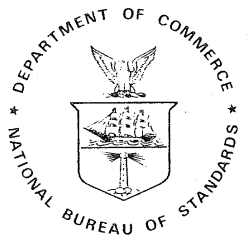

NBS SPECIAL PUBLICATION 260-63

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

Standard Reference Materials:

A REFERENCE METHOD FOR THE DETERMINATION OF POTASSIUM IN SERUM

Standard Reference Materials:

A REFERENCE METHOD FOR THE DETERMINATION OF POTASSIUM IN SERUM

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PREFACE

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

George A. Uriano, Acting 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 potassium 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 and sodium 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.

Philip D. LaFleur, Director
Center for Analytical Chemistry

NOTE

Because of concern for the usability of the NBS sodium and potassium methods, CDC personnel have proposed a procedure for the concurrent determination of potassium and sodium by FAES that differs from the separate potassium and sodium reference methods, described here and, for sodium, in NBS Special Publication 260-60. The CDC method includes semi-automated sample dilution, a different sample analysis format, and different standards preparation. CDC management believes its method will serve better as a "combined" reference method, and as a consequence, has declined to endorse the method described in this report and is proposing the CDC method as the reference method. While NBS supports the evolution of analytical methods and has agreed to participate in inter-laboratory exercises that are aimed toward establishing the transferability of the proposed CDC procedures, NBS management believes it important that the principles of analytical practice delineated in this present report be circulated in a timely manner. Since the method outlined in this report has been shown to satisfy the generally accepted criteria of a reference method, it should function as such until the efficacy of a subsequent method has been demonstrated. In addition, NBS will maintain its primary role in supplying SRM's and definitive methods.

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ABSTRACT

Guided by a committee of experts in clinical chemistry, a reference method was established for the determination of serum potassium based on flame atomic emission spectroscopy (FAES). Its accuracy was evaluated by comparing the values obtained by use of the method in 12 laboratories against the results obtained by a definitive analytical method based on isotope dilution-mass spectrometry (IDMS). Seven serum pools with potassium concentrations in the range 1.319 to 7.326 mmol/L were analyzed. Manual and semiautomated pipetting alternatives were tested using sample sizes of 5.0 and 0.25 mL, respectively.

The laboratories used several different FAES instruments. The results showed that the standard error for a single laboratory's performance of the procedure ranged from 0.049 to 0.063 mmol/L with a maximum bias of 0.065 mmol/L over the range of concentrations studied. These values were within the accuracy and precision goals that had been set by the committee. The results from the two pipetting techniques were similar. The calibration curve data showed excellent linearity over the total concentration range, with 20 of 22 curves having standard deviations of fit of 0.075 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 potassium values for pooled sera.

Key Words: Clinical analysis; clinical chemistry; definitive method; electrolytes; flame atomic emission spectroscopy; reference method; semiautomated pipetting; serum potassium analysis.

I. INTRODUCTION

Serum potassium can be determined by a wide variety of analytical methods; these include (1) separation by precipitation with measurement by photometry, gravimetry, or titrimetry, (2) separation by ion-exchange with measurement by photometry, and (3) direct analysis by use of ion-selective electrodes, gasometry, photometry, neutron activation, or flame atomic emission spectroscopy (FAES)[5] [1][6]. The use of flame atomic emission spectroscopy has been described as a standard method [2]. Whether the latter method or some other should be considered by clinical laboratories as the clinical reference method for serum potassium 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 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 accuracy of a clinical chemistry

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

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

method for total calcium and sodium in serum [3,4]. For calcium, the analytical procedure was based on the flame atomic absorption spectrometric (FAAS) method of Pybus, Feldman, and Bowers [5], while for sodium it was based on FAES. The accuracies of these methods were assessed by comparing the results obtained using them on several human serum pools for calcium and bovine serum pools for sodium in selected clinical laboratories against those obtained for the same pools by an isotope dilution-mass spectrometry (IDMS) method for calcium and an ion-exchange gravimetry method for sodium. These analyses were performed at the National Bureau of Standards (NBS) where the high accuracy of those methods[7] were established by the second approach of determining their systematic and random errors [4,6].

These studies, carried out with the guidance of clinical laboratory experts, used (a) Standard Reference Materials as the pure primary reference material to prepare standard solutions of calcium and sodium for all the analyses; (b) serum pools prepared at the Hartford Hospital (Hartford) and the Center for Disease Control (CDC, Atlanta); (c) definitive method analysis for calcium or sodium on these pools 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 the clinical reference method for total calcium [7] or sodium [8] in serum.

This same approach was adopted to develop clinical reference methods for a number of other serum electrolytes including rodium, chloride, lithium, and magnesium. This work was begun with the cooperation of individuals from the Standards Committees of the American Association for Clinical

[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.

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. We present in this report the development of a clinical reference method for serum potassium.

II. DEVELOPMENT OF THE SERUM POTASSIUM 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 development of these reference methods was organized by Dr. Robert Schaffer (NBS) aided by 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).

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

FAES 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.

---

[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.

On consideration of these quite similar analytical results and of alternative clinical laboratory procedures in use for the determination of serum potassium, it was concluded that FAES 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 potassium.

	- - - K in Serum, mmol/L - - -		
Pool	IDMS[a]	FAES	
		NBS[b]	CDC[c]
I	2.794	2.73	2.8
III	4.765	4.76	4.76
V	6.867	6.72	6.8

[a] Data from L. J. Moore (NBS).
[b] Data from R. Mavrodineanu (NBS).
[c] Data from J. White (CDC).

The experts agreed to serve as the Committee to oversee the development of the reference method for potassium (as well as for the other electrolytes discussed at the meeting). The Committee chose Dr. Bowers as chairman. Dr. White agreed to serve as the Committee's representative to work with those at NBS who would be involved in writing the protocol for the potassium reference method. The Committee agreed that the FAES method should use a concentration bracketing technique rather than calibration curves for determining potassium concentrations. However, calibration curve data would be obtained as a general check on the measurement system and to determine which of the primary standard solutions would be used to bracket the potassium levels in the samples being analyzed.

As goals for the candidate reference method, the maximum bias of the method and its one-standard deviation imprecision limit were set by the Committee at 0.2 and 0.1 mmol/L, respectively, for serum potassium at the 2.5 and 6.5 mmol/L levels. 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 FAES method according to the written protocol while NBS would provide potassium values 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 suppliers of test and control materials. One hospital was located outside the United States. The principal investigator at each laboratory is named in the list below. Other scientists in each of the laboratories who contributed to this study are acknowledged by name in Appendix A. The list includes three 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, Potassium Chloride (SRM 918, see Appendix B) was to be used as the pure primary reference material for all analyses [9]. Seven pools of homogeneous, sterile, bovine serum, having different concentrations of potassium, were prepared at the CDC by Dr. David Bayse and Miss Sue Lewis. Samples of each pool were supplied in approximately 7-mL volumes in stoppered 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 [10]. The pools were numbered in code from 1 to 7 according to increasing potassium concentration.

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

Table 2. Potassium concentrations for the seven serum pools as determined by IDMS, the definitive method.

Pool	$[K^+]$, mmol/L
1	1.319 ± 0.003[a]
2	2.540 ± 0.006
3	3.448 ± 0.009
4	4.323 ± 0.011
5	5.501 ± 0.014
6	6.092 ± 0.015
7	7.326 ± 0.018

[a] Estimated at ±0.25 percent for all concentrations at the 95 percent confidence limit.

C. Functions of the Various Groups

The interrelationships and functions of the various groups involved in developing FAES as a reference method for serum potassium 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.

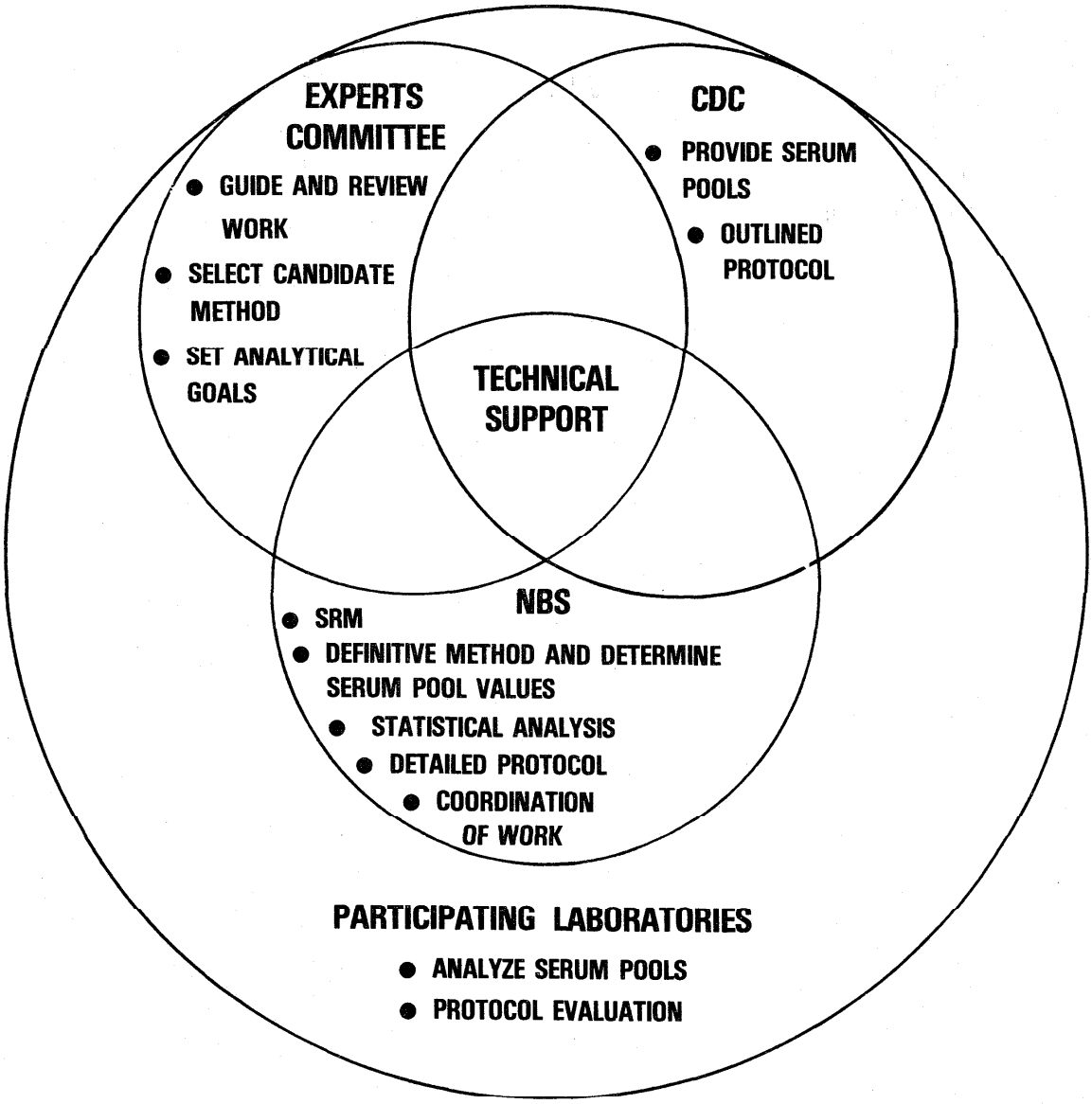

Figure 1. Interrelationships and functions of the various groups in the development of a clinical reference method for the determination of serum potassium.

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 round robin tests was established within the constraints imposed by protocol requirements and sample availability by Drs. John Mandel, Robert Paule, and R. A. Velapoldi. Dr. Velapoldi wrote the protocol for the candidate reference method from the outline provided by Dr. J. White. Drs. Mandel and Paule performed the statistical evaluation of the results from the interlaboratory tests. The definitive method was performed by Mr. Larry Machlan and Dr. John W. Gramlich.

D. Plan for Testing the Candidate Reference Method

The general plan was to evaluate the candidate reference method by performing a series of interlaboratory test exercises, which would consist of a preliminary round robin test (PRR) followed by successive round robin tests (RR) until the goals for the reference method were reached. A main objective of the PRR test was to allow participating laboratories to become familiar with and comment on the protocol. Since an evaluation of the bias was not sought in the PRR testing phase, normal bovine serum samples [11] not having definitive method analyses were to be used. However, interlaboratory imprecision was to be measured. If the imprecision of the results in the PRR was found to be small, round robin (RR) testing would begin on samples having definitive method potassium values.

In a RR, each participating laboratory would perform the same analyses on two separate days: i.e., analyze a pair of aliquots 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 RR tests using samples

from other pools would be conducted by following the protocol or a modified form of it until the bias and imprecision goals were reached. Revisions and modifications to the protocol could be made after a round robin test had been completed but would not be made after the final RR.

Three kinds of information were to be supplied by each participating laboratory after finishing a round robin:

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 FAES relative intensity values versus the potassium concentrations of the standards; 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 POTASSIUM

A. General

This protocol for the analysis of serum potassium by flame atomic emission spectroscopy provides for the optional use of either manual or semiautomated pipetting and also for one hundred-fold or two hundred-fold dilutions of samples to prepare working solutions. The pipetting alternatives are discussed separately in detail whereas the dilution alternatives are not discussed since they are prescribed by the instrument used.

B. Protocol Synopsis

The protocol must be followed exactly.

The reference method is used to analyze four aliquots of a serum: two on one day and the other two on a subsequent day.

1. Use an analytical balance to weigh the SRM KCl in appropriate quantities and to prepare a stock standard potassium solution;
2. Use either a single pipet or a pipettor-dilutor to dilute to the potassium concentrations that are used as working solutions for FAES a) aliquots of the serum to be analyzed, b) aliquots of the stock standard potassium solutions, and c) the solution used as a blank;
3. Obtain calibration curve data for the working blank and standards;
4. Measure the emission signals of the working solutions of the serum; select the pair of working standards whose emission signals most closely bracket the signal of each aliquot;
5. For each aliquot to be analyzed, obtain five valid measurement sets by measuring the emission signals obtained from repeated sequential measurements of the working solutions of the low bracketing standard, the sample, and the high bracketing standard;
6. Calculate the potassium concentration of the aliquot for each set in the valid measurement set by mathematical interpolation;
7. Average the five calculated values to obtain a 'single measurement' for that aliquot; (in the statistical analysis, each such average is designated a 'single measurement');
8. Perform steps (4) through (7) for each aliquot 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 aliquot;

10. Average the four values obtained by the replicate determinations to obtain the potassium concentration for each serum.

C. Detailed Protocol

The selection of the specific alternatives of the protocol to be used (i.e., the pipetting and the dilution) 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 0.01 MΩ·m at 23 ± 5 °C. At the time of use, this water should show a flame emission signal that is less than 0.1 percent of full scale at the instrumental settings used for the analysis. 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. Potassium Standard Solutions: Use Standard Reference Material, Potassium Chloride (originally issued as SRM 918, Certificate reproduced in Appendix B) [9] certified by the National Bureau of Standards. Dry

the SRM KCl at 110 °C for four hours in a loosely capped container and then store it in a desiccator containing $CaSO_4$ or an equivalent desiccant.

c. Lithium carbonate. sodium chloride, hydrochloric acid, nitric acid, chloroform, methanol and 95-percent ethanol meeting ACS [12] (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.

2. Glassware Specifications

a. All volumetric glassware (Appendix D, Note 2) should be of borosilicate material and meet NBS Class A [13] (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

If the instrument employed in the analyses does not use lithium as an internal standard, water is substituted for the aqueous lithium chloride diluent solution in this protocol.

a. Lithium Chloride Diluent Solution (LiCl Diluent, 15 mmol/L): The homogeneity of this solution is critical if an internal standard instrument is to

be used. The required volume may be prepared in eleven 2-liter batches and then mixed thoroughly.

For each 2-liter volume, weigh 1.1082 g of dried Li_2CO_3 (mw = 73.8912, Appendix D, Note 5b); however, if NBS SRM 924 is used, weigh 1.1092 g (see Appendix D, Note 5). Transfer the weighed Li_2CO_3 quantitatively into a 2-liter volumetric flask. Add water to just cover the bottom of the flask and, with swirling, carefully add 4 mL of concentrated HCl to dissolve the Li_2CO_3. Dilute to the calibration mark with water, stopper, and mix thoroughly by inverting the flask and shaking ten times. Repeat the inverting and shaking steps nine more times.

1) Manual pipetting alternative: Since all standards and samples are to be diluted with this reagent, approximately 22 liters will be needed on day 1 and 18 liters will be needed on day 2.

2) Semiautomated pipetting alternative: Prepare approximately six liters of the LiCl diluent.

b. Sodium Chloride Diluent Solution (NaCl Diluent 140 mmol/L): Weigh 8.182 g of NaCl (mw = 58.4428 Appendix D, Note 5b) and transfer it quantitatively to a one-liter volumetric flask. Dilute to the calibration mark with water, stopper, invert, and mix as described above. Four liters will be needed on day 1 and 2 liters will be needed on day 2.

c. Potassium Standard Stock Solutions: Weigh accurately (to 0.1 mg) approximately 5.96 g of dried potassium chloride (mw = 74.5513, Appendix D, Note 5b) and transfer it quantitatively into a 1-liter volumetric flask. Dissolve and dilute to the mark with the 140 mmol/L sodium chloride diluent solution. Mix thoroughly as described above. Repeat these steps

to prepare a second potassium standard stock solution. Label the solutions I and II. This weight will give a solution of approximately 80 mmol of potassium per liter. From the weighed quantities of KCl taken, calculate the exact potassium concentrations in mmol/L to three decimal places.

(1) Intercomparison of Standard Stock Solutions: Transfer by pipet, 25.00-mL of stock solution I into a one-liter volumetric flask. Dilute to the calibrated volume with LiCl diluent, stopper, invert, shake, and mix as described above. Transfer by pipet, 25-mL of the diluted solution to a one-liter volumetric flask. Dilute to the calibrated volume with LiCl diluent, stopper, invert, shake, and mix to give a working solution with a potassium concentration of 0.05 mmol/L. Repeat these dilution steps for stock solution II. [NOTE: Care must be exercised in the mixing step so that the analysts hands do not touch the rim of the flask since the solution, when poured out, will become contaminated with potassium.]

Immediately aspirate each of the 0.05 mmol/L potassium solutions and measure their relative intensity values under the instrumental settings used for this analysis. If the relative intensity values corrected for concentration differences for both solutions agree to within 0.5 percent, potassium stock standard I may be used for the analyses on day 1 and stock standard II may be used for the analyses on day 2 subject to temperature restrictions. If the relative intensity 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.

d. Diluted Potassium Standard Solution: Prepare the various diluted potassium standard solutions by transferring the appropriate volumes of the potassium stock standard solution listed in Table 3 to 250-mL volumetric flasks and dilute to the calibrated volume with the 140 mmol/L NaCl diluent. Mix thoroughly. [NOTE: These dilutions are made using volumetric pipets in the "to deliver" mode, rather than the "to contain" mode discussed in 4b-(3) below.]

Table 3. Volumes of potassium standard stock solution diluted to 250 mL that give the potassium diluted standard solutions.

Stock Solution to be Transferred, mL	Concentration of Diluted Standards KCl, mmol/L
3.00	0.96
5.00	1.60
10.00	3.20
15.00	4.80
20.00	6.40
25.00	8.00

4. Dilution and Pipetting Procedures

a. General: A one hundred-fold or two-hundred fold dilution is to be used as required by the instrument employed.

b. Manual Pipetting Alternative: The blank, the standard, and the sample solutions are diluted either one hundred-fold or two hundred-fold by employing only one 5-mL pipet with a wash-out technique and either 500-mL or 1-liter volumetric flasks. (The

working solutions are prepared with the one pipet and wash-out technique to eliminate errors that may be caused by differences in drainage between aqueous and serum solutions.) Two blanks are necessary with instruments using lithium as an internal standard: 1) the LiCl diluent (IIIC-3a) used as the blank for samples and standards, and 2) the NaCl diluent (IIIC-3b) diluted with the LiCl diluent used as a blank for the potassium standards (see Appendix D, Note 6).

(1) One Hundred-fold Dilutions: Transfer approximately 400 mL of LiCl diluent into a 500-mL volumetric flask and then add 5 mL of the sample or stock standard solution by the procedure described in step (3) below.

(2) Two Hundred-fold Dilutions: Transfer approximately 900 mL of LiCl diluent into a 1-liter volumetric flask and then add 5 mL of the sample or stock standard solution by the procedure described in step (3) below.

(3) Pipetting Procedure: Fill the 5-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 LiCl 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 5-mL volumetric pipet three times by filling with fresh LiCl 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 LiCl diluent and mix thoroughly.

(4) Preparation of Working Solutions:

(a) Working Blank Solution and Working Standard Solutions: Prepare the working solutions of the blank solution and the working 0.96-, 1.60-, 3.20-, 4.80-, 6.40-, and 8.00-mmol/L potassium standard solutions by making dilutions in appropriately labeled volumetric flasks in the order cited. Condition the 5-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 LiCl diluent, each time delivering the rinse solution into the volumetric flask. Fill the flask to the calibrated volume with the LiCl 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 5-mL pipet with some of the sample to be diluted in the following way: (1) Draw ~2 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-4b-(1) or (2) and (3), i.e., fill the 5-mL pipet with the sample, adjust volume to the mark, deliver, rinse three times into the volumetric flask with LiCl 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 (4)-(b).

c. Semiautomated Pipetting Alternative: To prepare working solutions, the blank, standard and sample solutions are diluted either one hundred-fold or two hundred-fold by using a pipettor-dilutor device to deliver either 0.250 or 0.500 mL into appropriately labeled 50-mL volumetric flasks. A single delivery tube on the pipettor-dilutor and the wash-out technique are used throughout. Two blanks are prepared for instruments using lithium as an internal standard: i.e., the LiCl diluent (Section IIIC-3a) is used as the blank for samples and standards and the NaCl diluent (IIIC-3b), diluted with the LiCl diluent, is used as a blank for the potassium standards (see Appendix D, Note 6).

(1) One Hundred-Fold Dilutions: Transfer approximately 20 mL of LiCl diluent (or water) into a 50-mL volumetric flask and then add 0.500 mL of

the appropriate solution by the procedure described in step (3) below.

(2) Two Hundred-Fold Dilutions: Transfer approximately 20 mL of LiCl diluent (or water) into a 50-mL volumetric flask and then add 0.250 mL of the appropriate solution by the procedure described in step (3) below.

(3) Procedure: The pipettor-dilutor is set to sample either 0.250 or 0.500 mL and to dilute with 5 mL of 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 (0.250 or 0.500 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 appropriate diluent, and mix.

(4) Solution Preparation:

(a) Prepare the working blank, standard, and sample solutions by the procedures described in Sections c(1), (2), and (3).

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

(1) For the Manual Pipetting Alternative:

(a) One (or two) working blank(s);
(b) Six working standards;
(c) A working solution for each serum sample to be analyzed.

(2) For the Semiautomated Pipetting Alternative:

(a) One (or two) working blank(s);
(b) Six working standards;
(c) A working solution for each serum sample to be analyzed.

5. Flame Atomic Emission 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 propane are used as oxidant and fuel, respectively. The instruments that are currently in use for FAES measurements may be classified into two groups: internal standard and non-internal standard instruments. Each group is considered briefly.

For the internal standard instruments, the concentration of the internal-standard LiCl must be kept uniform throughout the analysis since the potassium emission signal is measured relative to the lithium emission signal.

a. Internal Standard Instruments:

(1) Instrument Adjustment:

The most commonly used internal standard instruments employ filter 'monochromators', automatic gas-flow control systems and automatic ignition devices. Choose the correct series of filters for the analyses. After starting the instrument, turn on the air supply (adjust to manufacturer's recommended pressure), open the valve on the propane fuel tank, and allow the instrument to warm-up for at least 15 minutes while aspirating the LiCl diluent. Check the flame appearance and aspiration rate to assure that the nebulizer burner system is free of foreign materials.

(2) Instrument Stability:

Determine the stability and repeatability of the instrument as follows:

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

(b) Nebulize the working potassium standard solution obtained from the 8.000 mmol/L

standard solution and adjust direct read-out instruments so that a reading of 8.000 units is observed.

(c) Check the instrument zero with LiCl diluent and readjust as necessary.

(d) Repeat steps (2)(a)-(c) until stable conditions are achieved. Readings for the same solution should agree within 0.5 percent of full scale.

b. Non-Internal Standard Instruments:

(1) Instrumental Adjustments:

(a) After turning on the instrument and adjusting the wavelength to 766.5 nm, adjust the slit as recommended by the manufacturer.

(b) Open the propane and air supply valves and adjust the secondary regulators as recommended by the manufacturer.

(c) Ignite the gas and adjust the flow rates for the fuel and oxidant as recommended for the instrument. Check the flame appearance and nebulization rate to assure that the nebulizer burner system is free of foreign materials.

(d) Nebulize water into the flame for at least 10 min; then make a fine adjustment of wavelength by nebulizing one of the working standards and adjusting the wavelength selector until a maximum signal is obtained.

(2) Instrument Stability:

Determine the stability and repeatability of the instrument as follows:

(a) Adjust the instrument to zero while nebulizing water. [NOTE: Always nebulize water when measurements of working standard, blank, or sample solutions are not being made. Water should give a reading of 'zero' at all times.]

(b) Nebulize the working standard obtained from dilution of the 8.00 mmol/L standard and adjust the instrumental gain so that for digital read-out instruments a reading of at least 2.000 units is observed.

(c) Check the instrument zero with water and readjust as necessary.

(d) Repeat steps (2)(a)-(c) until stable conditions are achieved. Readings should reproduce within 0.5 percent of full scale.

c. Determination of the Calibration Curve:

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

(2) Subtract the value for the blank from the values obtained with the standard solutions, and plot these corrected relative intensity values versus the calculated potassium 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 1 percent or less.

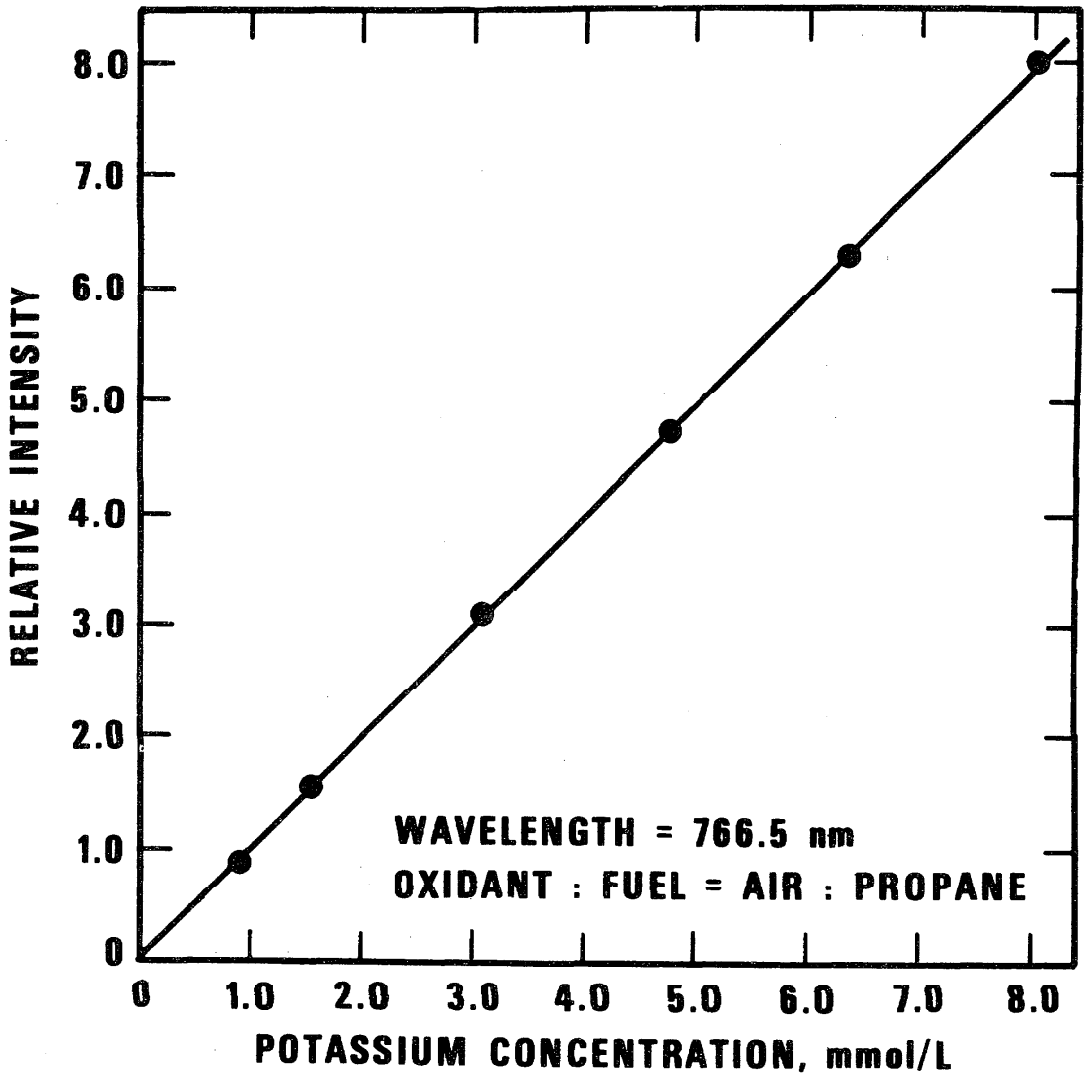

Figure 2. Typical calibration curve for the determination of serum potassium by flame atomic emission spectroscopy.

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 smooth curve drawn through the remaining points, remeasure that standard solution. If the value for the solution continues to exhibit the large deviation, prepare that standard solution again, remeasure it, and compare the values obtained, as in steps c(1) and (2). (See Statistical Analysis Section V-A-3d.)

d. Sample Measurements:

(1) Nebulize a working sample solution and select the two working standard solutions whose emission intensities 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 d(2) until 5 valid sets are obtained, as illustrated in section e, below.

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

e. Valid Sets of Readings:

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

The emission intensities 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: The first set measured is considered to be 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 intensities for the Low Standard (Set_2-Set_1 = +0.01), the Sample (Set_2-Set_1 = +0.01) and the High Standard (Set_2-Set_1 = -0.01) is less than 2 percent. Note, however that set 4 is not valid because two differences, i.e., between the Low Standard values (Set_4-Set_3 = +0.05), and Sample values (Set_4-Set_3 = +0.08), 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 intensity values for sets of readings using a direct read-out instrument.

Set	Low Standard 1.601 mmol/L	Sample	High Standard 3.201 mmol/L
1	1.61	1.98	3.22
2	1.62	1.99	3.22
3	1.61	2.00	3.20
4	1.66	2.08	3.21
5	1.61	1.99	3.21
6	1.60	2.00	3.22

f. Data Recording and Calculations:

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

(2) Calculate the concentration $\hat{C}$ of potassium 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 potassium in mmol/L,

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

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

Y is the relative emission intensity of the sample minus that of the blank (the LiCl diluent or water reading that was initially set at '0')

X_1 is the relative emission intensity of the low standard minus both blanks (the diluted sodium chloride solution blank and the LiCl diluent blank), and

X_2 is the relative emission intensity of the high standard minus both blanks.

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

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

IV. RESULTS AND STATISTICAL ANALYSIS

The main objective of the statistical analyses of the round robin 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 uncertainty 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 product of five valid flame atomic emission 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 "replication" 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 round robin is discussed separately; the final, detailed statistical analysis is reported for the results from RRII.

A. Round Robin Results

1. Preliminary Round Robin (Dates Run: June-August 1975).

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

b. Samples: Three vials, each containing a sample from the same serum pool. Each participating laboratory was to analyze a single portion of each sample within one day.

c. Procedure: The manual pipetting protocol was used.

d. Data: The three 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 those from laboratory 10 are within 4 percent of the collective average with a standard deviation of ±0.05 mmol/L. No explanation could be determined as to why the results from laboratory 10 were so different from the average. These results were considered to be outliers and were not included in the calculation of the standard deviation. (NOTE: see comments on protocol deviation after RRI.) No major problems were encountered in the performance of the protocol.

e. Direction: On examining these results with the statisticians and the Experts Committee, it was concluded that a round robin should be undertaken using samples with potassium concentration values determined by the definitive method. A semi-automated pipetting alternative was written into the protocol.

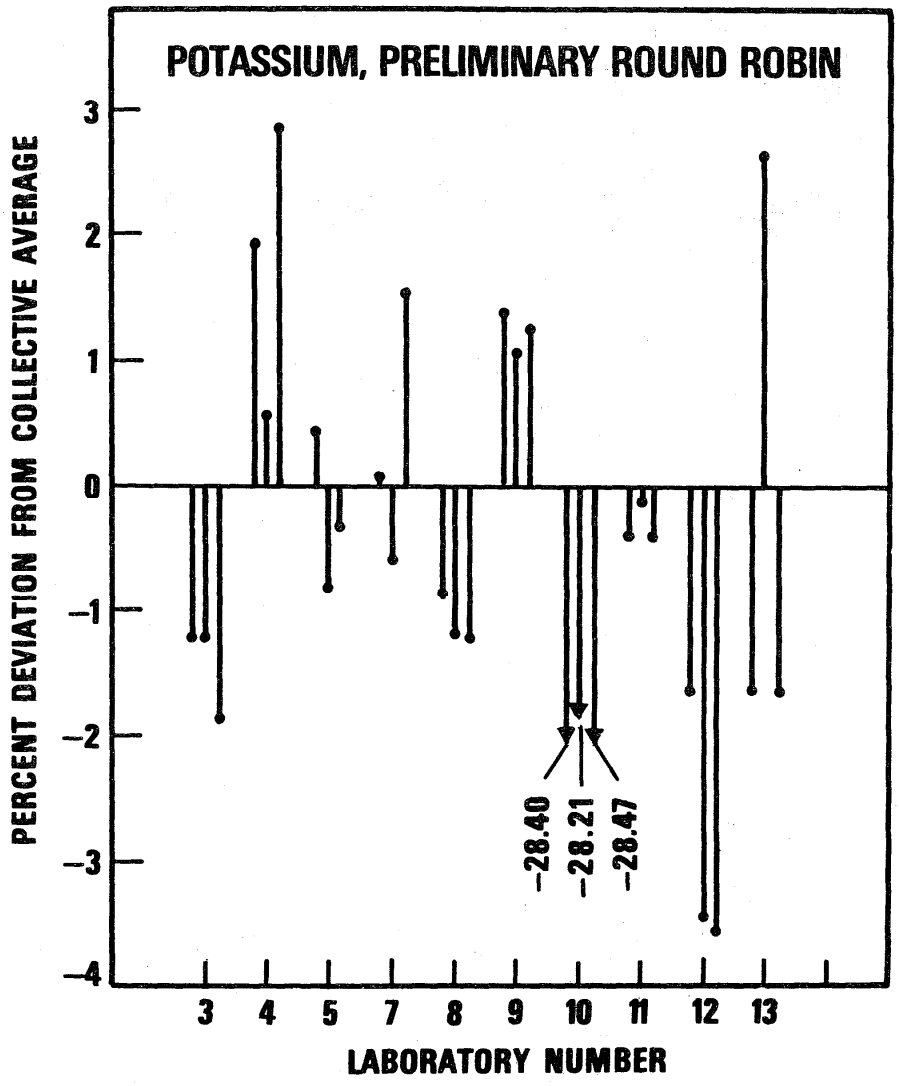

Figure 3. Percent deviations of individual results from the collective average of the measurements obtained in the Preliminary Round Robin test.

Table 5. Serum potassium concentrations reported by the participating laboratories for the Preliminary Round Robin, manual pipetting protocol.

	- - - - - - - - $[K^+]$, mmol/L[a] - - - - - - - -			
Laboratory	Vial 1	Vial 2	Vial 3	Laboratory Average
3	4.62	4.62	4.59	4.61
4	4.77	4.70	4.81	4.76
5	4.70	4.64	4.66	4.67
7	4.68	4.65	4.75	4.69
8	4.64	4.62	4.62	4.63
9	4.73	4.74	4.74	4.73
10	3.35	3.36	3.35	3.35
11	4.66	4.67	4.66	4.66
13	4.60	4.80	4.60	4.67
			Collective Average	4.68[b]

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

[b] Does not include value from laboratory 10.

2. Round Robin I (RRI. Dates Run: November 1975 – January 1976.)

a. General: The addition of the semiautomated pipetting alternative to RRI 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. Objectives: To test the manual and semiautomated pipetting alternatives on serum samples having a wider range of potassium values and determine the imprecision and bias of the test results.

c. Samples: RRI was a test series run on 12 samples – four vials 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.

d. Protocol: The manual and semiautomated pipetting protocols were used.

e. Data: The single-measurement data reported by the laboratories for both pipetting alternatives are summarized in Tables 6 and 7. The data are presented graphically in figures 4 and 5 as percent deviations of each one-day 'single measurement' average from the definitive method value. In general, the data reported by most laboratories were within 5 percent of the definitive method values, although several results for the manual pipetting alternative (lab 7 – pool 1 – day 1; lab 8 – pool 4 – day 1; lab 15 – pools 1, 4, 6 – day 2) were outside this limit. Excluding the excessively high values, statistical analysis of the remaining results showed that the imprecision and bias goals set by the Experts Committee had been reached in RRI testing. The

statistical analysis of the data for RRI yields imprecision and bias values that are similar to those obtained for RRII. [NOTE: The RRII data are discussed in Section IV-A-3.] It is interesting to note that the results that differed extensively from the definitive method values were all high — in fact, for each pool in RRI, the average difference for all the laboratories results from the definitive method values (i.e., $X_{obs}-X_{DM}$) were positive by an average of approximately 1.3 percent. One laboratory showed that contact of the analysts hand with the rim of the volumetric flasks used for the preparation of the working solutions can result in potassium contamination, which in turn, leads to potassium values that can be 10 percent high. It is suggested that care be exercised in sample and glassware handling.

A complete statistical analysis for the final round robin, RRII, is presented later; therefore no detailed tables summarizing these results are presented here. Considering the *caveat* that statistical sampling in terms of the number of participating laboratories and samples is considered to be 'limited', the following observations could be made for RRI: a) at the highest potassium concentration, the imprecision value approached the goal of 0.1 mmol/L; b) the imprecisions increase with increasing potassium concentration; and c) the bias becomes more positive as the potassium concentration increases.

Laboratory 15 repeated the analysis (data labelled 15R) since on the initial analysis instrumental and blank problems were encountered.

Table 6. Concentrations of serum potassium reported by the participating laboratories for Round Robin I, manual pipetting protocol.

Laboratory	[K], mmol/L[a]					
	Pool 1		Pool 4		Pool 6	
	Day 1	Day 2	Day 1	Day 3	Day 1	Day 2
4	1.325	1.306	4.323	4.332	6.130	6.127
	1.311	1.314	4.345	4.390	6.092	6.142
4X[c]	1.306	1.299	4.341	4.311	6.191	6.114
	1.311	1.310	4.362	4.305	6.134	6.118
5	1.292	1.312	4.386	4.370	6.034	6.204
	1.272	1.370	4.404	4.374	6.102	6.192
7	1.796	1.356	4.504	4.404	6.106	6.044
	1.656	1.316	4.334	4.338	6.172	6.098
8	1.398	1.374	4.568	4.468	6.388	6.198
	1.328	1.370	4.736	4.390	6.406	6.114
11	1.304[b]	1.301	4.293	4.352	6.109	6.182
	--- [b]	1.291	4.399	4.391	6.060	6.144
13	1.335	1.326	4.335	4.360	6.166	6.135
	1.350	1.326	4.344	4.320	6.159	6.120
15R[c]	1.352	1.652	4.420	4.932	6.098	6.855
	1.377	1.429	4.390	4.200	6.087	6.395
Definitive Method Values	1.319		4.323		6.092	

[a] Each value is the single measurement average of five valid FAES readings made on a single sample dilution.

[b] Value not reported.

[c] Repeated analysis, instrumental problems.

Table 7. Concentrations of serum potassium reported by the participating laboratories for Round Robin I, semiautomated pipetting protocol.

Laboratory	[K], mmol/L[a]					
	Pool 1		Pool 4		Pool 6	
	Day 1	Day 2	Day 1	Day 3	Day 1	Day 2
4	1.349	1.321	4.312	4.397	6.155	6.206
	1.343	1.319	4.291	4.412	6.095	6.246
4X[c]	1.311	1.298	4.373	4.353	6.030	6.183
	1.353	1.306	4.389	4.450	6.149	6.168
10	1.300	1.380	4.202	4.346	6.154	6.134
	1.300	1.304	4.316	4.358	6.214	6.136
11	1.264[b]	1.374	4.387	4.576	6.418	6.228
	--- [b]	1.409	4.387	4.576	6.251	6.284
15R[c]	1.297	1.371	4.372	4.337	6.167	6.150
	1.384	1.364	4.374	4.402	6.151	6.144
15A[d]	1.298	1.386	4.329	4.377	6.139	6.138
	1.312	1.299	4.337	4.380	6.137	6.125
Definitive Method Values	1.319		4.323		6.092	

[a] Each value is the single measurement average of five valid FAES readings made on a single sample dilution.

[b] Value not reported.

[c] Repeated analysis, instrument problems.

[d] Dilution was 1 in 201 and not 1 in 200.

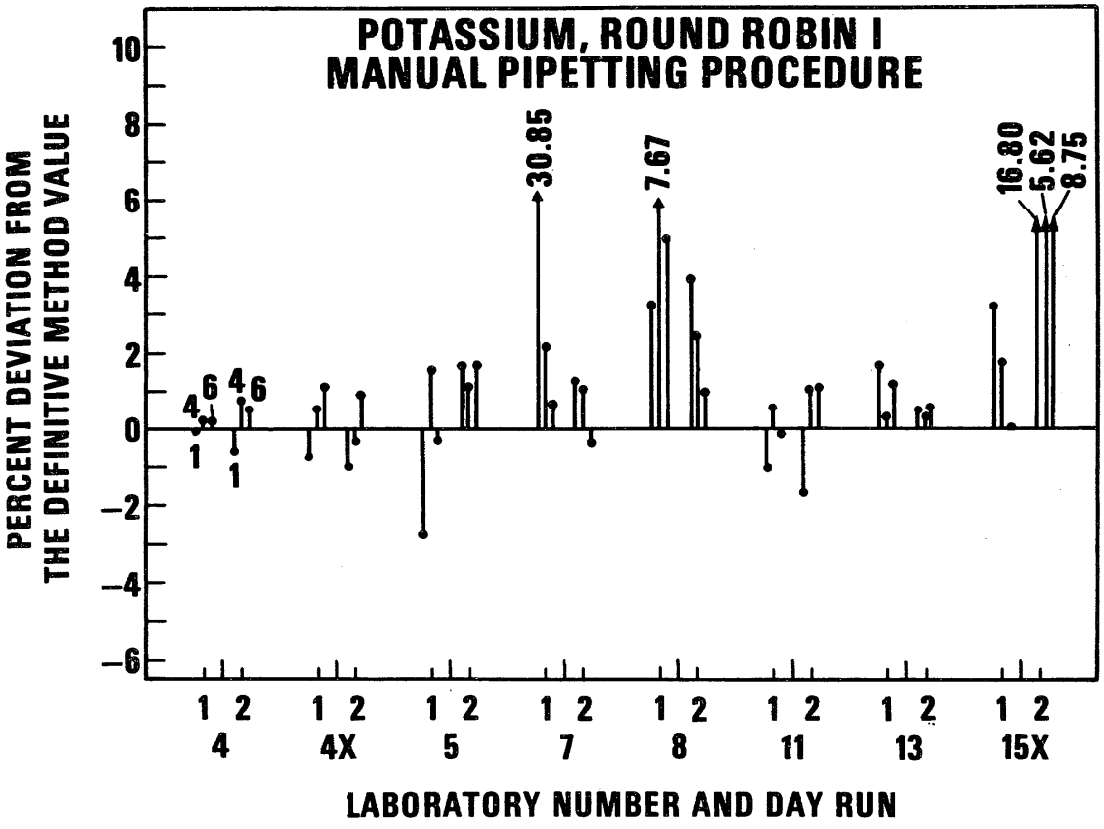

Figure 4. Percent deviations of the Round Robin I measurements using manual pipetting from the definitive method values. The analyzed pools are identified by the numbers 1, 4, and 6 next to the data from laboratory 4. The designations are similar for the remaining results. The numbers 1 and 2, placed directly above the laboratory number, designate the first day and subsequent day test results, respectively. The letter 'X' after the laboratory number designates a repeat analysis.

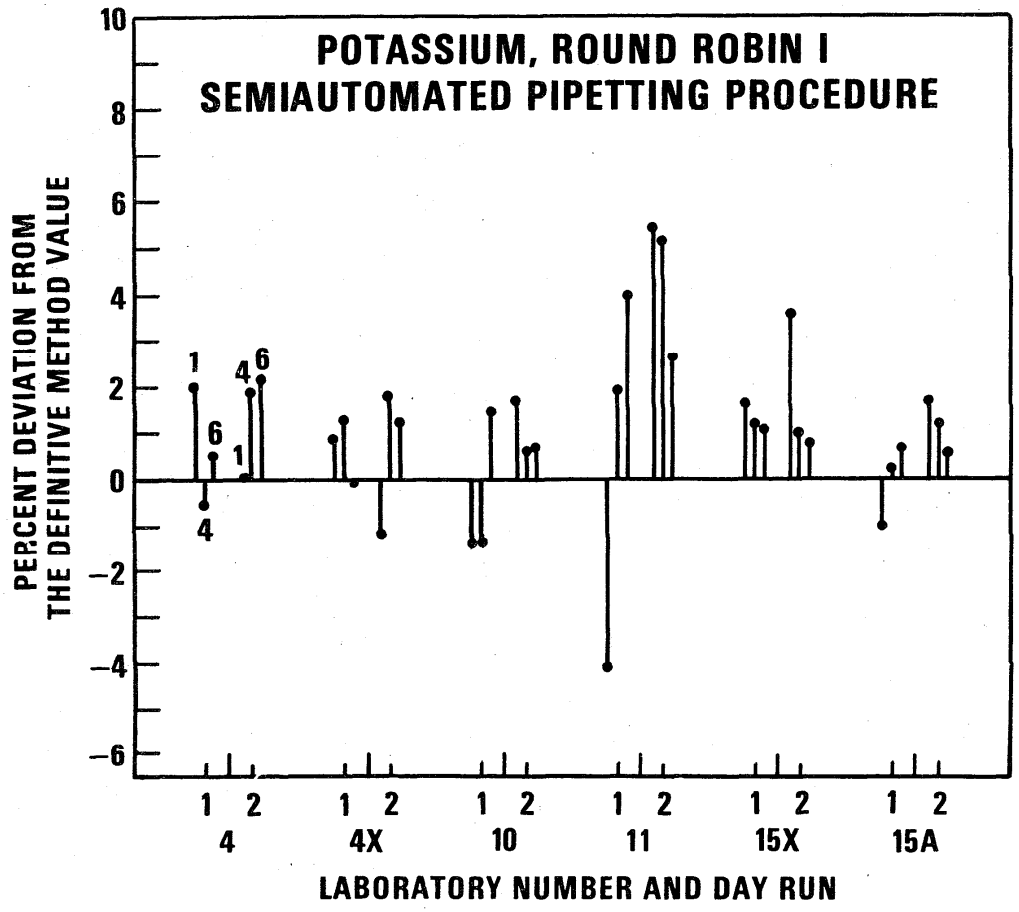

Figure 5. Percent deviations of the Round Robin I measurements using semiautomated pipetting from the definitive method values. The analyzed pools are identified by the numbers 1, 4, and 6 next to the data from laboratory 4. The designations are similar for the remaining results. The numbers 1 and 2, placed directly above the laboratory number, designate the first day and subsequent day test results, respectively. The letter 'X' after the laboratory number designates a repeat analysis and 'A' designates a 201-fold dilution.

f. Comments and Protocol Deviations: The following laboratory comments germane to changing the protocol or signifying deviations from the protocol were received:

(1) Lab 4: Encountered instrument problems; consequently, working samples and standards for day 1 manual procedure, had been prepared 8 days before being measured. The diluted samples had been stored at 4 °C. A similar problem was encountered during performance of the semiautomated pipetting alternative. RRI was repeated with new set of samples.

(2) Lab 7: Noted working standard solutions do not aspirate the same as working serum solutions.

(3) Lab 10: Dilutions of the stock standard solutions were made to 200 mL rather than 250 mL.

g. Direction: A second round robin test (RRII) was to be run using both the semiautomated and manual pipetting alternatives. Test samples would cover the full range of potassium concentrations.

2. Round Robin II: (RRII. Dates Run: July – November 1976.)

a. Objective: To test both the manual and semiautomated pipetting alternatives of the protocol on samples with potassium concentrations over the nominal range of 1.32 to 7.33 mmol/L.

b. Samples: RRII was a test series run on a total of 20 samples – four vials of each of five different potassium concentrations (Pools 1, 2, 4, 5, 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 manual and semiautomated pipetting versions of the protocol were used.

d. Data and Statistical Analysis: Results from RRII are given in Tables 8-9 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 1.3 to 7.3 millimoles of potassium 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 potassium concentrations in the sample pools are listed at the bottom of Tables 8-9.

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 10-11.

Table 8. Concentrations of serum potassium reported by the participating laboratories for Round Robin II, manual pipetting protocol.

	[K], mmol/L				
Laboratory[a]	Pool 1	Pool 2	Pool 4	Pool 5	Pool 7
1-1	1.319	2.572	4.375	5.565	7.495
	1.318	2.553	4.349	5.546	7.394
1-2	1.279	2.653	4.411	5.202	7.133
	1.353	2.697	4.416	5.631	7.306
2-1	1.318	2.628	4.368	5.514	7.386
	1.381	2.589	4.366	5.522	7.360
2-2	1.473	2.566	4.498	5.640	7.396
	1.421	2.601	4.340	5.660	7.409
5-1	1.278	2.520	4.299	5.532	7.347
	1.283	2.527	4.272	5.508	7.396
5-2	1.378	2.582	4.401	5.712	7.507
	1.359	2.592	4.355	5.507	7.460
7-1	1.340	2.573	4.334	5.539	7.386
	1.344	2.572	4.342	5.557	7.418
7-2	1.372	2.600	4.393	5.604	7.392
	1.363	2.600	4.394	5.595	7.386
8-1	1.324	2.552	4.338	5.508	7.362
	1.336	2.548	4.328	5.520	7.344
8-2	1.298	2.538	4.314	5.480	7.340
	1.290	2.532	4.304	5.488	7.316
9-1	1.292	2.509	4.257	5.543	7.467
	1.280	2.474	4.281	5.538	7.466
9-2	1.293	2.502	4.251	5.423	7.458
	1.274	2.509	4.202	5.423	7.420
10-1	1.306	2.560	4.440	5.586	7.340
	1.312	2.540	4.412	5.602	7.314
10-2	1.328	2.542	4.334	5.478	7.384
	1.334	2.536	4.336	5.480	7.380

continued

Continuation of Table 8.

	- - - - - - - - [K], mmol/L - - - - - - - -				
Laboratory[a]	Pool 1	Pool 2	Pool 4	Pool 5	Pool 7
11-1	1.323	2.597	4.443	5.613	7.393
	1.309	2.574	4.425	5.644	7.399
11-2	1.302	2.526	4.283	5.536	7.364
	1.297	2.563	4.258	5.515	7.360
13-1	1.344	2.580	4.338	5.611	7.480
	1.280	2.527	4.400	5.589	7.493
13-2	1.404	2.622	4.331	5.467	7.453
	1.417	2.588	4.387	5.465	7.383
Definitive Method Values	1.319	2.540	4.323	5.501	7.326

[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 9. Concentrations of serum potassium reported by the participating laboratories for Round Robin II, semiautomated pipetting protocol.

	- - - - - - - - - [K], mmol/L - - - - - - - - -				
Laboratory[a]	Pool 1	Pool 2	Pool 4	Pool 5	Pool 7
1-1	1.349	2.629	4.428	5.574	7.350
	1.359	2.626	4.420	5.588	7.395
1-2	1.261	2.456	4.285	5.346	7.082
	1.288	2.475	4.205	5.349	7.112
2-1	1.375	2.582	4.388	5.573	7.423
	1.372	2.528	4.355	5.548	7.390
2-2	1.398	2.586	4.378	5.573	7.425
	1.501	2.578	4.549	5.569	7.409
9-1	1.269	2.474	4.356	5.517	7.461
	1.235	2.467	4.289	5.505	7.474
9-2	1.206	2.441	4.211	5.553	7.465
	1.205	2.388	4.204	5.544	7.463
10-1	1.294	2.514	4.224	5.460	7.434
	1.292	2.484	4.168	5.506	7.382
10-2	1.240	2.496	4.312	5.518	7.294
	1.276	2.394	4.264	5.406	7.262
11-1	1.269	2.543	4.178	5.463	7.308
	1.292	2.588	4.280	5.424	7.415
11-2	1.274	2.462	4.295	5.451	7.359
	1.250	2.463	4.277	5.494	7.366
15-1	1.377	2.503	4.271	5.467	7.424
	1.430	2.482	4.241	5.513	7.390
15-2	1.291	2.577	4.320	5.497	7.350
	1.344	2.582	4.309	5.487	7.403
Definitive Method Values	1.319	2.540	4.323	5.501	7.326

[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.

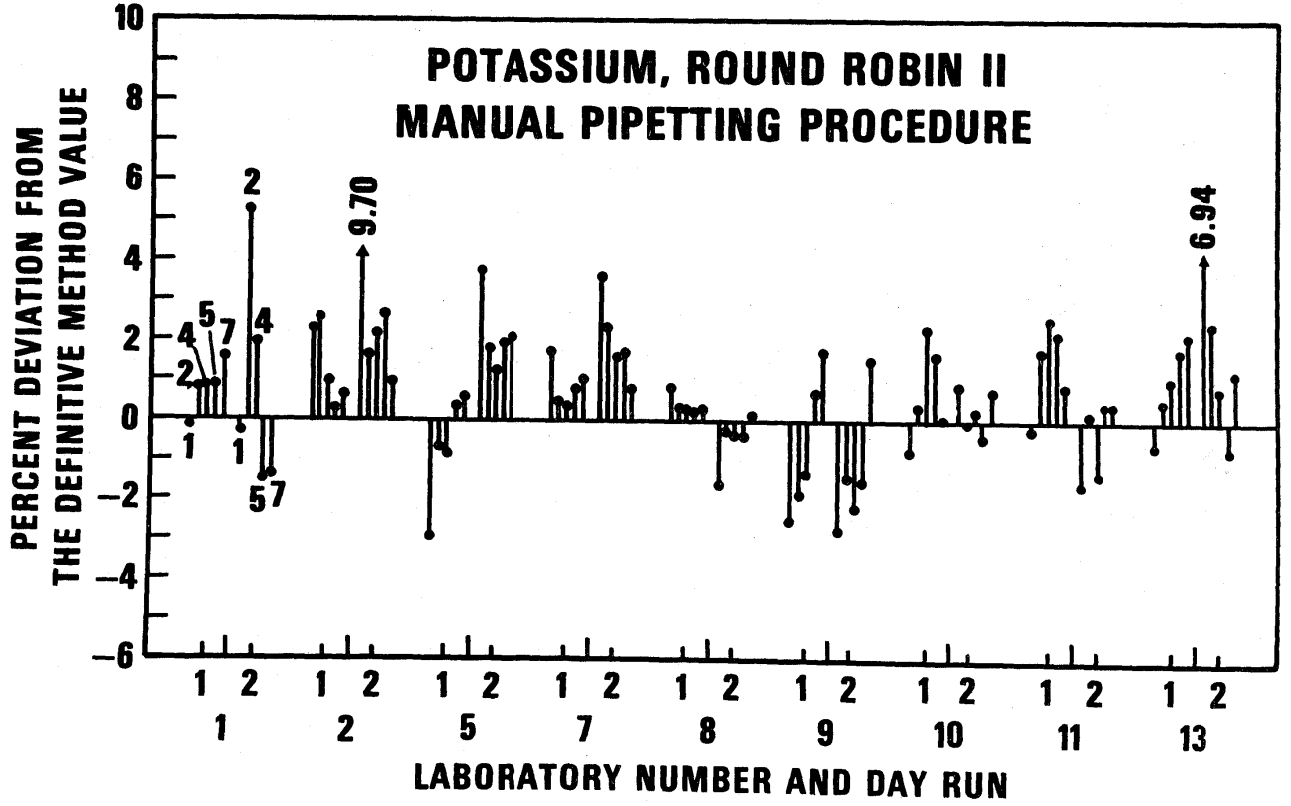

Figure 6. Percent deviations of the Round Robin II measurements using manual pipetting from the definitive method values. The analyzed pools are identified by the numbers 1, 2, 4, 5, and 7 near the results from laboratory 1. The designations are similar for the remaining results. The numbers 1 and 2 placed directly above the laboratory number, designate the first day and subsequent day test results, respectively.

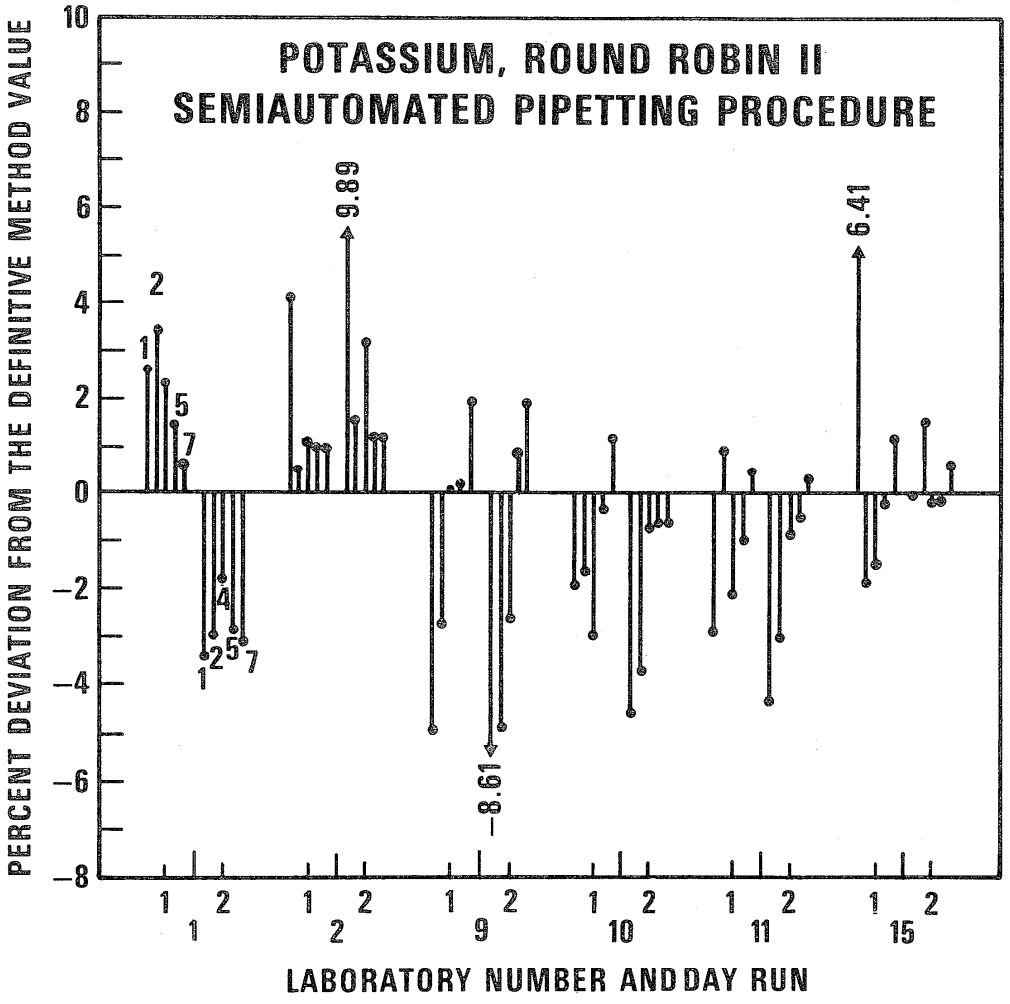

Figure 7. Percent deviations of the Round Robin II measurements using semiautomated pipetting from the definitive method values. The analyzed pools are identified by the numbers 1, 2, 4, 5, and 7 near the results from laboratory 1. The designations are the same for the remaining results. The numbers 1 and 2, placed directly above the laboratory number, designate the first day and subsequent day test results, respectively.

Table 10. Percent deviations from averages for potassium in serum from Round Robin II, manual pipetting protocol.

Laboratory[a]	Pool 1	Pool 2	Pool 4	Pool 5	Pool 7
1-1	-.95	-.10	.29	.33	.72
1-2	-1.14	4.28	1.48	-2.18	-2.32
2-1	1.38	1.69	.41	-.35	-.25
2-2	8.70	.72	1.60	2.04	.15
5-1	-3.81	-1.62	-1.47	-.31	-.27
5-2	2.80	.85	.66	1.30	1.25
7-1	.81	.29	-.26	.19	.14
7-2	2.73	1.36	1.02	1.12	-.03
8-1	-.09	-.59	-.37	-.42	-.52
8-2	-2.79	-1.17	-.93	-.96	-.86
9-1	-3.39	-2.87	-1.85	.06	1.02
9-2	-3.58	-2.32	-2.82	-2.06	.65
10-1	-1.67	-.59	1.76	1.02	-.87
10-2	-.01	-1.02	-.33	-1.05	-.13
11-1	-1.14	.79	1.95	1.65	.06
11-2	-2.38	-.80	-1.81	-.21	-.40
13-1	-1.44	-.45	.45	1.13	1.29
13-2	5.96	1.56	.22	-1.29	.36
Averages used in calculations	1.332	2.565	4.349	5.537	7.391

[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 11. Percent deviations from averages for potassium in serum from Round Robin II, semiautomated pipetting protocol.

Laboratory[a]	Pool 1	Pool 2	Pool 4	Pool 5	Pool 7
1-1	3.34	4.55	2.88	1.53	.06
1-2	-2.73	-1.90	-1.29	-2.72	-3.68
2-1	4.82	1.66	1.66	1.16	.52
2-2	10.62	2.74	3.80	1.35	.66
9-1	-4.45	-1.70	.52	.26	1.35
9-2	-8.00	-3.93	-2.16	.94	1.30
10-1	-1.32	-.57	-2.43	-.25	.54
10-2	-3.99	-2.72	-.29	-.63	-1.22
11-1	-2.27	2.08	-1.66	-.97	-.09
11-2	-3.69	-2.02	-.33	-.44	-.08
15-1	7.11	-.83	-1.03	-.13	.53
15-2	.55	2.64	.33	-.09	.11
Averages used in calculations	1.310	2.513	4.300	5.497	7.368

[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 12-13). If all of the participating laboratories were of the same population in regard to replication error, the standard deviation ratios reported in Tables 12-13

Table 12. Ratios of standard deviations to average standard deviations for potassium in serum from Round Robin II, manual pipetting protocol.

Laboratory[a]	Pool 1	Pool 2	Pool 4	Pool 5	Pool 7
1-1	.05	.90	.84	.40	2.79
1-2	3.45	2.08	.16	9.07	4.78
2-1	2.94	1.85	.06	.17	.72
2-2	2.42	1.66	5.11	.42	.36
5-1	.23	.33	.87	.51	1.35
5-2	.89	.47	1.49	4.34	1.30
7-1	.19	.05	.26	.38	.88
7-2	.42	.00	.03	.19	.17
8-1	.56	.19	.32	.25	.50
8-2	.37	.28	.32	.17	.66
9-1	.56	1.66	.78	.11	.03
9-2	.89	.33	1.58	.00	1.05
10-1	.28	.95	.90	.34	.72
10-2	.28	.28	.06	.04	.11
11-1	.65	1.09	.58	.66	.17
11-2	.23	1.75	.81	.44	.11
13-1	2.98	2.51	2.00	.47	.36
13-2	.61	1.61	1.81	.04	1.94
Average Standard Deviation, mmol/L	.0152	.0149	.0219	.0334	.0256

[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 13. Ratios of standard deviations to average standard deviations for potassium in serum for Round Robin II, semiautomated pipetting protocol.

Laboratory[a]	Pool 1	Pool 2	Pool 3	Pool 5	Pool 7
1-1	.33	.10	.15	.46	1.27
1-2	.88	.66	1.52	.10	.85
2-1	.10	1.86	.63	.83	.93
2-2	3.35	.28	3.25	.13	.45
9-1	1.11	.24	1.27	.40	.37
9-2	.03	1.83	.13	.30	.06
10-1	.07	1.03	1.06	1.52	1.47
10-2	1.17	3.52	.91	3.70	.91
11-1	.75	1.55	1.94	1.29	3.03
11-2	.78	.03	.34	1.42	.20
15-1	1.72	.72	.57	1.52	.96
15-2	1.72	.17	.21	.33	1.50
Average Standard Deviation, mmol/L	.0217	.0205	.0372	.0214	.0250

[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.

would be larger than 2.43 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 round robin II data were made on the data in the two-way tables using a weighted least squares fit to the following model [14]:

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

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 [15].

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 16 was used.) From the analyses it is possible to derive the following estimates for three components of variability, each characterized by its standard deviation:

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

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

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

These components of standard deviation are given in Table 14.

Table 14. Components of standard deviation in mmol/L for all Round Robin II potassium levels (1.3-7.3 mmol/L).

	$\hat{\sigma}_{\varepsilon(Repl)}$	$\hat{\sigma}_{Day}$	$\hat{\sigma}_{Lab}$
Manual Pipetting Protocol (Pooled results from 9 labs)	.039	.048	.030
Semiautomated Pipetting Protocol (Pooled results from 6 labs)	.034	.065	.039

From the analyses, it was observed that the ranges of values for the $\hat{\sigma}_\varepsilon$, $\hat{\sigma}_D$, and $\hat{\sigma}_L$ components of standard deviation were small, and that the values did not depend significantly on the potassium concentration. Because of this, only average $\hat{\sigma}_\varepsilon$, $\hat{\sigma}_D$, and $\hat{\sigma}_L$ values are reported.

Because of the relatively small size of the potassium round robin tests, the individual components of standard deviation are considered to be only advisory in nature. Nevertheless, they do seem to indicate that the three components ($\hat{\sigma}_\varepsilon$, $\hat{\sigma}_D$, and $\hat{\sigma}_L$) are all of about the same order of magnitude. The final, practical statements of uncertainty are made through the recombination of these components. One such final statement is $\hat{\sigma}_{within}$, the expected uncertainty within a single laboratory from running the complete protocol (2 replicates/day for 2 days). The $\hat{\sigma}_{within}$ results are reported in columns three and seven in the top section of Table 15, and are calculated as follows:

$$\hat{\sigma}_{within} = \sqrt{\frac{\hat{\sigma}_\varepsilon^2}{4} + \frac{\hat{\sigma}_D^2}{2}} \cdot \qquad (4)$$

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 15 list such standard deviations for the manual and semiautomated data from round robin II. 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 15. Summary of imprecision and bias results in mmol/L for potassium in serum, Round Robin II.

- - - - - - - - - - - - - 1σ Precision - - - - - - - - - - - - -

	Manual Pipetting Protocol				Semiautomated Pipetting Protocol		
K Level mmol/L	$\hat{\sigma}_{comp}$	$\hat{\sigma}_{within}$	$\hat{\sigma}_{total}$	Goal	$\hat{\sigma}_{total}$	$\hat{\sigma}_{within}$	$\hat{\sigma}_{comp}$
1.3-7.3	.016	.039	.049	.100	.063	.049	.026

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

	Manual Pipetting Protocol		Semiautomated Pipetting Protocol
K Level mmol/L	Round Robin Composite Bias $(X_{obs}-X_{DM})$	Goal	Round Robin Composite Bias $(X_{obs}-X_{DM})$
1.3	.013	±.200	-.009
2.5	.025	±.200	-.027
4.3	.026	±.200	-.023
5.5	.036	±.200	-.004
7.3	.065	±.200	.042

The standard errors of the round robin composite values are given in columns two and eight of the top section of Table 15. These standard errors are calculated from the components of standard deviation as follows:

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

where N represents the 9 or 6 laboratories participating in the manual or semiautomated procedures, respectively. The bottom section of Table 15 lists the observed biases between the reference method round robin composite values and the definitive method values. The observed biases are within the goals for the reference method.

Table 16 lists the composite round robin II 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 round robin results is within the recommended goal of the reference method. The biases tend to become more positive at the higher potassium concentrations. The biases, however, are very close to zero and are in general not significant.

Table 16. Summary of potassium in serum values ±2 standard errors.

Round Robin II - Composite Values (mmol/L)		Definitive Method Values (mmol/L)
Manual	Semiautomated	
1.332 ± .032	1.310 ± .052	1.319 ± .003[a]
2.565 ± .032	2.513 ± .052	2.540 ± .006
4.349 ± .032	4.300 ± .052	4.323 ± .011
5.537 ± .032	5.497 ± .052	5.501 ± .014
7.391 ± .032	7.368 ± .052	7.326 ± .018

[a] Estimated maximum error of 0.25 percent as reported by NBS Analytical Mass Spectrometry Section. This estimated maximum error includes both imprecision and an estimated upper bound for possible systematic errors. The estimated maximum error is believed to be equal to or greater than the true error for the 95 percent confidence limits.

Auxiliary Statistical Analysis

The protocol requires a check on the flame emission 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 sodium procedures were reported and are given in Tables 17-18. Straight line least square fits were made to these data and the resultant standard deviations of fit are given in Table 19. These standard deviations of fit are expressed in units of potassium concentration (mmol/L). Our

Table 17. Calibration curve data for potassium in serum, Round Robin II using manual pipetting.

Laboratory[a]		Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Std. 6
1-1	X[b]	.96	1.60	3.20	4.80	6.40	8.00
	Y[c]	.94	1.54	3.14	4.74	6.32	7.97
1-2	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	.92	1.57	3.18	4.97	6.36	8.03
2-1	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	1.00	1.70	3.24	4.83	6.42	7.99
2-2	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	.96	1.62	3.24	4.82	6.41	7.97
5-1	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	.98	1.59	3.22	4.78	6.37	7.99
7-1	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	1.91	3.20	6.40	9.68	12.83	15.99
8-1	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	.97	1.61	3.19	4.80	6.40	8.00
8-2	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	.97	1.62	3.20	4.82	6.42	8.00
10-1	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	.93	1.57	3.19	4.82	6.49	8.05
10-2	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	.96	1.59	3.18	4.79	6.40	7.95
11-1	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	.93	1.60	3.21	4.73	6.41	8.01
11-2	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	.94	1.62	3.21	4.80	6.45	8.02

[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 reading.

Table 18. Calibration curve data for potassium in serum, Round Robin II using semiautomated pipetting.

Laboratory[a]		Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Std. 6
1-1	X[b]	.96	1.60	3.20	4.80	6.40	8.00
	Y[c]	.94	1.60	3.16	4.74	6.44	8.05
1-2	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	.95	1.62	3.23	4.74	6.42	8.06
2-1	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	1.02	1.64	3.21	4.80	6.40	7.98
2-2	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	1.05	1.64	3.25	4.84	6.42	7.97
10-1	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	.95	1.58	3.21	4.85	6.44	8.01
10-2	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	.94	1.57	3.26	4.86	6.45	7.99
11-1	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	1.01	1.55	3.19	4.90	6.30	7.96
11-2	X	.96	1.60	3.20	4.80	6.40	8.00
	Y	.90	1.58	3.26	4.85	6.53	8.07
15-1	X	.959	1.598	3.197	4.795	6.394	7.992
	Y	.932	1.614	3.302	4.928	6.404	7.980
15-2	X	.959	1.598	3.197	4.795	6.394	7.992
	Y	.978	1.664	3.210	4.826	6.404	7.960

[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 reading.

Table 19. Calibration curve results for potassium 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	.025	1-1	.039
1-2	.086	1-2	.043
2-1	.025	2-1	.012
2-2	.023	2-2	.020
5-1	.018	10-1	.024
7-1	.019	10-2	.045
8-1	.007	11-1	.077
8-2	.011	11-2	.041
10-1	.024	15-1	.073
10-2	.017	15-2	.024
11-1	.035		
11-2	.021		

[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.

analysis indicates that if in the calibration step it is found that any calibration point deviates from the calibration curve by more than 0.08 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. However, in the case of potassium, the similarity of results obtained by White and Mavrodineanu using FAES and the similarity of their results with those obtained using the highly specific IDMS method, led the Committee to decide to proceed directly into the round robin testing phase with the FAES method, without further preliminary studies.

2. Specifications

In light of the prior experience [3,4,7,17], 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 Emission Spectroscopy

Specific instructions are not given for the use of flame emission instruments. In general, all the instruments used in the laboratories that participated in this study

provided excellent results. The FAES instruments that were used are listed in Table 20. Internal and non-internal standard instruments for which eight laboratories used 200-fold dilutions and two laboratories used 100-fold dilutions provided essentially similar results. One laboratory used air-acetylene rather than air-propane as oxidant-fuel without a problem. Thus specifications other than the requirement for stable instrument operating conditions are not 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 one-percent agreement for measurement sets to be considered valid. That requirement was changed to two percent at the July 1975 meeting of the representatives from the participating laboratories. 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. However, the precision of the round robin results was not significantly degraded due to this change.

Instrument linearity requirements were not included in the protocol since the bracketting method for obtaining valid measurements was used to minimize the errors attributable to instrumental drift. Nevertheless, on examination of the data reported for the calibration curves, excellent linearity was found over the range of potassium concentrations from 1.3 to 7.3 mmol/L. More than 85 percent of the calibration curves showed standard deviations of fit of about 0.04 mmol/L or less. A standard deviation of fit larger than 0.08 mmol/L would clearly warrant a laboratory's investigation of its operation of the procedure and/or preparation of the standard solutions.

Table 20. Instruments and operating conditions used by the participating laboratories in RRII.

Lab. #	Instrument[a]	Burner	λ, nm	Flow Rates—Pressure		Dilution	Pipetting Alternatives	
				Air	Propane		M[b]	SA[b]
1	IL143	Premix[c]	766	30 psig	110 psig	200	X	X
2	IL143	Premix[c]	766	0.45 L/min	1.42 L/min	200	X	X
5	IL343	Premix[c]	768	NR[d]	NR[d]	200	X	-
7	IL443	Premix[c]	NR[d]	7.5 SCF	NR[d]	200	X	-
8	IL343	Premix[c]	766	30 psi	25 psi	200	X	-
9	--[e]	PE-5 cn slot	766.5	7.34 L/min[f]	1.9 L/min[f]	200	X	X
10	Beckman, Klina	Total Consumption	768	5.8 L/min	0.34 L/min	100	X	X
11	IL443	Premix[c]	766	30 psi	30 psi	200	X	X
13	Beckman, Klina	Total Consumption	768	NR[d]	NR[d]	100	X	-
15	IL343	Premix[c]	766	0.5 SCF	NR[d]	200	-	X

[a]To describe instruments, it was necessary to identify commercial products by the 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 is necessarily the best available for that purpose.

[b]M = manual; SA = semiautomated.

[c]Glass chimney.

[d]Not reported.

[e]Instrument built in laboratory.

[f]Used air-acetylene.

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.) Much 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 in fact reached over the total concentration range by the laboratories using either the manual or semiautomated pipetting protocols. Additionally, there were no significant differences in the imprecision values obtained by the two pipetting alternatives as evident in Table 15. The imprecision values for both pipetting procedures are approximately twice as good as the original goal set for the reference method by the Experts Committee. Although the observed composite bias values listed in Table 15 for both the manual and semiautomated pipetting procedures are quite small and considered inconsequential, the manual pipetting procedure appears to show biases that are somewhat more positive. This could be due to increased human contact with the solution or glassware (manual pipetting) which results in potassium contamination. The bias values are about five times better than the goals, with the largest bias being about equal to σ_{total}. Thus the agreement between the reference

method values and the definitive method values are considered to be quite acceptable. From this statistical analysis, it is concluded that 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), imprecisions ($\hat{\sigma}_{total}$) within 0.063 mmol/L and biases within 0.065 mmol/L can be expected in the performance of this reference method (refer to Table 15).

VI. CONCLUSIONS

A 'candidate' reference method, specified by a written protocol for the determination of serum potassium by flame atomic emission spectroscopy was evaluated by analyzing serum and aqueous samples in a selected group of laboratories. The results for samples having potassium concentrations in the 1.3 to 7.3 mmol/L range showed a total imprecision of approximately 0.06 mmol/L or less and a maximum bias of 0.06 mmol/L as compared to definitive method values for these samples. The observed bias values, converted to a 'percent' bias, are in general approximately one percent or less over the total potassium concentration range. Similar imprecisions were found whether manual pipetting, requiring large sample volumes, or semiautomated pipetting, requiring small sample volumes, was used. 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 potassium values in the pooled sera.

Statistical analysis of the results shows that the candidate reference method can be carried out with the accuracy and precision expected of a reference method for serum potassium. 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 potassium

by comparative testing. It may also be used to determine reference serum potassium values. Each of these uses would require an appropriate experimental design to ensure achievement of the desired accuracy and precision goals.

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. D. Sue Lewis, CDC, for providing excellent, homogeneous serum pools used in the interlaboratory testing process.

The work at NBS was carried out in the Institute for Materials Research and we acknowledge Ms. Nancy Steele for initial reduction of the PRR and RRI data. We thank Dr. Philip D. LaFleur, Director, Center for Analytical Chemistry, and Mr. J. Paul Cali, Chief, Office of Standard Reference Materials, for their support and encouragement throughout this program. Special thanks go to Dr. Radu Mavrodineanu, Mr. Theodore Rains, and Dr. Michael Epstein for discussions and suggestions in the area of flame atomic emission spectroscopy. Acknowledgement is also made to Mr. John Matwey for help in sample handling. Special thanks are due to Mrs. Joy Shoemaker of the Text Editing Facility for her efforts in manuscript production which included formatting, editing, and typing.

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VII. REFERENCES

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[17] Clinical reference methods are also being developed at NBS for lithium, chloride, and magnesium.

APPENDIX A

Scientists not previously acknowledged who contributed to this study are:

Dr. Robert Moore
Hartford Hospital

Mrs. Harriet Bailey
Dr. Daniel Grisley
New England Deaconess Hospital

Dr. Johan Kofstad
University of Oslo

Mr. Orlando Flores
Mr. James North
Beckman Instruments

Mr. Richard Schlough
University of Wisconsin

Miss Mary Dassow
Mrs. Shirley Wertlake
University of California

Mr. Frank Doherty
Pennsylvania Department of Health

Mr. Claude Walker
Food and Drug Administration

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 918

Potassium Chloride

(Clinical Standard)

This standard reference material is certified as a chemical of known purity. It is intended primarily for use in the calibration and standardization of procedures employed in the determination of potassium and chloride ions in clinical analyses. The sample consists of highly purified potassium chloride. Chemical assay as well as analyses for specific impurities indicate that the material may be considered essentially pure, except for moisture due to occlusion.

Purity . . . . . 99.9 ± 0.0 percent

The above value for the purity of the material is based on a sample dried over magnesium perchlorate for 24 hours. Potassium chloride is hygroscopic when the relative humidity at room temperature exceeds 75 percent, but can be dried to the original weight by desiccation over freshly exposed P_2O_5 or $Mg(ClO_4)_2$ for 24 hours. The material should be stored with such a desiccant. The potassium was determined by a combination of gravimetric and isotope dilution analyses. More than 99 percent of the potassium was precipitated, filtered, and weighed as potassium perchlorate. The weight of potassium perchlorate was corrected for rubidium perchlorate. The soluble potassium was determined by isotope dilution mass spectrometry. Total potassium was the sum of the potassium from the potassium perchlorate and the potassium from the filtrate. The chloride was determined by a coulometric argentimetric procedure.

Based on 12 independent measurements for each ion, the sample was considered homogeneous. Material dried at 500 °C for 4 hours in a platinum or Vycor crucible (Pyrex is unsatisfactory) was assayed at 99.98 ± 0.01 percent. The loss of moisture by this procedure was about 0.07 percent.

The potassium chloride 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, T. J. Murphy, T. C. Rains, T. A. Rush, W. P. Schmidt, and V. C. Stewart.

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

The technical and support aspects concerning the preparation, certification, and issuance of this standard reference material were coordinated through the Office of Standard Reference Materials by J. L. Hague.

Washington, D. C. 20234
January 22, 1971
Revised November 23, 1973

J. Paul Cali, Chief
Office of Standard Reference Materials

(over)

This material was examined for compliance with the specifications for reagent grade potassium chloride as given in Reagent Chemicals, 4th edition, published by the American Chemical Society. The material met or exceeded the minimum requirements in every respect.

A semi-quantitative survey for trace contaminants by emission spectroscopy indicated the presence of less than 0.001 percent aluminum, copper, iron, and magnesium. A value of 0.24 parts per million (ppm) of magnesium was obtained by atomic absorption spectrometry. Flame emission spectrometry indicated the presence of the following elements: rubidium, 27 ppm; sodium, 9 ppm; lithium, 0.6 ppm; and cesium and calcium, less than 2 ppm.

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

This material is intended for use as a standard for potassium and, to a lesser degree, for chloride determination in clinical chemistry.

Potassium is most frequently determined by flame emission photometry. The operative details of this methodology vary from instrument to instrument and are discussed at length in their respective operating manuals. A standard solution of potassium chloride (10 mmol potassium per liter) may be prepared as follows: transfer 0.746 g of SRM 918 that has been dried to constant weight quantitatively to a 1-liter volumetric flask, dilute to volume with water, and make the solution uniform by inverting the flask at least 30 times. The concentrations required for analysis may be prepared by accurate dilution of this standard with deionized water.

A standard solution of chloride containing 100 mmol per liter may be prepared by transferring quantitatively 7.46 g of SRM 918 to a 1-liter volumetric flask and adding 3 ml of concentrated nitric acid (ACS Reagent Grade) and 100 ml of deionized water. After all the salt is dissolved, dilute to volume with deionized water. It should be noted that a chloride standard solution prepared from sodium chloride contains the respective ions in a ratio more nearly that of normal serum than a solution prepared from potassium chloride.

This Standard Reference Material should be stored in the well-closed original container under normal laboratory conditions. It is recommended that weighing and other manipulations of the solid SRM not be made when the relative humidity exceeds 75 percent.

The solutions of SRM 918 are stable indefinitely when stored in a well-stoppered, all-glass container. All such solutions should be clear and display no turbidity.

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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
Room B311, Chemistry Building
National Bureau of Standards
Washington, D. C. 20234

The date of issuance and certification of this Standard Reference Material was January 22, 1971.

APPENDIX C

Isotopic 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:

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

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 arise 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 with the same mass to charge ratio, and may be avoided by either selecting, where possible, an isotope of the element without isobaric interference, or by chemically removing the interfering element. Conveniently, 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 potassium concentrations are to be determined, isotopes ^{39}K and ^{41}K, which do not have isobaric interferences, are used.

(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 these errors can be eliminated or measured accurately for correction. However, random error components are present in the isotope ratio measurements for the analyte determination, the spike calibration, and the blank correction. If the blank correction is insignificant, then the total error in a careful determination reduces to the combined random errors for the spike calibration and the analyte determination. The error of either measurement is reflected in the precision of the isotopic measurements; for many analytes, it is on the order of 0.05 to 0.25 percent at the 95 percent confidence level. Therefore, absolute accuracies of 0.1 to 0.5 percent are possible even for very low concentrations in complex matrices. 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 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 far enough to allow the needle to enter the vial. Approximately 5 g samples, weighed to 0.01 mg, were transferred to 50- or 10-mL Teflon beakers. Weighed aliquots of ^{41}K separated-isotope solution sufficient to give a $^{39}K/^{41}K$ ratio of approximately one, were added to each sample. The samples then were decomposed by adding 5 g of HNO_3 and 5 g of $HClO_4$ 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 the solution was transferred to a 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 0.3 mol/L HCl was added as an eluting agent. The first 50 mL of the eluent was discarded and the next 40 mL of eluent which contained the K fraction was collected in a Teflon beaker. (Those volumes may vary depending on the particular lot of resin; nearly all of the Na should have been eluted and K should just be starting to elute with this volume; this can be checked with a flame test.) The solution was then evaporated to dryness. To aid in the decomposition of the organic material from the column, a few drops of HNO_3 were added and the sample was heated and evaporated to dryness. The residue was converted to the chloride form by adding a few drops of 5 mol/L HCl and evaporating to dryness. The residue was dissolved in enough 0.2 mol/L HCl to give a solution containing approximately 2.5 mg K/mL.

Mass Spectrometry

Isotopic ratios were determined by solid sample, thermal ionization mass spectrometry on 30-cm radius-of-curvature, 90°-analyzer tube, mass spectrometers equipped with thin-lens "Z"-focusing ion-sources and multielement, deep-bucket, faraday-cage collectors. Details of the procedure for measuring the isotopic K ratios have been published [3].

Results

The potassium concentrations determined by isotope dilution mass spectrometry on seven lots of serum are given in Table 1. Exclusive of Lot P3, the precisions (one standard deviation) of the concentration determinations are equal to or less than 0.05 percent. However, a realistic estimate of the imprecision must also include uncertainty components for the spike calibration, analytical blank variability and the effects of impurities [4]. The estimated uncertainty at the 95 percent confidence level for the potassium concentrations in Table 1 is ±0.25 percent and includes allowances for unknown sources of systematic error.

Table I

Lot	Sample No.	µg K/mL 23 °C
P1	1	51.583
	2	51.558
	3	51.574
	4	51.578
Average		51.573
σ		±.011 (0.02%)
P2	1	99.332
	2	99.364
	3	99.272
Average		99.323
σ		±.047 (0.05%)
P3	1	134.79
	2	134.70
	3	134.94
Average		135.81
σ		±.12 (0.09%)
P4	1	168.97
	2	168.99
	3	169.09
	4	169.02
Average		169.02
σ		±.05 (0.03%)
P5	1	215.04
	2	215.10
	3	215.10
Average		215.10
σ		±.03 (0.02%)
P6	1	238.12
	2	238.26
	3	238.20
Average		238.19
σ		±.07 (0.03%)
P7	1	286.34
	2	286.49
	3	286.49
Average		286.44
σ		±.09 (0.03%)

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 (1900).

[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 (1900).

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: (for one hundred-fold dilutions): one 2-L; seven 1-L; six 250-mL; seven 500-mL plus one additional 500-mL volumetric flask for each sample.

Volumetric Flasks: (for two hundred-fold dilutions): one 2-L; six 250-mL; fourteen 1-L plus one additional 1-L volumetric flask for each sample.

Pipets: two 5-mL, five 25-mL, and one each of 3-, 10-, 15-, and 20-mL.

b) Semiautomated pipetting alternative:

Volumetric Flasks: (for one hundred-fold or two hundred-fold dilutions): One 2-L; seven 1-L; six 250-mL; and seven 50-mL plus one 50-mL volumetric flask for each sample.

Pipets: five 25-mL; and one each of 3-, 5-, 10-, 15-, and 20-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 0.250 mL or 0.500 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 0.250 or 0.500 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 0.250 or 0.500 mL of water by allowing air to be sampled rather than water; thus only the 5 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 0.250-mL or 0.500-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

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	------	------	------	------	------	------	------

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:

1) Determine the volume of the nominally 0.250-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 0.0025 and the value for the appropriate water temperature read from Table 43.

2) Determine the volume of the nominally 0.500-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 0.0050 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 III-A2.

Sample Size, mL	Bias, mL	Imprecision, Relative Standard Deviation
0.250	±0.005	0.2%
0.500	±0.010	0.2%

Note 5:

The use of SRM Li_2CO_3 is not recommended for this purpose. However, if it is used, note the following:

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. Thus, more of the SRM 924 Li_2CO_3 is needed to obtain the lithium diluent solution with the desired concentration.

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

Note 6:

There can be two blanks for the standards. The lithium chloride diluent solution (or water) blank is nebulized to set the instrument reading to zero. If the reading of this blank is not zero, then its value and the blank for the working solution of the sodium chloride diluent are to be subtracted from the readings for the standards. Additionally, if the lithium chloride blank reading is not zero, then its value must also be subtracted from the readings obtained for the working samples.

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 round robin test.

ELECTROLYTES IN SERUM — CLINICAL REFERENCE METHOD

ION K

LABORATORY 2 ANALYST FD - LS

EXERCISE NO. RR II

DATE SAMPLES RECEIVED 7/7 DATES ANALYZED (1) 8/2 (2) 8/4

INSTRUMENT MANUFACTURER IL, Inc. MODEL 143-02

WAVELENGTH _____ NM SCAN _____ SIT ON PEAK MAX _____

SLIT WIDTH _____ μM

BURNER TYPE _____

OXIDANT Propane FLOW RATE 0.45 L/MIN

FUEL Air FLOW RATE 1.42 L/MIN

INSTRUMENT TIME CONSTANT _____ S

RECORDER TIME CONSTANT _____ S

READOUT: RECORDER _____ , DIGITAL _____ , OTHER _____

LABORATORY TEMPERATURE 24.0 °C TO 26.0 °C (VARIATION DURING ROUND ROBIN)

COMMENTS: Aspiration tube length - 90 mm; inside diameter - 0.5 mm. SRM 918 KCl dried over anhydrous Al_2O_3. See attached sheet for additional comments. [NOTE: Additional comments concerned the protocol].

DATA SHEET: STANDARD CURVE

Lab 2

PROTOCOL USED: MANUAL _____ SEMI-AUTOMATED X Day 1

STANDARD	CALCULATED ION CONCENTRATION, MMOL/L	RELATIVE INTENSITY VALUES	CORRECTED RELATIVE INTENSITY VALUES
1	0.960	1.04	1.02
2	1.600	1.66	1.64
3	3.200	3.23	3.21
4	4.800	4.82	4.80
5	6.400	6.42	6.40
6	8.000	8.00	7.98
DILUENT BLANK	0.02		
LITHIUM BLANK	0.00		

DATA REPORTING SHEET FOR VALID MEASUREMENTS

PROTOCOL USED: MANUAL _____ SEMI-AUTOMATED X

LAB 2 ION K ROUND ROBIN II DATE ANALYZED 8/2 OPERATOR FD - LS

SAMPLE # 236725

STANDARD CONCENTRATIONS MMOL/L (CALCULATED)	VALID SET	RELATIVE INTENSITIES LO STD (X_1)	SAMPLE (Y)	HI STD (X_2)	$\hat{C}$
LO 1.600 (C_1)	1.	1.59	2.57	3.18	2.586
HI 3.200 (C_2)	2.	1.57	2.57	3.19	2.588
	3.	1.58	2.56	3.19	2.574
	4.	1.59	2.57	3.19	2.580
	5.	1.59	2.57	3.19	2.580

SAMPLE # 328732

STANDARD CONCENTRATIONS MMOL/L (CALCULATED)	VALID SET	RELATIVE INTENSITIES LO STD (X_1)	SAMPLE (Y)	HI STD (X_2)	$\hat{C}$
LO 4.800 (C_1)	1.	4.80	5.57	6.41	5.565
HI 6.400 (C_2)	2.	4.78	5.57	6.41	5.575
	3.	4.78	5.57	6.41	5.575
	4.	4.78	5.57	6.43	5.566
	5.	4.78	5.57	6.39	5.585

SAMPLE # 9469

STANDARD CONCENTRATIONS MMOL/L (CALCULATED)	VALID SET	RELATIVE INTENSITIES LO STD (X_1)	SAMPLE (Y)	HI STD (X_2)	$\hat{C}$
LO 6.400 (C_1)	1.	6.43	7.44	8.02	7.416
HI 8.000 (C_2)	2.	6.43	7.47	8.02	7.447
	3.	6.44	7.43	8.03	7.396
	4.	6.42	7.46	8.05	7.421
	5.	6.43	7.46	8.02	7.436