National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 967

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 967 consists of four stoppered ampoules of frozen human serum, two ampoules each at two different creatinine concentration levels. Each ampoule contains 1.0 mL of human serum.

Certified Concentration Values: The certified concentrations of creatinine were determined using methods based upon isotope dilution gas chromatography/mass spectrometry (ID-GC/MS) and isotope dilution liquid chromatography/mass spectrometry (ID-LC/MS) at NIST. The concentrations and their uncertainties, expressed in both mmol/L and mg/dL, for the two concentration levels are listed in Table 1. The certified concentrations apply only to serum thawed to room temperature, 20 °C to 25 °C (see "Instructions for Use").

Table 1. Certified Concentrations and Uncertainties for Creatinine

Concentration Levels	mmol/L	mg/dL
Level 1	0.0665 ± 0.0019	0.753 ± 0.021
Level 2	0.3462 ± 0.0073	3.916 ± 0.083

The uncertainties in the certified values are calculated as $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the ISO Guide and NIST Guidelines [1], and k is a coverage factor. The values of u_c are intended to represent, at the level of one standard deviation, the uncertainties in mean concentration. The expanded uncertainty, $U = ku_c$, is defined as an interval estimated to have a level of confidence of at least 95 %. The effective degrees of freedom (ν_{eff}) is very large for each of the four levels, thus, $k = 2$.

Expiration of Certification: The certification of SRM 967 is valid, within the measurement uncertainties specified, until **31 December 2011**, provided the SRM is handled and stored in accordance with the instructions given in the certificate. However, the certification is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive 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 analytical measurements were performed by N.G. Dodder, L.T. Sniegoski, S.S-C. Tai, and M.J. Welch of the NIST Analytical Chemistry Division.

Design of the sampling protocol and statistical analysis of the data were performed by N.F. Zhang of the NIST Statistical Engineering Division.

The overall direction and coordination of the technical activities were under the chairmanship of M.J. Welch of the NIST Analytical Chemistry Division.

Stephen A. Wise, Chief
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Certificate Issue Date: 24 January 2007

Robert L. Watters, Jr., Chief
Measurement Services Division

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

NOTICE AND WARNINGS TO USERS

SRM 967 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 HbsAg, HIV-1 & 2 antibodies, HCV and syphilis. 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 or higher as recommended for any POTENTIALLY INFECTIOUS HUMAN SERUM OR BLOOD SPECIMEN in the Centers for Disease Control/National Institutes of Health Manual [2].

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.

Stability: The material is kept at –80 °C for long term storage at NIST. Under these conditions, the creatinine is expected to be stable. NIST will continue to monitor the stability of creatinine in this material and will notify purchasers of the material of any changes in the certified concentrations.

Instructions for Use: Ampoules 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 967 was prepared by Solomon Park Research Laboratories, Kirkland WA. Blood was collected from healthy post-menopausal adult females following National Committee for Clinical Laboratory Standards (NCCLS) 37A guidelines. This pool of approximately 3 L was split into Master Pool A (not spiked with additional creatinine) and Master Pool B (which was spiked with an appropriate amount of reagent grade creatinine to generate a level of approximately 4 mg/dL). Serum was filtered through a 0.2 micron filter. No preservatives were added. The serum was aliquoted into 1 mL aliquots in 3 mL amber vials, stoppered with Teflon stoppers, and capped with aluminum seals. The pH, TG, TP, ALB, A/G Ratio, glucose, bilirubin, and clotting element test of each pool were recorded. Homogeneity runs were performed and both pools passed using ANalysis Of VAriance (ANOVA) calculations.

Analytical Methods: Two independent methods were used for the certification of this SRM. The first method involved isotope dilution-gas chromatography/mass spectrometry (ID-GC/MS) and involves converting creatinine into the ethyl ester of N-(4,6-dimethyl-2-pyrimidinyl)-N-methylglycine [3]. The method is considered to be a "definitive" method for serum creatinine by the NCCLS [4] and is an approved higher order reference measurement procedure according to the Joint Committee on Traceability in Laboratory Medicine (JCTLM) [5]. The second 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 JCTLM as a higher order reference measurement procedure. Both methods were 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 manufacturing process. The results indicated that there was no apparent trend in the data when plotted against the sequence in which the ampoules were prepared.

[1] Any mention of commercial products in this certificate is for information only. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology.

Commutability Validation: A commutability validation was organized by the Laboratory Working Group of the National Kidney Disease Education Program. The study design followed the protocol recommended in Clinical and Laboratory Standards Institute (CLSI) document EP14-A2 [7]. Briefly, creatinine was measured in the SRM and 20 individual patients' serum samples using a routine laboratory measurement procedure and using the NIST LC/IDMS measurement procedure. The numeric relationship between results for the patients' serum samples was established by regression of results from each routine procedure on the reference procedure. The SRM was considered commutable with native patients'samples for a given routine procedure if the SRM results were within the 95 % prediction interval from the regression relationship as defined in CLSI EP14-A2. For a list of the procedures for which the SRM proved to be commutable, please see http://www.nkdep.nih.gov/. There were no procedures tested for which the SRM was found to be noncommutable according to the EP14-A2 definition.

REFERENCES

[1] ISO; G*uide 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/.

[2] CDC/NIH; *Biosafety in Microbiological and Biomedical Laboratories*, 2nd ed.; DHHA Publication No. (CDC) 88-8395; U.S. Department of Health and Human Services, Public Health Service; U.S. Government Printing Office: Washington, DC (1988).

[3] Welch, M.J.; Cohen, A.; Hertz, H.S.; Ng, K.J.; Schaffer, R.; Van Der Lijn, P.; White V, E.; *Determination of Serum Creatinine by Isotope Dilution Mass Spectrometry as a Candidate Definitive Method*; Anal. Chem., Vol. 58, pp. 1681–1685 (1986).

[4] CLSI/NCCLS NRSCL 1-A; *Development of Definitive Methods for the National Reference System for the Clinical Laboratory; Approved Guideline*; National Committee for Clinical Laboratory Standards (1991).

[5] JCTLM Web. http://www.bipm.org/utils/en/xls/jctlm_listI.xls (accessed Dec. 2006).

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

[7] CLSI EP14-A2; *Evaluation of Matrix Effects: Approved Guideline – 2nd Edition*; ISBN 1-56238-561-5; Clinical and Laboratory Standards Institute, Document EP14-A2, Vol. 25, No. 4 (2005).

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) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.