National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 955d

Toxic Metals and Metabolites in Frozen Human Blood

This Standard Reference Material (SRM) is intended for use in the validation of analytical methods for measuring toxic metals in human blood. A unit of SRM 955d consists of six vials of frozen human blood with two vials at each of three concentration levels. Each vial contains nominally 1.6 mL of whole blood.

Certified Mass Concentration Values: Certified values are provided 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 are metrologically traceable to the International System of Units (SI) through the gravimetric and volumetric primary standards and procedures used.

Table 1. Certified Mass Concentration Values for SRM 955d[(a)]

Constituent	Unit	Level 1	Level 2	Level 3
Lead (Pb)[(b,c,d,e)]	µg/dL	1.480 ± 0.026	4.947 ± 0.085	42.13 ± 0.63
Arsenic (As)[(c,d,e,f)]	µg/L	5.31 ± 0.76	277.5 ± 4.8	774 ± 13
Cadmium (Cd)[(b,c,d,e)]	µg/L	0.326 ± 0.010	5.343 ± 0.082	10.50 ± 0.11
Chromium (Cr)[(b,c,d,e)]	µg/L	0.886 ± 0.069	2.012 ± 0.037	42.5 ± 1.3
Cobalt (Co)[(c,d,e,f)]	µg/L	0.384 ± 0.051	1.610 ± 0.057	31.91 ± 0.50
Manganese (Mn)[(c,d,e,f)]	µg/L	48.8 ± 1.3	51.2 ± 1.3	75.9 ± 2.0
Mercury (Hg) (Total)[(c,d,e,g)]	µg/L	1.373 ± 0.081	6.83 ± 0.33	55.3 ± 1.6
Selenium (Se)[(c,e,f)]	µg/L	206.6 ± 9.5	263 ± 16	749 ± 62
Uranium (U)[(c,e,f)]	µg/L	0.0111 ± 0.0013	0.1016 ± 0.0095	1.024 ± 0.065
Ethylmercury (as Hg)[(h,i)]	µg/L	0.342 ± 0.034	0.649 ± 0.028	
Inorganic Mercury (as Hg)[(h,i)]	µg/L	0.405 ± 0.024	2.135 ± 0.043	
Methylmercury (as Hg)[(h,i)]	µg/L	0.626 ± 0.020	3.844 ± 0.077	

[(a)] Values are expressed as $x \pm U_{95\%}(x)$, where x is the certified value and $U_{95\%}(x)$ is the expanded uncertainty of the certified value. The true value of the analyte is believed to lie within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2].
[(b)] Isotope Dilution Inductively Coupled Plasma Mass Spectrometry (ID-ICP-MS) at NIST
[(c)] ICP-MS at Centers for Disease Control and Prevention (CDC)
[(d)] ICP-MS at Mayo Clinic
[(e)] ICP-MS at New York State Department of Health (NYSDOH)
[(f)] ICP-MS at NIST
[(g)] Isotope Dilution Cold Vapor ICP-MS at NIST
[(h)] Isotope Dilution Gas Chromatography (ID-GC) ICP-MS at CDC
[(i)] ID-GC-ICP-MS at CDC by NIST

Expiration of Certification: The certification of SRM 955d is valid, within the measurement uncertainty specified, until **01 February 2030**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 18 December 2020
Certificate Revision History on Last Page

Steven J. Choquette, Director
Office of Reference Materials

Coordination of the technical measurements leading to the certification of this SRM was performed by L.L. Yu of the NIST Chemical Sciences Division and S.E. Long, formerly of NIST.

Coordination of collaborative production and measurement of the SRM at CDC was performed by C.D. Ward, R.L. Jones, and K.L. Caldwell of the Inorganic and Radiation Analytical Toxicology Branch, Division of Laboratory Sciences, National Center for Environmental Health (Atlanta, GA). Coordination of collaborative measurement of the SRM at Mayo Clinic (Rochester, MN) was performed by P. Jannetto. Coordination of collaborative measurement at NYSDOH was performed by P.J. Parsons of the Division of Environmental Health Sciences, Laboratory of Inorganic and Nuclear Chemistry, Wadsworth Center (Albany, NY).

Maintenance of Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before expiration, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Analytical measurements were performed by G.C. Caceres, S.E. Long, K.E. Murphy, C.E. Bryan Sallee, T.W. Vetter, and L.L. Yu of NIST; M.A. Franklin, J. Castro Georgi, J.M. Jarrett, D.M. Jones, Z. Li, Y.L. Sommer, D.S. Tevis, and K. Wallon of CDC; M. Maras, S. Erdahl, C. Gilmer, M. Wermers, and H. Woldeysus of Mayo Clinic; and K.F. Mehigan, M. Morrissette, C.D. Palmer, and P.J. Parsons of NYSDOH.

Statistical analysis of the data was performed by C. Hagwood of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

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

SAFETY: SRM 955d is intended for research use. This is a human source material. Handle this product as a biohazardous material capable of transmitting infectious disease. The supplier has reported that each donor unit of blood used in the preparation of this product was tested by FDA-licensed tests and found to be negative for human immunodeficiency virus (HIV), HIV 1 antigen, hepatitis B surface antigen, and hepatitis C. However, no known test method can offer complete assurance that hepatitis B virus, hepatitis C virus, HIV, or other infectious agents are absent from this material. Accordingly, this human blood-based product should be handled at the Biosafety Level 2 [3].

INSTRUCTIONS FOR STORAGE AND USE

Storage: SRM 955d is shipped frozen (on dry ice) and, upon receipt, must be stored frozen at temperatures below –60 °C in the original packaging until use. The certification does not apply to contents of previously opened material, because the stability of the analytes has not been investigated under such conditions.

Use: SRM 955d 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 0.1 mL should be used for the values provided in this certificate to be valid.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source: This SRM was developed after an appropriate human subjects research determination by NIST.

Preparation: Bags of whole human blood from a commercial vendor were combined in three pre-cleaned 15 L high density polyethylene (HDPE) bottles to form the pools for SRM 955d, Level 1 through Level 3. The mass concentrations of toxic metals and mercury species were adjusted to the target levels by spiking with appropriate amounts of NIST SRM 3100 series single element standard solutions and commercial high purity methylmercury and ethylmercury. The contents were then homogenized with a magnetic stirrer for 24 h prior to dispensing into individual pre-screened polypropylene cryovials.

[(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.

Analysis: Measurements for value assignment of SRM 955d were performed at NIST, CDC, Mayo Clinic, New York State Department of Health, and ETH Zurich (Zurich, Switzerland) using methods listed in Table 1 and Table 3.

Homogeneity: Measurements for homogeneity assessment were made at CDC using methods listed in Table 1. The SRM was determined to be homogeneous based on the statistical analysis of between-vial variances.

Reference Values: Reference density values are provided in Table 2. A reference value is a noncertified value that is the best estimate of the true value based on available data [1]. These values do not meet NIST criteria for certification and are provided with associated uncertainties that may not include all sources of uncertainty.

Table 2. Blood Density Reference Values[(a)] for SRM 955d at 22 ºC

Level 1 (g/mL)	Level 2 (g/mL)	Level 3 (g/mL)
1.052147 ± 0.000088	1.052118 ± 0.000017	1.051285 ± 0.000040

[(a)] The density values are expressed as $x \pm U_{95\%}(x)$, where x is the mean of the measurement replication and $U_{95\%}(x)$ is the expanded uncertainty of the mean value with 95 % confidence. To propagate this uncertainty, treat the reference value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2]. Values are metrologically traceable to the density scale realized by the density meter and procedure used for the measurement.

Information Value: An information value for the mass concentration of thyroglobulin is provided in Table 3. An information value is considered to be of interest to the SRM user, but insufficient information is available to assess the uncertainty associated with the value, or only a limited number of analyses were performed [1]. Information values cannot be used to establish metrological traceability.

Table 3. Information Mass Concentration Value for Thyroglobulin in SRM 955d Level 1[(a)]

Constituent	Concentration (µg/L)
Thyroglobulin	29.9

[(a)] Enzyme-linked Immunosorbent Assay (ELISA) at ETH Zurich [4].

Additional Resource: Full details on the production, analysis, and statistical evaluation of SRM 955d is provided in: NIST Special Publication 260-206, *Certification of Standard Reference Material® 955d Toxic Metals and Metabolites in Frozen Human Blood.* This publication is available free of charge on the SRM web site https://www-s.nist.gov/srmors/view_detail.cfm?srm=955d.

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 (2000); available at https://www.nist.gov/system/files/documents/srm/SP260-136.PDF (accessed Dec 2020).

[2] Possolo, A; *Simple Guide for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1900; U.S. Government Printing Office: Washington, DC (2015); available at https://nvlpubs.nist.gov/nistpubs/TechnicalNotes/NIST.TN.1900.pdf (accessed Dec 2020).

[3] CDC/NIH; *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed.; HHS publication No. (CDC) 21-1112; Chosewood, L.C.; Wilson, D.E., Eds.; US Government Printing Office: Washington, D.C. (2009); available at https://www.cdc.gov/biosafety/publications/bmbl5/index.htm (accessed Dec 2020).

[4] Stinca, S.; Andersson, M.; Erhardt, J.; Zimmermann, M.; *Development and Validation of a New Low-Cost Enzyme-Linked Immunoassay for Serum and Dried Blood Spot Thyroglobulin*, Thyroid, Vol. 25, Issue 12, pp. 1297-1305 (2015).

Certificate Revision History: 18 December 2020 (Editorial changes); **22 July 2020** (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; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.