



Vitamin D Standardization Program (VDSP)

VDSP Reference Measurement System: NIST Components – Current and Future

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NIST and NIH-ODS History

NIST and NIH-ODS have collaborated since 2002 on:

- Development of standard reference materials (SRMs) and measurement procedures for:
 - Botanical dietary supplements
 - Fish and krill oils
 - Multivitamin/multielement tablets; calcium tablets, chromium tablets, iodized salt
 - Iodine status markers (thyroglobulin, T3 and T4)
 - Vitamin metabolites in serum
 - Vitamin D
 - Other vitamin metabolites (e.g., B₆ , B₁₂, folate vitamers)
- Development of measurement quality assurance programs for:
 - Dietary supplements
 - Vitamin D metabolites in serum
 - Fatty acids in serum

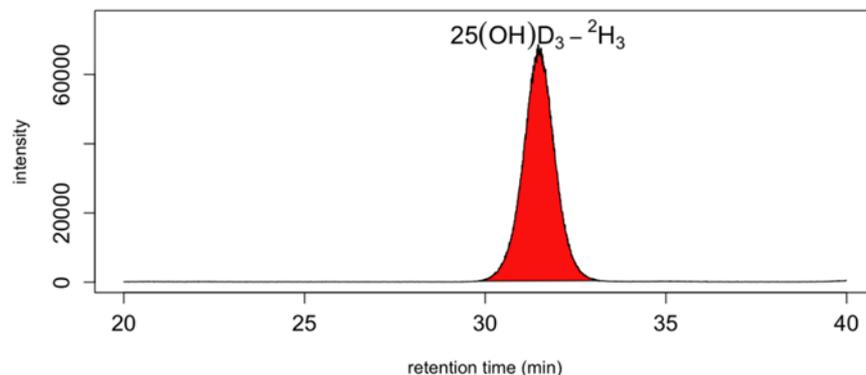
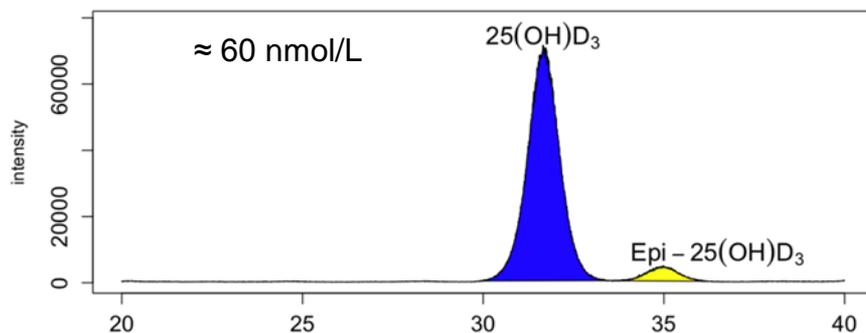


Current Role of NIST in the VDSP

- Development of reference materials and measurement procedures for vitamin D metabolites
- Development of measurement quality assurance programs for vitamin D metabolites (VitDQAP)
- Commutability Studies to Support VDSP
- Assignment of values to DEQAS materials using the NIST Reference Measurement Procedure (RMP) for 25-hydroxyvitamin D

Reference Measurement Procedure for 25(OH)D

SRM 972 Level 1



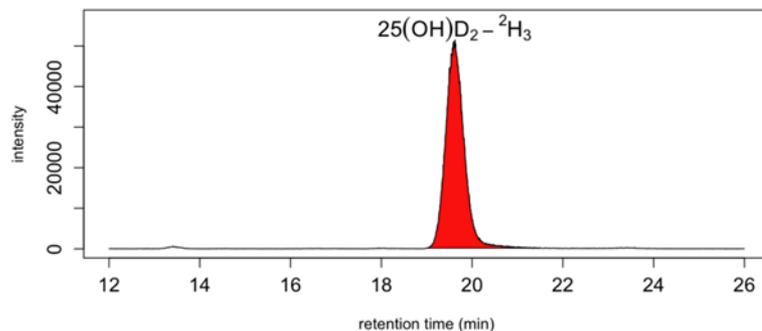
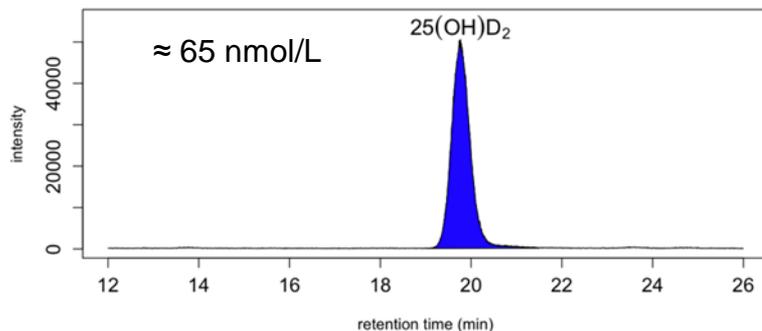
- Method validated as a candidate reference measurement procedure, as recognized by the JCTLM
- APCI in positive ionization mode, multiple reaction monitoring
- Methanol:water mobile phase, cyano column
- Good precision for all four levels of SRM 972 (CVs < 2%)
- 3-Epimer of 25OHD₃ is chromatographically resolved

Susan Tai, Mary Bedner, and Karen Phinney, *Anal. Chem.*, 82 (2010) 1942 – 1948



Reference Measurement Procedure for 25(OH)D

SRM 972 Level 3



C18 column used to maximize sensitivity for 25OHD_2 , higher methanol content in mobile phase than cyano column

Susan Tai, Mary Bedner, and Karen Phinney, *Anal. Chem.*, 82 (2010) 1942 – 1948

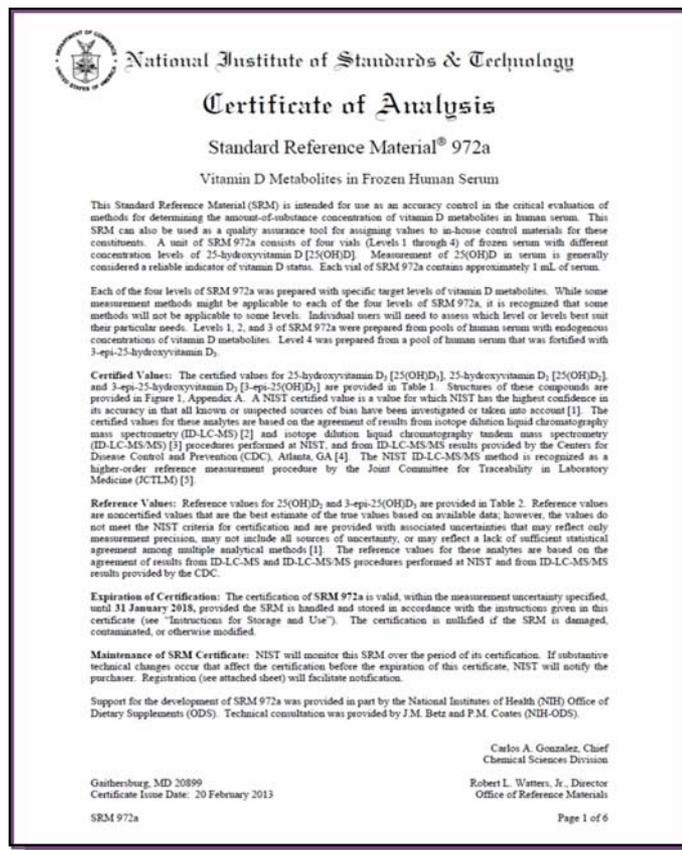
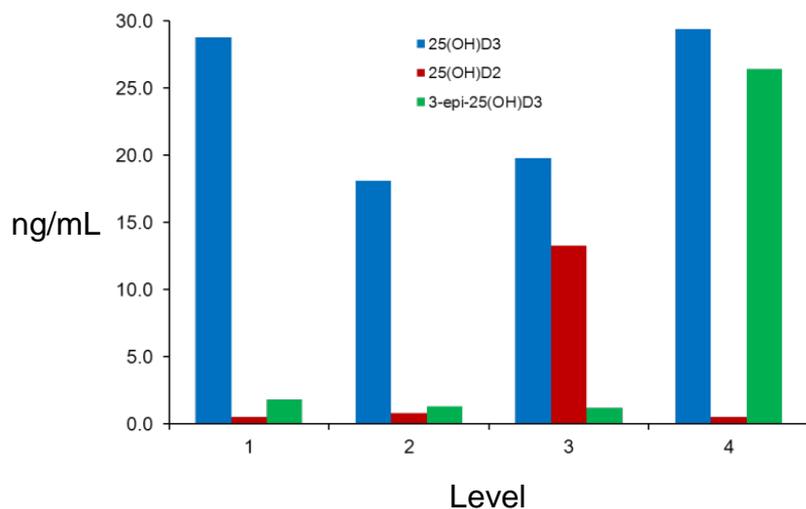
SRM 972 Vitamin D in Human Serum

- Four levels, each containing 1.0 mL of serum
 - Level 1 = endogenous normal level
 - Level 2 = level 1 serum pool diluted (2x) with horse serum
 - Level 3 = normal serum fortified with 25(OH)D₂
 - Level 4 = normal serum fortified with 3-*epi*-25(OH)D₃
- Certified and reference values for 25(OH)D₂, 25(OH)D₃, and 3-*epi*-25(OH)D₃
- Value assignment by isotope dilution (ID) LC/MS and LC-MS/MS using data from NIST and CDC
- Issued in 2009; sold over 700 units/year until supply depleted in late 2011



SRM 972a – Renewal of Vitamin D Metabolites in Frozen Human Serum

- Four levels, each containing 1.0 mL serum (pools)
 - Levels 1-2 = endogenous levels
 - Level 3 = endogenous from supplemented donors
 - Level 4 = fortified with 3-*epi*-25(OH)D₃
- Certified and reference values for 25(OH)D₂, 25(OH)D₃, and 3-*epi*-25(OH)D₃
- Value assignment by isotope-dilution LC-MS and LC-MS/MS using data from NIST and CDC



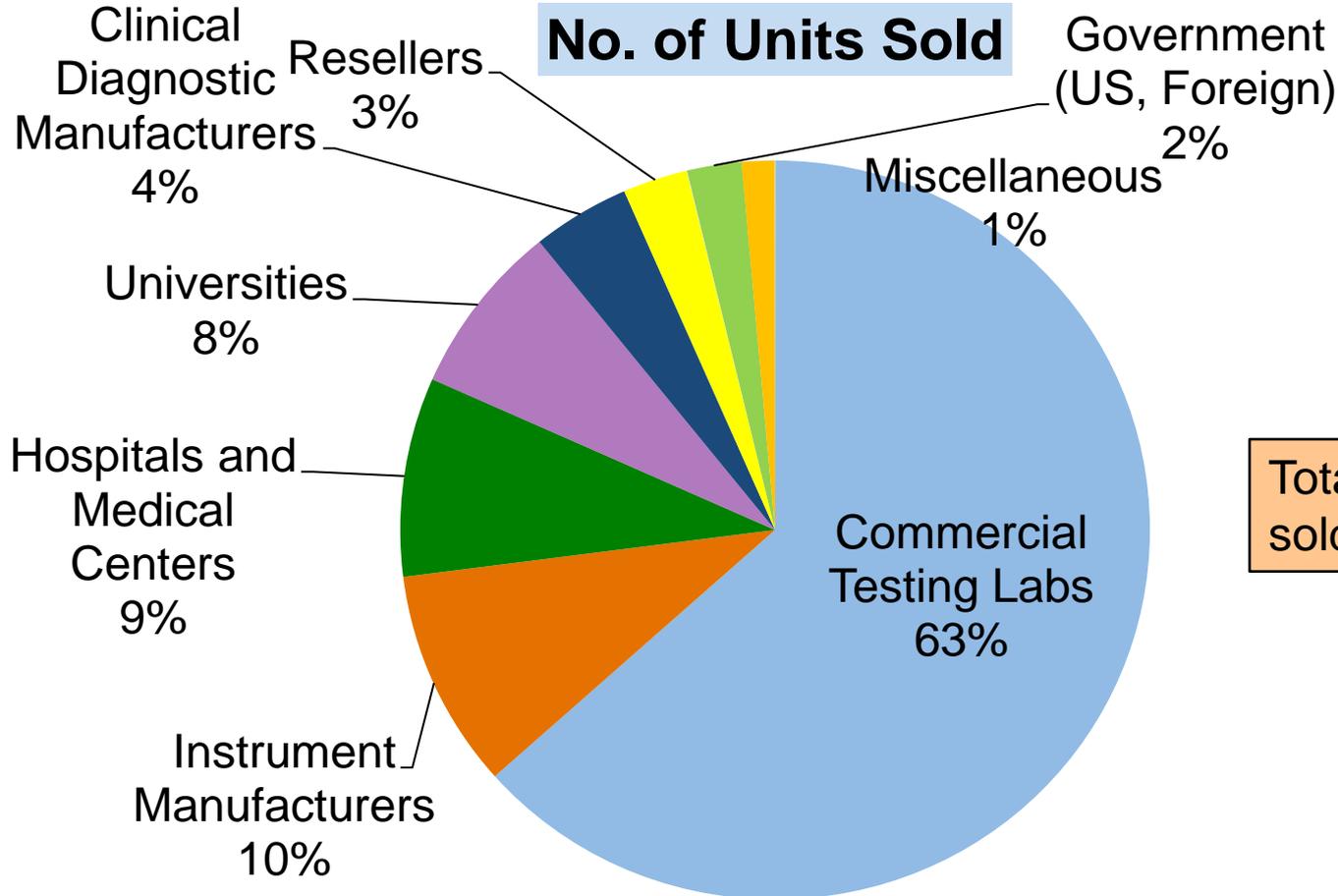
Assigned Values in SRM 972a

	25(OH)D ₂	25(OH)D ₃	3-epi-25(OH)D ₃
Level 1	0.52 ± 0.06	28.1 ± 1.1	1.80 ± 0.08
Level 2	0.80 ± 0.06	17.7 ± 0.4	1.26 ± 0.06
Level 3	13.0 ± 0.3	19.4 ± 0.4	1.15 ± 0.13
Level 4	0.54 ± 0.10	28.7 ± 0.9	25.8 ± 2.0



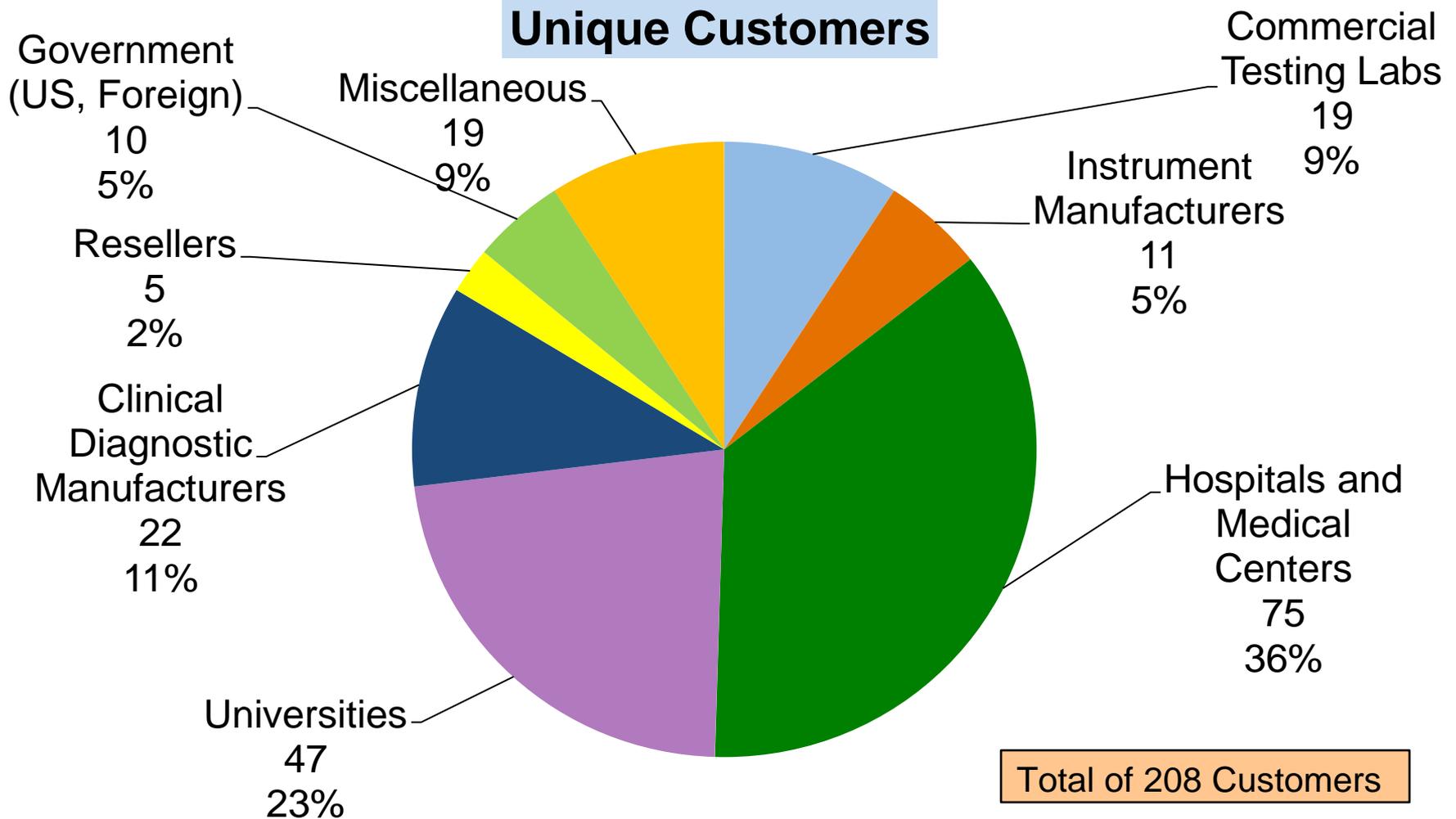
Certified and reference values obtained from combination of results from multiple methods: LC-MS (NIST), LC-MS/MS (NIST), and LC-MS/MS (CDC). Certified values are shown in bold. All values in ng/g.

Distribution of SRM 972



Total of 1610 units sold in 2 years

Distribution of SRM 972





SRM 2972 25-Hydroxyvitamin D₂ and D₃ Calibration Solutions

- Ethanol-based solutions, one each containing 25(OH)D₂ and 25(OH)D₃
- Certified values for both 25(OH)D₂ and 25(OH)D₃
- Can be diluted to prepare calibration curves
- SRM 2972 is being updated to SRM 2972a, which will include higher (2x) concentration solutions of 25(OH)D₂ and 25(OH)D₃ plus a solution of 3-*epi*-25(OH)D₃



Additional SRMs for Vitamin D Metabolites in Human Serum

- SRM 1950 Metabolites in Human Plasma
- SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum
- SRM 2973 Vitamin D Metabolites in Human Serum (high level) (in progress)





New NIST Activities in Support of the VDSP

- Development of SRMs and measurement procedures for vitamin D metabolites
 - SRM 2973 Vitamin D Metabolites in Human Serum (High Level)
 - Improved, rapid method for vitamin D metabolites in serum including additional metabolites
 - Comparison of labeled standards (^{13}C vs. deuterated) for determination of 25(OH)D in serum
- Design and coordination of a new commutability study
- Feasibility for blood spots as SRM matrix for vitamin D metabolites
- Methods and SRMs for 25(OH)D in food matrices



Contributors to the NIST Vitamin D Metabolites Activities

- Mary Bedner
- Carolyn Burdette
- Johanna Camara
- Katrice Lippa
- Karen Phinney
- Lane Sander
- Susan Tai