National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 965b

Glucose in Frozen Human Serum

This Standard Reference Material (SRM) is intended primarily for use in evaluating the accuracy of procedures for the determination of glucose in human serum. It is also intended for use in validating working or secondary reference materials. Because it is made from pools of human serum that have been modified to achieve the target concentrations, this material may not be commutable with natural human serum in all routine glucose measurement procedures. However, NIST is unaware of any commutability problems with the previous lots of this frozen serum material, which were similarly prepared. A unit of SRM 965b consists of eight flame-sealed ampoules of frozen human serum, two ampoules at each of four different glucose concentration levels. Each ampoule contains 2.00 mL ± 0.04 mL of human serum.

Certified Concentration Values: The certified concentration values of glucose 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 the NIST definitive isotope dilution gas chromatography – mass spectrometry (ID/GC/MS) method for glucose [2,3]. The concentrations and their expanded uncertainties for the four 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 Concentration Values for Glucose in SRM 965b[(a)]

Concentration Levels	mmol/L	mg/dL
Level 1	1.836 ± 0.027	33.08 ± 0.48
Level 2	4.194 ± 0.059	75.56 ± 1.06
Level 3	6.575 ± 0.094	118.5 ± 1.7
Level 4	16.35 ± 0.20	294.5 ± 3.6

[(a)] Each certified concentration value is the mean of the measurements made using the NIST definitive method for glucose. The uncertainty in the certified value, calculated according to the method described in the ISO Guide [4], 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 an approximately 95 % confidence [4].

Expiration of Certification: The certification of **SRM 965b** is valid, within the measurement uncertainty specified, until **31 December 2014**, 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.

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 leading to certification of this material were performed by K.W. Phinney of the NIST Analytical Chemistry Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

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Certificate Issue Date: 01 December 2009

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

The analytical measurements were performed by J.L. Prendergast, L.T. Sniegoski 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.

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

NOTICE AND WARNINGS TO USERS

SRM 965b 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 (HIV) 1 and 2 antibodies, and hepatitis C virus (HCV). 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 [5].

Storage: The SRM is stored at –80 °C at NIST. 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 glucose 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. **NOTE THAT AMPOULES ARE NOT PRESCORED**. To open, wear protective gloves to avoid injury, score the neck of the ampoule with a file or other suitable device, and snap open.

SOURCE, PREPARATION, AND ANALYSIS[1]

SRM 965b was prepared by Aalto Scientific, Carlsbad, CA. The material was prepared from normal human serum and its appearance is a clear amber solution free of particulate matter.

Analytical Methods: For the certification of this SRM, the NIST definitive method, ID/GC/MS, was used. This method involves the conversion of glucose into a dibutylboronate acetate derivative. The method is considered to be a definitive method [2] for serum glucose by the National Committee for Clinical Laboratory Standards (NCCLS) [3] and is approved as a higher-order reference measurement procedure by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) [6].

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 ampoules. There was no apparent trend in the data when plotted against the sequence in which the ampoules 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://www.cstl.nist.gov/nist839/NIST_special_publications.htm.

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

[3] White V, E.; Welch, M.J.; Sun, T.; Sniegoski, L.T.; Schaffer, R.; Hertz, H.S.; Cohen, A.; *The Accurate Determination of Serum Glucose by Isotope Dilution Mass Spectrometry - Two Methods*; Biomed. Mass Spectrom.; Vol. 9, pp. 395-405 (1982).

[4] 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; 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/.

[5] 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.

[6] *Joint Committee for Traceability in Laboratory Medicine*; http://www.bipm.org/en/committees/jc/jctlm/

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.