

Standard Reference Material[®] 2669

Arsenic Species in Frozen Human Urine

CERTIFICATE OF ANALYSIS

Purpose: This Standard Reference Material (SRM) is intended primarily for validating analytical methods and measurements for the determination of arsenic species in human urine.

Description: 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.

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.

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 in Table 1. Metrological traceability is to the SI derived unit for mass concentration (expressed as micrograms per liter).

Non-Certified Values: Non-certified values are provided in Appendix A.

Additional Information: Values of potential interest to users and additional information are provided in Appendix B.

Period of Validity: The certified values delivered by **SRM 2669** are valid within the measurement uncertainty specified until **31 December 2033**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Values: NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (<https://www.nist.gov/srm>) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

Safety: SRM 2669 IS INTENDED FOR RESEARCH USE. This is a human source material. SRM 2669 is a Biosafety Level 2 material and should be handled according to applicable federal, state, and/or local regulations and according to policies and procedures of recipient's organization.

Storage: The SRM should be stored at $-80\text{ }^{\circ}\text{C}$ 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 $2 \times 10^4\text{ g}_n$ for 5 min, or (2) filtration using a $0.45\text{ }\mu\text{m}$ 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.

REFERENCES

- [1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication 260-136, 2021 edition; U.S. Government Printing Office: Washington, DC (2021); available at <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf> (accessed July 2023).
- [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 https://www.bipm.org/utis/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed July 2023); 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/pubs/index.cfm> (accessed July 2023).
- [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 <https://www.cdc.gov/nchs/> (accessed July 2023).
- [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: 21 July 2023 (Updated format; change of period of validity; editorial changes); 19 January 2016 (Editorial changes); 04 November 2013 (Extension of certification period; editorial changes); 22 January 2009 (Original certificate date).

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at <https://www.nist.gov/srm>.

* * * * * End of Certificate of Analysis * * * * *

APPENDIX A

Non-Certified Values: Table A1 lists non-certified values with expanded uncertainties for total arsenic. Non-certified values are suitable for use in method development, method harmonization, and process control but do not provide metrological traceability to the International System of Units (SI) or other higher-order reference system. The non-certified 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.

Table A1. Non-Certified Values for Total Arsenic in SRM 2669^(a)

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

^(a) The non-certified values and the expanded uncertainty were calculated in accordance with NIST Technical Note 1297 [2].

Period of Validity: The non-certified values delivered by **SRM 2669** are valid within the measurement uncertainty specified until **31 December 2033**. The non-certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Appendix and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

***** End of Appendix A *****

APPENDIX B

Values of Potential Interest: Table B1 lists values for trimethylarsine oxide (TMAO) and arsenocholine (AC) in Level I. These are values that may be of interest to the SRM user, but insufficient information is available to assess the uncertainty associated with the value [1]. Values of potential interest cannot be used to establish metrological traceability.

Table B1. Values of Potential Interest for Arsenic Species in SRM 2669

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

Source and Preparation: 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). 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.

The urine pool used for the preparation of SRM 2669 was collected at CDC from volunteers in August 2005. 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 B2.

Table B2. 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.

Table B3. 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	

***** End of Appendix B*****