

# Isotope Dilution Mass Spectrometry and the National Reference System

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The National Reference System for the Clinical Laboratory (NRSL) was established in 1978 under the aegis of the National Committee for Clinical Laboratory Standards (NCCLS) to promote greater analytical accuracy of patient results across the United States (U1). Metrologists at the National Institutes of Standards and Technology (NIST) and elsewhere (U2-U15) etc. have utilized isotope dilution mass spectrometry (IDMS) as one of the primary technologies to achieve more accurate (closest approach to *true value*) chemical measurements. NIST's application of IDMS to standardization problems in clinical chemistry has clearly helped to improve the accuracy of measurements for a number of vital analytes present in human serum. This article gives information on the use of IDMS, primarily for assigning certified values to human serum reference materials within the standard reference material (SRM) program at NIST. These human serum SRMs provide for the National Reference System (NRSL/NCCLS) the accuracy base for about a dozen key analytes in human serum.

The task of improving the accuracy of patient test results in thousands of working laboratories all across this country requires a widespread voluntary consensus on standardization. To do this NRSL/NCCLS has defined an accuracy hierarchy of interrelated reference materials and reference methods that seek to promote the systematic transfer of accuracy from this accuracy base. This process of accuracy transfer was outlined by Cali (U16) and is now described in detail by guideline documents of the NRSL/NCCLS (U17-U21). It depends on the following: (A) a rational, self-consistent system of units of measurement [i.e., the SI base units (U22)], (B) materials to realize in daily practice the defined units and their derivatives [i.e., SRMs from NIST (U23) or other certified reference materials (CRMs) (U19)], (C) reference methods of proven accuracy [i.e., definitive methods (DMs) (U17) or reference methods (RMs) (U18)], (D) field or working methods, and (E) means to assure the long-term intralaboratory and interlaboratory integrity of the measurement system [i.e., internal quality control testing (U24) and external interlaboratory comparison programs (U25)].

NIST scientists have used the analytical power of IDMS technology to quantitatively measure the concentration of several analytes in human serum (i.e., SRMs 909, 909a, and 956). These certified values define accuracy for the pragmatic needs of the NRSL/NCCLS. For example, SRM 909a, a lyophilized human serum, carries IDMS/DM certified values for calcium, chloride, cholesterol, creatinine, glucose, lithium, magnesium, potassium, urea, and uric acid. Thus, SRM 909a is the artifact that transfers the accuracy inherent in NIST's IDMS measurements to other laboratories. These SRMs are purchased *primarily* by standardization and calibration laboratories in industrial, professional, and governmental organizations within the clinical laboratory community. *Secondarily*, they are purchased by working clinical laboratories that are seeking to independently improve and maintain the accuracy on a continual basis of analytical results on patient samples. SRM 909a purchases from October 1991 to October 1992 show that of the 731 units distributed 62% went to 42 industrial organizations, 19% went to 22 commercial (private) laboratories, 13% went to 44 clinical (hospital/clinic/university) laboratories, and 6% went to 13 governmental or professional organizations. Units were distributed to Austria, Canada, Denmark, England, France, Germany, Iceland, Ireland, Italy, Japan, Kuwait, Mexico, The Netherlands, Russia, South Africa, Spain, Sweden, Switzerland, Taiwan, and the United States (U26)].

George N. Bowers, Jr. is a life-long resident of Connecticut, having been born at Hartford Hospital where he has just retired after 33 years as the Director of Clinical Chemistry. After discharge from active service in Europe from the U.S. Army in 1946, he attended Colby College in Maine and graduated with a B.A. in chemistry. He received his M.D. from Yale University School of Medicine in 1954 and completed a full medical residency program at Hartford Hospital in 1958. This was followed by a postdoctoral year at the Pepper Laboratory of the University of Pennsylvania under Dr. John Reinhold, who was one of the fore-

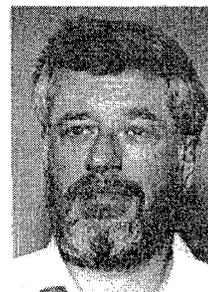
most clinical chemists. He returned to Hartford Hospital with a dual appointment in Pathology and Medicine in 1959 and rose to the Senior Staff in Pathology in 1970. Subsequently he was appointed Professor of Laboratory Medicine (Affiliated Staff at Hartford Hospital), University of Connecticut, Farmington. He has been active in the American Association for Clinical Chemistry (Standards, Editorial Board, and President), in the National Committee for Clinical Laboratory Standards (Board of Directors) and the National Reference System for the Clinical Laboratory (Member, Chair), and at the Centers for Disease Control (Medical Laboratory Advisory) and the National Institutes of Standards and Technology (NAS/NRC Evaluation Panel). He has been a persistent advocate for greater analytical accuracy in patient results, especially by the systematic use of isotope dilution mass spectrometry for value assignments to human serum Standard Reference Materials at the National Institutes of Standards and Technology.

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## ISOTOPE DILUTION MASS SPECTROMETRY (IDMS)

IDMS is accepted as a DM for both organic and inorganic analytes because of its precision and accuracy. However, the methodology and instrumentation for IDMS in the two fields



differ considerably. The following sections summarize the application of both organic and inorganic IDMS at NIST with special emphasis on the issues of accuracy faced by both.

**Inorganic IDMS.** The staff of the Chemical Science and Technology Laboratory at NIST have over the last few decades developed state-of-the-art IDMS technology to redetermine (add additional significant figures to) the atomic weight of calcium, potassium, chloride, and magnesium. The same fastidious chemical processing and thermal ionization mass spectrometric (TIMS) techniques were utilized for assigning DM values to these analytes in human serum SRMs. The major potential sources of systematic error are controlled by chemical purification of the analyte and by careful reproduction of instrumental ratio measurement conditions. The details of DM development have been published in a series of *NBS Special Publications (U27-U30)*.

TIMS is not a widely used technique in analytical chemistry, although its applicability to elements of the periodic table continues to broaden in terms of both sensitivity and number (*U31*). The precision in ratio measurement achieved by the technique is routinely 1 part in  $10^3$  and in some cases can be controlled to better than 1 part in  $10^5$ . From this high precision is derived very accurate and reproducible measurements of concentration by isotope dilution using enriched stable isotopes of the element (*U32*).

The impact of potential systematic errors is minimal in the application of ID-TIMS; this is its essential qualification as a DM technique. Nevertheless, evaluation of a DM requires the enumeration of potential systematic errors which in ID-TIMS can arise from both chemistry, that is, sample treatment, and mass spectrometry, that is, ratio measurement.

The two main sources of potential systematic error in the chemical treatment arise (1) from lack of equilibration of the sample analyte with the isotopic spike that is added and (2) contamination, or chemical blank, that is incurred during sample processing. Equilibration means the complete mixing of spike and sample and the ultimate disposition of the analyte in the same chemical state. Dissolution must be complete and selective loss of either sample or spike cannot be tolerated. Once equilibration is complete, total recovery of the analyte is not required, since it is the isotopic ratio that defines the concentration. This is one of the major advantages of isotope dilution.

The chemical blank can arise from containers, reagents, and the procedures used in the chemical processing of the samples and from the environment including the mass spectrometer, filament, etc. Ultrapure acids are used to minimize contamination from reagents (*U33*). Chemical clean rooms are used to control particulate contamination from the laboratory (*U34*). The typical blanks encountered depend on the analyte; for Li, Mg, Cl, K, and Ca in serum, the blank levels are smaller than 0.1% of the sample levels of those elements.

The main sources of systematic error in ratio measurement are (1) isobaric interferences (different elements with the same mass to charge) and (2) isotopic fractionation. The specificity of thermal ionization, coupled with the chemical processing of the sample, and the relatively large signals achievable for microgram amounts of material obviate serious concerns about interferences, once the mass spectrometry has been validated using standards. Fractionation, or the change in isotope ratios versus time during thermal ionization, will limit the reproducibility of the technique. Internal normalization is possible to minimize the effect of fractionation for elements with more than two measurable isotopes (e.g., Mg and Ca) (*U35*). For elements with only two isotopes, fractionation is externally normalized to isotopic standards determined in sequence with samples. External normalization requires careful attention to reproducing all details of sample loading, instrumental heating, and data collection protocols.

The specific analytes for which ID-TIMS DM have been developed at NIST are Li, Mg, Cl, K, and Ca. The procedures used for each of these analytes are reviewed below. The mass spectrometry procedure used originally for all of these analytes was a triple-filament procedure. In a triple-filament procedure, the sample is dried onto side filaments which are heated independently of the ionization filament in the mass spectrometer. New mass spectrometry procedures have been developed which are equally applicable to the analytes (*U36*).

**Lithium.** Li is characterized by a relatively large mass difference between the two stable isotopes  $^6\text{Li}$  and  $^7\text{Li}$ . This difference results in difficulty controlling isotopic fractionation and concern about fractionation of the two isotopes during chemical processing (*U30*). With the large amounts of isotopically separated Li that are found in commercial samples, it is also important to verify the isotopic composition of all Li samples, including reagents. The absolute abundances of reference samples of lithium have only recently been available although the isotopic composition of lithium reagents and a reference material had been compared (*U37*). New, higher precision mass spectrometry procedures for Li ratio measurement have been reported. Two procedures based on the evaporation of lithium metaborate have been described: a single-filament procedure in which  $\text{Li}_2\text{BO}_2^+$  is measured (*U38*) and a multiple-filament procedure in which  $\text{Li}^+$  is measured (*U39*). These new procedures have not yet been applied to serum DM analysis, but should provide a significant improvement over the original work (*U27*) in future development of the ID-TIMS/DM for Li.

**Magnesium.** Mg has three stable isotopes,  $^{24}\text{Mg}$ ,  $^{25}\text{Mg}$ , and  $^{26}\text{Mg}$ ;  $^{26}\text{Mg}$  is the spike isotope used in our laboratory. The triple-filament procedure developed for Mg (*U40*) suffers from significant fractionation (about 1%). This fractionation is corrected by normalizing the  $^{26}\text{Mg}/^{24}\text{Mg}$  ratio to its absolute natural value. More problems were reported for determination of Mg in serum than for other electrolytes (*U41*). These problems in all probability stem from the relatively low ionization efficiency for magnesium using the triple-filament technique. A single-filament technique has been reported for Mg with significantly improved efficiency (greater than 1000X) for Mg ionization (*U42*).

**Chloride.** The determination of chloride in serum (*U29*) was the first determination of chlorine using IDMS (*U41*), and probably the first analytical use of negative thermal ionization. In contrast to the determination of metals, wet ashing was not done for the chloride determination. The samples were spiked, diluted with  $\text{HNO}_3$  (0.1 mol/L), and heated for several hours. The protein was precipitated by the addition of ammonium molybdate, and the chloride in the protein-free filtrate was precipitated as  $\text{AgCl}$ . The  $\text{AgCl}$  was removed by centrifugation and repurified through a second crystallization. Samples were dissolved in ammonium hydroxide for mass spectrometry loading. TIMS of chlorine was hindered somewhat by instrumental memory. This problem was addressed by using different mass spectrometers for samples with vastly different ratios and by cleaning the instrument source more frequently.

**Potassium.** The measurement of K in serum showed a precision of 0.03% relative standard deviation using ID-TIMS (*U28*). Potassium is readily thermally ionized; the spike is  $^{41}\text{K}$ . The reproducibility is achieved by careful control of fractionation, since internal normalization is not possible. The vital factor in control of fractionation is the relative purity of the dried analyte on the filament (*U43*).

**Calcium.** The determination of calcium is accomplished by using  $^{44}\text{Ca}$  as the spike isotope and measurement of the  $^{44}\text{Ca}$  isotope in addition to  $^{42}\text{Ca}$  and  $^{40}\text{Ca}$  for internal normalization of fractionation. The use of internal normalization avoids the imprecision caused by fractionation which was encountered in the original development of the DM for Ca in serum (*U27, U44*).  $^{40}\text{Ca}$  has a potential interference from a very minor isotope of potassium. This potential interference is checked by monitoring  $^{41}\text{K}$  ( $^{40}\text{K}/^{41}\text{K} = 0.001774$ ).

Improvements and modifications to TIMS methodology have been developed. Expansion of the methodology to other important analytes has also occurred, as evidenced by the recent certification of Pb in blood, SRM 955a, using ID-ICP-MS (*U45*).

## ORGANIC IDMS

The technique and application of IDMS to the accurate measurement of clinically significant analytes has been reviewed (*U46-U48*). In principle, the method is simple and allows for knowledge or control of all of the variables which can lead to error. In practice, achieving accurate results requires careful design of the experiment and considerable attention to detail. The staff at NIST has developed a number

Table U-I. Characteristics of the ID GC/MS Definitive Methods in Use at NIST for Six Organic Analytes in Serum

analyte (refs)	internal standard <sup>a</sup>	workup procedure <sup>a</sup>	derivative for GC/MS <sup>a</sup>	precision <sup>a</sup> CV (%) <sup>b</sup>	applications (refs) <sup>c</sup>
cholesterol (total) (U50, U54)	cholesterol- <sup>13</sup> C <sub>3</sub> (cholesterol-5- $\alpha$ -25,26,27- <sup>13</sup> C <sub>3</sub> - $\beta$ 3ol)	hydrolysis with KOH in ethanol; extraction with hexane	cholesterol, trimethylsilyl ether	0.22	SRMs 909 (U50), 909a (U54), 1563 (U55), 1951 (U54), 1952 (U54); CDC serum pools (U56); comparison with another ID/MS method (U57); comparison with CDC reference method (U56); effect of lyophilization (U58)
creatinine (U59)	creatinine- <sup>13</sup> C <sub>2</sub> (2-amino-1,5-dihydro-1-(methyl- <sup>13</sup> C)-4H-imidazole-4-one-5- <sup>13</sup> C)	chromatography on weakly acidic ion exchange resin	N-(4,6-dimethyl-2-pyrimidinyl)-N-methylglycine, ethyl ester	0.15-0.27	SRMs 909, 909a; CAP survey materials
glucose (U49, U53)	glucose- <sup>13</sup> C <sub>6</sub> (uniformly labeled [ <sup>13</sup> C]glucose, 50% <sup>13</sup> C <sub>6</sub> )	deproteinization; deionization	$\alpha$ -D-glucopyranose cyclic 1,2,3,5-bis-(butylboronate)-6-acetate	0.29	SRMs 909, 909a; CDC serum pools (U59); CAP survey materials; WHO serum
triglycerides (U60)	glycerol- <sup>13</sup> C <sub>3</sub>	total glycerides; hydrolysis with KOH in ethanol; deionization. triglycerides: extraction, then as for total triglycerides sublimation	glycerol cyclic 1,2-butylboronate-3-trimethylsilyl ether		in progress
urea (U51)	urea- <sup>18</sup> O		6-methyl-2,4-bis-(trimethylsilyl)oxyl-pyrimidine	0.19	SRMs 909 (U51), 909a; CDC serum pools (U51); evaluation of AACC reference method (U62); CAP survey materials; WHO serum
uric acid (U61)	[1,3- <sup>15</sup> N <sub>2</sub> ]uric acid	anion exchange	uric acid tetrakis-( <i>tert</i> -butyl-dimethylsilyl) derivative	0.34-0.42	SRM 909 (U61), 909a (U61); CAP survey materials; WHO serum

<sup>a</sup> Internal standard, workup procedure, and precision are given for the most recently published method. <sup>b</sup> Typical coefficient of variation for a single measurement. <sup>c</sup> Applications with earlier versions of the method and with closely related methods are included.

of IDMS DMs for organic analytes in serum beginning with studies on glucose by Schaffer et al. in the mid-1970s (U49). These were modeled on the methods for the inorganic analytes discussed above. The organic analytes for which methods have been developed at NIST are listed in Table U-I.

The methods share some common steps: addition of stable isotope-labeled internal standard to serum samples; equilibration of the labeled and native analyte; isolation and at least partial purification of the analyte (labeled and unlabeled); formation of a derivative suitable for GC/MS; measurement, in accordance with a strict protocol of ion abundance ratios for the analyte/labeled analyte, bracketed in both time and ion abundance ratio by calibration standards. Multiple sets of independently prepared samples and calibration standards are prepared and measured. Measurements are made by combined GC/MS with the principal measurements by electron ionization. Tests for interferences are made by chemical ionization, electron ionization on ions of different masses, or by using different GC columns. Some of the factors considered in designing these methods are discussed below.

**The Analyte.** The choice of target analytes is made on the basis of a combination of perceived usefulness to the clinical community and the feasibility of developing a DM. Serum uric acid and urea, for example, are defined species, but "total" cholesterol is the sum of free cholesterol and cholesterol esters, the exact proportions of which vary from serum to serum. Thus a method for "total" cholesterol includes a well-characterized step to convert the esters to free cholesterol. In contrast, a method for glucose must free

no glucose from oligosaccharides or glycosylated compounds. Some analytes are not, at present, suitable for the development of a DM. Thus the bilirubin of clinical interest includes glucuronide conjugates and protein-bound bilirubin. The latter is a chemically undefined species and therefore cannot be measured by IDMS.

**The Unlabeled Standard and the Labeled Internal Standard.** IDMS is a technique which measures the concentration of an analyte relative to a standard of known concentration. At NIST these unlabeled standards have been SRMs, materials which are homogeneous and have well-established and documented purities. An internal standard for IDMS should ideally be indistinguishable from the analyte except for the mass difference, but it is a fact that the unlabeled and labeled molecules do not have identical physical properties or reaction rates. The labeled internal standard must, however, mimic the natural analyte well enough to avoid introducing unacceptable errors. For example, a cholesterol labeled with seven deuterium atoms was a satisfactory internal standard in our original method for cholesterol where packed GC columns were used (U50), but was unsatisfactory when capillary columns of much greater resolution were used (U51). Cholesterol, labeled with three carbon-13 atoms, as shown in Table U-I, avoided the substantial separation of the analyte from the internal standard.

**The Sample.** The methods described here, and summarized in Table U-I, are all for serum. The precision of the values assigned to samples in a pool may be limited by a lack of uniformity of vial fill weight or by instability of the analyte.

For example, very precise concentration values were assigned for all vials of SRM 909, human serum, and were applicable if a vial was reconstituted *only* after weighing its dry content. However, if the user skipped the weighing, the uncertainty of each assigned value was much greater. Refractive index measurements showed vial to vial differences in the weight of dried serum per vial. Over the 10 years that SRM 909 was studied some analytes, like creatinine and uric acid, were found to be very stable, and others, like cholesterol, showed a steady decline of about 0.1% per year. Glucose, however, declined about 0.7% per year with considerable vial to vial variation over time.

**Chemical Manipulations.** The results of an ideal isotope dilution method are independent of the extent of recovery of an analyte. Procedures are therefore tailored to eliminate possible sources of interference and obtain the analyte in the form best suited for analysis. Losses of either analyte or labeled material before equilibration, or fractionation after equilibration, will bias the results. Thus, during the development of a method for uric acid, several sets of calibration standards were prepared. Inconsistent results among sets were traced to decomposition of uric acid in ammonium hydroxide. Careful control of the ratio of ammonium hydroxide to uric acid solved the problem (U52). The procedures used at NIST to isolate an analyte from serum and to prepare it for GC/MS have ranged from very simple to extremely complex. For example the chemical manipulations for cholesterol (U50) include only hydrolysis, extraction, and formation of a derivative, while those for one of the two methods for glucose (U53) required a labor-intensive process, a condensation reaction followed by sublimation and three thin-layer chromatography steps.

**Instrumentation.** The GC/MS instrument used for all of the NIST organic DMs is a 1974-vintage single-focusing magnetic mass spectrometer which has been modified to improve the precision of the measurements (U51, U54). Attempts to transfer these methods to commercial quadrupole instruments, one a research-grade instrument, and another, much less expensive, gave results that were 3-10 times less precise. The GC columns used have included packed, support-coated open tubular (SCOT), and fused-silica capillary columns. Memory effects resulting from material remaining in the injector or on the column may occur and these lead to error since a portion of a previous sample remains to contaminate the next stage. For example the trimethylsilyl derivative of uric acid showed an unacceptable large memory effect, but the *tert*-butyldimethylsilyl derivative showed no detectable memory effect.

**Measurements.** All measurements of amount which must be quantitative are made by gravimetric rather than by volumetric techniques, a procedure necessary when volumetric measurements would contribute significantly to imprecision of the assigned value. A calibration method referred to as bracketing is used for the ion abundance ratio measurements. In this technique each measurement of a sample is immediately preceded and followed by measurements of calibration standards whose ion abundance ratios closely surround the ion abundance ratio of the sample. Approximately 10 calibration standards with ratios from about 0.8 to 1.2 are used. The measurements are repeated in reverse order on a different day to show that there is no statistically significant memory effect or any time-dependent changes. A strict protocol governs the acceptance, rejection, or repetition of measurements.

## SUMMARY

The clinical laboratory community of the United States, which is well represented by the NRSL/NCCLS, has endorsed the IDMS/DMs developed at NIST. These DMs provide the accuracy (*true value*) base for the U.S. National Reference System for a number of specific analytes in human serum. Fortunately, the U.S. government through (a) actions of NIST administrators and scientists, (b) financial support from NIH (NIGMS) and FDA, and (c) interagency agreements with CDC has accepted the responsibility for developing and maintaining IDMS/DMs for clinically important analytes as an essential part of this national measurement system infrastructure. Furthermore, it is important to note that several professional organizations, particularly, The American

Association for Clinical Chemistry (AACC) and The College of American Pathologists (CAP), have interacted heavily with NIST in full support of these national standardization activities. CAP supports three full-time Research Associates at NIST so that target values on serum samples used in its Interlaboratory Comparison Survey Programs may be traced to DMs. This remarkable cooperation and teamwork between government agencies and private sector organizations, as well as numerous individual scientists and physicians, which promotes greater accuracy of patient results, depends heavily upon the continued timely availability of IDMS/DM measurements. In short, NIST's value assignments on human serum samples (e.g., SRMs and materials for CLIA '88 proficiency testing programs) by this critical IDMS/DM metrology provide the pragmatic base for assuring accurate test results in medicine.

The resources required to support IDMS/DM technology at NIST over many decades are not trivial and from time to time require renewed R&D efforts to upgrade methodology and recapitalization in mass spectrometry instrumentation.

## LITERATURE CITED

- (U1) Boutwell, J. H., Ed. *A National understanding for the development of reference materials and methods for clinical chemistry*; AACC Press: Washington, DC, 1978.
- (U2) Bjorkhem, I.; Blomstrand, R.; Svensson, L. *Clin. Chim. Acta* **1974**, *54*, 185-93.
- (U3) Bjorkhem, I.; Blomstrand, R.; Lantto, O.; Svensson, L.; Ohman, G. *Clin. Chem.* **1976**, *22*, 1789-801.
- (U4) Vellion, C.; Wolf, W. R.; Guthrie, B. E. *Anal. Chem.* **1979**, *51*, 1022.
- (U5) Everson, S.; Patterson, C. *Clin. Chem.* **1980**, *26*, 1603.
- (U6) Reamer, C.; Vellion, C. *Anal. Chem.* **1981**, *53*, 2168.
- (U7) Heumann, K. G. *Int. J. Mass Spectrom. Ion Phys.* **1982**, *45*, 87.
- (U8) Sliu, K. W. M.; Bednas, M. E.; Berman, S. S. *Anal. Chem.* **1983**, *55*, 4473.
- (U9) Waldman, E.; Hilpert, K.; Schladot, J. D.; Stoeppler, M. *Fresenius' Z. Anal. Chem.* **1984**, *317*, 273.
- (U10) Broekman, A.; Van Raaphorst, J. G. *Fresenius' Z. Anal. Chem.* **1984**, *318*, 398.
- (U11) McLaren, J. W.; Myktyluk, A. P.; Willie, S. N.; Berman, S. S. *Anal. Chem.* **1985**, *57*, 2907.
- (U12) Beyrich, W.; Golly, W.; Spannagel, G.; De Bieve, P.; Wolters, W. H.; Lycke, W. *Nucl. Technol.* **1986**, *75*, 73.
- (U13) McLaren, J. W.; Beauchemin, D.; Berman, S. S. *Anal. Chem.* **1987**, *59*, 610.
- (U14) Peirce, P. L.; Hambidge, K. M.; Goss, C. H.; Miller, L. V.; Fennessey, P. K. *Anal. Chem.* **1987**, *59*, 2034.
- (U15) Aggarwal, S. K.; Kinter, M.; Willis, M. R.; Savory, J.; Herold, D. A. *Anal. Chem.* **1989**, *61*, 1099.
- (U16) Call, J. P. *Clin. Chem.* **1975**, *30*, 1800-3.
- (U17) National Committee for Clinical Laboratory Standards. *Development of definitive methods for the National Reference System for the Clinical Laboratory, Approved Guideline*; NCCLS Publication NRSL1-A; NCCLS: 771 E. Lancaster Ave., Villanova, PA 19085, 1991.
- (U18) National Committee for Clinical Laboratory Standards. *Development of reference methods for the National Reference System for the Clinical Laboratory, Approved Guideline*; NCCLS Publication NRSL2-A; NCCLS: Villanova, PA, 1991.
- (U19) National Committee for Clinical Laboratory Standards. *Development of certified reference materials for the National Reference System for the Clinical Laboratory, Approved Guideline*; NCCLS Publication NRSL3-A; NCCLS: Villanova, PA, 1991.
- (U20) National Committee for Clinical Laboratory Standards. *Development of methodological principles documents for the National Reference System for the Clinical Laboratory, Tentative Guideline (1989)*; NCCLS Publication NRSL6-T. NCCLS: Villanova, PA, 1991.
- (U21) National Committee for Clinical Laboratory Standards. *Nomenclature and Definitions for Analytes in the National Reference System for the Clinical Laboratory, Proposed Guideline (1985)*; NCCLS Publication NRSL8-P; NCCLS: Villanova, PA, 1991.
- (U22) Taylor, B. N., Ed. *The International System of Units (SI)*. *Natl. Inst. Stand. Tech. (U.S.) Spec. Publ.* **1991**, No. 330.
- (U23) Trahey, N. M., Ed. *Standard Reference Materials Catalog 1982-1993*. *Natl. Inst. Stand. Tech. (U.S.) Spec. Pub.* **260**. National Institute of Standards and Technology: Gaithersburg, MD 20899, 1992.
- (U24) National Committee for Clinical Laboratory Standards. *Internal Quality Control Testing: Principles and Definitions; Approved Guideline (1991)*; NCCLS Publication C24-A. NCCLS: 771 E. Lancaster Ave., Villanova, PA 19085, 1991.
- (U25) 1992 CAP Surveys, Chemistry Surveys-Set C3-C, Set C4-C, and Set C4-C. College of American Pathologists, Northfield, IL 60093-2750.
- (U26) Communication from: Ms. Jennifer Colbert, Project Manager, Standard Reference Materials Program, National Institute of Standards and Technology, Gaithersburg, MD 20899.
- (U27) Call, J. P.; Mandel, J.; Moore, L. J.; Young, D. S. *Natl. Bur. Stand. (U.S.) Spec. Publ.* **1972**, 260-36.
- (U28) Velapoldi, R. A.; Paule, R. C.; Schaffer, R.; Mandel, J.; Machlan, L. A.; Gramlich, J. W. *Natl. Bur. Stand. (U.S.) Spec. Publ.* **1979**, 260-83.
- (U29) Velapoldi, R. A.; Paule, R. C.; Schaffer, R.; Mandel, J.; Murphy, T. J.; Gramlich, J. W. *Natl. Bur. Stand. (U.S.) Spec. Publ.* **1979**, 260-67.

- (U30) Velapoldi, R. A.; Paule, R. C.; Schaffer, R.; Mandel, J.; Machian, L. A.; Garner, E. L.; Rains, T. C. *Natl. Bur. Stand. (U.S.) Spec. Publ.* **1980**, 260-69.
- (U31) Heumann, K. G. In *Inorganic Mass Spectrometry*, Adams, F., Gijbels, R., Van Grieken, R., Eds.; John Wiley and Sons, New York, 1988; Chapter 7.
- (U32) Fassett, J. D.; Paulsen, P. J. *Anal. Chem.* **1989**, *61*, 643A.
- (U33) Paulsen, P. J.; Beary, E. S.; Bushee, D. S.; Moody, J. R. *Anal. Chem.* **1989**, *61*, 827.
- (U34) Moody, J. R. *Anal. Chem.* **1982**, *54*, 1358A.
- (U35) Moore, L. J.; Machian, L. A.; Shields, W. R.; Garner, E. L. *Anal. Chem.* **1974**, *46*, 1082.
- (U36) Lamberty, A.; DeBievre, P. *Int. J. Mass Spectrom. Ion Processes* **1988**, *83*, 135.
- (U37) Lamberty, A.; Michiels, E.; DeBievre, P. *Int. J. Mass Spectrom. Ion Processes* **1987**, *79*, 311.
- (U38) Chan, L. H. *Anal. Chem.* **1987**, *59*, 2662.
- (U39) Xiao, Y. K.; Beary, E. S. *Int. J. Mass Spectrom. Ion Processes* **1989**, *94*, 110.
- (U40) Catanzaro, E. J.; Murphy, T. J.; Garner, E. L.; Shields, W. R. *J. Res. Natl. Bur. Stand., Sect. A* **1986**, *70A*, 453.
- (U41) Garner, E. L.; Machian, L. A.; Gramlich, J. W.; Moore, L. J.; Murphy, T. J.; Barnes, I. L. *Natl. Bur. Stand. (U.S.) Spec. Publ.* **422**, 1978, 951-80.
- (U42) Lee, T.; Papanastassiou, D. A.; Wasserburg, G. J. *Geochim. Cosmochim. Acta* **1977**, *41*, 1473.
- (U43) Gramlich, J. W.; Machian, L. A.; Brletic, K. A.; Kelly, W. R. *Clin. Chem.* **1982**, *28*, 1309.
- (U44) Moore, L. J.; Machian, L. A. *Anal. Chem.* **1972**, *44*, 2291.
- (U45) Vocke, R. D.; Murphy, K. E.; Paulsen, P. J. Proceedings, 40th ASMS Conference on Mass Spectrometry and Allied Topics, Washington, DC, 1992, p 798.
- (U46) Lawson, A. M.; Gaskell, S. J.; Hjelm, M. J. *Clin. Chem. Clin. Biochem.* **1985**, *23*, 433-41.
- (U47) De Leenheer, A. P.; Thienpont, L. M. *Mass Spectrom. Rev.* **1992**, *11*, 249-307.
- (U48) De Leenheer, A. P.; Thienpont, L. M. *Int. J. Mass Spectrom. Ion Processes* **1992**, *116/119*, 723-37.
- (U49) Schaffer, R. *Pure Appl. Chem.* **1976**, *48*, 75-9.
- (U50) Cohen, A.; Hertz, H. S.; Mandel, J.; Paule, R. C.; Schaffer, R.; Sniegowski, L. T.; Sun, T.; Welch, M. J.; White, E. V. *Clin. Chem.* **1980**, *28*, 854-60.
- (U51) Welch, M. J.; Cohen, A.; Hertz, H. S.; Ruegg, F. C.; Schaffer, R.; Sniegowski, L. T.; White, E. V. *Anal. Chem.* **1984**, *56*, 713-9.
- (U52) Ellerbe, P.; Cohen, A.; Welch, M. J.; White, E. V. *Clin. Chem.* **1988**, *34*, 2280-2.
- (U53) White, E. V.; Welch, M. J.; Sun, T.; Sniegowski, L. T.; Schaffer, R.; Hertz, H. S.; Cohen, A. *Biomed. Mass Spectrom.* **1982**, *9*, 395-405.
- (U54) Ellerbe, P.; Melselman, S.; Sniegowski, L. T.; Welch, M. J.; White, E., *Anal. Chem.* **1989**, *61*, 1718-23.
- (U55) Ellerbe, P.; Sniegowski, L. T.; Welch, M. J.; White, E., *V J. Agric. Food Chem.* **1989**, *37*, 954-7.
- (U56) Ellerbe, P.; Meyers, G. L.; Cooper, G. R.; Hertz, H. S.; Sniegowski, L. T.; Welch, M. J.; White, E., *V Clin. Chem.* **1990**, *36*, 370-5.
- (U57) Schaffer, R.; Sniegowski, L. T.; Welch, M. J.; White, E. V.; Cohen, A.; Hertz, H. S.; Mandel, J.; Paule, R. C.; Svensson, L.; Björkhem, I.; Blomstrand, R. *Clin. Chem.* **1982**, *28*, 5-8.
- (U58) Kroll, M. H.; Chesler, R.; Elin, R. J. *Clin. Chem.* **1989**, *35*, 1523-6.
- (U59) Welch, M. J.; Cohen, A.; Hertz, H. S.; Ng, K. W.; Schaffer, R.; Van Der Lijn, P. White, E., *V Anal. Chem.* **1986**, *58*, 1681-5.
- (U60) Unpublished work in progress, National Institute of Standards and Technology.
- (U61) Ellerbe, P.; Cohen, A.; Welch, M. J.; White, E., *V Anal. Chem.* **1990**, *62*, 2173-7.
- (U62) Schaffer, R.; Mandel, J.; Sun, T.; Cohen, A.; Hertz, H. S. *Natl. Bur. Stand. (U.S.) Spec. Publ.* **1982**, 260-80.