

Vitamin D Standardization Program (VDSP)

VDSP Reference Measurement System: NIST Components – Current and Future

Stephen A. Wise Chemical Sciences Division National Institute of Standards and Technology

> NIST National Institute of Standards and Technology U.S. Department of Commerce

stephen.wise@nist.gov

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., B_6 , B_{12} , folate vitamers)
- Development of measurement quality assurance programs for:
 - Dietary supplements
 - Vitamin D metabolites in serum
 - Fatty acids in serum

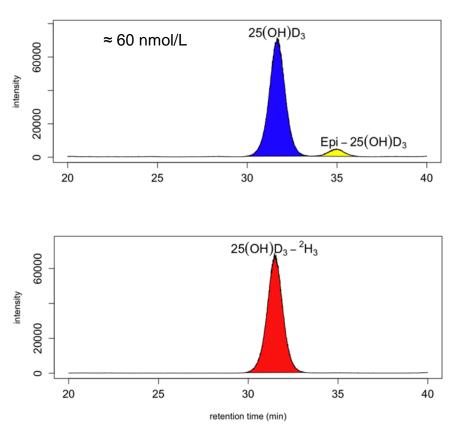


Current Role of NIST in the VDSP

- 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

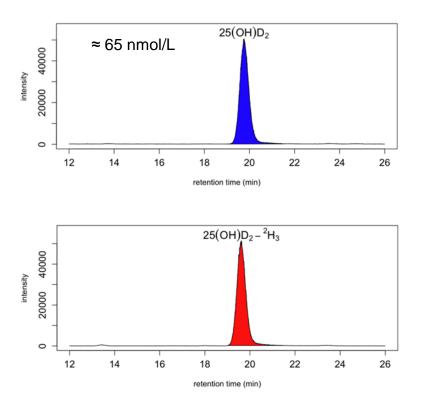


- Method validated as a candidate reference measurement procedure, as recognized by the JCTLM
- APCI in positive ionization mode, multiple reaction monitoring
- Methanol:water mobile phase, cyano column
- Good precision for all four levels of SRM 972 (CVs < 2%)
- 3-Epimer of 25OHD₃ is chromatographically 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 25OHD₂, 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 $25(OH)D_2$
 - Level 4 = normal serum fortified with $3-epi-25(OH)D_3$
- 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





Standard Edwardse Manuali (BMO) 972 is intradied for use is an accuracy control in the extitual evaluation or included for denominang the instance of obstances conconstruction of crisinan D methodies is its mass marked and the extited of the SEM can also be used in a quality assumed to the for any ingent quality of the extited of the extited of the extited of the extited of the concentration, bench of 2534 972 concents of their wide (Concent 1 decough 4 of forces series usis is generally concentration bench and concent of the extited of 2534 972 constants of previously in a feature of series of the extited of the

Each of the frow levels of SEM 972 was prepared with specific target levels of vinamia D metholizes. While some measurement methods might be explicitly to each of the Eou levels of VEM 972, at its recipition flat issues pecific beets may not be equivalent to a symmetrical, allowidant uses will metel to some which level on its most here that their particular meth. Level ($1 \leq SEM 977$ was prepared from "arming" issues areas which level on short here its level $3 \approx 10^{-10}$ km s⁻¹ sectors that the the state of the VEM 100 sectors are short level in the state is comming a second by Adheng Level $1 \leq V_{10} \leq V_{10}$

Confided Concentration Values: The certified concentration values for 32-bydroxyriamia D, [23/00102), 23 hydroxyriamas D, (23/00102), and 1-hyg-32-bydroxyrizman D, [1-hyg-32/00102) are provided in Table 1. Synchron of facto composition are provided in Figure 1. A NSH contradic value in a value for which NSH has the figure confidence in in scremery in the Values are surgested on stress with an even been meetingened to value an accurate [1]. The certified concentration values for factor matching and the surgested part of methods in the stress accurate [1]. The certified concentration values for factor matching and on the agreement of method from integra distants higher (decomposition) and the stress stress provided by the Centers for Disses Control and Prevention (CDC). Altans, 6A.

Reference Concentration Values: Reference consummities values for 25000 mol $l \rightarrow p25000$ more provided in 20040. Zubieven values are assumed for hardia > 2 zubieven values are summarized values for an the low sections are done available does, however, the values do not more the NST content for conclusion, and we provided with assumed more subscription of the section of the section of the section of the section of the subscription of the section of the section of the section of the section of the hardia of the section o

Exploration of Certification: The certification of SRM 972 in volid, within the measurement uncertainty specified, and 30 September 2015, previded the SEM in handled in accordance with the autorations given in this certificate use "horaccions for Use"). The certification is unlifted if the SEM in damaged, contaminated, or otherwise molified.

Maintenance of SEM Conflictor: VIST well monitor this SEM even the period of its certification. If substantive technical changes recore that afflect the certifications before the exploration of this certificate, NIST will northly the particlaser. Registration (see reached there) will facilize ausfloration.

Support for the development of SEM 972 was provided in part by the National lastitutes of Health (NIR) Office of Densy Supplements (IDIS). Technical consultation was provided by J.M. Betz and M.F. Picciane (NIR-ODS).

The overall direction and coordination of the preparation and analytical measurements leading to the certification of this SRM were performed by K.W. Phinney and S.A. Wire of the NIST Analytical Chemistry Division.

Gaithersburg, MD 20899 Certificate Issue Date: 9 June 2009

SRM 972

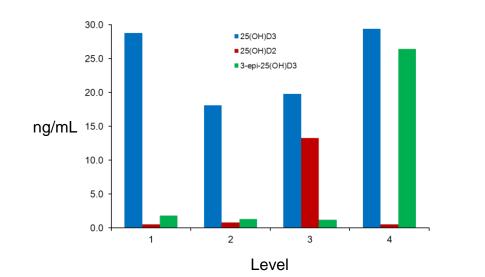
Stephen A. Wise, Chief Analytical Chemistry Division

Robert L. Watters, Jr., Chief superaent Services Division Page 1 of 9

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) D_3
- 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



Certificate of Analysis Standard Reference Material® 972a Vitamin D Metabolites in Frozen Human Serum This Standard Reference Material (SRM) is intended for use a sa accurscy control in the critical exhauton of methods for determining the anounced-of-butcance concentration of vitamic D methodics in human serum. This SRM can also be used as a quality assurance tool for assigning values to indouse control materials for three constitutions. A unit of SRM 723 consists of four value (Leyek Horoge 4) of focuses serum with different concentrations. Investment D123(ORD). Measurement of 25(ORD) in serum is greening considered a relative indicated or value of D24073 continua proprocementy 1 and of serum.

(National Institute of Standards & Technology

Each of the four levels of SEM 972a was prepared with specific target levels of vitamin D metabolites. While some mensurement methods might be applicable to each of the four levels of SRM 972a, it is recognized that some methods will not be applicable to some levels. Individual mere will need to assess which level or trets best unit their particular needs. Levels 1, 2, and 3 of SSM 972a were prepared from pools of human serum with neodopenous concentrations of vitamin D metabolites. Level 4 was prepared from a pool of human serum that was fortified with 3-en/2-3/mdrovity-winamin D.

Certified Values: The certified values for 33-hydroxyvitanii D₁ [250(HD₂)], 25-hydroxyvitanii D₁ [250(HD₂)], and 1-gei-33-hydroxyvitanii D₁ [2-gei-320(HD₂)] are provided in Table 1. Structure of these compounds are provided in Figure 1, Appendix A. A NIST certified value is a value for which NIST has the highest confidence in this occuracy in that all harvour compared sources of bias have been investigated or tables into accound [1]. The certified values for these analytes are based on the agreement of results from isotope dilution liquid chromatopraphy tands (1) and prevention (CD₂). Addition [14] and throw [14] The NIST Hout the implet contracted are (ID-LC-MS/MS) [3] procedures performed at NIST, and from ID-LC-MS/MS method is recognized as higher-order reference measurement procedure by the Joint Committee for Traceshilty in Laboratopy

Reference Values: Reference values for 25(OHD) and 3-epi-21(OHD) are provided in Table 2. Reference values are noncertified values that are the best estimate of the true values based on available data, however, the values do not meet the NST criteria for certification and are provided with noncitated uncertainties that may reflect only measurement precision, may not include all loweres of uncertainty, or may reflect a lack of sufficient statistical agreement amough multiple analysical method (11). The reflexence values for these analysis are based on the agreement of cessits from ID-LC-MS and ID-LC-MS/MS procedures performed at NIST and from ID-LC-MS/MS.

Exploration of Certifications: The certification of SRM 972 as valid, within the measurement uncertainty specified until 31 January 2018, provided the SRM is handled and stored in accontance with the instructions given in this certificate (see "Instructions for Storage and Use"). The certification is millified if the SRM is damaged, contaminated, or certification is distanged.

Maintenance of SRM Certificate: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will nonly the purchaser. Regulation (see attached sheet) will licitiste notification.

Support for the development of SRM 972a was provided in part by the National Institutes of Health (NIH) Office of Dietary Supplements (ODS). Technical consultation was provided by J.M. Betz and P.M. Coates (NIH-ODS).

Gaithersburg, MD Certificate Issue D

SRM 972a

	Carlos A. Gonzalez, Chier Chemical Sciences Division
20899 Nate: 20 February 2013	Robert L. Watters, Jr., Director Office of Reference Materials
	Page 1 of 6





Assigned Values in SRM 972a

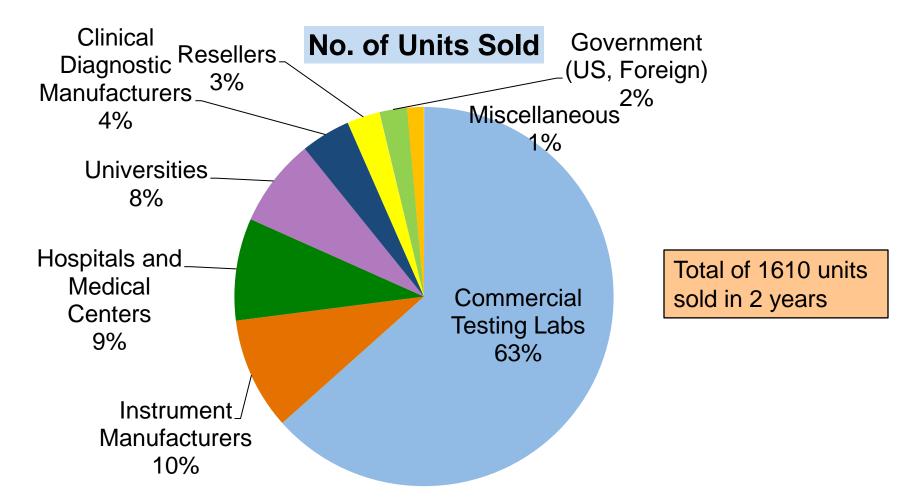
	25(OH)D ₂	25(OH)D ₃	3-epi-25(OH)D ₃
Level 1	0.52 ± 0.06	28.1 ± 1.1	1.80 ± 0.08
Level 2	0.80 ± 0.06	17.7 ± 0.4	1.26 ± 0.06
Level 3	13.0 ± 0.3	19.4 ± 0.4	1.15 ± 0.13
Level 4	0.54 ± 0.10	28.7 ± 0.9	25.8 ± 2.0



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.

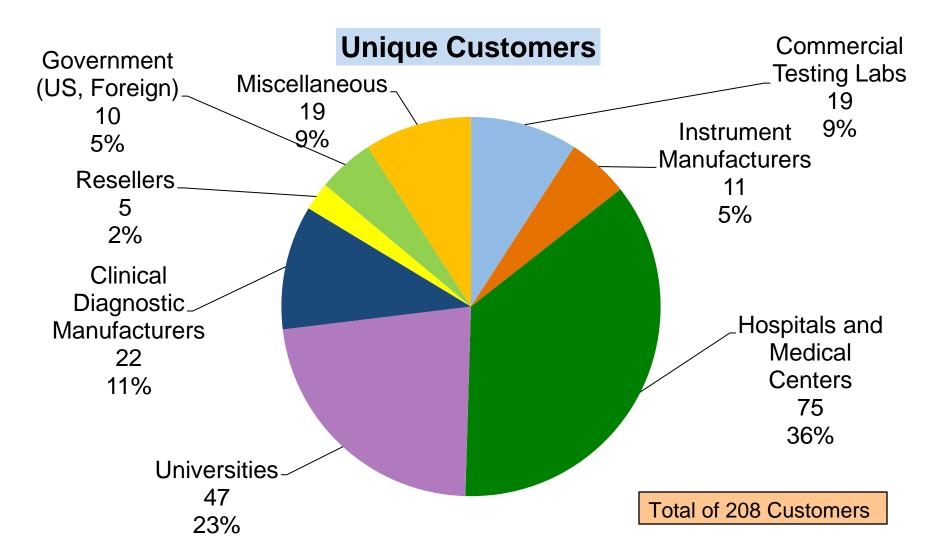


Distribution of SRM 972





Distribution of SRM 972



SRM 2972 25-Hydroxyvitamin D₂ and D₃ Calibration Solutions

- Ethanol-based solutions, one each containing $25(OH)D_2$ and $25(OH)D_3$
- 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 (¹³C 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