An Overview of the NIST/NIH Vitamin D Metabolites Quality Assurance Program (VitDQAP)

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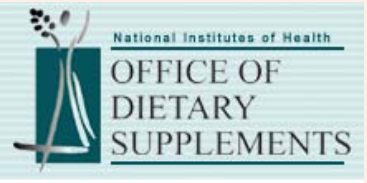
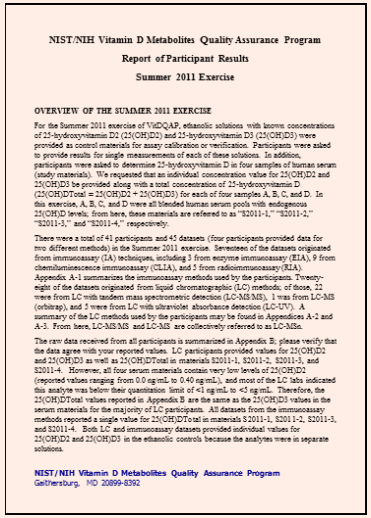
VitDQAP: Key Features

- Collaboration between NIST and NIH-ODS; initiated 2009
- No cost for participation
- First accuracy-based program for vitamin D metabolites
  - Performance evaluated relative to both NIST and participant consensus values
  - NOT a proficiency program – no pass/fail
- Participants receive summarized results, consultation as needed





NIST/NIH Vitamin D Metabolites Quality Assurance Program

Report of Participant Results

Summer 2011 Exercise

OVERVIEW OF THE SUMMER 2011 EXERCISE

For the Summer 2011 exercise of VitDQAP, ethanolic solutions with known concentrations of 25-hydroxyvitamin D2 (25(OH)D2) and 25-hydroxyvitamin D3 (25(OH)D3) were provided as control materials for assay calibration or verification. Participants were asked to provide results for single measurements of each of these solutions. In addition, participants were asked to determine 25-hydroxyvitamin D in four samples of human serum (study materials). We requested that an individual concentration value for 25(OH)D2 and 25(OH)D3 be provided along with a total concentration of 25-hydroxyvitamin D (25(OH)DTotal = 25(OH)D2 + 25(OH)D3) for each of four samples A, B, C, and D. In this exercise, A, B, C, and D were all blended human serum pools with endogenous 25(OH)D levels; from here, these materials are referred to as "S2011-1," "S2011-2," "S2011-3," and "S2011-4," respectively.

There were a total of 41 participants and 45 datasets (four participants provided data for two different methods) in the Summer 2011 exercise. Seventeen of the datasets originated from immunoassay (IA) techniques, including 3 from enzyme immunoassay (EIA), 9 from chemiluminescence immunoassay (CLIA), and 5 from radioimmunoassay (RIA). Appendix A-1 summarizes the immunoassay methods used by the participants. Twenty-eight of the datasets originated from liquid chromatographic (LC) methods; of those, 22 were from LC with tandem mass spectrometric detection (LC-MS/MS), 1 was from LC-MS (orbitrap), and 5 were from LC with ultraviolet absorbance detection (LC-UV). A summary of the LC methods used by the participants may be found in Appendices A-2 and A-3. From here, LC-MS/MS and LC-MS are collectively referred to as LC-MSn.

The raw data received from all participants is summarized in Appendix B; please verify that the data agree with your reported values. LC participants provided values for 25(OH)D2 and 25(OH)D3 as well as 25(OH)DTotal in materials S2011-1, S2011-2, S2011-3, and S2011-4. However, all four serum materials contain very low levels of 25(OH)D2 (reported values ranging from 0.0 ng/mL to 0.40 ng/mL), and most of the LC labs indicated this analyte was below their quantitation limit of <1 ng/mL to <5 ng/mL. Therefore, the 25(OH)DTotal values reported in Appendix B are the same as the 25(OH)D3 values in the serum materials for the majority of LC participants. All datasets from the immunoassay methods reported a single value for 25(OH)DTotal in materials S2011-1, S2011-2, S2011-3, and S2011-4. Both LC and immunoassay datasets provided individual values for 25(OH)D2 and 25(OH)D3 in the ethanolic controls because the analytes were in separate solutions.

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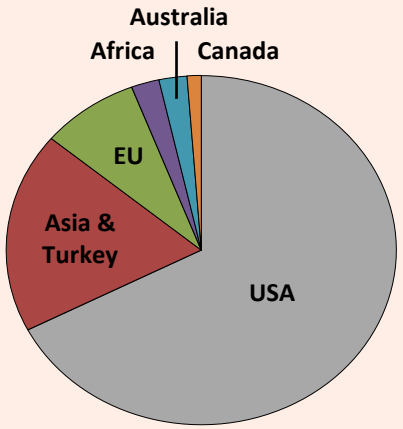
VitDQAP: Participation



- Participants identified by code number
- ≈ 90 participating labs
  - 60% US, 40% International
  - Government, academic, testing, hospital, research

- Participants report 25(OH)D_{Total} and 25(OH)D_2, 25(OH)D_3, 3-epi-25(OH)D_3 (LC only):
  - Immunoassay – 40%: CLIA, RIA, EIA
  - LC techniques – 60%: MS/MS, UV

VitDQAP Controls and Samples: SRMs /Pooled Serum

- SRM 2972: 25-Hydroxyvitamin D Calibration Solutions
- SRM 972: Vitamin D in Human Serum (L3 only)
- SRM 972a: Vitamin D Metabolites in Frozen Human Serum
- SRM 968d/e: Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum
- SRM 1950: Metabolites in Human Plasma
- VitDQAP-I, VitDQAP-II, and VitDQAP-III

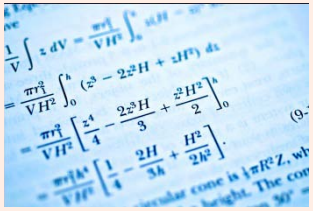
Comparability Studies / Data Analysis

- 7 completed studies (Winter 2010 – Summer 2013)

SRM 968d
Native 25(OH)D

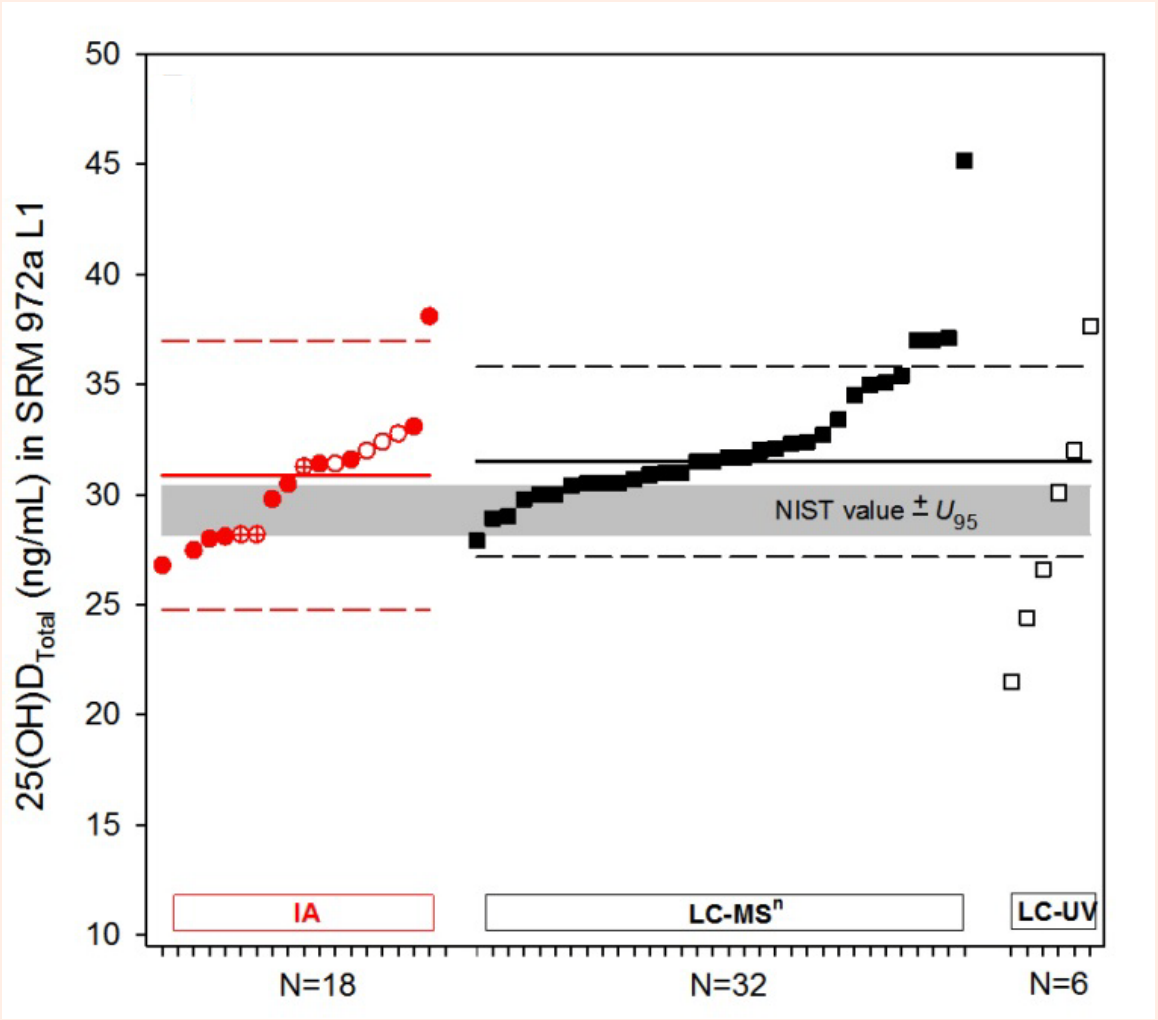
- Each study includes:
  - Control (ethanolic calibrants or serum SRM 968d L1)
  - Study samples - 2 to 4 vials of human serum or plasma

- For each control/sample consensus statistics (median, MADe, %CV) determined for the $25(OH)D_{Total}$ results for all methods, the IA methods only, and the LC methods only
  - Within-method, all-method variability



$25(OH)D_{Total}$ in SRM 972a Level 1

Native, predominantly $25(OH)D_3$



CLIA (●), EIA (⊕), RIA (○), LC-MS^n ( ), and LC-UV ( )

IA methods

between-lab:

CV%

≈ **10%**

Median > NIST range

LC methods

between-lab:

CV%

≈ **7%**

Median > NIST range

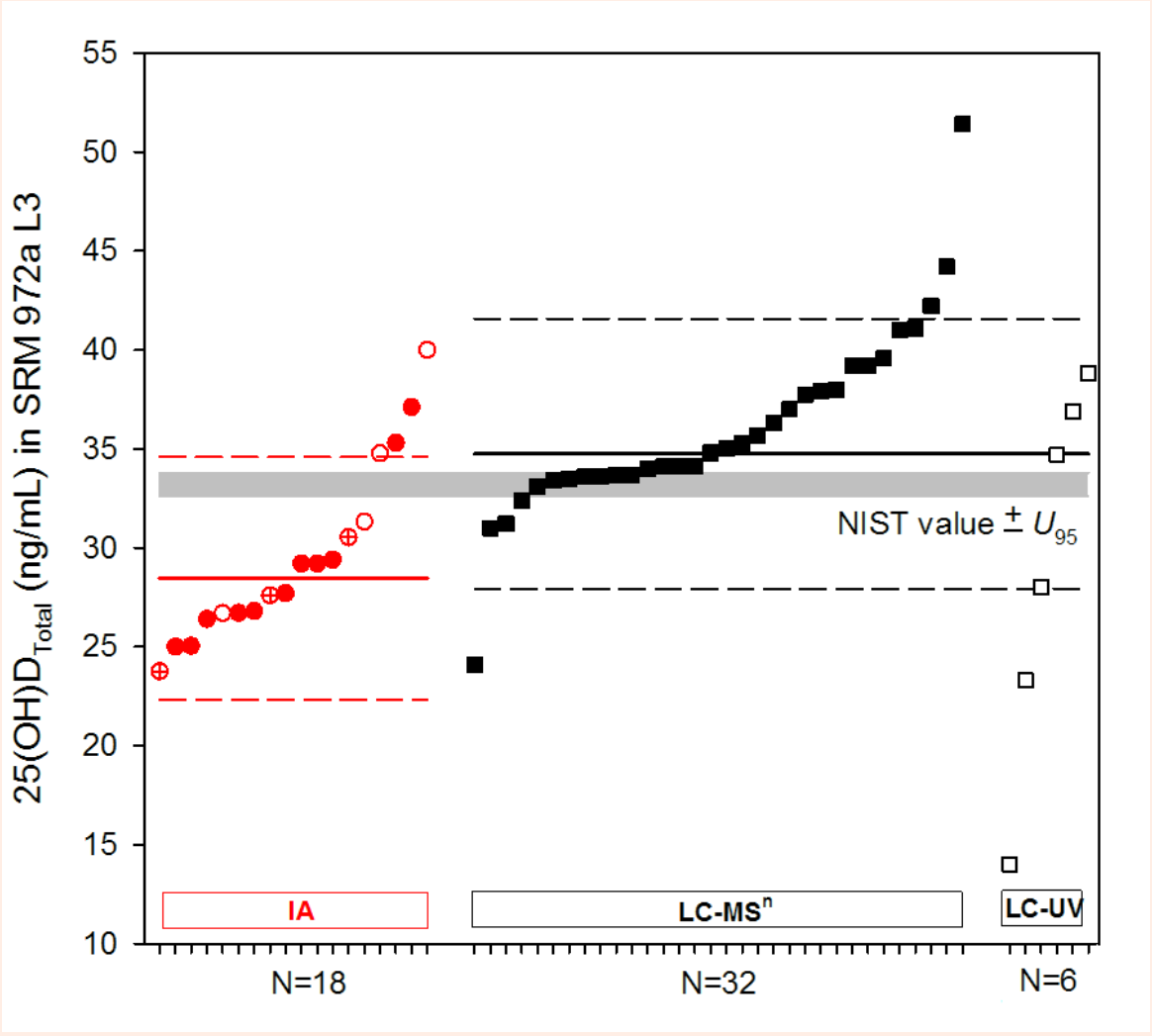
All methods

CV%

≈ 7%

$25(OH)D_{Total}$ in SRM 972a Level 3

Native, contains $25(OH)D_2$ and $25(OH)D_3$



CLIA (●), EIA (⊕), RIA (○), LC-MS^n ( ), and LC-UV ( )

IA methods

between-lab:

CV%

≈ **11%**

Median < NIST range

Median < LC median

LC methods

between-lab:

CV%

≈ **10%**

Median > NIST range

All methods

CV%

≈ 17%

VitDQAP Results Summary

- For serum and plasma materials with predominantly 25(OH)D_3
  - Median results for both IA and LC higher than the NIST expanded uncertainty range
  - All-method CV consistently in range 7% to 19%
- Results obtained for IA and LC methods not comparable for serum with high 25(OH)D_2 and 3-epi-25(OH)D_3 (all method CV 17% to 47%)
- Improvements in participant methodology needed to address biases from 25(OH)D_2 and 3-epi-25(OH)D_3

Questions
Comments
Information:

email: vitdqap@nist.gov

http://www.nist.gov/mml/csd/vitdqap.cfm

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