National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2668

Toxic Elements in Frozen Human Urine

This Standard Reference Material (SRM) is intended primarily for validating analytical methods and measurements for the determination of toxic elements in human urine. A unit of SRM 2668 consists of five vials of each of two levels. Each vial contains nominally 1.5 mL of urine. SRM 2668 is shipped on dry ice, and it should be stored at −70 °C until use.

The development of SRM 2668 was a collaboration between the National Institute of Standards and Technology (NIST) and the Centers for Disease Control and Prevention (CDC), National Centers for Environmental Health, Division of Laboratory Sciences, Atlanta, GA.

Certified Mass Concentration Values: Certified mass concentration values for 6 elements in Level I and 14 elements in Level II of SRM 2668 are listed 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]. Values were derived from the combination of results provided by NIST and collaborating laboratories. The certified values in this material are the weighted means [2–4] of the individual sets of measurements made by NIST and collaborating laboratories. The associated expanded uncertainties include between-laboratory, within-laboratory, and inhomogeneity components of uncertainty and are provided at the 95 % level of confidence [5].

Reference Mass Concentration Values: Reference mass concentration values for 17 elements in Level I and 9 elements in Level II are provided in Table 2. Reference values are non-certified 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 [1] and are provided with associated uncertainties that may not include all sources of uncertainty.

Information Values: Information values for mass concentration of creatinine are listed in Table 3. 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 2668** is valid, within the measurement uncertainty specified, until **31 December 2031**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Handling, 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.

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

Statistical analysis 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.

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Certificate Revision History on Last Page

Steven J. Choquette, Director
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Analytical measurements for certification of this SRM were performed by S.J. Christopher, K.E. Murphy, R.L. Paul, K.W. Pratt, L.J. Wood, and L.L. Yu of the NIST Chemical Sciences Division, E.A. Mackey of NIST, and R. Oflaz Spatz, B.E. Tomlin, formerly of NIST; K.L. Caldwell, J.M. Jarrett, R.L. Jones, A. Makhmudov, G. Mitchell, G. Shakirova, and G. Xiao of the CDC Inorganic and Radiation Analytical Toxicology Branch, Division of Laboratory Sciences, National Centers for Environmental Health (Atlanta, GA); M.M. Kimberly of the CDC Clinical Chemistry Branch, Division of Laboratory Sciences, National Centers for Environmental Health (Atlanta, GA); P. Olive of Battelle working at the CDC Clinical Chemistry Branch, Division of Laboratory Sciences, National Centers for Environmental Health (Atlanta, GA); J. Good and T. Moyer of Mayo Clinic (Rochester, MN); and A.J. Steuerwald, A.L. Roselan, C.N. Pellegri, and P.J. Parsons of the Division of Environmental Health Sciences, Laboratory of Inorganic and Nuclear Chemistry, Wadsworth Center, New York State Department of Health (NYSDOH), (Albany, NY).

Partial support for the development of this SRM was provided by the CDC, National Centers for Environmental Health, Division of Laboratory Sciences, under the direction of R.L. Jones of the Inorganic and Radiation Analytical Toxicology Branch.

NOTICE AND WARNING TO USERS

SRM 2668 IS INTENDED FOR RESEARCH USE. This is a human-source material. Handle product as a biohazardous material capable of transmitting infectious disease. This product should be handled at Biosafety Level 2 or higher as recommended in the Centers for Disease Control and Prevention/National Institutes of Health Biosafety in Microbiological and Biomedical Laboratories (5th edition) for human-derived products where the presence of an infectious agent may be unknown [6].

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

The SRM should be stored at –70 °C in the original unopened Mylar bag. The certification does not apply to contents of previously opened bags as the stability of all analytes has not been investigated under such conditions. SRM 2668 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. Each vial of the SRM should be homogenized by gently inverting the vial several times before a test portion is removed. A minimum test portion of 0.5 mL should be used for the values provided in this certificate to be valid.

PREPARATION AND ANALYSIS[(1)]

The urine used for the preparation of SRM 2668 was collected at CDC from volunteers in Spring 2007 using a protocol approved by the Institutional Review Board of the CDC. Each urine specimen, collected in a plastic cup, was screened for trace element contents and then combined in one of the two 17 L urine pools. While stirring, the urine pools were acidified slowly to contain 1 % nitric acid (volume fraction). The pooled urine was centrifuged for 60 min at 3700 g_n, and the precipitates discarded. The concentrations of trace elements in the two pools were adjusted to the target levels (see below) with addition of appropriate amounts of NIST SRM 3100 series single-element standards or the equivalent. On the day of production, aliquots of 1.8 mL from each urine pool were dispensed into 2 mL cryovials under class 100 clean room condition. The vials were capped, heat-sealed in Mylar bags, stored at –70 °C at CDC and at NIST following transfer (on dry ice).

The target levels of trace elements in Level I and Level II of the SRM were designed to represent the 50th to 95th and >95th percentiles, respectively, of the concentrations (with some adjustments) in the U.S. population based primarily on data from the National Report on Human Exposure to Environmental Chemicals (2005-2006 National Health and Nutrition Examination Survey) [7].

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

[(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.

Homogeneity: Except for creatinine, chlorine, fluoride, potassium, and sodium, measurements for homogeneity assessment were made at CDC using inductively coupled plasma mass spectrometry methods listed in Table 4. Measurements for homogeneity assessment of fluoride were made at NIST using the potentiometry (fluoride ion-selective electrode) method listed in Table 4. The SRM was determined inhomogeneous based on the analysis of within-vial and between-vial variances and on visual inspection. This observed vial-to-vial variability is incorporated into the expanded uncertainties of the certified and reference values using statistical Monte Carlo methods consistent with the methods suggested by Supplement 1 to the ISO Guide [8]. Although homogeneity of chlorine, potassium, and sodium was not assessed, a statistical prediction interval was leveraged when expressing the expanded uncertainties of these elements to reflect the material heterogeneity.

Stability: Except for that of creatinine, chlorine, fluoride, potassium, and sodium, stability was monitored at intervals of approximately every six months for a period of more than two years. All analytes under the stability monitoring program were determined to be stable by one of two methods: If the number of times (N) the stability monitoring measurements were conducted was less than or equal to 6, each stability measurement value had to overlap the certified or reference value to be considered stable. If $N > 6$, the measurement data were analyzed with linear regression. At 95 % confidence, the slope of the regression analysis had to include 0 for the analyte to be considered stable.

Certified values and reference values for trace elements are weighted means of results from NIST and collaborating laboratories, found by leveraging a linear, Gaussian random effects statistical model [2,3] and the methods of maximum likelihood estimation [4,9] or the DerSimonian-Laird procedure [2,10]. Maximum likelihood estimation was utilized when degrees of freedom were readily available, otherwise the DerSimonian-Laird procedure was used. The estimation procedures are supplemented by the parametric bootstrap [11] for uncertainty propagation. The associated uncertainty is expressed as an expanded uncertainty, U. 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 effect of between-laboratory, within-laboratory, and inhomogeneity components of uncertainty. The coverage factor (k) corresponds to approximately 95 % confidence for each analyte.

Table 1. Certified Mass Concentration Values

	Level I (µg/L)	Coverage Factor, k	Level II (µg/L)	Coverage Factor, k
Antimony (Sb)			22.4 ± 1.0	1.96
Arsenic (As)	10.81 ± 0.54	1.99	213.1 ± 4.4	2.02
Barium (Ba)			254.6 ± 3.2	2.07
Beryllium (Be)			54.5 ± 2.4	2.06
Cadmium (Cd)	1.056 ± 0.052	2.00	16.40 ± 0.25	2.07
Cesium (Cs)	4.90 ± 0.30	2.02	221 ± 12	2.06
Chromium (Cr)			27.7 ± 2.1	1.90
Cobalt (Co)	0.816 ± 0.058	1.89	51.8 ± 1.7	1.98
Copper (Cu)			134.1 ± 5.4	1.97
Lead (Pb)	1.234 ± 0.061	2.00	137.9 ± 3.6	2.02
Manganese (Mn)			47.6 ± 3.4	2.01
Molybdenum (Mo)			1687 ± 58	2.04
Nickel (Ni)			115.3 ± 5.2	2.05
Vanadium (V)	0.980 ± 0.086	1.97	48.5 ± 4.6	2.06

Table 2. Reference Mass Concentration Values

Trace Elements

	Level I (µg/L)	Coverage Factor, *k*	Level II (µg/L)	Coverage Factor, *k*
Antimony (Sb)	0.242 ± 0.031	1.93		
Barium (Ba)	1.96 ± 0.14	2.06		
Beryllium (Be)	1.073 ± 0.081	1.93		
Chromium (Cr)	1.08 ± 0.31	2.09		
Copper (Cu)	28.1 ± 2.0	2.05		
Manganese (Mn)	1.08 ± 0.16	1.97		
Molybdenum (Mo)	51.6 ± 1.8	1.95		
Nickel (Ni)	2.31 ± 0.32	2.04		
Platinum (Pt)	1.04 ± 0.12	1.87	117.0 ± 9.8	1.98
Thallium (Tl)	0.719 ± 0.029	2.15	115.2 ± 2.8	2.00
Tin (Sn)	1.69 ± 0.14	1.91	171.0 ± 9.0	1.96
Tungsten (W)	1.252 ± 0.080	2.00	62.5 ± 1.0	2.00
Uranium (U)	0.0340 ± 0.0024	2.05	13.37 ± 0.49	1.97

Major Components[(a)]

	Level I (mg/L)	Coverage Factor, *k*	Level II (mg/L)	Coverage Factor, *k*
Chlorine (Cl)	2730 ± 120	2.44	2620 ± 100	2.41
Fluoride (F^-)	12.25 ± 0.14	1.98	18.83 ± 0.92	2.26
Potassium (K)	1581 ± 86	2.57	1540 ± 110	2.57
Sodium (Na)	1840 ± 100	2.49	1802 ± 53	2.36

[(a)] Reference mass concentration values for major components are the mean results of one NIST method. The associated uncertainty is expressed as an expanded uncertainty, *U*. 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 effect of within-laboratory and inhomogeneity components of uncertainty. The coverage factor (*k*) corresponds to approximately 95 % confidence for each analyte. The associated expanded uncertainty was calculated in accordance with the ISO Guide [5].

Table 3. Information Values

	Level I (mg/L)	Level II (mg/L)
Creatinine	626	618

Table 4. Methods of Analysis Used for Characterization of SRM 2668

Method	Analytes Determined
Isotope dilution inductively coupled plasma mass spectrometry at NIST [12,13]	Cd, Cr[(a)], Pb
Inductively coupled plasma mass spectrometry (ICP-MS) at NIST	As, Ba, Be, Co, Cr, Cs, Cu, Mn, Mo, Ni, Pb, Pt, Sb, Sn, Tl, U, V, W
Instrumental neutron activation analysis (INAA) at NIST [14,15]	Cl, Co, Cs, K, Na, Sb[(a)]
Pre-concentration INAA at NIST [16]	Cu[(a)], V
Radiochemical neutron activation analysis at NIST [17]	As
Inductively coupled plasma optical emission spectrometry at NIST	Ba[(a)], Be[(a)], Mn[(a)], Mo[(a)], Ni[(a)]
Potentiometry (fluoride ion-selective electrode) at NIST	F
ICP-MS at CDC [18–20]	As, Ba, Be, Cd, Co, Cr, Cs, Cu, Mn, Mo, Ni, Pb, Pt, Sb, Sn, Tl, U, V, W
Automated enzymatic method at CDC [21]	Creatinine
ICP-MS at the Mayo Clinic	As, Ba, Be, Cd, Co, Cr, Cs, Cu, Mn, Mo, Ni, Pb, Pt, Sb, Sn, Tl, V, W
ICP-MS Method A at NYSDOH	As, Ba, Be, Co, Cs, Pb, Pt, Sb, Tl, U, W
ICP-MS Method B at NYSDOH [22]	As, Ba, Be, Co, Cr, Cs, Cu, Mn, Mo, Pb, Pt, Sb, Sn, Tl, U, W

[(a)] Only analytes in Level II were determined.

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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; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.