National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 965

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. A unit of SRM 965 consists of six flame sealed ampules of frozen human serum, two ampules at each of three 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 three 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 I	5.680 ± 0.046	102.33 ± 0.84
Level II	11.097 ± 0.196	199.93 ± 3.54
Level III	16.355 ± 0.184	294.65 ± 3.32

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 %. For users to propagate the uncertainty of calibration when SRM 965 is used as a calibrant, the combined standard uncertainty for each level, u_c, and its associated degrees freedom, ν_{eff}, are listed in Table 2. Since $\nu_{eff} = 2$ for each of the three levels, the coverage factor is the 97.5 percentile of the Student t-distibution with 2 degrees of freedom for each level; that is, $k = 4.303$.

The analytical measurements were performed in the NIST Analytical Chemistry Division by L.T. Sniegoski and P. Ellerbe (College of American Pathologists Research Associate).

Design of the sampling protocol and statistical analysis of the data were performed in the NIST Statistical Engineering Division by S.B. Schiller and M. Vangel.

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

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.

Gaithersburg, MD 20899
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Table 2. Combined Standard Uncertainties and Effective Degrees of Freedom

	Level I		Level II		Level III	
	u_c	ν_{eff}	u_c	ν_{eff}	u_c	ν_{eff}
mmol/L	0.011	2	0.046	2	0.043	2
mg/dL	0.19	2	0.82	2	0.77	2

NOTICE AND WARNINGS TO USERS

SRM 965 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.

Expiration of Certification: The certification of this SRM is valid until the date stamped on the exterior package label, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given on the certificate (see Storage and Instructions for Use). **Return of the attached registration card will facilitate notification.**

Instructions for Use: Ampules 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

SRM 965 was prepared by the Diagnostics Group, Bayer Corporation, Middletown, VA. The material was prepared from normal human serum and its appearance is a clear amber solution free of particulate matter. Donor units were collected, allowed to clot for a minimum of two hours 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 Level I and Level III subpools to adjust the concentrations of glucose to the desired levels. The Level II subpool was made from appropriate volumes of the Level I and Level III subpools and blended. The pH of each subpool was adjusted to 7.7 at 25 °C and filtered through a pre-sterilized 0.22 μm filter. Finally, 2.0 mL aliquots of each subpool were dispensed into 6.0 mL cryogenic glass ampules, 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 and involves converting glucose into a dibutylboronate acetate derivative. The method is considered to be "definitive" [1] for serum glucose by the National Committee for Clinical Laboratory Standards (NCCLS) [2].

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 ampules were prepared.

REFERENCES

[1] "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).

[2] White V, E., Welch, M.J., Sun, T., Sniegoski, L.T., Schaffer, R., Hertz, H.S., and Cohen, A., The Accurate Determination of Serum Glucose by Isotope Dilution Mass Spectrometry - Two Methods, Biomed. Mass Spectrom., **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. and Kuyatt, C.E., "Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results," NIST Technical Note 1297, U.S. Government Printing Office, Washington, D.C., (1994).

[4] U.S. Department of Health and Human Services, "Biosafety in Microbiological and Biomedical Laboratories," U.S. Government Printing Office, Washington, D.C., (1988).