National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 972a

Vitamin D Metabolites in Frozen Human Serum

This Standard Reference Material (SRM) is intended for use as an accuracy control in the critical evaluation of methods for determining the amount-of-substance concentration of vitamin D metabolites in human serum. This SRM can also be used as a quality assurance tool for assigning values to in-house control materials for these constituents. A unit of SRM 972a consists of four vials (Levels 1 through 4) of frozen serum with different concentration levels of 25-hydroxyvitamin D [25(OH)D] and 24R,25-dihydroxyvitamin D_3 [24R,25$(OH)_2D_3$]. Measurement of total 25(OH)D concentration in serum, the sum of 25-hydroxyvitamin D_2 [25$(OH)D_2$] and 25-hydroxyvitamin D_3 [25$(OH)D_3$], is generally considered a reliable indicator of vitamin D status. The concentration of 3-epi-25-hydroxyvitamin D_3 [3-epi-25$(OH)D_3$] is generally not included in total 25(OH)D, but this metabolite poses a potential measurement interference for some vitamin D metabolite assays. Measurement of 24R,25$(OH)_2D_3$ in serum is considered as a catabolism marker and an indicator of kidney disease. Each vial of SRM 972a contains approximately 1 mL of serum.

Each of the four levels of SRM 972a was prepared with a specific target level of 25(OH)D. While some measurement methods might be applicable to each of the four levels of SRM 972a, it is recognized that some methods may not be applicable to some levels. Individual users will need to assess which level or levels best suit their particular needs. Levels 1, 2, and 3 of SRM 972a were prepared from pools of human serum with endogenous concentrations of 25(OH)D. Level 4 was prepared from a pool of human serum that was fortified with 3-epi-25-hydroxyvitamin D_3.

Certified Values: The certified values for 25$(OH)D_3$, 25$(OH)D_2$, 3-epi-25$(OH)D_3$, and 24R,25$(OH)_2D_3$ are provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified values for 25$(OH)D_3$, 25$(OH)D_2$, and 3-epi-25$(OH)D_3$ are based on the consensus of results from isotope dilution liquid chromatography mass spectrometry (ID-LC-MS) [2] and isotope dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) [3] procedures performed at NIST, and from ID-LC-MS/MS results provided by the Centers for Disease Control and Prevention (CDC), Atlanta, GA [4]. The certified values for 24R,25$(OH)_2D_3$ are based on the results from a reference measurement procedure using ID-LC-MS/MS performed at NIST [5]. The NIST ID-LC-MS/MS methods are recognized as higher-order reference measurement procedures by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) [6].

Reference Values: Reference values for 25$(OH)D_2$ and 3-epi-25$(OH)D_3$ 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 NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [1]. The reference values for 25$(OH)D_2$ and 3-epi-25$(OH)D_3$ are based on the agreement of results from ID-LC-MS and ID-LC-MS/MS procedures performed at NIST and from ID-LC-MS/MS results provided by the CDC.

Certified and Reference Values for total 25(OH)D are provided in Tables 3 and 4 primarily for assays that are suitable for use with SRM 972a but do not measure the vitamin D metabolites separately.

Expiration of Certification: The certification of **SRM 972a** is valid, within the measurement uncertainty specified, until **31 January 2023**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Carlos A. Gonzalez, Chief
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Certificate Issue Date: 29 November 2017
Certificate Revision History on Last Page

Steven J. Choquette, Director
Office of Reference Materials

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 notify the purchaser. Registration (see attached sheet or register online) will facilitate 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 C.T. Sempos, J.M. Betz and P.M. Coates of NIH-ODS.

Overall direction and coordination of the analytical measurements leading to the certification of this SRM were performed by K.W. Phinney of the NIST Biomolecular Measurement Division, and S.S.-C. Tai and L.C. Sander of the NIST Chemical Sciences Division.

Acquisition of the material was performed by K.W. Phinney and K.E. Sharpless of the NIST Office of Special Programs. Certification measurements were performed by M. Bedner and S.S.-C. Tai of the NIST Chemical Sciences Division and R.S.C. Chia, a guest scientist at NIST from the Health Sciences Authority of Singapore. Certification measurements were also performed by K. Maw, S. Encisco, M. Chaudhary-Webb, and R.L. Schleicher at the CDC. Additional measurements in support of the development of SRM 972a were performed by M.A. Nelson, B.E. Lang, M.M. Schantz, and L.T. Sniegoski of the NIST Chemical Sciences Division.

Statistical analysis was provided by J.H. Yen of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

NOTICE AND WARNINGS TO USERS

Warning: SRM 972a IS INTENDED FOR LABORATORY USE ONLY. THIS IS A HUMAN-SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. The supplier of the serum has reported that each donor unit of serum used in the preparation of this product has been tested by a FDA-approved method and found non-reactive/negative for hepatitis B surface antigen (HbsAg), human immunodeficiency (HIV) 1 and 2 antibodies, and hepatitis C virus (HCV). However, no known test method can offer complete assurance that hepatitis B virus, hepatitis C virus, HIV, or other infectious agents are absent from this material. Accordingly, this human blood-based product should be handled at the Biosafety Level 2 or higher as recommended for any POTENTIALLY INFECTIOUS HUMAN SERUM OR BLOOD SPECIMEN in the Centers for Disease Control/National Institutes of Health Manual [7].

This SRM was developed after an appropriate human subjects research determination by NIST.

INSTRUCTIONS FOR STORAGE AND USE

Storage: Until required for use, SRM 972a should be stored in the dark at a temperature between –20 °C and –80 °C.

Use: SRM 972a is provided as a set of four vials of frozen serum. The vial (or vials) to be used should be allowed to thaw at room temperature for at least 30 min under subdued light. The contents of the vial should then be gently mixed prior to removal of a test portion for analysis. Precautions should be taken to avoid exposure to strong UV light and direct sunlight.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: SRM 972a was prepared by Solomon Park Research Laboratories (Kirkland, WA). Four serum pools were prepared. The naturally occurring concentrations of vitamin D metabolites in the human serum pools used to prepare Levels 1, 2, and 3 have not been modified. Level 4 is a human serum pool enriched with 3-epi-25(OH)D_3.

Analysis: Value assignment of the concentrations of 25(OH)D_3, 25(OH)D_2, and 3-epi-25(OH)D_3, in SRM 972a are based on the combination of results provided from two analytical methods at NIST (ID-LC-MS and ID-LC-MS/MS)

[(1)] Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

and from ID-LC-MS/MS at CDC. Value assignment of the concentrations of 24R,25(OH)$_2$D$_3$ are based on the results from ID-LC-MS/MS at NIST.

Measurement of 25(OH)D$_3$, 25(OH)D$_2$, and 3-epi-25(OH)D$_3$ by ID-LC-MS (NIST): Serum (450 mg) and an internal standard solution containing ^{2}H$_6$-25(OH)D$_3$, ^{2}H$_3$-25(OH)D$_2$, and ^{2}H$_3$-3-epi-25(OH)D$_3$ were combined in glass tubes, proteins were precipitated, and the metabolites were extracted into hexane twice. The hexane phases were combined and evaporated to dryness at 40 °C under nitrogen. The residues were reconstituted with methanol and vortex-mixed. Extracts were analyzed by using LC-MS with (1) an Ascentis Express F5 pentafluorophenylpropyl column (Supelco, Bellefonte, PA) and (2) a Zorbax SB-CN cyanopropyl stationary phase (Agilent Technologies, Palo Alto, CA). Analyses on the pentafluorophenylpropyl column were performed under isocratic conditions with a mobile phase of 26 % water and 74 % methanol at a flow rate of 0.8 mL/min. All solvent compositions represent volume fractions in percent. The column temperature was maintained at 15 °C. A step gradient to 100 % methanol was incorporated into the method at the end of the run to elute retained matrix components. Analyses on the cyanopropyl column were performed under isocratic conditions with a mobile phase of 33 % water and 67 % methanol (Levels 1, 2, and 4 of SRM 972a) or 34 % water and 66 % methanol (Level 3) at a flow rate of 1.0 mL/min. A step gradient to 100 % methanol was incorporated into the method at the end of the run to elute retained matrix constituents. The column temperature was maintained at 45 °C.

Atmospheric pressure chemical ionization (APCI) with positive polarity was used for both chromatographic methods. The $[M - H_2O + H]^+$ ions were monitored and used for quantification of all species. The ions monitored included *m/z* 383 for 25(OH)D$_3$ and for 3-epi-25(OH)D$_3$; *m/z* 386 for ^{2}H$_3$-3-epi-25(OH)D$_3$; *m/z* 389 for ^{2}H$_6$-25(OH)D$_3$; *m/z* 395 for 25(OH)D$_2$; and *m/z* 398 for ^{2}H$_3$-25(OH)D$_2$.

Measurement of 25(OH)D$_3$, 25(OH)D$_2$, and 3-epi-25(OH)D$_3$ by ID-LC-MS/MS (NIST): Serum (1.0 g to 2.0 g) was spiked with an appropriate internal standard solution [^{2}H$_6$-25(OH)D$_3$, ^{2}H$_3$-25(OH)D$_2$, or ^{2}H$_3$-3-epi-25(OH)D$_3$]. After equilibration at room temperature for 1 h, the pH of each sample was adjusted to pH 9.8 ± 0.2 with carbonate buffer. Analytes were extracted twice from the serum matrix with a mixture of hexane and ethyl acetate. The combined extracts were dried under nitrogen at 45 °C, and the residues were reconstituted with methanol for LC-MS/MS analysis. Extracts were analyzed using either an Ascentis Express F5 or a Zorbax SB-CN column under isocratic conditions with water:methanol mobile phases. APCI in the positive-ion mode and multiple reaction monitoring (MRM) mode were used. The following transitions were monitored: *m/z* 401 → *m/z* 383 for 25(OH)D$_3$ and 3-epi-25(OH)D$_3$; *m/z* 407 → *m/z* 389 for ^{2}H$_6$-25(OH)D$_3$; *m/z* 404 → *m/z* 386 for ^{2}H$_3$-3-epi-25(OH)D$_3$; *m/z* 413 → *m/z* 395 for 25(OH)D$_2$; and *m/z* 416 → *m/z* 398 for ^{2}H$_3$-25(OH)D$_2$.

Measurement of 25(OH)D$_3$, 25(OH)D$_2$, and 3-epi-25(OH)D$_3$ by ID-LC-MS/MS (CDC): Samples of SRM 972a (100 µL) were spiked with the following isotopically-labeled internal standards: ^{2}H$_6$-25(OH)D$_3$, ^{2}H$_3$-25(OH)D$_2$, and ^{2}H$_3$-epi-25(OH)D$_3$. Each serum sample was extracted using 1.5 mL hexane, and the supernatant was collected, dried under nitrogen at 25 °C, and reconstituted in 69 % methanol in water (volume fractions). Analytes were eluted from the extract (isocratic mobile phase same composition as the diluent) at a flow rate of 0.4 mL/min on a Hypersil GOLD pentafluorophenyl column (Thermo Fisher Scientific, Waltham, MA) at 28 °C and detected using APCI in positive-ion mode. Two transitions per vitamin D metabolite along with one transition per internal standard were monitored: 25(OH)D$_3$: *m/z* 383 → *m/z* 365 and *m/z* 383 → *m/z* 105; ^{2}H$_6$-25(OH)D$_3$: *m/z* 389 → *m/z* 377; epi-25(OH)D$_3$: *m/z* 383 → *m/z* 365 and *m/z* 383 → *m/z* 105; ^{2}H$_3$-epi-25(OH)D$_3$: *m/z* 386 → *m/z* 368; 25(OH)D$_2$: *m/z* 395 → *m/z* 377 and *m/z* 395 → *m/z* 209; ^{2}H$_3$-25(OH)D$_2$: *m/z* 398 → *m/z* 380. Analytes were quantitated using six-point linear calibration curves traceable to SRM 2972 25-Hydroxyvitamin D2 and D3 Calibration Solutions, and internal standards were used to correct for recovery.

Measurement of 24R,25(OH)$_2$D$_3$ by ID-LC-MS/MS (NIST): Serum (1.5 g to 2.0 g) was spiked with an internal standard solution containing ^{2}H$_6$-24R,25(OH)$_2$D$_3$. After equilibration at room temperature for 1 h, the pH of each sample was adjusted to pH 9.8 ± 0.2 with carbonate buffer. The 24R,25(OH)$_2$D$_3$ was extracted twice from the serum matrix with a mixture of hexane and ethyl acetate. The combined extracts were dried under nitrogen at 45 °C, and the residues were reconstituted with methanol for LC-MS/MS analysis. Extracts were analyzed using an Ascentis Express C_{18} column under isocratic conditions with a water:methanol mobile phase. APCI in the positive-ion mode and multiple reaction monitoring (MRM) mode were used. The following transitions were monitored: *m/z* 417 → *m/z* 381 for 24R,25(OH)$_2$D$_3$ and *m/z* 423 → *m/z* 387 for ^{2}H$_6$-24R,25(OH)$_2$D$_3$.

Homogeneity Analysis: The homogeneity assessment was made at the time the certification analyses were performed. A stratified sampling plan was devised to test for homogeneity across the lot of vials. There was no apparent trend in the data when plotted against the sequence in which the vials were prepared, with the exception of 3-epi-25(OH)D_3 in Level 4. An additional component of uncertainty related to possible inhomogeneity has been included in the expanded uncertainty for this analyte in Level 4.

Certified Values for 25(OH)D_2, 25(OH)D_3, 3-epi-25(OH)D_3, and 24R,25(OH)$_2D_3$: Values are weighted means of the results from analyses at NIST using ID-LC-MS and ID-LC-MS/MS and from CDC using ID-LC-MS/MS. The uncertainty provided with each certified value is an expanded uncertainty about the weighted mean to cover the measurand with approximately 95 % confidence; it incorporates Type B uncertainty components related to the analyses and expresses both the observed difference between the results from the methods and their respective uncertainties, consistent with the Guide to the Expression of Uncertainty in Measurement and with its Supplement 1 [8–11]. The expanded uncertainties are calculated as $U = ku_c$, where u_c is the combined uncertainty and k is a coverage factor corresponding to approximately 95 % confidence for each analyte [8]. For the certified values shown in Table 1, $k = 2$. The measurands are the total concentrations of the analytes listed in Table 1. Metrological traceability is to the SI derived units of mass fraction, expressed as nanograms per gram; mass concentration, expressed as nanograms per milliliter; and amount-of-substance concentration, expressed as nanomoles per liter.

Table 1. Certified Values for 25(OH)D_2, 25(OH)D_3, 3-epi-25(OH)D_3, and 24R,25(OH)$_2D_3$ in SRM 972a

	ng/g			ng/mL[(a)]			nmol/L[(b)]		
Level 1									
25-hydroxyvitamin D_3	28.1	±	1.1	28.8	±	1.1	71.8	±	2.7
3-epi-25-hydroxyvitamin D_3	1.77	±	0.10	1.81	±	0.10	4.5	±	0.2
24R,25-dihydroxyvitamin D_3	2.60	±	0.10	2.66	±	0.10	6.38	±	0.23
Level 2									
25-hydroxyvitamin D_2	0.80	±	0.06	0.81	±	0.06	2.0	±	0.2
25-hydroxyvitamin D_3	17.7	±	0.4	18.1	±	0.4	45.1	±	1.0
3-epi-25-hydroxyvitamin D_3	1.25	±	0.09	1.28	±	0.09	3.2	±	0.2
24R,25-dihydroxyvitamin D_3	1.38	±	0.05	1.41	±	0.05	3.39	±	0.12
Level 3									
25-hydroxyvitamin D_2	13.0	±	0.3	13.3	±	0.3	32.3	±	0.8
25-hydroxyvitamin D_3	19.4	±	0.4	19.8	±	0.4	49.5	±	1.1
24R,25-dihydroxyvitamin D_3	1.58	±	0.06	1.62	±	0.06	3.88	±	0.13
Level 4									
25-hydroxyvitamin D_3	28.8	±	0.9	29.4	±	0.9	73.4	±	2.3
3-epi-25-hydroxyvitamin D_3	25.4	±	2.1	26.0	±	2.2	64.8	±	5.4
24R,25-dihydroxyvitamin D_3	2.58	±	0.09	2.64	±	0.09	6.32	±	0.22

[(a)] Mass concentration levels were calculated from mass fractions using measured serum densities: Level 1, 1.02326 g/mL; Level 2, 1.02196 g/mL; Level 3, 1.02294 g/mL; and Level 4, 1.02295 g/mL.

[(b)] Molar concentration levels were calculated from mass concentration levels using the relative molecular masses. The relative molecular masses are 412.65 g/mol for 25(OH)D_2, 400.64 g/mol for 25(OH)D_3 and 3-epi-25(OH)D_3 and 416.64 g/mol for 24R,25(OH)$_2D_3$. The equivalent conversion factors are 2.4234 for 25(OH)D_2, 2.4960 for 25(OH)D_3 and 3-epi-25(OH)D_3 and 2.4002 for 24R,25(OH)$_2D_3$.

Reference Values for 25(OH)D_2 and 3-epi-25(OH)D_3 : Values are weighted means of the results from analyses at NIST using ID-LC-MS and ID-LC-MS/MS and from CDC using ID-LC-MS/MS. The measurands are the concentrations of analytes listed in Table 2 as determined by the indicated methods. Metrological traceability is to the ID-LC-MS/MS method, with values expressed in SI derived units of mass fraction, expressed as nanograms per gram; mass concentration, expressed as nanograms per milliliter; and amount-of-substance concentration, expressed as nanomoles per liter. The uncertainty provided with each reference value is an expanded uncertainty about the weighted mean to cover the measurand with approximately 95 % confidence; it incorporates Type B uncertainty components related to the analyses and expresses both the observed difference between the results from the methods and their respective uncertainties, consistent with the ISO/JCGM Guide and with its Supplement 1 [8–11]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty and k is a coverage factor corresponding to approximately 95 % confidence for each analyte [8]. For the reference values shown in Table 2, $k = 2$.

Table 2. Reference Values for 25(OH)D_2 and 3-epi-25(OH)D_3 in SRM 972a

	ng/g			ng/mL[(a)]			nmol/L[(b)]		
Level 1									
25-hydroxyvitamin D_2	0.52	±	0.06	0.54	±	0.06	1.3	±	0.2
Level 3									
3-epi-25-hydroxyvitamin D_3	1.14	±	0.14	1.17	±	0.14	2.9	±	0.4
Level 4									
25-hydroxyvitamin D_2	0.54	±	0.10	0.55	±	0.10	1.3	±	0.2

[(a)] Mass concentration levels were calculated from mass fractions using measured serum densities: Level 1, 1.02326 g/mL; Level 2, 1.02196 g/mL; Level 3, 1.02294 g/mL; and Level 4, 1.02295 g/mL.

[(b)] Molar concentration levels were calculated from mass concentration levels using the relative molecular masses. The relative molecular masses are 412.65 g/mol for 25(OH)D_2 and 400.64 g/mol for 25(OH)D_3 and 3-epi-25(OH)D_3. The equivalent conversion factors are 2.4234 for 25(OH)D_2 and 2.4960 for 25(OH)D_3 and 3-epi-25(OH)D_3.

Certified and Reference Values for Total 25(OH)D: Vitamin D levels in serum are typically reported as the total of 25(OH)D_3 and 25(OH)D_2. The values for total 25(OH)D, as the sum of the individual values for 25(OH)D_3 and 25(OH)D_2, are shown in Table 3 for certified values and Table 4 for reference values. The uncertainty provided with each value is an expanded uncertainty about the total 25(OH)D that covers the measurands with approximately 95 % confidence; it incorporates Type B uncertainty components related to the analyses and their respective uncertainties of the two analytes, consistent with the ISO/JCGM Guide and its Supplement 1 [8–11]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty and k is a coverage factor corresponding to approximately 95 % confidence for the analytes [8]. For the values shown in Table 3 and Table 4, $k = 2$.

Table 3. Certified Values for Total 25(OH)D in SRM 972a[(a)]

	ng/g			ng/mL[(b)]		
Level 2						
Total 25(OH)D	18.5	±	0.4	18.9	±	0.4
Level 3						
Total 25(OH)D	32.4	±	0.5	33.2	±	0.5

[(a)] Certified values for total 25(OH)D are based on the combination of certified values for both 25(OH)D_2 and 25(OH)D_3. The measurands are the total concentrations of the analytes listed. Metrological traceability is to the SI derived units of mass fraction, expressed as nanograms per gram; and mass concentration, expressed as nanograms per milliliter.

[(b)] Mass concentration levels were calculated from mass fractions using measured serum densities: Level 1, 1.02326 g/mL; Level 2, 1.02196 g/mL; Level 3, 1.02294 g/mL; and Level 4, 1.02295 g/mL.

Table 4. Reference Values for Total 25(OH)D in SRM 972a[(a)]

	ng/g			ng/mL[(b)]		
Level 1						
Total 25(OH)D	28.7	±	1.1	29.3	±	1.1
Level 4						
Total 25(OH)D	29.3	±	0.9	30.0	±	0.9

[(a)] Reference values for total 25(OH)D are based on the combination of a reference value for 25(OH)D_2 and a certified value for 25(OH)D_3. The measurands are the amounts of substance listed, as determined by the indicated method. Metrological traceability is to the ID-LC-MS/MS method, with values expressed in SI derived units of mass fraction expressed as nanograms per gram; and mass concentration ,expressed as nanograms per milliliter.

[(b)] Mass concentration levels were calculated from mass fractions using measured serum densities: Level 1, 1.02326 g/mL; Level 2, 1.02196 g/mL; Level 3, 1.02294 g/mL; and Level 4, 1.02295 g/mL.

REFERENCES

[1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136 (2000); available at https://www.nist.gov/sites/default/files/documents/srm/SP260-136.PDF (accessed Nov 2017).

[2] Bedner, M.; Phinney, K.W.; *Development and Comparison of Three Liquid Chromatography–Atmospheric Pressure Chemical Ionization/Mass Spectrometry Methods for Determining Vitamin D Metabolites in Human Serum*; J. Chromatogr. A, Vol. 1240, pp. 132–139 (2012).

[3] Tai, S.S.-C.; Bedner, M.; Phinney, K.W.; *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., Vol. 82, pp. 1942–1948 (2010).

[4] Schleicher, R.L.; Encisco, S.; Chaudhary-Webb, M.; Paliakov, E.; McCoy, L.F.; Pfeiffer, C.M.; *Isotope-Dilution Ultra Performance Liquid Chromatography–Tandem Mass Spectrometry Method for Simultaneous Measurement of 25-Hydroxyvitamin D_2, 25-Hydroxyvitamin D_3 and 3-epi-25-Hydroxyvitamin D_3 in Human Serum*; Clin. Chim. Acta, Vol. 412, pp. 1594–1599 (2011).

[5] Tai, S.S.-C.; Nelson, M.A.; *Candidate Reference Measurement Procedure for the Determination of 24R,25-Dihydroxyvitamin D3 in Human Serum using Isotope-Dilution Liquid Chromatography-Tandem Mass Spectrometry*; Anal. Chem., Vol. 87 (15), pp 7964–7970 (2015).

[6] Joint Committee for Traceability in Laboratory Medicine (JCTLM); available at http://www.bipm.org/en/committees/jc/jctlm/ (accessed Nov 2017).

[7] CDC/NIH; *Biosafety in Microbiological and Biomedical Laboratories, 5th ed.*; Richardson, J.; Barkley, W.E.; Richmond, J.; McKinney, R.W., Eds.; U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health; US Government Printing Office: Washington, D.C. (2009); available at http://www.cdc.gov/OD/OHS/biosfty/bmbl5/BMBL_5th_Edition.pdf (accessed Nov 2017).

[8] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Nov 2017); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at https://www.nist.gov/pml/nist-technical-note-1297 (accessed Nov 2017).

[9] JCGM 101:2008; *Evaluation of Measurement Data – Supplement 1 to the Guide to the Expression of Uncertainty in Measurement – Propagation of Distributions Using a Monte Carlo Method*; JCGM (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed Nov 2017).

[10] DerSimonian, R.; Laird, N.; *Meta-Analysis in Clinical Trials*; Controlled Clin. Trials, Vol. 7, pp. 177–188 (1986).

[11] Rukhin, A. L.; *Weighted Means Statistics in Interlaboratory Studies*; Metrologia, Vol. 46, pp. 323–331 (2009).

Certificate Revision History: 29 November 2017 (Correction to tables numbering; editorial changes); 11 September 2017 (Change from reference to certified values for 24R,25$(OH)_2D_3$; editorial changes); 15 September 2015 (Addition of reference values for 24R,25$(OH)_2D_3$; addition of certified and reference values for total 25(OH)D; update of certified and reference values for 25$(OH)D_3$ and 3-epi-25$(OH)D_3$; editorial changes); 20 February 2013 (Original certificate issue date).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.