National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2669

Arsenic Species in Frozen Human Urine

This Standard Reference Material (SRM) is intended primarily for validating analytical methods and measurements for the determination of arsenic species in human urine. A unit of SRM 2669 consists of five pouches, each pouch containing one vial of Level I and one vial of Level II Arsenic Species in Frozen Human Urine. Each vial contains nominally 1.5 mL of urine. SRM 2669 is shipped on dry ice, and it should be stored at –80 °C until use.

The development of SRM 2669 was a collaboration between NIST and the Centers for Disease Control and Prevention (CDC), National Centers for Environmental Health, Division of Laboratory Sciences, (Atlanta, GA).

Certified Values: Table 1 lists the certified values and expanded uncertainties for arsenic species in SRM 2669. The structural formulas of the arsenic species are shown in the appendix. 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 value is the unweighted average of the values from NIST and collaborating laboratories. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined uncertainty due to material variability and measurement uncertainty calculated according to the method described in the ISO/JCGM Guide [2] and reference 3. The coverage factor, k, for a 95 % confidence interval, equals 2 for all certified values in Table 1.

Reference Values: Table 2 lists reference values with expanded uncertainties for total arsenic. Reference values are non-certified values that are the best estimate of the true value; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may not include all sources of uncertainty [1]. The reference values are based on results obtained from a single NIST analytical method. The coverage factors for a 95 % confidence interval equal 2.33 and 2.59 for total arsenic in Level I and Level II, respectively.

Information Values: Table 3 lists information values for trimethylarsine oxide (TMAO) and arsenocholine (AC) in Level I. An information value is considered to be a value that will be of interest to the SRM user, but insufficient information is available to assess the uncertainty associated with the value [1].

Expiration of Certification: The certification of **SRM 2669** is valid, within the measurement uncertainty specified, until **31 December 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.

Maintenance of SRM Certification: 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) will facilitate notification.

The coordination of the technical measurements leading to the certification was under the direction of L.L. Yu and G.C. Turk of the NIST Chemical Sciences Division.

Analytical measurements for certification of this SRM were performed by W.C. Davis and L.L. Yu of the NIST Chemical Sciences Division; C.P. Verdon, C.G.K. Freeman, M. Fresquez, and G. Shakirova of the CDC Inorganic and Radiation Analytical Toxicology Branch; and B. Buckley and R. Xie of Rutgers, The State University of New Jersey, Environmental and Occupational Health Sciences Institute (Piscataway, NJ).

Carlos A. Gonzalez, Chief
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Gaithersburg, MD 20899
Certificate Issue Date: 04 November 2013
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Robert L. Watters Jr., Director
Office of Reference Materials

Partial support for the development of this SRM was provided under the direction of R.L. Jones of the CDC Inorganic and Radiation Analytical Toxicology Branch.

Statistical consultation for this SRM was provided by D.D. Leber 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 WARNING TO USERS

SRM 2669 IS INTENDED FOR IN-VITRO DIAGNOSTIC USE ONLY. THIS IS A HUMAN-SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. Proper protective equipment such as gloves, safety goggles, and laboratory coat should be worn when handling the material. The SRM and anything in contact with the SRM should be disinfected with 10 % bleach before being properly disposed.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored at –80 °C in the original unopened package. The certification does not apply to contents of previously opened pouches as the stability of all species has not been investigated under such conditions.

Use: SRM 2669 should be thawed at room temperature. The material should be used within 4 h after being thawed. Unused or remaining material should be discarded after the specified time. Once the pouches are cut open, each vial of the SRM should be homogenized by gently inverting the vial several times before a test portion is removed. To determine arsenic species in the SRM, particulates in the subsample should be removed. Recommended procedures for removal of particulates are: (1) extracting supernatant after centrifuging at 2×10^4 g_n for 5 min, or (2) filtration using a 0.45 µm syringe filter. To determine the total arsenic in SRM 2669, the entire subsample, including particulates, should be used. The recommended minimum sample size for speciation measurement is 0.2 mL. The recommended minimum sample size for total arsenic measurement is 1 mL.

PREPARATION AND ANALYSIS[(1)]

The urine pool used for the preparation of SRM 2669 was collected at CDC from volunteers in spring 2006. Each urine specimen, collected in plastic cups, was screened for total arsenic and arsenic species and then combined in one of three urine pools representing low, medium, and high arsenic levels. The three urine pools were blended to form two pools (Levels I and II) containing arsenic species at the target concentrations (see below). The urine pools were centrifuged at approximately 5 °C, and the precipitates discarded. The concentrations of the seven arsenic species in each pool were adjusted to the target levels (see below) with addition of appropriate amounts of arsenic species. The pools were stirred and were sparged continuously with nitrogen the day before production. On the day of production, the tubing for sparging was withdrawn to the surface of the urine pools to stop sparging while keeping the pools in the positively pressurized nitrogen environment. Aliquots of approximately 1.5 mL of urine from the pools were dispensed into 2 mL cryovials inside a glove box continuously purged with nitrogen to provide an anaerobic environment. The vials were heat-sealed in Mylar bags containing oxygen absorbers and stored at –80 °C at CDC and then NIST following transfer (on dry ice).

The target levels of arsenic species in Level I and Level II of the SRM were designed to represent approximately the 50th and 95th percentiles of the concentrations (with some adjustments) in the U.S. population based on preliminary data from the recent National Health and Nutrition Examination Survey (NHANES) [4].

Analytical determinations for certification of this SRM were performed at NIST, CDC, and Rutgers using the methods listed in Table 4.

[(1)]Certain commercial instruments, materials, or processes 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 instruments, materials, or processes identified are necessarily the best available for the purpose.

Table 1. Certified Values for Arsenic Species in SRM 2669[a,b]

Species	Level I, µg/L as Arsenic	Level II, µg/L as Arsenic
Arsenous acid (AsIII)	1.47 ± 0.10	5.03 ± 0.31
Arsenic acid (AsV)	2.41 ± 0.30	6.16 ± 0.95
Monomethylarsonic acid (MMA)	1.87 ± 0.39	7.18 ± 0.56
Dimethylarsinic acid (DMA)	3.47 ± 0.41	25.3 ± 0.7
Trimethylarsine oxide (TMAO)		1.94 ± 0.27
Arsenobetaine (AB)	12.4 ± 1.9	1.43 ± 0.08
Arsenocholine (AC)		3.74 ± 0.35

[a] The certified value and the expanded uncertainty were calculated using the method described in reference [3].
[b] The measurand is the total concentration for each arsenic species listed and the certified value is metrologically traceable to the unit microgram per liter.

Table 2. Reference Values for Total Arsenic in SRM 2669[a,b]

Element	Level I, µg/L	Level II, µg/L
Arsenic, total	22.2 ± 4.8	50.7 ± 6.3

[a] The reference value and the expanded uncertainty were calculated in accordance with NIST Technical Note 1297 [2].
[b] The measurand is the concentration for total arsenic as determined by the single method listed in Table 4, and the reference value is metrologically traceable to the unit microgram per liter.

Table 3. Information Values for Arsenic Species in SRM 2669

Species	Level I, µg/L as Arsenic
Trimethylarsine oxide (TMAO)	<0.8
Arsenocholine (AC)	<0.7

Table 4. Methods of Analysis for SRM 2669

Analyte	Methods[a]	Laboratory
Arsenous acid (AsIII)	Anion exchange LC – (H_2 dynamic reaction) ICPMS	CDC [5]
	Cation exchange LC – ICPMS	NIST [6]
	Anion exchange IC – (H_2/He collision) ICPMS	NIST [6]
	Anion exchange IC – ICPMS	Rutgers [7]
Arsenic acid (AsV)	Anion exchange LC – (H_2 dynamic reaction) ICPMS	CDC [5]
	Anion exchange LC – ICPMS	NIST [6]
	Anion exchange IC – (H_2/He collision) ICPMS	NIST [6]
	Anion exchange IC – ICPMS	Rutgers [7]
Monomethylarsonic acid (MMA)	Anion exchange LC – (H_2 dynamic reaction) ICPMS	CDC [5]
	Cation exchange LC – ICPMS	NIST [6]
	Anion exchange IC – (H_2/He collision) ICPMS	NIST [6]
	Anion exchange IC – ICPMS	Rutgers [7]
Dimethylarsinic acid (DMA)	Anion exchange LC – (H_2 dynamic reaction) ICPMS	CDC [5]
	Cation exchange LC – ICPMS	NIST [6]
	Anion exchange IC – (H_2/He collision) ICPMS	NIST [6]
	Cation exchange IC – ICPMS	Rutgers [7]

Trimethylarsine oxide (TMAO)	Cation exchange LC – ICPMS	NIST [6]
	Anion exchange IC – (H_2/He collision) ICPMS	NIST [6]
	Cation exchange IC – ICPMS	Rutgers [7]
Arsenobetaine (AB)	Cation exchange LC – ICPMS	NIST [6]
	Anion exchange IC – (H_2/He collision) ICPMS	NIST [6]
	Cation exchange IC – ICPMS	Rutgers [7]
Arsenocholine (AC)	Anion exchange LC – (H_2 dynamic reaction) ICPMS	CDC [5]
	Cation exchange LC – ICPMS	NIST [6]
	Anion exchange IC – (H_2/He collision) ICPMS	NIST [6]
Total arsenic	(H_2/He collision) ICPMS after complete digestion of urine.	NIST

[(a)] LC: Liquid Chromatography; IC: Ion Chromatography; ICPMS: Inductively Coupled Plasma Mass Spectrometry.

REFERENCES

[1] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260–136, U.S. Government Printing Office: Washington, DC (2000); available at http://www.nist.gov/srm/upload/SP260-136.PDF (accessed Nov 2013).

[2] 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 2013); 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 http://www.nist.gov/pml/pubs/index.cfm (accessed Nov 2013).

[3] Levenson, M.S.; Banks, D.L.; Eberhardt, K.R.; Gill, L.M.; Guthrie, W.F.; Liu, H.-K.; Vangel, M.G.; Yen, J.H.; Zhang, N.F.; *An Approach to Combining Results from Multiple Methods Motivated by the ISO GUM*; J. Res. Natl. Inst. Stand. Technol., Vol. 105, pp. 571–579 (2000).

[4] National Health and Nutrition Examination Survey 2003-2004; *Documentation, Codebook, and Frequencies. Laboratory Component: Total and Speciated Arsenics*; November 2007; available at http://www.cdc.gov/nchs/ (accessed Nov 2013).

[5] Verdon, C.P.; Caldwell, K.L.; Fresquez, M.R.; Jones, R.L.; *Determination of Seven Arsenic Compounds in Urine by HPLC-ICP-DRC-MS: A CDC Population Biomonitoring Method*; Anal. Bioanal. Chem., Vol. 393, pp. 939–947 (2009).

[6] Davis, W.C.; Zeisler, R.; Sieber, J.R.; Yu, L.L.; *Methods for the Separation and Quantification of Arsenic Species in SRM 2669: Arsenic Species in Frozen Human Urine*; Anal. Bioanal. Chem., Vol. 396, pp. 3041–3050 (2010).

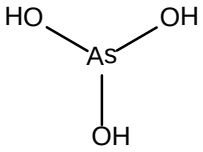
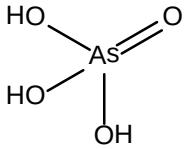
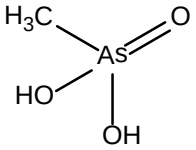
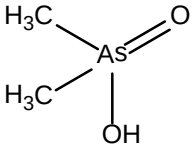
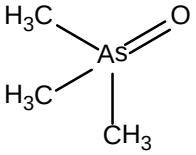
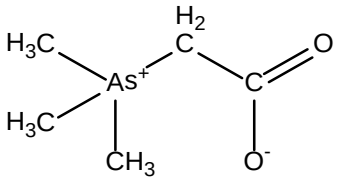
[7] Xie, R.; Johnson, W.; Spayd, S.; Hall, G.S.; Buckley, B; *Arsenic Speciation Analysis of Human Urine Using Ion Exchange Chromatography Coupled to Inductively Coupled Plasma Mass Spectrometry*; Anal. Chim. Acta, Vol. 578, pp. 186–194 (2006).

Certificate Revision History: 04 November 2013 (Extension of certification period; editorial changes); 22 January 2009 (Original certificate 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.

APPENDIX

Structural Formulas for Arsenic Species in SRM 2669

Name (Abbreviation)	CAS Registry Number	Structural Formula
Arsenous acid (AsIII)	13464-58-9	
Arsenic acid (AsV)	7778-39-4	
Monomethylarsonic acid (MMA)	124-58-3	
Dimethylarsinic acid (DMA)	75-60-5	
Trimethylarsine oxide (TMAO)	4964-14-1	
Arsenobetaine (AB)	64436-13-1	
Arsenocholine (AC)	39895-81-3