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Standard Reference Materials®

Compilation of NIST Higher-Order Methods for the Determination of Electrolytes in Clinical Materials

Stephen E. Long
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NIST National Institute of Standards and Technology • Technology Administration • U.S. Department of Commerce

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Abstract

This manual comprises higher-order analytical methods developed and used at the National Institute of Standards and Technology (NIST) for the determination of electrolytes in clinical materials. Higher order methods are used to certify Standard Reference Materials (SRM) such as the 909, 956 (blood serum) and 2670 (Urine) series for clinical analytes and to provide a basis for evaluating the accuracy and comparability of other reference methods. Several of these methods are listed in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database. The manual comprises comprehensive descriptions, evaluation and method performance data for the major electrolytes calcium, chloride, lithium, magnesium, potassium and sodium.

Foreword

Higher order or "definitive" methods have been in use at NIST for several decades to establish certified values for electrolytes and other trace elements in clinical Standard Reference Materials (SRMs). Although some of these methods have been described in various NIST 260 series documents, there has not been, until now, a single information resource providing comprehensive descriptions of the methods in a format which facilitates systematic updates for new methods, performance improvements to existing methods and removal of methods which are considered obsolete. This manual attempts to address this issue and provides a unified collection of all methods currently in use within the Analytical Chemistry Division for the measurement of electrolytes in clinical samples. This resource should prove useful not only for personnel within the Division but also to external customers who are involved in clinical testing. For example, the manual provides comprehensive information on the traceability basis of methods used to certify higher-order reference materials, which are an essential component of the European Union In-Vitro Diagnostic Medical Devices Directive (IVD), which became effective in December 2003.

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Please send any comments or notification of errors and omissions found in this document to selong@nist.gov.

Glossary of Terms Used in This Manual

Accuracy: The degree of agreement of the measured test result with the true value or accepted reference value.

Certified Reference Material (CRM): A reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence [ISO Guide 30, Terms and Definitions Used in Connection with Reference Materials:1992 and ISO International Vocabulary of basic and General Terms in Metrology (VIM):1993].

Electrolyte: Positively and negatively charged species present in extra-cellular and intra-cellular systems, plasma and fluids. Electrolytes are responsible for maintaining various critical functions within the body.

Inductively Coupled Plasma – Mass Spectrometry (ICP-MS): Instrumental technique in which the analyte is ionized in an argon plasma and the ions separated and measured by a mass spectrometer as a function of their mass to charge ratio.

Isotope Dilution: A highly accurate method in which a separated isotope of the analyte of interest is added to the sample and the perturbed isotopic composition measured. The analyte concentration can be calculated from the perturbed isotope ratios and the known mass of added spike.

National Metrology Institute (NMI): Nationally designated laboratory considered to have the greatest competence in the measurement of physical and chemical quantities using the highest-order metrological measurement methods available.

NIST: Is an agency of the Department of Commerce. Its role as the National Metrology Institute (NMI) for the United States is to develop, and maintain custody of the national standards of measurement, to provide the means and methods for making measurements consistent with those standards and to assure the compatibility of United States national measurement standards with those of other nations.

NIST SRM Certificate: Document stating the intended purpose and application of an SRM, its certified property value(s) with associated uncertainty(ies), and any other technical information deemed necessary for its proper use. In accordance with ISO Guide 31:1996, a NIST SRM certificate bears the logo of the U.S. Department of Commerce, the name of NIST as certifying body, and the name and title of the NIST officer authorized to accept responsibility for its contents.

Plasma: The liquid component of blood, containing suspended blood cells and platelets.

Precision: A measure of the dispersion of the sample distribution.

Procedure Blank: A measure of the amount of the analyte contributed to the sample aliquot from the measurement process. The procedure blank is an absolute quantity.

Reference Material: Material or substance, one or more of whose property values are sufficiently homogeneous to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials [ISO VIM:1993].

Serum: Liquid component of plasma with clotting factors removed.

Standard Reference Material (SRM): A CRM issued by NIST that also meets additional NIST-specific certification criteria and is issued with a certificate or certificate of analysis that reports the results of its characterizations and provides information regarding the appropriate use(s) of the material [NIST SP 260-136].

Thermal Ionization Mass Spectrometry (TIMS): Mass spectrometric technique in which the sample is loaded onto a metal filament (usually rhenium) and ionized by heating the filament to a high temperature. A very stable ion beam is produced which can be processed by the mass spectrometer.

Traceability: The property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons, all having stated uncertainties [ISO VIM:1993].

NIST Standard Reference Materials® (SRMs)

One of the primary functions of the Inorganic Chemical Metrology Group of the NIST Analytical Chemistry Division is to assign values for target analytes in Standard Reference Materials for chemical measurements. These measurements are principally carried out by using higher-order or definitive methods. Reference methods are also used for some measurements. Values appearing on certificates fall into three categories [NIST SP 260-136]:

NIST Certified Value - Value and its uncertainty assigned by NIST in conformance with the NIST uncertainty policy. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been fully investigated and accounted for by NIST. A certified value is obtained by one or more of the following measurement modes:

1. Certification at NIST using a single primary method with confirmation by other method(s).
2. Certification at NIST using two independent critically-evaluated methods.
3. Certification/value assignment using one method at NIST and different methods by outside collaborating laboratories.

NIST Reference Value – Is a best estimate of the true value provided by NIST where all known or suspected sources of bias have not been fully investigated by NIST.

NIST Information Value – Is a value that will be of interest and use to the SRM/RM user, but insufficient information is available to assess the uncertainty associated with the value.

For additional information see
http://ts.nist.gov/ts/htdocs/230/232/ABOUT/definitions.htm

Contents

1 Introduction

1.1 Clinical Electrolyte Measurements

Determinations of electrolytes in serum and urine samples are among the most commonly ordered panels for clinical diagnosis and point-of-care testing. Electrolytes in body fluids occur either as free ions or partly bound to proteins. The maintenance of correct electrolyte concentrations in body fluids is vital and disorders in electrolyte balance are used to diagnose changes in health status (such as renal and metabolic function) and fluid balance. The determination of the major electrolytes in body fluids within the clinical laboratory setting is therefore well established. Traditionally, inductively coupled plasma emission spectrometry (ICP-AES) or flame photometry have been used, but electrochemical systems, especially automated ion-selective electrode (ISE) systems which can rapidly measure a panel of electrolytes, have gained in popularity. Methods based on spectrophotometry are becoming less common than they once were but are still important for some electrolytes such as the determination of total calcium and magnesium in serum.

1.2 The IVD Directive

In an effort to unify the regulatory status of the medical device and diagnostics industry, the In-Vitro Diagnostics (IVD) Directive was adopted by the European Union Council of Ministers on October 5, 1998, and formally published on December 7, 1998. This, the last of three directives in this industry, mandates a *Conformite Europeene* (CE) marking system for manufacturers of diagnostic medical devices which encompass reagents, calibrators and instruments intended for ascertaining the status of patient health, and which are destined for sale in the European Union. The Directive came into effect five years later in December 2003. One important requirement of the IVD Directive is the provision of a risk analysis. It must be demonstrated that products are suitable for their intended purpose and that the risks associated with their use be fully evaluated and mitigated as far as possible within the limits of the current state of the art knowledge. Another important requirement is that "the traceability of values assigned to calibrators and/or control materials must be assured through available reference measurement procedures and /or reference materials of a higher order." It is predicted that this requirement will increase demand for a variety of appropriate certified clinical reference materials from National Metrology

Institutes (NMIs). For example, sales of the NIST material SRM 956a (Electrolytes in Frozen Human Serum) increased dramatically at the time the IVD Directive came into effect.

1.3 JCTLM

The Joint Committee on Traceability in Laboratory Medicine (JCTLM) was formed in June 2002. The aim of the Joint Committee is to meet the need for a worldwide platform to promote and give guidance on comparability, reliability and equivalence of measurement results in Laboratory Medicine and traceability to appropriate measurement standards for the purpose of improving health care. The four principal promoters of the JCTLM are the International Bureau of Weights and Measures (BIPM), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), the International Laboratory Accreditation Cooperation (ILAC) and the World Health Organization (WHO). Several other key stakeholders are also involved including reference material producers, representatives of the IVD industry, quality assurance organizations and the International Organization for Standardization (ISO). The JCTLM is currently divided into two working groups, one assigned to reference measurement methods and reference materials, with the purpose of identifying reference materials and reference procedures and endorsing those appropriate to meet the requirements of the IVD Directive, the other responsible for collecting information on existing and candidate reference measurement laboratories and to encourage and facilitate the formation of networks of laboratories for different groups of measurable quantities.

1.4 NIST Clinical Measurement Methods - Philosophy and Trends

Where possible, NIST clinical measurement methods for electrolytes have traditionally employed isotope dilution mass spectrometry [1]. When correctly used, this is a highly accurate analytical method with direct traceability to the mole. Before the advent of inductively coupled plasma mass spectrometry (ICP-MS), isotope dilution thermal ionization mass spectrometry (TIMS) was widely employed for these measurements [2]. While this offers extremely good isotope ratio measurement repeatability, sample throughput tends to be rather slow when using the NIST designed solid-source mass spectrometers. Therefore, in common with many other laboratories, NIST clinical measurement methods have gradually been transferred to ICP-MS systems which offer higher sample throughput and more cost-effective measurement options. The trade-off in slightly poorer isotope ratio measurement

repeatability is not a significant disadvantage for clinical reference material certification. The emergence of "cool" (shielded) plasma, and more recently, collision cell ICP-MS systems have now permitted some of the more problematic elements such as calcium and potassium to be accurately measured. All of the common clinical electrolytes are now measured by ICP-MS, with the exception of chloride, which currently still remains the domain of TIMS.

1.5 About This Manual

As a principal stakeholder in the JCTLM, NIST has several assigned responsibilities which include the maintenance of primary measurement methods and reference materials of a higher metrological order, participation in global reference laboratory networks, providing reference laboratory measurement services and working with other NMIs and organizations to establish equivalence of measurement services. This manual provides a collection of higher-order methods currently used at NIST for the measurement of electrolytes in clinical materials. The manual has been organized in such a way that the methods can be updated with revised versions, newer methods added, and obsolete methods deleted as necessary to meet these responsibilities. The manual is divided into a section for each of the principal electrolytes measured at NIST. Each section comprises a brief summary of the electrolyte, the context of clinical measurement together with detailed descriptions of all analytical measurement methods currently in active use at NIST.

1.6 References

[1] Bowers G.N., Fassett J.D. and White V.E., "*Isotope Dilution Mass Spectrometry and the National Reference System.*" Clin. Chem. **65**, pp. 475R-479R (1993).

[2] Moore L.J. and Machlan L.A., *"High Accuracy Determination of Calcium in Blood Serum by Isotope Dilution Mass Spectroscopy."* Anal. Chem. **44**, p. 2291 (1972).

2 Calcium

2.1 Clinical Significance

The total calcium body content in humans is approximately 1 kg, the majority of which is localized in the skeletal system as hydroxyapatite. A small amount of calcium is present in both the intra-cellular and extra-cellular fluid compartments, with approximately 50 % of the extra-cellular calcium present in blood plasma. About 40 % of plasma or serum calcium is bound to proteins (mostly to albumin), 10 % is complexed with inorganic ions and the remainder is so-called ionized calcium. Approximately 15 % of the total serum calcium is in a biologically active free form. Calcium has many critically important functions aside from its structural role, among which are the proper functioning of the neuromuscular system, myocardial contractility, the structure of cell membranes and the production of clotting factors. The level of calcium in plasma, therefore, is under strict homeostatic control. Serum calcium levels are regulated by a complex mechanism involving renal excretion and tubular renal re-absorption. Parathyroid hormone (PTH) stimulates re-absorption and therefore increases calcium activity. Calcitonin on the other hand, reduces the calcium activity by inhibiting the osteoclastic release of calcium and phosphate from bone to the extra-cellular fluid and decreases renal re-absorption. In *hypocalcemia*, calcium serum levels are depressed. Two common causes are renal failure and pancreatitis. *Hypercalcemia*, a condition where serum calcium is elevated, is commonly encountered in hyperparathyroidism, where too much PTH is produced by the parathyroid gland.

2.2 Normal Range and Reporting Units

The normal range for total calcium in serum or plasma is 9.2-11.0 mg/dL in children aged 4 to 20, and 8.8-10.2 mg/dL in adults, with a mean of 9.4 mg/dL. The reference interval for ionized calcium in adults is about 4.7-5.3 mg/dL. Calcium clinical results are normally reported in mg/dL.
To convert from mmol/L to mg/dL multiply by 4.00780.
To convert from mg/dL to mmol/L multiply by 0.24951.

2.3 Routine Clinical Measurement Methods

Blood and urine are the most commonly used media for assessing patient health status. For the measurement of total calcium, flame atomic absorption

spectrometry (FAAS), using the 422.7 nm resonance line, has been the accepted reference method, however, in the routine clinical laboratory, colorimetric methods adapted for use on automated analyzer systems are more commonly found. One of these involves reaction with *o*-cresolphthalein complexone in a buffered solution, which produces an intense red-purple color at 570 nm. The method exhibits linearity up to about 20 mg/dL. A more recent method utilizes the reaction with arsenazo III, which purportedly provides better specificity, sensitivity, stability and freedom from interferences than the *o*-cresolphthalein method. The calcium complex produces a blue-purple color at around pH 8, which is measured at 650 nm. The method is also linear up to approximately 20 mg/dL, which reduces the need for excessive sample dilutions.

2.4 Available Standard Reference Materials

Two blood serum SRMs are available with certified values for calcium, which are **SRM 909b (*Human Serum*)**, a two-level lyophilized material, and **SRM 956b (*Electrolytes in Frozen Human Serum*)**, which is a three-level frozen material intended for standardizing and calibrating ion selective electrode analyzers. There is also a reference value for calcium in **SRM 2670a (*Toxic Elements in Freeze-Dried Urine*)**, a two level, freeze-dried material. **SRM 915b (*Calcium Carbonate, Clinical Standard*)** is sold as a clinical assay standard which is intended for the purpose of instrument calibration and standardization. Certificate information for these SRMs is summarized in Table 2-1.

Table 2-1. Clinical Standard Reference Materials for Calcium

SRM	Description	Value (mg/dL)	Expanded Uncertainty (mg/dL)
909b Level 1	Human Serum	8.890	0.063
909b Level 2	Human Serum	14.16	0.11
956b Level 1	Electrolytes in Frozen Human Serum	11.82	0.08
956b Level 2	Electrolytes in Frozen Human Serum	9.844	0.059
956b Level 3	Electrolytes in Frozen Human Serum	7.911	0.053
2670a Level 1	Toxic Elements in Urine (Freeze-Dried)	(2.9)	(0.2)
2670a Level 2	Toxic Elements in Urine (Freeze-Dried)	(3.0)	(0.2)
SRM	**Clinical Standard**	**Purity (% mass fraction)**	**Expanded Uncertainty (%)**
915b	Calcium Carbonate (Clinical Standard)	99.907	0.021

( ) Values in Parentheses are Reference Values

Version Number	1.0
Approved	S E Long
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CA-1
TOTAL CALCIUM BY ISOTOPE DILUTION INDUCTIVELY COUPLED PLASMA – MASS SPECTROMETRY

1. Applicability

The method is applicable to the determination of total calcium at physiological levels in blood plasma, serum (frozen and lyophilized) and urine.

2. Principle of Measurement

The method is based on isotope dilution mass spectrometry using a quadrupole inductively coupled plasma – mass spectrometer (ICP-MS) operated in "cool plasma" (shielded) mode [1]. A stable isotopic spike of ^{42}Ca is added to the sample, equilibrated by oxidation with nitric acid in a microwave digestion system, and the $^{40}Ca/^{42}Ca$ isotope ratio measured using low power plasma conditions. The method is extremely accurate and selective for calcium.

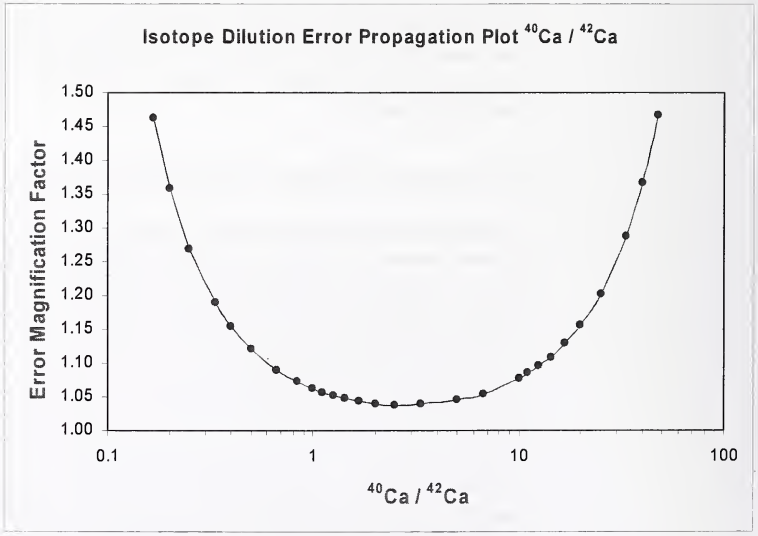
3. Measurement Strategy and Experimental Design

The determination of calcium in biological fluids by isotope dilution ICP-MS is not simple because of the presence of potential spectral interferences from ^{40}Ar, ^{40}K , $^{23}Na^{16}O^{1}H$ and $^{24}Mg^{16}O$ which interfere with ^{40}Ca, and $^{40}Ar^{1}H_2$ and $^{26}Mg^{16}O$, which interfere with ^{42}Ca. The use of "cool" or shielded plasma conditions effectively attenuates the Ar-based spectral background interferences. The other interferences are minor, except for the $^{23}Na^{16}O^{1}H$ interference which is significant because of the relatively high level of sodium in biological fluids. For this reason, a chemical separation of calcium and sodium is necessary. For convenience, potassium (which is similarly afflicted) and calcium can be separated, re-combined and measured together. The isobaric interference from ^{40}K on ^{40}Ca is negligible for the potassium levels normally found in biological fluids. Treatment of the sample with nitric acid in a microwave digestion system is necessary to oxidize

protein-bound calcium. Normally, ^{42}Ca is used as the spike, which is available in approximately 95 % isotopic purity.

The amount of spike added to the sample prior to digestion needs to be optimized. The error propagation has been calculated and plotted in Figure 3-1 for a typical measurement system employing a ^{42}Ca spike of 95 % purity. The calculated error magnification factor (EM) is at a minimum (EM = 1.04) for a $^{40}Ca/^{42}Ca$ ratio of 2.8. However, although this is the ideal ratio, it is considered better to overspike the system to yield an isotope ratio closer to unity. Under these conditions, the effects of detector dead-time and pulse counting statistics, which are important aspects of ICP-MS measurement systems, are minimized. As is evident from Figure 3-1, at a ratio of approximately unity, the EM factor is about 1.06, which is acceptable. An additional approach to measurement is to closely match the ratios of the spike calibration mixtures to the sample measurement mixtures, and run them in the same temporal measurement space. This requires a fairly accurate knowledge of the concentration of calcium in the unknown sample prior to spiking.

FIGURE 3-1



4. Safety

Clinical fluids are biohazardous materials which may contain pathogenic substances. The usual safety precautions should be taken to prevent exposure during handling, including the use of gloves at all times. Although most materials will have been screened for such substances, a clinical monitoring regimen, including screening for HIV 1 and 2 and vaccination against hepatitis B is recommended for personnel handling biological specimens on an extended basis.

5. Required Reagents

5.1 ^{42}Ca Isotopic Spike

A spike having an isotopic purity ≥ 95 % can be obtained through Oak Ridge National Laboratory (ORNL) or other stable-isotope supplier in a form such as $^{42}CaCO_3$. A stock solution can be prepared by dissolving a small amount of the $^{42}CaCO_3$ in 2 % (volume fraction) high-purity nitric acid to yield a concentration of about 50 µg/g (1.2 µmol/g) and storing in a cleaned Teflon® fluoropolymer bottle. Tracking the tare weight of the bottle is useful for long-term storage and monitoring the concentration of the spike on a multiple-use basis.

5.2 SRM 915b, Calcium Carbonate, Clinical Standard

The material should be dried according to the directions on the certificate in a laboratory oven at 200 °C - 210 °C for 4 h.

The certified purity of the material is 99.907 % (mass fraction) with an expanded uncertainty of 0.021 %. The relative mass fraction of calcium in SRM 915b adjusted for the purity is 0.400104.

Alternatively, Calcium Standard Solution (SRM 3109a), which has a concentration of 10.025 mg/g ± 0.017 mg/g (0.2 % relative) can be used.

5.3 High-Purity Nitric Acid

High-purity acid is essential to minimize the extent of calcium contamination from reagent sources. High-purity acid containing less than 100 pg/g calcium may be obtained from a number of commercial suppliers.

5.4 High-Purity Hydrochloric Acid

For chemical separation of calcium from sample matrix. High-purity acid is essential to minimize the extent of calcium contamination from reagent sources. High-purity acid containing less than 100 pg/g calcium may be obtained from a number of commercial suppliers.

5.5 High-Purity Water

De-ionized or quartz-distilled water, which has been tested and is low in calcium contamination. High-purity water is used for sample dilution. At a minimum, the water should meet or exceed the specifications of CAP/NCCLS Type I water [2].

5.6 Ion-Exchange Resin

AG 50-X8, 100-200 mesh (75-150 µm) cation-exchange resin, or equivalent for chemical separation of calcium from the sample matrix. The resin should be bulk cleaned, packed into columns and batch cleaned twice by alternating between 25 mL, 5 mol/L hydrochloric acid and sufficient high-purity de-ionized water to achieve neutral pH.

6. Equipment

6.1 ICP-MS System

The method utilizes a standard ICP-MS system employing a quadrupole mass spectrometer and an automated shielded torch system operating at low RF forward power ("cool-plasma") for reducing argon-based spectral interferences. The instrument should be capable of operating in the pulse counting detection mode, should be fitted with a low dead-volume, cooled spray chamber and a high-efficiency pneumatic nebulizer system, if available.

6.2 Microwave Digestion System

A commercial system should be used which is capable of reaching at least 70 bar (7 MPa) vessel pressure and a maximum vessel temperature of at least 200 °C using either PTFE or quartz reaction vessels. The system should be fitted with industry-standard safety interlocks. A continuous (un-pulsed) variable power delivery system is advantageous in maintaining optimum reaction control.

6.3 Teflon® Fluoropolymer Beakers

Clean 50 mL Teflon® fluoropolymer beakers for solution evaporation prior to column separations.

6.4 Ion-Exchange Columns

Clean plastic or glass ion-exchange columns containing a cation-exchange resin bed (see Reagents) of approximate dimensions 10 cm long x 0.7 cm wide. This bed should contain sufficient capacity and column resolution for most clinical samples.

6.5 Sample Bottles

Clean 30 mL polyethylene (Nalgene) sample bottles (or equivalent material) for sample storage and analysis.

> **Note on Sample Bottles**: Polyethylene or Teflon® fluoropolymer sample bottles formulated for low trace-metal content should be used. These contain very low levels of electrolyte contaminants. Containers such as polypropylene centrifuge tubes should not be used for this purpose, as they frequently contain appreciable levels of contaminants. Clean the sample bottles prior to use by soaking in dilute nitric acid for a minimum of 24 h.

6.6 Micro-Pipette

Automatic or manual micro-pipette for dispensing sample, spike and standard aliquots. A capped plastic syringe fitted with a Teflon® fluoropolymer uptake tube may also be used for this purpose, depending on the weighing method employed.

6.7 Analytical Balance

Five-place analytical balance for weighing sample, spike and standard aliquots. The balance should be calibrated and verified to be functioning correctly prior to weighing measurements.

7. Sample Handling, Preparation and Quality Control

7.1 Analytical Sample

The analytical sample should be equilibrated to room temperature prior to measurement. Follow supplier instructions for reconstitution of lyophilized material or equilibration from freezer temperature. All sample preparation should be carried out in a Class 10 clean environment with the use of protective clothing and gloves.

7.2 Aliquoting and Acidification

Approximately 0.7 g of the liquid sample should be weighed accurately, using a five-place analytical balance, into a cleaned microwave digestion vessel (manufactured from quartz or Teflon® fluoroploymer) and spiked with an aliquot of ^{42}Ca stock (approximately 1.3 g for normal blood serum) to yield a $^{40}Ca/^{42}Ca$ ratio of approximately unity. Six grams of high-purity nitric acid should be added to each vessel and the vessels then sealed using the appropriate procedure specified by the manufacturer.

7.3 Digestion

The prepared solutions should be placed into a high-pressure microwave digestion system and digested using the manufacturers recommended conditions, or user-developed digestion protocol. A vessel pressure approaching 70 bar (7 MPa) for at least 30 min will usually ensure complete oxidation of the sample, and conversion of all carbon in the sample to carbon dioxide.

7.4 Evaporation and Chemical Separations

The digested samples should be evaporated to near dryness using the microwave digestion system (if this is a feature), or alternatively by heating on a hot-plate in 50 mL Teflon® fluoropolymer beakers. Re-dissolve in a small quantity of dilute 2 % (volume fraction) nitric acid. Transfer to cation-exchange columns. Elute the sodium using approximately 40 mL of 0.3 mol/L hydrochloric acid. Elute the potassium with approximately 20 mL of 0.5 mol/L hydrochloric acid, elute the magnesium with approximately 20 mL of 1 mol/L hydrochloric acid and finally elute the calcium fraction with approximately 20 mL of 1.75 mol/L hydrochloric acid. Evaporate the calcium fraction to dryness and re-dissolve in 2 % (volume fraction) nitric acid to a concentration suitable for ICP-MS measurement. Store in clean 30 mL polyethylene bottles.

7.5 Stability

The prepared solutions are stable for several days. However, determinations should be carried out as soon as possible to avoid the risk of contamination, losses or stability issues.

7.6 Spike Calibration Mixtures

The concentration of the ^{42}Ca spike stock solution must be measured by preparing at least four independent spike calibration mixtures. Prepare two different stock calibration solutions of natural calcium from SRM 915b or SRM 3109a. Prepare two spike calibration mixtures from each of the natural calcium calibration solutions by aliquoting known amounts of the calibration stock and ^{42}Ca spike into sample bottles and diluting with 2 % (volume fraction) nitric acid to a concentration suitable for measurement by ICP-MS. The aliquots should be adjusted to yield $^{40}Ca/^{42}Ca$ isotope ratios as similar as possible to the analytical samples. A five-place analytical balance should be used for all weighing operations.

7.7 Procedure Blanks

Prepare a minimum of three procedure blanks by adding approximately 100 ng of the ^{42}Ca spike to microwave digestion vessels and carrying them through the entire preparation and analysis procedure.

7.8 Composition Sample

Prepare a composition sample by aliquoting approximately 0.7 g of sample into a microwave digestion vessel and carrying it through the same preparation procedure as the samples. The composition sample is not spiked with ^{42}Ca and is used to assess the presence or absence of spectral interferences in the ICP-MS measurement process.

7.9 Controls

To assess the accuracy of the measurements, prepare at least two different SRM control materials having certified values for calcium, preferably of the same matrix type as the samples, and carry the controls through the same sample preparation and measurement procedures as the samples.

8. Analytical Measurement

8.1 Instrument Preparation

Prior to analytical measurements, the ICP-MS system should be equilibrated for 30 min. The sample introduction system should be rigorously cleaned before use, including the sampler and skimmer cones, peristaltic pump tubing, plasma torch and spray chamber. These procedures are designed to mitigate the background from calcium as much as possible. It is recommended that new peristaltic pump tubing be installed prior to measurements. A dilute nitric acid solution (2 % volume fraction) should be pumped through the tubing for a while to remove any leachable calcium from the tubing.

8.2 Instrument Optimization

The instrument should be optimized for maximum ion transmission and minimum background at m/z 40 (maximum signal to background ratio) using a standard solution of calcium and adjusting ion lens voltages, plasma gas flows, torch position (relative to the sample cones) and rf forward power.

8.3 Run Sequence

Software acquisition menus should be prepared for measuring the ^{40}Ca and ^{42}Ca isotopes. The run order for the samples should be established according to the following protocol:

Analytical procedure blanks should be run at the beginning of the sequence, followed by the composition sample and then the analytical samples (including control materials) in randomized order. Instrument mass discrimination measurement standards (*see note*) should be run after every three to four samples depending on the stability of the instrument.

> **Note on Mass Discrimination Measurement:** The measured $^{40}Ca/^{42}Ca$ ratios should be corrected for the ICP-MS mass discrimination. This can be achieved by measurement of a calibration standard (SRM 915b) consisting of natural calcium. However, the natural ratio of $^{40}Ca/^{42}Ca$ is approximately 150, which is very different from the measured sample ratios. A better way to assess discrimination and measurement run discrimination drift is to carefully calibrate a solution matched more closely to the samples. A spike calibration solution can conveniently be used for this purpose.

8.4 Isotope Ratio Acquisition

The isotope ratio $^{40}Ca/^{42}Ca$ should be acquired in peak jumping mode. A typical acquisition scheme would be five to seven sets of ratios with a total acquisition time of 60 s per set, using a dwell-time of 10 ms per isotope. The isotope ratio data should be dead-time corrected and downloaded to a spreadsheet program such as Microsoft Excel. Inspect the ratios and remove any considered to be statistical outliers.

9. Data Reduction

9.1 Mass Discrimination Corrections

Convert the experimentally measured $^{40}Ca/^{42}Ca$ isotope ratios to absolute ratios using the following equation:

$$\left[\frac{^{40}Ca}{^{42}Ca}\right]_{CORR} = \frac{\left[\frac{^{40}Ca}{^{42}Ca}\right]_{TRUE}}{\left[\frac{^{40}Ca}{^{42}Ca}\right]_{MEAS}}$$

where, $\left[\frac{^{40}Ca}{^{42}Ca}\right]_{CORR}$ is the discrimination corrected sample ratio

$\left[\frac{^{40}Ca}{^{42}Ca}\right]_{TRUE}$ is the true ratio for the isotopic standard

$\left[\frac{^{40}Ca}{^{42}Ca}\right]_{MEAS}$ is the measured ratio for the isotopic standard

The measured ratios for the isotopic standard can be applied to sample discrimination corrections in a number of ways, which will depend on the discrimination drift as a function of time: (1) the ratio can be calculated by averaging the two standard measurements bracketing the samples of interest, (2) a more sophisticated approach is to mathematically model the drift in the standard ratio as a function of time and interpolate the ratio for each sample. This may require more frequent measurement of the isotopic standard to define the drift function adequately.

9.2 Spike Calibration

Calculate the concentration of the ^{42}Ca spike in each spike calibration mixture using the following equation:

$$C_{SPK} = \left[\frac{M_{STD}(BR - A)}{M_s K(A_s - B_s R)}\right]$$

where, C_{SPK} is the concentration of calcium in the spike in µg/g
M_{STD} is the mass of calcium added to the spike mix (µg)
B is the natural abundance of the spike isotope
R is the corrected $^{40}Ca/^{42}Ca$ ratio in the spike mix
A is the natural abundance of the reference isotope
M_S is the mass of ^{42}Ca spike aliquot added to the mix (g)
K is the natural to spike relative atomic mass ratio
A_S is the abundance of the reference isotope in the spike
B_S is the abundance of the spike isotope in the spike

Calculate the mean and standard deviation of the spike calibration mixtures. Blank corrections to the spike calibration are not normally necessary because they are simple mixtures prepared in dilute acid with minimal sample processing.

9.3 Calculation of Calcium Sample Concentrations

Calculate the calcium concentration (µg/g) in the sample using the following equation:

$$C = \left[\frac{M_s K(A_s - B_s R)}{(BR - A)} - S_B \right] \times \frac{1}{W}$$

where, **C** is the concentration of calcium in the sample (µg/g)
M_s is the absolute mass of ^{42}Ca spike added (µg)
K is the natural to spike relative atomic mass ratio
A_s is the abundance of the reference isotope in the spike
B_s is the abundance of the spike isotope in the spike
R is the corrected $^{40}Ca/^{42}Ca$ ratio
B is the natural abundance of the spike isotope
A is the natural abundance of the reference isotope
S_B is the absolute mean measured blank (µg)
W is the mass of sample aliquot taken (g)

An alternative approach, if desired, is to calculate the spike and sample concentrations in mol/g. In this case the equations are slightly modified and the relative atomic masses of natural calcium and the spike are not required for the calculations.

10. Uncertainty Analysis

The expanded uncertainty for the set of samples should be calculated according to ISO guidelines [3] by combining both Type A and Type B uncertainties. Typical Type A uncertainty components for this measurement might be the variability of the measured concentrations of calcium in the samples, the measurement of the spike concentration and the variability of the correction for the blank. Typical Type B uncertainty components might be the uncertainty of the assay of the primary calibrant, weighing measurements, conversion correction for the density of the sample and instrumental parameters such as detector dead-time correction, instrument mass discrimination correction and instrument background correction.

For a 95 % confidence level, combine the Type A and Type B components and calculate the total expanded uncertainty using the following equation:

$$U = k \left[s_1^2/df_1 + \ldots + s_n^2/df_n + B_1^2 + \ldots + B_n^2 \right]^{1/2}$$

where, *U* is the expanded uncertainty
k is the coverage factor
s is the observed standard deviation of (n) Type A components
df is the degrees of freedom associated with each component

B is the Type B component (n components) with infinite degrees of freedom.

Report the expanded uncertainty in the same units as the sample concentrations.

11. Data Reporting

Convert data from a mass/mass basis to a mass/volume basis using the measured density of the serum. **For clinical use, report calcium data in units of mg/dL**. To convert data from µg/mL to mg/dL divide by 10. To convert from mg/dL to mmol/L multiply by 0.24951.

12. Performance Statement

12.1 Measurement Repeatability

The isotope ratio measurement repeatability is usually limited by detector counting statistics. Typical measurement repeatability on a quadrupole ICP-MS system for a $^{40}Ca/^{42}Ca$ ratio approaching unity is 0.1-0.2 %.

12.2 Detection Limit

The instrument detection limit is influenced by the instrument background. A typical instrument detection limit for a well-optimized quadrupole ICP-MS instrument is 5 pg/mL. For a 0.7 g sample aliquot this equates to a matrix detection limit of approximately 0.04 ng/mL, which is far below the calcium concentrations typically found in clinical fluids.

12.3 Instrument Background and Procedure Blank

The ICP-MS instrument background tends to be very high for calcium owing to the direct isobaric interference of argon species. This can be very effectively reduced by means of "cool plasma" technology. It should be possible to reduce the background for ^{40}Ca to around 100 counts per second by judicious choice of operating conditions.

A typical procedure blank using this method is in the range 50-100 ng (1-2 nmol), depending on the purity of the reagents, cleanliness of containers and processing conditions. This is low in relation to the calcium content in clinical fluids. For example, using this method for the determination of calcium in blood serum, the relative blank correction would be ≤ 0.1 %.

12.4 Performance Summary

A summary of typical method performance parameters expected for use of this method are provided in Table 12-1.

Table 12-1: Method Performance Summary

Parameter	Typical Value or Range
Isotope ratio measurement repeatability	0.1-0.2 %
Applicable concentration range	All ranges found in clinical specimens
Instrument background / sample ratio	≈ 0.01 %
Procedure blank	≈ 50-100 ng
Sample throughput (measurement)	10 samples per hour

13. Reference Citations

1. Murphy K.E., Long S.E., Rearick M.S. and Ertas O.S., *"The Accurate Determination of Potassium and Calcium using Isotope Dilution Inductively Coupled "Cold" Plasma Mass Sectrometry."* J. Anal. At. Spectrom. **17**, pp. 469-477 (2002).

2. *Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline – Third Edition*, NCCLS Document C3-A3, NCCLS, Wayne, Pennsylvania (1997).

3. *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed. ISO, Switzerland, 1993.

CA-1 Revision History

Revision	Date	Author	Record of Changes and Comments
1.0	9/2006	S E Long	Initial draft method

3 Chloride

3.1 Clinical Significance

Chloride is the most abundant anion in extra-cellular fluid. The principle function is to provide charge neutrality. Therefore, the concentration of chloride is closely related to that of sodium as the major cation in extra-cellular fluid. Homeostatic maintenance of chloride is achieved through dietary intake and renal excretion. An elevation in the chloride concentration in serum (*hyperchloremia*) over normal levels may be caused by excessive intake, dehydration, renal failure and renal tubular acidosis. Correspondingly, a reduction in chloride activity over normal levels (*hypochloremia*) may be caused by excessive loss through sweating or vomiting or other conditions, such as metabolic alkalosis and Addison's disease.

3.2 Normal Range and Reporting Units

The normal range for chloride in serum or plasma is about 96-106 mmol/L. Clinical data for chloride are normally reported in mmol/L. The unit mEq/L is also sometimes used.
To convert from mmol/L to mg/dL multiply by 3.54527.
To convert from mg/dL to mmol/L multiply by 0.28207.

3.3 Routine Clinical Measurement Methods

Chloride is commonly measured in serum, plasma, urine and sweat. Several methods are used for the determination of chloride. Electrochemical methods are commonly employed which are based predominantly on amperometry/coulometry and ion-selective electrodes. Another common method is mercurimetric titration in which the chloride reacts with mercuric nitrate to form insoluble mercuric chloride. Diphenylcarbazone, which complexes with free Hg^{2+} to form a blue/violet color, is used as an indicator of the end-point. A spectrophotometric method, based on complexometry, utilizes a reaction of the chloride with mercuric thiocyanate to quantitatively release free thiocyanate. The free thiocyanate is reacted with Fe^{3+} to form a red complex at 480 nm.

3.4 Available Standard Reference Materials

Two primary blood serum SRMs are available with certified values for chloride which are **SRM 909b (*Human Serum*)**, a two-level lyophilized material, and **SRM 956b (*Electrolytes in Frozen Human Serum*)**, which is a three-level frozen material intended for standardizing and calibrating ion selective electrode analyzers. Two clinical assay standards may be used for chloride: **SRM 919a (*Sodium Chloride, Clinical Standard*)** and **SRM 918a (Potassium Chloride, Clinical Standard)** which are sold as clinical primary standards intended for instrument calibration and standardization. Certificate information for these SRMs is summarized in Table 3-1.

Table 3-1. Clinical Standard Reference Materials for Chloride

SRM	Description	Value (mmol/L)	Expanded Uncertainty (mmol/L)
909b Level 1	Human Serum	89.11	0.57
909b Level 2	Human Serum	119.43	0.85
956b Level 1	Electrolytes in Frozen Human Serum	99.01	0.66
956b Level 2	Electrolytes in Frozen Human Serum	111.88	0.82
956b Level 3	Electrolytes in Frozen Human Serum	126.85	0.81
SRM	**Clinical Standard**	**Purity (% mass fraction)**	**Expanded Uncertainty (%)**
919a	Sodium Chloride (Clinical Standard)	99.89	0.03
918a	Potassium Chloride (Clinical Standard)	99.9817	0.0084

( ) Values in Parentheses are Reference Values

Version Number	1.0
Approved	S E Long
Effective Date	Sep 2006

CL-1
CHLORIDE BY ISOTOPE DILUTION THERMAL IONIZATION MASS SPECTROMETRY

1. Applicability

The method is applicable to the determination of chloride at physiological and therapeutic levels in blood plasma, serum (frozen and lyophilized), sweat and urine.

2. Principle of Measurement

The method is based on isotope dilution mass spectrometry using a solid source magnetic sector thermal ionization mass spectrometer (TIMS). A stable isotopic spike of ^{37}Cl is added to the sample and the chloride precipitated as silver chloride, which is deposited onto a rhenium filament after re-dissolving in ammonia. The $^{35}Cl/^{37}Cl$ isotope ratio is measured in negative ion mode. The method is extremely accurate and very selective for chloride.

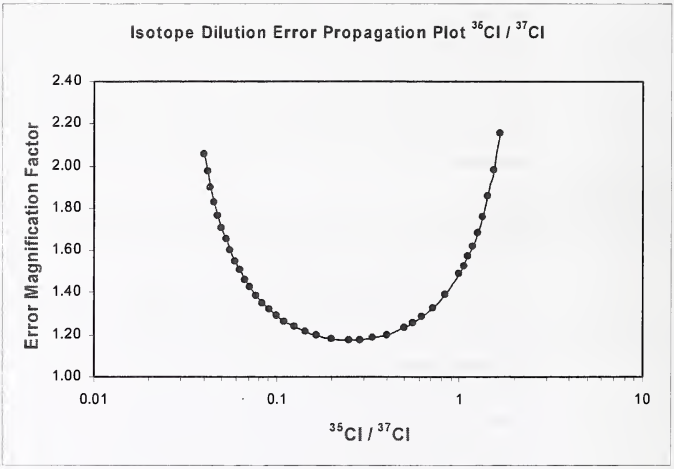
3. Measurement Strategy and Experimental Design

Thermal ionization mass spectrometry (TIMS) has been used at NIST since the late 1960s to determine a variety of analytes, including chloride, in clinical reference materials. Eventually TIMS will be superceded by inductively coupled plasma – mass spectrometry (ICP-MS) methods when suitable methodology is established. However, TIMS is still the principal analytical method for certification of chloride in NIST SRMs at the present time. The use of isotope dilution analysis for the determination of chloride is straightforward, except that, because of the relatively high first ionization potential of chlorine (13.01 V), the measurement is made using negative ion mode. The attainable analytical measurement repeatability is, however, slightly poorer than for positive ion measurements. The principal component of uncertainty in the method is the process blank, which is relatively high. The blank comes principally from reagent sources, but stringent precautions also need to be taken to reduce external contamination from the laboratory

environment. In order to maintain a high sample to blank ratio, the amount of chloride in the sample aliquot needs to be high. For clinical samples such as serum, a minimum of 0.3 g is recommended. As is typical with TIMS methods, the chloride needs to be chemically separated from the rest of the sample matrix, which is achieved by precipitating any protein from the material, and then adding silver chloride to precipitate the chloride as silver chloride. The silver chloride is re-dissolved in ammonium hydroxide and deposited onto rhenium filaments. The filaments are then mounted into the mass spectrometer as a triple filament assembly.

The amount of spike added to the sample prior to separation, needs to be optimized. The error propagation has been calculated and plotted in Figure 3-1 for a typical measurement system employing a ^{37}Cl spike of 98 % purity. The calculated error magnification factor (EM) is at a minimum (EM = 1.18) for a $^{35}Cl/^{37}Cl$ ratio of approximately 0.25. However, ratios in the range 0.06 to about 1.2 would also yield valid measurements.

FIGURE 3-1



The TIMS method described here is written specifically for use with the NIST 12 in series 90° sector solid source mass spectrometers. These mass spectrometers permit only single sample loading and measurement, and therefore limit the sample throughput to about 5 to 6 samples per day. Although this is relatively slow, this is not too restrictive for small sample

loads such as those associated with SRM certification measurements or international comparisons.

4. Safety

Clinical fluids are biohazardous materials which may contain pathogenic substances. The usual safety precautions should be taken to prevent exposure during handling, including the use of gloves at all times. Although most materials will have been screened for such substances, a clinical monitoring regimen, including screening for HIV 1 and 2 and vaccination against hepatitis B is recommended for personnel handling biological specimens on an extended basis.

5. Required Reagents

5.1 ^{37}Cl Isotopic Spike

A spike having an isotopic purity ≥ 98 % can be obtained through Oak Ridge National Laboratory (ORNL) or other stable-isotope supplier in a form such as $Na^{37}Cl$. A stock solution can be prepared by dissolving a small amount of the spike in high-purity de-ionized water to yield a concentration of about 800 µg/g (22 µmol/g) and storing in a cleaned PTFE bottle. Tracking the tare weight of the bottle is useful for long-term storage and monitoring the concentration of the spike on a multiple-use basis.

5.2 SRM 919a, Sodium Chloride, Clinical Standard

The material should be dried according to the directions on the SRM certificate, which specify 3 h at 110 ºC in a convection oven. Allow to cool. Store in a desiccator at room temperature when not in use. The purity of the material is 99.89 %. The mass fraction of chloride in the sodium chloride, adjusted for the purity, is 0.60596.

5.3 SRM 918a, Potassium Chloride, Clinical Standard

The material should be dried according to the directions on the SRM certificate, which specify 2 h at 110 ºC in a convection oven. Allow to cool. Store in a desiccator at room temperature when not in use. The purity of the material is 99.9817 %. The mass fraction of chloride in the potassium chloride, adjusted for the purity, is 0.47546.

5.4 SRM 975a, Isotopic Standard for Chlorine

Isotopic standard having a natural ratio $^{35}Cl/^{37}Cl = 3.1279 \pm 0.0047$ (Relative Atomic Mass 35.45265) for assessing mass fractionation during sample measurements.

5.5 Ammonium Molybdate

Obtain from a commercial supplier as ammonium heptamolybdate tetrahydrate. High-purity reagent is essential to minimize the extent of chloride contamination. Prepare a fresh solution containing 50 g/L in high-purity de-ionized water.

5.6 Silver Nitrate

Obtain from a commercial supplier. High-purity reagent is essential to minimize chloride contamination. Prepare a fresh solution containing 20 g/L in high-purity de-ionized water. When not in use, store in a dark place.

5.7 Ammonium Hydroxide

High-purity ammonium hydroxide is essential to minimize chloride contamination. It may be obtained from a number of commercial suppliers. Prepare a 500 mL/L (50 % volume fraction) solution in de-ionized water and store in a clean bottle.

5.8 Nitric Acid

High-purity acid is essential to minimize the extent of chloride contamination from reagent sources. High-purity acid may be obtained from a number of commercial suppliers.

5.9 High-Purity Water

De-ionized or quartz-distilled water, which has been tested and is low in chloride contamination, is used for sample dilution. The water should meet or exceed the specifications of CAP/NCCLS Type I water [1].

6. Equipment

6.1 Thermal Ionization Mass Spectrometer

The method utilizes a NIST designed 12 in, 90° sector field solid source mass spectrometer, operating in the negative ion mode. A modified open ion source designed specifically for use with the negative ion mode is beneficial in producing a stable ion beam. The instrument is equipped with a manual triple-filament control, optical pyrometer for setting

filament temperature and a computer control system for measuring and recording $^{35}Cl/^{37}Cl$ isotope ratios.

6.2 Filament Degasser

Vacuum chamber system fitted with a current source and readout, ion collector and pressure readout for degassing filaments.

6.3 Centrifuge

A general purpose laboratory centrifuge capable of reaching at least 1000 g relative centrifugal force (RCF). The system should be fitted with industry standard safety interlocks and a rotor which is capable of holding several 15 mL capacity centrifuge tubes.

6.4 Centrifuge Tubes

Polystyrene (or similar material with low chloride contamination) centrifuge tubes of 15 mL capacity for use in centrifuge system. Tubes must be cleaned prior to use by soaking in dilute nitric acid (2 % volume fraction), and rinsing thoroughly with de-ionized water.

6.5 Micro-Pipette

Automatic or manual micro-pipette for dispensing sample, spike and standard aliquots. A capped plastic syringe fitted with a Teflon® fluoropolymer uptake tube may also be used for this purpose.

6.6 Analytical Balance

Five-place analytical balance for weighing sample, spike and standard aliquots. The balance should be calibrated and verified to be functioning correctly prior to weighing measurements.

7. Sample Handling, Preparation and Quality Control

7.1 Analytical Sample Preparation

The analytical sample should be equilibrated to room temperature prior to measurement. Follow supplier instructions for reconstitution of lyophilized material or equilibration from freezer temperature. All sample preparation should be carried out in a Class 10 clean environment with mandatory use of protective clothing and gloves.

7.2 Sample Aliquoting and Spiking

The amount of analytical sample to be taken depends on the level of chloride present. In order to maintain a high sample to blank ratio, the sample aliquot needs to contain at least 1000 µg of chloride. For

example, in a serum material, which typically contains about 100 mmol/L chloride, a sample size of 0.3 g should suffice. Weigh the sample aliquot accurately by difference into a clean 15 mL centrifuge tube by means of a pipette or syringe using a five-place analytical balance. Add an accurately known amount of ^{37}Cl spike (approximately 1 g of the spike solution prepared in section 5.1). Dilute mixture to 6 mL with dilute nitric acid (1% volume fraction).

7.3 Protein Separation

If protein material is present in the sample, it must be removed by precipitation. Add ammonium molybdate to the cooled sample mixture (1 mL of a 50 g/L solution) and mix. Separate the precipitate by centrifugation at approximately 2000 rpm (500 RCF) for 10 min and remove the supernatant containing the chloride by decantation. Transfer to a clean 15 mL centrifuge tube.

7.4 Chloride Separation

Add silver nitrate to the sample (1 mL of a freshly prepared 20 g/L solution) and allow to stand in the dark for 10 min. Centrifuge at approximately 2000 rpm (500 RCF) for 10 min. Discard the supernatant by decantation. Wash the precipitate with a small amount of dilute nitric acid (1 % volume fraction) and re-dissolve the precipitate in ammonium hydroxide solution (1 mL of a 500 mL/L solution). Re-precipitate the chloride by drop-wise addition of nitric acid (10 % volume fraction) and dilute back to 6 mL with high-purity de-ionized water. Centrifuge again and discard the supernatant by decantation. Wash the precipitate with dilute nitric acid (1 % volume fraction), remove, and dissolve the precipitate in ammonium hydroxide (0.75 mL of a 500 mL/L solution). Store the prepared samples at freezer temperature (-20 °C) in the dark.

7.5 Stability

The prepared solutions are stable for at least two weeks. However, determinations should be carried out as soon as possible to avoid the risk of contamination, losses or stability issues. Silver chloride solutions are unstable, particularly in the presence of light.

7.6 Spike Calibration Mixtures

The concentration of the ^{37}Cl spike stock solution must be accurately measured by preparing at least four independent spike calibration mixtures. Prepare one stock calibration solution of natural chloride from SRM 919a (Sodium Chloride, Clinical Standard). Prepare two spike calibration mixtures from the natural stock by aliquoting known amounts of the calibration stock and ^{37}Cl spike into sample bottles and process as

for the samples. Prepare two more mixtures using SRM 918a (Potassium Chloride, Clinical Standard). A five-place analytical balance should be used for all weighing operations.

7.7 Procedure Blanks

Prepare a minimum of three procedure blanks by adding approximately 10 µg or less of the ^{37}Cl spike to a clean 15 mL centrifuge tube and processing them through the entire sample preparation procedure.

7.8 Composition Sample

Prepare a composition sample by aliquoting approximately 0.3 g of sample into a 15 mL centrifuge tube and carrying it through the same preparation procedure as the samples. The composition sample is not spiked with ^{37}Cl and is used to assess the isotopic composition of the material being measured and to demonstrate the validity of isotopic measurements by the mass spectrometer.

7.9 Controls

To assess the accuracy of the measurements, prepare at least two separate SRM control materials having certified values for chloride, preferably of the same matrix type as the samples, and carry the controls through the same sample preparation and measurement procedures as the samples. An additional control material, consisting of an accurately known amount of standard, should be prepared and carried through the whole measurement and calculation process. The gravimetric recovery of the standard facilitates assessment of the accuracy of the measurement process and veracity of the calculations.

8. Analytical Measurement

8.1 Instrument Preparation

If not already configured, switch the TIMS instrument from positive ion to negative ion mode. Prior to analytical measurements, warm up the electronic components of the instrument for a minimum period of 30 min.

8.2 Filament Preparation

For each sample prepare a set of rhenium filaments, consisting of two side filaments and one center filament. Outgas the filaments by placing them in a vacuum chamber degasser and passing a current of 4 A through them for a period of 30 min. Allow to cool to ambient temperature, remove them from the vacuum chamber and transfer to a closed container which will protect them from dust and damage.

8.3 Filament Loading

Remove the sample from the freezer and transfer to a Class 10 clean area. Load approximately 10 µg of chloride onto each of two rhenium side filaments using a suitable micro-pipette. Dry the filaments under an infra-red heat-lamp for 30 min, after which a thin film of silver chloride should be present on each filament.

8.4 Filament Mounting and Isotope Ratio Measurement

Assemble the two loaded side filaments in a suitable filament block former along with a center filament. Place in the source-can of the mass spectrometer, connect the electrical leads and evacuate the chamber. After a suitable vacuum has been attained, add liquid nitrogen to the source-can cold-finger and open the beam valve. Heat the center filament to 1500 °C, and then slowly ramp the side filaments to produce a stable ion beam. Optimum signals are usually obtained for a relatively narrow temperature range for the side filaments, corresponding to approximately 0.5–0.6 A filament current. After approximately 30 min conditioning and background stabilization, acquire several blocks of $^{35}Cl/^{37}Cl$ isotope ratios. Assess the mass fractionation of the measurements by measuring SRM 975a, Isotopic Standard for Chlorine, at periodic intervals.

8.5 Run Sequence

To reduce the effects of memory carry over, which are of significant concern in the measurement of chloride by thermal ionization, all samples, standards, blanks and control materials should be divided into separate batches having essentially similar isotope ratios. Memory effects should be assessed at the end of each measurement run by turning off the side filaments and acquiring a final block of data with reduced signal. The measured ratios should not differ significantly from the initial sample ratios.

9. Data Reduction

9.1 Mass Fractionation Corrections

Convert the experimentally measured $^{35}Cl/^{37}Cl$ isotope ratios to absolute ratios using the following equation:

$$\left[\frac{^{35}Cl}{^{37}Cl}\right]_{CORR} = \frac{\left[\frac{^{35}Cl}{^{37}Cl}\right]_{TRUE}}{\left[\frac{^{35}Cl}{^{37}Cl}\right]_{MEAS}}$$

where, $\left[\frac{^{35}Cl}{^{37}Cl}\right]_{CORR}$ is the fractionation corrected sample ratio

$\left[\frac{^{35}Cl}{^{37}Cl}\right]_{TRUE}$ is the true ratio for the isotopic standard

$\left[\frac{^{35}Cl}{^{37}Cl}\right]_{MEAS}$ is the measured ratio for the isotopic standard

The measured ratios for the isotopic standard can be applied to sample fractionation corrections in a number of ways, which will depend on the fractionation drift as a function of time: (1) the ratio can be calculated by averaging the two standard measurements bracketing the samples of interest, (2) a more sophisticated approach is to mathematically model the fractionation in the standard ratio as a function of time and interpolate the ratio for each sample. This may require more frequent measurement of the isotopic standard to define the fractionation adequately.

9.2 Spike Calibration

Calculate the concentration of the ^{37}Cl spike in each spike calibration mixture using the following equation:

$$C_{SPK} = \left[\frac{M_{STD}(BR - A)}{K(A_s - B_s R)} - S_B\right] \times \frac{1}{M_S}$$

where,
- C_{SPK} is the concentration of chloride in the spike in µg/g
- M_{STD} is the mass of chloride added to the spike mix (µg)
- **B** is the natural abundance of the spike isotope (37)
- **R** is the corrected $^{35}Cl/^{37}Cl$ ratio in the spike mix
- **A** is the natural abundance of the reference isotope (35)
- M_S is the mass of ^{37}Cl spike aliquot added to the mix (g)
- **K** is the natural to spike relative atomic mass ratio
- A_S is the abundance of the reference isotope in the spike
- B_S is the abundance of the spike isotope in the spike
- S_B is the absolute mean measured blank (µg)

Calculate the mean and standard deviation of the spike calibration mixtures. Unlike ICP-MS measurements, where the spike calibration is normally made on simple mixtures without extensive sample processing, a spike calibration procedure blank must be accounted for and should be subtracted from the data for the spike calibration.

9.3 Calculation of Chloride Sample Concentrations

Calculate the chloride concentration (µg/g) in the sample using the following equation:

$$C = \left[\frac{M_s K(A_s - B_s R)}{(BR - A)} - S_B \right] \times \frac{1}{W}$$

where,
- **C** is the concentration of chloride in the sample (µg/g)
- $\mathbf{M_s}$ is the absolute mass of ^{37}Cl spike added (µg)
- **K** is the natural to spike relative atomic mass ratio
- $\mathbf{A_s}$ is the abundance of the reference isotope in the spike
- $\mathbf{B_s}$ is the abundance of the spike isotope in the spike
- **R** is the corrected $^{35}Cl/^{37}Cl$ ratio
- **B** is the natural abundance of the spike isotope
- **A** is the natural abundance of the reference isotope
- $\mathbf{S_B}$ is the absolute mean measured blank (µg)
- **W** is the mass of sample aliquot taken (g)

An alternative approach, if desired, is to calculate the spike and sample concentrations in mol/g. In this case the equations are slightly modified.

10. Uncertainty Analysis

The expanded uncertainty for the set of samples should be calculated according to ISO guidelines [2] by combining both Type A and Type B uncertainties. Typical Type A uncertainty components for this measurement might be the variability of the measured concentrations of chloride in the samples, the measurement of the spike concentration and the variability of the correction for the blank. Typical Type B uncertainty components might be the uncertainty of the assay of the primary calibrant, weighing measurements, conversion correction for the density of the sample and instrumental parameters such as drift in the instrument mass discrimination correction and instrument background correction.

For a 95 % confidence level, combine the Type A and Type B components and calculate the total expanded uncertainty using the following equation:

$$U = k\,[s_1^2/df_1 + \ldots + s_n^2/df_n + B_1^2 + \ldots + B_n^2\,]^{1/2}$$

where,
- U is the expanded uncertainty
- k is the coverage factor
- s is the observed standard deviation of (n) Type A components

df is the degrees of freedom associated with each component
B is the Type B component (n components) with infinite degrees of freedom.

Report the expanded uncertainty in the same units as the sample concentrations.

11. Data Reporting

Convert data from a mass/mass basis to a mass/volume basis using the measured density of the serum. **For clinical use, report data in units of mmol/L**. To convert from mmol/L to mg/dL multiply by 3.54527.

12. Performance Statement

12.1 Measurement Repeatability

The isotope ratio measurement repeatability is limited by short term drift in the ^{35}Cl and ^{37}Cl signal amplitudes, which tends to be higher in the negative ion mode of operation. Typical measurement repeatability that can be obtained on a NIST TIMS instrument is around 0.1 %.

12.2 Instrument Background Correction and Procedure Blank

The instrument background is fairly low and is not a significant factor in the measurement process under normal conditions. Sample-to-sample carryover may arise from contamination of the ion source or other components, and needs to be minimized by correct sampling protocols.

A typical procedure blank using this method is approximately 1 µg, depending on the purity of the reagents and cleanliness of the sample containers. This represents approximately a 0.1 % correction to the sample data, using aliquot sizes described in this method.

12.3 Performance Summary

A summary of typical method performance parameters expected for use of this method are provided in Table 12-1.

Table 12-1: Method Performance Summary

Parameter	Typical Value or Range
Isotope ratio measurement repeatability	0.1 %
Applicable concentration range	All ranges found in clinical specimens
Procedure blank	≈ 1 µg
Procedure blank to sample ratio	≈ 0.1 %
Sample throughput (measurement)	5-6 samples per day

13. Reference Citations

1. *Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline – Third Edition*, NCCLS Document C3-A3, NCCLS, Wayne, Pennsylvania (1997).

2. *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed. ISO, Switzerland, 1993.

CL-1 Revision History

Revision	Date	Author	Record of Changes and Comments
1.0	9/2006	S E Long	Initial draft method

4 Lithium

4.1 Clinical Significance

Although lithium is normally present in blood plasma or serum, clinical measurements are usually made in connection with its administration as a first-line agent in the treatment of affective disorders, such as manic depressive psychosis (bipolar disorder). In this respect, lithium is highly effective, the usual pharmaceutical dosage forms being lithium carbonate or lithium citrate. Lithium is absorbed from the gastro-intestinal tract within approximately eight hours following administration, however, intracellular transport, which is necessary for therapeutic mechanism, is relatively poor, which necessitates the use of high dosages to obtain therapeutic benefit. There is a narrow margin between these therapeutic dosages and the onset of toxic effects, which therefore mandates a system of intensive patient monitoring for both physical status and serum lithium concentrations. Serum lithium measurements are normally made approximately twelve hours after a previous dose has been taken. The therapeutic interval should lie within the range 0.4–1.4 mmol/L. Higher levels than this are considered to be toxic, which may result in a number of effects which include hand tremor, thirst, nausea, vomiting and slurred speech. Some studies which were made in the late 1970s following FDA approval of lithium for the treatment of manic depressive disorder and maintenance therapy, presented some evidence for long-term renal damage from extended use. More recent studies have shown that this is not the case and that the incidence rate among patients taking lithium medication is typically low.

4.2 Normal Range and Reporting Units

The normal therapeutic range for lithium in serum or plasma is about 0.4–1.4 mmol/L (levels > 1.5 mmol/L are considered toxic). Lithium clinical data are normally reported in mmol/L.

4.3 Routine Clinical Measurement Methods

Classical reference methods for the determination of lithium in serum or plasma involve atomic absorption spectrometry and atomic emission spectrometry (flame photometry). These methods require transport of the sample to a specialized laboratory and a relatively slow turnaround time for

the transmission of utility data back to the patient. The use of more rapid point-of-care testing methods are advantageous, given the need for the frequent monitoring of lithium levels to obtain the correct therapeutic index in the patient. Thus ion selective electrodes are now extensively used for the measurement of lithium in serum and plasma. A rapid colorimetric system based on the formation of a lithium porphyrin complex, which absorbs at 505 nm, is also used.

4.4 Available Standard Reference Materials

Two blood serum SRMs are available with certified values for lithium, which are **SRM 909b (*Human Serum*)**, a two-level lyophilized material, and **SRM 956b (*Electrolytes in Frozen Human Serum*)**, which is a three-level frozen material intended for standardizing and calibrating ion selective electrode analyzers. One clinical assay standard may be used for lithium: **SRM 924a (*Lithium Carbonate, Clinical Standard*)** is sold as a clinical primary standard intended for instrument calibration and standardization. Certificate information for these SRMs is summarized in Table 4-1.

Table 4-1, Clinical Standard Reference Materials for Lithium

SRM	Description	Value (mmol/L)	Expanded Uncertainty (mmol/L)
909b Level 1	Human Serum	0.6145	0.0050
909b Level 2	Human Serum	2.600	0.023
956b Level 1	Electrolytes in Frozen Human Serum	1.920	0.027
956b Level 2	Electrolytes in Frozen Human Serum	1.207	0.017
956b Level 3	Electrolytes in Frozen Human Serum	0.486	0.007
SRM	**Clinical Standard**	**Purity (% mass fraction)**	**Expanded Uncertainty (%)**
924a	Lithium Carbonate (Clinical Standard)	99.867	0.017

Version Number	1.0
Approved	S E Long
Effective Date	Sep 2006

LI-1
LITHIUM BY ISOTOPE DILUTION INDUCTIVELY COUPLED PLASMA – MASS SPECTROMETRY

1. Applicability

The method is applicable to the determination of lithium at physiological and therapeutic levels in blood plasma, serum (frozen and lyophilized) and urine.

2. Principle of Measurement

The method is based on isotope dilution mass spectrometry using a quadrupole inductively coupled plasma – mass spectrometer (ICP-MS). A stable isotopic spike of ^{6}Li is added to the sample, equilibrated by oxidation with nitric acid in a microwave digestion system, and the $^{7}Li/^{6}Li$ isotope ratio measured under optimized conditions. The method is extremely accurate and very selective for lithium.

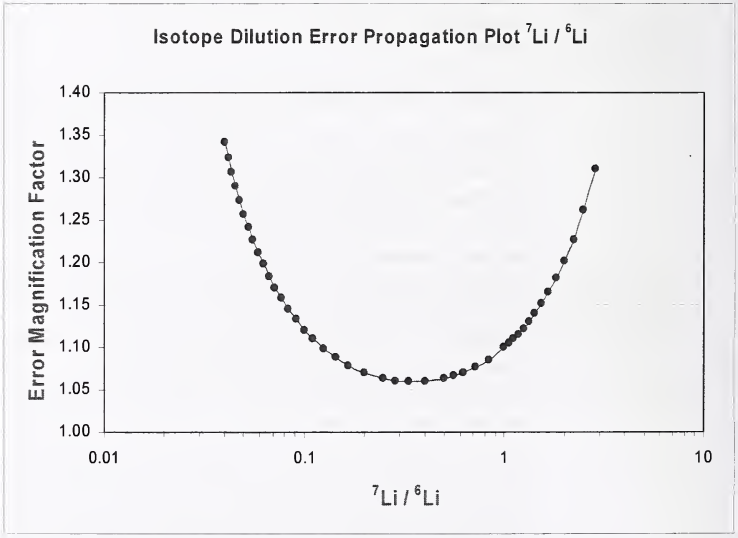
3. Measurement Strategy and Experimental Design

The determination of lithium in biological fluids by isotope dilution ICP-MS has not been widely employed in the past because of the difficulties associated with memory effects, high instrument background and relatively large instrument mass discrimination. The lithium isotopes at masses 6 and 7 are free from spectral interferences but, because they are very light isotopes, the mass discrimination is usually high and unstable on a typical ICP-MS instrument. For these reasons, the determination of lithium in biological fluids at NIST has usually been carried out by TIMS. In this ICP-MS method, the plasma power is slightly reduced to mitigate secondary ionization effects from lithium on the skimmer cone. The instrument background is then fairly low and washout between samples is very rapid. The voltages on the lens system are adjusted specifically to reduce the instrument discrimination to less than 5 %. This does result in some minor ion transmission loss, but the instrument detection limit for lithium is so low that this is of no consequence. Under these conditions, drift in the mass discrimination is low. Oxidation with nitric acid in a microwave digestion system is necessary to chemically

equilibrate the ^{6}Li isotopic spike with the lithium in the sample. Chemical separation of the lithium is not necessary. Large dilution factors are employed which reduces the incidence of matrix effects from other components, such as sodium, in the sample.

The amount of spike added to the sample prior to oxidation needs to be optimized. The error propagation has been calculated and plotted in Figure 3-1 for a typical measurement system employing a ^{6}Li spike of 99 % isotopic purity. The calculated error magnification factor (EM) is at a minimum (EM = 1.06) for a $^{7}Li/^{6}Li$ ratio of approximately 0.35. However, although this is the ideal ratio, it is considered better to underspike the system to yield an isotope ratio closer to unity. Under these conditions, the effects of detector dead-time and pulse counting statistics, which are important aspects of ICP-MS measurement systems, are minimized. As is evident from Figure 3-1, at a ratio of approximately unity, the EM factor is about 1.10, which is totally acceptable. The other approach to measurement would be to exactly match the ratios of the spike calibration mixtures to the sample measurement mixtures, and run them in the same temporal space. This is not easily achieved and does not circumvent the counting statistics limitation.

FIGURE 3-1



4. Safety

Clinical fluids are biohazardous materials which may contain pathogenic substances. The usual safety precautions should be taken to prevent exposure during handling, including the use of gloves at all times. Although most materials will have been screened for such substances, a clinical monitoring regimen, including screening for HIV 1 and 2 and vaccination against hepatitis B is recommended for personnel handling biological specimens on an extended basis.

5. Required Reagents

5.1 ^{6}Li Isotopic Spike

A spike having an isotopic purity >99 % can be obtained through Oak Ridge National Laboratory (ORNL), or other stable-isotope supplier, in a form such as $^{6}Li_2CO_3$. A stock solution can be prepared by dissolving a small amount of the spike in 2 % (volume fraction) high-purity nitric acid to yield a concentration of about 10 µg/g (1.7 µmol/g) and storing in a cleaned PTFE bottle. Tracking the tare weight of the bottle is useful for long-term storage and monitoring the concentration of the spike on a multiple-use basis.

5.2 SRM 924a, Lithium Carbonate, Clinical Standard

The material should be dried according to the directions on the SRM certificate, which specify 4 h at 200 °C in a convection oven. Allow to cool. Store in a desiccator at room temperature when not in use. The purity of the material is 99.867 % with an expanded uncertainty of 0.017 %. The mass fraction of lithium in SRM 924a, adjusted for the purity, is 0.18763.

5.3 High-Purity Nitric Acid

High-purity acid is essential to minimize the extent of lithium contamination from reagent sources. High-purity acid containing less than 20 pg/g lithium may be obtained from a number of commercial suppliers.

5.4 High-Purity Water

De-ionized or quartz-distilled water, which has been tested and is low in lithium contamination, is used for sample dilution. The water should meet or exceed the specifications of CAP/NCCLS Type I water [1].

6. Equipment

6.1 ICP-MS

The method utilizes a standard ICP-MS system employing a quadrupole mass spectrometer. The instrument should be capable of operating in the pulse counting detection mode, and should be fitted with a high-efficiency pneumatic nebulizer system, if available. The use of a sample introduction system composed of an inert material is beneficial in reducing instrument background and improving sample washout.

6.2 Microwave Digestion System

A commercial system should be used which is capable of reaching at least 70 bar (7 MPa) vessel pressure and a maximum vessel temperature of at least 200 °C using either PTFE or quartz reaction vessels. The system should be fitted with industry-standard safety interlocks. A continuous (un-pulsed) variable power delivery system is advantageous in maintaining optimum reaction control.

6.3 Sample Bottles

Clean 60 mL polyethlylene sample bottles (or equivalent material) for sample analysis and storage.

> **Note on Sample Bottles**: Polyethylene or Teflon® fluoropolymer sample bottles formulated for low trace-metal content should be used. These contain very low levels of electrolyte contaminants. Containers such as polypropylene centrifuge tubes should not be used for this purpose, as they frequently contain appreciable levels of contaminants.

6.4 Micro-Pipette

Automatic or manual micro-pipette for dispensing sample, spike and standard aliquots. A capped plastic syringe fitted with a Teflon® fluoropolymer uptake tube may also be used for this purpose.

6.5 Analytical Balance

Five-place analytical balance for weighing sample, spike and standard aliquots. The balance should be calibrated and verified to be functioning correctly prior to weighing measurements.

7. Sample Handling, Preparation and Quality Control

7.1 Analytical Sample

The analytical sample should be equilibrated to room temperature prior to measurement. Follow supplier instructions for reconstitution of lyophilized material or equilibration from freezer temperature. All sample

preparation should be carried out in a Class 10 clean environment with the use of protective clothing and gloves.

7.2 Aliquoting and Acidification

Approximately 0.5 g of the liquid sample should be weighed accurately, using a five-place analytical balance, into a cleaned microwave digestion vessel manufactured from quartz or Teflon® fluoropolymer and spiked with an aliquot of ^{6}Li stock (ranging from 0.1 g to 0.4 g depending on the level in the material) to yield a $^{7}Li/^{6}Li$ ratio of approximately unity. Five grams of high-purity nitric acid should be added to each vessel, and the vessels then sealed using the appropriate procedure specified by the manufacturer.

7.3 Digestion

The prepared solutions should be placed into a high-pressure microwave digestion system and digested using the manufacturers recommended conditions, or user-developed digestion protocol. A vessel pressure approaching 70 bar (7 MPa) for at least 30 min will usually ensure complete oxidation of the sample and conversion of all carbon in the sample to carbon dioxide.

7.4 Dilution

The digested samples should be carefully removed from the digestion vessels and diluted with high-purity water to a concentration level appropriate to the ICP-MS instrumentation used for the measurements. Typically, the working concentration is around 5 ng/g.

7.5 Stability

The prepared solutions are stable for several days. However, determinations should be carried out as soon as possible to avoid the risk of contamination, losses or stability issues.

7.6 Spike Calibration Mixtures

The concentration of the ^{6}Li spike stock solution must be measured by preparing at least four independent spike calibration mixtures. Prepare two stock calibration solutions of natural lithium from SRM 924a. Prepare two spike calibration mixtures from each of the natural lithium calibration solutions by aliquoting known amounts of the calibration stock and ^{6}Li spike into sample bottles and diluting with 2 % (volume fraction) nitric acid to a concentration suitable for measurement by ICP-MS. The aliquots should be adjusted to yield $^{7}Li/^{6}Li$ isotope ratios as similar as possible to the analytical samples. A five-place analytical balance should be used for all weighing operations.

7.7 Procedure Blanks

Prepare a minimum of three procedure blanks by adding approximately 0.05 µg or less of the ^{6}Li spike to microwave digestion vessels and carry them through the entire preparation procedure.

7.8 Composition Sample

Prepare a composition sample by aliquoting approximately 0.5 g of sample into a microwave digestion vessel and carrying it through the same preparation procedure as the samples. The composition sample is not spiked with ^{6}Li and is used to assess the isotopic composition of the material being measured. The isotopic composition of lithium has natural variability and must be measured to assure accuracy of the data. The measured isotopic composition data are used in the calculations of the sample concentrations. Isotopic composition data for some relevant NIST SRMs are compiled in Appendix A.

7.9 Controls

To assess the accuracy of the measurements, prepare at least two separate SRM control materials having certified values for lithium, preferably of the same matrix type as the samples, and carry the controls through the same sample preparation and measurement procedures as the samples. An additional control material, consisting of an accurately known amount of standard, should be prepared and carried through the whole measurement and calculation process. The gravimetric recovery of the standard facilitates assessment of the accuracy of the measurement process and veracity of the calculations.

8. Analytical Measurement

8.1 Instrument Preparation

Prior to analytical measurements, the ICP-MS system should be equilibrated for at least 30 min. The sample introduction system should be cleaned rigorously before use, including the sampler and skimmer cones, peristaltic pump tubing, plasma torch and spray chamber. These procedures are designed to mitigate the instrument lithium background as much as possible. It is recommended that new peristaltic pump tubing be installed prior to measurements. A dilute nitric acid solution (2 % volume fraction) should be pumped through the new tubing for a while to remove any leachable lithium from the tubing.

8.2 Instrument Optimization

In order to reduce instrument background, the plasma rf power should be reduced slightly from the normal setting. For an instrument operating normally at 1350 W, a setting of 1100 W would be typical. The instrument should be optimized for maximum ion transmission at m/z 7 by nebulizing a standard solution of lithium and adjusting ion lens voltages, plasma gas flows and torch position (relative to the sample cone). The ion lens(es) should then be adjusted slightly to minimize as far as possible the mass discrimination. Although this results in some loss of sensitivity, this should not compromise measurements of lithium in clinical materials.

8.3 Run Sequence

Software acquisition menus should be prepared for measuring the ^{6}Li and ^{7}Li isotopes. The run order for the samples should be established according to the following protocol:

Analytical procedure blanks should be run at the beginning of the sequence, followed by the composition sample and then the analytical samples (including control materials) in randomized order. Instrument mass discrimination measurement standards (*see note*) should be run after every three to four samples depending on the stability of the instrument.

> **Note on Mass Discrimination Measurement:** The measured $^{7}Li/^{6}Li$ ratios should be corrected for the ICP-MS mass discrimination. This can be achieved by periodic measurement of an isotopic calibration standard consisting of natural lithium. Suitable calibration materials are IRMM (Institute for Reference Materials and Measurements, Geel, Belgium), IRMM-016 which has a ^{7}Li / ^{6}Li ratio of 12.177 (molar mass 6.9401 g/mol) and NIST LSVEC (RM 8545). The LSVEC material is identical to the IRMM material. The natural ratio is very different from the working sample ratios. A more elegant way to assess discrimination and discrimination drift is to carefully cross-calibrate a solution matched more closely to the sample ratios. A spike calibration solution can conveniently be used for this purpose.

8.4 Isotope Ratio Acquisition

The isotope ratio $^{7}Li/^{6}Li$ should be acquired in peak jumping mode. A typical acquisition scheme would be five to seven sets of ratios with a total acquisition time of 60 s per set, using a dwell-time of 10 ms per isotope. The isotope ratio data should be downloaded to a spreadsheet program such as Microsoft Excel. Inspect the ratios and remove any considered to be statistical outliers.

9. Data Reduction

9.1 Mass Discrimination Corrections

Convert the experimentally measured $^{7}Li/^{6}Li$ isotope ratios to absolute ratios using the following equation:

$$\left[\frac{^{7}Li}{^{6}Li}\right]_{CORR} = \frac{\left[\frac{^{7}Li}{^{6}Li}\right]_{TRUE}}{\left[\frac{^{7}Li}{^{6}Li}\right]_{MEAS}}$$

where, $\left[\frac{^{7}Li}{^{6}Li}\right]_{CORR}$ is the discrimination corrected sample ratio

$\left[\frac{^{7}Li}{^{6}Li}\right]_{TRUE}$ is the true ratio for the isotopic standard

$\left[\frac{^{7}Li}{^{6}Li}\right]_{MEAS}$ is the measured ratio for the isotopic standard

The measured ratios for the isotopic standard can be applied to sample discrimination corrections in a number of ways, which will depend on the discrimination drift as a function of time: (1) the ratio can be calculated by averaging the two standard measurements bracketing the samples of interest, (2) a more sophisticated approach is to mathematically model the drift in the standard ratio as a function of time and interpolate the ratio for each sample. This may require more frequent measurement of the isotopic standard to define the drift function adequately.

9.2 Spike Calibration

Calculate the concentration of the ^{6}Li spike in each spike calibration mixture using the following equation:

$$C_{SPK} = \left[\frac{M_{STD}(BR - A)}{M_s K(A_s - B_s R)}\right]$$

where, C_{SPK} is the concentration of lithium in the spike in µg/g
M_{STD} is the mass of lithium added to the spike mix (µg)
B is the natural abundance of the spike isotope (6)
R is the corrected $^{7}Li/^{6}Li$ ratio in the spike mix
A is the natural abundance of the reference isotope (7)

M_S is the mass of ^6Li spike aliquot added to the mix (g)
K is the natural to spike relative atomic mass ratio
A_S is the abundance of the reference isotope in the spike
B_S is the abundance of the spike isotope in the spike

Calculate the mean and standard deviation of the spike calibration mixtures. Blank corrections to the spike calibration are not normally necessary because they are simple mixtures prepared in dilute acid.

9.3 Calculation of Lithium Sample Concentrations

Calculate the lithium concentration (µg/g) in the sample using the following equation:

$$C = \left[\frac{M_s K(A_s - B_s R)}{(BR - A)} - S_B \right] \times \frac{1}{W}$$

where, C is the concentration of lithium in the sample (µg/g)
M_s is the absolute mass of ^6Li spike added (µg)
K is the natural to spike relative atomic mass ratio
A_s is the abundance of the reference isotope in the spike
B_s is the abundance of the spike isotope in the spike
R is the corrected $^7Li/^6Li$ ratio
B is the natural abundance of the spike isotope
A is the natural abundance of the reference isotope
S_B is the absolute mean measured blank (µg)
W is the mass of sample aliquot taken (g)

An alternative approach, if desired, is to calculate the spike and sample concentrations in mol/g. In this case the equations are slightly modified.

10. Uncertainty Analysis

The expanded uncertainty for the set of samples should be calculated according to ISO guidelines [2] by combining both Type A and Type B uncertainties. Typical Type A uncertainty components for this measurement might be the variability of the measured concentrations of lithium in the samples, the measurement of the spike concentration and the variability of the correction for the blank. Typical Type B uncertainty components might be the uncertainty of the assay of the primary calibrant, weighing measurements, conversion correction for the density of the sample and instrumental parameters such as detector dead-time correction, instrument mass discrimination correction and instrument background correction.

For a 95 % confidence level, combine the Type A and Type B components and calculate the total expanded uncertainty using the following equation:

$$U = k\,[s_1^2/df_1 + \ldots + s_n^2/df_n + B_1^2 + \ldots + B_n^2\,]^{1/2}$$

where, U is the expanded uncertainty
k is the coverage factor
s is the observed standard deviation of (n) Type A components
df is the degrees of freedom associated with each component
B is the Type B component (n components) with infinite degrees of freedom.

Report the expanded uncertainty in the same units as the sample concentrations.

11. Data Reporting

Convert data from a mass/mass basis to a mass/volume basis using the measured density of the serum. **For clinical use, report data in units of mmol/L**. To convert from mmol/L to mg/dL multiply by the relative atomic mass of the material and divide by ten. To convert data from µg/mL to mg/dL divide by ten.

12. Performance Statement

12.1 Measurement Repeatability

The isotope ratio measurement repeatability is usually limited by detector counting statistics and instrument drift. Typical measurement repeatability on a quadrupole ICP-MS system for a $^7Li/^6Li$ ratio approaching unity is 0.2-0.3 %.

12.2 Detection Limit

The instrument detection limit is influenced by the instrument background and the operating conditions chosen for the analytical measurements. A typical instrument detection limit (3 sigma) for a quadrupole ICP-MS instrument is 0.5 pg/mL. For a 0.5 g sample aliquot this corresponds to a detection limit of approximately 0.1 ng/mL which is far below the lithium concentrations typically found in clinical fluids.

12.3 Instrument Background and Procedure Blank

The ICP-MS instrument background is average to high for lithium. The background is typically in the range from 0–1000 counts per second depending on the cleanliness of the system and history of use.

A typical procedure blank using this method is approximately 100 pg depending on the purity of the reagents and storage containers. This is extremely low in relation to the lithium content in clinical fluids. For example, the blank correction for the determination of lithium in blood serum should be ≤ 0.01 %.

12.4 Performance Summary

A summary of typical method performance parameters expected for this method are provided in Table 12-1.

Table 12-1: Method Performance Summary

Parameter	Typical Value or Range
Isotope ratio measurement repeatability	0.2 %
Applicable concentration range	All ranges found in clinical specimens
Instrument background / sample ratio	≈ 0.1 %
Procedure blank	≈ 100 pg
Sample throughput (measurement)	10 samples per hour

13. Reference Citations

1. *Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline – Third Edition*, NCCLS Document C3-A3, NCCLS, Wayne, Pennsylvania (1997).

2. *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed. ISO, Switzerland, 1993.

LI-1 Revision History

Revision	Date	Author	Record of Changes and Comments
1.0	9/2006	S E Long	Initial draft method

APPENDIX A

Measured Absolute Lithium Isotopic Compositions for Selected NIST Clinical SRMs

SRM 924a, Lithium Carbonate	
$^6Li/^7Li$	0.08082
	Atom (%)
^6Li	7.4779
^7Li	92.5221
Relative Atomic Mass	6.9412

SRM 956a, Serum Level 1,2,3	
$^6Li/^7Li$	0.08200
	Atom (%)
^6Li	7.5790
^7Li	92.4210
Relative Atomic Mass	6.9402

SRM 956b, Serum Level 1,2,3	
$^6Li/^7Li$	0.080823
	Atom (%)
^6Li	7.5816
^7Li	92.4184
Relative Atomic Mass	6.9401

SRM 909b, Serum	
$^6Li/^7Li$	0.080823
	Atom (%)
^6Li	7.4779
^7Li	92.5221
Relative Atomic Mass	6.9412

5 Magnesium

5.1 Clinical Significance

Magnesium is an essential element which plays a critical role in metabolic processes. Like calcium, the majority of magnesium is localized within the skeletal system, while approximately 2 % is present in the extra-cellular fluid compartment. Of the magnesium found in plasma, just over half is in the active ionized form, while one third is bound to protein material. The remainder is bound to other free ligands such as citrate and phosphate. The ionized fraction is maintained under homeostatic control by renal excretion with replenishment through dietary sources. Magnesium is important in many physiological processes, which include, principally, the storage and use of energy, neuromuscular function, activation of enzyme reactions involving phosphate, and carbohydrate, lipid and protein metabolism. A reduced magnesium level (*hypomagnesemia*) can result from lowered dietary absorption of magnesium or excessive renal excretion, and often occurs in tandem with *hypokalemia* and *hypocalcemia,* and is associated with a variety of pathological conditions including cardiovascular disease. The condition presents a variety of symptoms which range from muscle weakness to increased reflexes, cramps, tremors and convulsions. Conversely, high levels of magnesium (*hypermagnesemia*) are predominantly associated with renal failure, but occasionally with impaired renal function combined with abnormally high intake, such as would result from excessive consumption of magnesium-containing pharmaceuticals such as antacids or laxatives.

5.2 Normal Range and Reporting Units

The normal range for total magnesium in serum or plasma is about 1.8–2.5 mg/dL. Magnesium clinical data are conventionally reported in mg/dL.
To convert from mmol/L to mg/dL multiply by 2.43050.
To convert from mg/dL to mmol/L multiply by 0.41144.

5.3 Routine Clinical Measurement Methods

Both flame atomic absorption and atomic emission systems are accurate, specific and sensitive for the measurement of magnesium in a variety of clinical media. The use of ion selective electrodes for such measurements has so far been limited because of the lack of specificity particularly with respect to calcium ions which are almost always present in the sample. An

important class of routine determinations is spectrophotometry. A variety of reactions have been employed. One of the most widely used is Calmagite which forms a red colored complex with magnesium, absorbing at 530 nm. Other systems based on chlorophosphonazo III and Magon dye complex reactions are also employed.

5.4 Available Standard Reference Materials

Two blood serum SRMs are available with certified values for magnesium, which are **SRM 909b (*Human Serum*)**, a two-level lyophilized material, and **SRM 956b (*Electrolytes in Frozen Human Serum*)**, which is a three-level frozen material intended for standardizing and calibrating ion selective electrode analyzers. There are also reference values for magnesium in **SRM 2670a (*Toxic Elements in Freeze-Dried Urine*)**, a two-level, freeze-dried material. One clinical assay standard may be used for magnesium: **SRM 929a (*Magnesium Gluconate, Clinical Standard*)** which is sold as a clinical primary standard intended for instrument calibration and standardization. Certificate information for these SRMs is summarized in Table 5-1.

Table 5-1. Clinical Standard Reference Materials for Magnesium

SRM	Description	Value (mg/dL)	Expanded Uncertainty (mg/dL)
909b Level 1	Human Serum	1.855	0.012
909b Level 2	Human Serum	4.661	0.051
956b Level 1	Electrolytes in Frozen Human Serum	3.699	0.048
956b Level 2	Electrolytes in Frozen Human Serum	2.417	0.031
956b Level 3	Electrolytes in Frozen Human Serum	1.113	0.014
2670a Level 1	Toxic Elements in Urine (Freeze-Dried)	(2.10)	(0.02)
2670a Level 2	Toxic Elements in Urine (Freeze-Dried)	(2.12)	(0.02)
SRM	**Clinical Standard**	**Purity (% mass fraction)**	**Expanded Uncertainty (%)**
929a	Magnesium Gluconate (Clinical Standard)	5.362	0.027

( ) Values in Parentheses are Reference Values.

Version Number	1.0
Approved	S E Long
Effective Date	Sep 2006

MG-1
MAGNESIUM BY ISOTOPE DILUTION INDUCTIVELY COUPLED PLASMA – MASS SPECTROMETRY

1. Applicability

The method is applicable to the determination of magnesium at physiological levels in blood plasma, serum (frozen and lyophilized) and urine.

2. Principle of Measurement

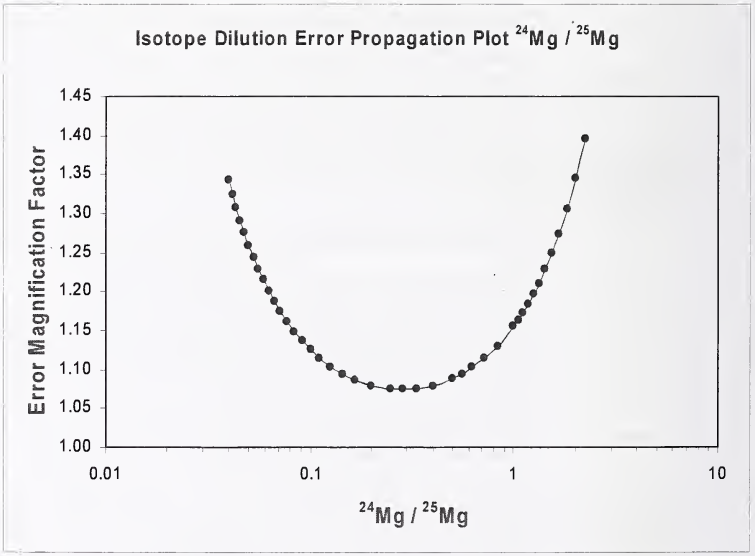
The method is based on isotope dilution mass spectrometry using a quadrupole inductively coupled plasma – mass spectrometer (ICP-MS). A stable isotopic spike of ^{25}Mg is added to the sample, equilibrated by oxidation with nitric acid in a microwave digestion system, and the $^{24}Mg/^{25}Mg$ isotope ratio measured under standard conditions. The method is extremely accurate and very selective for magnesium.

3. Measurement Strategy and Experimental Design

The determination of magnesium in biological fluids by isotope dilution ICP-MS is a relatively straightforward application. Oxidation with nitric acid in a microwave digestion system is necessary to avoid potential interferences on the isotopes ^{24}Mg (C_2^+) and ^{25}Mg (C_2^+, C_2H^+) arising from carbon in the sample. The potential for an additional interference on ^{24}Mg from $^{48}Ca^{2+}$ does exist, but the natural abundance of ^{48}Ca is so low that the interference is negligible. Two spikes could theoretically be used for the isotope dilution measurement, namely ^{25}Mg and ^{26}Mg. These are available in approximately 99% isotopic purity and both inexpensive when purchased from commercial sources. The isotope ^{25}Mg is considered to be a better choice for the measurement of magnesium because of its proximity in mass to the reference isotope ^{24}Mg. This reduces the effects of mass discrimination in the measurement system relative to ^{26}Mg. Mass discrimination in this region of the mass scale tends to be high on a quadrupole ICP-MS system.

The amount of spike added to the sample prior to oxidation needs to be optimized. The error propagation has been calculated and plotted in Figure 3-1 for a typical measurement system employing a ^{25}Mg spike of 99 % isotopic purity. The calculated error magnification factor (EM) is at a minimum (EM = 1.07) for a $^{24}Mg/^{25}Mg$ ratio of 0.28. However, although this is the ideal ratio, it is considered better to underspike the system to yield an isotope ratio closer to unity. Under these conditions, the effects of detector dead-time and pulse counting statistics, which are important aspects of ICP-MS measurement systems, are minimized. As is evident from Figure 3-1, at a ratio of approximately unity, the error magnification factor is about 1.15, which is totally acceptable. The other approach to measurement would be to exactly match the ratios of the spike calibration mixtures to the sample measurement mixtures, and run them in the same temporal space. This is not easily achieved and does not circumvent the counting statistics limitation.

FIGURE 3-1



4. Safety

Clinical fluids are biohazardous materials which may contain pathogenic substances. The usual safety precautions should be taken to prevent

exposure during handling, including the use of gloves at all times. Although most materials will have been screened for such substances, a clinical monitoring regimen, including screening for HIV 1 and 2 and vaccination against hepatitis B is recommended for personnel handling biological specimens on an extended basis.

5. Required Reagents

5.1 ^{25}Mg Isotopic Spike

A spike having an isotopic purity >99 %, can be obtained through Oak Ridge National Laboratory (ORNL) or other stable-isotope supplier in a form such as ^{25}MgO. A stock solution can be prepared by dissolving a small amount of the ^{26}MgO in 2 % (volume fraction) high-purity nitric acid to yield a concentration of about 7.5 µg/g (0.3 µmol/g) and storing in a cleaned Teflon® fluoropolymer bottle. Tracking the tare weight of the bottle is useful for long-term storage and monitoring the concentration of the spike on a multiple-use basis.

5.2 SRM 929a, Magnesium Gluconate, Clinical Standard

The material should be dried according to the directions on the certificate for 24 h over magnesium perchlorate in a desiccator.

The relative mass fraction of magnesium is 5.362 % with an expanded uncertainty of 0.027 %.

Alternatively, magnesium standard solution (SRM 3131a), which has a concentration of 9.99 mg/g ± 0.02 mg/g (0.2 % relative) can be used.

5.3 High-Purity Nitric Acid

High-purity acid is essential to minimize the extent of magnesium contamination from reagent sources. High-purity acid containing less than 100 pg/g magnesium may be obtained from a number of commercial suppliers.

5.4 High-Purity Water

De-ionized or quartz-distilled water, which has been tested and is low in magnesium contamination, is used for sample dilution. The water should meet or exceed the specifications of CAP/NCCLS Type I water [1].

6. Equipment

6.1 ICP-MS

The method utilizes a standard ICP-MS system employing a quadrupole mass spectrometer. The instrument should be capable of operating in

the pulse counting detection mode, and should be fitted with a high-efficiency pneumatic nebulizer system, if available. The use of a sample introduction system composed of an inert material is beneficial in reducing instrument background.

6.2 Microwave Digestion System

A commercial system should be used which is capable of reaching at least 70 bar (7 MPa) vessel pressure and a maximum vessel temperature of at least 200 °C using either PTFE or quartz reaction vessels. The system should be fitted with industry-standard safety interlocks. A continuous (un-pulsed) variable power delivery system is advantageous in maintaining optimum reaction control.

6.3 Sample Bottles

Clean 60 mL polyethylene (Nalgene) sample bottles (or equivalent material) for sample storage and analysis.

> **Note on Sample Bottles**: Polyethylene or Teflon® fluoropolymer sample bottles formulated for low trace-metal content should be used. These contain very low levels of electrolyte contaminants. Containers such as polypropylene centrifuge tubes should not be used for this purpose, as they frequently contain appreciable levels of contaminants.

6.4 Micro-Pipette

Automatic or manual micro-pipette for dispensing sample, spike and standard aliquots. A capped plastic syringe fitted with a Teflon® fluoropolymer uptake tube may also be used for this purpose.

6.5 Analytical Balance

Five-place analytical balance for weighing sample, spike and standard aliquots. The balance should be calibrated and verified to be functioning correctly prior to weighing measurements.

7. Sample Handling, Preparation and Quality Control

7.1 Analytical Sample

The analytical sample should be equilibrated to room temperature prior to measurement. Follow supplier instructions for reconstitution of lyophilized material or equilibration from freezer temperature. All sample preparation should be carried out in a Class 10 clean environment with the use of protective clothing and gloves.

7.2 Aliquoting and Acidification

Approximately 0.5 g of the liquid sample should be weighed accurately, using a five-place analytical balance, into a cleaned microwave digestion vessel manufactured from quartz or Teflon® fluoropolymer and spiked with an aliquot of ^{25}Mg stock to yield a $^{24}Mg/^{25}Mg$ ratio of approximately unity. Five grams of high-purity nitric acid should be added to each vessel and the vessels then sealed using the appropriate procedure specified by the manufacturer.

7.3 Digestion

The prepared solutions should be placed into a high-pressure microwave digestion system and digested using the manufacturers recommended conditions or user-developed digestion protocol. A vessel pressure approaching 70 bar (7 MPa) for at least 30 min will usually ensure complete oxidation of the sample and conversion of all carbon in the sample to carbon dioxide.

7.4 Dilution

The digested samples should be carefully removed from the digestion vessels and diluted with high-purity water to a concentration level appropriate to the ICP-MS instrumentation used for the measurements.

7.5 Stability

The prepared solutions are stable for several days. However, determinations should be carried out as soon as possible to avoid the risk of contamination, losses or stability issues.

7.6 Spike Calibration Mixtures

The concentration of the ^{25}Mg spike stock solution must be measured by preparing at least four independent spike calibration mixtures. Prepare two stock calibration solutions of natural magnesium from SRM 929a or SRM 3131a. Prepare two spike calibration mixtures from each of the natural magnesium calibration solutions by aliquoting known amounts of the calibration stock and ^{25}Mg spike into sample bottles and diluting with 2 % (volume fraction) nitric acid to a concentration suitable for measurement by ICP-MS. The aliquots should be adjusted to yield $^{24}Mg/^{25}Mg$ isotope ratios as similar as possible to the analytical samples. A five-place analytical balance should be used for all weighing operations.

7.7 Procedure Blanks

Prepare a minimum of three procedure blanks by adding approximately 0.5 µg or less of the ^{25}Mg spike to microwave digestion vessels and carrying them through the entire preparation procedure.

7.8 Composition Sample

Prepare a composition sample by aliquoting approximately 0.5 g of sample into a microwave digestion vessel and carrying it through the same preparation procedure as the samples. The composition sample is not spiked with ^{25}Mg and is used to assess the presence or absence of spectral interferences in the ICP-MS measurement process.

7.9 Controls

To assess the accuracy of the measurements, prepare at least two different SRM control materials (preferably of the same matrix as the samples) having certified values for magnesium, and carry them through the same sample preparation and measurement procedures as the samples.

8. Analytical Measurement

8.1 Instrument Preparation

Prior to analytical measurements, the ICP-MS system should be equilibrated for 30 min. The sample introduction system should be rigorously cleaned before use, including the sampler and skimmer cones, peristaltic pump tubing, plasma torch and spray chamber. Magnesium background from the instrument is usually quite high and these procedures are designed to mitigate the background as much as possible. It is recommended that new peristaltic pump tubing be installed prior to measurements. A dilute nitric acid solution (2 % volume fraction) should be pumped through the tubing for a while to remove any leachable magnesium from the tubing.

8.2 Instrument Optimization

The instrument should be optimized for maximum ion transmission at m/z 24 by nebulizing a standard solution of magnesium and adjusting ion lens voltages, plasma gas flows, torch position (relative to the sample cones) and rf forward power.

8.3 Run Sequence

Software acquisition menus should be prepared for measuring the ^{24}Mg and ^{25}Mg isotopes. The run order for the samples should be established according to the following protocol:

Analytical procedure blanks should be run at the beginning of the sequence, followed by the composition sample and then the analytical samples (including control materials) in randomized order. Instrument mass discrimination measurement standards (*see note*) should be run after every three to four samples depending on the stability of the instrument.

> **Note on Mass Discrimination Measurement:** The measured $^{24}Mg/^{25}Mg$ ratios should be corrected for the ICP-MS mass discrimination. This can be achieved by periodic measurement of a calibration standard consisting of natural magnesium. The natural ratio of $^{24}Mg/^{25}Mg$ is approximately 7.9, which is very different from the sample ratios. A more elegant way to assess discrimination is to carefully calibrate a solution having a ratio closer to that of the samples. A spike calibration solution can conveniently be used for this purpose.

8.4 Isotope Ratio Acquisition

The isotope ratio $^{24}Mg/^{25}Mg$ should be acquired in peak jumping mode. A typical acquisition scheme would be five to seven sets of ratios with a total acquisition time of 60 s per set, using a dwell-time of 10 ms per isotope. The isotope ratio data should be downloaded to a spreadsheet program such as Microsoft Excel. Inspect the ratios and remove any considered to be statistical outliers.

9. Data Reduction

9.1 Mass Discrimination Corrections

Convert the experimentally measured $^{24}Mg/^{25}Mg$ isotope ratios to absolute ratios using the following equation:

$$\left[\frac{^{24}Mg}{^{25}Mg}\right]_{CORR} = \frac{\left[\frac{^{24}Mg}{^{25}Mg}\right]_{TRUE}}{\left[\frac{^{24}Mg}{^{25}Mg}\right]_{MEAS}}$$

where, $\left[\frac{^{24}Mg}{^{25}Mg}\right]_{CORR}$ is the discrimination corrected sample ratio

$\left[\frac{^{24}Mg}{^{25}Mg}\right]_{TRUE}$ is the true ratio for the isotopic standard

$\left[\frac{^{24}Mg}{^{25}Mg}\right]_{MEAS}$ is the measured ratio for the isotopic standard

The measured ratios for the isotopic standard can be applied to sample discrimination corrections in a number of ways, which will depend on the discrimination drift as a function of time: (1) the ratio can be calculated by averaging the two standard measurements bracketing the samples of interest, (2) a more sophisticated approach is to mathematically model the drift in the standard ratio as a function of time and interpolate the ratio for each sample. This may require more frequent measurement of the isotopic standard to define the drift function adequately.

9.2 Spike Calibration

Calculate the concentration of the ^{24}Mg spike in each spike calibration mixture using the following equation:

$$C_{SPK} = \left[\frac{M_{STD}(BR - A)}{M_s K(A_s - B_s R)}\right]$$

where, C_{SPK} is the concentration of magnesium in the spike in µg/g
M_{STD} is the mass of magnesium added to the spike mix (µg)
B is the natural abundance of the spike isotope
R is the corrected $^{24}Mg/^{25}Mg$ ratio in the spike mix
A is the natural abundance of the reference isotope
M_S is the mass of ^{25}Mg spike aliquot added to the mix (g)
K is the natural to spike relative atomic mass ratio
A_S is the abundance of the reference isotope in the spike
B_S is the abundance of the spike isotope in the spike

Calculate the mean and standard deviation of the spike calibration mixtures. Blank corrections to the spike calibration are not normally necessary because they are simple mixtures prepared in dilute acid.

9.3 Calculation of Magnesium Sample Concentrations

Calculate the magnesium concentration (µg/g) in the sample using the following equation:

$$C = \left[\frac{M_s K(A_s - B_s R)}{(BR - A)} - S_B \right] \times \frac{1}{W}$$

where, **C** is the concentration of magnesium in the sample (µg/g)
M_s is the absolute mass of ^{25}Mg spike added (µg)
K is the natural to spike relative atomic mass ratio
A_s is the abundance of the reference isotope in the spike
B_s is the abundance of the spike isotope in the spike
R is the corrected ^{24}Mg/^{25}Mg ratio
B is the natural abundance of the spike isotope
A is the natural abundance of the reference isotope
S_B is the absolute mean measured blank (µg)
W is the mass of sample aliquot taken (g)

An alternative approach, if desired, is to calculate the spike and sample concentrations in mol/g. In this case the equations are slightly modified and the relative atomic masses of natural magnesium and the spike are not required for the calculations.

10. Uncertainty Analysis

The expanded uncertainty for the set of samples should be calculated according to ISO guidelines [2] by combining both Type A and Type B uncertainties. Typical Type A uncertainty components for this measurement might be the variability of the measured concentrations of magnesium in the samples, the measurement of the spike concentration and the variability of the correction for the blank. Typical Type B uncertainty components might be the uncertainty of the assay of the primary calibrant, weighing measurements, conversion correction for the density of the sample and instrumental parameters such as detector dead-time correction, instrument mass discrimination correction and instrument background correction.

For a 95 % confidence level, combine the Type A and Type B components and calculate the total expanded uncertainty using the following equation:

$$U = k \left[s_1^2/df_1 + \ldots + s_n^2/df_n + B_1^2 + \ldots + B_n^2 \right]^{1/2}$$

where, *U* is the expanded uncertainty
k is the coverage factor
s is the observed standard deviation of (n) Type A components
df is the degrees of freedom associated with each component

B is the Type B component (n components) with infinite degrees of freedom.

Report the expanded uncertainty in the same units as the sample concentrations.

11. Data Reporting

Convert data from a mass/mass basis to a mass/volume basis using the measured density of the serum. **For clinical use, report data in units of mg/dL**. To convert data from µg/mL to mg/dL divide by 10. To convert from mg/dL to mmol/L multiply by 0.41144.

12. Performance Statement

12.1 Measurement Repeatability

The isotope ratio measurement repeatability is usually limited by detector counting statistics. Typical measurement repeatability on a quadrupole ICP-MS system for a $^{24}Mg/^{25}Mg$ ratio approaching unity is 0.1-0.2 %.

12.2 Detection Limit

The instrument detection limit is influenced by the instrument background. A typical instrument detection limit for a well-optimized quadrupole ICP-MS instrument is 5 pg/mL. For a 0.5 g sample aliquot this equates to a detection limit of approximately 1 ng/mL which is far below the magnesium concentrations typically found in clinical fluids.

12.3 Instrument Background and Procedure Blank

The ICP-MS instrument background tends to be fairly high for magnesium. The background can vary from a few hundred counts per second to several thousand counts per second depending on the cleanliness of the system and history of use.

A typical procedure blank using this method is approximately 5 ng, depending on the purity of the reagents and storage containers. This is low in relation to the magnesium content in clinical fluids. For example, the blank correction for the determination of magnesium in blood serum should be ≤ 0.1 %.

12.4 Performance Summary

A summary of typical method performance parameters expected for this method are provided in Table 12-1.

Table 12-1: Method Performance Summary

Parameter	Typical Value or Range
Isotope ratio measurement repeatability	0.1-0.2 %
Applicable concentration range	All ranges found in clinical specimens
Instrument background / sample ratio	≈ 0.2 %
Procedure blank	≈ 5 ng
Sample throughput (measurement)	10 samples per hour

13. Reference Citations

1. *Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline – Third Edition*, NCCLS Document C3-A3, NCCLS, Wayne, Pennsylvania (1997).

2. *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed. ISO, Switzerland, 1993.

MG-1 Revision History

Revision	Date	Author	Record of Changes and Comments
1.0	9/2006	S E Long	Initial draft method

6 Potassium

6.1 Clinical Significance

Potassium is the most abundant cation in intra-cellular fluid. It has a major role in maintaining the resting membrane potential of cells, which is dependent on the concentration gradient of potassium inside and outside the cell. Cell membranes are highly permeable to potassium ions, so that there is free exchange of potassium ions across the membrane. The majority of the potassium in the body is in the intra-cellular fluid, with approximately 2 % of the total in the extra-cellular fluid. The physiological action of potassium is intimately associated with that of sodium and, like sodium, is regulated by aldosterone and other hormones. Potassium homeostasis is maintained by dietary intake and renal excretion. A lowered potassium level (*hypokalemia*) results when there is excessive loss of potassium, such as renal losses during diuretic treatment and in both chronic and acute metabolic alkalosis. Losses can also occur by dehydration resulting from fluid and electrolyte losses from burns and hemorrhages, and from gastrointestinal losses associated with excessive vomiting and diarrhea. In some cases, extreme losses of potassium can be life threatening. Elevated potassium levels (*hyperkalemia*) can occur during renal failure, from reduced aldosterone output associated with Addison's disease, dehydration and from movement of potassium out of cells as a result of large muscle injury or tissue damage.

6.2 Normal Range and Reporting Units

The normal range for potassium in serum or plasma is 3.5–5.3 mmol/L.
Potassium clinical data are conventionally reported in mmol/L.
To convert from mmol/L to mg/dL multiply by 3.90983.
To convert from mg/dL to mmol/L multiply by 0.25577.

6.3 Routine Clinical Measurement Methods

Blood and urine are the most commonly used media for assessing patient health status. Flame photometry and flame atomic absorption spectrometry are reference methods used for the measurement of potassium, providing accuracy and specificity. For routine use, especially in point-of-care environments, ion selective electrodes are now well established. These systems, which typically measure potassium and sodium in tandem, are capable of providing accurate and consistent data in a very short

measurement time frame and are therefore extremely useful in environments where rapid measurements are important such as intensive care units and operating theaters.

6.4 Available Standard Reference Materials

Two blood serum SRMs are available with certified values for potassium, which are **SRM 909b (*Human Serum*)**, a two-level lyophilized material, and **SRM 956b (*Electrolytes in Frozen Human Serum*)**, which is a three-level frozen material intended for standardizing and calibrating ion selective electrode analyzers. There are also reference values for potassium in **SRM 2670a (*Toxic Elements in Freeze-Dried Urine*)**, a two-level, freeze-dried material. One clinical assay standard may be used for potassium: **SRM 918a (Potassium Chloride, Clinical Standard)** which is sold as a clinical primary standard intended for instrument calibration and standardization. Certificate information for these SRMs is summarized in Table 6-1.

Table 6-1. Clinical Standard Reference Materials for Potassium

SRM	Description	Value (mmol/L)	Expanded Uncertainty (mmol/L)
909b Level 1	Human Serum	3.424	0.025
909b Level 2	Human Serum	6.278	0.052
956b Level 1	Electrolytes in Frozen Human Serum	5.973	0.045
956b Level 2	Electrolytes in Frozen Human Serum	3.983	0.029
956b Level 3	Electrolytes in Frozen Human Serum	1.987	0.014
2670a Level 1	Toxic Elements in Urine (Freeze-Dried)	(10.49)	0.26
2670a Level 2	Toxic Elements in Urine (Freeze-Dried)	(10.61)	0.26
SRM	**Clinical Standard**	**Purity (% mass fraction)**	**Expanded Uncertainty (%)**
918a	Potassium Chloride (Clinical Standard)	99.9817	0.0084

( ) Values in Parentheses are Reference Values.

Version Number	1.0
Approved	S E Long
Effective Date	Sep 2006

K-1
POTASSIUM BY ISOTOPE DILUTION INDUCTIVELY COUPLED PLASMA – MASS SPECTROMETRY

1. Applicability

The method is applicable to the determination of potassium at physiological levels in blood plasma, serum (frozen and lyophilized) and urine.

2. Principle of Measurement

The method is based on isotope dilution mass spectrometry using a quadrupole inductively coupled plasma – mass spectrometer (ICP-MS) operated in "cool plasma" (shielded) mode [1]. A stable isotopic spike of ^{41}K is added to the sample, equilibrated by oxidation with nitric acid in a microwave digestion system, and the $^{39}K/^{41}K$ isotope ratio measured using low power plasma conditions. The method is extremely accurate and selective for potassium.

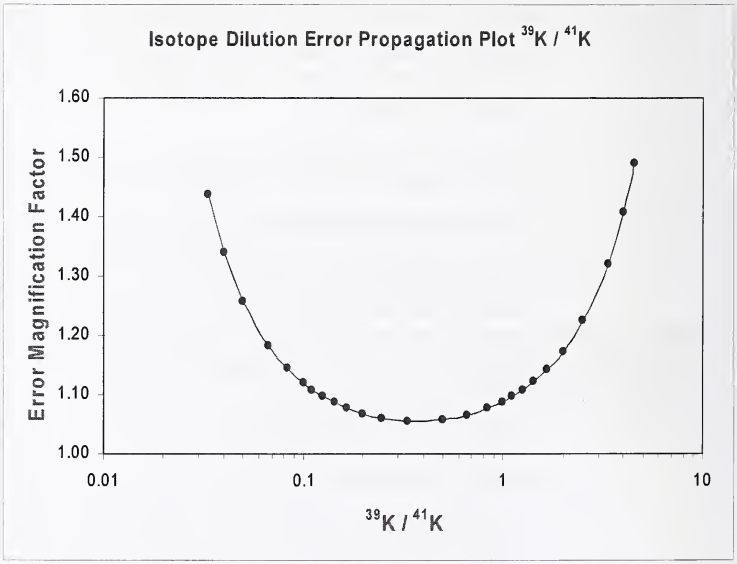
3. Measurement Strategy and Experimental Design

The determination of potassium in biological fluids by isotope dilution ICP-MS is complicated by the presence of potential spectral interferences from $^{38}Ar^{1}H$ which interferes with ^{39}K, and $^{40}Ar^{1}H$, $^{23}Na^{18}(H_2O)$ and $^{25}Mg^{16}O$ which interfere with ^{41}K. The use of "cool" or shielded plasma conditions effectively attenuates the Ar based spectral background interferences. The other interferences are minor, except for the $^{23}Na^{18}(H_2O)$ interference which is significant because of the relatively high level of sodium in biological fluids. For this reason, a chemical separation of potassium and sodium is necessary. For convenience, potassium and calcium (which is similarly afflicted) can be separated, re-combined and determined together in the same measurement system if desired. The isobaric interference from ^{40}K on ^{40}Ca is negligible for the potassium levels normally found in clinical fluids. Treatment of the sample with nitric acid in a microwave digestion system is

necessary to digest the sample matrix. Normally, ^{41}K is used as the spike, which is available in approximately 99 % isotopic purity.

The amount of spike added to the sample prior to digestion needs to be optimized. The error propagation has been calculated and plotted in Figure 3-1 for a typical measurement system employing a ^{41}K spike of 99 % purity. The calculated error magnification factor (EM) is at a minimum (EM = 1.06) for a $^{39}K/^{41}K$ ratio of 0.37. However, although this is the ideal ratio, it is considered better to underspike the system to yield an isotope ratio closer to unity. Under these conditions, the effects of detector dead-time and pulse counting statistics, which are important aspects of ICP-MS measurement systems, are minimized. As is evident from Figure 3-1, at a ratio of approximately unity, the EM factor is about 1.09, which is acceptable. An additional approach to measurement is to closely match the ratios of the spike calibration mixtures to the sample measurement mixtures, and run them in the same temporal measurement space. This requires a fairly accurate knowledge of the concentration of potassium in the unknown sample prior to spiking.

FIGURE 3-1



4. Safety

Clinical fluids are biohazardous materials which may contain pathogenic substances. The usual safety precautions should be taken to prevent exposure during handling, including the use of gloves at all times. Although most materials will have been screened for such substances, a clinical monitoring regimen, including screening for HIV 1 and 2 and vaccination against hepatitis B is recommended for personnel handling biological specimens on an extended basis.

5. Required Reagents

5.1 ^{41}K Isotopic Spike

A spike having an isotopic purity ≥ 99 %, can be obtained through Oak Ridge National Laboratory (ORNL) or other stable-isotope supplier in a form such as ^{41}KCl. A stock solution can be prepared by dissolving a small amount of the ^{41}KCl in 1 % (volume fraction) high-purity nitric acid to yield a concentration of about 275 µg/g (6.7 µmol/g) and storing in a cleaned PTFE bottle. Tracking the tare weight of the bottle is useful for long-term storage and monitoring the concentration of the spike on a multiple-use basis.

5.2 SRM 918a, Potassium Chloride, Clinical Standard

The material should be dried according to the directions on the certificate in a laboratory oven at 110-120 °C for 2 h.

The certified purity of the material is 99.9917 % (mass fraction) with an expanded uncertainty of 0.0084 %. The relative mass fraction of potassium in SRM 918a, adjusted for the purity, is 0.52435.

Alternatively, potassium standard solution (SRM 3141a), which has a concentration of 10.295 mg/g ± 0.023 mg/g (0.2 % relative) can be used.

5.3 High-Purity Nitric Acid

High-purity acid is essential to minimize the extent of potassium contamination from reagent sources. High-purity acid containing less than 100 pg/g potassium may be obtained from a number of commercial suppliers.

5.4 High-Purity Hydrochloric Acid

For chemical separation of potassium from sample matrix. High-purity acid is essential to minimize the extent of potassium contamination from reagent sources. High-purity acid containing less than 100 pg/g potassium may be obtained from a number of commercial suppliers.

5.5 High-Purity Water

De-ionized or quartz-distilled water, which has been tested and is low in potassium contamination, is used for sample dilution. At a minimum the water should meet or exceed the specifications of CAP/NCCLS Type I water [2].

5.6 Ion-Exchange Resin

AG 50-X8, 100-200 mesh (75-150 µm) cation-exchange resin, or equivalent for chemical separation of potassium from the sample matrix. The resin should be bulk cleaned, packed into columns and batch cleaned twice by alternating between 25 mL of 5 mol/L hydrochloric acid and sufficient high-purity de-ionized water to achieve neutral pH.

6. Equipment

6.1 ICP-MS System

The method utilizes a standard ICP-MS system employing a quadrupole mass spectrometer and an automated shielded torch system operating at low RF forward power ("cool-plasma") for reducing argon-based spectral interferences. The instrument should be capable of operating in the pulse counting detection mode, should be fitted with a low dead-volume, cooled spray chamber, and a high-efficiency pneumatic nebulizer system, if available.

6.2 Microwave Digestion System

A commercial system should be used which is capable of reaching at least 70 bar (7 MPa) vessel pressure and a maximum vessel temperature of at least 200 °C using either PTFE or quartz reaction vessels. The system should be fitted with industry-standard safety interlocks. A continuous (un-pulsed) variable power delivery system is advantageous in maintaining optimum reaction control.

6.3 Teflon® Fluoropolymer Beakers

Clean 50 mL Teflon® fluoropolymer beakers for solution evaporation prior to column separations.

6.4 Ion-Exchange Columns

Clean plastic or glass ion-exchange columns containing a cation-exchange resin bed (see Reagents) of approximate dimensions 10 cm long x 0.7 cm wide. This bed should contain sufficient capacity and column resolution for most clinical samples.

6.5 Sample Bottles

Clean 30 mL polyethylene (Nalgene) sample bottles (or equivalent material) for sample storage and analysis.

> **Note on Sample Bottles**: Polyethylene or Teflon® fluoropolymer sample bottles formulated for low trace-metal content should be used. These contain very low levels of electrolyte contaminants. Containers such as polypropylene centrifuge tubes should not be used for this purpose as they frequently contain appreciable levels of contaminants. Clean the sample bottles prior to use by soaking in dilute nitric acid for a minimum of 24 h.

6.6 Micro-Pipette

Automatic or manual micro-pipette for dispensing sample, spike and standard aliquots. A capped plastic syringe fitted with a Teflon® fluoropolymer uptake tube may also be used for this purpose, depending on the weighing method employed.

6.7 Analytical Balance

Five-place analytical balance for weighing sample, spike and standard aliquots. The balance should be calibrated and verified to be functioning correctly prior to weighing measurements.

7. Sample Handling, Preparation and Quality Control

7.1 Analytical Sample

The analytical sample should be equilibrated to room temperature prior to measurement. Follow supplier instructions for reconstitution of lyophilized material or equilibration from freezer temperature. All sample preparation should be carried out in a Class 10 clean environment with the use of protective clothing and gloves.

7.2 Aliquoting and Acidification

Approximately 0.7 g of the liquid sample should be weighed accurately, using a five-place analytical balance, into a cleaned microwave digestion vessel manufactured from quartz or Teflon® fluoropolymer and spiked with an aliquot of ^{41}K stock (approximately 0.4–0.5 g for normal blood serum) to yield a $^{39}K/^{41}K$ ratio of approximately unity. Six grams of high-purity nitric acid should be added to each vessel and the vessels then sealed using the appropriate procedure specified by the manufacturer.

7.3 Digestion

The prepared solutions should be placed into a high-pressure microwave digestion system and digested using the manufacturers recommended conditions or user-developed digestion protocol. A vessel pressure approaching 70 bar (7 MPa) for at least 30 min will usually ensure complete oxidation of the sample and conversion of all carbon in the sample to carbon dioxide.

7.4 Evaporation and Chemical Separations

The digested samples should be evaporated to near dryness using the microwave digestion system (if this is a feature), or alternatively by heating on a hot-plate in 50 mL Teflon® fluoropolymer beakers. Re-dissolve in a small quantity of dilute 2 % (volume fraction) nitric acid. Transfer to cation-exchange columns. Elute the sodium using approximately 40 mL of 0.3 mol/L hydrochloric acid. Elute the potassium with approximately 20 mL of 0.5 mol/L hydrochloric acid. Evaporate the potassium fraction to dryness and re-dissolve in 2 % (volume fraction) nitric acid to yield a concentration suitable for ICP-MS measurement. Store in clean 30 mL polyethylene bottles.

7.5 Stability

The prepared solutions are stable for several days. However, determinations should be carried out as soon as possible to avoid the risk of contamination, losses or stability issues.

7.6 Spike Calibration Mixtures

The concentration of the ^{41}K spike stock solution must be measured by preparing at least four independent spike calibration mixtures. Prepare two different stock calibration solutions of natural potassium from SRM 918a or SRM 3141a. Prepare two spike calibration mixtures from each of the natural potassium calibration solutions by aliquoting known amounts of the calibration stock and ^{41}K spike into sample bottles and diluting with 2 % (volume fraction) nitric acid to a concentration suitable for measurement by ICP-MS. The aliquots should be adjusted to yield $^{39}K/^{41}K$ isotope ratios as similar as possible to the analytical samples. A five-place analytical balance should be used for all weighing operations.

7.7 Procedure Blanks

Prepare a minimum of three procedure blanks by adding approximately 100 ng of the ^{41}K spike to microwave digestion vessels and carrying them through the entire preparation and analysis procedure.

7.8 Composition Sample

Prepare a composition sample by aliquoting approximately 0.7 g of sample into a microwave digestion vessel and carrying it through the same preparation procedure as the samples. The composition sample is not spiked with ^{41}K and is used to assess the presence or absence of spectral interferences in the ICP-MS measurement process.

7.9 Controls

To assess the accuracy of the measurements prepare at least two different SRM control materials (preferably of the same matrix type as the samples) having certified values for potassium, and carry the controls through the same sample preparation and measurement procedures as the samples.

8. Analytical Measurement

8.1 Instrument Preparation

Prior to analytical measurements, the ICP-MS system should be equilibrated for 30 min. The sample introduction system should be rigorously cleaned before use, including the sampler and skimmer cones, peristaltic pump tubing, plasma torch and spray chamber. These procedures are designed to mitigate the background from potassium as much as possible. It is recommended that new peristaltic pump tubing be installed prior to measurements. A dilute nitric acid solution (2 % volume fraction) should be pumped through the tubing for a while to remove any leachable potassium from the tubing.

8.2 Instrument Optimization

The instrument should be optimized for maximum ion transmission and minimum background at m/z 39 and 41 (maximum signal to background ratio) using a suitable potassium standard, and adjusting ion lens voltages, plasma gas flows, torch position (relative to the sample cones) and rf forward power as necessary.

8.3 Run Sequence

Software acquisition menus should be prepared for measuring the ^{39}K and ^{41}K isotopes. The run order for the samples should be established according to the following protocol:

Analytical procedure blanks should be run at the beginning of the sequence, followed by the composition sample and then the analytical samples (including control materials) in randomized order. Instrument

mass discrimination measurement standards (*see note*) should be run after every three to four samples depending on the stability of the instrument.

Note on Mass Discrimination Measurement: The measured $^{39}K/^{41}K$ ratios should be corrected for the ICP-MS mass discrimination. This can be achieved by measurement of a calibration standard (SRM 918a) consisting of natural potassium. However, the natural ratio of $^{39}K / ^{41}K$ is approximately 14, which is very large and different from the measured sample ratios. A better way to assess discrimination and measurement run discrimination drift is to carefully calibrate a solution having a ratio matched more closely to the samples. A spike calibration solution can conveniently be used for this purpose.

8.4 Isotope Ratio Acquisition

The isotope ratio $^{39}K/^{41}K$ should be acquired in peak jumping mode. A typical acquisition scheme would be five to seven sets of ratios with a total acquisition time of 60 s per set, using a dwell-time of 10 ms per isotope. The isotope ratio data should be dead-time corrected and downloaded to a spreadsheet program such as Microsoft Excel. Inspect the ratios and remove any considered to be statistical outliers.

9. Data Reduction

9.1 Mass Discrimination Corrections

Convert the experimentally measured $^{39}K/^{41}K$ isotope ratios to absolute ratios using the following equation:

$$\left[\frac{^{39}K}{^{41}K}\right]_{CORR} = \frac{\left[\frac{^{39}K}{^{41}K}\right]_{TRUE}}{\left[\frac{^{39}K}{^{41}K}\right]_{MEAS}}$$

where, $\left[\frac{^{39}K}{^{41}K}\right]_{CORR}$ is the discrimination corrected sample ratio

$\left[\frac{^{39}K}{^{41}K}\right]_{TRUE}$ is the true ratio for the isotopic standard

$\left[\frac{^{39}K}{^{41}K}\right]_{MEAS}$ is the measured ratio for the isotopic standard

The measured ratios for the isotopic standard can be applied to sample discrimination corrections in a number of ways, which will depend on the discrimination drift as a function of time: (1) the ratio can be calculated by averaging the two standard measurements bracketing the samples of interest, (2) a more sophisticated approach is to mathematically model the drift in the standard ratio as a function of time and interpolate the ratio for each sample. This may require more frequent measurement of the isotopic standard to define the drift function adequately.

9.2 Spike Calibration

Calculate the concentration of the ^{41}K spike in each spike calibration mixture using the following equation:

$$C_{SPK} = \left[\frac{M_{STD}(BR - A)}{M_s K(A_s - B_s R)} \right]$$

where, C_{SPK} is the concentration of potassium in the spike in µg/g
M_{STD} is the mass of potassium added to the spike mix (µg)
B is the natural abundance of the spike isotope
R is the corrected $^{39}K/^{41}K$ ratio in the spike mix
A is the natural abundance of the reference isotope
M_S is the mass of ^{41}K spike aliquot added to the mix (g)
K is the natural to spike relative atomic mass ratio
A_S is the abundance of the reference isotope in the spike
B_S is the abundance of the spike isotope in the spike

Calculate the mean and standard deviation of the spike calibration mixtures. Blank corrections to the spike calibration are not normally necessary because they are simple mixtures prepared in dilute acid with minimal sample processing.

9.3 Calculation of Potassium Sample Concentrations

Calculate the potassium concentration (µg/g) in the sample using the following equation:

$$C = \left[\frac{M_s K(A_s - B_s R)}{(BR - A)} - S_B \right] \times \frac{1}{W}$$

where, C is the concentration of potassium in the sample (µg/g)
M_s is the absolute mass of ^{41}K spike added (µg)

K	is the natural to spike relative atomic mass ratio
A_s	is the abundance of the reference isotope in the spike
B_s	is the abundance of the spike isotope in the spike
R	is the corrected $^{39}K/^{41}K$ ratio
B	is the natural abundance of the spike isotope
A	is the natural abundance of the reference isotope
S_B	is the absolute mean measured blank (µg)
W	is the mass of sample aliquot taken (g)

An alternative approach, if desired, is to calculate the spike and sample concentrations in mol/g. In this case the equations are slightly modified and the relative atomic masses of natural potassium and the spike are not required for the calculations.

10. Uncertainty Analysis

The expanded uncertainty for the set of samples should be calculated according to ISO guidelines [3] by combining both Type A and Type B uncertainties. Typical Type A uncertainty components for this measurement might be the variability of the measured concentrations of potassium in the samples, the measurement of the spike concentration and the variability of the correction for the blank. Typical Type B uncertainty components might be the uncertainty of the assay of the primary calibrant, weighing measurements, conversion correction for the density of the sample and instrumental parameters such as detector dead-time correction, instrument mass discrimination correction and instrument background correction.

For a 95 % confidence level, combine the Type A and Type B components and calculate the total expanded uncertainty using the following equation:

$$U = k\,[s_1^2/df_1 + \ldots + s_n^2/df_n + B_1^2 + \ldots + B_n^2\,]^{1/2}$$

where, *U* is the expanded uncertainty
k is the coverage factor
s is the observed standard deviation of (n) Type A components
df is the degrees of freedom associated with each component
B is the Type B component (n components) with infinite degrees of freedom.

Report the expanded uncertainty in the same units as the sample concentrations.

11. Data Reporting

Convert data from a mass/mass basis to a mass/volume basis using the measured density of the serum. **For clinical use, report potassium data in units of mmol/L**. To convert data from µg/mL to mg/dL divide by 10. To convert from mmol/L to mg/dL multiply by 3.90983.

12. Performance Statement

12.1 Measurement Repeatability

The isotope ratio measurement repeatability is usually limited by detector counting statistics. Typical measurement repeatability on a quadrupole ICP-MS system for a $^{39}K/^{41}K$ ratio approaching unity is 0.1-0.2 %.

12.2 Detection Limit

The instrument detection limit is influenced by the instrument background. A typical instrument detection limit for a well-optimized quadrupole ICP-MS instrument is 15 pg/mL. For a 0.7 g sample aliquot this equates to a matrix detection limit of approximately 0.1 ng/mL, which is far below the potassium concentrations typically found in clinical fluids.

12.3 Instrument Background and Procedure Blank

The ICP-MS instrument background tends to be very high for potassium owing to the direct isobaric interference of argon species. This can be very effectively reduced by means of "cool plasma" technology. It should be possible to reduce the background for ^{41}K to around 1000 counts per second or lower by judicious choice of operating conditions.

A typical procedure blank using this method is of the order of 50-100 ng (1-2 nmol), depending on the purity of the reagents, cleanliness of the containers and processing conditions. This is extremely low in relation to the normal potassium content in clinical fluids. For example, the blank correction for the determination of potassium in blood serum should be ≤ 0.1 %.

12.4 Performance Summary

A summary of typical method performance parameters expected for this method are provided in Table 12-1.

Table 12-1: Method Performance Summary

Parameter	Typical Value or Range
Isotope ratio measurement repeatability	0.1-0.2 %
Applicable concentration range	All ranges found in clinical specimens
Instrument background / sample ratio	≈ 0.1 %
Procedure blank	≈ 50 - 100 ng
Sample throughput (measurement)	10 samples per hour

13. Reference Citations

1. Murphy K.E., Long S.E., Rearick M.S. and Ertas O.S., *"The Accurate Determination of Potassium and Calcium using Isotope Dilution Inductively Coupled "Cold" Plasma Mass Sectrometry."* J. Anal. At. Spectrom. **17**, pp. 469-477 (2002).

2. *Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline – Third Edition*, NCCLS Document C3-A3, NCCLS, Wayne, Pennsylvania (1997).

3. *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed. ISO, Switzerland, 1993.

K-1 Revision History

Revision	Date	Author	Record of Changes and Comments
1.0	9/2006	S E Long	Initial draft method

7 Sodium

7.1 Clinical Significance

Sodium is the predominant cation in serum or plasma. The majority of the sodium, approximately 140 mmol/L, is distributed in the extra-cellular fluid, while the intra-cellular fluid contains less than 10 mmol/L. The gradient between the two is closely associated with potassium, the major intra-cellular cation, through a sodium-potassium ion pump. Sodium controls the osmotic pressure in the extra-cellular fluid and therefore influences the amount of water retained in the extra-cellular fluid. Sodium plays a critical role in the function of the renal, circulatory and neuro-muscular systems of the body. The concentration of sodium in blood is maintained through dietary intake, balanced by renal excretion, which is modified by the hormone aldosterone. Elevated concentrations (>145 mmol/L) of sodium (*hypernatremia*) can result from excessive loss of water from the body through dehydration or excess ingestion. Equally, depressed concentrations (<130 mmol/L) of sodium (*hyponatremia*) may result from excessive loss through sweating, insufficient dietary intake, renal tubular acidosis (such as that resulting from burns), renal impairment or disorders of the gastro-intestinal system.

7.2 Normal Range and Reporting Units

The normal range for sodium in serum or plasma is 130–145 mmol/L.
Sodium clinical data are conventionally reported in mmol/L. Occasionally mEq/L is also used.
To convert from mmol/L to mg/dL multiply by 2.29898.
To convert from mg/dL to mmol/L multiply by 0.43498.

7.3 Routine Clinical Measurement Methods

In the clinical laboratory, the classical method for measuring sodium is flame atomic emission (flame photometry). Sodium has a very intense emission doublet consisting of the D lines at 589.00 nm and 589.59 nm. This is a very selective method but is not as easily automated or as rapid as some of the potentiometric analyzers. Ion selective electrodes are now widely employed for electrolyte measurements. These systems can provide analytical data in minutes with accuracy which is competitive with flame photometry. The

compact size of some of these systems makes them amenable to portable use in a variety of settings including emergency care.

7.4 Available Standard Reference Materials

Two blood serum SRMs are available with certified values for sodium, which are **SRM 909b (*Human Serum*)**, a two-level lyophilized material, and **SRM 956b (*Electrolytes in Frozen Human Serum*)**, which is a three-level frozen material intended for standardizing and calibrating ion selective electrode analyzers. There are also reference values for sodium in **SRM 2670a (*Toxic Elements in Freeze-Dried Urine*)**, a two-level, freeze-dried material. **SRM 919a (*Sodium Chloride, Clinical Standard*)** is sold as a clinical primary standard which is intended for the purpose of instrument calibration and standardization. Certificate information for these SRMs is summarized in Table 7-1.

Table 7-1. Clinical Standard Reference Materials for Sodium

SRM	Description	Value (mmol/L)	Expanded Uncertainty (mmol/L)
909b Level 1	Human Serum	120.76	0.92
909b Level 2	Human Serum	141.0	1.3
956b Level 1	Electrolytes in Frozen Human Serum	120.1	1.4
956b Level 2	Electrolytes in Frozen Human Serum	141.0	1.6
956b Level 3	Electrolytes in Frozen Human Serum	160.7	1.8
2670a Level 1	Toxic Elements in Urine (Freeze-Dried)	(37.2)	(0.7)
2670a Level 2	Toxic Elements in Urine (Freeze-Dried)	(41.0)	(0.9)
SRM	**Clinical Standard**	**Purity (% mass fraction)**	**Expanded Uncertainty (%)**
919a	Sodium Chloride (Clinical Standard)	99.89	0.03

( ) Values in Parentheses are Reference Values

Version Number	1.0
Approved	S E Long
Effective Date	Sep 2006

NA-1
SODIUM BY STABLE-ISOTOPE INTERNAL STANDARD INDUCTIVELY COUPLED PLASMA – MASS SPECTROMETRY

1. Applicability

The method is applicable to the determination of sodium at physiological levels in blood serum (frozen and lyophilized), sweat and urine.

2. Principle of Measurement

The method is based on measurement of the sodium ion at m/z 23 using an inductively coupled plasma – quadrupole mass spectrometer (ICP-MS). The instrument is operated in analog detection mode, which reduces the contribution from the instrument background which would otherwise be a significant portion of the measured ion signal. As the ions are separated on the basis of their mass to charge ratio, the method is extremely selective for sodium. There are no spectral interferences at this mass from species normally found in clinical fluids. A separated stable-isotope of ^{26}Mg is added to the sample as an internal standard.

3. Measurement Strategy and Experimental Design

Sodium has not commonly been measured by pulse counting detection ICP-MS because of high residual background contamination, which comprises a substantial fraction of the total instrument dynamic range. Because of the relatively high concentration of sodium in clinical materials, however, it is possible to measure sodium using analog ion detection. This approach has a much more effective dynamic range as well as immunity to detector dead-time effects and the limitations of counting statistics. Since sodium is monoisotopic, it is not possible to use isotope dilution. However, a ^{26}Mg enriched isotope can be used as an internal standard with measurement of the $^{23}Na/^{26}Mg$ isotope ratio. ^{26}Mg is used for the internal standard because it is close in mass to sodium and similar in ionization behavior. The ^{26}Mg isotope is used because clinical materials also contain a small amount of

magnesium and the background contribution from this is minimized by the use of ^{26}Mg as the internal standard rather than natural magnesium, which is predominantly ^{24}Mg. All of the $^{23}Na/^{26}Mg$ ratios are internally corrected by concurrent measurement of the $^{24}Mg/^{26}Mg$ ratio and subtracting out the small contribution from the ^{26}Mg component indigenous to the sample.

4. Safety

Clinical fluids are biohazardous materials which may contain pathogenic substances. The usual safety precautions should be taken to prevent exposure during handling, including the use of gloves at all times. Although most materials will have been screened for such substances, a clinical monitoring regimen, including screening for HIV 1 and 2 and vaccination against hepatitis B is recommended for personnel handling clinical specimens on an extended basis.

5. Required Reagents

5.1 ^{26}Mg Isotopic Spike

A spike having an isotopic purity >99 % can be obtained through Oak Ridge National Laboratory (ORNL) or other stable-isotope supplier in the form of ^{26}MgO. A stock solution can be prepared by dissolving 100 mg of the ^{26}MgO in 20 mL of 2 % (volume fraction) high-purity nitric acid and storing in a cleaned Teflon® fluoropolymer bottle.

5.2 SRM 919a, Sodium Chloride Clinical Standard

Dry for 3 h in a convection oven at 110 ºC. Store in a desiccator when not in use.

The certified purity of the material is 99.89 % (mass fraction) with an expanded uncertainty of 0.03 %. The relative mass fraction of sodium in SRM 919a adjusted for the purity is 0.39294.

5.3 High-Purity Nitric acid

High-purity acid is essential to minimize the extent of sodium contamination from reagent sources. High-purity acid may be obtained from a number of commercial suppliers.

5.4 High-Purity Water

De-ionized or quartz-distilled water which has been tested and is low in sodium contamination is used for sample dilution. The water should meet or exceed the specifications of CAP/NCCLS Type I water [1].

6. Equipment

6.1 ICP-MS

The method utilizes a standard ICP-MS system employing a quadrupole mass spectrometer. The instrument should be capable of operating in the analog detection mode using a high-efficiency pneumatic nebulizer system. The use of a sample introduction system using an inert material is beneficial in reducing instrument background.

6.2 Polyethylene Bottles

Clean 30 mL polyethlylene sample bottles for sample preparation and analysis.

Note on Sample Bottles: Polyethylene sample bottles formulated for low trace-metal content should be used. These contain very low levels of electrolyte contaminants. Containers such as polypropylene centrifuge tubes should not be used for this purpose as they frequently contain appreciable levels of contaminants.

6.3 Micro-Pipette

A 1 mL capacity automatic or manual micro-pipette for dispensing sample and standard aliquots. A capped plastic syringe fitted with a Teflon® fluoropolymer uptake tube may also be used for this purpose.

6.4 Analytical Balance

Five-place analytical balance for weighing sample and standard aliquots. The balance should be calibrated and verified to be functioning correctly prior to weighing measurements.

7. Sample Handling and Preparation

7.1 Analytical Sample

The analytical sample should be equilibrated to room temperature prior to measurement. Follow provider instructions for reconstitution of lyophilized material or equilibration from freezer temperature. All sample preparation should be carried out in a Class 10 clean environment with the use of protective clothing and gloves.

7.2 Aliquoting and Dilution

Approximately 0.25 g of the liquid sample should be weighed accurately using a five-place analytical balance into a 30 mL polyethylene bottle by means of a transfer pipette or syringe. The sample is diluted with high-purity water to approximately 25 mL. The exact volume is not critical. An

accurately weighed aliquot of ^{26}Mg spike (5000 µg/g as ^{26}MgO) is then added to the solution. The aliquot volume is typically 0.25 g but the optimum volume will vary depending on the characteristics of the ICP-MS system and the concentration of sodium in the matrix. The spike should not be added to the sample until dilution has taken place because the acid in the spike solution could otherwise precipitate proteins in the sample.

7.3 Acidification

The solution should be acidified to 0.25 % (volume fraction) nitric acid by adding approximately 60 µL of high-purity nitric acid and mixing thoroughly. On no account should the acid concentration be greater than this as there is a risk of precipitating proteins from the sample. Although the sample could still be analyzed, it is better to avoid doing this. Addition of a small amount of acid is necessary to improve sample washout during the measurement process.

7.4 Stability

The prepared solutions should be stable for several days. However, determinations should be carried out as soon as possible to avoid the risk of contamination, losses or stability issues.

7.5 Calibration Solution

Prepare an instrument calibration solution from SRM 919a which falls within the expected range of concentrations of the samples, and spike this solution as for the samples from the same stock of ^{26}Mg isotope.

7.6 Procedure Blanks

Prepare a minimum of three procedure blanks by adding approximately 0.25 g of the ^{26}Mg spike to a 30 ml polyethylene bottle and treating in an identical manner to the samples.

8. Analytical Measurement

8.1 Instrument Preparation

Prior to analytical measurements, the ICP-MS system should be equilibrated for 30 min. The sample introduction system should be rigorously cleaned before use, including the sampler and skimmer cones, peristaltic pump tubing, plasma torch and spray chamber. Sodium background from the instrument is usually very high and these procedures are designed to mitigate the background as much as possible. It is recommended that new peristaltic pump tubing be installed

prior to measurements. A dilute nitric acid solution (2 % volume fraction) should be pumped through the tubing for a while to remove any leachable sodium from the tubing.

8.2 Instrument Optimization

The instrument should be optimized for maximum ion transmission at m/z 23 by nebulizing a standard solution of sodium and adjusting ion lens voltages, plasma gas flows and rf forward power. The voltage on the analog stage of the detector should be adjusted such that the signal to background ratio is maximized.

Note on Optimization of Instrument Conditions: The RF forward power to the plasma should be maintained as high as possible to ensure complete decomposition of protein material in the sample. The use of low RF powers (typically less than 1300 W) will result in significantly degraded measurement precision.

8.3 Run Sequence

Software acquisition menus should be prepared for measuring the ^{23}Na, ^{24}Mg and ^{26}Mg isotopes. The run order for the samples should be established according to the following protocol:

Analytical procedure blanks should be run at the beginning of the sequence, followed by the composition sample and then the analytical samples (including control materials) in randomized order. Instrument calibration standards should be run after every three to four samples depending on the mass discrimination stability of the instrument.

8.4 Isotope Ratio Acquisition

The isotope ratios $^{23}Na/^{26}Mg$ and $^{24}Mg/^{26}Mg$ should be acquired in peak jumping mode. A typical acquisition scheme would be five to seven sets of ratios with a total acquisition time of 60 s per set, using a dwell-time of 10 ms per isotope. Dead-time corrections to the data are not necessary as analog detection mode is used. The isotope ratio data should be downloaded to a spreadsheet program such as Microsoft Excel. Inspect the ratios and remove any considered to be statistical outliers.

9. Data Reduction

9.1 Correction Equations for Magnesium in the Sample

Convert the measured isotope ratio $^{23}Na/^{26}Mg_{meas}$ to the true ratio $^{23}Na/^{26}Mg_{true}$ which is corrected for the magnesium sample contribution using the following equations:

$$^{26}I_{meas} = {}^{26}I_{true} + C_{nat} \cdot {}^{24}I_{serum} \quad (1)$$

where, $^{26}I_{meas}$ is the measured signal intensity at m/z 26
$^{26}I_{true}$ is the true signal intensity for ^{26}Mg spike
$^{24}I_{serum}$ is the signal intensity for serum ^{24}Mg
C_{nat} is the correction for the natural composition $^{24}Mg/^{26}Mg$

$$^{24}I_{serum} = {}^{24}I_{meas} - C_{spk} \cdot {}^{26}I_{true} \quad (2)$$

where, $^{24}I_{meas}$ is the measured signal intensity at m/z 24
C_{spk} is the ratio correction for the spike composition $^{24}Mg/^{26}Mg$

$$^{26}I_{meas} = {}^{26}I_{true} + C_{nat} ({}^{24}I_{meas} - C_{spk} \cdot {}^{26}I_{true}) \quad (3)$$

$$^{26}I_{meas} = (1 - C_{nat} \cdot C_{spk}) \, {}^{26}I_{true} + C_{nat} \cdot {}^{24}I_{meas} \quad (4)$$

$$^{26}I_{true} = ({}^{26}I_{meas} - C_{nat} \cdot {}^{24}I_{meas}) \cdot (1 - C_{nat} \cdot C_{spk})^{-1} \quad (5)$$

The magnitude of this correction process is small, typically amounting to < 0.1 % for a sample sodium concentration in the region of 140 mmol/L.

9.2 Calculation of Sodium Concentration

Calculate the sodium concentration (µg/g) in the sample using the following equation:

$$C_{smp} = \{ (R_{smp} / R_{std} \times M_{std} \times M_{IS\,smp} / M_{IS\,std}) - B_{avg} \} \times (M_{smp})^{-1}$$

where, C_{smp} is the concentration of sodium in the sample in µg/g
R_{smp} is the corrected $^{23}Na/^{26}Mg$ ratio in the sample
R_{std} is the $^{23}Na/^{26}Mg$ ratio in the standard
M_{std} is the absolute mass of Na in the prepared standard (µg)
$M_{IS\,smp}$ is the mass of ^{26}Mg spike added to the sample (g)
$M_{IS\,std}$ is the mass of ^{26}Mg spike added to the standard (g)
B_{avg} is the mean measured procedure blank (µg)
M_{smp} is the mass of sample taken (g)

10. Uncertainty Analysis

The expanded uncertainty for the set of samples should be calculated according to ISO guidelines [3] by combining both Type A and Type B uncertainties. Typical Type A uncertainty components for this measurement might be the variability of the measured concentrations of sodium in the samples, the instrument calibration process and the variability of the contribution of the procedure blank correction. Typical Type B uncertainty components might be the uncertainty of the assay of the SRM 919a calibrant, the weighing measurements, the calibration standard response

(discrimination) drift, the ICP-MS instrument background subtraction, the correction for the magnesium present in the sample and the conversion correction for the density of the sample.

For a 95 % confidence level, combine the Type A and Type B components and calculate the total expanded uncertainty using the following equation:

$$U = k\,[s_1^2/df_1 + \ldots + s_n^2/df_n + B_1^2 + \ldots + B_n^2]^{1/2}$$

where, U is the expanded uncertainty
k is the coverage factor
s is the observed standard deviation of (n) Type A components
df is the degrees of freedom associated with each component
B is the Type B component (n components) with infinite degrees of freedom.

Report the expanded uncertainty in the same units as the sample concentrations.

11. Data Reporting

Convert data from a mass/mass basis to a mass/volume basis using the measured density of the serum. **Report data in units of mmol/L**. To convert data from µg/mL to mmol/L, multiply by 0.043498.

12. Performance Statement

12.1 Measurement Reproducibility

The isotope ratio measurement reproducibility is not limited by counting statistics. Typical measurement repeatability for both of the ratios, $^{24}Mg/^{26}Mg$ and $^{23}Na/^{26}Mg$ is 0.1-0.2 % [2].

12.2 Linearity

Analog mode detection is subject to non-linearity at high concentrations. The linearity of the system has been tested [2] using four sodium calibration standards covering the range 20-75 µg/mL which were spiked with a constant amount of natural Mg and the ratio $^{23}Na/^{24}Mg$ measured as a function of the sodium concentration. The resulting calibration curve was fitted through zero and clearly indicated that the measurement system is linear (r = 0.999995) at least to this limit.

12.3 Instrument Background and Procedure Blank

The use of analog mode ion detection dramatically reduces the effective measured instrument background. By careful optimization, the magnitude of background corrections to the measurement data can be limited to approximately 0.1 % or less. The instrument background under these conditions is very stable. A typical procedure blank [2] is 400 ng (n=7, 1s = 69 ng), which represents a very small source of uncertainty.

12.4 Method Validation Data using NIST SRM 909b

The method has been tested using SRM 909b [2]. Nine statistically selected bottles of SRM 909b Level 1 were tested using the method. Four separate bottles were tested using a primary gravimetric method. In addition, six bottles of the Level 2 material were tested by the ICP-MS method and five separate bottles were tested by gravimetry. The analytical data obtained by both methods are compiled in Appendix A, Table A-1, together with the expanded uncertainties and the certified values listed in the SRM 909b Certificate of Analysis. Excellent agreement between the methods was obtained. For Level 1, the relative difference between the arithmetic means of the methods was 0.14 %, and for Level 2 the relative difference was 0.07 %. In both cases the means of the two methods were within the calculated expanded uncertainties of each method.

12.5 Method Validation Data using NIST SRM 956a

The method has been tested using SRM 956a [2]. Five measurements from separate ampoules were made for each of the three concentration levels. Gravimetric measurements were used to certify the sodium concentrations and were made independently on six ampoules each of Levels 1 and 2 and five ampoules of Level 3. Analytical data obtained by both methods are summarized in Appendix A, Table A-2, together with the expanded uncertainties and the certified values in the SRM Certificate of Analysis. Again excellent agreement was obtained between the two methods for all of the concentration levels. The difference between the two methods was 0.16 %, 0.05 % and 0.13 % respectively for Levels 1, 2 and 3, and the means of the two methods were within the calculated expanded uncertainties of each method.

12.6 Performance Summary

A summary of typical method performance parameters expected for this method are provided in Table 12-1.

Table 12-1: Method Performance Summary

Parameter	Typical Value or Range
Isotope ratio measurement precision	0.1-0.2 %
Applicable concentration range	120-160 mmol/L
Instrument background / sample ratio	≤ 0.1 %
Procedure blank	≤ 400 ng
Sample throughput (measurement)	10 samples per hour

13. Reference Citations

1. *Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline – Third Edition*, NCCLS Document C3-A3, NCCLS, Wayne, Pennsylvania (1997).

2. Long S.E. and Vetter T.W., *"Determination of Sodium in Blood Serum by Inductively Coupled Plasma Mass Spectrometry."* J. Anal. Atom.Spectrom., **17** (12), pp.1589-1594 (2002).

3. *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed. ISO, Switzerland, 1993.

NA-1 Revision History

Revision	Date	Author	Record of Changes and Comments
1.0	9/2006	S E Long	Initial draft method

Appendix A

Table A-1. ICP-MS and Gravimetric Data for Sodium in SRM 909b

	Level 1, mmol/L		Level 2, mmol/L	
Sample	**ICP-MS**	**Gravimetry**	**ICP-MS**	**Gravimetry**
1	120.73	120.75	141.13	141.21
2	121.31	120.90	140.92	140.95
3	120.94	120.53	141.61	141.02
4	120.97	120.83	140.41	140.94
5	120.74	--	141.23	141.08
6	120.76	--	141.55	--
7	121.27	--	--	--
8	120.84	--	--	--
9	120.73	--	--	--
Mean	**120.92**	**120.75**	**141.14**	**141.04**
Standard Deviation	0.23	0.16	0.44	0.11
RSD (%)	0.19	0.13	0.31	0.08
Expanded Uncertainty[a]	0.45	0.31	0.62	0.26
Certified Value[b]	120.76 ± 0.92		141.0 ± 1.3	

[a] Expanded uncertainty calculated at a 95 % confidence interval. [b]NIST certificate value and expanded uncertainty for a 95 % statistical tolerance interval reflecting the effects of measurement uncertainty and variability in the mass of dry serum fill mass.

Table A-2. ICP-MS and Gravimetric Data for Sodium in SRM 956a

	Level 1, mmol/L		Level 2, mmol/L		Level 3, mmol/L	
Sample	**ICP-MS**	**Gravimetry**	**ICP-MS**	**Gravimetry**	**ICP-MS**	**Gravimetry**
1	121.51	121.45	140.51	141.00	160.07	161.10
2	120.64	121.59	141.24	141.05	160.09	160.83
3	120.95	121.46	141.08	140.91	161.40	160.88
4	121.42	121.40	140.61	141.02	161.12	160.89
5	121.59	121.35	141.41	141.36	160.73	160.75
6	--	121.22	--	140.89	--	--
Mean	**121.22**	**121.41**	**140.97**	**141.04**	**160.68**	**160.89**
Standard Deviation	0.41	0.12	0.39	0.17	0.60	0.13
RSD (%)	0.34	0.10	0.28	0.12	0.37	0.08
Expanded Uncertainty[a]	0.59	0.31	0.62	0.29	0.82	0.30
Certified Value[b]	121.4 ± 0.3		141.0 ± 0.3		160.9 ± 0.4	

[a]Expanded uncertainty calculated at a 95 % confidence interval. [b]NIST certificate value and expanded uncertainty for a 95 % statistical confidence interval.