NBS SPECIAL PUBLICATION 260-80

U.S. DEPARTMENT OF COMMERCE/National Bureau of Standards

Standard Reference Materials:

Evaluation by an ID/MS Method of the AACC Reference Method for Serum Glucose

NATIONAL BUREAU OF STANDARDS

The National Bureau of Standards[1] was established by an act of Congress on March 3, 1901. The Bureau's overall goal is to strengthen and advance the Nation's science and technology and facilitate their effective application for public benefit. To this end, the Bureau conducts research and provides: (1) a basis for the Nation's physical measurement system, (2) scientific and technological services for industry and government, (3) a technical basis for equity in trade, and (4) technical services to promote public safety. The Bureau's technical work is performed by the National Measurement Laboratory, the National Engineering Laboratory, and the Institute for Computer Sciences and Technology.

THE NATIONAL MEASUREMENT LABORATORY provides the national system of physical and chemical and materials measurement; coordinates the system with measurement systems of other nations and furnishes essential services leading to accurate and uniform physical and chemical measurement throughout the Nation's scientific community, industry, and commerce; conducts materials research leading to improved methods of measurement, standards, and data on the properties of materials needed by industry, commerce, educational institutions, and Government; provides advisory and research services to other Government agencies; develops, produces, and distributes Standard Reference Materials; and provides calibration services. The Laboratory consists of the following centers:

Absolute Physical Quantities[2] — Radiation Research — Chemical Physics — Analytical Chemistry — Materials Science

THE NATIONAL ENGINEERING LABORATORY provides technology and technical services to the public and private sectors to address national needs and to solve national problems; conducts research in engineering and applied science in support of these efforts; builds and maintains competence in the necessary disciplines required to carry out this research and technical service; develops engineering data and measurement capabilities; provides engineering measurement traceability services; develops test methods and proposes engineering standards and code changes; develops and proposes new engineering practices; and develops and improves mechanisms to transfer results of its research to the ultimate user. The Laboratory consists of the following centers:

Applied Mathematics — Electronics and Electrical Engineering[2] — Manufacturing Engineering — Building Technology — Fire Research — Chemical Engineering[2]

THE INSTITUTE FOR COMPUTER SCIENCES AND TECHNOLOGY conducts research and provides scientific and technical services to aid Federal agencies in the selection, acquisition, application, and use of computer technology to improve effectiveness and economy in Government operations in accordance with Public Law 89-306 (40 U.S.C. 759), relevant Executive Orders, and other directives; carries out this mission by managing the Federal Information Processing Standards Program, developing Federal ADP standards guidelines, and managing Federal participation in ADP voluntary standardization activities; provides scientific and technological advisory services and assistance to Federal agencies; and provides the technical foundation for computer-related policies of the Federal Government. The Institute consists of the following centers:

Programming Science and Technology — Computer Systems Engineering.

[1]Headquarters and Laboratories at Gaithersburg, MD, unless otherwise noted; mailing address Washington, DC 20234.
[2]Some divisions within the center are located at Boulder, CO 80303.

Standard Reference Materials:

Evaluation by an ID/MS Method of the AACC Reference Method for Serum Glucose

R. Schaffer, J. Mandel, T. Sun,
A. Cohen and H. S. Hertz

National Measurement Laboratory
National Bureau of Standards
Washington, DC 20234

J. W. Neese

Centers for Disease Control, Atlanta, GA 30333

In conjunction with:

R. J. L. Bondar, C. Fasce, N. Gochman,
G. Kessler, R. B. McComb, W. T. Ryan,
and R. E. Vanderlinde

U.S. DEPARTMENT OF COMMERCE, Malcolm Baldrige, Secretary
NATIONAL BUREAU OF STANDARDS, Ernest Ambler, Director

Issued October 1982

Library of Congress Catalog Card Number: 82-600618

National Bureau of Standards Special Publication 260-80
Natl. Bur. Stand. (U.S.), Spec. Publ. 260-80, 55 pages (Oct. 1982)
CODEN: XNBSAV

U.S. GOVERNMENT PRINTING OFFICE
WASHINGTON: 1982

For Sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.
Price $4.75
(Add 25 percent for other than U.S. mailing.)

PREFACE

Standard Reference Materials (SRM's) as defined by the National Bureau of Standards are well-characterized materials produced in quantity and certified for one or more physical or chemical properties. They are used to assure the accuracy and compatibility of measurements throughout the Nation. SRM's are widely used as primary standards in many diverse fields in science, industry, and technology, both within the United States and throughout the world. They are also used extensively in the fields of environmental and clinical analysis. In many applications, traceability of quality control and measurement processes to the national measurement system are carried out through the mechanism and use of SRM's. For many of the Nation's scientists and technologists it is therefore of more than passing interest to know the details of the measurements made at NBS in arriving at the certified values of the SRM's produced. An NBS series of papers, of which this publication is a member, called the NBS Special Publication - 260 Series, is reserved for this purpose.

This 260 Series is dedicated to the dissemination of information on different phases of the preparation, measurement, certification and use of NBS-SRM's. In general, much more detail will be found in these papers than is generally allowed, or desirable, in scientific journal articles. This enables the user to assess the validity and accuracy of the measurement processes employed, to judge the statistical analysis, and to learn details of techniques and methods utilized for work entailing the greatest care and accuracy. These papers also should provide sufficient additional information not found on the certificate so that new applications in diverse fields not foreseen at the time the SRM was originally issued will be sought and found.

Inquiries concerning the technical content of this paper should be directed to the authors. Other questions concerned with the availability, delivery, price, and so forth will receive prompt attention from:

Office of Standard Reference Materials
National Bureau of Standards
Washington, D.C. 20234

George A. Uriano, Chief
Office of Standard Reference Materials

OTHER NBS PUBLICATIONS IN THIS SERIES

Catalog of NBS Standard Reference Materials (1981-83 edition), R. W. Seward, ed., NBS Spec. Publ. 260 (November 1981).

Michaelis, R. E., and Wyman, L. L. Standard Reference Materials: Preparation of White Cast Iron Spectrochemical Standards. NBS Misc. Publ. 260-1 (June 1964). COM74-11061**

Michaelis, R. E., Wyman, L. L., and Flitsch, R., Standard Reference Materials: Preparation of NBS Copper-Base Spectrochemical Standards. NBS Misc. Publ. 260-2 (October 1964). COM74-11063**

Michaelis, R. E., Yakowitz, H., and Moore, G. A., Standard Reference Materials: Metallographic Characterization of an NBS Spectrometric Low-Alloy Steel Standard. NBS Misc. Publ. 260-3 (October 1964). COM74-11060**

Hague, J. L. Mears, T. W., and Michaelis, R. E., Standard Reference Materials: Sources of Information, NBS Misc. Publ. 260-4 (February 1965). COM74-11059

Alvarez, R., and Flitsch R., Standard Reference Materials: Accuracy of Solution X-Ray Spectrometric Analysis of Copper-Base Alloys. NBS Misc. Publ. 260-5 (March 1965). PB168068**

Shultz, J. I., Standard Reference Materials: Methods for the Chemical Analysis of White Cast Iron Standards, NBS Misc. Publ. 260-6 (July 1975). COM74-11068**

Bell, R. K., Standard Reference Materials: Methods for the Chemical Analysis of NBS Copper-Base Spectrochemical Standards. NBS Misc. Publ. 260-7 (October 1965). COM74-11067**

Richmond, M.S., Standard Reference Materials: Analysis of Uranium Concentrates at the National Bureau of Standards. NBS Misc. Publ. 260-8 (December 1965). COM74-11066**

Anspach, S. C., Cavallo, L. M. Garfinkel, S. B. Hutchinson, J. M. R., and Smith, C. N., Standard Reference Materials: Half Lives of Materials Used in the Preparation of Standard Reference Materials of Nineteen Radioactive Nuclides Issued by the National Bureau of Standards NBS Misc. Publ. 260-9 (November 1965). COM74-11065**

Yakowitz, H., Vieth, D. L., Heinrich, K. F. J., and Michaelis, R. E., Standard Reference Materials: Homogeneity Characterization on NBS Spectrometric Standards II: Cartridge Brass and Low-Alloy Steel, NBS Misc. Publ. 260-10 (December 1965). COM74-11064**

Napolitano, A., and Hawkins, E. G., Standard Reference Materials: Viscosity of Standard Lead-Silica Glass, NBS Misc. Publ. 260-11 (November 1966). NBS Misc. Publ. 260-11**

Yakowitz, H., Vieth, D. L., and Michaelis, R. E., Standard Reference Materials: Homogeneity Characterization of NBS Spectrometric Standards III: White Cast Iron and Stainless Steel Powder Compact, NBS Misc. Publ. 260-12 (September 1966). NBS Misc. Publ. 260-12**

Spijkerman, J. L., Snediker, D. K., Ruegg, F. C., and DeVoe, J. R., Standard Reference Materials: Mossbauer Spectroscopy Standard for the Chemical Shift of Iron Compounds, NBS Misc. Publ. 260-13 (July 1967). NBS Misc. Publ. 260-13**

Menis, O., and Sterling, J. T., Standard Reference Materials: Determination of Oxygen in Ferrous Materials - SRM 1090, 1091, and 1092, NBS Misc. Publ. 260-14 (September 1966). NBS Misc. Publ. 260-14**

Passaglia, E., and Shouse, P. J. Standard Reference Materials: Recommended Method of Use of Standard Light-Sensitive Paper for Calibrating Carbon Arcs Used in Testing Textiles for Colorfastness to Light, NBS Misc. Publ. 260-15 (June 1967). (Replaced by NBS Spec. Publ. 260-41.)

Yakowitz, H., Michaelis, R. E., and Vieth, D. L., Standard Reference Materials: Homogeneity Characterization of NBS Spectrometric Standards IV: Preparation and Microprobe Characterization of W-20% MO Alloy Fabricated by Powder Metallurgical Methods, NBS Spec. Publ. 260-16 (January 1969). COM74-11062**

Catanzaro, E. J., Champion, C. E., Garner, E. L., Marinenko, G., Sappenfield, K. M., and Shields, W. R. Standard Reference Materials: Boric Acid; Isotopic and Assay Standard Reference Materials, NBS Spec. Publ. 260-17 (February 1970). Out of Print

Geller, S. B., Mantek, P.A., and Cleveland, N. G., Standard Reference Materials: Calibration of NBS Secondary Standard Magnetic Tape (Computer Amplitude Reference) Using the Reference Tape Amplitude Measurement "Process A, "NBS Spec. Publ. 260-18 (November 1969). (See NBS Spec. Publ. 260-29.)

Paule, R. C., and Mandel, J., Standard Reference Materials: Analysis of Interlaboratory Measurements on the Vapor Pressure of Gold (Certification of Standard Reference Material 745). NBS Spec. Publ. 260-19 (January 1970). PB190071**

Paule, R. C., and Mandel, J., Standard Reference Materials: Analysis of Interlaboratory Measurements on the Vapor Pressures of Cadmium and Silver, NBS Spec. Publ. 260-21 (January 1971). COM74-11359**

Yakowitz, H., Fiori, C. E., and Michaelis, R. E., Standard Reference Materials: Homogeneity Characterization of Fe-3 Si Alloy, NBS Spec. Publ. 260-22 (February 1971). COM74-11357**

Napolitano, A., and Hawkins, E. G., Standard Reference Materials: Viscosity of a Standard Borosilicate Glass, NBS Spec. Publ. 260-23 (December 1970). COM71-00157**

Sappenfield, K. M., Marineko, G., and Hague, J. L., Standard Reference Materials: Comparison of Redox Standards, NBS Spec. Publ. 260-24 (January 1972). COM72-50058**

Hicho, G. E., Yakowitz, H., Rasberry, S. D., and Michaelis, R. E., Standard Reference Materials: A Standard Reference Material Containing Nominally Four Percent Austenite, NBS Spec. Publ. 260-25 (February 1971). COM74-11356**

Martin, J. F., Standard Reference Materials: National Bureau of Standards-US Steel Corportion Joint Program for Determining Oxygen and Nitrogen in Steel, NBS Spec. Publ. 260-26 (February 1971). 85 cents* PB 81176620

Garner, E. L., Machlan, L. A., and Shields, W. R., Standard Reference Materials: Uranium Isotopic Standard Reference Materials, NBS Spec. Publ. 260-27 (April 1971). COM74-11358**

Heinrich, K. F. J., Myklebust, R. L., Rasberry, S. D., and Michaelis, R. E., Standard Reference Materials: Preparation and Evaluation of SRM's 481 and 482 Gold-Silver and Gold-Copper Alloys for Microanalysis, NBS Spec. Publ. 260-28 (August 1971). COM71-50365**

Geller, S. B., Standard Reference Materials: Calibration of NBS Secondary Standard Magnetic Tape (Computer Amplitude Reference) Using the Reference Tape Amplitude Measurement "Process A-Model 2," NBS Spec. Publ. 260-29 (June 1971). COM71-50282

Gorozhanina, R. S., Freedman, A. Y., and Shaievitch, A. B. (translated by M. C. Selby), Standard Reference Materials: Standard Samples Issued in the USSR (A Translation from the Russian). NBS Spec. Publ. 260-30 (June 1971). COM71-50283**

Hust, J. G., and Sparks, L. L., Standard Reference Materials: Thermal Conductivity of Electrolytic Iron SRM 734 from 4 to 300 K, NBS Spec. Publ. 260-31 (November 1971). COM71-50563**

Mavrodineanu, R., and Lazar, J. W., Standard Reference Materials: Standard Quartz Cuvettes, for High Accuracy Spectrophotometry, NBS Spec. Publ. 260-32 (December 1973). 55 cents* SN003-003-01213-1

Wagner, H. L., Standard Reference Materials: Comparison of Original and Supplemental SRM 705, Narrow Molecular Weight Distribution Polystyrene, NBS Spec. Publ. 260-33 (May 1972). COM72-50526**

Sparks, L. L., and Hust, J. G., Standard Reference Materials: Thermoelectric Voltage, NBS Spec. Publ. 260-34, (April 1972). COM72-50371**

Sparks, L. L., and Hust, J. G., Standard Reference Materials: Thermal Conductivity of Austenitic Stainless Steel, SRM 735 from 5 to 280 K, NBS Spec. Publ. 260-35 (April 1972.) 35 cents* COM72-50368**

Cali, J. P., Mandel, J., Moore, L. J., and Young, D. S., Standard Reference Materials: A Referee Method for the Determination of Calcium in Serum, NBS SRM 915, NBS Spec. Publ. 260-36 (May 1972). COM72-50527**

Shultz, J. I. Bell., R. K. Rains, T. C., and Menis, O., Standard Reference Materials: Methods of Analysis of NBS Clay Standards, NBS Spec. Publ. 260-37 (June 1972). COM72-50692**

Richmond, J. C., and Hsia, J. J., Standard Reference Materials: Preparation and Calibration of Standards of Spectral Specular Reflectance, NBS Spec. Publ. 260-38 (May 1972). COM72-50528**

Clark, A. F., Denson, V.A., Hust, J. G., and Powell, R. L., Standard Reference Materials The Eddy Current Decay Method for Resistivity Characterization of High-Purity Metals, NBS Spec. Publ. 260-39 (May 1972). COM72-50529**

McAdie, H. G., Garn, P.D., and Menis, O., Standard Reference Materials: Selection of Thermal Analysis Temperature Standards Through a Cooperative Study (SRM 758, 759, 760), NBS Spec. Publ. 260-40 (August 1972.) COM72-50776**

Wood, L. A., and Shouse, P. J., Standard Reference Materials: Use of Standard Light-Sensitive Paper for Calibrating Carbon Arcs Used in Testing Textiles for Colorfastness to Light, NBS Spec. Publ. 260-41 (August 1972) COM72-50775**

Wagner, H. L. and Verdier, P. H., eds., Standard Reference Materials: The Characterization of Linear Polyethylene, SRM 1475, NBS Spec. Publ. 260-42 (September 1972). COM72-50944**

Yakowitz, H., Ruff, A. W., and Michaelis, R. E., Standard Reference Materials: Preparation and Homogeneity Characterization of an Austenitic Iron-Chromium-Nickel Alloy, NBS Spec. Publ. 260-43 (November 1972). COM73-50760**

Schooley, J. F., Soulen, R. J., Jr., and Evans, G. A., Jr., Standard Reference Materials: Preparation and Use of Superconductive Fixed Point Devices, SRM 767, NBS Spec. Publ. 260-44 (December 1972). COM73-50037**

Greifer, B., Maienthal, E. J. Rains, T. C., and Rasberry, S. D., Standard Reference Materials: Powdered Lead-Based Paint, SRM 1579, NBS Spec. Publ. 260-45 (March 1973). COM73-50226**

Hust, J. G., and Giarratano, P. J., Standard Reference Materials: Thermal Conductivity and Electrical Resistivity Standard Reference Materials: Austenitic Stainless Steel, SRM's 735 and 798, from 4 to 1200 k, NBS Spec. Publ. 260-46 (March 1975). SN003-003-01278-5

Hust, J. G., Standard Reference Materials: Electrical Resistivity of Electrolytic Iron, SRM 797, and Austenitic Stainless Steel, SRM 798, from 5 to 280 K, NBS Spec. Publ. 260-47 (February 1974). COM74-50176**

Mangum, B. W., and Wise, J. A., Standard Reference Materials: Description and Use of Precision Thermometers for the Clinical Laboratory, SRM 933 and SRM 934, NBS Spec. Publ. 260-48 (May 1974). 60 cents* SN003-003-01278-5

Carpenter, B. S., and Reimer, G. M., Standard Reference Materials Calibrated Glass Standards for Fission Track Use, NBS Spec. Publ. 260-49 (November 1974). COM74-51185

Hust, J. G., and Giarratano, P. J., Standard Reference Materials: Thermal Conductivity and Electrical Resistivity Standard Reference Materials: Electrolytic Iron, SRM's 734 and 797 from 4 to 1000 K, NBS Spec. Publ. 260-50 (June 1975). $1.00* SN003-003-01425-7

Mavrodineanu, R., and Baldwin, J. R., Standard Reference Materials: Glass Filters As a Standard Reference Material for Spectrophotometry; Selection; Preparation; Certification; Use-SRM 930, NBS Spec. Publ. 260-51 (November 1975). $1.90* SN003-003-01481-8

Hust, J. G., and Giarratano, P. J., Standard Reference Materials: Thermal Conductivity and Electrical Resistivity Standard Reference Materials 730 and 799, from 4 to 3000 K, NBS Spec. Publ. 260-52 (September 1975). $1.05* SN003-003-01464-8

Durst, R. A., Standard Reference Materials: Standardization of pH Measurements, NBS Spec. Publ. 260-53 (December 1975, Revised). $1.05 SN003-003-01551-2

Burke, R. W., and Mavrodineanu, R. Standard Reference Materials: Certification and Use of Acidic Potassium Dichromate Solutions as an Ultraviolet Absorbance Standard, NBS Spec. Publ. 260-54 (August 1977). $3.00* SN003-003-01828-7

Ditmars, D. A., Cezairliyan, A., Ishihara, S., and Douglas, T. B., Standard Reference Materials: Enthalpy and Heat Capacity; Molybdenum SRM 781, from 273 to 2800 K, NBS Spec. Publ. 260-55 (September 1977). $2.20* SN003-003-01836-8

Powell, R. L., Sparks, L. L., and Hust, J. G., Standard Reference Materials: Standard Thermocouple Materials, Pt.67: SRM 1967, NBS Spec. Publ. 260-56 (February 1978). $2.20* SN003-003-018864

Cali, J. P. and Plebanski, T., Guide to United States Reference Materials, NBS Spec. Publ. 260-57 (February 1978). $2.20* PB 277173

Barnes, J. D., and Martin, G. M., Standard Reference Materials: Polyester Film for Oxygen Gas Transmission Measurements SRM 1470, NBS Spec. Publ. 260-58 (June 1979) $2.00* SN003-003-02077

Chang, T., and Kahn, A. H. Standard Reference Materials: Electron Paramagnetic Resonance Intensity Standard; SRM 2601, NBS Spec. Publ. 260-59 (August 1978) $2.30* SN003-003-01975-5

Velapoldi, R. A., Paule, R. C., Schaffer, R., Mandel, J., and Moody, J. R., Standard Reference Materials: A Reference Method for the Determination of Sodium in Serum, NBS Spec. Publ. 260-60 (August 1978). $3.00* SN003-003 01978-0

Verdier, P. H., and Wagner. H. L., Standard Reference Materials: The Characterization of Linear Polyethylene (SRM 1482, 1483, 1484), NBS Spec. Publ. 260-61 (December 1978). $1.70* SN003-003-02006-1

Soulen, R. J., and Dove, R. B., Standard Reference Materials: Temperature Reference Standard for Use Below 0.5 K (SRM 768). NBS Spec. Publ. 260-62 (April 1979). $2.30* SN003-003-02047-8

Velapoldi, R. A., Paule, R. C., Schaffer, R. Mandel, J., Machlan, L. A., and Gramlich, J. W., Standard Reference Materials: A Reference Method for the Determination of Potassium in Serum. NBS Spec. Publ. 260-63 (May 1979). $3.75* SN003-003-02068

Velapoldi, R. A., and Mielenz, K. D., Standard Reference Materials: A Fluorescence Standard Reference Material Quinine Sulfate Dihydrate (SRM 936), NBS Spec. Publ. 260-64 (January 1980). $4.25* SN003-003-02148-2

Marinenko, R. B., Heinrich, K. F. J., and Ruegg, F. C., Standard Reference Materials: Micro-Homogeneity Studies of NBS Standard Reference Materials, NBS Research Materials, and Other Related Samples. NBS Spec. Publ. 260-65 (September 1979). $3.50* SN003-003-02114-1

Venable, W. H., Jr., and Eckerle, K. L., Standard Reference Materials: Didymium Glass Filters for Calibrating the Wavelength Scale of Spectrophotometers (SRM 2009, 2010, 2013). NBS Spec. Publ. 260-66 (October 1979). $3.50* SN003-003-02127-0

Velapoldi, R. A., Paule, R. C., Schaffer, R., Mandel, J., Murphy, T. J., and Gramlich, J. W., Standard Reference Materials: A Reference Method for the Determination of Chloride in Serum, NBS Spec. Publ. 260-67 (November 1979). $3.75* SN003-003-02136-9

Mavrodineanu, R. and Baldwin, J.R., Standard Reference Materials: Metal-On-Quartz Filters as a Standard Reference Material for Spectrophotometry-SRM 2031, NBS Spec. Publ. 260-68 (April 1980). $4.25* SN003-003-02167-9

Velapoldi, R. A., Paule, R. C., Schaffer, R., Mandel, J., Machlan, L. A., Garner, E. L., and Rains, T. C., Standard Reference Materials: A Reference Method for the Determination of Lithium in Serum, NBS Spec. Publ. 260-69 (July) 1980). $4.25* SN003-003-02214-4

Marinenko, R. B., Biancaniello, F., Boyer, P. A., Ruff, A. W., DeRobertis, L., Standard Reference Materials: Preparation and Characterization of an Iron-Chromium-Nickel Alloy for Microanalysis, NBS Spec. Publ. 260-70 (May 1981). $2.50* SN003-003-02328-1

Seward, R. W., and Mavrodineanu, R., Standard Reference Materials: Summary of the Clinical Laboratory Standards Issued by the National Bureau of Standards, NBS Spec. Publ. 260-71 (November 1981). $6.50* SN003-003-02381-7

Reeder, D.J., Coxon, B., Enagonio, D., Christensen, R. G., Schaffer, R., Howell, B. F., Paule, R. C., Mandel, J., Standard Reference Materials: SRM 900, Antiepilepsy Drug Level Assay Standard, NBS Spec. Publ. 260-72 (June 1981). $4.25* SN003-003-02329-9

Interrante, C. G., and Hicho, G. E., Standard Reference Materials: A Standard Reference Material Containing Nominally Fifteen Percent Austenite (SRM 486), NBS Spec. Publ. 260-73 (January 1982). $2.75* SN003-003-02386-8

Marinenko, R. B., Standard Reference Materials: Preparation and Characterization of K-411 and K-414 Mineral Glasses for Microanalysis: SRM 470. NBS Spec. Publ. 260-74 (April 1982). $3.50 SN003-003-023-95-7

Weidner, V. R., Hsia, J. J., Standard Reference Materials: Preparation and Calibration of First Surface Aluminum Mirror Specular Reflectance Standards (SRM 2003a), NBS Spec. Publ. 260-75 (May 1982). $3.75 SN003-003-023-99-0

Hicho, G. E. and Eaton, E. E., Standard Reference Materials: A Standard Reference Material Containing Nominally Five Percent Austenite (SRM 485a), NBS Spec. Publ. 260-76 (in press).

Furukawa, G. T., Riddle, J. L., Bigge, W. G., and Pfieffer, E. R., Standard Reference Materials: Application of Some Metal SRM's as Thermometric Fixed Points, NBS Spec. Publ. 260-77 (in press).

Hicho, G. E. and Eaton, E. E., Standard Reference Materials: Standard Reference Material Containing Nominally Thirty Percent Austenite (SRM 487), NBS Spec. Publ. 260-78 (in press).

Richmond, J. C., Hsia, J. J., Weidner, V. R., and Wilmering, D. B., Standard Reference Materials: Second Surface Mirror Standards of Specular Spectral Reflectance (SRM's 2023, 2024, 2025), NBS Spec. Publ. 260-79 (in press).

Schaffer, R., Mandel, J., Sun, T., Cohen, A., and Hertz, H. S., Standard Reference Materials: Evaluation by an ID/MS Method of the AACC Reference Method for Serum Glucose, NBS Spec. Publ. 260-80 (in press).

* Send order with remittance to Superintendent of Documents, US Government Printing Office Washington, DC 20402. Remittance from foreign countries should include an additional one-fourth of the purchase price for postage.

** May be ordered from: National Technical Information Services (NTIS). Springfield Virginia 22151.

Table of Contents

List of Tables

EVALUATION BY AN ID/MS METHOD OF THE AACC REFERENCE METHOD FOR SERUM GLUCOSE

R. Schaffer, J. Mandel, T. Sun*, A. Cohen, and H. S. Hertz

National Bureau of Standards
Washington, DC 20234

J. W. Neese

Centers for Disease Control, Atlanta, GA 30333

In conjunction with:

R. J. L. Bondar[1], C. Fasce[2], N. Gochman[3], G. Kessler[4], R. B. McComb[5], W. T. Ryan[6], and R. E. Vanderlinde[7]

In conjunction with a study group of the Committee on Standards of the American Association for Clinical Chemistry working to establish a reference method for glucose in serum, the authors from NBS developed an isotope dilution/mass spectrometric method (ID/MS) for providing essentially bias-free, precise serum glucose analyses. This method, which is too elaborate for clinical laboratory use as a reference method, involves addition of a known amount of D-glucose-U-^{13}C to a serum sample, conversion of the labeled and unlabeled glucose in the sample into 1,2:5,6-di-O-isopropylidene-D-glucose (DAG), and measurement of the ratio of labeled to unlabeled DAG as the corresponding $(M+1)^+$ ions, by

---

*Present address: U.S. Department of Agriculture, Athens, GA
[1]Diagnostic Systems, Inc., Freehold, NJ
[2]City of Kingston Laboratory, Kingston, NY
[3]San Diego Veterans Adminstration Hospital, San Diego, CA
[4]Jewish Hospital, St. Louis, MO
[5]Hartford Hospital, Hartford, CT
[6]Beckman Instruments, Fullerton, CA
[7]New York State Department of Health, Albany, NY
(present address, Hahnemann Medical College and Hospital, Philadelphia, PA).

isobutane-chemical ionization mass spectrometry. Five serum pools having glucose concentrations ranging from 0.4 to 3.0 g/L were analyzed. The relative standard deviation among single measurements made on different samples of the same pool was found to range from 0.34 to 0.46 percent for four of the pools, and was 0.79 percent for the pool with the highest glucose concentration. Pool concentrations were also determined from the same DAG samples using electron impact mass spectrometry and monitoring the ratios of corresponding $(M-15)^+$ ions, and the results were similar. There was no evidence of bias.

These serum pools were used by the study group for a statistically controlled interlaboratory test to evaluate a hexokinase/glucose-6-phosphate dehydrogenase method using a protein-free filtrate as the reference method for glucose. Investigators at the Centers for Disease Control (Atlanta) had found that method the most appropriate of the several glucose methods that were studied as possible reference methods. [J. W. Neese et al. HEW Publication No. (CDC) 77-8330.]. Statistical analysis of the multilaboratory results showed that the relative standard deviations among single measurements made in different laboratories decreased as glucose concentrations increased. With manual pipetting used for performing the candidate reference method, the relative standard deviation ranged from 4.4 to 1.2 percent; with semi-automated pipetting, the range was 2.8 to 0.8 percent. Compared to the ID/MS results, the mean values found by the candidate reference method were about 1 percent higher at the 0.4 g/L level and changed linearly to about 2 percent lower at the 3.0 g/L level. We conclude that the candidate reference method fulfilled our prechosen criterion for acceptance as a reference method for serum glucose.

Key words: clinical analysis; glucose in serum; glucose reference method; isotope dilution/mass spectrometry; reference method; statistical analysis.

1. Introduction

A study group* of the Committee on Standards of the American Association for Clinical Chemistry was organized in 1972 for the purpose of establishing a reference method for serum glucose determinations. This required the study group to a) consider the limits for bias and imprecision it judged would be acceptable in a glucose reference method for clinical chemistry, b) identify the potential (i.e., candidate) reference method, from such evidence as low susceptibility to possible sources of interference and amenability to precise performance, c) obtain several serum pools whose glucose levels are determined by an essentially bias-free, highly precise (i.e., definitive) method, and d) use the serum pools in a statistically designed, multilaboratory study to evaluate the candidate method as the reference method. Cali et al. [1] employed that approach in attempting to establish the reference method for total calcium, and it was subsequently used for the serum sodium [2], potassium [3], chloride [4], and lithium [5] reference methods.

Studies performed at the Centers for Disease Control (CDC) led to the development of a modified form of Slein's method [6] as a choice candidate reference method for serum glucose. The modified method involves the use of a protein-free filtrate and reactions catalyzed by hexokinase (EC 2.7.1.1 ATP:D-hexose-6-phosphotransferase) and D--glucose-6-phosphate dehydrogenase (EC 1.1.1.49 D-glucose-6-phosphate:NAD oxidoreductase). CDC's experimental work and description of the candidate method have been published [7]. The study group undertook to evaluate it as the reference method, following a multilaboratory testing plan that was largely organized at the CDC [7]. As the criterion for acceptability as a glucose reference method, the study group considered a limit of bias of ±3 percent or ±30 mg/L from the definitive glucose method, whichever was larger.

The development of a definitive method for serum glucose, carried out at the National Bureau of Standards (NBS), involved isotope dilution mass spectrometry (ID/MS) although, according to the literature current when the work was begun, "definitive quality" analyses using ID/MS had

---

*R. Schaffer, Chairman. Participants in addition to the authors: B. Tejeda, Food and Drug Adminstration, Washington, DC; C. Burtis, Oak Ridge National Laboratory, TN; P. D. Schroff, Warner-Lambert Co., Morris Plains, NJ.

been attained only for certain elements [8], but not for complex compounds like glucose [9]. The use of ID/MS offered promising advantages: Specific ion monitoring would provide the needed ratios of the unlabeled and labeled forms of the analyte as highly specific measurements. With only very small quantities of the labeled and unlabeled analyte mixture required for individual ratio measurements, replicate ratio measurements could be performed on samples for demonstrating precision. Furthermore, small losses of analyte that might occur prior to making ratio measurements would not affect analytical accuracy as long as the initial ratio of labeled and unlabeled analyte remained unaltered; hence, analyte recovery in isolation steps need not be quantitative and the purification procedure could be extensive if necessary to remove interfering substances. Our preliminary experiments using the ID/MS method have been described [10]: D-glucose-U-^{13}C or, alternatively, D-glucose-6,6-d_2 was added to the serum sample; time was allowed for complete mixing and equilibration with the serum glucose; and the mixture of glucose forms was converted into 1,2:5,6-di-O-isopropylidene-D-glucose (DAG). Samples of the purified DAG were introduced into the mass spectrometer via the spectrometer's direct insertion probe, and subjected either to electron-impact mass spectrometry (EIMS) or chemical ionization mass spectrometry (CIMS) for measuring the ratio of unlabeled-to-labeled glucose derivative. The two differently labeled glucoses were used as alternatives in an attempt to reveal evidence of interferences in the measurements. With ions of different mass being measured, interferences should affect the ratios and the results would be different. The initial data indicated (incorrectly, as found later when our measurement techniques improved) that a systematic error associated with use of the glucose-6,6-d_2 had occurred, and only glucose-U-^{13}C was used thereafter. The evidence showing the absence of interferences in the method was then obtained, as described in the present publication, by measuring the same DAG samples by CIMS and alternatively by EIMS.

By the end of 1975, both the ID/MS method described in the present publication and the multilaboratory analyses with the candidate reference method had been applied to the same five bovine serum pools. However, the results and their statistical analysis which was performed at NBS were not published then because some additional analytical results not in agreement with earlier data were being obtained both at NBS and at CDC and needed to be investigated. They are discussed, in turn.

At NBS, some remaining samples of the serum pools which were being stored at −20 °C were analyzed for evidence of the long-term reproducibility of ID/MS method results. However, the new results were lower than those obtained earlier. To establish whether this was due to changes in the stored serum samples or to defects in the method, three separate actions were undertaken. The first was to modify the ID/MS method in order to improve its precision [11]: For that packed-column gas chromatography (GC) was used in place of the direct insertion probe for introduction of samples into the mass spectrometer and measurements were carried out with rigid adherence to an individual sample bracketing protocol like the one described with the definitive ID/MS method for cholesterol [12]. The second action was to use the modified ID/MS method over a 6-month period to analyze samples of six human serum pools that were also being stored at −20 °C. (The supply of bovine serum samples was exhausted.) These results showed a gradual decrease in glucose values, about 1.2 percent in 6 months [11]. The third action was to develop an alternative ID/MS method that would permit independent confirmation of the DAG method results: For that a different glucose derivative was used, namely, glucose 1,2:3,5-bis(butylboronate)-6-acetate (glucose BBA) which is synthesized under different reaction conditions than those required for DAG synthesis. This compound had been used in a previously reported glucose ID/MS method [13], but the two methods differ. In the NBS version [11] the glucose BBA is purified by capillary column GC and the ratios are measured by following an individual sample bracketing protocol [12]. Details of the modified DAG and the NBS glucose BBA ID/MS methods and evidence showing the comparability of their results were recently published [11]. The correspondence of results from the original and modified DAG ID/MS methods is described in the present publication.

At the CDC, additional analyses of stored samples of the original bovine serum pools also revealed that the glucose levels were decreasing. This rate of decrease, about 2 percent per year, was later observed also with the six human serum pools stored at -20 °C. In studies conducted with these aged human serum pools, it was found that 50 percent to 75 percent of this loss could be recovered by preincubating the specimens at 25 °C for 1 to 2 days before analysis. Normally, the samples were thawed and preincubated at 25 °C for about one hour before analysis. CDC's study showed that these phenomena were

related to storage conditions. By lowering the storage temperature of freshly prepared pools to -50 °C, glucose deterioration was prevented [14].

The present publication covers a) the original DAG ID/MS method and evidence showing its accuracy, and b) the statistical analysis of the multilaboratory candidate reference method and ID/MS method data. The data evaluated were obtained by both the ID/MS and candidate reference methods during May and June 1975; the small glucose level changes in the samples in that period are considered to have only a minor effect on our evaluation.

2. Experimental

2.1 Materials and Methods for the ID/MS Method

2.1.1 Serum Samples

Vials containing samples of bovine and human serum pools were provided by the CDC where they had been prepared and stored at −20 °C. At NBS, most pools were stored at −20 °C; others, as indicated, were kept at −50 °C.

2.1.2 D-Glucose

NBS Standard Reference Material (SRM) 917, which is certified as 99.9 ± 0.1% pure D-glucose, was used as the primary standard material.

2.1.3 D-Glucose-U-^{13}C

Crystalline D-glucose-U-^{13}C was supplied by Drs. D. Ott and T. W. Whalley of the Los Alamos Scientific Laboratory (Los Alamos, NM 87544). The sugar showed no evidence of impurities by thin layer chromatography (TLC). EIMS of samples of DAG prepared from the labeled sugar (details given below) showed that the principal ions at high mass correspond to the DAG-fragment ion $(M-15)^+$, the molecular ion minus a methyl radical, and occurred in a cluster from m/z 245 to m/z 253; the most prevalent (0.3 of the total cluster) was at m/z 250. The $(M-15)^+$ ion from unlabeled DAG was at m/z 245. By CIMS with isobutane, the $(M+1)^+$ ions were most intense, and the most prevalent from the labeled and unlabeled DAG were at m/z 266 and m/z 261, respectively.

2.1.4 Calibration Mixtures

Known quantities of SRM glucose and glucose-U-^{13}C were combined in a series of mixtures that ranged in proportion from about 0.85 to 1.15. The ratios of $(M-15)^+$ ions at m/z 245 to 250 observed for these mixtures by EIMS are shown in Table 1. The mixtures were prepared from standard solutions of the two sugars (each about 0.4 g/L of water). Weighings were made to 1 part in 10,000. The aliquots required for the mixtures were delivered from burets that had been "conditioned" by filling with one of the standard solutions, draining the solution going back into its original container, and repeating the filling and draining again. As each aliquot of a standard solution was transferred into a tared 300-mL flask, the flask and contents were weighed, so that proportions in the calibration mixtures could be calculated from the weighed quantities. After the aliquots from both solutions were added, water was added to the flask to wash remnants of the aliquots from the walls into the mixture and the contents were swirled to ensure mixing. The combinations of aliquots and washings, with total volumes about 55-75 mL each, were freeze-dried in the flasks. The dried glucose residues were converted into DAG, as described below under DAG synthesis.

Table 1. Relative weights of unlabeled and labeled glucose in standards and the relative measured intensities of $(M-15)^+$ ions at m/z 245 and 250 from DAG.

Standard No.	Relative Weights	Relative Ion-Intensities
1	0.26672	0.8647
2	0.30388	0.9566
3	0.31878	1.0032
4	0.32727	1.0288
5	0.34378	1.0727
6	0.35158	1.0940
7	0.36888	1.1431
8	0.37467	1.1554

2.1.5 Sample Preparation

(The ID/MS method requires the ratio of labeled to unlabeled sugar in samples to be within the range of the calibration mixtures; hence, approximate glucose concentrations in samples must be known beforehand, for example, by use of a routine glucose method.) A serum aliquot

containing between 1.3 and 1.7 mg of glucose, weighed to 1 part per 1,000 or better, was combined with an exactly known amount (of the order of 4.5 mg) of glucose-U-^{13}C, as follows: A weighed aliquot of the standard solution of glucose-U-^{13}C from the buret was added to a 300-mL flask. The serum aliquot added was taken from a vial that had been allowed to warm to room temperature and gently inverted several times for homogenizing the serum. A plastic syringe that was twice alternately filled and emptied of serum from the vial, was used to add the aliquot to the flask. The amount of serum transferred to the flask was obtained by weighing the syringe when refilled with sample and then after delivering the aliquot. About 60 mL of water from a wash bottle was used to wash any droplets of labeled glucose solution or serum from the upper walls of the flask into the mixture. The contents were swirled for mixing and left at room temperature for 3 h to allow the distribution of labeled and unlabeled glucose to reach equilibrium. Then the solution was freeze-dried. The glucose in the dried residue was converted into DAG, as described next.

2.1.6 DAG Synthesis

The conversion of the freeze-dried glucose (calibration mixtures or samples) into DAG was carried out by adding 1 g of anhydrous $CuSO_4$, 1 g of anhydrous $CaSO_4$, and 25 mL of acetone to the flask containing the glucose; then, while swirling the slurry, adding about 0.1 mL of concentrated H_2SO_4 dropwise; and finally shaking the securely stoppered flask vigorously for 2 days on a shaking machine. The reaction mixture was neutralized (test with pH paper) by adding 1-2 g of Na_2CO_3 and stirring; then it was filtered, and the filtrate was concentrated by evaporation at reduced pressure. The concentrate was transferred, with $CHCl_3$ used for rinsing, into a sublimation apparatus, where the solution was evaporated with gentle warming under a gentle stream of N_2 gas. Sublimation was performed at reduced pressure (7 Pa; about 0.05 mm Hg) and 88 ± 2 °C. The DAG in the sublimate was separated from acetone self-condensation products and the diacetone derivatives of other hexoses possibly present in serum samples by performing three sequential TLC steps: the first on a 20 x 20-cm, 500-µm SiO_2 GF plate, with benzene-methanol (90:7.5 by vol.) used as developer; the second on a 20 x 20-cm, 500 µm Al_2O_3 G plate, with $CHCl_3$ as developer; and the third TLC on a 20 x 20-cm, 500 µm SiO_2 GF plate, by double-development using benzene-methanol (4:1 by vol.). The DAG was extracted from the SiO_2 with $CHCl_3$

and methanol and the solution was filtered and then blown with N_2 gas down to a concentration of about 1 mg of DAG per mL of $CHCl_3$ for mass spectrometry. The DAG in the calibration mixtures required only the first TLC step for its purification, because no other hexoses were present.

The location of the DAG on SiO_2-coated plates was detected indirectly, by gently pressing a strip of adhesive-coated, transparent tape to the SiO_2 coating, to remove a thin covering of SiO_2 from the plate, and then charring the tape after spraying the adhering SiO_2 with H_2SO_4. On an Al_2O_3-coated plate, the DAG was detected indirectly by pressing a SiO_2-coated plate to the wet Al_2O_3-coated plate as soon as the development was finished, so that the SiO_2-coated plate became wet with developer. When dry, the SiO_2-coated plate was charred with H_2SO_4.

2.1.7 MS Instrumentation*

We used a Model CH 7A mass spectrometer (Varian MAT; now, Finnigan MAT) equipped with a combined chemical ionization/electron impact ion-source and a multi-ion selection device, with modifications and additions, as noted. The standard ion-detection system for the CH 7A was used, including the electron multiplier, preamplifier, and amplifier. The output of this amplifier is connected to three devices in parallel: a mass-peak display, the multi-ion selection device (the output of which was used only for qualitative data), and a multi-channel scaler for quantitative data.

The mass-peak display device permitted the selection and observation of a region of the ion beam about one atomic mass unit wide. From a low-frequency function generator, we applied a voltage of triangular wave form (55 Hz, with ±20 V maximum amplitude) to the beam-deflection plates located in front of the exit slit, so that an ion-intensity signal was obtained by sweeping a small mass range. The signal was displayed on an oscilloscope, providing continuous visual monitoring of peak shapes and positions. The advantages for quantitative application of combining continuous display with selected ion monitoring have been previously noted for systems having other methods of display [15,16].

---

*Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

The multi-ion selection device controlled the switching of the magnetic field, for monitoring two masses, and the timing, for acquisition of intensity data. The device also provided for acquisition of intensity data for each mass selected, and for the data to be displayed in the form of gas-chromatographic peak profiles.

Data for quantification were collected in parallel by two channels of an eight-channel scaler-timer (built at NBS), which converts voltage to frequency and then counts, giving 100 counts per millivolt-second. Two masses were monitored and the separately accumulated counts were transferred to a Model 9830A Calculator (Hewlett-Packard) for data reduction.

2.1.8 MS Measurements

The direct insertion probe of the mass spectrometer was used for introduction of DAG samples. About 3 μg of purified DAG in $CHCl_3$ solution was pipetted into a short gold capillary tube, sealed at one end. When the $CHCl_3$ had evaporated, the tube was inserted in the probe, and the latter was introduced into the ion-source. For isobutane-chemical-ionization measurements, the ion-source temperature was 200 °C, the source manifold pressure was about 5×10^{-5} mm Hg, the electron energy was 400 eV, and the probe heating current was varied to volatilize the DAG over a 0.5 to 1.0 min interval. For measurements made in the electron-impact mode, the ion-source was at 200 °C and the probe-heating current was raised to volatilize the DAG at a fairly constant source pressure (about 3×10^{-6} mm Hg) to allow the volatilization to proceed between 0.5 to 1.0 min. The multi-ion selection device was set to monitor the two selected ions alternately by switching the magnetic field every second. The peak-display device was operated simultaneously, with the width of the display set so that, for each mass peak, all of that peak and some of the adjacent baseline on each side were displayed. Data were collected from the onset of sample ionization until the ion-intensities were very low (about 1 percent of maximum). Measurements using electron-impact ionization were carried out using the same measurement procedure and calculation method as for isobutane-chemical ionization.

2.1.9 Measurement Procedure

As a preliminary for each sample, the intensity ratio of the selected labeled to unlabeled DAG ions was measured once. The pair of calibration standards whose previously measured intensity ratios of the

same selected ions most closely bracketed the ratio observed for each sample were identified. The duplicate ion-intensity ratios of the two standards and the sample were measured, usually not in sequential order, and measurements were accepted only if duplicates agreed within 1 percent.

2.1.10 Calculations

RW, the weight-ratio of unlabeled to labeled glucose for a calibration standard or sample can be equated to a constant b plus the product of a constant m by the quantity RI, the unlabeled to labeled ion intensity ratio for that standard or sample; thus, RW = b + mRI. The values of m and b are obtained from RW and RI values for the calibration standards, and are then used for calculating RW for the sample. The product of RW and the amount (mass) of glucose-U-^{13}C added to that sample gives the concentration of glucose in the sample, in terms of mass of glucose per gram of serum. The specific gravity of the serum is used to obtain the glucose concentration in terms of mass per unit volume of serum.

2.1.11 ID/MS Analyses on Five Serum Pools

Single vials of bovine serum pools labeled A, CGSP, 2774, 2974, and 3074 respectively were warmed to room temperature (from −20 °C), weighed aliquots were taken from each, and these were spiked with labeled glucose on days 1, 8, 15, 140, and 290. The spiked samples were allowed to equilibrate, then freeze-dried and treated to convert the glucose into DAG. Four replicate CIMS measurements of each DAG sample were obtained within a two-day period. Later, two to four replicate EIMS measurements were made on the same DAG samples. Only the CIMS data from days 1, 8, and 15 were used for calculating the target values for the serum pools, because the glucose concentrations in the stored samples were slowly falling. The EIMS values were used only for confirming the accuracy of the CIMS-derived results.

2.2 Hexokinose/Glucose-6-Phosphate Dehydrogenase Method Using a Protein-Free Filtrate for the Determination of Glucose. The Candidate Reference Method

The method with all details has been published [7]; hence, the description here is a synopsis. Included with the published method are: a) recommendations and precautions for sampling, handling, and storage of glucose standard solutions and biologic specimens; b) specifications for pipets and semi-automated pipetting and diluting devices, spectrophotometric instruments and cuvetts, other glassware, and reagent chemicals;

c) procedures for preparing stock and working solutions of D-glucose (SRM 917a is used), standard solutions of protein precipitating reagents, and buffers; and d) pre-assay test procedures for the components of the enzyme reagents and for the enzyme reagent.

The method is performed either with glass pipets (pipetting manually) or with a semi-automated pipetor/dilutor. With the former, the sample volume is 1,000 µL and is only 500 µL with the latter – but then the reagent volumes are proportionally less.

Vials of frozen serum are thawed and kept in a 25 °C water bath for about 1 h before aliquots are removed. The aliquot of sample is mixed with a tenfold larger volume of standardized barium hydroxide solution and immediately thereafter also with a like volume of standardized zinc sulfate. The mixture is centrifuged and placed in the 25 °C water bath. A 1-mL aliquot of the deproteinized supernatant solution is incubated with 5 mL of enzyme reagent, and after 30 min (when the absorbance is no longer changing) the photometric measurement at 340 nm is made. Calibration data are collected by running the series of working solutions of standard glucose before and again after the samples. Tests for the validity of the calibration data are applied before the sets of calibration data are considered acceptable.

3. Multilaboratory Study of the Candidate Reference Method

3.1 Round-Robin I (RR I)

Participating laboratories were supplied with six standard glucose solutions, prepared enzyme and protein-precipitating reagents, and samples of five unknown specimens. The materials had been prepared at the CDC according to the directions given in the procedure for the candidate reference method and were distributed by the CDC. The laboratories analyzed the standard solutions in duplicate and the unknowns in quadruplicate in a single run. Only manual pipetting was used with the method.

3.2 Round-Robin II (RR II)

The participating laboratories were supplied by the CDC with six standard glucose solutions, prepared enzyme reagent, samples of 5 unknowns and 2 controls, and with crystalline $Ba(OH)_2$ and $ZnSO_4$. The laboratories prepared their own $Ba(OH)_2$ and $ZnSO_4$ solutions as directed in the procedure for the method. They analyzed the standard solutions

in duplicate and the unknowns in quadruplicate in a single run. Manual pipetting and, alternatively, semi-automated pipetting were used.

3.3 Round-Robin III (RR III)

The participating laboratories were supplied by the CDC with samples of 5 unknowns. Each laboratory prepared its own standard solutions and reagents, following the procedures in the candidate method. The laboratories analyzed the standard solutions in duplicate and the unknowns in quadruplicate on five separate days. Manual and, alternatively, semi-automated pipetting were used.

4. Results and Statistical Analysis

4.1 ID/MS

The target values used for evaluating the candidate reference method were obtained by the ID/MS procedure described in the present report. The complete data obtained with the use of CIMS are shown in Table 2. However, only the data obtained on days 1, 8, and 15 were used for the target values, because of the instability of the glucose in the stored serum over longer periods. A statistical analysis was made of these data for the purpose of calculating the components of variance due to within-day measurement-replication error and between-day (sample-preparation and measurement) error. Table 3 gives four standard deviations for each of the five levels: s_e is the standard deviation among replicate measurements on the same DAG sample; s_p is the square root of the component of variance due to sample preparation and measurement effects (different DAG preparations from a pool); s_T is the total standard deviation (for single measurements made on different samples of a pool), where $s_T = \sqrt{s_e^2 + s_p^2}$; and, s_{av} is the standard error of the average value over all replicate measurements and samples. Since the average value at each level is the average of 12 individual measurements and 3 samples of a pool, $s_{av} = \sqrt{\frac{s_e^2}{12} + \frac{s_p^2}{3}}$

The EIMS measurements of the same DAG samples are summarized in Table 4. Only averages and not the components of variance of these measurements are given, because the number of replicate measurements differed (from 2 to 4) from sample to sample. The precision of the EIMS measurements appears to be similar to the precision of the CIMS measurements.

Table 2. Isotope dilution mass spectrometry results (in mg/L) from CIMS measurements.

	Pools				
Day	Pool A	CGSP	2774	2974	3074
1	408.5	789.9	1366	1980	2945
	407.5	786.6	1341	1981	2983
	406.9	784.5	1372	1986	2989
	406.9	794.7	1358	1975	2987
8	410.8	786.7	1340	1979	2940
	413.0	788.6	1349	1982	2963
	412.5	793.7	1349	1978	3001
	411.3	793.0	1335	1978	2997
15	413.0	789.1	1344	1988	3016
	412.8	790.4	1357	1987	3014
	412.9	787.1	1352	1982	3014
	413.5	791.0	1351	1982	3011
140[a]	403.7	775.6	1338	1957	2969
	403.5	774.8	1337	1951	2953
	402.8	775.0	1338	1948	2969
	403.8	775.6	1338	1953	2953
290[a]	400.5	778.5	1325	1940	2936
	405.4	776.7	1322	1923	2918
	406.7	781.6	1334	1945	2930
	406.8	785.1	1322	1938	2932

[a]Data obtained on days 140 and 290 were not used for the target value calculations.

Table 3. ID/MS precision parameters[a] (mg/L).

Pool	Average	s_e	s_p	s_T	s_{av}
Pool A	410.8	1.4	1.2	1.9	0.8
CGSP	789.6	2.1	1.7	2.7	1.2
2774	1351.2	3.7	2.9	4.7	2.1
2974	1981.5	7.0	5.2	8.8	3.8
3074	2988.3	19.6	13.3	23.6	10.1

[a]Based on data shown in Table 2 for days 1, 8, and 15.

Table 4. ID/MS results from EIMS measurements. Average concentrations (mg/L).

	Pools				
Day	Pool A	CGSP	2774	2974	3074
1	409.3	784.5	1346	1982	2962
8	410.8	786.4	1338	1966	2993
15	413.3	787.3	1360	1967	3013
140	405.4	775.6	1330	1947	2956
Ave. EIMS[a]	411.1	786.1	1348.0	1971.7	2989.3
Ave. CIMS[a]	410.8	789.6	1351.2	1981.5	2988.3

[a]Only data from days 1, 8, and 15.

Other results giving evidence of the accuracy of the ID/MS method, are the following: a) Four, weighed, 1.5-mL aliquots of a dialyzed serum were analyzed after adding about 1.5 mg (exactly weighed) of SRM glucose to each aliquot and also adding to two of the aliquots about 0.03 g each of D-fructose, D-galactose, and D-mannose. The recoveries of glucose were 100.4, 100.5, 100.2, and 100.3 percent for the four samples, respectively; b) Two 2.2-mL aliquots (exactly weighed) of Pool GCSP were analyzed after 1.5 mg (exactly weighed) of SRM glucose was added to each. These analyses were begun on day 150 in the time sequence indicated in Table 2. By assuming 100 percent recovery of the added glucose, the glucose levels in the serum at day 150 was 774 and 776 mg/L. The average CGSP values at 140 days from Table 2 is 775.2 mg/L and from Table 4 is 775.6 mg/L.

4.2 Candidate Reference Method

Tables 5-9 exhibit all the multilaboratory sample values obtained in the three round robins of this study. These values were derived from the calibration experiments (see below), using a least squares linear fit for the derivation of the blank and the conversion factor. The data are presented as a series of two-way tables in which the rows represent laboratories (and, where applicable, days within laboratories) and the columns represent samples or pools. The laboratories are designated by code numbers from 1 to 11. The code is used consistently for the three round robins. The replicates obtained on a particular day, for a particular sample, in a particular laboratory constitute a "cell" of the two-way table.

Table 5. Round Robin I - individual measurements: concentration in mg/L.

	Pools				
	I-1	I-2	I-3	I-4	I-5
Laboratory					
1	429.2	820.7	1101.9	2143.4	5856.8
	429.2	820.7	1101.9	2147.2	5856.8
	429.2	813.1	1098.1	2147.2	5879.6
	429.2	824.5	1094.3	2154.8	5853.0
2	464.4	849.4	1118.2	2040.9	5702.3
	468.0	831.3	1103.7	2055.4	5651.5
	457.1	831.3	1114.6	2062.6	5684.1
	453.5	820.4	1107.3	2044.5	5666.0
3	415.8	781.1	1068.0	2063.2	5574.2
	419.5	781.1	1053.1	2078.1	5555.6
	419.5	788.5	1060.6	2059.5	5555.6
	427.0	773.6	1068.0	2074.4	5533.2
4	421.7	809.2	1111.4	2111.0	5753.0
	425.6	820.8	1099.8	2091.6	5799.5
	352.0	754.9	1010.6	2037.4	5660.0
	359.7	754.9	1026.1	2045.1	5784.0
5	407.3	799.6	1071.4	2074.9	5705.6
	388.1	789.7	1062.1	2083.8	5695.0
	404.5	792.5	1062.1	2074.9	5695.0
	406.2	790.1	1066.4	2055.4	5673.7
6	421.5	799.9	1080.9	2100.0	5614.4
	406.5	799.9	1084.6	2107.5	5839.2
	417.7	781.1	1077.1	2081.3	5558.2
	414.0	769.9	1092.1	2096.2	5876.7
7	412.2	778.0	1063.9	2069.9	5667.3
	416.0	778.0	1079.1	2081.3	5644.4
	412.2	778.0	1067.7	2077.5	5655.9
	412.2	781.9	1063.9	2085.1	5499.6

Table 6. Round Robin II - manual: individual measurements, concentration in mg/L.

Laboratory	Pools				
	II-1	II-2	II-3	II-4	II-5
1	410.8	755.4	1321.3	2057.6	4513.3
	414.4	748.2	1324.9	2043.1	4513.3
	410.8	748.2	1324.9	2054.0	4516.9
	418.1	748.2	1332.2	2064.9	4513.3
2	408.7	760.4	1377.8	2063.4	4540.1
	423.0	785.5	1374.2	2084.9	4514.9
	423.0	774.8	1385.0	2049.0	4536.5
	405.1	778.4	1385.0	2102.9	4532.9
3	397.3	739.3	1339.7	2038.3	4501.6
	397.3	759.3	1332.4	2023.7	4501.6
	400.9	750.2	1317.9	2034.7	4519.8
	393.6	750.2	1325.1	2041.9	4487.1
4	440.7	791.5	1357.1	2119.6	4442.8
	444.3	766.4	1317.7	2015.7	4457.1
	437.1	773.6	1338.0	2015.7	4457.1
	429.9	762.9	1339.2	2090.9	4503.6
5	408.4	760.9	1351.9	2077.8	4498.0
	409.5	767.6	1352.6	2065.3	4501.6
	410.2	756.9	1344.8	2061.8	4512.9
	407.4	766.9	1359.1	2077.8	4512.3
6[a]	372.9	724.7	1339.2	2100.6	4547.2
	369.1	720.8	1339.2	2085.2	4585.9
	365.2	728.5	1327.6	2073.6	4558.8
	365.2	724.5	1335.3	2089.0	4585.9
6[a]	388.9	753.8	1318.0	2089.1	4425.0
	400.2	738.8	1325.5	1995.1	4575.5
	392.7	738.8	1321.8	2062.8	4537.9
	381.4	738.8	1325.5	2092.9	4537.9
7	413.1	749.6	1344.8	2051.0	4376.4
	402.1	760.7	1348.5	2002.9	4365.3
	405.8	764.4	1315.2	2069.4	4383.8
	420.5	764.4	1333.7	1999.2	4413.4
8	471.1	810.6	1401.9	2073.4	4549.3
	425.3	795.4	1340.9	2088.6	4564.5
	455.8	806.8	1409.6	2096.3	4610.3
	429.1	864.0	1352.3	2035.2	4511.1

[a]Different spectrophotometers.

Table 7. Round Robin II - semi-automated: individual measurements, concentration in mg/L.

	Pools				
Laboratory	II-1	II-2	II-3	II-4	II-5
1	412.1	763.2	1337.6	2072.8	4498.1
	408.4	759.6	1366.8	2065.5	4516.4
	412.1	759.6	1337.6	2065.5	4516.4
	412.1	759.6	1337.6	2065.5	4516.4
3	404.9	756.9	1306.8	2021.6	4510.8
	401.3	760.5	1321.4	2036.3	4525.5
	401.3	749.5	1317.7	2029.0	4514.5
	404.9	756.9	1325.1	2047.3	4532.8
7	442.6	767.7	1396.3	2075.5	4470.7
	413.7	753.3	1334.9	2050.2	4564.6
	413.7	756.9	1342.1	2032.2	4539.3
	410.1	760.5	1338.5	2071.9	4546.6
8	420.5	754.7	1323.9	2003.3	4416.1
	420.5	762.0	1320.2	2036.4	4441.8
	413.1	754.7	1323.9	1984.9	4460.2
	416.8	758.3	1327.6	2008.2	4452.8

Table 8. Round Robin III - manual: individual measurements, concentration in mg/L.

		Pools				
		III-1	III-2	III-3	III-4	III-5
Laboratory	Day					
1	1	412.1	780.9	1336.5	1948.0	2940.0
		408.5	789.8	1340.1	1948.0	2940.9
1	2	416.3	786.6	1336.5	1940.4	2928.9
		412.7	786.6	1336.5	1940.4	2928.9
1	3	410.8	783.5	1328.3	1937.7	2923.3
		410.8	780.0	1324.8	1934.1	2919.7
1	4	415.2	784.9	1330.5	1940.7	2927.8
		415.2	784.9	1334.1	1933.5	2927.8
1	5	413.7	782.8	1331.9	1940.1	2911.0
		413.7	782.8	1331.0	1932.9	2921.8
2	1	402.5	800.1	1363.5	1959.9	3005.6
		420.0	807.5	1374.5	2048.3	3005.6
2	2	406.0	800.9	1364.0	1930.7	2950.8
		417.0	789.9	1364.0	1949.0	2950.8
2	3	399.1	781.4	1321.9	1972.6	2935.8
		391.7	774.1	1336.6	1910.1	2928.5
2	4	393.1	792.6	1331.5	1907.0	2918.7
		385.7	767.0	1320.5	1910.7	2918.7
2	5	420.0	799.3	1351.8	1941.2	2909.8
		420.0	795.7	1348.1	1944.8	2935.6
3	1	424.3	817.0	1348.2	1925.7	2927.5
		428.4	799.2	1358.9	1940.0	2920.4
3	2	411.9	795.4	1345.3	1964.0	2962.5
		408.3	788.1	1370.6	1953.1	2948.1
3	3	424.3	802.7	1352.5	1955.9	2930.5
		431.4	777.7	1341.8	1941.6	2916.2
3	4	405.0	804.4	1340.6	1919.9	2927.5
		408.6	790.0	1333.4	1934.3	2949.1
3	5	402.3	786.8	1327.9	1904.6	2897.8
		405.8	772.5	1324.3	1901.0	2905.0
4	1	397.4	823.5	1305.1	1990.5	2909.3
		390.0	849.4	1305.1	1975.6	2994.4
4	2	416.6	815.6	1395.9	1940.0	2970.1
		434.7	822.8	1374.2	1987.2	2966.5
4	3	430.3	813.1	1352.7	1961.5	3018.7
		415.7	805.8	1334.4	1961.5	2960.4
4	4	435.4	802.6	1358.7	1958.5	2943.6
		417.2	806.2	1355.1	1958.5	2958.1
4	5	414.7	782.4	1330.3	1982.8	2981.4
		432.7	789.6	1337.5	1975.6	2927.3

Continuation of Table 8.

Laboratory	Day	Pools III-1	III-2	III-3	III-4	III-5
5	1	414.8	787.2	1339.5	1993.4	2943.0
		422.3	804.5	1338.4	1947.3	2958.1
5	2	410.8	781.2	1328.0	1915.1	2917.7
		413.0	782.0	1347.1	1936.7	2960.9
5	3	411.9	778.2	1336.5	1930.1	2911.4
		408.0	773.9	1338.7	1898.8	2938.3
5	4	415.5	782.6	1306.3	1960.2	2930.1
		410.3	784.0	1331.6	1938.5	2955.5
5	5	414.6	788.2	1341.4	1960.3	2929.5
		413.9	786.4	1341.4	1942.2	2953.0
6	1	424.6	828.5	1380.5	1980.8	2951.5
		420.9	810.0	1369.4	1984.5	3003.4
6	2	427.9	795.1	1355.1	1947.9	2929.7
		431.5	791.5	1362.4	1958.8	2977.0
6	3	440.1	826.7	1360.6	1968.1	2888.6
		425.4	808.3	1342.2	1979.1	2918.0
6	4	399.4	796.0	1379.6	2023.1	2958.4
		410.7	777.3	1364.7	1978.2	2950.9
6	5	416.7	794.5	1366.8	1975.8	2958.9
		420.4	790.9	1359.5	1986.8	2977.2
7	1	405.4	792.3	1347.4	1942.0	2930.5
		416.2	803.0	1351.0	1934.8	2944.8
7	2	409.1	805.6	1359.1	1935.9	3012.7
		416.3	823.4	1348.4	1957.4	2939.5
7	3	399.8	794.2	1326.6	1936.8	2886.6
		408.7	778.3	1303.6	1928.0	2900.7
7	4	383.1	772.6	1317.7	1921.7	2911.7
		397.4	774.4	1314.1	1918.1	2900.9
7	5	336.0	741.0	1302.5	1876.7	2873.8
		330.6	712.2	1316.9	1865.9	2872.0
8	1	439.8	804.1	1336.3	1964.8	2982.7
		427.3	800.6	1336.3	1939.8	2939.8
8	2	426.5	802.6	1357.8	1959.5	2940.9
		426.5	813.4	1366.7	1991.7	2955.2
8	3	440.7	816.9	1332.9	1952.7	3002.5
		426.4	791.8	1343.6	2063.8	2966.7
8	4	426.1	785.4	1354.6	1966.9	2939.5
		451.0	796.1	1354.6	1959.4	2966.1
8	5	419.0	792.7	1337.2	1967.1	2974.2
		415.5	803.4	1365.6	1952.8	2949.3

Table 9. Round Robin III - semi-automated: individual measurements, concentration in mg/L.

		Pools				
		III-1	III-2	III-3	III-4	III-5
Laboratory	Day					
1	1	419.0	794.1	1329.7	1938.6	2929.1
		423.6	794.1	1337.0	1938.6	2936.4
1	2	412.6	788.9	1340.6	1932.5	2915.3
		412.6	788.9	1340.6	1936.2	2908.0
1	3	412.2	788.0	1327.9	1926.3	2914.9
		419.5	791.6	1331.6	1926.3	2914.9
1	4	410.4	785.4	1339.0	1918.0	2908.5
		414.9	781.8	1324.4	1918.0	2919.5
1	5	415.9	778.7	1328.0	1921.0	2917.7
		418.6	778.7	1328.0	1921.0	2903.1
3	1	427.8	795.0	1325.7	1932.7	2932.4
		409.6	791.3	1340.2	1936.4	2936.0
3	2	405.2	791.2	1344.7	1941.9	2932.5
		408.8	783.9	1337.4	1931.0	2921.5
3	3	404.4	789.2	1326.4	1921.7	2927.1
		404.4	771.0	1326.4	1921.7	2923.5
3	4	426.7	771.9	1320.6	1909.3	2912.2
		412.2	771.9	1320.6	1916.5	2926.7
3	5	402.8	778.9	1315.8	1925.8	2922.8
		424.7	778.9	1330.4	1940.4	2933.8
6	1	420.0	798.1	1355.9	1966.2	2939.6
		408.7	794.4	1355.9	1969.9	2939.6
6	2	410.4	787.8	1359.6	1957.5	2932.8
		414.1	787.8	1359.6	1961.2	2929.1
6	3	416.4	795.0	1366.6	1964.2	2895.9
		401.5	791.3	1366.6	1930.8	3007.3
6	4	423.9	808.7	1374.5	1970.5	2992.7
		423.9	812.4	1363.2	1970.5	2981.4
6	5	409.6	791.7	1359.3	1979.8	2967.3
		417.1	799.3	1359.3	1960.8	2940.8
7	1	405.4	797.2	1306.2	1958.0	2902.7
		379.3	764.3	1320.8	1910.4	2953.9
7	2	426.7	802.6	1387.7	1958.6	2955.0
		430.2	788.4	1359.3	1923.1	2912.5
7	3	445.3	820.9	1342.5	1914.1	2957.0
		427.8	799.4	1324.6	1932.0	2900.2
7	4	411.4	777.3	1358.4	1914.3	2900.7
		418.6	780.9	1329.7	1925.1	2925.8
7	5	396.1	784.4	1323.8	1917.1	2931.1
		424.8	780.8	1341.8	1913.5	2895.2

Continuation of Table 9.

Laboratory	Day	Pools				
		III-1	III-2	III-3	III-4	III-5
8	1	467.2	802.1	1333.2	1937.2	2926.2
		435.1	798.6	1351.0	1939.0	2933.2
8	2	423.1	794.5	1349.8	1928.6	2943.8
		426.6	783.7	1360.7	1955.7	2954.6
8	3	384.7	813.9	1303.0	1940.5	2931.2
		395.4	742.4	1296.7	1901.2	2916.9
8	4	432.8	798.0	1342.2	1958.1	2967.7
		432.8	801.6	1342.2	1961.6	2946.2
8	5	425.1	782.6	1333.2	1919.5	2934.9
		460.8	793.3	1343.9	1951.7	2938.5
9	1	418.5	816.1	1356.9	1947.9	2932.8
		447.2	787.4	1339.8	1947.9	2950.7
9	2	408.7	785.3	1341.3	1947.4	2923.0
		408.7	785.3	1341.3	1947.4	2923.0
9	3	419.3	796.4	1342.4	1949.5	2930.1
		422.9	796.4	1346.0	1953.1	2937.2
9	4	416.6	782.2	1361.5	1951.7	2951.0
		420.2	778.6	1405.0	1958.9	2925.6
9	5	402.5	780.2	1337.7	1913.3	2920.5
		420.5	780.2	1348.5	1931.3	2920.5
10	1	426.4	789.4	1346.8	1940.8	2930.9
		415.4	793.1	1346.8	1940.8	2930.9
10	2	421.0	792.5	1340.5	1925.3	2907.2
		428.4	785.1	1344.2	1925.3	2918.3
10	3	414.4	790.3	1328.8	1925.1	2890.0
		414.4	786.7	1343.2	1925.1	2890.0
10	4	415.7	778.5	1324.5	1932.8	2918.5
		426.7	789.5	1324.5	1932.8	2911.2
10	5	428.5	800.4	1349 0	1949.1	2932.2
		446.9	800.4	1349.0	1949.1	2932.2
11	1	411.3	789.3	1343.7	1952.1	2945.6
		407.7	803.7	1343.7	1944.9	2949.2
11	2	421.2	790.9	1350.8	1950.2	2933.6
		414.0	787.3	1340.0	1935.8	2926.4
11	3	409.4	798.7	1339.3	1941.3	2950.4
		409.4	805.9	1339.3	1926.8	2932.4
11	4	407.5	793.1	1335.7	1939.1	2931.0
		421.8	782.4	1332.2	1932.0	2931.0
11	5	411.3	786.5	1343.8	1911.9	2930.1
		414.8	797.2	1336.7	1929.7	2908.6

The method of analysis applied to each two-way table is the "weighted linear model" analysis [17,18], where, however, the method for the calculation of weights was a modified version of that given in [19]. The rationale underlying this method of analysis is simple. Essentially, a plot is made of the results obtained by a particular laboratory on a particular day versus the corresponding target values or, in a study of precision, versus the average values obtained for each pool by all laboratories. Ideally, the points of such a plot would be expected to fall on a straight line going through the origin and of unit slope. In practice, the line may deviate from this ideal line both in location and in slope. Thus, each day-laboratory combination is characterized by an intercept and a slope. The variability between the lines representing the various laboratory-day combinations is the systematic between-laboratory (and/or day) variability. In addition to the systematic component of variability, one must consider another component resulting from the scatter of the observed points about the lines fitted to them. This scatter is only partly explained by the replication error, i.e., the variability among replicate measurements (same day, same laboratory). The portion of the scatter that is not explained by the replication error is referred to as lambda variability [19] and constitutes the random part of between-laboratory (and/or day) variability. When combined with the systematic component of between-laboratory (and/or day) variability, it yields the total between-laboratory (and/or day) variability. The combined component is then partitioned into two parts: between-days within-laboratories and between-laboratories. To summarize: at each level, the imprecision is made up of three components: 1) the replication error (within-days within-laboratories), 2) the between-days within-laboratories component, and 3) the between-laboratories component.

One important aspect of this analysis must be mentioned. In each of the individual round robins, five samples are used as representatives of five different levels, covering a wide range of concentrations of glucose, and the statistical analysis used a smoothing procedure in order to express each component of variability as a function of level only. In other words, the idiosyncrasies of the individual samples are considered unimportant, since each sample merely represents a particular concentration level and is not an object of specific interest.

4.2.1 Calibration

The calibration curve for the conversion of optical absorbance into glucose concentration is based on absorbance measurements made after performing the analysis on aqueous glucose solutions of known concentration. The protocol for RR I prescribed a specific calculation procedure for the determination of the blank and conversion factor. However, the least squares method can be used for fitting a straight line to the absorbance-concentration points, and the least squares method was subsequently adopted in the final version of the protocol. In the present report, we include for RR I a comparison between the concentration data for the five unknown pools derived from both methods of calibration calculations. A similar comparison for RR II and the corresponding data and calculations are available, but are not provided in this report. A detailed comparison of all calibration curves obtained in RR III in which each laboratory performed calibrations on each of five days, is included in this report.

4.2.2 Round Robin I

Each calibration line is based on the linear relation: $A = B + k \cdot C$, where C is concentration in mg/L, A is absorbance, B represents the blank and is also the intercept of the calibration line, and k is the slope of the calibration line. For the derivation of concentration C from absorbance measurement A, we write: $C = \frac{A-B}{k} = F(A-B)$, where $F = \frac{1}{k}$. F is the conversion factor of absorbance (corrected for blank) to concentration. Values for B and F, for the two calibrations in each laboratory, obtained by the least squares fitting procedure, are shown in Table 10. It is apparent that with the exception of laboratory 7, the two calibrations by each laboratory are in excellent agreement with each other. We discarded the first calibration curve for laboratory 7, and the concentration values given in Table 5 for this laboratory were based on the data from the second calibration curve exclusively. For all other laboratories, the conversion to concentration was based on the averages of the blanks and conversion factors of the two curves. Table 11 exhibits the averages obtained by both methods of calculation. It is seen that the two methods of calculation give similar, though not identical, results.

Table 10. Round Robin I - pre- and post-analysis calibration results by least squares method.

Lab	Calibration Time	Intercept (B) abs[a]	Conversion Factor (F) mg/L/abs
1	Before	.056	3770
	After	.058	3830
2	Before	.052	3610
	After	.052	3610
3	Before	.064	3730
	After	.065	3730
4	Before	.061	3890
	After	.057	3860
5	Before	.096	3570
	After	.097	3530
6	Before	.061	3750
	After	.062	3750
7	Before	.074	3980
	After	.077	3810

[a]abs = absorbance units

Table 11. Round Robin I - comparison of two methods for calculation of calibration parameters.

	Least Squares Method		RR I-Protocol Method	
Lab	B abs[a]	F mg/L/abs	B abs	F mg/L/abs
1	.057	3800	.055	3780
2	.052	3630	.051	3640
3	.064	3730	.062	3710
4	.059	3870	.055	3820
5	.097	3550	.095	3530
6	.062	3750	.064	3770
7[b]	.074	3980	.076	4010
7[c]	.077	3810	.078	3810

[a]abs = absorbance unit.

[b]Before sample measurements.

[c]After sample measurements.

Two tables can be constructed from the data in Table 5: One containing the 35 cell-averages and the other the 35 standard deviations within cells. The latter forms the basis for the calculation of the replication error at each of the five levels of concentration. The table of cell-averages forms the basis for the between-laboratory comparisons. The calculations were carried out with the data in Table 5 and with the analogous data obtained by using the RR I protocol method for the calculation of the calibration parameters. These results are shown in Table 12, where parameter s_e is the standard deviation of replication error (i.e., the standard deviation among replicate analyses made at the same time, in the same location, on the same pool); parameter s_L is the square root of the component of variance due to laboratory-to-laboratory variability; and parameter s_T is the square root of the sum of the squares of s_e and s_L, that is, $s_T = \sqrt{s_e^2 + s_L^2}$. The parameter s_T thus represents the standard deviation among single measurements obtained on the same pool in different laboratories. For all practical purposes, the two methods of calculation of calibration factors are seen to give identical results, and henceforth only the results based on the least squares fit of calibration data are discussed. It is important to note that all sets of standard deviations are functions of the concentration level of the sample, and to recall that the values shown are "smoothed" values, in which all effects of the samples due to factors other than their concentration levels have been eliminated. Later in this report, a comparison of all three round robins is made in terms of coefficients of variation as well as standard deviations.

Table 12. Round Robin I - precision parameters and sample concentrations in mg/L, calculated by two methods.

	Least Squares Method				Protocol			
Pools	Average	s_e	s_L	s_T	Average	s_e	s_L	s_T
I-1	420.5	10	22	24	421.2	10	21	23
I-2	800.1	14	20	25	798.7	14	21	25
I-3	1082.9	18	20	27	1079.9	17	22	28
I-4	2092.0	29	29	41	2081.8	29	30	42
I-5	5714.3	69	87	111	5680.1	69	83	108

Table 13 was prepared to provide a more detailed picture of the laboratory-to-laboratory differences in RR I. It shows the extent by which each laboratory average differs from the overall average of all laboratories at every concentration level. The deviations are expressed as percentages of the overall average at each level.

Table 13. Round Robin I - percent deviations of individual laboratory averages from all-labs average.

	Pools				
Laboratory	I-1	I-2	I-3	I-4	I-5
1	2.55	2.86	1.87	3.09	2.90
2	10.09	4.54	2.97	-1.58	- .35
3	.46	-1.99	-1.53	- .72	-2.48
4	-6.88	-1.50	-1.57	- .60	.93
5	-4.06	- .50	-1.24	- .55	- .07
6	- .86	-1.16	.44	.60	.46
7	-1.29	-2.25	- .95	- .25	-1.39
Average all labs mg/L	420.5	800.1	1082.9	2092.0	5714.3

4.2.3 Round Robin II

A statistical analysis was made separately for the data from the so-called manual and semi-automated methods. Table 14 lists the parameters B, F and standard deviation of fit s_f (generally referred to in the statistical literature as standard error of estimate) for each calibration curve. The calibration data consists of two sets of single measurements for each of 7 solutions. The least squares fit is therefore a linear regression of absorbance on concentration made on 14 experimental points. It is seen that, with the exception of laboratory 6, all calibrations were made with comparable precision (average standard deviation approximately 0.0030 A, or approximately 10 mg of glucose per liter per point). The calibration precision for laboratory 6 (which made measurements with two different spectrophotometers) was poorer, with a standard deviation of the order of 50 mg/L per point. The results reveal little difference in the precision obtained in calibration between the manual and the semi-automated methods.

Table 14. Round Robin II - calibration results.

Lab	B abs[a]	F mg/L/abs	s_f abs
1M[b]	.052	3630	.0018
2M	.053	3590	.0043
3M	.059	3640	.0026
4M	.057	3580	.0047
5M	.097	3570	.0030
6M[c]	.084	3860	.0136
6M[c]	.084	3760	.0162
7M	.051	3700	.0043
8M	.060	3810	.0032
1SA[d]	.053	3660	.0032
3SA	.054	3660	.0018
7SA	.057	3610	.0014
8SA	.056	3670	.0034

[a] abs = absorbance unit.
[b] M = manual.
[c] Different spectrophotometers.
[d] SA = semi-automated.

Table 15 lists the precision parameters s_e, s_L, and s_T derived from the data of Tables 6 and 7. Examination shows that both for within- and between-laboratory comparisons, the precision of the serum glucose values determined with the semi-automated method is somewhat better than with the manual method. Tables 16 and 17 exhibit, at each concentration level, the percent deviations of individual laboratory averages from the all-laboratories average for the manual and semi-automated method, respectively.

Table 15. Round Robin II - precision parameters for calculated sample concentrations (mg/L).

Pools	Average	s_e	s_L	s_T
		Manual		
II-1	409.7	12	25	28
II-2	762.0	14	22	26
II-3	1343.9	17	20	26
II-4	2060.8	20	24	31
II-5	4505.7	32	59	67
		Semi-Automated		
II-1	413.0	8	6	10
II-2	758.4	9	9	13
II-3	1334.9	12	14	18
II-4	2041.6	15	21	26
II-5	4501.3	25	42	49

Table 16. Round Robin II - manual: percent deviations of individual laboratory averages from all-labs average.

	Pools				
Laboratory	II-1	II-2	II-3	II-4	II-5
1	.94	-1.57	-1.34	- .28	.19
2	1.28	1.67	2.72	.69	.56
3	-3.03	-1.61	-1.12	-1.27	-.07
4	6.90	1.52	- .44	- .01	-.90
5	- .20	.14	.61	.48	.01
6[a]	-10.15	-4.78	- .64	1.28	1.42
6[a]	- 4.61	-2.56	-1.58	- .04	.30
7	.17	- .30	- .62	-1.46	-2.68
8	8.70	7.50	2.40	.61	1.18
Average all labs (mg/L)	409.7	762.0	1343.9	2060.8	4505.7

[a]Different spectrophotometers.

Table 17. Round Robin II - semi-automated: percent deviations of individual laboratory averages from all-labs average.

Laboratory	Pools				
	II-1	II-2	II-3	II-4	II-5
1	-.44	.28	.75	1.25	.23
3	-2.40	-.32	-1.28	-.39	.48
7	1.70	.16	1.35	.78	.64
8	1.14	-.12	-.82	-1.63	-1.30
Average all labs (mg/L)	413.0	758.4	1334.9	2041.6	4501.3

4.2.4 Round Robin III

In this round robin the manual and semi-automated methods were performed in seven laboratories. Each laboratory analyzed the five samples in duplicate on each of five days, resulting in 35 analytical runs for each method. New calibration curves were made each day. Each calibration consisted of measurements made on seven standard solutions, run before and again after the serum samples. The manual and the semi-automated methods were calibrated in the same way. All the data were used for the statistical analysis although the calibration curves for eight runs by the manual method and three runs by the semi-automated method did not meet all the criteria for acceptability.

Tables 18 and 19 are summaries of the calibration results for the manual and semi-automated methods, respectively. For each laboratory, the calibration parameters $\underline{B}$, $\underline{F}$ and $s_{\underline{f}}$ were calculated for each of the five days. As in the previous round robins, the precision of calibration by the manual method, from the standard deviations of fit, is quite uniform over all labs, with the exception of laboratory 6, for which the values of $s_{\underline{f}}$ are somewhat higher than for the other laboratories. It is also apparent from Tables 18 and 19 that systematic differences exist between the calibration lines of the various laboratories, with much smaller variations existing between calibrations made in the same laboratory on different days.

Table 18. Round Robin III - manual: calibration results.

Lab		Day 1	Day 2	Day 3	Day 4	Day 5
1	B	.044	.044	.044	.043	.044
	F	3600	3590	3580	3590	3580
	s_f	.0013	.0012	.0014	.0017	.0009
2	B	.048	.049	.046	.058	.056
	F	3680	3660	3680	3670	3680
	s_f	.0042	.0042	.0052	.0031	.0029
3	B	.058	.050	.058	.060	.062
	F	3570	3620	3570	3600	3560
	s_f	.0036	.0019	.0022	.0016	.0019
4	B	.050	.055	.052	.064	.058
	F	3710	3630	3650	3640	3600
	s_f	.0044	.0061	.0052	.0078	.0036
5	B	.044	.046	.045	.048	.047
	F	3660	3660	3590	3620	3620
	s_f	.0020	.0060	.0035	.0094	.0045
6	B	.053	.054	.052	.061	.058
	F	3710	3640	3680	3740	3670
	s_f	.0066	.0061	.0101	.0067	.0030
7	B	.057	.059	.060	.059	.076
	F	3580	3570	3540	3570	3600
	s_f	.0022	.0069	.0105	.0062	.0192
8	B	.043	.041	.042	.046	.041
	F	3570	3580	3580	3560	3560
	s_f	.0042	.0028	.0065	.0022	.0036

Table 19. Round Robin III - semi-automated: calibration results.

Lab		Day 1	Day 2	Day 3	Day 4	Day 5
1	B	.044	.045	.045	.045	.045
	F	3670	3650	3650	3640	3640
	s_f	.0010	.0010	.0016	.0012	.0014
3	B	.063	.058	.052	.063	.062
	F	3640	3640	3630	3630	3650
	s_f	.0018	.0011	.0013	.0024	.0030
6	B	.050	.055	.055	.055	.061
	F	3740	3740	3710	3770	3780
	s_f	.0054	.0040	.0117	.0042	.0054
7	B	.080	.059	.056	.059	.060
	F	3660	3550	3570	3590	3600
	s_f	.0099	.0045	.0043	.0051	.0054
8	B	.049	.048	.042	.039	.041
	F	3560	3610	3580	3580	3580
	s_f	.0049	.0053	.0024	.0022	.0025
9	B	.035	.041	.044	.044	.048
	F	3580	3590	3590	3610	3600
	s_f	.0046	.0027	.0026	.0029	.0046
10	B	.053	.050	.055	.056	.054
	F	3670	3680	3610	3660	3680
	s_f	.0040	.0055	.0054	.0024	.0021
11	B	.055	.056	.053	.056	.055
	F	3660	3590	3660	3570	3570
	s_f	.0036	.0039	.0030	.0025	.0026

Table 20 lists the precision parameters for the calculated concentration values for both the manual and semi-automated methods, at each concentration level. The parameters are: s_e, $s_{D/L}$, the square root of the component of variance due to day-to-day variability within laboratories, s_L, and s_T, where $s_T = \sqrt{s_e^2 + s_{D/L}^2 + s_L^2}$. Thus, s_T represents the standard deviation among single measurements made on samples of the same pool in different laboratories. Percent deviations from the all-laboratories averages were also calculated for each laboratory at each level. These results are given in Tables 21 and 22 for the manual and the semi-automated methods, respectively.

Table 20. Round Robin III - precision parameters for calculated sample concentrations (mg/L).

Pools	Average	s_e	$s_{D/L}$	s_L	s_T
		Manual			
Pool A	413.4	7	14	9	18
CGSP	793.4	9	15	9	20
2774	1343.3	13	16	10	23
2974	1950.5	17	17	13	27
3074	2941.8	23	19	18	34
		Semi-Automated			
Pool A	417.9	9	9	0	12
CGSP	789.9	10	9	0	13
2774	1341.4	12	8	6	15
2974	1937.5	13	8	11	19
3074	2930.1	16	6	18	25

Table 21. Round Robin III - manual: percent deviations of individual laboratory averages from all-labs average.

	Pools				
Laboratory	Pool A	CGSP	2774	2974	3074
1	- .13	-1.04	- .77	- .56	- .50
2	-1.90	- .33	.33	- .16	.14
3	.40	0	.08	- .84	- .45
4	1.22	2.22	.12	.96	.72
5	.04	-1.09	- .62	- .42	- .07
6	2.01	1.06	1.55	1.43	- .32
7	-5.61	-1.73	-1.08	-1.47	- .83
8	3.98	.91	.39	1.07	.68
Average all labs (mg/L)	413.4	793.4	1343.3	1950.5	2941.8

Table 22. Round Robin III - semi-automated: percent deviations of individual laboratory averages from all-labs average.

Laboratory	Pools				
	Pool A	CGSP	2774	2974	3074
1	- .49	- .37	- .65	- .51	- .45
3	-1.25	- .97	- .94	- .51	- .11
6	- .80	.85	1.54	1.32	.77
7	- .30	- .04	- .14	- .56	- .22
8	2.50	.14	- .42	.09	.32
9	.15	- .14	.79	.38	.05
10	1.41	.08	- .12	- .15	- .47
11	-1.20	.45	- .06	- .06	.13
Average all labs (mg/L)	417.9	789.9	1341.4	1937.5	2930.1

4.3 Comparison of Results of Round Robin III with ID/MS Values

Table 23 presents the averages obtained at each concentration level by ID/MS and the RR III manual and semi-automated candidate reference methods. The standard error of each average is also listed. Table 24 lists the differences between the RR III results and the target values, together with their standard errors.

Table 23. Comparison of Round Robin III results with ID/MS target values: averages and standard errors (mg/L).

ID/MS		Round Robin III			
		Manual		Semi-automated	
Average	Std. Error	Average	Std. Error	Average	Std. Error
410.8	0.8	413.4	3.9	417.9	1.7
789.6	1.2	793.4	4.1	789.9	1.8
1351.2	2.1	1343.3	4.7	1341.4	2.8
1981.5	3.8	1950.5	5.6	1937.5	4.2
2988.3	10.1	2941.8	7.4	2930.1	6.8

Table 24. Comparison of Round Robin III results with ID/MS target values: differences and standard errors in mg/L.

	(ID/MS)-(Manual)		(ID/MS)-(Semi-Automated)	
Pool	Difference	Std. Error of Diff.	Difference	Std. Error of Diff.
Pool A	-2.6	4.0	-7.1	1.9
CGSP	-3.8	4.3	-0.3	2.2
2774	7.7	5.1	9.6	3.5
2974	31.5	6.8	44.5	5.7
3074	46.2	12.5	57.9	12.2

For a laboratory-by-laboratory comparison with the target values, the individual percent deviations of each laboratory average from the corresponding target value are listed in Tables 25 and 26.

Table 25. Round Robin III - manual: percent deviations from target value.

	Target Value (mg/L)				
Laboratory	410.8	789.6	1351.2	1981.5	2988.3
1	0.51	-0.56	-1.35	-2.12	-2.05
2	-1.27	0.16	-0.26	-1.72	-1.42
3	1.04	0.48	-0.51	-2.40	-2.00
4	1.87	2.72	-0.47	-0.62	-0.85
5	0.68	-0.61	-1.21	-1.98	-1.62
6	2.67	1.56	0.95	-0.16	-1.24
7	-5.00	-1.25	-1.66	-3.02	-2.38
8	4.64	1.41	-0.20	-0.51	-0.89

Table 26. Round Robin III - semi-automated: percent deviations from target value.

	Target Value (mg/L)				
Laboratory	410.8	789.6	1351.2	1981.5	2988.3
1	1.23	-0.33	-1.37	-2.72	-2.39
3	0.45	-0.92	-1.66	-2.71	-2.06
6	0.92	0.89	0.80	-0.92	-1.19
7	1.43	0.00	-0.86	-2.77	-2.17
8	4.27	0.19	-1.15	-2.13	-1.64
9	1.88	-0.10	0.56	-1.85	-1.90
10	3.16	0.13	-0.85	-2.37	-2.41
11	0.50	0.49	-0.79	-2.28	-1.82

Table 27 is a summary of the precision results by the manual method for all three round robins. The table lists the glucose contents of all samples in increasing order of magnitude and exhibits the corresponding coefficients of variation, both for the replication error and for the total error (single determinations made in different laboratories). Table 28 shows the same information for the semi-automated results for RR II and RR III.

Table 27. Summary of precision data - manual: coefficient of variation (%).

Glucose (mg/L)	Replication[a]			Total[b]		
	RR I	RR II	RR III	RR I	RR II	RR III
409.7		2.9			6.8	
413.4			1.7			4.4
420.5	2.4			5.7		
762.0		1.8			3.4	
793.4			1.1			2.5
800.1	1.8			3.1		
1082.9	1.7			2.5		
1343.3			1.0			1.7
1343.9		1.3			1.9	
1950.5			0.9			1.4
2060.8		1.0			1.5	
2092.0	1.4			2.0		
2941.8			0.8			1.2
4505.7		0.7			1.5	
5714.3	1.2			1.9		

[a] Corresponds to s_e.

[b] Corresponds to s_T.

Table 28. Summary of precision data - semi-automated: coefficient of variation (%).

Glucose (mg/L)	Replication[a]		Total[b]	
	RR II	RR III	RR II	RR III
413.0	1.9		2.4	
417.9		2.2		2.9
758.4	1.2		1.7	
789.9		1.3		1.6
1334.9	0.9		1.4	
1341.4		0.9		1.1
1937.5		0.7		1.0
2041.6	0.7		1.3	
2930.1		0.6		0.9
4501.3	0.6		1.1	

[a] Corresponds to s_e.

[b] Corresponds to s_T.

5. Discussion

5.1 ID/MS Method

Some of the practices employed for optimizing ID/MS analyses for elemental composition [8] were adapted to the glucose method, e.g., a) a magnetic sector mass spectrometer was used; b) each analysis involved weighed aliquots of serum and labeled glucose solution; c) the proportions of labeled and unlabeled glucose in mixtures were controlled so that the signal intensities of the two monitored ions, corresponding to the labeled and unlabeled DAG, were approximately alike (within ±10 percent); and d) the measurements were made on purified DAG samples inserted directly into the mass spectrometer. In addition, the DAG samples were measured together with standards having ion-intensity ratios bracketing those of the samples, so that interpolation of the known weight-ratios of a pair of standards bracketing a sample and the measured ion-intensity ratios for those standards and that sample, would give the weight-ratio of unlabeled to labeled glucose in the sample. The analyst monitored the replicate ion-intensity ratios as acquired, for acceptability according to a simple protocol.

The precision of the ID/MS analyses is evident from the entries in Tables 2 and 3 for days 1, 8, and 15. The standard deviations for single determinations were less than 1 percent of the average glucose

content of the pool; and the standard errors were less than 0.5 percent of the pool averages.

The small variability in these results lies mainly between the values for the successively analyzed samples of the pools. There was no directly observable evidence of interferences in the CIMS measurements; nor were interference effects evident by comparison with results obtained using EIMS (Table 4). The latter results agreed closely with the CIMS values; even the pattern of between-sample variability was similar. Although some of the variability must be due to random effects that occurred during sample preparation, the same sample preparation method was subsequently used for the 6 human serum pools that were analyzed by the revised ID/MS method, and there was much less variation in those results [11]. We believe the somewhat better precision obtained with these human serum samples was due in part to the use of the gas chromatograph coupled to the mass spectrometer and in part to the use of a measurement protocol requiring the immediate bracketing of each sample with standards.

The accuracy of the ID/MS method was assessed from the following results: 1) The near identity of the CIMS and EIMS results for the serum pools; 2) The 100.2–100.5 percent glucose recoveries of the quantities added to four samples of dialyzed serum, two of which also contained three other hexoses as possible interfering substances. In calculating these results, it was assumed that no glucose remained in the serum after dialysis; 3) The finding in two samples of Pool CGSP to which glucose had been added on day 150 (as numbered in Table 2), of 774 and 776 mg of glucose per liter, essentially the value that had been directly determined by ID/MS for that pool at day 140. For this calculation it was assumed that 100.0 percent of the added glucose was recovered.

The original and revised DAG ID/MS methods have not been compared directly. However, the results from each have been verified independently by EIMS to CIMS comparisons as described here for the original method and in reference [11] for the revised method. The revised DAG method has been used also for determining the glucose in several pools whose glucose content had been measured at the CDC by the candidate reference method; but that data will be discussed after we consider the multilaboratory, candidate reference method results, and compare them with the DAG method results.

5.2 Candidate Reference Method

The method and organization of the interlaboratory testing were discussed at length in the CDC publication [7]; here we consider the data from the three round robins, for evaluating precision and bias. The first two round robins were run primarily for familiarizing laboratory participants with the reagent and instrumental requirements and with the performance of the method. The data from those early rounds showed on statistical analysis that the method run using manual pipetting was fairly precise, and that did not change from RR I to RR II. Also, although based on data obtained in only 4 of the 8 laboratories, the statistical analysis showed that somewhat more precise results were obtained in RR II when the method was run using semi-automated pipetting. (The number of laboratories performing the method with each alternative pipetting version was equalized for RR III. Before participating, the additional laboratories familiarized themselves with the method.)

In RR III, probably because the laboratories ran the method for 5 days and developed more skill with the procedure, the precision was better than observed in the preliminary rounds, variances declining more for the method run with manual pipetting. Although the differences are not spectacular in RR III, the semi-automated version provided somewhat better precision.

5.3 Comparison of Results from the Candidate Reference and ID/MS Method

The differences between the round robin III results and the ID/MS values, and the corresponding standard errors, which are listed in Table 24, reveal that at low concentration levels, the differences are generally of the order of their standard errors. At high concentrations, they are appreciably larger. It would be erroneous to conclude that the candidate reference method is biased only at high concentration. When the average results obtained in round robin III are plotted against the ID/MS values, straight lines result, both for the manual and for the semi-automated methods, but the lines have non-zero, positive intercepts and slopes that are significantly less than unity. The least squares fits yield the following equations, where x is the ID/MS value and y the average obtained in round robin III:

$$\text{Manual:} \quad y = 1.6 + 0.979\,x$$

$$\text{Semi-automated:} \quad y = 2.0 + 0.973\,x$$

The pattern of increasing differences with increasing glucose level was found also with the six human serum pools that were being stored at −20 °C and were analyzed at the same time by the candidate reference method and the revised DAG ID/MS method, ID/MS giving the higher results (Table 29). In another comparison [11] involving three human serum pools which were related by spiking with added glucose and where the thawed samples were left at room temperature for 20 hours before analysis by the reference method and revised DAG ID/MS method, the revised DAG ID/MS to reference method differences were −0.2 mg/L at 700 mg/L, +8.3 mg/L at 1840 mg/L and +7.8 mg/L at 2980 mg/L. Also, with a bovine serum pool (i.e., the WHO reference serum) having glucose at 980 mg/L, the ID/MS value was higher by 9.3 mg/L [11]. In general the relationship between the revised ID/MS results and CDC results with the candidate reference method is in comformity with the differences found between the original ID/MS method and the multilaboratory study. The bias in the candidate reference method is less than 1 percent with serum glucose concentrations below 1500 mg/L and below 2 percent at higher concentrations. From the precision, given in Table 20, and the bias just recounted we conclude that the candidate reference method meets our prechosen criterion for an acceptable glucose reference method.

Table 29. Comparison of six human serum pools by the revised DAG ID/MS and candidate reference methods (data from [12 and 15]).

Pool No.	Method		Difference
	ID/MS mg/L	Reference mg/L	ID/MS−Reference mg/L
3077	667.7	665.6	2.1
3177	1180.7	1171.9	8.8
3277	1682.9	1672.1	10.8
3377	2194.3	2171.7	22.6
3477	2872.2	2848.0	24.2

Laboratories that intend to perform the reference method, must carry out the method in all detail, as described [7]. As initial evidence of appropriate performance of the method, calibration results should be examined in terms of closeness to straight line fits, in the light of the data given in Tables 18 and 19. When a laboratory performs the reference method for 5 days on different serum pools and also on the Human Serum Reference Material (SRM 909) for which NBS provides a

definitive glucose value and has results that are comparable in precision and accuracy to those in this report, they may wish to reduce the amount of testing per sample by using Table 30 as a guide. Table 30 exhibits the standard error that may be expected for the average in a laboratory that is able to perform the reference method as well as those that participated in this study, when running four or eight replicate analyses on only one or two days. The standard errors were calculated from the following equation $s_T = \sqrt{\frac{s_e^2}{n} + \frac{s_{D/L}^2}{m} + s_L^2}$ in which the values for s_e, $s_{D/L}$, and s_L are from Table 20, n is the total number of replicate analyses, and m is the number of days.

Table 30. Expected standard error of average for the candidate reference method on performance of four or eight replicate determinations on one or two days (mg/L).

		With Manual Pipetting		
Sample	Average	4 in 1 day	4 in 2 days	8 in 2 days
III-1	413.4	17	14	14
III-2	793.4	18	15	14
III-3	1343.3	20	16	16
III-4	1950.5	23	20	19
III-5	2941.8	29	25	24
		With Semi-Automated Pipetting		
III-1	419.9	10	8	7
III-2	789.9	11	8	7
III-3	1341.4	12	10	9
III-4	1937.5	15	14	13
III-5	2930.1	21	20	19

The Bureau of Medical Devices and Diagnostic Products of the Food and Drug Administration provided support for the work performed by the National Bureau of Standards.

6. References

[1] Cali, J. P.; Mandel, J.; Moore, L. J.; Young, D. S. A referee method for the determination of calcium in serum. Nat. Bur. Stand. (U.S.) Spec. Publ. 260-36; 1972.

[2] Velapoldi, R. A.; Paule, R. C.; Schaffer, R.; Mandel, J.; Moody, J. R. A reference method for the determination of sodium in serum. Nat. Bur. Stand. (U.S.) Spec. Publ. 260-60; 1978.

[3] Velapoldi, R. A., Paule, R. C.; Schaffer, R.; Mandel, J.; Machlan, L. A.; Gramlich, J. W. A reference method for the determination of potassium in serum. Nat. Bur. Stand. (U.S.) Spec. Publ. 260-63; 1979.

[4] Velapoldi, R. A.; Paule, R. C.; Schaffer, R.; Mandel, J.; Murphy, T. J.; Gramlich, J. W. A reference method for the determination of chloride in serum. Nat. Bur. Stand. (U.S.) Spec. Publ. 260-67; 1979.

[5] Velapoldi, R. A.; Paule, R. C.; Schaffer, R.; Mandel, J.; Machlan, L. A.; Garner, E. L.; Rains, T. C. A reference method for the determination of lithium in serum. Nat. Bur. Stand. (U.S.) Spec. Publ. 260-69; 1980.

[6] Slein, M. W. D-Glucose determination with hexokinase and glucose-6-phosphate dehydrogenase, chapter in Methods of Enzymatic Analysis, 2nd ed. H. U. Bergmeyer, ed. New York: Academic Press; 1965. 117-122.

[7] Neese, J. W.; Duncan, P.; Bayse, D.; Robinson, M.; Cooper, T; Stewart, C. Development and evaluation of a hexokinase/glucose-6-phosphate dehydrogenase procedure for use as a national glucose reference method. U. S. Dept. Health, Education, and Welfare Publ. No. (CDC) 77-8330; 1976.

[8] Moore, L. J.; Machlan, L. A. High accuracy determinations of calcium in blood serum by isotope dilution mass spectrometry. Anal. Chem. 44:2291-2296; 1972.

[9] Björkhem, I.; Blomstrand, R.; Lantto, O.; Svensson, L.; Öhman, G. Toward absolute methods in clinical chemistry: application of mass fragmentography to high-accuracy analysis. Clin. Chem. 22:1789-1801; 1976.

[10] Schaffer, R. The development of a clinical reference method for glucose in serum. Pure Appl. Chem. 45:75-79; 1976.

[11] White V, E. W.; 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. (in press).

[12] Cohen, A.; Hertz, H. S.; Mandel, J.; Paule, R. C.; Schaffer, R.; Sniegoski, L. T.; Sun, T.; Welch, M. J.; White, V, E. Total serum cholesterol by isotope dilution/mass spectrometry: A candidate definitive method. Clin. Chem. 26:854-860; 1980.

[13] Bier, D. M.; Leake, R. D.; Gruenke, L. D.; Sperling, M. A. The in vivo measurement of alanine and glucose turnover with deuterium labeled metabolites. Klein, P. D.; Peterson, S. V., eds. Proceedings of the first international conference on stable isotopes in chemistry, biology and medicine; 1973.

[14] Neese, J. W.; Roark, B. R.; Turner, W. E. Use of the glucose reference method to characterize biological reference materials. Clin. Chem. 28:1655; 1982.

[15] Klein, P. D.; Haumann, J. R.; Eisler, W. J. Gas chromatograph-mass spectrometer-accelerating voltage alternator system for the measurement of stable isotope ratios in organic molecules. Anal. Chem. 44: 490-493; 1972.

[16] Gruenke, L. D.; Craig, J. C.; Bier, S. M. Multiple ion detection by acceleration voltage alternations in conjunction with voltage sweeping. Biomed. Mass Spectrom. 1:418-422; 1974.

[17] Mandel, J. Models, transformations of scale, and weighting. J. Quality Technol. 8(2):86-97; 1976.

[18] Mandel, J. The interlaboratory evaluation of testing methods. ASTM Bulletin 239:53-61 and 133; 1959.

[19] Mandel, J. Estimation of weighting factors in linear regression and analysis of variance. Technometrics 6:1-25; 1964.

☆ U.S. GOVERNMENT PRINTING OFFICE : 1982 O—360-997 (2280)

NBS-114A (REV. 2-8C)

U.S. DEPT. OF COMM. BIBLIOGRAPHIC DATA SHEET (See instructions)	1. PUBLICATION OR REPORT NO. NBS SP 260-80	2. Performing Organ. Report No.	3. Publication Date October 1982

4. TITLE AND SUBTITLE

Standard Reference Materials:
Evaluation by an ID/MS Method of the AACC Reference Method for Serum Glucose

5. AUTHOR(S)

R. Schaffer, J. Mandel, T. Sun, A. Cohen, and H. S. Hertz

6. PERFORMING ORGANIZATION (If joint or other than NBS, see instructions)

NATIONAL BUREAU OF STANDARDS
DEPARTMENT OF COMMERCE
WASHINGTON, D.C. 20234

7. Contract/Grant No.

8. Type of Report & Period Covered

Final

9. SPONSORING ORGANIZATION NAME AND COMPLETE ADDRESS (Street, City, State, ZIP)

Same as 6.

10. SUPPLEMENTARY NOTES

Library of Congress Catalog Card Number: 82-600618

☐ Document describes a computer program; SF-185, FIPS Software Summary, is attached.

11. ABSTRACT (A 200-word or less factual summary of most significant information. If document includes a significant bibliography or literature survey, mention it here)

In conjunction with a study group of the Committee on Standards of the American Association for Clinical Chemistry working to establish a reference method for glucose in serum, the authors from NBS developed an isotope dilution/mass spectrometric method (ID/MS) for providing essentially bias-free, precise serum glucose analyses. This method, which is too elaborate for clinical laboratory use as a reference method, involves addition of a known amount of D-glucose-U^{13}C to a serum sample, conversion of the labeled and unlabeled glucose in the sample into 1,2:5,6-di-O-isopropylidine-D-glucose (DAG), and measurement of the ratio of labeled to unlabeled DAG as the corresponding $(M+1)^+$ ions, by isobutane-chemical ionization mass spectrometry. Five serum pools having glucose concentrations ranging from 0.4 to 3.0 g/L were analyzed. The relative standard deviation among single measurements made on different samples of the same pool was found to range from 0.34 to 0.46 percent for four of the pools, and was 0.79 percent for the pool with the highest glucose concentration. Pool concentrations were also determined from the same DAG samples using electron impact mass spectrometry and monitoring the ratios of corresponding $(M-15)^+$ ions, and the results were similar. There was no evidence of bias.

These serum pools were used by the study group for a statistically controlled interlaboratory test to evaluate a hexokinase/glucose-6-phosphate dehydrogenase method as the reference method for glucose. Investigators at the Centers for Disease Control (Atlanta) had found that method the most appropriate of the several clinical glucose methods that were studied as possible reference methods. [J. W. Neese et al. HEW Publication No. (CDC) 77-8330.]. Statistical analysis of the multilaboratory results showed that the relative standard deviations among single measurements made in different laboratories decreased as glucose concentrations increased. With manual pipetting used for performing the candidate reference method, the relative standard deviation ranged from 4.4 to 1.2 percent; with semi-automated pipetting, the range was 2.8 to 0.8 percent. Compared to the ID/MS results, the mean values found by the candidate reference method were about 1 percent higher at the 0.4 g/L level and changed linearly to about 2 percent lower at the 3.0 g/L level. We conclude that the candidate reference method fulfilled our prechosen criterion for acceptance as a reference method for serum glucose.

12. KEY WORDS (Six to twelve entries; alphabetical order; capitalize only proper names; and separate key words by semicolons)

clinical analysis; glucose in serum; glucose reference method; isotope dilution/mass spectrometry; reference method; statistical analysis.

13. AVAILABILITY

☒ Unlimited
☐ For Official Distribution. Do Not Release to NTIS
☒ Order From Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.
☐ Order From National Technical Information Service (NTIS), Springfield, VA. 22161

14. NO. OF PRINTED PAGES 55

15. Price $4.75

USCOMM-DC 6043-P80

NBS TECHNICAL PUBLICATIONS

PERIODICALS

JOURNAL OF RESEARCH—The Journal of Research of the National Bureau of Standards reports NBS research and development in those disciplines of the physical and engineering sciences in which the Bureau is active. These include physics, chemistry, engineering, mathematics, and computer sciences. Papers cover a broad range of subjects, with major emphasis on measurement methodology and the basic technology underlying standardization. Also included from time to time are survey articles on topics closely related to the Bureau's technical and scientific programs. As a special service to subscribers each issue contains complete citations to all recent Bureau publications in both NBS and non-NBS media. Issued six times a year. Annual subscription: domestic $18; foreign $22.50. Single copy, $4.25 domestic; $5.35 foreign.

NONPERIODICALS

Monographs—Major contributions to the technical literature on various subjects related to the Bureau's scientific and technical activities.

Handbooks—Recommended codes of engineering and industrial practice (including safety codes) developed in cooperation with interested industries, professional organizations, and regulatory bodies.

Special Publications—Include proceedings of conferences sponsored by NBS, NBS annual reports, and other special publications appropriate to this grouping such as wall charts, pocket cards, and bibliographies.

Applied Mathematics Series—Mathematical tables, manuals, and studies of special interest to physicists, engineers, chemists, biologists, mathematicians, computer programmers, and others engaged in scientific and technical work.

National Standard Reference Data Series—Provides quantitative data on the physical and chemical properties of materials, compiled from the world's literature and critically evaluated. Developed under a worldwide program coordinated by NBS under the authority of the National Standard Data Act (Public Law 90-396).

NOTE: The principal publication outlet for the foregoing data is the Journal of Physical and Chemical Reference Data (JPCRD) published quarterly for NBS by the American Chemical Society (ACS) and the American Institute of Physics (AIP). Subscriptions, reprints, and supplements available from ACS, 1155 Sixteenth St., NW, Washington, DC 20056.

Building Science Series—Disseminates technical information developed at the Bureau on building materials, components, systems, and whole structures. The series presents research results, test methods, and performance criteria related to the structural and environmental functions and the durability and safety characteristics of building elements and systems.

Technical Notes—Studies or reports which are complete in themselves but restrictive in their treatment of a subject. Analogous to monographs but not so comprehensive in scope or definitive in treatment of the subject area. Often serve as a vehicle for final reports of work performed at NBS under the sponsorship of other government agencies.

Voluntary Product Standards—Developed under procedures published by the Department of Commerce in Part 10, Title 15, of the Code of Federal Regulations. The standards establish nationally recognized requirements for products, and provide all concerned interests with a basis for common understanding of the characteristics of the products. NBS administers this program as a supplement to the activities of the private sector standardizing organizations.

Consumer Information Series—Practical information, based on NBS research and experience, covering areas of interest to the consumer. Easily understandable language and illustrations provide useful background knowledge for shopping in today's technological marketplace.

Order the **above** *NBS publications from: Superintendent of Documents, Government Printing Office, Washington, DC 20402.*

Order the **following** *NBS publications—FIPS and NBSIR's—from the National Technical Information Services, Springfield, VA 22161.*

Federal Information Processing Standards Publications (FIPS PUB)—Publications in this series collectively constitute the Federal Information Processing Standards Register. The Register serves as the official source of information in the Federal Government regarding standards issued by NBS pursuant to the Federal Property and Administrative Services Act of 1949 as amended, Public Law 89-306 (79 Stat. 1127), and as implemented by Executive Order 11717 (38 FR 12315, dated May 11, 1973) and Part 6 of Title 15 CFR (Code of Federal Regulations).

NBS Interagency Reports (NBSIR)—A special series of interim or final reports on work performed by NBS for outside sponsors (both government and non-government). In general, initial distribution is handled by the sponsor; public distribution is by the National Technical Information Services, Springfield, VA 22161, in paper copy or microfiche form.

U.S. Department of Commerce
National Bureau of Standards

Washington, D.C. 20234
Official Business
Penalty for Private Use $300

POSTAGE AND FEES PAID
U.S. DEPARTMENT OF COMMERCE
COM-215

THIRD CLASS
BULK RATE