



Certificate of Analysis

Standard Reference Material[®] 970

Ascorbic Acid in Frozen Human Serum

This Standard Reference Material (SRM) is intended primarily for use in validating methods for determining ascorbic acid in human serum and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 970 consists of four ampoules of frozen human serum, two ampoules each of Level I (high normal) and Level II (low normal). Each ampoule contains approximately 2.2 mL of solution, a 1:1 mixture of human serum and 100 g/L (10 % mass concentration) aqueous metaphosphoric acid (MPA). The MPA is present to stabilize and preserve the ascorbic acid.

Certified Concentration Values: Certified concentration values of total ascorbic acid (TAA), the combination of ascorbic acid and dehydroascorbic acid, 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 values combine results of a liquid chromatographic (LC) assay performed at NIST with summary results from a series of interlaboratory studies coordinated by NIST. Values are reported as the concentration of TAA in the 1:1 solutions of human serum and 100 g/L (10 % mass concentration) MPA.

Expiration of Certification: The certification of **SRM 970** is valid, within the measurement uncertainty specified, until **30 June 2019**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for 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 re-certification of this SRM was performed by J. Brown Thomas and S.A. Wise of the NIST Chemical Sciences Division.

The SRM 970 materials were prepared by S.A. Margolis formerly of the NIST Chemical Sciences Division. Analytical measurements at NIST were performed by J. Brown Thomas and S.A. Margolis. Analyses used for value assignment were also performed by the laboratories (see Appendix A) that participated in the NIST Micronutrients Measurement Quality Assurance Program coordinated by J. Brown Thomas.

Experimental design and data evaluation were provided by D.L. Duewer of the NIST Chemical Sciences Division and W.F. Guthrie of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

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NOTICE AND WARNING TO USERS

SRM 970 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 the source materials used to prepare this product found the materials to be non-reactive when tested for Hepatitis B surface antigen (HBsAg), human immunodeficiency virus (HIV), Hepatitis C virus (HCV), and human immunodeficiency virus 1 antigen (HIV-1Ag) by Food and Drug Administration (FDA) licensed tests. However, because no test method can offer complete assurance that HIV, hepatitis viruses, or other infectious agents are absent, this SRM should be handled at the Biosafety Level 2 for any potentially infectious human serum or blood specimen [2].

INSTRUCTIONS FOR STORAGE AND USE

Stability and Storage: The ampoules are sealed under dry nitrogen and should be stored at or below $-50\text{ }^{\circ}\text{C}$ until ready for use. Stability studies at NIST indicate that TAA degradation in MPA-preserved serum or plasma is slow (less than 1 % per year) under storage at $-70\text{ }^{\circ}\text{C}$ [3]. However, the material may rapidly degrade when in contact with contaminants, such as transition metals, present in even trace amounts on the inner surface of containers used in the measurement process [4].

Use: Remove the ampoule from the freezer, defrost the material for 10 min in a water bath at $20\text{ }^{\circ}\text{C}$, and then store at refrigerator temperatures from $4\text{ }^{\circ}\text{C}$ to $8\text{ }^{\circ}\text{C}$ until ready for use, preferably within 2 h [5].

PREPARATION AND ANALYSIS

Preparation: In 1998, fourteen units of human serum were combined into two separate lots and placed into 5-liter bottles. An equal volume of 100 g/L (10 % mass concentration) aqueous MPA was added to each lot of serum with constant stirring. A known amount of ascorbic acid in 50 g/L (5 % mass concentration) aqueous MPA was added to each lot solution at $4\text{ }^{\circ}\text{C}$ and stirred for 30 min. Each lot of solution was dispensed in 2.2 mL aliquots into washed and dried, nitrogen-filled ampoules which were subsequently flame sealed and stored at $-70\text{ }^{\circ}\text{C}$. The TAA concentrations of the SRM 970 Level I and II materials were designed to be approximately equal to the high and low normal quintiles of the distribution of serum ascorbic acid found by the NHANES III study [6]. See reference 7 for further details.

NIST Certification Analyses: SRM 970 was originally certified in 2000 on the basis of NIST-performed gravimetric preparation and LC analyses [7]. The SRM 970 units were stored at $-70\text{ }^{\circ}\text{C}$ at NIST in two separate facilities. In 2003, two ampoules of each level from each storage facility were analyzed in triplicate for TAA using LC [8]. The TAA concentration in both of the SRM 970 materials was confirmed to have decreased from the originally certified values. Within the measurement performance characteristics of the LC method, there was no evidence for TAA heterogeneity in the Level I material. However, statistically significant TAA heterogeneity was observed in the Level II material.

Micronutrients Measurement Quality Assurance Program Interlaboratory Studies: Both SRM 970 Level I and Level II have been evaluated in eight interlaboratory studies conducted from the fall of 1998 through the spring of 2004. Excluding NIST, a total of 31 laboratories participated in these studies. An average of 15 laboratories participated in each of these studies. All participating laboratories are listed in Appendix A. Most participants have used some form of LC analysis; see reference 7 for a detailed description of the methods used in the initial two studies. Due to the presence of a minority population of analytically suspect results, robust statistics have been used in the analysis of these interlaboratory data [9].

Certified Values: The certified values for SRM 970 Levels I and II are equally weighted averages of the mean value of the 2003 NIST certification measurements and the mean of eight interlaboratory median values [1].

95 % Confidence Intervals on the Certified Values: Because of the unusually large number of interlaboratory results available for the analysis of this material, the uncertainties for the SRM 970 Level I and II materials include the method-to-method and/or participant-to-participant variability observed in the interlaboratory studies as well as the variability observed in the NIST LC measurements. Because there was good agreement between the NIST means and the interlaboratory medians, no allowance for variation between the NIST and the interlaboratory values was required. An allowance for material heterogeneity is included in the uncertainty of the SRM 970 Level I material. The individual uncertainty components are considered to be independent and have been combined and expanded to provide approximately 95 % coverage in accordance with the ISO/JCGM Guide [10]. The confidence intervals for the true value of TAA are based upon the assumptions that the performance characteristics (bias and precision) [11,12] of the methods used by all participants in the interlaboratory studies were about the same, that

there were about the same number of participants in all of the interlaboratory studies, and that the median values from the interlaboratory studies are approximately independent and normally distributed. With about 95 % confidence, the true value of the concentration of TAA in a randomly selected vial of either SRM 970 level is expected to be within its certified 95 % confidence interval.

95 % Prediction Intervals for a Future Measurement: The prediction intervals for a future measurement of TAA in the SRM 970 Level I and II materials are based upon the same assumptions as above. Assuming the use of a measurement process that has about the same performance characteristics (bias and precision [11,12]) as those used by the participants in the interlaboratory studies, with about 95 % confidence, a future measurement on a randomly selected vial of either SRM 970 level is expected to be within its certified 95 % prediction interval.

Table 1. Certified Concentration Values of Total Ascorbic Acid (TAA)
(Ascorbic Acid + Dehydroascorbic Acid)

| Levels | Value | Concentration, $\mu\text{mol/L}$ of solution ^(a) | |
|--------|-------|---|--------------------------------|
| | | 95 % Confidence ^(b) | 95 % Prediction ^(c) |
| I | 8.41 | 7.75 to 9.07 | 4.92 to 11.91 |
| II | 28.05 | 27.56 to 28.54 | 20.58 to 35.51 |

| Levels | Value | Concentration, mg/L of solution ^(a) | |
|--------|-------|---|--------------------------------|
| | | 95 % Confidence ^(b) | 95 % Prediction ^(c) |
| I | 1.48 | 1.37 to 1.60 | 0.87 to 2.10 |
| II | 4.94 | 4.85 to 5.03 | 3.62 to 6.26 |

^(a) The SRM 970 solutions are 1:1 mixtures of human serum and 100 g/L (10 % mass concentration) aqueous metaphosphoric acid. The certified concentrations are given in terms of the total volume of solution, not the volume of human serum in the solution.

^(b) With about 95 % confidence, the true value of the concentration of TAA in a randomly selected vial of either SRM 970 Level is expected to be within its certified 95 % confidence interval.

^(c) Assuming the use of a measurement process that has about the same performance characteristics as those used by the participants in the NIST-coordinated interlaboratory studies of 1998 through 2004, with about 95 % confidence, a future measurement on a randomly selected vial of either SRM 970 Level is expected to be within its certified 95 % prediction interval.

REFERENCES

- [1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136 (2000); available at <http://www.nist.gov/srm/upload/SP260-136.PDF> (accessed April 2014).
- [2] U.S. Department of Health and Human Services; *Biosafety in Microbiological and Biomedical Laboratories*; U.S. Government Printing Office: Washington, DC (1988).
- [3] Margolis, S.A.; Duewer, D.L.; *Measurement of Ascorbic Acid in Human Plasma and Serum: Stability Interlaboratory Repeatability and Interlaboratory Reproducibility*; Clin. Chem., Vol. 42, pp. 1257–1262 (1996).
- [4] Margolis, S.A.; Park, E.; *Stability of Ascorbic Acid in Solutions Stored in Autosampler Vials*; Clin. Chem., Vol. 47, pp. 1463–1464 (2001).
- [5] Margolis, S.A.; Paule, R.; Ziegler, R.; *Ascorbic Acid and Dehydroascorbic Acid Measured in Plasma Preserved with Dithiothreitol or Metaphosphoric Acid*; Clin. Chem., Vol. 36, pp. 1750–1755 (1990).
- [6] NCHS; Third National Health and Nutrition Examination Survey (NHANES III); *NHANES III Vitamin and Mineral Supplements Data File Documentation*, Series 11, No. 2A; Atlanta, GA (1998); available at <http://www.cdc.gov/nchs/nhanes/nh3data.htm> (accessed April 2014).
- [7] Margolis, S.A.; Vangel, M.; Duewer, D.L.; *Certification of SRM 970, Ascorbic Acid in Serum, and the Analysis of Associated Interlaboratory Bias in the Measurement Process*; Clin. Chem., Vol. 49, pp. 463–469 (2003).
- [8] Margolis, S.A.; Schapira, R.; *Liquid Chromatographic Measurement of L-Ascorbic Acid and D-Ascorbic Acid in Biological Samples*; J. Chromatogr. B, Vol. 690, pp. 25–33 (1997).
- [9] Analytical Methods Committee; *Robust Statistics - How Not to Reject Outliers, Part 1, Basic Concepts*; Analyst, Vol. 114, pp. 1693–1698 (1989).
- [10] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement (GUM 1995 with Minor Corrections)*; Joint Committee for Guides in Metrology (2008); available at http://www.bipm.org/utis/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed April 2014); 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://www.nist.gov/pml/pubs/tn1297/index.cfm> (accessed April 2014).
- [11] JCGM 200:2012; *International Vocabulary of Metrology - Basic and General Concepts and Associated Terms (VIM)* (2008 version with Minor Corrections), 3rd ed.; JCGM (2012); available at http://www.bipm.org/utis/common/documents/jcgm/JCGM_200_2012.pdf (accessed April 2014).
- [12] Duewer, D.L.; Kline, M.C.; Sharpless, K.E.; Brown Thomas, J.; Gary, K.T.; Sowell, A.L; *Micronutrients Measurement Quality Assurance Program: Helping Participants Use Interlaboratory Comparison Exercise Results to Improve Their Long-Term Measurement Performance*; Anal. Chem., Vol. 71, pp. 1870–1878 (1999).

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| <p>Certificate Revision History: 11 April 2014 (Extension of certification period; editorial changes); 29 June 2009 (Extension of certification period); 18 October 2004 (This revision reflects the recertified values for total ascorbic acid); 18 May 2000 (Original certificate date).</p> |
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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) 948-3730; e-mail srminfo@nist.gov; or via the Internet at <http://www.nist.gov/srm>.

APPENDIX A

Participants in the 1998 to 2004 NIST-Coordinated Micronutrients Measurement Quality Assurance Program Interlaboratory Studies of Total Ascorbic Acid in Human Serum

Barbara Ann Karmanos Cancer Institute; Detroit, MI, USA
Bio-Reference Laboratories; Elmwood Park, NJ, USA
Cancer Research Center of Hawai'i - Cancer Etiology, University of Hawai'i at Manoa; Honolulu, HI, USA
Craft Technologies, Inc.; Wilson, NC, USA
Département de Biologie Intégrée, Batiment B – CHU; La Tronche, Grenoble, France
Department of Biomedical Research, Our Lady of Mercy Medical Center; Bronx, NY, USA
Department of Food and Food Supplement Analysis, TNO Nutrition and Food Research; Zeist, The Netherlands
Department of Laboratory Medicine and Pathology, University of Minnesota; Minneapolis, MN, USA
Department of Laboratory Medicine, Harborview Medical Center; Seattle, WA, USA
Department of Pathology, LSU Medical Center; New Orleans, LA, USA
Department of Pathology, School of Medicine, University of New Mexico; Albuquerque, NM, USA
GRECC Program, 11G, Veterans Administration; South Minneapolis, MN, USA
Inorganic Toxicology and Nutrition Branch, Centers for Disease Control and Prevention; Atlanta, GA, USA
Institut Suisse des Vitamines; Epalinges/Lausanne, Switzerland
JM Human Nutrition Research Center on Aging at Tufts University, USDA; Boston, MA, USA
Kronos Science; Phoenix, AZ, USA
Lab Central Division, Pathology Department, Parkland Memorial Hospital; Dallas, TX, USA
LabCorp; Burlington, NC, USA
Laboratoire de Biochimie A, CHU Bichat - Claude Bernard; Paris, France
Laboratoire de Biochimie Générale et Nutritionnelle, Hôpital Purpan; Toulouse, France
Micronutrient & Lipid Metabolism Division, Rowett Research Institute; Aberdeen, Scotland
Nutrition Division, Institute of Food Research; Norfolk, England
Public Health Sciences Core Labs, Fred Hutchinson Cancer Research Center; Seattle, WA, USA
Quality and Compliance Coordinator, ARUP Laboratories; Salt Lake City, UT, USA
Quality Assurance Department, Quest Diagnostics Nichols Institute; Chantilly, VA, USA
Roche Vitamins AG; Basel, Switzerland
Servei de Bioquímica Clínica, Hospital Clínic i Provincial de Barcelona; Barcelona, Spain
UCLA Center for Human Nutrition; Los Angeles, CA, USA
Unidad de Vitaminas. Sección de Nutrición, Hospital Universitario Puerta de Hierro; Madrid, Spain
Unité de Vitaminologie, Laboratoire Marcel Mérieux; Lyon, France
Western Human Nutrition Research Center, U.S. Department of Agriculture; San Francisco, CA, USA