



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material[®] 965a

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 for all routine glucose measurement procedures. However, NIST is unaware of any commutability problems with the previous lot of this frozen serum material. A unit of SRM 965a 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 ± 0.04 mL of human serum.

Certified Concentration Values: The certified concentrations of glucose were determined using the NIST definitive method for glucose [1,2]. The concentrations and their uncertainties, expressed in both mmol/L and mg/dL, 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 Concentrations and Uncertainties for Glucose

Concentration Levels	mmol/L	mg/dL
Level 1	1.918 ± 0.020	34.56 ± 0.36
Level 2	4.357 ± 0.048	78.50 ± 0.86
Level 3	6.777 ± 0.073	122.1 ± 1.3
Level 4	16.24 ± 0.19	292.6 ± 3.5

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 [3], 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}) are very large for each of the four levels, thus, $k = 2$.

Expiration of Certification: The certification of this SRM is valid until **31 December 2008**, within the measurement uncertainties specified, 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.

The analytical measurements were performed by L.T. Sniegowski and M.J. Welch of the NIST Analytical Chemistry Division.

The overall direction and coordination of the technical activities were under the chairmanship of 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.

Willie E. May, Chief
Analytical Chemistry Division

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Certificate Issue Date: 20 February 2004

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The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the Standard Reference Materials Program by J.C. Colbert and B.S. MacDonald of the NIST Measurement Services Division.

NOTICE AND WARNINGS TO USERS

SRM 965a 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 [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 -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.

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

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 ANALYSIS¹

SRM 965a was prepared by Euro-trol b.v., Wageningen, The Netherlands. The material was prepared from normal human serum and its appearance is a clear amber solution free of particulate matter. Donor units were collected and allowed to clot for a minimum of 2 h at room temperature using no additives to assist in the clot process. The serum pool was frozen at -20 °C, thawed, and filtered through an Avicel Cellulose slurry under vacuum to remove fibrin. Gentamicin sulfate was added as an antibacterial agent. The appropriate amounts of dextrose monohydrate were added to the four subpools to adjust the concentrations of glucose to the desired levels. The pH of each subpool was adjusted to 7.5 at 37 °C and filtered through a pre-sterilized 0.22 µm filter. Finally, 2.0 mL aliquots of each subpool were dispensed into 4.5 mL Duran glass ampoules, flame-sealed, and stored at -50 °C.

Analytical Methods: For the certification of this SRM, the method used was isotope dilution/gas chromatography/mass spectrometry (ID/GC/MS) and involves converting glucose into a dibutylboronate acetate derivative. The method is considered to be a *definitive* method [1] for serum glucose by the National Committee for Clinical Laboratory Standards (NCCLS) [2] and is an approved higher order reference measurement procedure according to the Joint Committee on Traceability in Laboratory Medicine (JCTLM) [5].

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.

¹Certain commercial equipment, instruments, or materials are identified in this certificate in order 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.

REFERENCES

- [1] *Development of Definitive Methods for the National Reference System for the Clinical Laboratory, Approved Guideline*, NCCLS Publication NRSLC 1-A; National Committee for Clinical Laboratory Standards: Wayne, PA (1991).
- [2] White V.E.; Welch, M.J.; Sun, T.; Sniegowski, 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).
- [3] *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed., ISO, Geneva, Switzerland, (1993): see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing Uncertainty of NIST Measurement Results, NIST Technical Note 1297*; U.S. Government Printing Office: Washington, DC (1994).
- [4] *Biosafety in Microbiological and Biomedical Laboratories*; U.S. Department of Health and Human Services; U.S. Government Printing Office: Washington, DC (1988).
- [5] *Joint Committee on Traceability in Laboratory Medicine*; <http://www.bipm.fr/en/committees/jc/jctlm/> (accessed 20 January 2004).

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>.