National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 971

Hormones in Frozen Human Serum

This Standard Reference Material (SRM) is intended primarily for use in evaluating the accuracy of procedures for the determination of the steroid hormones cortisol, progesterone, and testosterone in human serum. It is also intended for use in validating working or secondary reference materials. A unit of SRM 971 consists of two materials: one from a pool of healthy, premenopausal adult females and one from a pool of healthy adult males. Both materials are unfortified. Each vial contains 5.0 mL of human serum.

Certified Concentration Values: The certified concentrations of cortisol (female and male sera), testosterone (female and male sera), and progesterone (female serum only) and their uncertainties are listed in Table 1. Values were determined using results from NIST reference methods based upon isotope dilution liquid chromatography tandem mass spectrometry (ID-LC/MS/MS) [1-3], from the Physikalisch-Technische Bundesanstalt (PTB) in Braunschweig, Germany (cortisol and progesterone) using isotope dilution gas chromatography/mass spectrometry (ID-GC/MS), and from LGC Limited in Teddington, UK using ID-LC/MS/MS (cortisol only). 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 [4]. The NIST methods have been listed in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database as reference measurement procedures of a higher order [5]. The certified concentrations apply only to serum thawed to room temperature, 20 °C to 25 °C (see "Instructions for Handling, Storage, and Use").

Reference Concentration Value: The reference concentration value for progesterone in the male serum is listed in Table 2. The reference concentration of progesterone in the male serum was determined from measurements at NIST using the ID-LC/MS/MS-based reference method [2]. Reference values are noncertified 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 [4] and are provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Expiration of Certification: The certification of **SRM 971** is valid, within the measurement uncertainty specified, until **01 January 2015**, provided the SRM is handled and stored in accordance with the 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) will facilitate notification.

Coordination of the technical measurements leading to the certification of this SRM was performed by K.W. Phinney and M.J. Welch of the NIST Analytical Chemistry Division.

Analytical measurements at NIST were performed by S.S-C. Tai of the NIST Analytical Chemistry Division. Additional measurements were performed at PTB by C. Gollub, K. Schild, R. Ohlendorf, and A. Henrion, and at LGC Limited by C. Mussell and G. O'Connor.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 29 June 2011
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Robert L. Watters, Jr., Chief
Measurement Services Division

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

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

NOTICE AND WARNINGS TO USERS

SRM 971 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 FDA-licensed methods and found non-reactive/negative for hepatitis B surface antigen, hepatitis C virus, human immunodeficiency virus (HIV), and human immunodeficiency virus antigen 1. 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 and Prevention/National Institutes of Health Manual [6].

INSTRUCTIONS FOR HANDLING, STORAGE, AND 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.

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 for up to one week. If a longer storage time is anticipated, the material should be stored at or below −50 °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 the hormone concentrations.

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

SOURCE, PREPARATION AND ANALYSIS

Source of Material[(1)]: SRM 971 was prepared by Bioreclamation Inc. (Hicksville, NY). Off-clot serum was collected from healthy, adult donors, ages 21 years to 40 years (males) and 21 years to 39 years (premenopausal females), who were not taking prescription medications. The serum was processed according to Clinical Laboratory Standards Institute (CLSI) Publication C37-A [7] to reduce matrix effects.

Determination of Cortisol: The NIST reference method [1] for cortisol involves spiking the serum with cortisol-d_3, acidifying the sample, putting the sample through a solid-phase extraction cartridge (C_{18}), performing a liquid-liquid extraction, drying the sample, and reconstituting in 1 mL/L acetic acid in water-methanol (59 : 41 by volume). LC/MS/MS was performed using a C_{18} column and monitoring two transitions each for the unlabeled and labeled forms: *m/z* 363 → *m/z* 327 and *m/z* 363 → *m/z* 121 (unlabeled), and *m/z* 366 → *m/z* 330 and *m/z* 366 → *m/z* 121 (labeled). Calibration of the measurements was carried out by using SRM 921. The PTB method for cortisol involved spiking the serum with cortisol-d_4, a liquid-liquid extraction, followed by a cleanup step using liquid chromatography. The cortisol was then derivatized in a two-step process, prior to analysis by GC/MS, monitoring *m/z* 605 and *m/z* 609. The LGC method involved spiking the serum with cortisol-d_3, a liquid-liquid extraction, and LC/MS/MS analysis using the transitions at *m/z* 363 → *m/z* 121 and *m/z* 366 → *m/z* 121 for the unlabeled and labeled forms, respectively [8].

Determination of Progesterone: The NIST reference method for progesterone [2] involves spiking the serum with progesterone-$^{13}C_2$, a liquid-liquid extraction, followed by analysis using LC/MS/MS with a C_{18} column and using the transitions at *m/z* 315 → *m/z* 97 and *m/z* 317 → *m/z* 99 for the unlabeled and labeled forms, respectively. The PTB method involves spiking the serum with progesterone-$^{13}C_2$, two liquid-liquid extractions, followed by derivatization. GC/MS measurements were performed using *m/z* 510 and *m/z* 512, for the unlabeled and labeled forms, respectively [9].

[(1)] Certain commercial equipment, instruments, or materials are identified in this certificate 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.

Determination of Testosterone: The NIST reference method for testosterone [3] involves spiking the serum with testosterone-d_3, acidifying the sample, putting the sample through a solid-phase extraction cartridge (C_{18}), performing a liquid-liquid extraction, drying the sample, and reconstituting in methanol containing 0.5 mL/L acetic acid. Analysis by LC/MS/MS was performed by monitoring the transitions at *m/z* 289 → *m/z* 97 and *m/z* 292 → *m/z* 97 for the unlabeled and labeled forms, respectively.

Homogeneity Analysis: Homogeneity was assessed at the time the certification analyses were performed. A stratified sampling plan was devised to test for homogeneity across the entire lot. No appreciable vial-to-vial differences were detected.

Commutability: The commutability of this material for routine clinical methods has not been evaluated. Plans are underway to conduct such a study and the certificate will be updated as appropriate. Based upon previous experience with reference materials for other analytes prepared following the CLSI C37a protocol, this material should be commutable on most clinical assays.

Table 1. Certified Concentrations in SRM 971 Hormones in Human Serum

	Female		Male	
	(nmol/L)[(a)]	(ng/g)	(nmol/L)[(a)]	(ng/g)
Cortisol[(b)]	250.1 ± 5.8	88.5 ± 2.1	296.8 ± 4.1	105.0 ± 1.5
Progesterone[(b)]	6.20 ± 0.22	1.903 ± 0.068		
Testosterone[(c)]	0.961 ± 0.022	0.271 ± 0.006	22.31 ± 0.51	6.279 ± 0.143

(a) Molar concentrations were calculated from the mass fractions using the relative molecular masses for each compound and the measured serum densities for the two serum pools, which were 1.0241 g/mL (female) and 1.0247 g/mL (male).

(b) 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 [10], using a random effect model for combining results from multiple methods [11], and $k = 2$ is the 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 %.

(c) 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 [10], and $k = 2$ is the 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 %.

Table 2. Reference Concentration for Progesterone in the Male Serum[(a)]

Material	(nmol/L)[(b)]	(ng/g)
Male	0.131 ± 0.020	0.0403 ± 0.0062

(a) The uncertainty in the reference value is calculated as $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the ISO Guide [10], and $k = 2$ is the 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 %.

(b) The molar concentration was calculated from the mass fraction using the relative molecular mass of progesterone and the measured serum density (1.0247 g/mL).

REFERENCES

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Certificate Revision History: 29 June 2011 (Addition of certified values for testosterone; update of certified and reference values for cortisol and progesterone; extension of certification period; editorial changes); **19 December 2008** (Original certificate issue 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; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.