays, respectively. Similar plots for the remaining assays are provided in the Supplementary Material as Figs. S2 through S8. The results of the linear regression analysis (slope, intercept, R^2, and 95 % prediction intervals) are provided in Tables S11 and S12 in the Supplementary Material for the ligand binding assays and the LC-MS/MS assays, respectively. The plots for results from four LC-MS/MS assays are shown in Fig. 3. The results from the NIST RMP for 25(OH)D is shown in Fig. S8C and represent assays with a slope near unity and zero intercept, and similar results were obtained for Labs 6, 7, 12, 16, 17, 22, 25-1, and 25-2 (see Table S7 and Figs. S6 to S8 in Supplementary Material). The plots in Figs. 3B and 3C represent LC-MS/MS assays that have only slight deviations from the equivalence (identity) line (e.g., Labs 11, 33, 36, 37, and 38). The plot for Lab 28 (Fig. 3B) illustrates a greater deviation from the equivalence line with a slope near unity but with a constant positive offset from the equivalence line.

For the ligand binding assays, the plots of the results for the frozen versus ambient samples (Fig. 4) showed more instances of deviations from a slope of unity and zero intercept. The Abbott assay (Fig. 4A) is representative of an assay with equivalent response for both the frozen and ambient samples (i.e., slope of unity and intercept near zero). All four laboratories using the Abbott assay reported similar results (see Fig. 4A and Figs. S2A, S2B, and S2C). Two other assays demonstrated similar behavior to the Abbott assay, i.e., bioMérieux (Figs S2D and S3A) and Beckman Coulter (Fig. S4B). The plots for DIAsource (Fig. 4B), SNIBE (Fig. 4C) and Siemens (Fig. 4D) showed the greatest deviation from the equivalence line among the assays evaluated. The remaining two plots in Fig. 4 are for the DiaSorin

(E) and Roche (F) assays which represent a group of assays with moderate deviations from the equivalence line including Bio-Rad (Fig. S3B), IDS-iSYS (Fig. S4C and S4D), and IDS-EIA (Figs. S4A). The assays that show deviations in the linear regression assessment are the same assays in which differences in the ambient and frozen sample measurements were found to be significant using the paired *t*-test, with the exception of Bio-Rad.

Conclusions and recommendations

Based on the LC-MS/MS results, including the NIST RMPs, the concentrations of the 25(OH)D_2 and 25(OH)D_3, and therefore serum total 25(OH)D, were shown to be equivalent in the ambient versus frozen samples, and thus the analytes appear to be stable during shipment at ambient conditions. However, the response of 5 of the 11 distinct ligand binding assays for serum total 25(OH)D evaluated (DiaSorin, DIAsource, Roche, Siemens, and SNIBE prototype) is statistically different for the ambient- and frozen-shipped samples indicating that some non-specific property(ies) of the serum matrix may have changed under ambient conditions resulting in different responses. It should be noted that the DEQAS samples used in this study were not freshly prepared (i.e., 14 month to 18 months since used in DEQAS studies) and fresh DEQAS samples may not exhibit these differences. Based on this study, the VDSP recommends that additional research should be conducted to investigate these observed assay differences in ambient versus frozen distributed samples, including research by the 25(OH)D assay manufacturers whose assays were impacted by the shipping conditions, to confirm and to understand this assay behavior and to evaluate its impact in vitamin D research, public health surveillance through national nutrition surveys, and in clinical medicine.

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Declarations

Ethics approval

The National Institute of Standards and Technology Research Protections Office reviewed the protocol for this project and determined it is "not human subjects research" as defined in 15 CFR 27, the Common Rule for the Protection of Human Subjects. The laboratory study participants agreed to the publication of their measurement data, laboratory identification, and measurement assay platform identification.

Conflict of interest

S.A. Wise is an Editor of the journal of ***Analytical and Bioanalytical Chemistry*** and was not involved in peer reviewing this manuscript. The following coauthors are employees of companies that produced the assays evaluated in this study: Christian Popp, Christian Beckert, and Jan Schultess (Abbott Laboratories); Carole Tourneur and Camille Pease (bioMérieux); Ravi Kaul and Alfredo Villarreal (Bio-Rad Laboratories); Ralf Fischer (Chromsystems Instruments & Chemicals GmbH); James Freeman and Neil Parker (Siemens-Healthineers); and Jinyun Yuan (SNIBE). There are no financial or nonfinancial competing interest for any of the remaining coauthors.

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Certain commercial equipment or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology or the National Institutes of Health, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

References

1. Carter GD, Berry J, Durazo-Arvizu R, Gunter E, Jones G, Jones J, et al. Hydroxyvitamin D assays: An historical perspective from DEQAS. J Steroid Biochem Mol Biol. 2018;177:30-5. https://doi.

org/10.1016/j.jsbmb.2017.07.018.

2. Burdette CQ, Camara JE, Nalin F, Pritchett J, Sander LC, Carter GD, et al. Establishing an accuracy basis for the Vitamin D External Quality Assessment Scheme (DEQAS). J AOAC Int. 2017;100(5):1277-87. https://doi.org/10.5740/jaoacint.17-0306.
3. Tai SSC, Bedner M, Phinney KW. Development of a candidate reference measurement procedure for the determination of 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2 in human serum using isotope-dilution liquid chromatography-tandem mass spectrometry. Anal Chem. 2010;82(5):1942-8. https://doi.org/10.1021/ac9026862.
4. Mineva EM, Schleicher RL, Chaudhary-Webb M, Maw KL, Botelho JC, Vesper HW, et al. A candidate reference measurement procedure for quantifying serum concentrations of 25-hydroxyvitamin D-3 and 25-hydroxyvitamin D-2 using isotope-dilution liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2015;407(19):5615-24. https://doi.org/10.1007/s00216-015-8733-z.
5. Erdman P, Palmer-Toy DE, Horowitz G, Hoofnagle A. Accuracy-based vitamin D survey six years of quality improvement guided by proficiency testing. Arch Pathol Lab Med. 2019;143(12):1531-8. https://doi.org/10.5858/arpa.2018-0625-CP.
6. Hoofnagle A. (University of Washington, Seattle, WA, USA), Personal Communication, 2020.
7. College of American Pathologists (CAP); https://elss.cap.org/elss/ShowProperty?nodePath=/UCMCON/Contribution%20Folders/WebApplications/pdf/istore-shipping.pdf.
8. Sempos CT, Vesper HW, Phinney KW, Thienpont LM, Coates PM, VDSP. Vitamin D status as an international issue: National surveys and the problem of standardization. Scand J Clin Lab Invest. 2012;72:32-40. https://doi.org/10.3109/00365513.2012.681935.
9. Wise SA, Tai SSC, Burdette CQ, Camara JE, Bedner M, Lippa KA, et al. Role of the National Institute of Standards and Technology (NIST) in support of the vitamin D initiative of the National Institutes of Health, Office of Dietary Supplements. J AOAC Int. 2017;100(5):1260-76. https://doi.org/10.5740/jaoacint.17-0305.
10. Camara JE, Wise SA, Hoofnagle AN, Williams EL, Carter GD, Jones J, et al. Assessment of serum total 25-hydroxyvitamin D assay commutability of Standard Reference Materials and College of American Pathologists Accuracy-Based Vitamin D (ABVD) Scheme and Vitamin D External

Quality Assessment Scheme (DEQAS) materials: Vitamin D Standardization Program (VDSP) Commutability Study 2. Anal Bioanal Chem. 2021;413:5067-84. https://doi.org/10.1007/s00216-021-03470-w.

11. Wise SA, Camara JE, Sempos CT, Burdette CQ, Hahm G, Nalin F, et al. Interlaboratory comparison of 25-hydroxyvitamin D assays: Vitamin D Standardization Program (VDSP) intercomparison study 2 – Part 1 Liquid chromatography – tandem mass spectrometry (LC-MS/MS) assays – Impact of 3-epi-25-hydroxyvitamin D_3 on assay performance. Anal Bioanal Chem. 2022 in press, https://doi.org/10.1007/s00216-021-03576-1.
12. Wise SA, Camara JE, Sempos CT, Burdette CQ, Hahm G, Nalin F, et al. Interlaboratory comparison of 25-hydroxyvitamin D assays: Vitamin D Standardization Program (VDSP) intercomparison study 2 - Part 2 Ligand binding assays – Impact of 25-hydroxyvitamin D_2 and 24R,25-dihydroxyvitamin D_3 on assay performance. Anal Bioanal Chem. 2022, in press, https://doi.org/10.1007/s00216-021-03577-0.
13. Wise SA, Camara JE, Sempos CT, Lukas P, Le Goff C, Peeters S, et al. Vitamin D Standardization Program (VDSP) Intralaboratory study for the assessment of 25-hydroxyvitamin D assay performance. J Steroid Biochem Mol Biol. 2021;212:105917. https:// doi.org/10.1016/j.jsbmb.2021.105917.
14. Tai SSC, Nelson MA. Candidate reference measurement procedure for the determination of (24R),25-dihydroxyvitamin D_3 in human serum using isotope-dilution liquid chromatography-tandem mass spectrometry. Anal Chem. 2015;87(15):7964-70. https://doi.org/10.1021/acs.analchem.5b01861.
15. Rabe-Hesketh S, Skrondal A. Multilevel and Longitudinal Modeling Using Stata. Volume I: Continuous Responses: Strata Press; 2012.
16. Snijders TAB, Bosker RJ. Multilevel Analysis: An Introduction to Basic and Advanced Multilevel Modeling. 2nd ed: Sage Publications Ltd.; 2012.
17. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate - A Practical and Powerful Approach to Multiple Testing. J Royal Statistical Soc Series B-Statistical Methodology. 1995;57(1):289-300.https://doi-org.ezproxy.nihlibrary.nih.gov/10.1111/j.2517-6161.1995.tb02031.x
18. Rosner B. Fundamentals of Biostatistics. 8th ed: Cengage Learning; 2016.

Table 1. Participants and Assays Used in VDSP Study of Frozen vs. Ambient DEQAS Samples (Grouped by Assay Alphabetically)

Lab No.	Participant	Assay Manufacturer	Assay/Instrument Model or LC-MS/MS Instrumentation (LC column)	Storage Temp.[a]	Est. Storage Time (days)[b]
1	Abbott Diagnostics, DE	Abbott	Architect 25-OH Vitamin D; Architect i2000SR	-20 °C	80
18	Golwilkar Metropolis Health Services Pvt. Ltd., IN	Abbott	Architect 25-OH Vitamin D; Architect i2000 SR	N.A.	60
23	Imperial College Healthcare, UK	Abbott	Architect 25-OH Vitamin D; Architect i2000 SR	-20 °C	40
27	National University of Medical Sciences, PK	Abbott	Architect 25-OH Vitamin D; Architect CIA 12000 ST	-80 °C	10
26	National Institute of Public Health, NL	Beckman Coul.	Access 25(OH) Vitamin D Total; Access-2	-80 °C	35
3	bioMérieux, FR	bioMérieux	VIDAS 25 OH Vitamin D Total; Vidas Legacy	-80 °C	15
34	University of Chester, UK	bioMérieux	VIDAS 25 OH Vitamin D Total; Mini-Vidas	-80 °C	15
4	Bio-Rad Laboratories, Inc., US	Bio-Rad	BioPlex 2200 25(OH) Vitamin D; BioPlex 2200	-80 °C	35
9	CHU de Liège, University of Liège, BE	DiaSorin	Liaison 25 OH Vitamin D Total; Liaison XL	-80 °C	5
24	Imperial College Healthcare, UK	DiaSorin	Liaison 25 OH Vitamin D Total; Liaison XL	-20 °C	50
2	Awareness Technology, US	DIAsource	25OH Vitamin D Total ELISA; ChemWell 2910	-20 °C	35
21	Immunodiagnostic Systems (IDS), UK	IDS	IDS-EIA 25-Hydroxy Vitamin D^S EIA	-80 °C	20
20	Immunodiagnostic Systems (IDS), UK	IDS	IDS-iSYS 25VitD^S (IDS-iSYS-2)[c]	-80 °C	20
39	Yale University, US	IDS	IDS-iSYS 25-Hydroxy Vitamin D^S (IDS-iSYS-1)[c]	-70 °C	25
19	Hospital Israelita Albert Einstein, BR	Roche	Vitamin D Total II; Modular Analytics E-170	-70 °C	30
29	St. Vincent's University Hospital, IE	Roche	Vitamin D Total II; Cobas e602	-80 °C	65
30	Siemens-Healthineers, US	Siemens	Vitamin D Total (VitD); ADVIA Centaur XP	< -60 °C	15
40	CHU de Liège, University of Liège, BE	Siemens	Vitamin D Total (VitD); ADVIA Centaur XPT	-80 °C	5
31	SNIBE, CN	SNIBE[d,e]	Prototype MAGLUMI 25-OH Vitamin D; MAGLUMI 2000	-20 °C	35
5	Care S.r.l., IT	SNIBE[d,e]	Prototype MAGLUMI 25-OH Vitamin D; MAGLUMI 2000	N.A.	30
6	Manchester Royal Infirmary (MRI), UK	Chromsystems	ABSciex 5500 (Chromsystems column)	-80 °C	195

7	Chromsystems Instruments & Chemicals, GmbH, DE	Chromsystems	ABSciex API4000 (Chromsystems column)	-70 °C	10
11	Canisius Wilhelmina Hospital, NL	LC-MS/MS	Waters Quattro Premier XE (HSS PFP 2.1 x 100 mm, 1.8 µm)	-80 °C	35
12	Prince of Wales Hospital, HK	LC-MS/MS	Waters Xevo TQ-S micro ACQUITY UPLC (CSH Fluoro-Phenyl, 2.1 x 100 mm, 1.7 µm)	-80 °C	30
16	Endoceutics Inc., CA	LC-MS/MS	ABSciex (C18-PFP 3 x 100 mm, 2 µm)	N.A.	30
17	Endocrine Sciences (LabCorp), US	LC-MS/MS	Sciex API5000 (Proprietary)	< -60 °C	10
22	Imperial College Healthcare, UK	LC-MS/MS	Waters Acquity TQD (BEH Phenyl 2.1 x 50 mm, 1.8 µm)	-20 °C	55
25-1	NIHR BRC Nutritional Biomarker Laboratory, MRC Epidemiology Unit, Univ. of Cambridge, UK	LC-MS/MS	AB Sciex 4000, Waters Acquity UPLC (Hypersil PFP 2.1 x 100 mm, 1.9 µm)	-80 °C	30
25-2	NIHR BRC Nutritional Biomarker Laboratory, MRC Epidemiology Unit, Univ. of Cambridge, UK	LC-MS/MS	AB Sciex 5500, Waters Acquity UPLC (Hypersil PFP 2.1 x 100 mm, 1.9 µm)	-80 °C	35
28	Penn State University – College of Medicine, US	LC-MS/MS	Agilent 1260 HPLC and 6460 QQQ (Poroshell 120 EC-18, (2.1 x 50 mm, 2.7 µm)	-80 °C	30
33	University of California at San Diego, US	LC-MS/MS	Waters XevoTSQ (HSS T3 2.1 x 75 mm, 2.5 µm)	-80 °C	20
36	University of Washington, US	LC-MS/MS	Quattro Micro, Acquity UPLC, (PFP, 3.2 x100 mm, 2.5 µm)	-70 °C	10
37	University of Western Australia, AU	LC-MS/MS	Agilent 6460 QQQ (PFP x 2)	-80 °C	120
38	Waters Technologies Ireland Ltd., IE	LC-MS/MS	Waters Xevo TQD Acquity UPLC (BEH Phenyl, 2.1 x 50 mm, 1.7 µm)	-70 °C	10

[a] Temperature at which DEQAS samples were stored until analysis; N.A. = Storage information not available.

[b] Estimated days (within ±5 days) in frozen storage until samples analyzed.

[c] Two different IDS-iSYS kits were used in the study, 25-Hydroxy Vitamin DS and 25VitDS, which are designated as IDS-iSYS 1 and IDS-iSYS 2.

[d] SNIBE = Shenzhen New Industries Biomedical Engineering Co., Ltd.

[e] The MAGLUMI 25-OH Vitamin D kit used in this study was a prototype kit, which is not equivalent to current MAGLUMI 25-OH Vitamin D kit per personal communication from SNIBE.

Table 2. Summary of NIST Determination of 25(OH)D_2, 25(OH)D_3, and Total 25(OH)D in Frozen and Ambient DEQAS Samples

DEQAS Samples[a]	25(OH)D_2 (nmol/L)			25(OH)D_3 (nmol/L)			Total 25(OH)D (nmol/L)		
	Mean[b]	SD[b]	%CV[b]	Mean[b]	SD[b]	%CV[b]	Mean[b]	SD[b]	%CV[b]
DEQAS-1F	1.92	0.20	10.4	38.2	0.3	0.7	40.1	0.3	0.8
DEQAS-1A	1.88	0.10	5.2	37.3	0.9	2.4	39.2	1.0	2.4
DEQAS-2F	2.89	0.02	0.8	60.9	0.3	0.4	63.8	0.3	0.5
DEQAS-2A	2.97	0.05	1.7	61.9	0.2	0.4	64.9	0.2	0.3
DEQAS-3F	1.46	0.06	3.8	76.6	0.7	0.9	78.0	0.7	0.9
DEQAS-3A	1.39	0.05	3.4	75.8	1.1	1.4	77.2	1.1	1.4
DEQAS-4F	1.29	0.04	3.3	73.0	0.6	0.9	74.3	0.7	0.9
DEQAS-4A	1.21	0.04	3.8	73.6	0.8	1.1	74.8	0.8	1.1
DEQAS-5F	1.33	0.03	2.1	28.8	0.8	3.0	30.2	0.8	2.8
DEQAS-5A	1.25	0.11	8.4	29.5	0.3	1.0	30.8	0.4	1.2
DEQAS-6F	0.77	0.03	3.5	99.0	0.5	0.5	99.8	0.5	0.5
DEQAS-6A	0.76	0.06	8.3	99.3	1.2	1.2	100.1	1.2	1.2
DEQAS-7F	1.20	0.01	1.2	71.3	1.1	1.5	72.5	1.1	1.5
DEQAS-7A	1.13	0.06	5.5	70.6	0.3	0.4	71.7	0.3	0.5
DEQAS-8F	1.94	0.05	2.4	42.7	0.4	0.9	44.6	0.4	0.8
DEQAS-8A	1.84	0.05	2.8	42.4	0.4	1.1	44.2	0.4	0.9
DEQAS-9F	57.0	0.63	1.1	44.8	0.8	1.7	101.8	1.2	1.1
DEQAS-9A	57.1	0.79	1.4	46.6	1.4	3.0	103.6	1.0	1.0

[a] Samples designated as F were shipped frozen on dry ice and samples designated as A were shipped at ambient conditions.
[b] Two sample preparations and replicate measurements (injections) of each sample preparation.

Table 3. Paired *t*-Test for Significance of DEQAS Ambient and Frozen Samples for 25(OH)D Assays Based on Mean of Two Replicates

						***t*-Test Paired Two Sample for Means**			
Lab No.	**Assay**	**Mean (nmol/L) Frozen (F)**	**Mean (nmol/L) Ambient (A)**	**Δ (F-A) Mean**	**SE Δ Mean**	***t*-Statistic**	***t*-Critical[a]**	***p*-Value[a]**	**Significant Difference[b]**
LIGAND BINDING ASSAYS									
1	Abbott	68.16	68.98	-0.822	0.49	1.66	2.306	0.1344	No
18	Abbott	68.10	67.93	0.167	0.60	-0.28	2.306	0.7892	No
23	Abbott	68.76	69.03	-0.272	0.48	0.57	2.306	0.5829	No
27	Abbott	69.32	68.45	0.872	0.63	-1.38	2.306	0.2045	No
26	Beckman Coulter	70.85	70.44	0.411	0.66	-0.63	2.306	0.5481	No
3	bioMérieux	71.92	71.07	0.847	0.69	-1.22	2.306	0.2569	No
34	bioMérieux	76.32	77.06	-0.739	1.12	0.66	2.306	0.5263	No
4	Bio-Rad	66.41	66.24	0.168	0.88	-0.19	2.306	0.8543	No
9	DiaSorin	62.96	61.22	1.736	0.32	-5.44	2.306	0.00061	Yes
24	DiaSorin	76.51	74.82	1.687	0.51	-3.30	2.306	0.01089	Yes
2	DIAsource	87.90	82.27	5.633	1.15	-4.88	2.306	0.00122	Yes
21	IDS-EIA[b]	78.32	74.72	3.594	1.82	-1.98	1.859	0.0831	Yes
20	IDS-iSYS	79.36	75.57	3.796	1.84	-2.07	1.859	0.0726	Yes
39	IDS-iSYS	77.33	80.31	-2.972	1.56	1.91	1.859	0.0925	Yes
19	Roche	76.20	73.29	2.908	0.98	-2.95	2.306	0.01830	Yes
29	Roche	75.59	75.51	0.084	0.53	-0.16	2.306	0.8768	No
30	Siemens	71.15	79.32	-8.172	1.54	5.32	2.306	0.00071	Yes
40	Siemens	68.37	71.24	-2.880	1.03	2.78	2.365	0.02714	Yes
31	SNIBE prototype	84.51	73.16	11.35	2.07	-5.48	2.306	0.00059	Yes
5	SNIBE prototype	85.73	77.34	8.388	2.17	-3.87	2.306	0.00474	Yes
	Mean Ligand binding assays	**74.19**	**72.34**	**1.289**					
LC-MS/MS									
NIST	LC-MS/MS (RMP)	67.23	67.40	-0.165	0.95	0.52	2.306	0.62	No

6	Chromsystems	79.77	79.53	0.239	0.59	-0.41	2.306	0.6942	No
7	Chromsystems	75.38	75.28	0.106	0.54	-0.20	2.306	0.8503	No
11	LC-MS/MS	67.27	66.47	0.800	0.62	-1.30	2.306	0.2323	No
12	LC-MS/MS	69.48	69.74	-0.259	0.23	1.10	2.306	0.2987	No
16	LC-MS/MS	66.72	66.59	0.130	0.90	-0.14	2.306	0.8896	No
17	LC-MS/MS	63.82	63.88	-0.066	0.78	0.08	2.306	0.9343	No
22	LC-MS/MS	75.76	76.07	-0.311	0.69	0.45	2.306	0.6633	No
25-1	LC-MS/MS	69.83	69.47	0.356	0.59	-0.61	2.306	0.5616	No
25-2	LC-MS/MS	68.36	68.01	0.356	1.01	-0.35	2.306	0.6305	No
28	LC-MS/MS	75.65	74.95	0.70	0.41	-1.7	2.306	0.1217	No
33	LC-MS/MS	69.51	70.59	-1.083	0.53	2.05	1.859	0.0742	Yes
36	LC-MS/MS	70.09	71.16	-1.076	0.81	1.32	2.306	0.2216	No
37	LC-MS/MS	66.59	66.63	-0.040	0.79	0.05	2.306	0.9612	No
38	LC-MS/MS	70.08	69.27	0.805	0.78	-1.04	2.306	0.3297	No
	Mean LC-MS/MS[c]	**70.59**	**70.55**	**0.057**					

[a] Critical value p < 0.05 two-tailed *t*-test based on number of pairs. The number of pairs (observations) = 9 except for Lab 40 for which the number of pairs/observations = 8.

[b] Results highlighted in yellow are significant at $p < 0.05$; results highlighted in blue are significant at $p < 0.10$ with a critical *t*-value of 1.859.

[c] Mean excluding NIST results.

Table 4. False Discovery Rate (FDR) Testing for DEQAS Ambient and Frozen Samples for 25(OH)D Assays[a]

Lab No.	Assay	Rank (*i*)	*p*-Value	q_i	FDR_i	Reject[b]
31	SNIBE prototype	1	0.00059	0.2009	0.008055	Yes
9	DiaSorin	2	0.00061	0.1044	0.008055	Yes
30	Siemens	3	0.00071	0.008055	0.008055	Yes
2	DIAsource	4	0.00122	0.01036	0.01036	Yes
5	SNIBE prototype	5	0.00474	0.03221	0.03221	Yes
24	DiaSorin	6	0.01089	0.06170	0.06170	No
19	Roche	7	0.01830	0.8888	0.8888	No
40	Siemens	8	0.02714	0.1153	0.1153	No
20	IDS-iSYS	9	0.07258	0.2742	0.2524	No
33	LC-MS/MS	10	0.07424	0.2524	0.2524	No
21	IDS-EIA[b]	11	0.0831	0.2569	0.2569	No
39	IDS-iSYS	12	0.0925	0.2621	0.2621	No
28	LC-MS/MS	13	0.1217	0.3183	0.3183	No
1	Abbott	14	0.1344	0.3262	0.3262	No
27	Abbott	15	0.2045	0.4636	0.4636	No
36	LC-MS/MS	16	0.2216	0.4710	0.4646	No
11	LC-MS/MS	17	0.2323	0.4646	0.4646	No
3	bioMérieux	18	0.2569	0.4853	0.4853	No
12	LC-MS/MS	19	0.2987	0.5346	0.5346	No
38	LC-MS/MS	20	0.3297	0.5605	0.5605	No
34	bioMérieux	21	0.5263	0.8521	0.8258	No
26	Beckman Coulter	22	0.5481	0.8470	0.8258	No
25-1	LC-MS/MS	23	0.5616	0.8302	0.8258	No
23	Abbott	24	0.5829	0.8258	0.8258	No
25-2	LC-MS/MS	25	0.6305	0.8575	0.8575	No
22	LC-MS/MS	26	0.6633	0.8674	0.8674	No
6	Chromsystems	27	0.6942	0.8742	0.8742	No
18	Abbott	28	0.7892	0.9583	0.9451	No
7	Chromsystems	28	0.8503	0.9969	0.9451	No
4	Bio-Rad	30	0.8543	0.9682	0.9451	No
29	Roche	31	0.8768	0.9616	0.9451	No
16	LC-MS/MS	32	0.8896	0.9451	0.9451	No
17	LC-MS/MS	33	0.9343	0.9626	0.9612	No
37	LC-MS/MS	34	0.9612	0.9612	0.9612	No

[a] FDR Testing: k = number of test (34); $q_i = kp_i/i$ where i = rank of p-values among k tests; FDR_i = false-discovery rate for the ith test defined by min (q_i…..,q_k) [18].

[b] Reject H_0 (null hypothesis) for FDR > 0.05.

Figure Captions

Fig. 1. Plot of the mean of results for the determination of 25(OH)D in nine DEQAS samples shipped frozen versus ambient for the different LC-MS/MS assays (black squares) and ligand binding assays (red circles). The ligand binding assays are identified by laboratory number in parentheses. Only NIST is identified among the LC-MS/MS due to space limitations. The linear regression fit for the LC-MS/MS (black dashed line) and ligand binding assays (red dashed line) are shown. The identity (equivalence) line (y = x) is shown as blue dotted line.

Fig. 2. Plots of measurements for $25(OH)D_2$, $25(OH)D_3$, 3-epi-$25(OH)D_3$, and $24,25(OH)_2D_3$ for the frozen versus the ambient DEQAS samples using: (A) NIST RMP for $25(OH)D_2$, (B) NIST RMP for $25(OH)D_3$, (C) NIST ID LC-MS/MS for 3-epi-$25(OH)D_3$, and (D) NIST RMP for $24,25(OH)_2D_3$. Note that the x-axis in (A) is from 0 nmol/L to 4 nmol/L and does not include DEQAS09 which has a concentration of 57 nmol/L. Numbers within the circles indicate the DEQAS sample number. The linear regression fit line is shown as a black dashed line. The identity (equivalence) line (y = x) is shown as blue dotted line.

Fig. 3. Plots of measurements for 25(OH)D for frozen versus ambient DEQAS samples for four LC-MS/MS assays. (A) Lab 11, (B) Lab 28, (C) Lab 33, and (D) Lab 36. Numbers within the circles indicate the DEQAS sample number. The linear regression fit line is shown as a black dashed line. The identity (equivalence) line (y = x) is shown as blue dotted line.

Fig. 4. Plots of measurements for 25(OH)D for frozen versus ambient DEQAS samples for six different ligand binding assays. (A) Abbott (Lab 1), (B) DIAsource (Lab 2), (C) SNIBE (Lab 31), (D) Siemens (Lab 30), (E) DiaSorin (Lab 9), and (F) Roche (Lab 19). Numbers within the circles indicate the DEQAS sample number. The linear regression fit line is shown as a black dashed line. The identity (equivalence) line (y = x) is shown as blue dotted line.

Fig. 1.

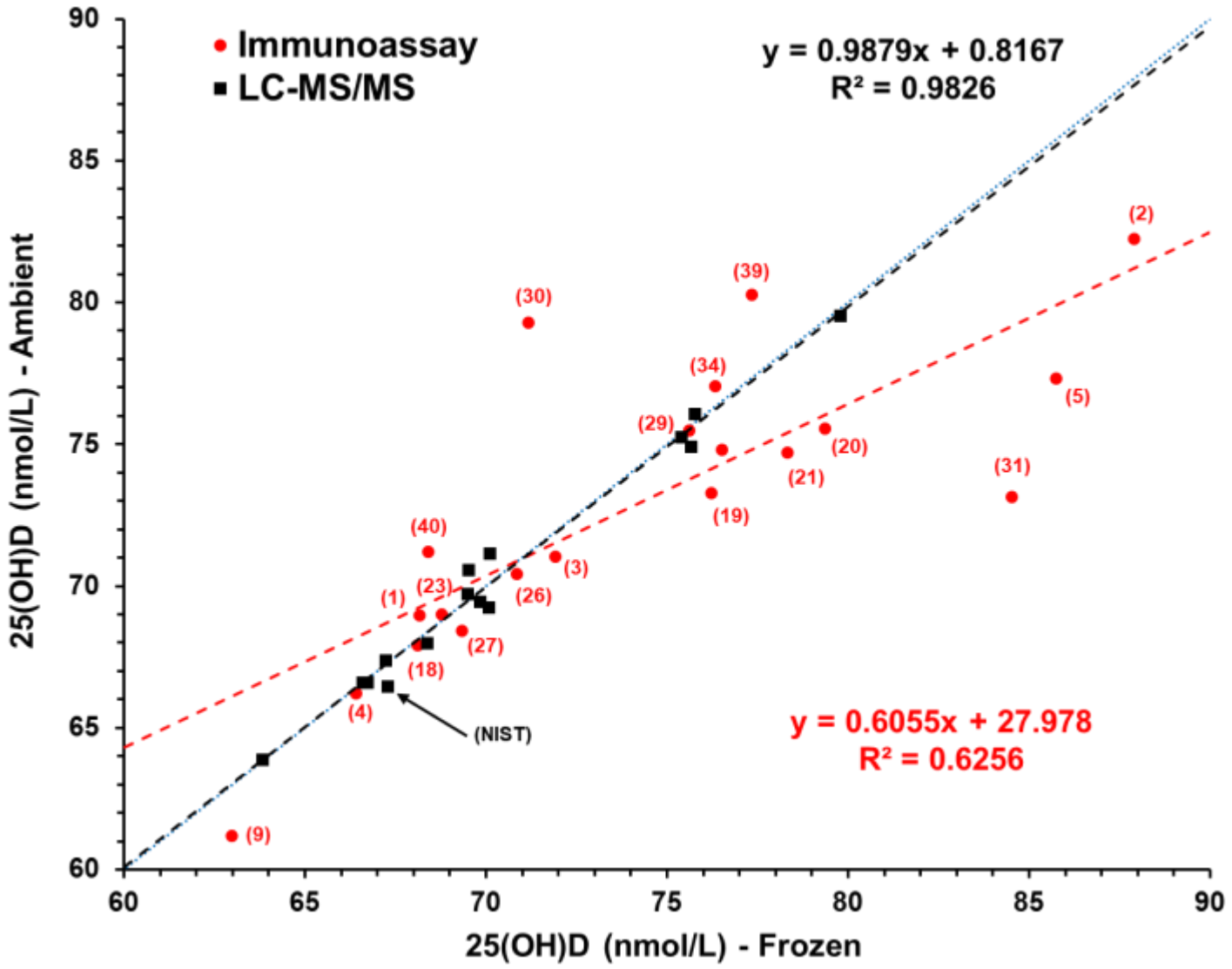

Immunoassay
LC-MS/MS
y = 0.9879x + 0.8167
R² = 0.9826
y = 0.6055x + 27.978
R² = 0.6256
25(OH)D (nmol/L) - Ambient
25(OH)D (nmol/L) - Frozen
(NIST)
(1)
(2)
(3)
(4)
(5)
(9)
(18)
(19)
(20)
(21)
(23)
(26)
(27)
(29)
(30)
(31)
(34)
(39)
(40)

Fig. 2.

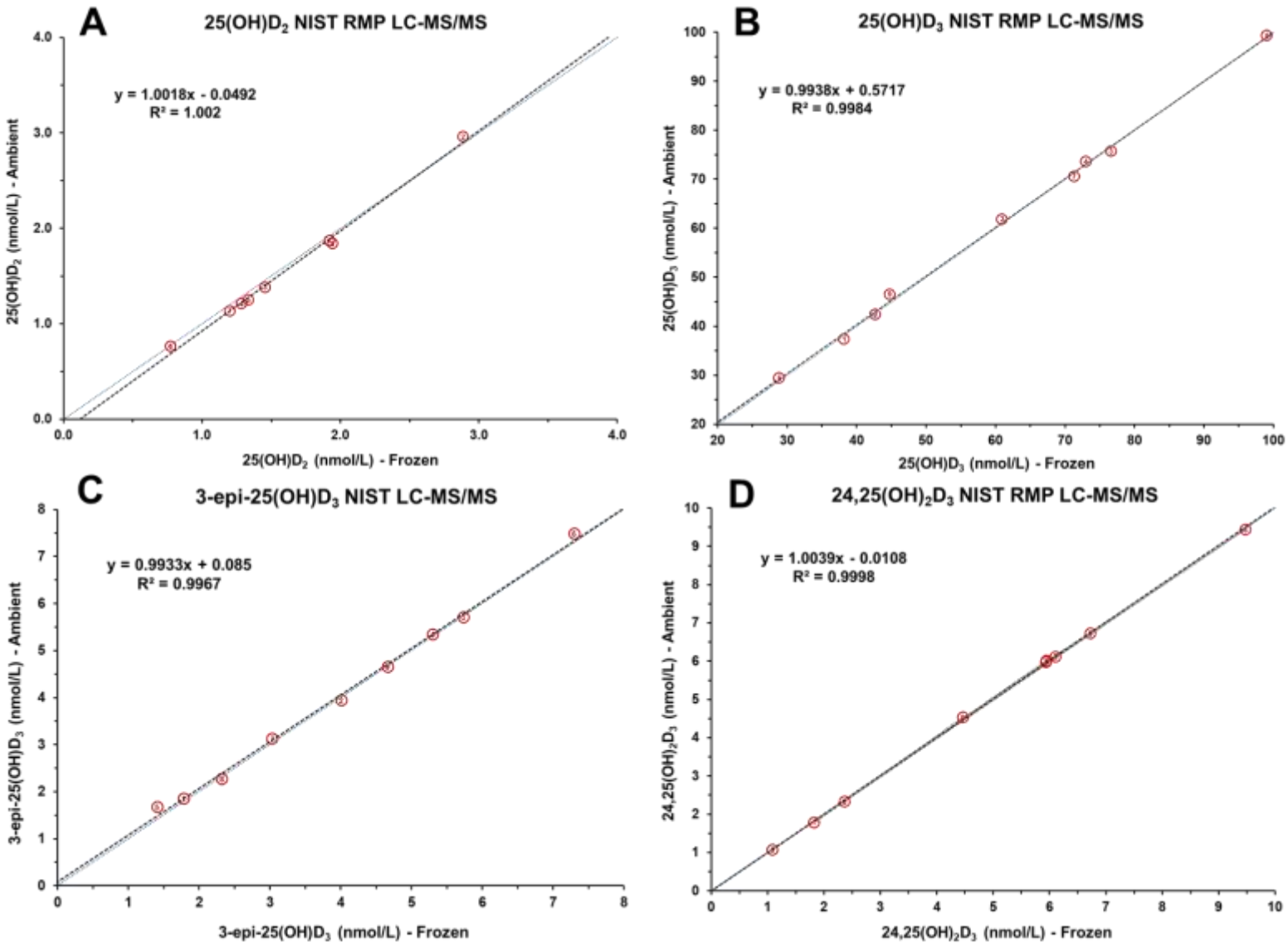

A
25(OH)D2 NIST RMP LC-MS/MS
y = 1.0018x - 0.0492
R² = 1.002
25(OH)D2 (nmol/L) - Ambient
25(OH)D2 (nmol/L) - Frozen
B
25(OH)D3 NIST RMP LC-MS/MS
y = 0.9938x + 0.5717
R² = 0.9984
25(OH)D3 (nmol/L) - Ambient
25(OH)D3 (nmol/L) - Frozen
C
3-epi-25(OH)D3 NIST LC-MS/MS
y = 0.9933x + 0.085
R² = 0.9967
3-epi-25(OH)D3 (nmol/L) - Ambient
3-epi-25(OH)D3 (nmol/L) - Frozen
D
24,25(OH)2D3 NIST RMP LC-MS/MS
y = 1.0039x - 0.0108
R² = 0.9998
24,25(OH)2D3 (nmol/L) - Ambient
24,25(OH)2D3 (nmol/L) - Frozen

Fig. 3.

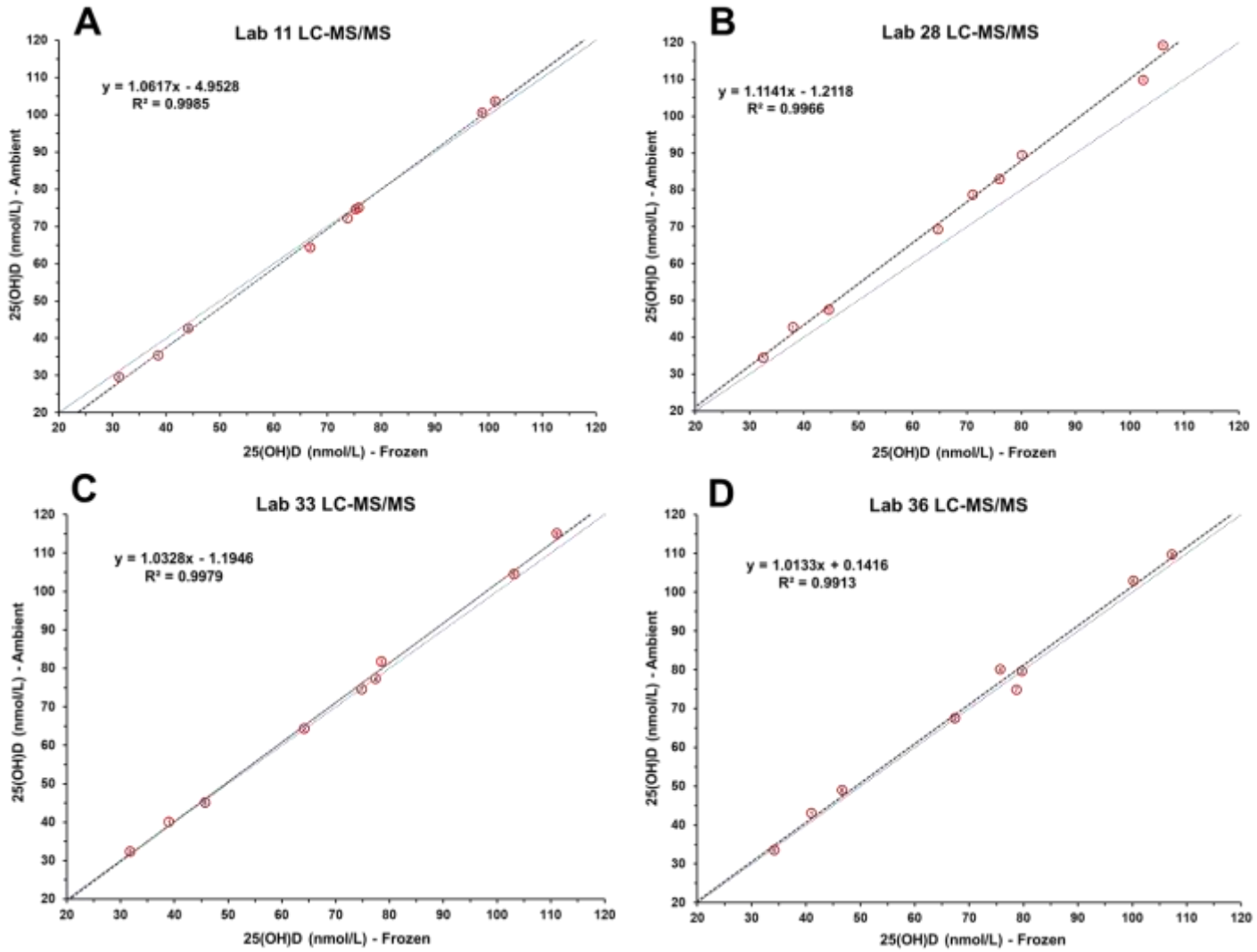
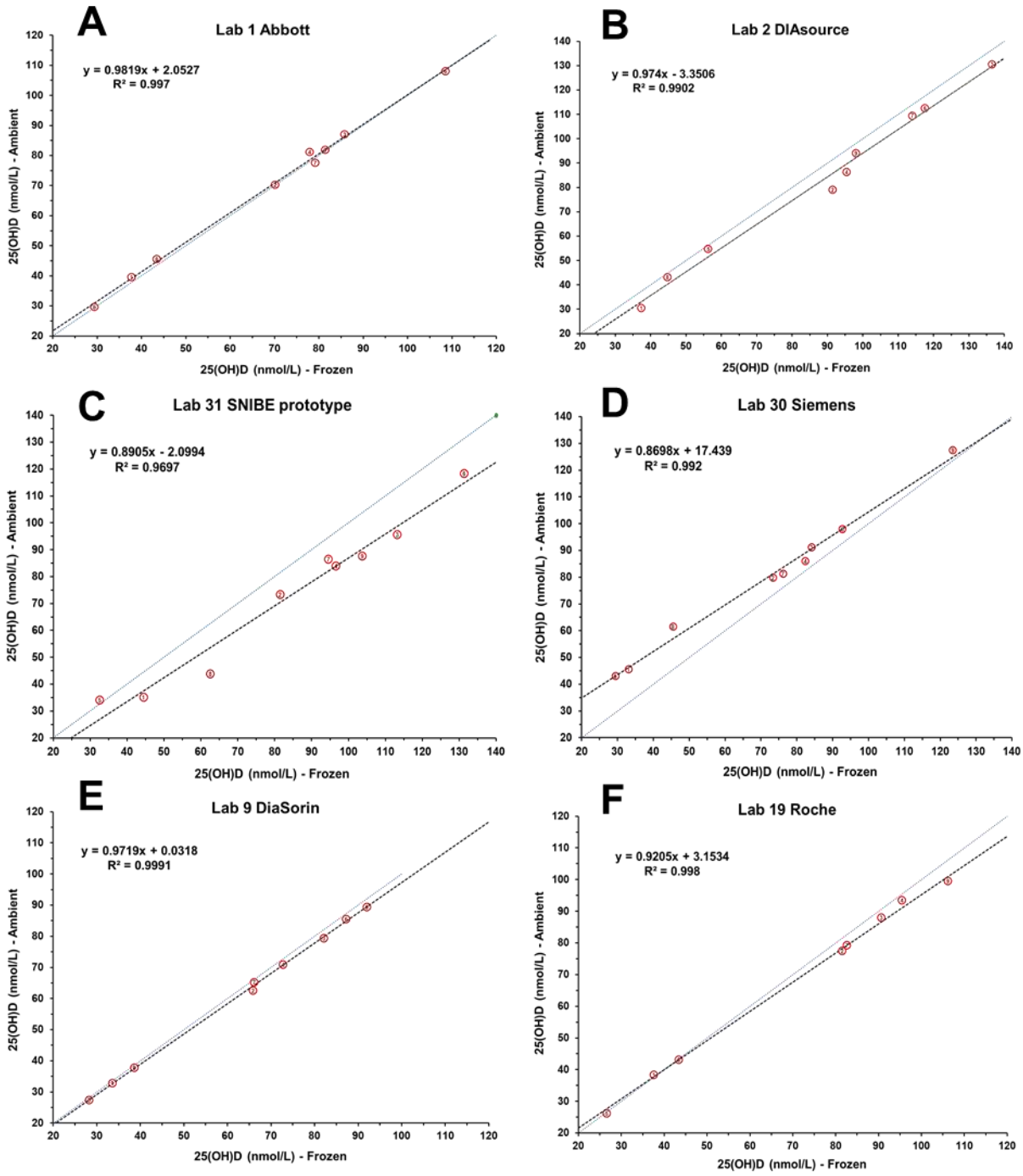

A
Lab 11 LC-MS/MS
y = 1.0617x - 4.9528
R² = 0.9985
25(OH)D (nmol/L) - Ambient
25(OH)D (nmol/L) - Frozen
B
Lab 28 LC-MS/MS
y = 1.1141x - 1.2118
R² = 0.9966
25(OH)D (nmol/L) - Ambient
25(OH)D (nmol/L) - Frozen
C
Lab 33 LC-MS/MS
y = 1.0328x - 1.1946
R² = 0.9979
25(OH)D (nmol/L) - Ambient
25(OH)D (nmol/L) - Frozen
D
Lab 36 LC-MS/MS
y = 1.0133x + 0.1416
R² = 0.9913
25(OH)D (nmol/L) - Ambient
25(OH)D (nmol/L) - Frozen

Fig. 4.


A
Lab 1 Abbott
y = 0.9819x + 2.0527
R² = 0.997
B
Lab 2 DIAsource
y = 0.974x - 3.3506
R² = 0.9902
C
Lab 31 SNIBE prototype
y = 0.8905x - 2.0994
R² = 0.9697
D
Lab 30 Siemens
y = 0.8698x + 17.439
R² = 0.992
E
Lab 9 DiaSorin
y = 0.9719x + 0.0318
R² = 0.9991
F
Lab 19 Roche
y = 0.9205x + 3.1534
R² = 0.998
25(OH)D (nmol/L) - Ambient
25(OH)D (nmol/L) - Frozen