Standard Reference Materials:

A REFERENCE METHOD FOR THE DETERMINATION OF LITHIUM IN SERUM

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PREFACE

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

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

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George A. Uriano, Chief
Office of Standard Reference Materials

FOREWORD

A fundamental requirement for assuring adequate patient care is the need for the accurate analysis of constituents in body fluids. Two major functions of the National Bureau of Standards (NBS) are to provide certified Standard Reference Materials for the calibration of measurement systems and to develop new or improved analytical methods. The results presented in this NBS Special Publication provide a methodology of known accuracy for the determination of lithium in serum. The evaluation of a reference method by comparison to a definitive method, used for the first time at NBS in the development of reference methods for calcium, sodium, potassium, and chloride in serum, also was applied to this work. This hierarchy of analytical procedures has been accepted as a valid format for developing reference methods by the clinical community at a recent Conference on an Understanding for a National Reference System in Clinical Chemistry.

In an undertaking of this magnitude, extensive collaboration with a committee of experts, the Center for Disease Control, the Food and Drug Administration, and a wide spectrum of participating analytical laboratories that included Federal, State, hospital, industrial, and academic laboratories was essential to establish a widely accepted reference method. It is hoped that this work will provide an additional basis for the development of future clinical reference methods through continued collaboration and the concerted efforts of the individual participants.

Curt W. Reimann, Director
Center for Analytical Chemistry

NOTE

Because of concern for the useability of this lithium reference method, Center for Disease Control management has declined to endorse the method described in this report. However, the authors believe the present method should function as the reference method until the efficacy of a subsequent improved method has been demonstrated, since this procedure has been shown to satisfy the generally accepted criteria of a reference method. NBS supports the evolution of analytical methods and believes it important that the principles of analytical practice delineated in the present report be circulated in a timely manner.

NBS will continue to participate in interlaboratory exercises that are aimed toward establishing the transferability of proposed reference procedures and will maintain its primary role in supplying SRM's and definitive methods.

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ABSTRACT

A reference method was established for the determination of serum lithium based on flame atomic absorption spectroscopy (FAAS). Its accuracy was evaluated by comparing the values obtained by use of the method in 14 laboratories against the results obtained by a definitive analytical method based on isotope dilution-mass spectrometry (IDMS). Ten serum pools with lithium concentrations in the range 0.534 to 2.954 mmol/L were analyzed. Manual and semiautomated pipetting alternatives were tested using sample sizes of 4.00 and 2.00 mL, respectively.

The laboratories used several different FAAS instruments. The results showed that the standard error for a single laboratory's performance using either pipetting procedure was about 1.5 percent with a negative bias of about 2.0 percent over the range of serum lithium concentrations studied. These values are within the accuracy and precision goals that had been set. The calibration curve data showed excellent linearity over the total concentration range, with 24 of 25 curves having standard deviations of fit of 0.025 mmol/L or less.

With appropriate experimental design, the reference method may be used to establish the accuracy of field methods as well as to determine reference lithium values for pooled sera.

Key Words: Accuracy; clinical analysis; clinical chemistry; definitive method; electrolytes; flame atomic absorption spectroscopy; interlaboratory testing; precision; reference method; semiautomated pipetting; serum lithium analysis; statistical analysis.

I. INTRODUCTION

Lithium was first used by Cade [1][5] to treat and prevent the recurrence of manic-depressive psychosis and is widely used today. However, the margin between therapeutic dosage and toxicity is not large [2]. Toxic symptoms have been observed in patients with serum lithium levels of 1.6 mmol/L, a value quite close to the 1.0-1.5 mmol/L serum level generally attained for immediate treatment [3]. It is thus important to have an accurate analysis for serum lithium in addition to observing the clinical symptoms of the patient during treatment.

The quantitative analysis of serum lithium by early analytical techniques was difficult. Attempts included separation by precipitation [4], paper chromatography [5], and a semiquantitative colorimetric procedure [6]. The advent of flame photometric techniques, including flame atomic absorption spectroscopy (FAAS)[6] and flame atomic emission spectroscopy (FAES)[6] has led to simple, quantitative techniques for determining serum lithium concentrations [7].

The use of flame atomic absorption spectroscopy has been described as a standard method [8]. Whether this FAAS method or some other should be considered by clinical laboratories as the reference method for serum lithium has not been proven; the accuracy of none of these methods is known.

Two approaches may be used for establishing the accuracy of analytical methods. In the first, the results obtained from the methods in use for that analyte are compared using typical samples and selected samples containing known interferences for the analyses. Statistical correlations are used

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[5]The bracketed numerals refer to the references listed at the end of this paper.

[6]Official name, International Union of Pure and Applied Chemistry, Information Bulletin Number 27, Nov. 1972.

to express the interrelationships of the methods. A technique is then considered to be accurate to the degree established by knowledge of the sources of error and the agreement of results. In the second, a single candidate method is selected (possibly the 'best' of the methods recognized by the first approach) and studied in detail. Each step of the candidate method is optimized and examined so that the systematic and the random errors can be quantitatively expressed.

Studies have been organized using a combination of these approaches to establish the accuracies of reference methods for total calcium, sodium, potassium, and chloride in serum [9-12]. For calcium, the analytical procedure was based on the FAAS method of Pybus, Feldman, and Bowers [13], for sodium and potassium the methods were based on FAES, and for chloride, the method was based on coulometric titration - amperometric end-point determination. The accuracies of these methods were assessed by comparing the results obtained in selected clinical laboratories in statistically controlled studies against those obtained for the same serum pools by use of isotope dilution-mass spectrometry (IDMS) methods for calcium, potassium, and chloride and an ion-exchange - gravimetry method for sodium. The latter analyses were performed at the National Bureau of Standards (NBS) where the high accuracy of those methods[7] was established by determining their systematic and random errors [14].

Those studies, carried out with the guidance of clinical laboratory experts, used (a) Standard Reference Materials as pure, primary reference materials to prepare standard solutions of calcium, sodium, potassium, and chloride; (b) serum pools prepared at the Hartford Hospital (Hartford) and the Center for Disease Control (CDC, Atlanta); (c) definitive

[7]Such methods are referred to as definitive methods because of their high accuracy and utility for evaluating the accuracy of a candidate reference method.

method analyses performed at NBS; (d) statistical analysis of the data at NBS; and (e) accuracy and precision goals as performance standards that the methods would have to meet to be recommended as reference methods [15]. This same approach was adopted to develop a clinical reference method for serum lithium.

This work was begun with the cooperation of individuals from the Standards Committees of the American Association for Clinical Chemistry (AACC) and the College of American Pathologists (CAP), the CDC and the NBS. The Food and Drug Administration (FDA) provided major suppport for the NBS work. Progress with this program was reported periodically to the AACC Standards Committee. We present in this report the development of a clinical reference method for serum lithium.

II. DEVELOPMENT OF THE SERUM LITHIUM REFERENCE METHOD

A. Organization

A panel of experts in clinical chemistry was invited to meet at NBS in March 1974 to consider the development of reference methods for five serum electrolytes, namely, potassium, sodium, chloride, lithium, and magnesium. The overall program for the development of these reference methods was organized by Dr. Robert Schaffer (NBS) and Dr. Rance A. Velapoldi (NBS). The invited experts were Dr. George N. Bowers, Jr. (Hartford Hospital), Dr. Bradley E. Copeland (New England Deaconess Hospital), Dr. Denis O. Rodgerson (Center for Health Sciences, University of California in Los Angeles), and Dr. James M. White[8] (CDC).

[8]Dr. James White died after this program was well underway. He was recommended for membership on this Experts Committee on electrolytes by Dr. Joseph H. Boutwell (CDC). Dr. White made significant contributions to the protocol for the reference method. His knowledge, advice, and cooperation in all phases of this work contributed greatly to the success of the program.

Prior to the meeting, several bovine serum pools prepared at the CDC had been analyzed for lithium by FAES, FAAS, and IDMS. The results, summarized in Table 1, were presented at the meeting as follows:

FAAS as obtained at the CDC, by Dr. J. White,

FAES as obtained at the NBS by Dr. R. Mavrodineanu, and

IDMS as obtained at the NBS, by Dr. L. Moore.

On consideration of these quite similar analytical results, of the relative advances in FAAS instrumentation over those for FAES instrumentation, and of alternative clinical laboratory procedures in use for the determination of serum lithium, it was concluded that FAAS was the appropriate candidate methodology to evaluate as the reference method and that its evaluation should be made using IDMS as the definitive method.

Table 1. Preliminary results from NBS and CDC for the determination of serum lithium.

	- - - - - Li, mmol/L - - - - -		
Pool	IDMS	FAES	FAAS
		NBS	CDC
I	1.004	0.99	1.00
III	1.969	1.96	1.96
V	2.954	2.96	2.90

The panel of experts agreed to serve as the Committee to oversee the development of the reference method for lithium (as well as for the other electrolytes under consideration at the meeting). The Committee chose Dr. Bowers as chairman. Dr. Bowers agreed to serve as the Committee's representative to work with those at NBS who would be involved in writing the protocol for the lithium reference method. The Committee agreed that the FAAS method should be based on the method of Pybus and Bowers (using a 10-fold sample dilution) [8,16], and should use a concentration bracketing technique rather

than calibration curves for determining lithium concentrations. However, calibration curve data should be obtained as a general check on the measurement system and to determine which of the primary standard solutions to use for bracketing the lithium levels in the samples being analyzed.

As goals for the candidate reference method, the maximum bias of the method and the one-standard deviation imprecision limit were set by the Committee at 0.2 and 0.1 mmol/L, respectively, for serum lithium at the 2.0 mmol/L level. These goals were to be achieved by controlled, interlaboratory tests involving a selected group of clinical chemistry laboratories which would perform the analyses by the FAAS method according to the written protocol. NBS would provide lithium values for the pools by the definitive method.

B. Participating Laboratories, Standards, Serum Samples, and Definitive Method

The laboratories that were asked to participate in the interlaboratory study were chosen to represent a wide spectrum of clinical chemistry interests and included government (federal and state) and hospital laboratories, and laboratories associated with suppliers of instruments and of test and control materials. Two hospitals were located outside the United States. The principal investigators at these laboratories are named in the following list. Other scientists in each of the laboratories who contributed to this study are acknowledged by name in Appendix A. The list includes two laboratories that participated only in the concluding interlaboratory work. They were added to maintain a minimum number of laboratories when some of the original laboratories were unable to continue their participation. In alphabetical order of the principal investigator, the laboratories that participated in the interlaboratory studies are:

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NBS Standard Reference Material Lithium Carbonate (SRM 924, see Appendix B) was to be used as the pure, primary reference material for all analyses [17]. Ten pools of

homogeneous, sterile, bovine serum, having different concentrations of lithium, were prepared at the CDC by Dr. David Bayse and Ms. Sue Lewis. Samples of each pool were supplied in approximately 7-mL volumes in sealed vials that were labeled with computer-generated random numbers. The samples, packed in dry ice, were shipped to NBS by air and within 24 h of packing were placed in freezers kept at -50 °C [18]. The pools were numbered in the codes 1a, 5a, and 7a for the first series of serum pools and from 1 to 7 for the second series of serum pools according to increasing lithium concentration.

A definitive method based on IDMS was developed at NBS and is given in Appendix C. The lithium concentrations for the ten serum pools were determined by this procedure and the results obtained are summarized in Table 2.

Table 2. Lithium concentrations of the serum pools determined by IDMS, the definitive method.

Pool	$[Li^+]$, mmol/L
1	0.534 ± 0.003[a]
1a	1.004 ± 0.005[a]
2	1.031 ± 0.005[a]
3	1.290 ± 0.006[a]
4	1.546 ± 0.008[a]
5	1.809 ± 0.009[a]
5a	1.969 ± 0.010[a]
6	2.042 ± 0.010[a]
7	2.572 ± 0.013[a]
7a	2.954 ± 0.015[a]

[a]Estimated maximum total error of 0.5 percent of the value. This estimated error is the sum of errors due to measurement imprecisions of ±0.3 percent (±2 sigma interval for the ramdom error of the mean) and an estimated upper bound of 0.2 percent for possible systematic errors.

C. Functions of the Various Groups

The interrelationships and functions of the various groups involved in developing FAAS as a reference method for serum lithium are represented in figure 1. The Committee, CDC, and NBS provided guidance and technical support for the program and also served as participating laboratories. The Experts Committee selected the candidate reference method, set maximum bias and imprecision goals for an acceptable reference method, assisted NBS in selecting other participating laboratories, and reviewed all analytical results. The CDC provided the serum pools. The participating laboratories provided the interlaboratory test data and critiques of the candidate reference method protocol.

At NBS, Dr. R. Schaffer served as the Reference Method Program Manager and Dr. R. A. Velapoldi served as the coordinator. The format of the interlaboratory exercises (IE)[9] was established within the constraints imposed by protocol requirements and sample availability by Drs. John Mandel, Robert Paule, and Rance Velapoldi. Dr. Velapoldi wrote the protocol for the candidate reference method from the outline provided by Dr. G. Bowers, Jr. Drs. Mandel and Paule performed the statistical evaluation of the results from the interlaboratory tests. The definitive method was performed by Mr. Lawrence A. Machlan and Mr. Ernest L. Garner.

D. Plan for Testing the Candidate Reference Method

The general plan was to evaluate the candidate reference method by performing a series of interlaboratory exercises, which would consist of a preliminary test (IE-P) followed by successive interlaboratory exercises until the goals for the reference method were reached. A main objective of the IE-P

[9] In previous reports, the Interlaboratory Exercises were called Round Robin Tests.

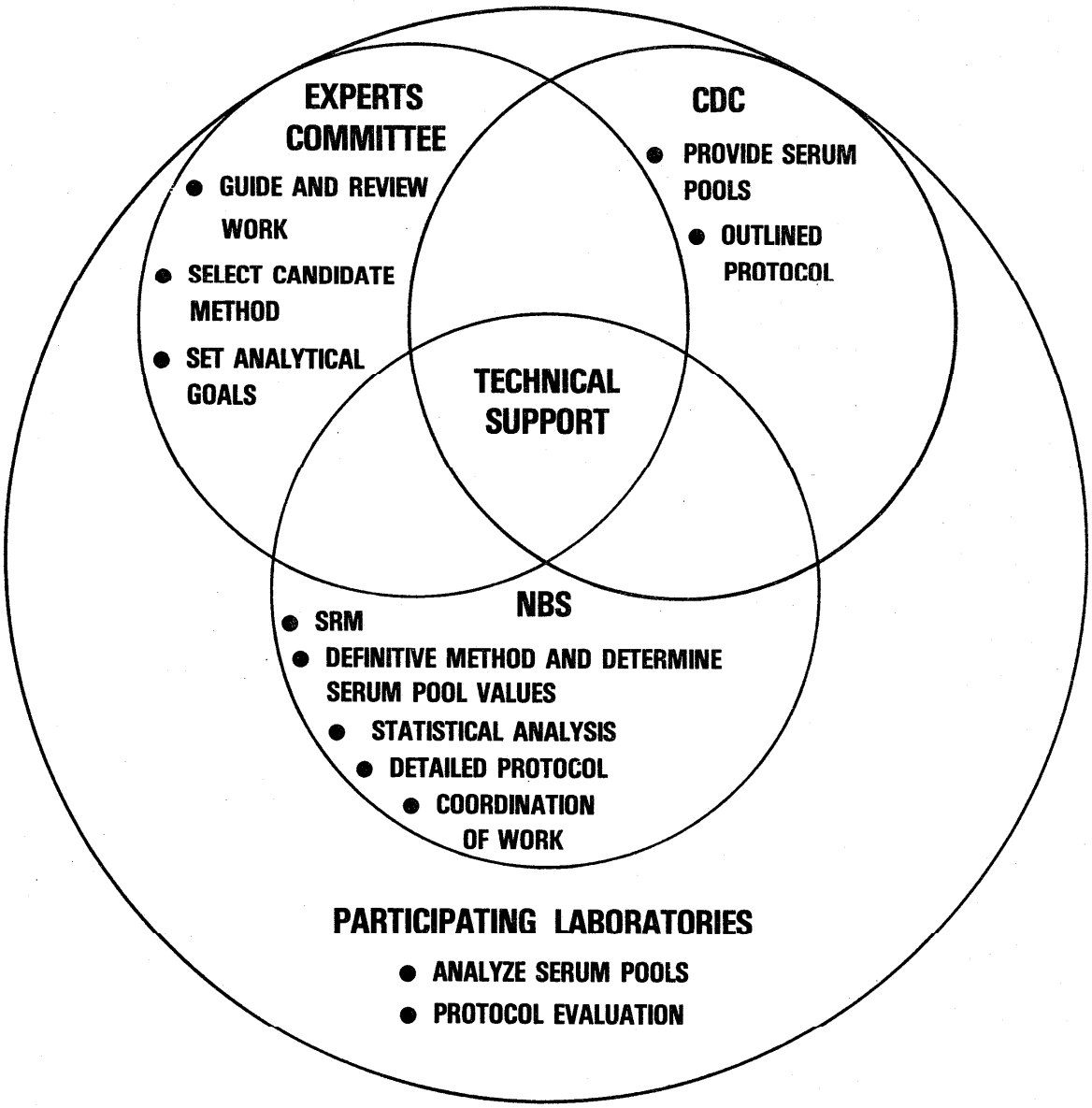

Figure 1. Interrelationships and functions of the various groups in the development of a clinical reference method for the determination of serum lithium.

was to allow participating laboratories to become familiar with and comment on the protocol. An evaluation of the bias was not sought in the IE-P testing phase since no definitive method lithium values were available at the time the IE-P was run. Thus, only interlaboratory imprecision was to be measured. If the imprecision of the results in the IE-P was found to be small, interlaboratory testing would begin on samples having definitive method lithium values.

In an IE, each participating laboratory would perform the same analyses on two separate days: i.e., analyze pairs of samples from each serum pool on each of two days where a minimum of one day or a maximum of seven days were to elapse between the two series of analyses. The bias and imprecision values obtained by statistical analysis would then be compared to the goals set by the Committee for the reference method. If the goals were not met, additional IE's using samples from other pools would be conducted by following the protocol or a modified version until the bias and imprecision goals were reached. Revisions and modifications to the protocol could be made after an interlaboratory exercise had been completed but would not be made after the final IE.

Three kinds of information were to be supplied by each participating laboratory after finishing an interlaboratory exercise:

1. General Data – a list of the instrumental parameters used and comments on the protocol including problems encountered during the analysis;
2. Calibration Curve Data – a list of the FAAS relative absorbance values versus the lithium concentrations of the standards calculated from the Li_2CO_3 used; and
3. Valid Measurement Data – a list of the sets of data that constituted the five 'valid measurements' (see section IIIC-5e for discussion).

Examples of the data sheets on which the information was collected are shown in Appendix D, Note 8.

III. REFERENCE METHOD PROTOCOL FOR THE DETERMINATION OF SERUM LITHIUM

A. General

This protocol for the analysis of serum lithium by flame atomic absorption spectroscopy provides for the optional use of either manual or semiautomated pipetting.

B. Protocol Synopsis

The protocol must be followed exactly. The reference method is used to analyze four samples of a serum or pool: two on one day and the other two on a subsequent day. Approximately 10 mL of serum is needed to carry out the semi-automated pipetting procedure and 25-30 mL of serum is needed to carry out the manual pipetting procedure.

1. Use an analytical balance to weigh the SRM Li_2CO_3 in appropriate quantities to prepare a stock standard lithium solution;
2. To dilute to the lithium concentrations that are used as working solutions for FAAS, use either a single pipet or a pipettor-dilutor to obtain a) aliquots of the serum to be analyzed, b) aliquots of the stock standard lithium solutions, and c) the solution used as a blank;
3. Obtain calibration curve data on the working blank and standards for use as a check on standards preparation and instrument stability and linearity;
4. Measure the FAAS signals of the working solutions of the serum sample; select the pair of working standards whose signals most closely bracket the signal for each sample;
5. For each sample to be analyzed, obtain five valid measurement sets from repeated sequential measurements of the

working solutions of the low bracketing standard, the sample, and the high bracketing standard;

6. Calculate the lithium concentration of the sample for each set by mathematical interpolation;
7. Average the five calculated values to obtain a 'single measurement' of that sample; (in the statistical analysis, each such average is designated a 'single measurement');
8. Perform steps (4) through (7) for each sample to be analyzed on the first day;
9. Repeat steps (1) through (8) on the subsequent day to obtain the second pair of measurements needed for each sample;
10. Average the four values obtained by the replicate determinations to obtain the lithium concentration for each serum pool.

C. Detailed Protocol

The selection of the specific alternatives of the protocol to be used dictates the glassware and diluent volumes needed. These needs are summarized in the protocol or in Appendix D notes. Stock solutions and working solutions are to be prepared at and maintained at a room temperature that is constant within ±2 °C (see Appendix D, Note 1).

1. Reagent Specifications

a. Water: At the time of preparation, the distilled and/or deionized water used should exhibit a specific resistance of at least 10 kΩ•m at 23 ± 2 °C. At the time of use, this water should show a FAAS signal that is less than 0.1 percent of the expanded absorbance scale (Section IIIC-7c-(2)). A large quantity of this water (more than 50 L) must be available for use as diluent and for the final

rinsings of all glassware and other apparatus that come in contact with the solutions involved. Unless specified otherwise, the water referred to in this protocol is this tested water.

b. Lithium Standard Solutions: Use Standard Reference Material Lithium Carbonate (originally issued as SRM 924, Certificate reproduced in Appendix B) [17] certified by the National Bureau of Standards. (Note: see Appendix D, Note 5.) Dry the SRM Li_2CO_3 at 200 °C for four hours in a loosely capped container, allow to cool, and then store it tightly capped in a desiccator containing $CaSO_4$ or an equivalent desiccant.

c. Potassium and sodium chlorides, hydrochloric and nitric acids, chloroform, methanol and 95-percent ethanol (v:v) meeting ACS [20] or equivalent specifications are to be used.

d. Dilute nitric acid (0.77 mol/L) is prepared by making a twenty-fold dilution of concentrated HNO_3 (15.4 mol/L) with water.

e. Dilute hydrochloric acid (2.3 mol/L is prepared by making a five-fold dilution of concentrated HCl (11.6 mol/L) with diluent solution (see IIIC-3a). Prepare approximately 25 mL.

2. Glassware Specifications

a. Volumetric glassware (Appendix D, Note 2) should be of borosilicate material and meet NBS Class A [21] or equivalent specifications. All glass or plastic surfaces that come into contact with reagents, water, diluent, or sample must be clean (Appendix D, Note 3).

b. Pipettor-dilutor Device: The volumetric delivery of the pipettor-dilutor device must have a tested maximum inaccuracy of 2 percent and a maximum imprecision of ±0.2 percent relative standard deviation at the pump setting used. (The test procedures are in Appendix D, Note 4.)

3. Preparation of Reagents

a. Sodium Chloride-Potassium Chloride Diluent Solution (NaCl - 140 mmol/L and KCl - 5.0 mmol/L Diluent: Add 16.36 g of NaCl and 0.746 g of KCl to a 2-liter volumetric flask, dissolve in water, and dilute to the calibrated volume with water. Stopper and invert the flask and shake 10 times; repeat the inversion and shaking 10 times. (NOTE: Approximately 6 liters of this solution are needed.)

b. Lithium Standard Stock Solutions (10 mmol/L): Prepare separately two stock solutions each containing approximately 10.0 mmol/L LiCl. Weigh accurately (to 0.1 mg) approximately 0.74 g of lithium carbonate (molecular weight = 73.94855, Appendix D, Note 5b) and transfer quantitatively into a 2-liter volumetric flask. Dissolve the Li_2CO_3 by just covering the bottom of the flask with diluent solution (IIIC-3a) and slowly adding 10 mL of dilute HCl (IIIC-1e). Swirl until dissolved. Fill to the calibrated mark using diluent solution and mix thoroughly. Repeat these steps to prepare the second lithium standard stock solution. Label the solutions I and II. From the weighed quantities of Li_2CO_3 taken, calculate their lithium concentrations in mmol/L to four decimal places.

(1) Intercomparison of Standard Stock Solutions: Transfer by pipet using the "to deliver mode", 5.00-mL of stock solution I into a one-liter volumetric flask. Dilute to the calibrated volume with water, stopper, invert, shake, and mix as described above to give a working solution with a lithium concentration of 0.05 mmol/L. Repeat these dilution steps for stock solution II.

Immediately aspirate each of the 0.05 mmol/L lithium solutions and measure their expanded absorbance values (see Section IIIC-7d) under the instrumental settings used for this analysis. If the expanded absorbance values corrected for concentration differences agree to within 0.5 percent for both solutions, lithium stock standard I may be used for the analyses on the first day and stock standard II may be used for the analyses on the subsequent day subject to temperature restrictions. If the values do not agree within 0.5 percent, discard both stock standard solutions and repeat their preparation and the intercomparison test until the requirement of 0.5 percent agreement is obtained.

c. Diluted Lithium Standard Solution: Prepare the various diluted lithium standard solutions by transferring the volumes of the lithium stock standard solution listed in Table 3 into 100-mL volumetric flasks and diluting each to the calibrated volume with the NaCl-KCl diluent. Mix thoroughly. (NOTE: These dilutions are made using volumetric pipets in the "to deliver" mode (Section IIIC-4) - not the "to contain" mode, Section IIIC-5.

Table 3. Volumes of lithium standard stock solution diluted to 100 mL to give the diluted lithium standard solutions.

Stock Solution to be Transferred, mL	Concentration of Diluted Standards LiCl, mmol/L
5.00	0.50
10.00	1.00
15.00	1.50
20.00	2.00
25.00	2.50
30.00[a]	3.00

[a]See Appendix D, Note 6.

4. Pipetting and Diluting Procedures to Prepare Dilute Lithium Standard Solutions (To Deliver Mode):

a. General: Transfer of the lithium stock standard solution to prepare the diluted lithium standard solutions is performed by using the appropriate Class A pipet in the to deliver mode (see Table 3).

b. Procedure: Add approximately 15 mL of the NaCl-KCl diluent solution to each 100-mL volumetric flask. Condition the pipet as in Section IIIC-5b-(3)(b) with approximately 3 mL of the lithium standard stock solution to be transferred and discard this solution. Repeat twice more. Fill the pipet to approximately 1.0 cm above its calibration mark. Withdraw the pipet from the container, and wipe the delivery end of the pipet with a clean, absorb paper. Contact the tip of the pipet with the side of the container or a clean waste container and deliver excess solution until the meniscus is at the calibrated mark on the pipet. Remove the pipe

from contact with the container and direct the delivery end of the pipet into the receiver. Deliver the solution into the volumetric flask by allowing the solution to run down the side of the flask. When the solution level has reached the low end of the pipet (just below the bulb), contact the pipet tip to the side of the flask. (NOTE: As a minimum, allow the pipet to drain for the delivery time inscribed on the pipet.) After drainage is complete, remove the tip from contact with the flask wall, dilute to volume using the diluent solution, and mix. Repeat these steps until the six diluted lithium standard solutions are prepared and labeled appropriately.

5. Pipetting and Diluting Procedures to Prepare Working Solutions ('to contain' mode):

a. General: A 25-fold dilution is to be used.

b. Manual Pipetting Alternative: The blank, the standard, and the sample solutions are diluted by employing only one 4-mL pipet with a wash-out technique and 100-mL volumetric flasks. Working solutions are prepared with the one pipet and the wash-out technique to eliminate errors that may be caused by differences in drainage between aqueous and serum solutions.

(1) Twenty-five-fold Dilutions: Transfer approximately 25 mL of NaCl-KCl diluent into a 100-mL volumetric flask and then add a 4 mL aliquot of the sample or diluted lithium standard solution by the procedure described in step (2) below.

(2) Pipetting Procedure: Fill the 4-mL pipet to approximately 1.0 cm above its calibration mark, withdraw the pipet from the container, and wipe the

delivery tip with a clean, absorbent paper. Contact the tip to the side of a clean waste container and allow excess solution to drain until the meniscus is at the calibrated mark on the pipet. Remove the pipet from contact with the container and direct the delivery tip of the pipet into the receiver. Deliver the sample by contact of the pipet tip with the wall inside the volumetric flask and allow the solution to drain fully. After drainage stops, gently expel the residual liquid. Wash off the outside of the pipet tip into the receiver with about 4 mL of NaCl-KCl diluent delivered, for example, from a wash bottle or a disposable Pasteur or similar pipet. (Caution: New, disposable pipets need to be cleaned.) Rinse the 4-mL volumetric pipet three times by filling with fresh NaCl-KCl diluent from a separate beaker, each time delivering the contents into the receiver by drainage against the inner wall of the flask above the liquid level. Dilute to the calibrated volume with the NaCl-KCl diluent and mix thoroughly.

(3) Preparation of Working Solutions:

(a) Working Blank Solution and Working Standard Solutions: Prepare the working solutions of the blank and of the 0.50-, 1.00-, 1.50-, 2.00-, 2.50-, and 3.00-mmol/L lithium standards by making dilutions in appropriately labeled volumetric flasks in the order cited. Condition the 4-mL pipet by filling it with the solution to be diluted. Discard this pipetful and repeat filling and discarding twice more. Then refill the pipet with the solution, adjust to the calibrated volume, and deliver into the volumetric flask to be used for the dilution. Rinse the pipet by filling it three

times with the NaCl-KCl diluent, each time delivering the rinse solution into the volumetric flask. Fill the flask to the calibrated volume with the NaCl-KCl diluent. Wash out the pipet three times with water (see Appendix D, Note 7) and expel the residual liquid.

(b) Working Sample Solutions: Condition the 4-mL pipet with some of the sample to be diluted in the following way: 1) Draw ∿1 mL of the sample into the pipet, 2) withdraw the pipet from the container, 3) wipe off the tip with a clean, absorbent paper, 4) tilt the pipet to a horizontal position, 5) allow a small volume of air to leak in and rotate the pipet so that the conditioning liquid wets all the internal surface to approximately 0.5 cm above the calibration mark, 6) discard this conditioning solution, and 7) repeat steps (1-6). Then prepare the working solutions as described in sections IIIC-5b-(1) and (2), i.e., fill the 4-mL pipet with the sample, adjust volume to the mark, deliver, rinse three times into the volumetric flask with NaCl-KCl diluent, dilute to the calibrated volume, and mix. Finally, wash out the pipet three times with water (Appendix D, Note 7). For each of the next sample solutions to be diluted, repeat step IIIC-5b-(3)(b).

c. Semiautomated Pipetting Alternative: To prepare working solutions, the blank, standard, and sample solutions are diluted by using a pipettor-dilutor device to deliver 2.000 mL into appropriately labeled 50-mL volumetric flasks. A single delivery tube on the pipettor-dilutor and the wash-out technique are used throughout.

(1) Twenty-five-fold Dilutions: Transfer approximately 15 mL of NaCl-KCl diluent into a 50-mL volumetric flask and then add 2.000 mL of the appropriate solution by the procedure described in step (2) below.

(2) Procedure: The pipettor-dilutor is set to sample 2.000 mL and to dilute with 5 mL of NaCl-KCl diluent. After conditioning the pipettor-dilutor as in Appendix D, Note 3b, dip the delivery tip of the pipettor-dilutor into the solution to be transferred. Draw up the desired volume of solution (2.000 mL). Care must be taken to avoid air bubbles in the tubing before or during this operation. Withdraw the tip of the delivery tube from the solution, touch the tip to the container side, and remove the container. With care not to touch the open end of the tip of the tube, wipe the outside of the delivery tube, direct the tip of the tube into the 50-mL volumetric flask, and deliver the aliquot and diluent solution into the flask. Rinse the delivery tube twice more by delivering two additional 5-mL portions of diluent through the tube into the 50-mL volumetric flask. (NOTE: To minimize foaming and spattering, deliver the stream of solution and diluent on the wall inside the neck of the flask.) After delivery is complete, touch the tip of the tube to the inside wall of the flask to transfer any solution remaining outside the tube. Remove the volumetric flask, dilute to the calibrated volume with the NaCl-KCl diluent, and mix.

(3) Preparation of Working Solutions:

(a) Prepare the working blank, standard, and sample solutions using the semiautomated pipetting procedure described above.

6. Total Solutions:

At the conclusion of the dilution procedure, appropriately labeled flasks with the following working solutions should be ready for analysis:

a. For the Manual and/or Semiautomated Pipetting Alternatives:

(1) One working blank;
(2) Six working standards; and
(3) A working solution for each serum sample to be analyzed.

7. Flame Atomic Absorption Spectroscopy Measurement Procedures

It is not possible to provide detailed instructions for each type of instrument to assure necessary instrument stability, linearity, flame conditions, etc. The operator must be familiar with the instrument used. The instrument should meet all the manufacturer's specifications. In general, the accuracy of the method cannot be attained unless the instrument is in optimum operating condition. Air and acetylene are used as oxidant and fuel, respectively.

a. Instrument and Electrical Adjustment. Prepare the atomic absorption spectrometer for operation according to instructions provided in the operator's manual. Select the optimum current for lamp, and allow ample "warm-up" time for the lamp to become stable. Adjust the monochromator slit and set the

wavelength selector to the lithium resonance line at 670.8 nm (6708 Å). Adjust the photomultiplier dynode voltage to give optimum current output with minimum dark current.

b. Flame Condition. Adjust the secondary regulators for the air and acetylene and adjust the air-acetylene flow rates to those recommended for the instrument. Stabilize the temperature of the burner head by aspirating water into the flame for 10 min before proceeding to the next step. (NOTE: A fuel-rich air-acetylene flame gives optimum sensitivity for the measurement of lithium; however, it may be difficult to obtain the precision specified in this method with a fuel-rich flame. Therefore, it is suggested that a stoichiometric or only slightly fuel-rich (slight yellow streaking) flame be used to obtain the highest precision for lithium in serum.) Check the flame appearance and aspiration rate to assure that the nebulizer burner system is free of foreign materials.

c. Instrument Stability. Determine the stability and repeatability of the instrument as follows:

(1) Adjust the instrument to a zero absorbance reading while nebulizing the NaCl-KCl diluent. (NOTE: Always nebulize NaCl-KCl diluent when measurements of the working blank, standard, or sample solutions are not being made. Adjust the instrument so that the NaCl-KCl diluent reads 'zero' at all times.)

(2) Nebulize the working lithium standard solution obtained from the 3.000 mmol/L standard solution, maximize the signal by adjusting the appropriate parameters, and adjust direct read-out instruments

so that a relative absorbance of at least 1.500 is observed.

(3) Check the instrument zero with NaCl-KCl diluent and readjust as necessary.

(4) Repeat steps (c)(1)-(3) until stable conditions are achieved. Readings for the same solution should agree within 0.5 percent of full scale.

d. Determination of the Calibration Curve.

(1) Nebulize the working solutions of the blank and the lithium standards and record their relative absorbance values. (A typical data sheet is given in Appendix D.)

(2) Subtract the value for the blank (if any) from the values obained with the standard solutions, and plot these corrected relative absorbance values versus the calculated lithium concentrations on rectilinear graph paper. A typical calibration curve is shown in figure 2. The calibration curve, using a least squares linear fit, should show a standard deviation of fit of 0.03 mmol/L or less.

(3) The standard deviation of fit can be calculated from the deviations, d_i, of the N points from the least squares fitted calibration line:

$$S_{fit} = \sqrt{\sum_{i=1}^{N} (d_i^2)/(N-2)}. \qquad (1)$$

If on visual inspection, one point of the plot exhibits a large residual from a straight line drawn through the remaining points, remeasure that standard solution. If the remeasured value for

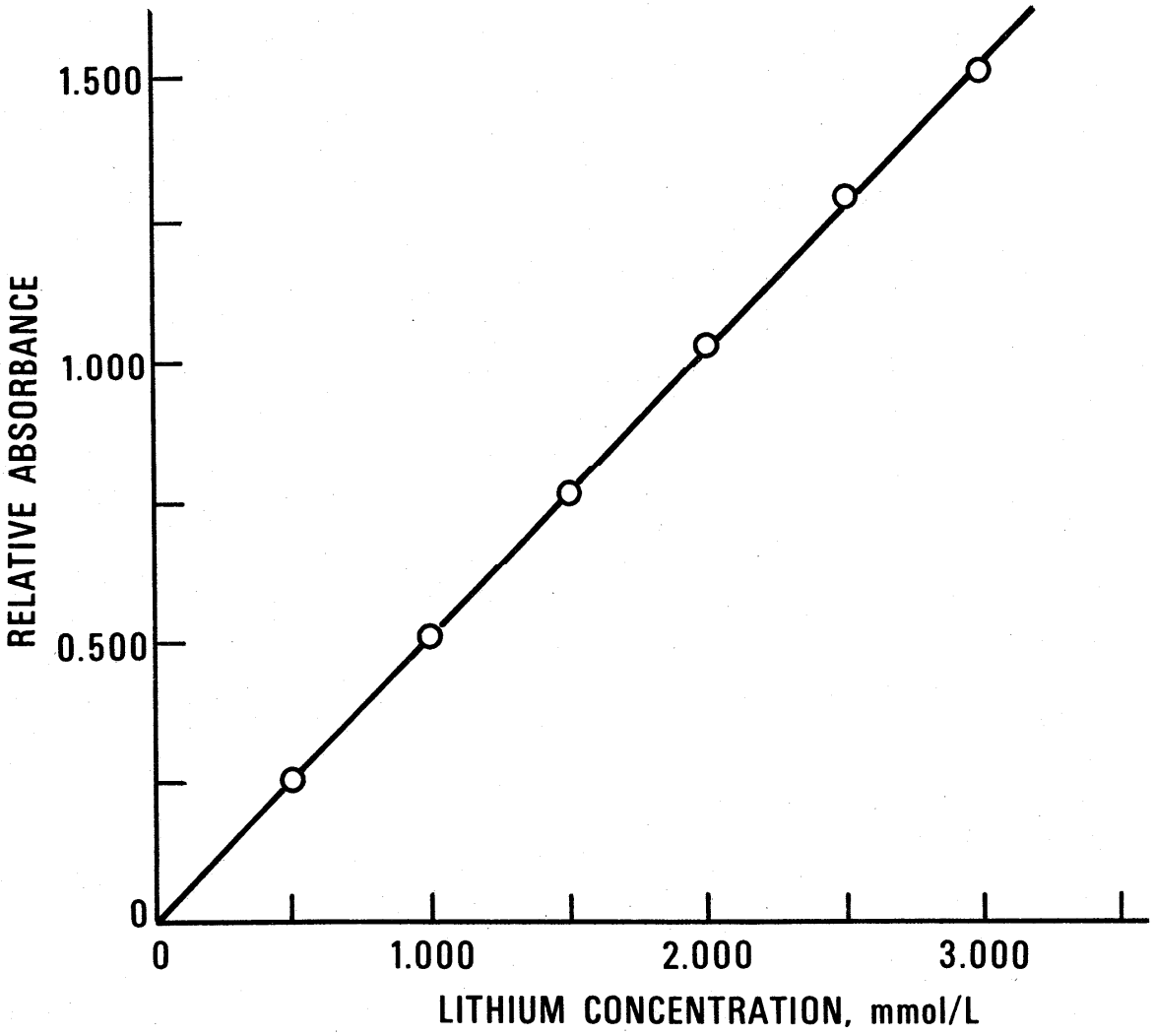

Figure 2. Typical calibration curve for the determination of serum lithium by flame atomic absorbance spectroscopy; wavelength = 670.8 nm; oxidant-fuel = air-acetylene.

the solution continues to exhibit the large deviation, prepare that standard solution again, remeasure it, and compare the values obtained, as in steps d-(1) and (2). (See Results and Statistical Analysis Section IVA-4f.)

e. Sample Measurements.

(1) Nebulize a working sample solution and select the two working standard solutions whose relative absorbance values most closely bracket that of the sample.

(2) Nebulize the lower working standard, the working sample, and the higher working standard in that order and record each reading in the set.

(3) Repeat step e-(2) until five valid sets are obtained, as illustrated in section f, below.

(4) Repeat steps e-(1), (2), and (3) for all of the samples.

f. Valid Sets of Readings.

Sets of readings are considered valid if the following condition is met:

The relative absorbance values for the sample and the two standards in a set may not differ by more than 2 percent from any of the corresponding values in the previous valid set. (NOTE: Initially, the first set measured is considered to be valid, but may be discarded if subsequent sets prove it to be non-valid. Non-valid sets are discarded.)

Five valid sets must be obtained to complete a measurement. For example: In Table 4, set 2 is valid since each difference between the relative absorbances for the Low Standard (Set_2-Set_1 = -0.001), the Sample (Set_2-Set_1 = +0.005) and the

2 percent. Note, however that set 4 is not valid because two differences, i.e., between the Low Standard values (Set_4-Set_3 = +0.015), and Sample values (Set_4-Set_3 = +0.018), are outside the 2 percent limit. Just one such difference would have disqualified set 4. Thus, sets 1, 2, 3, 5, and 6 comprise the group of 5 valid sets.

Table 4. Example of relative absorbance values for sets of readings using a direct read-out instrument.

Set	Low Standard 1.001 mmol/L	Sample	High Standard 1.501 mmol/L
1	0.523	0.639	0.753
2	0.522	0.644	0.758
3	0.525	0.641	0.750
4	0.539	0.659	0.753
5	0.530	0.643	0.752
6	0.539	0.645	0.748

f. Data Recording and Calculations:

(1) On the data sheet, record the concentrations of the standard solutions in mmol/L of lithium to four significant figures and the measured relative absorbance values to as many figures as given by the instrument.

(2) Calculate the concentration $\hat{C}$ of lithium present in the sample in mmol/L by mathematical interpolation as follows:

$$\hat{C} = C_1 + \frac{(C_2-C_1)(Y-X_1)}{(X_2-X_1)} \qquad (2)$$

where

$\hat{C}$ is the sample concentration of lithium in mmol/L,

C_1 is the low standard concentration of lithium in mmol/L,

C_2 is the high standard concentration of lithium in mmol/L,

Y is the relative absorbance value of the sample minus that of the blank (the NaCl-KCl diluent reading that was initially set at '0')

X_1 is the relative absorbance value of the low standard minus that of the blank (the NaCl-KCl diluent blank), and

X_2 is the relative absorbance value of the high standard minus the blank.

(3) Record the $\hat{C}$ values calculated to four significant figures in the column provided on the data sheet.

(4) Average the results for the four samples of the serum pool analyzed to obtain the 'final concentration'.

IV. RESULTS AND STATISTICAL ANALYSIS

The main objective of the statistical analyses of the interlaboratory exercise data is to derive measures of precision and accuracy for the manual and semiautomated versions of the reference method. Precision is characterized by the variability of the protocol measurements within a single laboratory, $\hat{\sigma}_{within}$, and by the total variability of a laboratory's protocol measurements, $\hat{\sigma}_{total}$. This latter uncertaint includes the variability of 'between laboratory' measurements. Accuracy relates to the comparison between reference method and definitive method values and is related to the magnitude of the bias.

Each reported data point (test result) is the end produc of five valid flame atomic absorbance spectrometer reading sets, the number of valid readings specified by the protocol. For simplicity of discussion, each reported data point is referred to as a single measurement, meaning that each is the product of a single run-through of the protocol. When "repli ation" is mentioned, replication of the entire protocol process is meant, and "replication error" thus refers to the variability among the end results of repeated run-throughs of the protocol. Each interlaboratory exercise is discussed separately; the final, detailed statistical analysis is reported for the results from IE-III.

A. Interlaboratory Exercise Results

1. Preliminary Interlaboratory Exercise (Dates Run: October-January 1975).

   a. Objectives: To allow the participating laboratories to become familiar with and comment on the protocol and to determine interlaboratory precision

b. Samples: Six vials, two of three different pools each containing a sample from the same serum pool. Each participating laboratory was to analyze a single vial of each concentration on each of two days, with seven days between the two analyses.

c. Procedure: The manual pipetting protocol was used.

d. Comments and Protocol Deviations: The following laboratory comments or protocol deviations were received:

(1) Lab 3: Called attention to error in manuscript, corrected same.

(2) Lab 6: Prefer use of a single stock standard solution with volumetric dilutions. (Original protocol called for weighings and dissolution to prepare each standard solution. Subsequently changed as presented in this protocol.)

(3) Lab 8: Performed extra work. Showed that using a 10-fold dilution, a difference between sample and standard with same lithium concentrations may be observed due to protein effect.

(4) Lab 9: Instrumental problems. Dilutions for two samples, 2nd day analyses stored overnight in refrigerator.

(5) Lab 11: One sample had solid suspended in it, and showed 'many long gram positive rods'.

e. Data: The six data points reported by the individual laboratories are summarized in Table 5. The data are presented graphically in figure 3 as the percent differences from the collective average of the

reported values. All reported values except one from laboratory 11 for pool B are within three percent of the collective average with a standard deviation of ±0.03 mmol/L.

Table 5. Serum lithium concentrations reported by the participating laboratories for the Preliminary Interlaboratory Exercise.

	[Li], mmol/L[a]					
Laboratory	Pool A		Pool B		Pool C	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	1.021	0.996	1.524	1.477	1.985	1.960
2	0.99	1.00	1.49	1.46	1.95	1.91
3	0.99	0.96	1.46	1.46	1.95	1.91
4	0.999	0.990	1.502	1.468	1.941	1.942
6	1.026	1.00	1.505	1.493	1.991	1.968
7	1.00	0.95	1.49	1.46	1.98	2.02
8	1.043	1.01	1.504	1.50	1.997	1.998
9	1.019	1.013	1.511	1.492	1.990	1.962
10	1.033	1.008	1.502	1.488	1.971	1.960
11	1.03	1.03	1.59	1.53	2.04	2.02
13	0.995	0.995	1.481	1.500	1.956	1.960
$\bar{X}$	1.004		1.495		1.971	
S_x	0.022		0.029		0.033	

[a]Each value represents a single measurement on one sample.

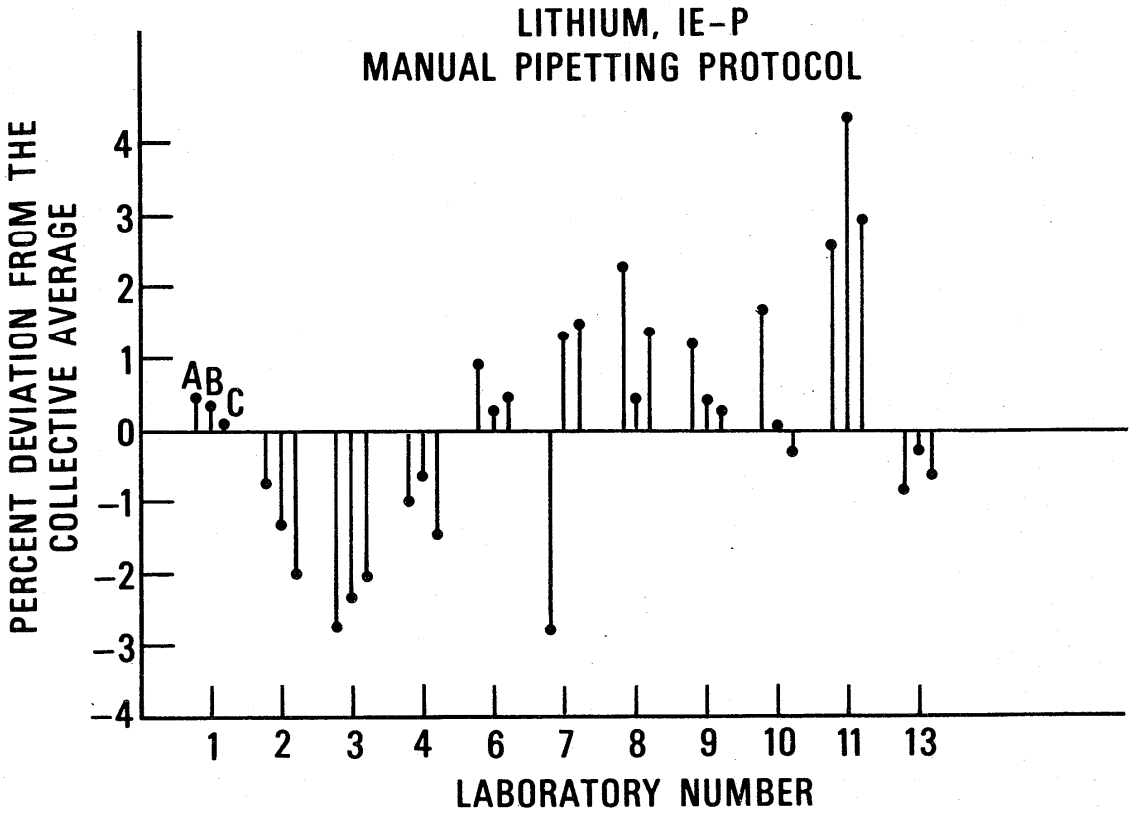

Figure 3. Percent deviations of the two day average results from the collective average of the measurements obtained in the Preliminary Interlaboratory Exercise. The letters A, B, and C next to the results from Lab 1 refer to three different sample pools.

f. Based on the good interlaboratory agreement for IE-P and discussions with the statisticians and Experts Committee, it was concluded that an interlaboratory exercise should be undertaken using samples with lithium concentration values determined by the definitive method. (NOTE: IDMS values for the serum pools were not available at this time, so a preliminary estimation of the bias could not be made. It was expected that few problems would be encountered in the lithium analyses.)

2. Interlaboratory Exercise I (IE-I. Dates Run: January 1975 – May 1975.)

a. Objectives. To test the manual pipetting protocol on serum samples for which definitive lithium values had been obtained and determine the imprecision and bias of the test results.

b. Samples: IE-I was a test series run on 12 samples – four vials (samples) of each of three different concentrations (Pools 1, 4, and 5). Each laboratory was to analyze two vials of each pool on one day and the remaining pairs of samples on a subsequent day with the requirement that a minimum of one day and a maximum of seven days should elapse between analyses.

c. Protocol: A revised manual pipetting protocol was used. A 10-fold dilution for samples and standards was used (same as IE-P). Five valid measurement sets rather than ten were required.

d. Comments and Protocol Deviations: The following laboratory comments or protocol deviations were received:

(1) Lab 1: Many readings were taken to obtain 'valid measurement sets' using 0.5 percent agreement criteria. (NOTE: Requirement changed to 1.0 percent for IE-II and to 2.0 percent for IE-III.)

(2) Lab 7: Instrument noisy if scale expansion is large.

(3) Lab 10: Fifty mL of working standard insufficient volume; although only five valid measurement sets required, agreement of 0.5 percent too stringent; use of single slot burner head resulted in more stable operation compared to stability with use of triple slot head.

e. Data: The single-measurement data reported by the laboratories for this procedure are summarized in Table 6. The data are presented graphically in figure 4 as percent deviations of each one-day 'single measurement' average from the definitive method value.

Table 6. Concentrations of serum lithium reported by the participating laboratories for Interlaboratory Exercise I, manual pipetting protocol.

Laboratory	[Li], mmol/L[a]					
	Pool 1		Pool 4		Pool 5	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	0.496	—	1.451	—	1.730	—
	0.516	—	—	—	—	—
2	0.500	0.498	1.483	1.493	1.738	1.719
	0.502	0.500	1.461	1.485	1.738	1.720
3	0.500	0.538	1.458	1.500	1.726	1.786
	0.500	0.538	1.458	1.500	1.726	1.771
4	0.518	0.520	1.497	1.502	1.752	1.749
	0.518	0.519	1.496	1.490	1.751	1.747
6	0.511	0.511	1.505	1.493	1.773	1.766
	0.521	0.510	1.505	1.511	1.788	1.758
7	0.495	0.524	1.437	1.540	1.762	1.783
	0.499	0.511	1.445	1.595	1.725	1.823
9	0.524	0.524	1.514	1.508	1.768	1.758
	0.525	0.521	1.506	1.507	1.755	1.761
10	0.514	0.505	1.476	1.474	1.737	1.739
	0.511	0.508	1.456	1.492	1.760	1.777
13	0.512	0.524	1.488	1.488	1.731	1.744
	0.512	0.524	1.488	1.488	1.731	1.750
14	0.513	0.517	1.500	1.510	1.762	1.759
	0.516	0.515	1.505	1.487	1.760	1.758
Definitive Method Values	0.534		1.546		1.809	

[a]Each value represents a single measurement on one sample.

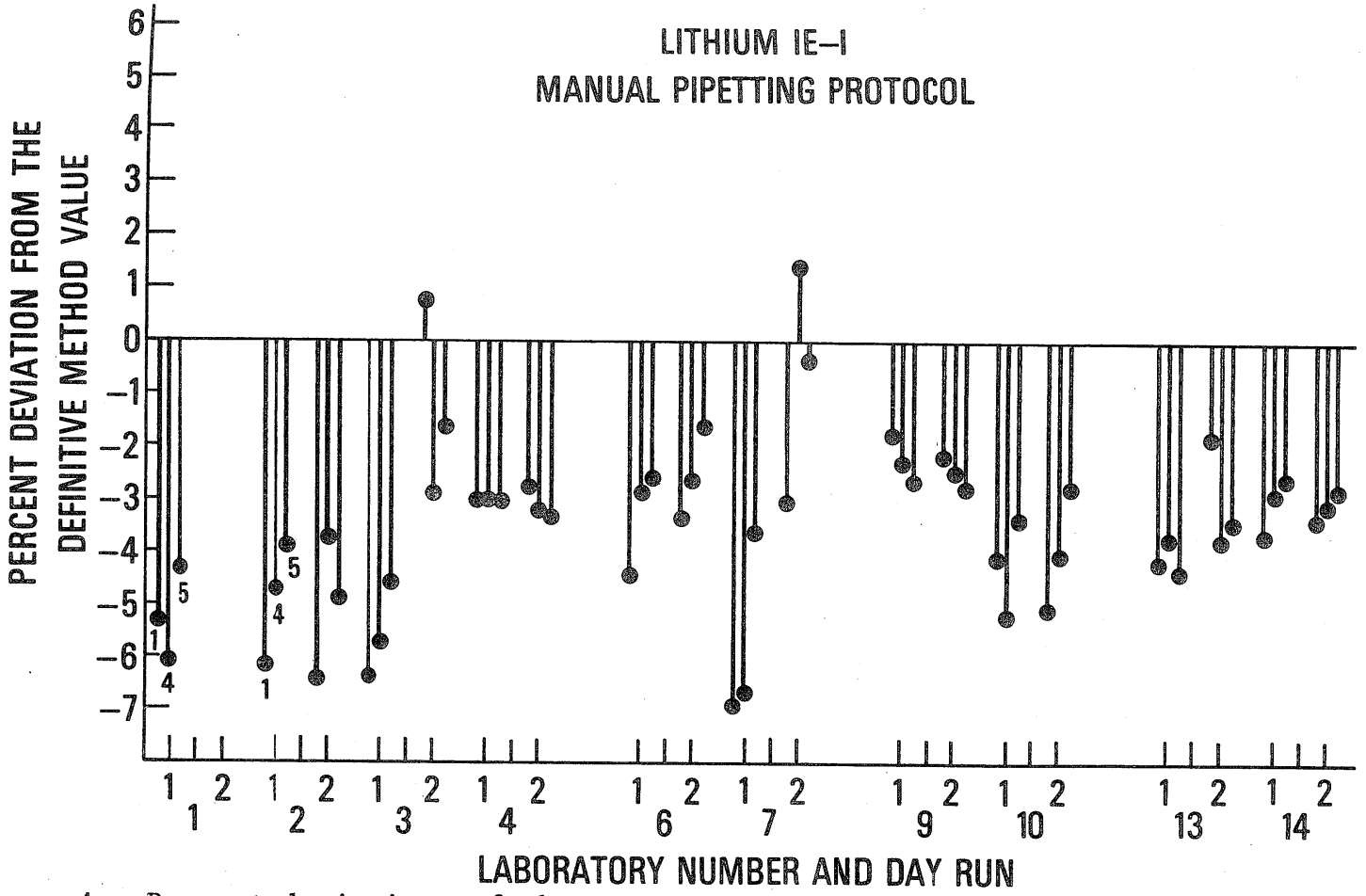

Figure 4. Percent deviations of the Interlaboratory Exercise I measurements using manual pipetting from the definitive method values. The analyzed pools are identified by the numbers 1, 4, and 5 near the results from laboratory 1. The presentation is similar for the remaining results. The numbers 1 and 2 placed directly above the laboratory number, designate the first day and subsequent day test results, respectively.

f. Discussion: Except for a few scattered values, all the results from the laboratories showed an average, negative bias of approximately five percent. This was surprising in view of the results reported earlier [8].

Although estimation of the bias and imprecision values for the results from IE-I show the values to be within the original goals set by the Experts Committee, the negative bias is somewhat disconcerting. Upon inquiry, it was found that the results presented in Table 1 for FAAS and FAES were obtained using a 25-fold dilution of sample and standard rather than the expected 10-fold dilution as performed in references 8 and 16.

Based on this observation, and the observation of Laboratory 8 while performing extra work during IE-P and IE-I which showed a possible protein effect, a ministudy to determine the effect of dilution was performed by Laboratory 9. The results are summarized in Table 7. As can be seen, increasing the dilution ratios decreases the negative bias. Similar studies performed at two other laboratories support this observation. A 25-fold dilution was chosen to be tested rather than a 50-fold dilution since the latter required a large electronic scale expansion resulting in greatly increased instrument noise.

Table 7. The effect of sample and standard dilution on measured serum lithium concentrations.

Dilution	[Li], mmol/L		% Difference
	FAAS	IDMS	
50:1	1.999	2.000	-0.05
25:1	1.992	2.000	-0.40
12.5:1	1.963	2.000	-1.85
25:1	1.010 ± 0.007	1.004	+0.55
25:1	1.999 ± 0.004	1.969	+1.50
25:1	1.025 ± 0.007	1.032	-0.67
10:1[a]	1.016 ± 0.005	1.032	-1.55
25:1	1.535 ± 0.010	1.542	-0.45
10:1[a]	1.500 ± 0.009	1.542	-2.72
25:1	2.092 ± 0.006	2.041	+2.45
10:1[a]	1.970 ± 0.007	2.041	-3.48

[a]From IE-P.

g. Direction: Based on the results of the dilution minitests by Lab 9 and supporting data from Labs 7 and 8, the statisticians and Experts Committee decided to incorporate a 25-fold dilution step into the protocol and to run IE-II using the manual pipetting protocol.

3. Interlaboratory Exercise II: (IE-II. Dates run: May – July 1975.)

a. Objective: To test the revised manual pipetting protocol on samples with lithium concentrations determined by the definitive method.

b. Samples: IE-II was a test series run on a total of 12 samples – four vials (samples) of each of three different lithium concentrations (Pools 3, 4, and 7). Each laboratory was to analyze two vials of each concentration on the first day and the remaining pairs of samples after the elapse of a minimum of one day and a maximum of seven days.

c. Protocol: The revised (25-fold dilution) manual pipetting protocol was used.

d. Comments and Protocol Deviations: The following laboratory comments or protocol deviations were received:

   (1) Lab 3: All samples were run on one day.

   (2) Lab 4: Suggested semiautomated procedure be added to protocol. (NOTE: See general comments for IE-III.)

e. Data: Results from IE-II are given in Table 8 and illustrated in figure 5 in the usual format.

f. Discussion: The majority of laboratories returned results that showed a negative bias of ∿3 percent. Laboratories 9 and 10 showed excellent agreement with the IDMS values; this was expected in Lab 9 since it performed the mini-test to determine the effect of dilution. No explanation could be found to explain the results obtained by Lab 8. All other laboratories verified that they performed the test using the revised protocol with the 25-fold dilution. The average negative bias did decrease from about -5 to about -2.3 percent, a decrease in the right direction but certainly not as large as expected.

Table 8. Concentration of serum lithium reported by the participating laboratories for Interlaboratory Exercise II, manual pipetting protocol.

Laboratory	[Li], mmol/L					
	Pool 3		Pool 4		Pool 7	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1	1.253	1.274	1.490	1.487	2.428	2.461
	1.240	1.241	1.492	1.497	2.440	2.483
2	1.250	1.250	1.453	1.400	2.441	2.523
	1.223	1.261	1.473	1.420	—	2.403
3	1.250	1.250	1.429	1.464	2.500	2.465
	1.250	1.214	1.464	1.464	2.465	2.500
4	1.273	1.265	1.511	1.491	2.494	2.490
	1.272	1.263	1.513	1.505	2.502	2.484
6	1.271	1.274	1.510	1.521	2.497	2.525
	1.267	1.292	1.504	1.519	2.509	2.537
7	1.273	1.265	1.514	1.465	2.523	2.419
	1.266	1.264	1.504	1.459	—	2.419
8	1.350	1.345	1.580	1.584	2.626	2.629
	1.350	1.336	1.580	1.580	2.625	2.626
9	1.292	1.294	1.539	1.538	2.552	2.539
	1.291	1.292	1.540	1.530	2.550	2.541
10	1.296	1.308	1.525	1.538	2.547	2.569
	1.302	1.307	1.543	1.553	2.533	2.567
11	1.251	1.253	1.475	1.495	2.480	2.499
	1.247	1.261	1.488	1.493	2.495	2.480
13	1.259	1.284	1.468	1.506	2.469	2.518
	1.239	1.288	1.474	1.506	2.473	2.516
14	1.259	1.264	1.492	1.496	2.491	2.488
	1.256	1.261	1.491	1.498	2.494	2.491
Definitive Method Values	1.291		1.546		2.572	

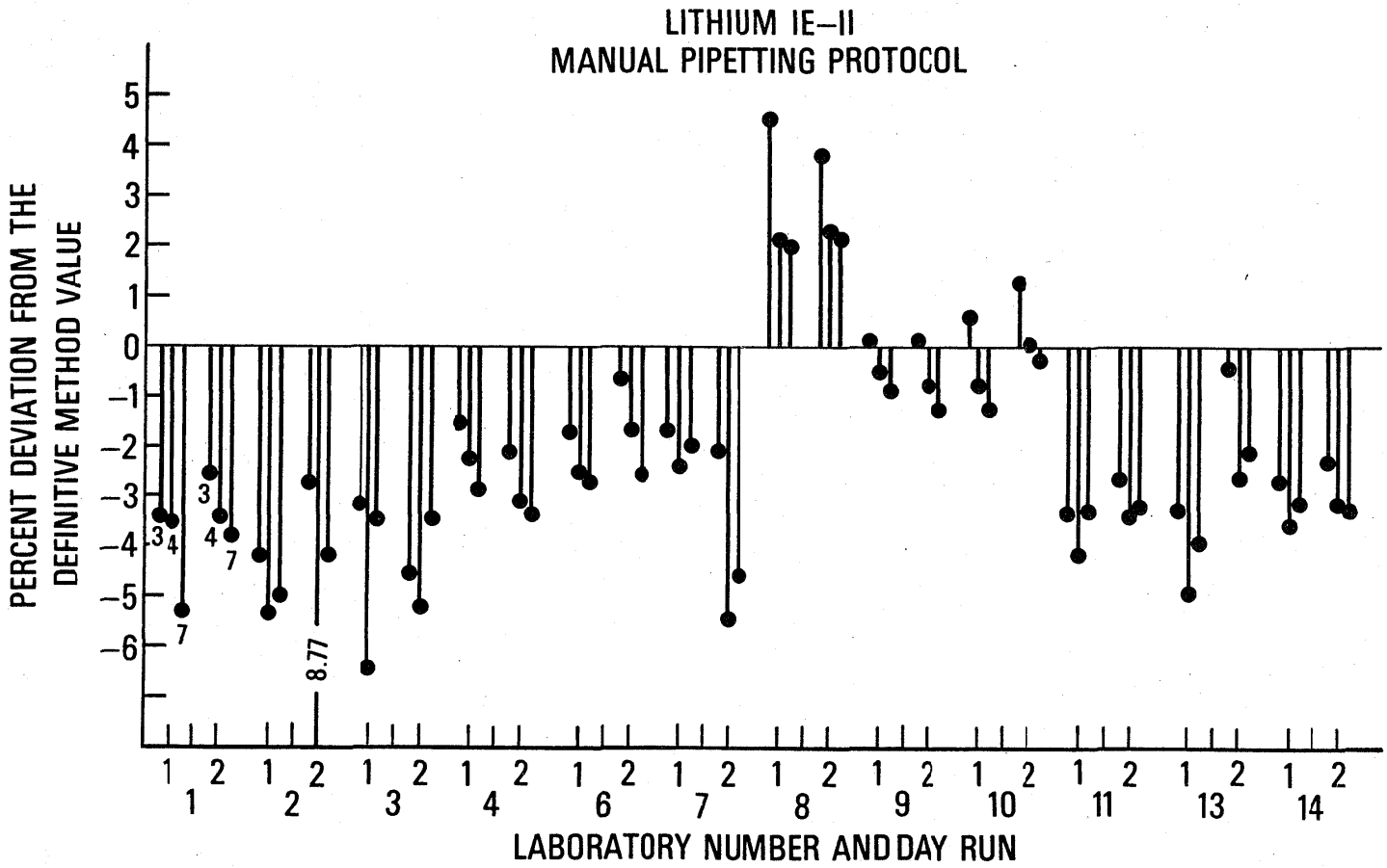

Figure 5. Percent deviations of the Interlaboratory Exercise II measurements using manual pipetting from the definitive method values. The analyzed pools are identified by the numbers 3, 4, and 5 near the results from laboratory 1. The presentation is similar for the remaining results. The numbers 1 and 2 placed directly above the laboratory number, designate the first day and subsequent day test results, respectively.

Variations in instruments, burner heads, hollow cathode sources, and bandpasses do not appear to be the cause of the slight negative bias found during IE-II as shown in Table 9. All laboratories used acetylene as the fuel with variable air-acetylene pressures and flow rates.

Table 9. Summary of FAAS instruments[a] and selected experimental parameters used for IE-II.

Lab	Average % Bias	Instrument	Burner	HC	BP, nm[b]
1	-3.71	IL 453	Hi Solid	Varian	0.24
2	-5.02	PE 303	3 slot	Sing. Ele.	4.0
3	-4.39	IL 153	3 slot	Pyrex-neon	0.43
4	-2.50	PE 403	1 slot	PE	1.4
6	-1.80	IL 353	Boling Laminar Flow	IL	0.43
7	-3.46	IL 402	3 slot	Low. Int.	—
8	+2.50	TECH AA5	1 slot	TECH	—
9	-0.49	PE 403	3 slot	J.A.	1.4
10	-0.05	PE 403	1 slot	WE	1.4
11	-3.32	PE 503	1 slot	PE	1.4
13	-2.85	PE 403	1 slot	PE	1.4
14	-2.94	PE 403	3 slot	PE	1.4

[a]To describe instruments, it was necessary to identify commercial products by manufacturer's name. In no instances does such identification imply endorsement by the National Bureau of Standards, nor does it imply that the particular product or equipment is necessarily the best available for that purpose.

[b]Bandpass.

Additional testing performed by Lab 9 showed that the diameter of the aspiration tubing and the aspiration rate of the solution affects the lithium analysis and could decrease the bias by approximately one percent, Table 10.

Table 10. Effect of aspiration tube diameter on measured serum lithium concentrations at several dilutions.

Tube Diameter cm	Dilution	[Li], mmol/L		% Difference
		FAAS	IDMS	
0.038	50:1	1.519 ± 0.005[a]	1.546	-1.75
	25:1	1.506 ± 0.009	1.546	-2.59
	12.5:1	1.496 ± 0.006	1.546	-3.23
	6.25:1	1.482 ± 0.008	1.546	-4.14
0.058	50:1	1.525 ± 0.011	1.546	-1.36
	25:1	1.516 ± 0.005	1.546	-1.94
	12.5:1	1.502 ± 0.006	1.546	-2.85
	6.25:1	1.474 ± 0.013	1.546	-4.66
0.086	50:1	1.530 ± 0.005	1.546	-1.03
	25:1	1.529 ± 0.004	1.546	-1.10
	12.5:1	1.537 ± 0.003	1.546	-0.58
	6.25:1	1.498 ± 0.018	1.546	-3.10

[a]Standard deviation of a single measurement.

g. Direction: Based on the results of IE-II, the studies by Lab 9, and the laboratory comments, the statisticians and Experts Committee decided to run one additional interlaboratory exercise, IE-III which would include a semiautomated pipetting procedure as well as a manual pipetting procedure. An aspiration tube with ID of ∿0.076 cm was supplied with the samples with instructions for its use as instructions to maximize the solution aspiration rate to ∿8-10 mL/min. It was emphasized again that the flame should be stoichiometric or slightly fuel-rich since Lab 9 demonstrated that a fuel-lean flame caused an error of -1.3 percent in lithium concentration compared to the results from a slightly fuel-rich flame.

4. Interlaboratory Exercise III (IE-III. Dates Run: December 1975 – March 1976.)

a. General: The addition of the semiautomated pipetting alternative to IE-III was considered advantageous because the manual and semiautomated pipetting versions could be evaluated simultaneously on the same serum samples. The semiautomated version would be used in suitably equipped laboratories with consequent economies in reagents and labor; whereas the manual version would be used in laboratories having equipment basic to the method but lacking the appropriate seimautomated sampling device.

   A review and test of the capabilities of positive displacement pipettor-dilutors demonstrated that the precision and accuracy requirements listed in the protocol could be met. Consequently, a method for testing the pipettor-dilutor was included in the protocol.

b. Objective: To test the revised manual and semiautomated pipetting protocols on serum samples having a wider range of lithium values and to determine the imprecision and bias of the test results.

c. Samples: IE-III was a test series run on the total of 16 samples – four vials (samples) of each of four different lithium concentrations (Pools 1, 1a, 5, and 7a). Each laboratory was to analyze two vials of each concentration on the first day and the remaining pairs of samples after the elapse of a minimum of one day and a maximum of seven days.

d. Protocol: The manual and semiautomated pipetting versions of the protocol were used.

e. Data: Results from IE-III are given in Tables 11-12 and illustrated in figures 6-7. The data are presented as two-way tables in which the rows represent the different participating laboratories and the columns represent the different sample pools. The sample pool concentrations ranged from approximately 0.5 to 3.0 millimoles of lithium per liter of serum. The results for the manual procedure and for the semiautomated procedure are listed separately, and all single measurements reported are included in the tables. The definitive method values for the lithium concentrations in the sample pools are listed at the bottom of Tables 11-12.

A detailed statistical analysis was made. First the data were inspected by calculating the percent deviation of each day's results for each pool from an average for that sample pool. These percent deviation values for all laboratories and the two pipetting procedures are listed in Tables 13-14.

Table 11. Concentraton of serum lithium reported by the participating laboratories for Interlaboratory Exercise III, manual method.

	[Li], mmol/L			
Laboratory[a]	Pool 1	Pool 1a	Pool 5	Pool 7a
1-1	.566	.987	1.772	3.078
	.520	.991	1.744	2.904
1-2	.525	1.000	1.760	2.918
	.520	.989	1.750	2.898
2-1	.500	.987	1.771	2.973
	.510	.995	1.790	3.012
2-2	.500	1.013	1.772	2.962
	.506	.984	1.762	2.993
4-1	.517	.983	1.750	2.873
	.517	.976	1.743	2.866
4-2	.521	.988	1.769	2.914
	.520	.990	1.768	2.918
5-1	.533	.981	1.820	2.896
	.504	1.035	1.749	2.996
5-2	.500	1.022	1.827	2.954
	.523	1.008	1.785	2.896
8-1	.533	1.035	1.850	3.007
	.539	1.028	1.837	3.032
8-2	.537	1.029	1.826	3.046
	.535	1.030	1.826	3.057
9-1	.521	.998	1.778	2.922
	.524	.990	1.784	2.930
9-2	.521	.997	1.776	2.934
	.525	.995	1.769	2.918
10-1	.523	1.008	1.786	2.952
	.529	1.004	1.893	2.947
10-2	.528	1.000	1.770	2.948
	.525	.998	1.768	2.959

continued

Continuation of Table 11.

Laboratory[a]	[Li], mmol/L Pool 1	Pool 1a	Pool 5	Pool 7a
13-1	.520	.977	1.776	2.916
	.520	.982	1.767	2.913
13-2	.522	.983	1.770	2.902
	.519	.985	1.770	2.898
14-1	.521	.986	1.767	2.898
	.520	.982	1.759	2.899
14-2	.524	.985	1.764	2.900
	.522	.986	1.763	2.904
15-1	.522	.981	1.765	2.899
	.518	.977	1.760	2.887
15-2	.521	.988	1.761	2.895
	.521	.992	1.760	2.890
Definitive Method Values	.534	1.004	1.809	2.954

[a]The laboratory designation consists of two parts: the initial digit(s) represents the assigned laboratory number and the last digit represents either the first or the second day's results.

Table 12. Concentration of serum lithium reported by the participating laboratories for Interlaboratory Exercise III, semiautomated pipetting protocol.

	[Li], mmol/L			
Laboratory[a]	Pool 1	Pool 1a	Pool 5	Pool 7a
4-1	.513	.986	1.759	2.901
	.517	.981	1.759	2.906
4-2	.517	.981	1.744	2.888
	.516	.976	1.741	2.874
6-1	.542	1.015	1.805	2.965
	.536	1.014	1.805	2.964
6-2	.525	.994	1.802	2.927
	.520	.996	1.801	2.937
9-1	.523	.991	1.786	2.931
	.524	.996	1.773	2.921
9-2	.525	.997	1.775	2.924
	.521	.998	1.775	2.924
10-1	.535	1.011	1.792	2.942
	.520	.998	1.786	2.942
10-2	.525	.995	1.779	2.950
	.525	.988	1.777	2.985
11-1	.520	.977	1.766	2.873
	.517	.973	1.752	2.865
11-2	.520	.995	1.762	2.891
	.516	.991	1.773	2.911
15-1	.521	.986	1.759	2.914
	.521	.988	1.757	2.910
15-2	.520	.985	1.759	2.972
	.517	.986	1.753	2.898
Definitive Method Values	.534	1.004	1.809	2.954

[a]The laboratory designation consists of two parts: the initial digit(s) represents the assigned laboratory number and the last digit represents either the first or the second day's results.

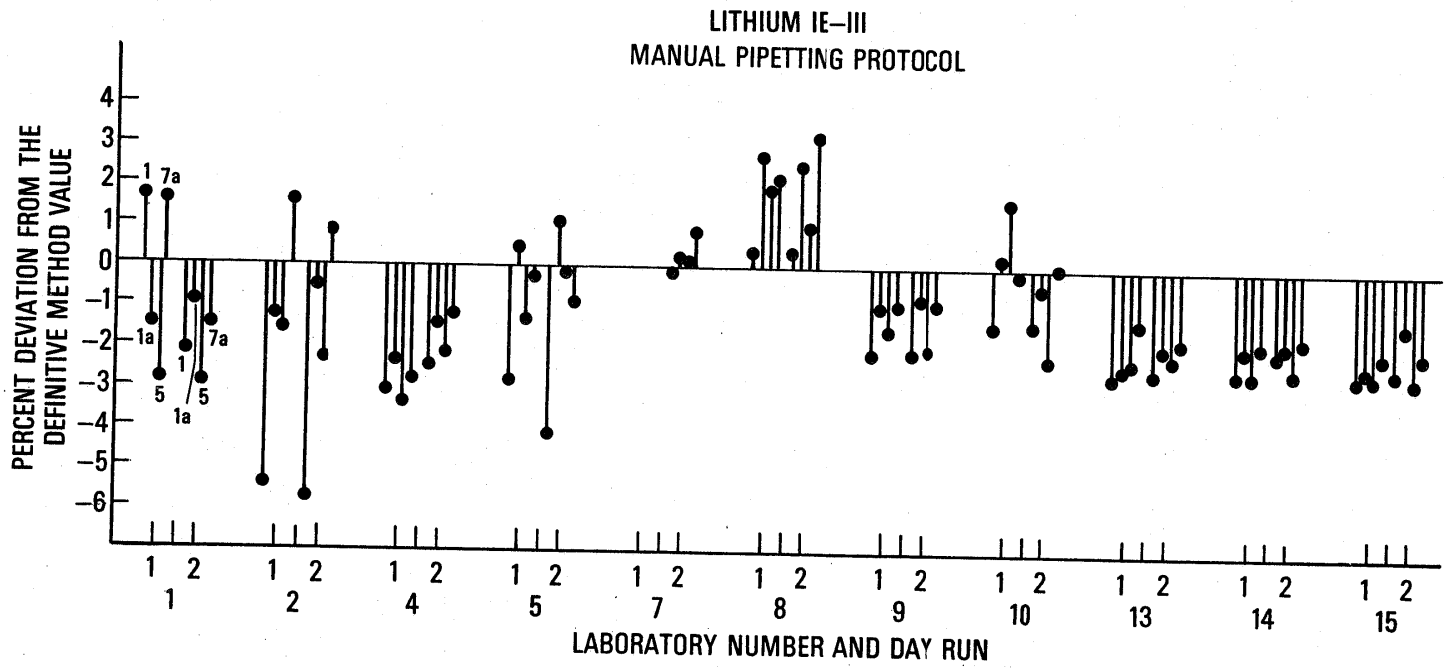

Figure 6. Percent deviations of the Interlaboratory Exercise III measurements using manual pipetting from the definitive method values. The analyzed pools are identified by the numbers 1, 1a, 5, and 7a near the results from laboratory 1. The presentation is similar for the remaining results. The numbers 1 and 2 placed directly above the laboratory number, designate the first day and subsequent day test results, respectively.

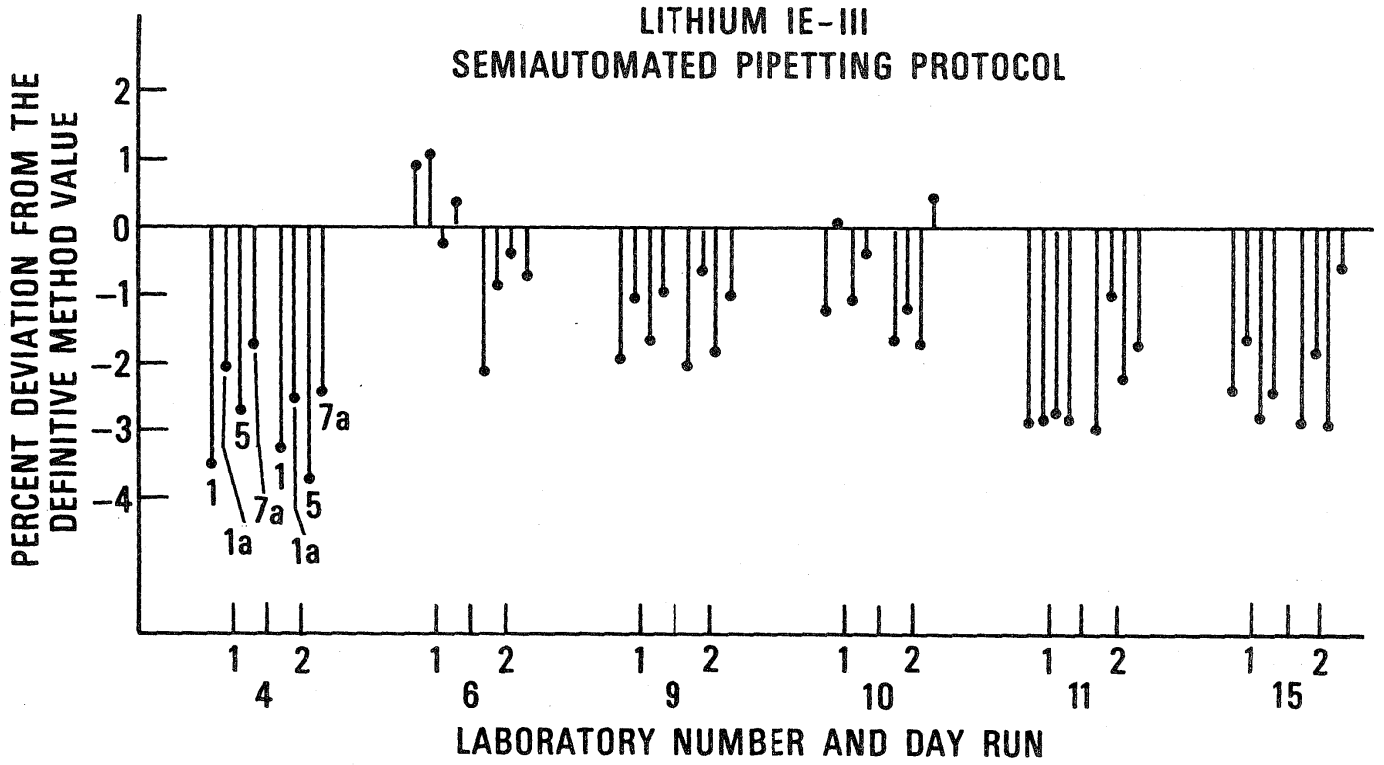

Figure 7. Percent deviations of the Interlaboratory Exercise III measurements using semiautomated pipetting from the definitive method values. The analyzed pools are identified by the numbers 1, 1a, 5, and 7a near the results from laboratory 1. The presentation is similar for the remaining results. The numbers 1 and 2 placed directly above the laboratory number, designate the first day and subsequent day test results, respectively.

Table 13. Percent deviations from averages for lithium in serum from Interlaboratory Exercise III, manual pipetting protocol.

Laboratory[a]	Pool 1	Pool 1a	Pool 5	Pool 7a
1-1	4.06	-.72	-1.19	1.82
1-2	.13	-.16	-1.36	-1.01
2-1	-3.22	-.51	.07	1.87
2-2	-3.60	-.68	-.68	1.36
4-1	-.92	-1.67	-1.84	-2.32
4-2	-.25	-.72	-.60	-.74
5-1	-.63	1.19	.30	.29
5-2	-1.97	1.89	1.51	-.43
8-1	2.72	3.55	3.62	2.79
8-2	2.72	3.35	2.63	3.88
9-1	.13	-.21	.10	-.39
9-2	.23	-.01	-.38	-.39
10-1	.80	.99	3.39	.41
10-2	.90	.29	-.57	.54
13-1	-.34	-1.67	-.71	-.79
13-2	-.25	-1.22	-.52	-1.28
14-1	-.25	-1.22	-.91	-1.33
14-2	.23	-1.07	-.88	-1.21
15-1	-.34	-1.72	-.94	-1.52
15-2	-.15	-.61	-1.05	-1.54
Average used in calculations, mmol/L	.522	.996	1.779	2.938

[a]The laboratory designation consists of two parts: the initial digit(s) represents the assigned laboratory number and the last digit represents either the first or second day's results.

Table 14. Percent deviations from averages for lithium in serum from Interlaboratory Exercise III, semiautomated pipetting protocol.

Laboratory[a]	Pool 1	Pool 1a	Pool 5	Pool 7a
4-1	-1.40	-.82	-.76	-.61
4-2	-1.12	-1.32	-1.69	-1.38
6-1	3.19	2.31	1.83	1.47
6-2	.03	.34	1.64	.36
9-1	.22	.19	.39	.16
9-2	.13	.60	.14	.09
10-1	.99	1.30	.93	.70
10-2	.51	-.01	.31	1.58
11-1	-.73	-1.67	-.76	-1.80
11-2	-.83	.14	-.28	-.70
15-1	-.26	-.46	-.82	-.32
15-2	-.73	-.61	-.93	.46
Average used in calculations, mmol/L	.522	.992	1.773	2.921

[a]The laboratory designation consists of two parts: the initial digit(s) represents the assigned laboratory number and the last digit represents either the first or second day's results.

A comparison was next made of the ability of each laboratory to replicate its values relative to that of the average replication ability of all laboratories. This was done by comparing the standard deviation for each day's measurements for each pool against the laboratory averaged standard deviation for that pool (see Tables 15-16). If all of the participating laboratories were of the same population in regard to replication error, the standard deviation ratios reported in Tables 15-16 would be

Table 15. Ratios of standard deviations to average standard deviation for lithium in serum from Interlaboratory Exercise III, manual pipetting protocol.

Laboratory[a]	Pool 1	Pool 1a	Pool 5	Pool 7a
1-1	5.97	.46	1.65	6.47
1-2	.65	1.27	.59	.74
2-1	1.30	.92	1.12	1.45
2-2	.78	3.35	.59	1.15
4-1	.00	.81	.41	.26
4-2	.13	.23	.06	.15
5-1	3.77	6.24	4.19	3.72
5-2	2.99	1.62	2.48	2.16
8-1	.78	.81	.77	.93
8-2	.26	.12	.00	.41
9-1	.39	.92	.35	.30
9-2	.52	.23	.41	.59
10-1	.78	.46	6.31	.19
10-2	.39	.23	.12	.41
13-1	.00	.58	.06	.11
13-2	.39	.23	.00	.15
14-1	.13	.46	.47	.04
14-2	.26	.12	.06	.15
15-1	.52	.46	.29	.45
15-2	.00	.46	.06	.19
Average Standard Deviation, mmol/L	.00545	.00612	.01199	.01902

[a]The laboratory designation consists of two parts: the initial digit(s) represents the assigned laboratory number and the last digit represents either the first or second day's results.

Table 16. Ratios of standard deviations to average standard deviation for lithium in serum from Interlaboratory Exercise III, semiautomated pipetting protocol.

Laboratory[a]	Pool 1	Pool 1a	Pool 5	Pool 7a
4-1	1.04	1.20	.00	.33
4-2	.26	1.20	.62	.93
6-1	1.57	.24	.00	.07
6-2	1.30	.48	.21	.66
9-1	.26	1.20	2.69	.66
9-2	1.04	.24	.00	.00
10-1	3.91	3.12	1.24	.00
10-2	.00	1.68	.41	2.32
11-1	.78	.96	2.90	.53
11-2	1.04	.96	2.28	1.33
15-1	.00	.48	.41	.27
15-2	.78	.24	1.24	4.91
Average Standard Deviation, mmol/L	.00271	.00295	.00342	.01067

[a]The laboratory designation consists of two parts: the initial digit(s) represents the assigned laboratory number and the last digit represents either the first or second day's results.

larger than 2.44 and 2.36, respectively, only about one percent of the time. In practice, it is not too uncommon to encounter a few standard deviation ratios that are somewhat larger as this is a reflection of some heterogeneity of the laboratory population in regard to replication error. (As long as the standard deviation ratios are not too large, this is normally not used as a reason for rejection of a laboratory. It is advised, however, that

laboratories with large standard deviation ratios should reexamine their procedures for possible sources of excessive replication error.)

The calculations on IE-III data were made on the data in the two-way tables using a weighted least squares fit to the following model [22]:

$$Y_{ijk} = \mu_i + \beta_i(X_j - \tilde{X}) + \lambda_{ij} + \varepsilon_{ijk} \qquad (3)$$

where:

Y_{ijk} = the sample concentration reported by the i^{th} laboratory, for the j^{th} sample, and for the k^{th} replicate measurement,

μ_i = a constant factor associated with the average bias for laboratory i,

β_i = a slope factor for laboratory i, expressing the relation of bias to concentration,

X_j = the observed average concentration for sample pool j (this average is taken over all laboratories),

$\tilde{X}$ = the weighted average concentration for all samples (this average is taken over all laboratories and over all sample pools),

λ_{ij} = a random sample interference factor (matrix effect) for laboratory i and sample pool j, and

ε_{ijk} = a random replication error.

The above model is quite general and extensive experience has shown that it is well suited to describe a number of measurement factors in interlaboratory tests [23].

Weighted analyses of variance were made on the data in the two-way tables using the fits to the above model. (A modified version of the weighting procedure reported in reference 24 was used.) From the analyses it is possible to derive the following estimates for three components of variability, each characterized by its standard deviation:

$\hat{\sigma}_{\varepsilon} = \hat{\sigma}_{\varepsilon(\text{Repl})}$ = the uncertainty observed for replicate measurements in a given laboratory on a given day,

$\hat{\sigma}_{D} = \hat{\sigma}_{\text{Day}}$ = the additional uncertainty that is observed when measurements are made on different days within the same laboratory, and

$\hat{\sigma}_{L} = \hat{\sigma}_{\text{Lab}}$ = the additional uncertainty that is observed when measurements are made by different laboratories.

All three of these components of standard deviation were observed to systematically increase with increasing lithium concentration. The smoothed values of these components of standard deviation are given in Table 17.

Table 17. Components of standard deviation in mmol/L for lithium in serum, Interlaboratory Exercise-III.

Manual Pipetting Protocol
(Pooled Results from 10 Laboratories)

Lithium Level, mmol/L	$\hat{\sigma}_{\varepsilon(\text{Repl.})}$	$\hat{\sigma}_{\text{Day}}$	$\hat{\sigma}_{\text{Lab}}$
.5	.008	0	.007
1.0	.013	0	.013
1.8	.022	0	.026
2.9	.034	.003	.044

Semiautomated Pipetting Protocol
(Pooled Results from 6 Laboratories)

Lithium Level, mmol/L	$\hat{\sigma}_{\varepsilon(\text{Repl.})}$	$\hat{\sigma}_{\text{Day}}$	σ_{Lab}
.5	.002	.004	.005
1.0	.004	.006	.009
1.8	.009	.009	.016
2.9	.016	.012	.027

Because of the relatively small size of the lithium interlaboratory exercises, the individual components of standard deviation are considered to be only advisory in nature. The final, practical statements of uncertainty are made through the recombination of these components. One such final statement is $\hat{\sigma}_{\text{within}}$, the expected uncertainty within a single laboratory from running the complete protocol (2 replicates/day for 2 days). The $\hat{\sigma}_{\text{within}}$ results are reported in columns three and seven in the top section of Table 18, and are calculated as follows:

$$\hat{\sigma}_{within} = \sqrt{\frac{\hat{\sigma}_{\varepsilon}^2}{4} + \frac{\hat{\sigma}_{D}^2}{2}} \quad . \qquad (4)$$

These are the expected uncertainties that a single average laboratory could see by repeating the complete protocol a number of times and observing the variability of its results. This $\hat{\sigma}_{within}$ is not the total uncertainty since there is also a "between laboratory" component, $\hat{\sigma}_{Lab}$. The standard deviation of the total uncertainty expected as a result of a single laboratory running the complete protocol is calculated as follows:

$$\sigma_{Total} = \sqrt{\frac{\hat{\sigma}_{\varepsilon}^2}{4} + \frac{\hat{\sigma}_{D}^2}{2} + \hat{\sigma}_{L}^2} \quad . \qquad (5)$$

Columns four and six in the top section of Table 18 list such standard deviations for the manual and semiautomated data from IE-III. The precision goal for the reference method is listed in column five. Comparison of the tabulated standard deviations and the goal shows that the precision goals have been met.

Table 18. Summary of imprecision and bias results in mmol/L for lithium in serum, Interlaboratory Exercise-III.

	Manual Pipetting Protocol				Semiautomated Pipetting Protocol		
Li Level	$\hat{\sigma}_{comp}$	$\hat{\sigma}_{within}$	$\hat{\sigma}_{total}$	Goal	$\hat{\sigma}_{total}$	$\hat{\sigma}_{within}$	$\hat{\sigma}_{comp}$
0.5	0.002	0.004	0.008	0.1	0.005	0.003	0.003
1.0	.005	.006	.014	0.1	.010	.004	.004
1.8	.009	.011	.028	0.1	.017	.007	.007
2.9	.015	.017	.047	0.1	.029	.011	.012

- - - - - - - - - - - - Accuracy - - - - - - - - - - - -

	Manual Pipetting Protocol		Semiautomated Pipetting Protocol
Li Level	Interlaboraotry Exercise Composite Bias $(X_{obs}-X_{DM})$	Goal	Interlaboratory Exercise Composite Bias $(X_{obs}-X_{DM})$
0.5	-.012	±.2	-.012
1.0	-.008	±.2	-.012
1.8	-.030	±.2	-.036
2.9	-.016	±.2	-.033

The standard errors of the IE-III composite values are given in columns two and eight of the top section of Table 18. These standard errors are calculated from the components of standard deviation as follows:

$$\hat{\sigma}_{comp} = \sqrt{\left(\frac{1}{N}\right)\left[\frac{\hat{\sigma}_{\varepsilon}^{2}}{4} + \frac{\hat{\sigma}_{D}^{2}}{2} + \hat{\sigma}_{L}^{2}\right]} \qquad (6)$$

where N represents the 10 or 6 laboratories participating in the manual or semiautomated procedures, respectively. The bottom section of Table 18 lists the observed biases between the reference method interlaboratory exercise composite values and the definitive method values. A consistent, small negative bias is observed. The observed biases, however, are easily within the goals for the reference method.

Table 19 lists the composite IE-III sample averages ± twice the standard error for the manual and for the semiautomated versions, and for the corresponding definitive method values.

The accuracy of the IE-III results is within the recommended goal of the reference method.

Table 19. Summary of lithium in serum values.

IE-III - Composite Values (mmol/L)		Definitive Method Values (mmol/L)
Manual	Semiautomated	
0.522 ± 0.004[a]	0.522 ± 0.006[a]	0.534 ± 0.003[b]
0.996 ± 0.010	0.992 ± 0.008	1.004 ± 0.005
1.779 ± 0.018	1.773 ± 0.014	1.809 ± 0.009
2.938 ± 0.030	2.921 ± 0.024	2.954 ± 0.015

[a]Twice the standard error of the composite average, i.e., $2\hat{\sigma}_{comp}$.

[b]Estimated maximum error of 0.5 percent of the value. This estimated error is the sum of errors due to measurement imprecisions of ±0.3 percent (±2 sigma interval for the random error of the mean) and an estimated upper bound of 0.2 percent for possible systematic errors.

f. Auxiliary Statistical Analysis: The protocol requires a check on the flame atomic absorption spectrometer by running a calibration curve each day using freshly prepared standard solutions. The necessity of these curves also provides a check on the correct preparation of the standard solutions. The data reported here on the calibration curve check are advisory in nature since in the actual analytical procedure only the pair of calibrating solutions nearest to the unknown concentration is used. The calibration curve data for the manual and semiautomated lithium procedures were reported and are given in Tables 20 and 21. Straight line least squares fits were made to these data and the resultant standard deviations of fit are given in Table 22. These standard deviations of fit are expressed in units of lithium concentration (mmol/L). Our analysis indicates that if in the calibration

Table 20. Calibration curve data for lithium in serum, Inter-laboratory Exercise III, manual pipetting protocol.

Lab[a] No.		Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Std. 6
1-1	X[b]	0.5000	1.0000	1.5000	2.0000	2.5000	3.0000
	Y[c]	0.254	0.505	0.761	1.034	1.293	1.519
1-2	X	0.5000	1.0000	1.5000	2.0000	2.5000	3.0000
	Y	0.254	0.507	0.765	1.020	1.269	1.509
2-1	X	0.5001	1.0002	1.5003	2.0004	2.5005	3.0000
	Y	0.288	0.527	0.788	1.028	1.280	1.516
2-2	X	0.5001	1.0002	1.5003	2.0004	2.5005	3.0000
	Y	0.289	0.530	0.785	1.025	1.277	1.510
4-1	X	0.5000	1.0000	1.5000	2.0000	2.5000	3.0000
	Y	0.2595	0.5225	0.7775	1.035	1.2865	1.5535
4-2	X	0.5000	1.0000	1.5000	2.0000	2.5000	3.0000
	Y	0.257	0.5175	0.779	1.036	1.300	1.5565
5-1	X	0.5001	1.0002	1.5003	2.0004	2.5005	3.0006
	Y	0.0381	0.0716	0.1090	0.1475	0.1884	0.2328
8-1	X	0.500	1.000	1.500	2.000	2.500	3.000
	Y	0.100	0.200	0.300	0.398	0.500	0.601
8-2	X	0.500	1.000	1.500	2.000	2.500	3.000
	Y	0.100	0.200	0.300	0.399	0.500	0.602
9-1	X	0.500	1.000	1.500	2.000	2.500	3.000
	Y	0.265	0.531	0.796	1.059	1.326	1.585
10-1	X	0.5000	1.0000	1.5000	2.0000	2.5000	3.0001
	Y	0.299	0.591	0.880	1.165	1.458	1.744
10-2	X	0.5000	1.0000	1.5000	2.0000	2.5000	3.0001
	Y	0.284	0.554	0.831	1.104	1.373	1.642
13-1	X	0.5000	1.0000	1.5000	2.0000	2.5000	3.0001
	Y	0.255	0.510	0.764	1.015	1.260	1.505
14-1	X	0.5000	1.0000	1.5000	2.0000	2.5001	3.0001
	Y	0.259	0.518	0.770	1.022	1.273	1.524
14-2	X	0.5000	1.0000	1.5000	2.0000	2.5001	3.0001
	Y	0.255	0.512	0.762	1.011	1.260	1.512

continued

Continuation of Table 20.

Lab. No.[a]		Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Std. 6
15-1	X	0.5001	1.0001	1.5002	2.0002	2.5003	3.0003
	Y	0.015	0.0305	0.045	0.0605	0.075	0.090
15-2	X	0.4999	0.9999	1.4998	1.9998	2.4997	2.9997
	Y	0.016	0.031	0.046	0.061	0.075	0.089

[a]The laboratory designation consists of two parts: the initial digit(s) represents the assigned laboratory number and the last digit represents either the first or second day's results.

[b]X = Standard solution values in mmol/L.

[c]Y = Instrument readings.

Table 21. Calibration curve data for lithium in serum, Interlaboratory Exercise III, semiautomated pipetting protocol.

Lab. No.[a]		Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Std. 6
4-1	X[b]	0.5000	1.0000	1.5000	2.0000	2.5000	3.0000
	Y[c]	0.259	0.5125	0.768	1.0165	1.274	1.534
4-2	X	0.5000	1.0000	1.5000	2.0000	2.5000	3.0000
	Y	0.2565	0.5075	0.764	1.0195	1.269	1.5285
6-1	X	0.5000	1.0000	1.5000	2.0000	2.5000	3.0000
	Y	0.231	0.647	1.056	1.430	1.855	2.238
6-2	X	0.5000	1.0000	1.5000	2.0000	2.5000	3.0000
	Y	0.489	0.918	1.338	1.815	2.205	2.622
10-1	X	0.5000	1.0000	1.5000	2.0000	2.5000	3.0001
	Y	0.293	0.572	0.842	1.104	1.400	1.665
10-2	X	0.5000	1.0000	1.5000	2.0000	2.5000	3.001
	Y	0.261	0.5126	0.773	1.029	1.279	1.507
11-1	X	0.5000	1.0000	1.5000	2.0000	2.5000	3.0000
	Y	0.252	0.503	0.756	0.993	1.237	1.494
11-2	X	0.5000	1.0000	1.5000	2.0000	2.5000	3.0000
	Y	0.255	0.509	0.766	1.003	1.255	1.510

[a]The laboratory designation consists of two parts: the initial digit(s) represents the assigned laboratory number and the last digit represents either the first or second day's results.

[b]X = Standard solution values in mmol/L.

[c]Y = Instrument readings.

Table 22. Calibration curve results for lithium in serum as standard deviation of fit (s_{fit}) in mmol/L.

Manual		Semiautomated	
Laboratory Number[a]	s_{fit}	Laboratory Number[a]	s_{fit}
1-1	0.0248	4-1	0.0064
1-2	0.0124	4-2	0.0047
2-1	0.0127	6-1	0.0159
2-2	0.0111	6-1	0.0240
4-1	0.0076	10-1	0.0146
4-2	0.0033	10-2	0.0213
5-1	0.0482	11-1	0.0111
8-1	0.0054	11-2	0.0106
8-2	0.0048		
9-1	0.0039		
10-1	0.0036		
10-2	0.0052		
13-1	0.0095		
14-1	0.0055		
14-2	0.0051		
15-1	0.0094		
15-2	0.0178		

[a]The laboratory designation consists of two parts: the initial digit(s) represents the assigned laboratory number and the last digit represents either the first or second day's results.

step it is found that any calibration point deviates from the calibration curve by more than 0.03 mmol/L, then the standard solutions and the instrument should be checked for sources of excessive error before proceeding further into the analysis.

V. DISCUSSION

A. Candidate Protocol:

1. Preliminary Tests

Generally, in the development of a reference method where the state of analytical knowledge leaves an uncertainty in the choice of a 'candidate' reference method, it is essential that investigations be undertaken to assure optimized analytical conditions, minimized interferences, and freedom from other sources of bias. Such preparation helps avoid initiating the interlaboratory testing process with inappropriate procedures. In the case of lithium, the similarity of results obtained by White and Mavrodineanu using FAES and FAAS, the similarity of their results with those obtained using the highly specific IDMS method, and previous work [8] led the Committee to decide to proceed directly to the interlaboratory exercise phase with the FAAS method, without further preliminary studies. With this electrolyte, however, the interlaboratory exercises revealed a negative bias unless at least 25-fold dilutions of sample and standard are made and slightly fuel-rich flame, high aspiration rate, and large aspiration tube operating conditions are used.

2. Specifications

In keeping with prior experience [9-12], the written protocol is explicit as to reagent and glassware specifications, pipetting, and directions for dilution of the standard and sample. Thus, Class A or equivalent glassware, reagent grade or equivalent chemicals, 'tested' water, analytical balances with a ±0.1 mg weighing capability, and a pipettor-dilutor with tested accuracy and precision are specified. In addition, the reference method provides for the use of analytical techniques that should reduce the combined error due to weighing, pipetting, and dilution to below one percent.

3. Flame Atomic Absorption Spectroscopy

In general, all the FAAS instruments used in the laboratories that participated in this study provided excellent results. The instruments that were used are listed in Table 23 and encompass several types. Thus, specific instructions are not given for the use of flame atomic absorption instruments; only a requirement for stable instrument operating conditions is presented. As in sample preparation and handling, the human element in achieving accuracy and precision is critical. It is essential that operators be thoroughly familiar with their instruments and alert to the onset of instrumental difficulties.

The protocol initially required a 10-fold sample dilution and a one-percent agreement for measurement sets to be considered valid. The dilution of sample and standards was subsequently changed to 25-fold in order to decrease the negative bias that was observed and the one percent requirement was changed to two percent at the July 1975 meeting of the representatives from the participating laboratories to facilitate the analysis. In the discussion that led to this protocol change, the representatives affirmed that if their instruments were operating optimally, agreement of successive sets of readings could be obtained to within 0.5 percent. It was found that the overall precision of the interlaboratory exercise results did not significantly degrade due to this change.

Instrument linearity requirements were not included in the protocol since the bracketing method for obtaining valid measurements was used to minimize the errors attributable to instrumental drift. Examination of the calibration data which were requested showed excellent linearity over the range of lithium concentrations from 0.5 to 3.0 mmol/L. More than 95 percent (24 of 25) of the calibration curves showed standard deviations of fit of about 0.025 mmol/L or

Table 23. Instruments and operating conditions used by the participating laboratories in Interlaboratory Exercises II and III.

Lab. No.	Instrument[a]	Burner	λ, nm	Flow Rate-Pressure		Pipetting Alternative	
				Air	Acetylene	Manual	Semiautomated
1	IL 453	3 slot	670.8	–	–	X	
2	IL 253	3 slot	670.8	17 L/min	5.5 L/min	X	
3	IL 153	3 slot	670.8	–	–	X	
4	PE 403	3 slot	670.8	25 L/min	6.4 L/min	X	X
6	IL 353	3 slot	670.7	6.5 PSIG	3.8 PSIG		X
7	IL 140	3 slot	670.8	–	–	X	
8	TECH AA5	1 slot	670.7	6.8 L/min	2.2 L/min	X	
9	PE 403	3 slot	670.8	24.5 L/min	8.5 L/min	X	X
10	PE 403	1 slot	670.8	27.5 L/min	5.5 L/min	X	X
11	PE 503	1 slot	670.8	21.8 L/min	3.74L/min		X
13	PE 403	1 slot	670.8	60 L/min	35 L/min	X	
14	PE 460	3 slot	670.8	27 L/min	4.7 L/min	X	
15	PE 503	3 slot	670.8	30 PSIG	6 PSIG	X	X

[a] See footnote a, Table 9, page 43.

less. A standard deviation of fit larger than 0.03 mmol/L would clearly warrant a laboratory's investigation of its operation of the procedure and/or preparation of the standard solutions.

The use of the bracketing criterion for valid sets determined that a 50-mL minimum volume of working sample was needed for the semiautomated pipetting protocol. About 25 mL of working solution is required to obtain five sets of valid measurements, assuming a nebulization rate of 2-4 mL/min for approximately 45 s to obtain a single reading. (That time-interval is necessary for the instrument and flame to be stabilized and for actual integration of the signal.) Larger volumes of diluted sample were available with the manual pipetting protocol because of the large aliquot volumes taken to ensure pipetting accuracy.

4. Statistical Analysis

All of the results discussed here are based on the analysis of four replicate samples analyzed as pairs on two separate days. Adherence to this pattern of replicate analysis helped assure the reliable performance of the reference method.

The imprecision and bias goals of 0.1 and 0.2 mmol/L, respectively, were reached over the total concentration range by the laboratories using either the manual or semi-automated pipetting protocols. As is evident from Table 18, the imprecision values for both pipetting procedures are at least twice as good as the original goal set for the reference method by the Experts Committee.

The $\sigma_{(total)}$ precision values were constant over the lithium concentration range of 0.5-3.0 mmol/L when expressed as CV and were 1.6 and 1.0 percent for the manual and semi-automated pipetting protocols, respectively. A negative bias of one to two percent of the serum lithium concentration was observed for both pipetting protocols. This bias is

small when compared to the original accuracy goal of ±10 percent. The agreement between the reference method values and the definitive method values are considered to be acceptable.

The above precision and accuracies can be expected for laboratories in the population typical of those participating in this study (i.e., clinical laboratories that have practiced the reference method and are in good quality control).

VI. CONCLUSIONS

A 'candidate' reference method, specified by a written protocol for the determination of serum lithium by flame atomic absorption spectroscopy was evaluated by analyzing serum and aqueous samples in a selected group of laboratories. The results for samples having lithium concentrations in the 0.53 to 2.95 mmol/L range showed a total imprecision of about 1.5 percent and a negative bias of about 2 percent of the serum lithium concentration as compared to definitive method values for these samples. Slightly smaller imprecisions were found for the semiautomated pipetting procedure compared to the manual pipetting procedure. The imprecision and bias values for both pipetting procedures were well within the goals set by the experts committee. An isotope dilution – mass spectrometric procedure was used as the definitive method to determine lithium values in the pooled sera.

Statistical analysis of the results shows that the flame atomic absorption candidate reference method can be carried out with the accuracy and precision expected of a reference method for serum lithium. Hence, the 'candidate' method should be considered to be the reference method. This reference method may be used to establish the accuracy of field methods for lithium by comparative testing. It may also be used to determine reference serum lithium values. Each of these uses would require an appropriate experimental

design to ensure its achievement of accuracy and precision equal to those demonstrated here.

We would like to especially thank the principal investigators and other scientists in the participating laboratories (listed in Appendix A), who, through their efforts, made this work meaningful and possible. We thank Dr. David Bayse and Ms. Sue Lewis, CDC, for providing excellent, homogeneous serum pools used in the interlaboratory testing process.

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APPENDIX A

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U. S. Department of Commerce
Frederick B. Dent
Secretary

National Bureau of Standards
Richard W. Roberts, Director

National Bureau of Standards Certificate of Analysis

Standard Reference Material 924

Lithium Carbonate

This Standard Reference Material is certified as a chemical of known purity. It is intended primarily for use in calibration and standardization of procedures employed in clinical analysis and for the routine critical evaluation of daily working standards used in these procedures.

Purity . . . . . $100.0_5 \pm 0.0_2$ Percent

The purity shown is based on the determination of the carbonate ion by coulometric acidimetry. The molecular weight for lithium carbonate employed in the calculations is 73.9486. This value is based on a mass-spectrometrically determined value of 6.9696 for the atomic weight of lithium in this sample. The uncertainty shown represents the 95-percent confidence interval of the mean based on 16 determinations. The assay in excess of 100 percent may be due to anion impurities of lower molecular weight than the carbonate ion, e.g., hydroxide.

This Standard Reference Material is of limited certification because no actual determination of lithium content was made. The certification is based on the analysis of the carbonate anion and the proven absence (or presence in trace quantities) of metallic cations.

The lithium carbonate used for this Standard Reference Material was obtained from the J. T. Baker Chemical Company of Phillipsburg, New Jersey. Analyses were performed by G. Marinenko, M. Darr, E. L. Garner, T. C. Rains, and T. A. Rush.

The overall direction and coordination of technical measurements leading to certification were under the chairmanship of R. A. Durst.

The technical and support aspects concerning preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by T. W. Mears.

Washington, D. C. 20234
February 23, 1972
Revised November 23, 1973

J. Paul Cali, Chief
Office of Standard Reference Materials

(over)

The lithium carbonate meets or exceeds the specifications for reagent grade lithium carbonate as given in Reagent Chemicals, 4th edition, published by the American Chemical Society. A semi-quantitative survey for trace contaminants by emission spectroscopy showed no significant metallic impurities. Atomic absorption and flame emission spectrometry showed neither alkali metal nor alkaline-earth impurities in excess of 1 ppm except calcium (4 ppm).

This Standard Reference Material is intended for "in vitro" diagnostic use only.

This material is intended for use as a standard for determination of lithium in clinical chemistry. For best results using either atomic absorption spectroscopy or flame emission photometry it is necessary that lithium be determined against a background of sodium and potassium.

A "standard" solution containing 1.00 mmol of lithium per liter may be prepared as follows. Dry SRM 924 for 4 hours at 200 °C, then cool to room temperature in a desiccator. Dissolve 73.91 mg of SRM 924 in 50 ml of deionized water and 20 ml of 0.1N HCl (ACS Reagent Grade). Dilute to the mark with deionized water and mix well in a 2-liter class-A volumetric flask.

A "blank" solution containing 140 mmol of sodium per liter and 5 mmol of potassium per liter may be prepared as follows: Dissolve 8.18 g of sodium chloride (SRM 919) and 0.373 g of potassium chloride (SRM 918) in deionized water. Bring to the mark of a 1-liter flask with deionized water and mix well.

Working standards containing 0.10 or 0.20 mmol of lithium, 14 mmol of sodium and 0.5 mmol of potassium per liter may be prepared as follows. To each of two 100-ml volumetric flasks add 10 ml of "blank solution". Add exactly 10.00 ml of "standard solution" to one flask and exactly 20.00 ml of "standard solution" to the second flask using class-A volumetric pipettes. Dilute each flask to the mark with deionized water and shake well.

This Standard Reference Material should be stored in the well-closed original bottle under normal laboratory conditions.

The solutions of SRM 924 are stable indefinitely when stored in a well-stoppered, all-glass container. All such solutions should be clear and display no turbidity.

References:

[1] J. Pybus and G. N. Bowers, Jr., Serum lithium determination by atomic absorption spectroscopy, in Standard Methods of Clinical Chemistry, Vol. 6, R. P. McDonald, editor-in-chief, pp 189-192, Academic Press, Inc., New York, N. Y. (1970).

[2] A. L. Levy and E. M. Katz, A comparison of serum lithium determinations using flame photometry and atomic absorption. Clin. Chem. 15, 787 (1969).

This Standard Reference Material has been measured and certified at the laboratories of the National Bureau of Standards, Gaithersburg, Maryland. All inquiries should be addressed to:

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

The date of issuance and certification of this Standard Reference Material was February 23, 1972.

APPENDIX C

Isotope Dilution Mass Spectrometry

The use of thermal-ionization mass spectrometry for isotope analysis has a valid and well-described theoretical foundation. The methodology has been experimentally evaluated so that results from the procedure have negligible or accurately known systematic errors and high levels of precision. It is regarded at NBS as a definitive method.

Isotope dilution analyses are performed by measuring the change in the relative magnitude of two isotopes of the analyte when a measured amount of one of these isotopes is added to the sample. The method consists of the following steps:

(1) The addition of a known amount of a separated isotope (spike) of the analyte to be determined to a weighed serum sample. For high accuracy, this addition is made as a weighed portion of a spike solution having known isotopic composition and analyte concentration.

(2) Dissolution of the sample by appropriate means and thorough mixing of the resulting solution to ensure equilibration of the separated isotope with the analyte in the sample. This may involve chemical treatment to convert the analyte and the separated isotope to the same oxidation state.

(3) Chemical separation of the isotopically altered analyte from possible interfering elements and into a form suitable for mass spectrometric analysis. A major advantage of isotope dilution mass spectrometry (IDMS) is the fact that recoveries need not be quantitative since only the ratios of the isotopes are measured.

(4) Measurement of the altered isotopic ratio by thermal ionization mass spectrometry.

(5) Calculation of the amount of the analyte in the sample using equation 1:

$$\text{Concentration, } \mu g/g = \frac{W_{sp}\ C[A_{sp} - RB_{sp}]}{BR - A} \cdot \frac{M}{W_s} \qquad (1)$$

where:

W_{sp} = Weight of spike solution, grams
C = Concentration of spike, μmoles/gram of solution
A_{sp} = Atomic fraction of isotope A in spike
B_{sp} = Atomic fraction of isotope B in spike
A = Atomic fraction of isotope A in sample
B = Atomic fraction of isotope B in sample
R = Experimentally measured ratio
M = Atomic weight of element
W_s = Weight of sample, grams

This calculated concentration must be corrected for the blank.

The possible sources of systematic error in isotope dilution mass spectrometry (IDMS) are:

(1) Error in the calibration of the concentration of the spike isotope. The spike solution is calibrated against at least two different solutions of the pure analyte containing 'natural' isotopic abundances by what might be called reverse isotope dilution. Whenever possible, NBS Standard Reference Materials are used as the 'natural' material. The error from this source will be the same as for the analyte being determined and is due to the imprecision of the ratio measurement.

(2) Chemical errors. In a well designed analysis, an undetected chemical error should not occur if adequate precautions are taken against the following

potential sources of error. Errors might be caused by:

a) Incomplete decomposition or dissolution of the sample, a problem common to all wet analytical methods.

b) Loss of the analyte from the sample or spike due to volatility or adsorption during dissolution. These losses can usually be detected by spiking some samples before dissolution and others after dissolution.

c) Incomplete mixing or equilibration of the spike and the 'natural' analyte. This can be caused by differences in oxidation state or the presence of the 'natural' analyte in a complex or chelated form. This source of error can be eliminated by proper chemical treatment, for example, by oxidation or reduction and wet-ashing.

d) Isotope fractionation in the chemical treatment if the separation is not quantitative. This is seldom a problem but can occur with some techniques. Fractionation can be detected by isotopic analysis of small amounts of 'natural' materials before and after being subjected to the non-quantitative separation procedure.

(3) Contamination or blank. Sources of contamination or blank may be reagents, apparatus, or fall-out from the laboratory atmosphere. The problem can be minimized by carrying out the chemical operations in a carefully controlled atmosphere and by using special, high-purity reagents. The total blank may be estimated by carrying a number of 'blanks' through all the steps of the analysis. The average blank value can be treated as a systematic error

and the average value obtained for the analyte in the 'blanks' is used as a correction. The uncertainty of this correction is equal to the randomness of its measurement, i.e., its coefficient of variation. For concentrations where the blank amounts to a significant fraction of an analytical value, the blank may become the largest source of error.

(4) Interferences. Interference usually occurs between elements with isobars; i.e., isotopes of different elements that have the same mass to charge ratio, and may be avoided by either selecting for the analysis, where possible, an isotope of the element without isobaric interference, or by chemically removing the interfering element. Fortunately, most of the elements containing isobars are in different groups of the Periodic Table and separations are not difficult. Thus, a concealed systematic error should not arise from this source. [For example, although ^{40}Ca and ^{40}K are isobaric, Ca can be separated easily from K by cation exchange chromatography. To ensure that the amount of ^{40}K is insignificant when measuring ^{40}Ca, the mass spectromist can monitor for ^{39}K which is four orders of magnitude more abundant than ^{40}K in natural potassium.] In the present case, where lithium concentrations are to be determined, there are no isobaric interferences with the two lithium isotopes, ^{6}Li and ^{7}Li.

(5) Instrumental errors. Instrumental errors may be caused by mass discrimination or fractionation, but usually cancel since the same percent error is present in the ratio measurement for the spike calibration. With some analytes, impurities in a sample can cause a different fractionation pattern from the pure material. These effects are usually

small (less than 0.1%) and can be corrected by repurifying the sample.

This review of possible sources of systematic error shows that for IDMS these errors can be eliminated or measured accurately for correction. Thus the absolute accuracy for the analyte concentration is determined by the random error (imprecision) components in the measurements. The imprecision components are present in the isotope ratio measurements for the analyte, the spike calibration, and the blank correction. If the blank correction is insignificant, as is the case for lithium, the total error in a careful determination reduces to the combined imprecisions for the spike calibration and the analyte determination. The imprecision of the analyte determination for Li has been determined to be on the order of 0.30 percent (relative standard deviation of a single measurement). For an average of five replicates (as done in these measurements) the imprecision of the mean, due to analytical replication error, was $0.3/\sqrt{5}$ or 0.13 percent. The standard deviation for a single spike calibration was 0.12 percent and for four replicate calibrations was thus 0.06 percent. Quadrature addition of these two random error sources results in a combined standard deviation of 0.14 percent. The ± 2-sigma interval for the random error of the mean is approximately 0.3 percent. To this is added an estimated upper bound for possible systematic errors of 0.2 percent to give the estimated maximum total error of 0.5 percent used in Table 1. When the blank correction is significant, the uncertainty from this source must be added to the uncertainties from the ratio measurements.

Reagents, Columns, and Clean Laboratory

(1) Reagents: All acids and water were purified by a sub-boiling distillation technique utilizing quartz stills [1].

(2) Cation Exchange Column: A 0.7-cm ID ion exchange column filled to an approximately 10-cm height with 100-200 mesh, strongly-acidic, cation-exchange resin (Dowex 50x8AG) having eight percent crosslinkage was used for the separations. The column of resin was cleaned by eluting with 60 g of 5 mol/L HCl, followed by 10 g of H_2O.

(3) Clean Laboratory: To reduce particulate contamination, all the chemical preparations were carried out in a Class-100, clean-air hood located in a vertical flow clean room [2].

Procedure

The frozen serum samples were allowed to come to room temperature and mixed by repeated (~20) careful inversions of the vials. A sample was quickly withdrawn from each vial through a platinum needle (18 gauge) into a 10-mL plastic syringe after the septum was opened just enough to allow the needle to enter the vial. Approximately 5 g samples, weighed to 0.01 mg, were transferred to 50- or 100-mL Teflon beakers. Weighed aliquots of ^6Li separated isotope solution sufficient to give a $^6Li/^7Li$ ratio of approximately 1-1.5, were added to each sample. The samples then were decomposed by adding 5 g of HNO_3 (15.6 mol/L) and 5 g of $HClO_4$ (11.7 mol/L) and heating in the covered beakers. After decomposition, the covers were removed and the samples were evaporated to dryness. The acid on the sides of the beakers was rinsed down with a minimum amount of H_2O and the samples were again evaporated to dryness. Each residue was dissolved in 10 mL of H_2O and

transferred to a cleaned cation exchange column. Approximately 10 mL of H_2O was used to rinse the beaker and complete the transfer of the sample to the column. Then 0.2 mol/L HCl was added as an eluting agent. The first 12 mL of eluent was discarded and the next 12 mL[1] of eluent, containing the Li fraction, was collected in a Teflon beaker. The Li fractions were evaporated to dryness. To aid in the decomposition of organic material that elutes from the column, a few drops of HNO_3 (15.6 mol/L) were added and the sample was heated and evaporated to dryness. The Li residues were converted to the chloride form by adding a few drops of 5 mol/L HCl and evaporating to dryness. The residues were dissolved in enough 0.005 mol/L HCl to give a solution containing approximately 10 µg Li/mL.

Mass Spectrometry

Isotopic ratios were determined by solid sample, thermal ionization mass spectrometry on 15-cm-radius of curvature, 60°-analyzer tube, mass spectrometers equipped with thin-lens "Z"-focusing ion-sources and multielement, deep-bucket, faraday-cage collectors. The mass spectrometric technique for lithium is similar to a tantalum triple-filament procedure developed for potassium analysis [3]. The sample size per analysis was reduced to approximately 50 ng.

[1]The volumes may vary depending on the particular lot of resin and on the sample loading; most of the Li should have been eluted before Na starts; the start of Na elution can be checked by a flame test.

Reagents, Columns, and Clean Laboratory

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[1]The volumes may vary depending on the particular lot of resin and on the sample loading; most of the Li should have been eluted before Na starts; the start of Na elution can be checked by a flame test.

Results

The lithium concentrations determined by isotope dilution mass spectrometry on seven lots of serum are given in Table 1. The blank correction based on three blank determinations was less than 0.1 ng or less than 0.001 percent of the amount of lithium present in any sample. Sources of error in the lithium concentration determination are calibration of the ^{6}Li spike, the ratio measurement of the spiked sample, and the effects of the impurities [4]. The ^{6}Li spike solution was calibrated with two natural solutions prepared from SRM 924, Lithium Carbonate. The 95 percent confidence limits for a single analysis of the spike calibration is 0.3 percent. While this is a relatively large uncertainty in the calibration of a spike solution, it is consistent with the precision of the $^{6}Li/^{7}Li$ ratio of the standard. The chief source of imprecision is the inability to adequately control the isotopic fractionation between analyses. The estimated maximum error for the measured lithium concentrations in Table 1 is ±0.5 percent which includes allowances for measurement imprecision and systematic errors.

Table 1. Lithium concentrations in CDC bovine serum.

Pool	Sample No.	µg Li/g	mmol/L
1	1	3.592	
	2	3.628	
	3	3.608	
	4	3.615	
		$\bar{x}$ = 3.611	0.534 ± 0.003[a]
1a	1	6.817	
	2	6.783	
	3	6.810	
	4	6.809	
	5	6.804	
	6	6.788	
		$\bar{x}$ = 6.802	1.004 ± 0.005[a]
2	1	6.994	
	2	6.987	
	3	6.963	
	4	6.960	
		$\bar{x}$ = 6.976	1.031 ± 0.005[a]
3	1	8.720	
	2	8.741	
	3	8.710	
	4	8.732	
		$\bar{x}$ = 8.726	1.290 ± 0.006[a]

continued

Continuation of Table 1.

Pool	Sample No.	μg Li/g	mmol/L
4	1a	10.419	
	1b	10.440	
	2a	10.493	
	2b	10.453	
	3a	10.433	
	3b	10.445	
	4a	10.494	
	4b	10.474	
		$\bar{x}$ = 10.456	1.546 ± 0.008[a]
5	1	12.249	
	2	12.186	
	3	12.219	
	4	12.326	
		$\bar{x}$ = 12.245	1.809 ± 0.009[a]
5a	1	13.331	
	2	13.364	
	3	13.366	
	4	13.357	
	5	13.363	
	6	13.378	
		$\bar{x}$ = 13.360	1.969 ± 0.010[a]
6	1	13.764	
	2	13.765	
	3	13.888	
	4	13.823	
		$\bar{x}$ = 13.812	2.042 ± 0.010[a]

continued

Continuation of Table 1.

Pool	Sample No.		µg Li/g	mmol/L
7	1		17.287	
	2		17.426	
	3		17.425	
	4		17.465	
		$\bar{x}$ =	17.401	2.572 ± 0.013[a]
7a	1		20.058	
	2		20.005	
	3		20.072	
	4		20.002	
	5		20.020	
	6		20.020	
		$\bar{x}$ =	20.030	2.954 ± 0.015[a]

[a]Estimated maximum error of 0.5 percent of the value. This estimated error is the sum of errors due to measurement imprecisions of ±0.3 percent (± 2-sigma interval for the random error of the mean) and an estimated upper bound of 0.2 percent for possible systematic errors.

The isotopic composition of lithium was determined for each lot of serum and found to be experimentally identical to the reference standard, 7.562 atom percent ^{6}Li and 92.438 atom percent ^{7}Li. A significant amount of the high purity lithium that is commercially available is depleted in ^{6}Li; e.g., as much as 50 percent depletion has been found on a limited number of samples. Thus, the only methods for obtaining reliable knowledge of the isotopic composition in samples of this element are by isotopic analysis or use of a standard of known composition.

References

[1] Kuehner, E. C., Alvarez, R., Paulsen, P. J., and Murphy, T. J., Anal. Chem., 44, 2050 (1972).

[2] Murphy, T. J., The Role of the Analytical Blank in Accurate Trace Analysis, Nat. Bur. Stand. (U.S.) Special Publication 422, Accuracy in Trace Analysis: Sampling, Sample Handling, and Analysis, p. 509-539 (1976).

[3] Garner, E. L., Murphy, T. J., Gramlich, J. W., Paulsen, P. J., and Barnes, I. L., J. Res. Nat. Bur. Stand. (U.S.) 79A (Phys. and Chem.) 713-725 (Nov.-Dec. 1975).

[4] Garner, E. L., Machlan, L. A., Gramlich, J. W. Moore, L. J., Murphy, T. J., and Barnes, I. L., An Accurate Determination of Electrolyte Concentrations in Blood Serum by Isotope Dilution Mass Spectrometry, Nat. Bur. Stand. Special Publication 422, 951-960 (1976).

APPENDIX D

Note 1:

A temperature range of room ±2 °C is designated as the operating temperature. In this temperature range the maximum difference in aqueous solution volumes due to thermal expansion of the liquid is 0.102 percent and the difference in volume due to the volumetric glassware is very small since the coefficient of expansion for borosilicate glass is 0.00001 per °C. (J. Lembeck, "Calibration of Small Volumetric Laboratory Glassware", NBSIR Report 74-461, 1974, Institute for Basic Standards, National Bureau of Standards, Washington, D.C. 20234). We judge these errors to be acceptable for this reference method. Larger temperature variations may necessitate appropriate correction.

Note 2:

Glassware Required:

a) Manual pipetting alternative:

Volumetric Flasks: three 2-L; two 1-L; seven 100- plus one additional 100-mL volumetric flask for each sample.

Pipets: two 5-mL, and one each of 4-, 10-, 15-, 20-, 25-, and 30-mL.

b) Semiautomated pipetting alternative:

Volumetric Flasks: three 2-L; two 1-L; and seven 50-mL plus one 50-mL volumetric flask for each sample.

Pipets: two 5-mL; and one each of 10-, 15-, 20-, 25-, and 30-mL.

Note 3:

Cleaning of Glassware and the pipettor-dilutor:

a) Clean the glassware in the following manner:

   (1) Soak glassware for 60 min in 0.77 mol/L HNO_3.
   (2) Rinse six times with a volume of water equal to at least 10 percent of the container volume.
   (3) Use immediately or air dry (inverted in a dust-free environment) for later use.

b) Clean the pipettor-dilutor device as follows:

   (1) Rinse the tubing with water by delivering at least four 5-mL water samples.
   (2) Rinse the tubing with 0.77 mol/L HNO_3 by drawing into the delivery tube a volume of HNO_3 equal to the volume of sample pipetted and then delivering four 5-mL portions of HNO_3 through the system.
   (3) Repeat step (2) using H_2O, ethanol, and H_2O sequentially.
   (4) Repeat step (2) with the diluent to be used for preparing the working solutions of the sample, standards, and blank. The pipettor-dilutor is then ready for the preparation of the working solutions.

Note 4:

Procedure for Testing Pipettor-Dilutor Devices: The accuracy and precision of the device is determined by weighing fixed volumes of water repetitively delivered by the device.

1. The water that is delivered in tared, stoppered flasks is to be weighed on an analytical balance capable of being read to the nearest one-tenth milligram. Measure the temperature of the delivered water to the nearest 0.1 °C just before or after delivery.

2. Test the delivery of the 2.00 mL volume (as will be used) as follows:

   a. (1) Number and tare ten, clean, dry, stoppered, glass or plastic weighing bottles of approximately 10-20 mL volume.
   (2) Sample 2.00 mL of water and deliver it together with 5 mL of diluent water into the first bottle. Stopper immediately.
   (3) Repeat step '2' with the remaining 9 bottles.
   (4) Weigh each of the 10, filled bottles.
   (5) Calculate the weight of each aliquot plus diluent.

   b. Repeat steps 1-5 of part a, but in step 2 omit the sampling of the 2.00 mL of water by allowing air to be sampled rather than water; thus only the 5.00 mL of diluent water is collected in the tared bottles. Calculation then gives the weights of diluent.

   c. Calculate from part b the mean weight for the diluent.

   d. Calculate the differences between the individual weighings obtained in part a step (5) and the mean weight of the diluent (from part c) to obtain the weights of the water aliquots delivered at the 2.00-mL setting that was used.

   e. Calculate the mean and standard deviation for the weights of water samples (from part d).

   f. Use the attached table (#43) from Circular #19, "Standard Density and Volume Tables," [National Bureau of Standards, Washington, D.C. 20234] to convert the mean of the diluent weights (from part c) and the mean of the sample weights (from part e) into volumes at 20 °C, in the following manner:

Table 43. — Indicated capacity 100 mL.

Temperature in degrees C.	Tenths of degrees									
	0	1	2	3	4	5	6	7	8	9
15	0.207	0.208	0.210	0.211	0.212	0.213	0.215	0.216	0.217	0.219
16	.220	.221	.223	.224	.225	.227	.228	.230	.231	.232
17	.234	.235	.237	.238	.240	.241	.243	.244	.246	.247
18	.249	.250	.252	.253	.255	.257	.258	.260	.261	.263
19	.265	.266	.268	.270	.272	.273	.275	.277	.278	.280
20	.282	.284	.285	.287	.289	.291	.293	.294	.296	.298
21	.300	.302	.304	.306	.308	.310	.312	.314	.315	.317
22	.319	.321	.323	.325	.327	.329	.331	.333	.336	.338
23	.340	.342	.344	.346	.348	.350	.352	.354	.357	.359
24	.361	.363	.365	.368	.370	.372	.374	.376	.379	.381
25	.383	.386	.388	.390	.392	.395	.397	.399	.402	.404
26	.406	.409	.411	.414	.416	.418	.421	.423	.426	.428
27	.431	.433	.436	.438	.440	.443	.446	.448	.451	.453
28	.456	.458	.461	.463	.466	.469	.471	.474	.476	.479
29	.482	.484	.487	------	------	------	------	------	------	------

(1) Determine the volume of the nominally 2.000-mL sample at 20 °C by adding to the mean value of the delivered sample, (from part e) an amount equal to the product of 0.020 and the value for the appropriate water temperature read from Table 43. The sums obtained are in milliliters.

3. The requirements for the bias and imprecision of the pipettor-dilutor are listed in Table 1. The pipettor-dilutor may be used in the semiautomated pipetting alternative if these requirements are fulfilled.

Table 1. Bias and imprecision requirements for the volume of sample delivered by the pipettor-dilutor device, Section IIIC-2b.

Sample Size, mL	Bias, mL	Imprecision, Relative Standard Deviation
2.00	0.04	0.2%

Note 5:

a) The Li_2CO_3 in NBS SRM 924 has been depleted in the ^6Li isotope. Thus the atomic weight of lithium in this SRM is 6.9696 rather than the usual 6.941, and the molecular weight of this Li_2CO_3 is 73.9484 rather than 73.8912.

b) The atomic weights used in this report are those reported in: Pure and Applied Chemistry, 47, 75 (1976).

Note 6:

In some cases, two pipets will be needed to transfer the desired volume; e.g., a 30-mL aliquot may be transferred by using a combination of a 20-mL pipet and a 10-mL pipet. The statistical limit of error for this dual pipetting is 0.032 mL or 0.107 percent of the total volume transferred

and is not very different from the limit of error in using one pipet, 0.03 mL or 0.10 percent for a 30-mL pipet. No estimate of operator error is included in this calculation.

Note 7:

If the wash solution does not drain cleanly from the pipet, wash with 0.77 mol/L HNO_3, H_2O, MeOH, 70:30 v/v $CHCl_3$:MeOH, MeOH, and H_2O in that order. Then repeat the water wash and check that the pipet does drain properly.

Note 8:

The three following pages are examples of the data sheets returned from each laboratory after each inter-laboratory exercise.

ELECTROLYTES IN SERUM — CLINICAL REFERENCE METHOD

ION Li

LABORATORY 10 ANALYST XY

EXERCISE NO. IE-II

DATE SAMPLES RECEIVED 12/9/75 DATES ANALYZED (1) 12/16/75 (2) 12/18/75

INSTRUMENT MANUFACTURER Perkin-Elmer MODEL 403

WAVELENGTH 670.8 NM SLIT WIDTH 1.4 nm Bandpass µM

TYPE HOLLOW CATHODE LAMP Westinghouse

CURRENT 12 MA

BURNER TYPE Single Slot

OXIDANT Air FLOW RATE 27.5 L/MIN

FUEL Acetylene FLOW RATE 5.5 L/MIN

INSTRUMENT TIME CONSTANT NA S SCALE EXPANSION

RECORDER TIME CONSTANT NA S

READOUT: RECORDER , DIGITAL X , OTHER

LABORATORY TEMPERATURE 23 °C TO 26 °C (VARIATION DURING IE)

BACKGROUND CORRECTION? No HOW?

COMMENTS: Use plastic aspiration tube supplied. Aspiration rate = 8.0 mL/min.

DATA SHEET: STANDARD CURVE

PROTOCOL USED: MANUAL _____ SEMIAUTOMATED X

STANDARD	CALCULATED ION CONCENTRATION, MMOL/L	EXPANDED ABSORBANCE VALUES	CORRECTED EXPANDED ABSORBANCE VALUES
1.	0.5000	0.293	0.293
2.	1.0000	0.572	0.572
3.	1.5000	0.842	0.842
4.	2.0000	1.104	1.104
5.	2.5000	1.400	1.400
6.	3.0001	1.665	1.665
DILUENT BLANK	0.000		

DATA REPORTING SHEET FOR VALID MEASUREMENTS

PROTOCOL USED: MANUAL X SEMIAUTOMATED _____

LAB 10 ION Li IE III DATE ANALYZED 5/12/75 OPERATOR XY

SAMPLE # 802876

STANDARD CONCENTRATIONS MMOL/L (CALCULATED)	VALID SET	EXPANDED ABSORBANCES LO STD (X_1)	SAMPLE (Y)	HI STD (X_2)	$\hat{C}$
LO 0.5000 (C_1)	1.	0.277	0.200	0.557	0.5232
HI 1.0000 (C_2)	2.	0.281	0.292	0.556	0.5200
	3.	0.279	0.296	0.564	0.5298
	4.	0.277	0.297	0.559	0.5354
	5.	0.278	0.295	0.550	0.5312

SAMPLE # 76331

STANDARD CONCENTRATIONS MMOL/L (CALCULATED)	VALID SET	EXPANDED ABSORBANCES LO STD (X_1)	SAMPLE (Y)	HI STD (X_2)	$\hat{C}$
LO 1.5000 (C_1)	1.	0.870	1.020	1.159	1.7595
HI 2.0000 (C_2)	2.	0.876	1.022	1.154	1.7692
	3.	0.874	1.018	1.132	1.7790
	4.	0.859	1.005	1.126	1.7734
	5.	0.854	1.003	1.129	1.7709

SAMPLE # 35

STANDARD CONCENTRATIONS MMOL/L (CALCULATED)	VALID SET	EXPANDED ABSORBANCES LO STD (X_1)	SAMPLE (Y)	HI STD (X_2)	$\hat{C}$
LO 2.5000 (C_1)	1.	1.412	1.654	1.700	2.9202
HI 3.0001 (C_2)	2.	1.406	1.643	1.689	2.9506
	3.	1.399	1.654	1.671	2.9688
	4.	1.400	1.662	1.674	2.9781
	5.	1.397	1.658	1.672	2.9746