National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 966

Toxic Metals in Bovine Blood

This Standard Reference Material (SRM) is intended for use in evaluating the accuracy of lead, cadmium, total mercury, inorganic mercury, and methylmercury concentration determinations in whole blood. It can also be used for validating analytical methods and for providing traceability to working or secondary blood reference materials containing these constituents. SRM 966 consists of frozen whole bovine blood with the aforementioned components at two concentration levels: a base level and an elevated level that contains spiked cadmium, mercury, and methylmercury. A unit consists of two vials of each level; each vial contains approximately 2 mL of whole blood.

Certified Concentration Values and Uncertainties: Certified values for lead, cadmium, and total mercury concentrations in Levels 1 and 2 are provided in Tables 1 and 2, respectively. 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 are based on measurements using two or more independent analytical methods or a single NIST primary method. Analytical methods used for the characterization of this SRM are given in Table 3. All values are reported in concentration units of micrograms per liter except for lead, which has units of micrograms per deciliter. The expanded uncertainties are 95 % confidence intervals and reflect the combined effects of measurement uncertainty, blanks, and any systematic differences between techniques when more than one method has been used [2].

The certified values for lead and total mercury concentrations in both levels were determined by primary methods, isotope dilution inductively coupled plasma mass spectrometry (ID-ICPMS) and isotope dilution cold-vapor inductively coupled plasma mass spectrometry (ID-CV-ICPMS), respectively. The certified value for the cadmium concentration in Level 1 was also determined by ID-ICPMS while the certified value for cadmium in the Level 2 was the average of measurements from ID-ICPMS and radiochemical neutron activation analysis (RNAA). The certified values for lead, cadmium, and total mercury were also confirmed by measurements made by graphite furnace atomic absorption spectrometry (GFAAS) and flow injection atomic absorption spectrometry (FIAAS) at the Centers for Disease Control and Prevention (CDC, Atlanta, GA) [3,4]. The analytical methods used in the value assignment for each analyte are listed in Table 3.

Reference Concentration Value and Uncertainty: Reference values for constitutents in Level 2 are provided in Table 2. Reference values for the inorganic mercury (defined as mercury not in the form of alkyl mercury) and methylmercury concentrations in Level 2 were obtained by extraction with selective reduction ID-CV-ICPMS and solid-phase microextraction gas chromatography inductively coupled plasma mass spectrometry (SPME-GC-ICPMS) using the method of standard additions, respectively. Reference values are noncertified values that represent a best estimate of the true value; however, all known or suspected sources of bias have not been fully investigated at NIST [1]. The uncertainty for the reference concentration of inorganic mercury is reported as an expanded uncertainty at the 95 % confidence limit and reflects the combined effects of measurement uncertainty and variability in concentrations between vials.

Expiration of Certification: The certification of SRM 966 is valid, within the measurement uncertainty specified, until **31 December 2015**, provided the SRM is handled in accordance with instructions given in this certificate (see "Instructions for Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Stephen A. Wise, Chief
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Gaithersburg, MD 20899
Certificate Issue Date: 26 March 2009
See Certificate Revision History on Last Page

Robert L. Watters, Jr., Chief
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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 overall direction and coordination of the technical measurements leading to the certification of this SRM were carried out by R.D. Vocke, Jr. of the NIST Analytical Chemistry Division.

Analyses were performed by S.J. Christopher, W.C. Davis, S.E. Long, E.A. Mackey, K.E. Murphy, M.S. Rearick, and R.D. Vocke, Jr. of the NIST Analytical Chemistry Division and M. Chaudhary-Webb, H.P. Chen, and D.C. Paschal of the CDC (Atlanta, GA).

The experimental design and statistical analysis were provided by W.F. Guthrie, W.S. Liggett, and M.G. Vangel of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

NOTICE AND WARNING TO USERS

Note: This material is intended for "in vitro" diagnostic use only. It was derived from whole bovine blood collected at a USDA-licensed establishment. The supplier of this material has reported that this material was produced under sanitary conditions and was derived from clinically healthy animals

Storage: This SRM should be kept in its original vials and stored frozen at or below −20 °C. The vials should be stored in their original box and aluminized bag. Frost-free freezers should not be employed for storage because of temperature fluctuations.

INSTRUCTIONS FOR USE

The frozen blood in the vial should be allowed to thaw at room temperature (22 °C) before use. The vial should then be mixed by gently rolling, **NOT** shaking, to remix any water that might have separated on freezing. Shaking will cause unwanted bubbles to form at the top of the sample. **DO NOT** use if the blood is clotted. The contents of a vial may be refrozen after having been thawed and a sample withdrawn. Due to possible evaporative losses, it is advisable to discard this SRM if less than one-third of the original blood volume remains (< 0.6 mL).

SOURCE, PREPARATION, AND ANALYSIS[1]

Source of Material: This SRM was prepared in collaboration with the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health of the CDC under the direction of E.J. Sampson, D.T. Miller, and D.C. Paschal. The bovine blood, obtained from a University of Wisconsin facility, came from animals that were bled after oral dosing with gelatin capsules containing lead nitrate.

Preparation of Material: At the CDC, the collected blood was analyzed for lead by GFAAS [4] and pooled to give the base and elevated levels of endogenous lead. The pools were then treated with tripotassium ethylenediaminetetraacetic acid (EDTA) at a concentration of 1.5 mg/mL as an anticoagulant. The Level 2 pool was also spiked with methylmercury iodide, inorganic mercury, and inorganic cadmium. The two levels were then dispensed into 3 mL polyethylene vials. Homogeneity was assessed by analysis of every 200th vial in sequence at each level using standard CDC analytical methods.

Analytical Methods: At NIST, up to 10 randomly selected vials from each level were analyzed by each method together with controls. The entire content of a vial was weighed and analyzed. The results on a per-mass basis were converted to a per-volume basis using the experimentally determined density of the material: Level 1 – 1.0504 g/mL; Level 2 – 1.0544 g/mL. The element-specific analytical methods used in the value assignment of constituents in this SRM are listed in Table 3.

[1]Certain commercial equipment, instruments, or materials are identified in this certificate in order to specify adequately 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.

Table 1. Concentration Values for SRM 966 Toxic Metals in Bovine Blood, Level 1

Element	Certified Values [a]
Lead	1.459 µg/dL ± 0.013 µg/dL
Cadmium	0.032 µg/L ± 0.018 µg/L
Mercury (Total)	0.0445 µg/L ± 0.0033 µg/L

[a] The uncertainties in the certified values are expressed as expanded uncertainties, *U*, at the 95% level of confidence, and are calculated according to the method described in the ISO Guide [2]. The expanded uncertainties are calculated as $U = ku_c$ where u_c is intended to represent, at the level of one standard deviation, the effect of within-technique components of uncertainty. The coverage factor, *k*, for each analyte is determined from the Student's *t*-distribution corresponding to the appropriate associated degrees of freedom and 95 % level of confidence is 2.00, 2.78, and 2.12 for lead, cadmium, and total mercury, respectively.

Table 2. Concentration Values for SRM 966 Toxic Metals in Bovine Blood, Level 2[a]

Element	Certified Values	Reference Values
Lead	25.27 µg/dL ± 0.22 µg/dL	
Cadmium	5.22 µg/L ± 0.16 µg/L	
Mercury (Total)	31.4 µg/L ± 1.7 µg/L	
Mercury (Inorganic)		14.87 µg/L ± 0.93 µg/L
Mercury (Methlymercury)		16.4 µg/L ± 1.4 µg/L

[a] The uncertainties in the certified values and the reference value are expressed as expanded uncertainties, *U*, at the 95 % level of confidence, and are calculated according to the method described in the ISO Guide [2]. The expanded uncertainties are calculated as $U = ku_c$ where u_c is intended to represent, at the level of one standard deviation, the combined effects of between-technique and within-technique components of uncertainty when two or more independent techniques are used for value assignment. The coverage factor, *k*, for each analyte is determined from the Student's *t*-distribution corresponding to the appropriate associated degrees of freedom and 95 % level of confidence and is 2.20, 2.00, 2.78 , 2.00, and 2.23 for lead, cadmium, total mercury, inorganic mercury, and methylmercury, respectively.

Table 3. Methods Used in the Analysis of SRM 966[(a)]

Cadmium	**Isotope dilution inductively coupled plasma mass spectrometry (ID-ICPMS)** **Radiochemical neutron activation aAnalysis (RNAA)** Graphite furnace atomic absorption spectrometry (GFAAS)
Lead	**Isotope dilution inductively coupled plasma mass spectrometry (ID-ICPMS)** Graphite furnace atomic absorption spectrometry (GFAAS)
Total Mercury	**Isotope dilution cold-vapor inductively coupled plasma mass spectrometry (ID-CV-ICPMS)** Cold-vapor atomic absorption spectrometry (CVAAS) Flow injection atomic absorption spectrometry (FIAAS)
Inorganic Mercury	**Extraction with selective reduction isotope dilution cold-vapor inductively coupled plasma mass spectrometry (ID-CV-ICPMS)** Flow injection atomic absorption sSpectrometry (FIAAS)
Methylmercury	**Solid-phase microextraction gas chromatography inductively coupled plasma mass spectrometry (SPME-GC-ICPMS)**

[(a)] Methods used for value assignment are shown in a bold faced type; methods used to corroborate the assigned values are shown in normal faced type.

REFERENCES

[1] May, W.E., Gills, T.E., Parris, R., Beck, II, C.M., Fassett, J.D., Gettings, R.J., Greenberg, R.R., Guenther, F.R., Kramer, G., MacDonald, B.S., and Wise, S.A., "Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements," NIST Special Publication 260-136, (1999); available at http://ts.nist.gov/MeasurementServices/ReferenceMaterials/upload/SP260-136.PDF.

[2] ISO; *Guide to the Expression of Uncertainty in Measurement*; ISBN 92-67-10188-9, 1st ed., International Organization for Standardization: Geneva, Switzerland (1993); 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://physics.nist.gov/Pubs/.

[3] Chen, H.P.; Paschal, D.C.; Miller, D.T.; Morrow, J.C.; *Determination of Total and Inorganic Mercury in Whole Blood by Online Digestion with Flow Injection*; Atomic Spectroscopy, Vol. 19, pp. 176–179 (1998).

[4] Miller, D.T.; Paschal, D.C.; Gunter, E.W.; Stroud, P.E.; D'Angelo, J.; *Determination of Lead in Blood Using Electrothermal Atomization Atomic Absorption Spectrometry with a L'vov Platform and Matrix Modifier*; Analyst, Vol. 112, pp. 1701–1704 (1987).

Certificate Revision History: 26 March 2009 (This technical revision reports the revision of the certified value and associated uncertainty for lead in Level 1); 21 July 2006 (Editorial changes); 26 January 2006 (This technical revision reports the addition of a certified value for cadmium and total mercury in Level 1, the addition of a reference value for inorganic mercury and methylmercury in Level 2, and an extension of the certification period); 21 January2004 (Editorial changes); 22 December 2000 (Original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-2200; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.