



Certificate of Analysis

Standard Reference Material[®] 3669

Arsenic Species in Frozen Human Urine (Elevated Levels)

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 3669 consists of five pouches each containing a vial of arsenic species in frozen human urine at elevated levels. Each vial contains nominally 1.5 mL of urine. SRM 3669 is shipped on dry ice, and it should be stored at $-80\text{ }^{\circ}\text{C}$ until use.

The development of SRM 3669 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 3669. 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 reported certified values are the weighted means of the individual sets of measurements made by NIST and CDC, estimated using a Gaussian random effects model [2] and the DerSimonian-Laird procedure [3,4]. The associated measurement uncertainty was evaluated by the application of the parametric statistical bootstrap, consistent with the ISO/JCGM Guides and its Supplement 1 [5–7]. The expanded uncertainty, U , is calculated as $U = ku_c$, where u_c represents, at the level of one standard deviation, the combined effects of between-laboratory, within-laboratory, and inhomogeneity components of uncertainty. The coverage factor, k , corresponds to an approximately 95 % level of confidence.

Expiration of Certification: The certification of **SRM 3669** is valid, within the measurement uncertainty specified, until **30 June 2024**, 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 or register online) will facilitate notification.

Coordination of the technical measurements leading to the certification was under the direction of L.L. Yu of the NIST Chemical Sciences Division.

Analytical measurements for certification of this SRM were performed by W.C. Davis, R.L. Paul, and L.L. Yu of the NIST Chemical Sciences Division; N.D. Hilliard and C.D. Ward of the CDC Inorganic and Radiation Analytical Toxicology Branch.

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.

Carlos A. Gonzalez, Chief
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Office of Reference Materials

NOTICE AND WARNING TO USERS

SRM 3669 IS INTENDED FOR RESEARCH USE. THIS IS A HUMAN-SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. THE RECONSTITUTED URINE SHOULD BE HANDLED WITH PRECAUTIONS SUITABLE FOR FRESH URINE. Accordingly, this human source product should be handled at the Biosafety Level 2 or higher as recommended for any potentially infectious human serum or blood specimen by the Centers for Disease Control and Prevention (CDC) Office of Safety, Health, and Environment and the National Institutes of Health (NIH) [8].

INSTRUCTIONS FOR STORAGE AND USE

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: Unopened pouches of SRM 3669 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 SRM 3669, particulates in the subsample should be removed. Recommended procedures for removal of particulates are: (1) extracting supernatant after centrifuging at $2 \times 10^4 g_n$ for 5 min, or (2) filtration using a $0.45\text{ }\mu\text{m}$ syringe filter. The recommended minimum sample size for speciation measurement is 0.2 mL.

To determine the total arsenic in SRM 3669, the entire subsample, including particulates, should be used. 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 3669 was collected at CDC from volunteers in spring 2011. Each urine specimen, collected in plastic cups, was screened for arsenic species and then pooled. The urine pool was centrifuged at approximately $4\text{ }^{\circ}\text{C}$, and the precipitates discarded. The concentrations of the five arsenic species in the pool were adjusted to the target levels (see below) with addition of appropriate amounts of arsenic species. The pool was stirred and was 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 pool to stop sparging while keeping the pool in the positively pressurized nitrogen environment. Aliquots of approximately 1.5 mL of urine from the pool 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 $-80\text{ }^{\circ}\text{C}$ at CDC and then NIST following transfer (on dry ice).

The target levels were determined from the values of SRM 2669 Arsenic Species in Frozen Human Urine [9] and from a CDC database of arsenic levels of 1495 individuals unintentionally exposed to arsenic hazard. The target levels represent the 95th percentile of the values in the database or three times the mass concentration of the arsenic species in Level II of SRM 2669 [9], whichever is greater.

Analytical determinations for certification of this SRM were performed at NIST and CDC using the methods listed in Table 2.

⁽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 and Arsenic Species in SRM 3669^(a)

Species	Arsenic ($\mu\text{g/L}$)	<i>k</i> -factor
Arsenous acid (AsIII)	14.28 \pm 0.65	1.99
Arsenic acid (AsV)	17.8 \pm 1.0	2.00
Monomethylarsonic acid (MMA)	21.6 \pm 1.0	1.98
Dimethylarsinic acid (DMA)	77.1 \pm 1.6	2.00
Arsenobetaine (AB)	51.0 \pm 2.2	1.99
Arsenic (As)	185.1 \pm 9.9	1.98

^(a) The measurand is the total concentration of arsenic or each arsenic species listed, and the certified value is metrologically traceable to the SI derived unit for mass concentration (expressed as micrograms per liter).

Table 2. Methods of Analysis for SRM 3669

Analyte	Methods ^(a)	Laboratory
Arsenous acid (AsIII)	Anion exchange LC – (H ₂ dynamic reaction) ICPMS	CDC [10]
	Cation exchange LC – ICPMS	NIST [11]
	Anion exchange IC – (H ₂ /He collision) ICPMS	NIST [11]
Arsenic acid (AsV)	Anion exchange LC – (H ₂ dynamic reaction) ICPMS	CDC [10]
	Anion exchange LC – ICPMS	NIST [11]
	Anion exchange IC – (H ₂ /He collision) ICPMS	NIST [11]
Monomethylarsonic acid (MMA)	Anion exchange LC – (H ₂ dynamic reaction) ICPMS	CDC [10]
	Cation exchange LC – ICPMS	NIST [11]
	Anion exchange IC – (H ₂ /He collision) ICPMS	NIST [11]
Dimethylarsinic acid (DMA)	Anion exchange LC – (H ₂ dynamic reaction) ICPMS	CDC [10]
	Cation exchange LC – ICPMS	NIST [11]
	Anion exchange IC – (H ₂ /He collision) ICPMS	NIST [11]
Arsenobetaine (AB)	Anion exchange LC – (H ₂ dynamic reaction) ICPMS	CDC [10]
	Cation exchange LC – ICPMS	NIST [11]
	Anion exchange IC – (H ₂ /He collision) ICPMS	NIST [11]
Total arsenic	Radiochemical neutron activation analysis	NIST [12]
	(H ₂ /He collision) ICPMS after complete digestion of urine	NIST [13]

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

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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>.

