National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 967a

Creatinine in Frozen Human Serum

This Standard Reference Material (SRM) is intended primarily for use in evaluating the accuracy of procedures for the determination of creatinine in human serum. It is also intended for use in validating working or secondary reference materials. A unit of SRM 967a consists of four stoppered vials of frozen human serum, two vials each at two different creatinine concentration levels. Each vial contains 1.0 mL of human serum.

Certified Concentration Values: The certified concentration values of creatinine 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]. The certified concentration values for each level are based on isotope dilution liquid chromatography/mass spectrometry (ID-LC/MS) at NIST [2]. The concentrations and their uncertainties, expressed in both mmol/L and mg/dL, for the two concentration levels are listed in Table 1. These values were calculated from mass fractions using the measured serum density of 1.02291 g/mL. The certified concentrations apply only to serum thawed to room temperature, 20 °C to 25 °C (see "Instructions for Use").

Table 1. Certified Concentration Values for Creatinine[(a)]

Concentration Levels	mmol/L	mg/dL
Level 1	0.0749 ± 0.0016	0.847 ± 0.018
Level 2	0.3427 ± 0.0072	3.877 ± 0.082

[(a)] Each certified concentration value is the mean of the measurements made using the NIST definitive method for creatinine. The uncertainty in the certified value, calculated according to the method described in the ISO Guide [3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent the standard uncertainty of the mean concentration. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence [3].

Expiration of Certification: The certification of **SRM 967a** is valid, within the measurement uncertainties specified, until **31 December 2014**, provided the SRM is handled and stored in accordance with the instructions given in the certificate (see "Storage" and "Instructions for 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) will facilitate notification.

The overall direction and coordination of the technical activities were performed by K.W. Phinney of the NIST Analytical Chemistry Division.

The analytical measurements were performed by J. Camara of the NIST Analytical Chemistry Division.

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Certificate Issue Date: 23 March 2010
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Measurement Services Division

Design of the sampling protocol and statistical analysis of the data were performed by N.F. Zhang 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 WARNINGS TO USERS

SRM 967a IS INTENDED FOR IN-VITRO DIAGNOSTIC USE ONLY. THIS IS A HUMAN SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. The supplier of this serum has reported that each donor unit of serum or plasma used in the preparation of this product has been tested by an FDA approved method and found non-reactive/negative for hepatitis B surface antigen (HbsAg), human immunodeficiency virus (HIV) 1 and 2 antibodies, hepatitis C virus (HCV), and syphilis. However, no known test method can offer complete assurance that hepatitis B virus, HCV, HIV, or other infectious agents are absent from this material. Accordingly, this human blood-based product should be handled at the Biosafety Level 2 or higher as recommended for any POTENTIALLY INFECTIOUS HUMAN SERUM OR BLOOD SPECIMEN in the Centers for Disease Control/National Institutes of Health Manual [4].

Storage: The serum is shipped frozen (on dry ice) and, upon receipt, should be stored frozen until ready for use. A freezer temperature of –20 °C is acceptable for storage up to one week. If a longer storage time is anticipated, the material should be stored at or below –60 °C. The SRM should not be exposed to sunlight or ultraviolet radiation. Storage of thawed material at room or refrigerator temperatures may result in changes in creatinine concentrations.

Instructions for Use: Vials of the SRM to be analyzed should be removed from the freezer and allowed to stand at room temperature (20 °C to 25 °C) until thawed. After the material is thawed, it should be used immediately. The material should be swirled gently to mix it before aliquots are withdrawn.

SOURCE, PREPARATION, AND ANALYSIS[1]

SRM 967a was prepared by Solomon Park Research Laboratories (Kirkland, WA). Blood was collected from healthy adult males and females, following National Committee for Clinical Laboratory Standards (NCCLS) C-37A guidelines for preparation of commutable frozen serum pools to be used as reference materials. The pool of serum was split into two sub-pools, with one having normal levels of creatinine (Level 1, 0.8 mg/dL ± 0.1 mg/dL), and the other spiked with crystalline creatinine to achieve an elevated level of creatinine (Level 2, 4.0 mg/dL ± 0.2 mg/dL).

Analytical Methods: For the certification of this SRM, a NIST definitive method was used [5]. The method involved isotope dilution/liquid chromatography/mass spectrometry (ID-LC/MS) and is similar to a method [6] developed at the Laboratory of the Government Chemist (LGC) and approved by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) as a higher-order reference measurement procedure [7]. The method involves spiking with labeled creatinine, extraction into ethanol, and analysis using a Luna C18(2) column (Phenomenex, Torrance, CA) with mass spectrometric detection. This method was calibrated using SRM 914a Creatinine.

Homogeneity Analysis: The homogeneity assessment was made at the time the certification analyses were performed. A stratified sampling plan was devised to test for homogeneity across the lot of material received. There was no apparent trend in the data when plotted against the sequence in which the vials were prepared.

[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.

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, U.S. Government Printing Office, Gaithersburg, MD (2000); available at http://ts.nist.gov/MeasurementServices/ReferenceMaterials/PUBLICATIONS.cfm (accessed Mar 2010).

[2] NCCLS Publication NRSCL 1-A; *Development of Definitive Methods for the National Reference System for the Clinical Laboratory Approved Guideline*; National Committee for Clinical Laboratory Standards: Wayne, PA (1991).

[3] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (ISO GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Mar 2010); 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/ (accessed Mar 2010).

[4] CDC/NIH; *Biosafety in Microbiological and Biomedical Laboratories,* 5th ed.; Richardson, J.; Barkley, W.E.; Richmond, J.; McKinney, R.W., Eds.; U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health; US Government Printing Office: Washington, D.C. (2007); available at http://www.cdc.gov/OD/OHS/biosfty/bmbl5/BMBL_5th_Edition.pdf (accessed Mar 2010).

[5] Dodder, N. G.; Tai, S.; Sniegoski, L.T.; Zhang, N. F.; Welch, M.J.; *Certification of Creatinine in a Human Serum Reference Material by GC-MS and LC-MS*; Clin. Chem., Vol. 53, pp 1694–1699 (2007).

[6] Stokes, P.; O'Connor, G.; *Development of a Liquid Chromatograpy-Mass Spectrometry Method for the High-Accuracy Determination of Creatinine in Serum*; J. Chromatogr. B., Vol. 794, pp 125–136 (2003).

[7] Joint Committee for Traceability in Laboratory Medicine; http://www.bipm.org/en/committees/jc/jctlm/ (accessed Mar 2010).

Certificate Revision History: 23 March 2010 (This technical revision reports a correction in Table 1 to the certified concentration value and associated uncertainty in mmol/L of creatinine for concentration Level 1 and Level 2); 22 December 2009 (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.