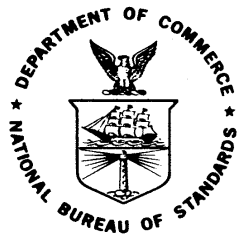

NBS SPECIAL PUBLICATION 260-60

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

Standard Reference Materials:

A REFERENCE METHOD FOR THE DETERMINATION OF SODIUM IN SERUM

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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 sodium 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 a reference method for calcium 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

PREFACE

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

This 260 Series is dedicated to the dissemination of information on all phases of the preparation, measurement, and certification 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. It is also hoped that these papers will provide sufficient additional information not found on the certificate so that new applications in diverse fields not foreseen at the time the SRM was originally issued will be sought and found.

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

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

J. Paul Cali, Chief
Office of Standard Reference Materials

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ABSTRACT

Guided by a committee of experts in clinical chemistry, a reference method was established for the determination of serum sodium based on flame atomic emission spectroscopy (FAES). Its accuracy was evaluated by comparing the values obtained by use of the method in twelve laboratories against the results obtained by a definitive analytical method based on an ion-exchange sodium separation followed by gravimetry as Na_2SO_4. Seven serum pools with sodium concentrations in the range 113.2 to 158.6 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 0.46 to 0.86 mmol/L with a maximum bias of 1.0 mmol/L over the range of concentrations studied. These values were within the accuracy and precision goals that had been set by the committee. There were no significant differences in the results from the two pipetting techniques. The calibration curve data showed excellent linearity over the total concentration range, with 21 of 26 curves having standard deviations of fit of 0.5 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 sodium values for pooled sera.

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

I. INTRODUCTION

Serum sodium 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, neutron activation, or flame atomic emission spectroscopy (FAES) [1] [1] [2]. 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 sodium 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.

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

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

Cali et al. [3] organized a study using a combination of these approaches to establish the accuracy of a clinical chemistry method for total calcium in serum, based on the flame atomic absorption spectrometric (FAAS) method of Pybus, Feldman, and Bowers [4]. The accuracy of the FAAS method was assessed by comparing the results obtained using it on several human serum pools in selected clinical laboratories against those obtained for the same pools by an isotope dilution-mass spectrometry method (IDMS). The IDMS method was performed at the National Bureau of Standards (NBS) where the high accuracy of that method[3] was established by the second approach of determining its systematic and random errors [5].

The Cali et al. study, carried out with the guidance of clinical laboratory experts, used (a) Standard Reference Material Calcium Carbonate (SRM 915) 99.9+ percent pure as the pure primary reference material to prepare standard solutions for all the analyses; (b) serum pools prepared at the Hartford Hospital (Hartford) and at the Center for Disease Control (CDC, Atlanta); (c) pools analyzed for calcium by IDMS at NBS; (d) statistical analysis of the data at NBS; and (e) accuracy and precision goals as performance standards that the FAAS method would have to meet to be considered acceptable as the clinical reference method for total calcium in serum [6].

This same approach was adopted to develop clinical reference methods for a number of other serum electrolytes including sodium, potassium, chloride, lithium, and magnesium. This work was begun with the cooperation of individuals from the Standards Committees of the American Association for Clinical Chemistry (AACC) and the College of American Pathologists (CAP), the CDC and the NBS. The Food and Drug

[3]Such a method is referred to as a definitive method because of its high accuracy and utility for evaluating the accuracy of a candidate reference method.

Administration (FDA) provided major suppport for the NBS work. We present in this report the development of a clinical reference method for serum sodium.

II. DEVELOPMENT OF THE SERUM SODIUM 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, sodium, potassium, 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 Copeland (New England Deaconess Hospital), Dr. Denis Rodgerson (Center for Health Sciences, University of California in Los Angeles), and Dr. James White[4] (CDC).

Prior to the meeting, several bovine serum pools prepare at the CDC had been analyzed for sodium by FAES, neutron activation analyses (NAA), and ion-exchange separation of the sodium followed by its gravimetric measurement as sodium sulfate (IEG). 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,
NAA as obtained at the NBS, by Dr. H. Rook, and
IEG as obtained at the NBS, by Dr. O. Menis.

[4]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 sodium, it was concluded that FAES was the appropriate candidate methodology to evaluate as the reference method and that its evaluation should be made using either NAA or IEG as the definitive method; NBS, on the basis of continued study, was to choose between NAA and IEG.

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

	- - - - - - - - - Na in Serum, mmol/L - - - - - - - - -			
			Flame Atomic Emission Spectroscopy	
Pool	Ion-Exchange-Gravimetry[a]	Neutron Activation[b]	NBS[c]	CDC[d]
I	124.2	121.8	121.1	123.2
III	138.1	135.5	137.3	137.5
V	154.4	150.1	152.5	153.0

[a] Data from O. Menis, R. K. Bell, M. Epstein, and J. Shultz (NBS).

[b] Data from J. E. Suddueth, R. M. Morris, and H. L. Rook (NBS).

[c] Data from R. Mavrodineanu (NBS).

[d] Data from J. White (CDC).

The experts agreed to serve as the Committee to oversee the development of the reference method for sodium (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 sodium reference method. The Committee agreed that the FAES method should use a concentration bracketing technique rather than calibration curves for determining sodium 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 sodium 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 2.0 mmol/L and 1.5 mmol/L, respectively, for serum sodium at the 140 mmol/L level. These goals were to be achieved by controlled, interlaboratory tests involving a selected group of clinical chemistry laboratories which would perform the analyses by the FAES method according to the written protocol while NBS would provide sodium 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:

Dr. George N. Bowers, Jr.
Hartford Hospital
Hartford, CT 06115

Dr. Bradley E. Copeland
New England Deaconess Hospital
Boston, MA 02215

Professor Lorentz Eldjarn
Rikshospitalet, University of Oslo
Oslo, Norway

Dr. Ronald E. Laessig
State Laboratory of Hygiene, University of Wisconsin
Madison, WI 53706

Mr. Theodore C. Rains
Dr. Michael Epstein
National Bureau of Standards
Gaithersburg, MD 20760

Dr. Denis O. Rodgerson
Center for Health Sciences, University of California
Los Angeles, CA 90025

Mr. William Ryan
Beckman Instruments
Fullerton, CA 92634

Mr. Leonard Sideman
Pennsylvania Department of Health
Philadelphia, PA 19130

Dr. Barbara Tejeda
Food and Drug Administration
Washington, D. C. 20250

Dr. James White
Dr. Richard Carter
Center for Disease Control
Atlanta, GA 30333

Ms. Peg T. Whittemore
Instrumentation Laboratories
Lexington, MA 02173

Dr. Charles E. Willis
College of American Pathologists, Cleveland Clinic
Cleveland, OH 44106

NBS Standard Reference Material Sodium Chloride (SRM 919, see Appendix B) was to be used as the pure primary reference material for all analyses [7]. Seven pools of homogeneous, sterile, bovine serum, having different concentrations of sodium, 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 were placed in freezers kept at -50 °C [8]. The pools were numbered in code from 1 to 7 according to increasing sodium concentration.

A definitive method based on ion-exchange separation of sodium followed by its gravimetric determination as sodium sulfate was developed at NBS. The definitive method is given in Appendix C. The sodium concentrations for the seven serum pools were determined by this procedure and the results obtained are summarized in Table 2. The sodium concentrations for the serum pools were also determined by NAA (procedure also outlined in Appendix C) and these supportive results are also summarized in Table 2.

Table 2. Sodium concentrations for the seven serum pools as determined by ion-exchange-gravimetry (IEG, the definitive method), and neutron activation analysis (NAA).

Pool	$[Na^+]$, mmol/L	
	IEG[a], Definitive Method	NAA[b]
1	113.2	114.2
2	121.0	121.7
3	129.9	130.7
4	136.6	137.4
5	146.3	148.6
6	153.8	154.7
7	158.6	158.7

[a] ±0.6 mmol/L for all concentrations at a 95 percent confidence limit. Data from J. Moody (NBS).

[b] Imprecision values at the 95 percent confidence limit ranged from ±0.8 to 1.2 mmol/L. Data from J. E. Suddueth, R. M. Morris, and H. L. Rook (NBS).

C. Functions of the Various Groups

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

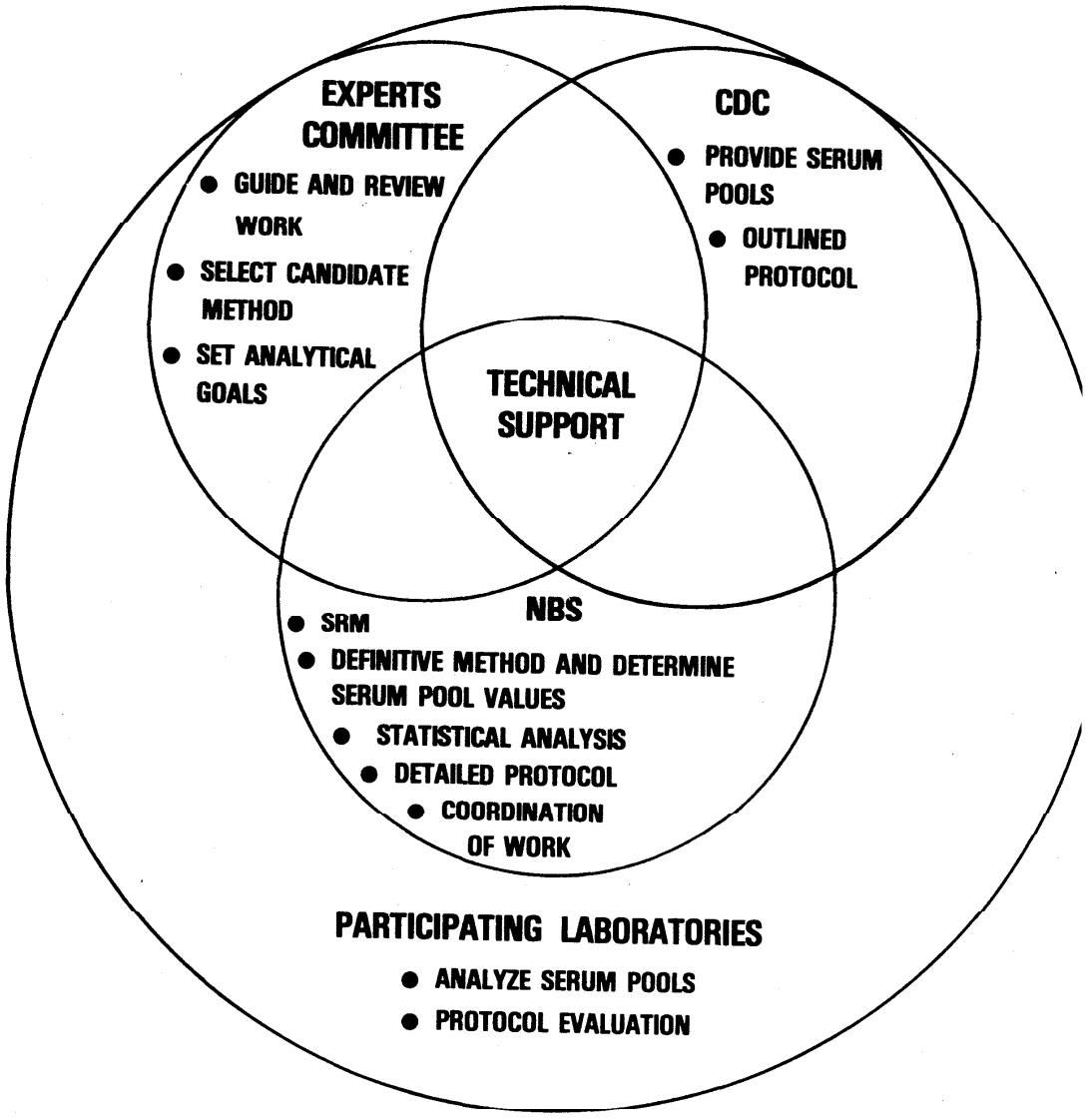

Figure 1. Interrelationships and functions of the various groups in the development of a clinical referenc method for the determination of serum sodium.

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 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.

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 [9] 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 sodium 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 sodium 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 SODIUM

A. General

This protocol for the analysis of serum sodium 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 NaCl in appropriate quantities and to prepare a series of stock standard sodium solutions;
2. Use either a single pipet or a pipettor-dilutor to dilute to the sodium concentrations that are used as working solutions for FAES a) aliquots of the serum to be analyzed, b) aliquots of the stock standard sodium 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 signals of the aliquots;
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 sodium 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 sodium 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. Sodium Standard Solutions: Use Standard Reference Material Sodium Chloride (originally issued as SRM 919, Certificate reproduced in Appendix B) [7] certified by the National Bureau of Standards.

The SRM NaCl should be dried by heating at 110 °C for four hours in a loosely capped container and then stored in a desiccator containing $CaSO_4$ or an equivalent desiccant.

c. Lithium carbonate, potassium chloride, hydrochloric acid, nitric acid, chloroform, methanol and 95-percent ethanol meeting ACS [10] (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 [11] (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 i ten 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, 16 to 20 liters of the LiCl diluent should be prepared.

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

b. Potassium Chloride Diluent Solution (KCl Diluent, 4.5 mmol/L): Weigh 0.336 g of KCl (MW = 74.5513, 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.

c. Sodium Standard Stock Solutions: Weigh accurately (to 0.1 mg) approximately 0.64, 0.70, 0.76, 0.82, 0.88, and 0.94 g of dried sodium chloride (MW = 58.44277, Appendix D, Note 5b) and transfer each quantitatively into separate 100-mL volumetric flasks. Dissolve and dilute to the mark with the KCl diluent. Mix thoroughly as described above. From the weighed quantities of NaCl taken, calculate

the sodium concentrations in mmol/L to one decimal place (an example of this step is given in Table 3).

Table 3. Sodium chloride standard solutions.

Solution	NaCl Concentration[a] mmol/L	NaCl[a], g
1	110.1	0.6432
2	120.0	.7010
3	130.0	.7598
4	140.1	.8186
5	150.1	.8772
6	160.0	.9350

[a] The NaCl concentrations were calculated using atomic weights from the literature reference cited in Appendix D, Note 5b.

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 solutions, the standard solutions, and the samples 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 KCl diluent (IIIC-3b) diluted with the LiCl diluent used as a blank for the sodium 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 110-, 120-, 130-, 140-, 150-, and 160-mmol/L sodium 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 KCl diluent (IIIC-3b), diluted with the LiCl diluent, is used as a blank for the sodium 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 sodium 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 sodium standard solution obtained from the 160 mmol/L standard solution and adjust direct read-out instruments so that a reading of 160.0 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 589 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 burner 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 160 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 sodium 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 sodium 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.

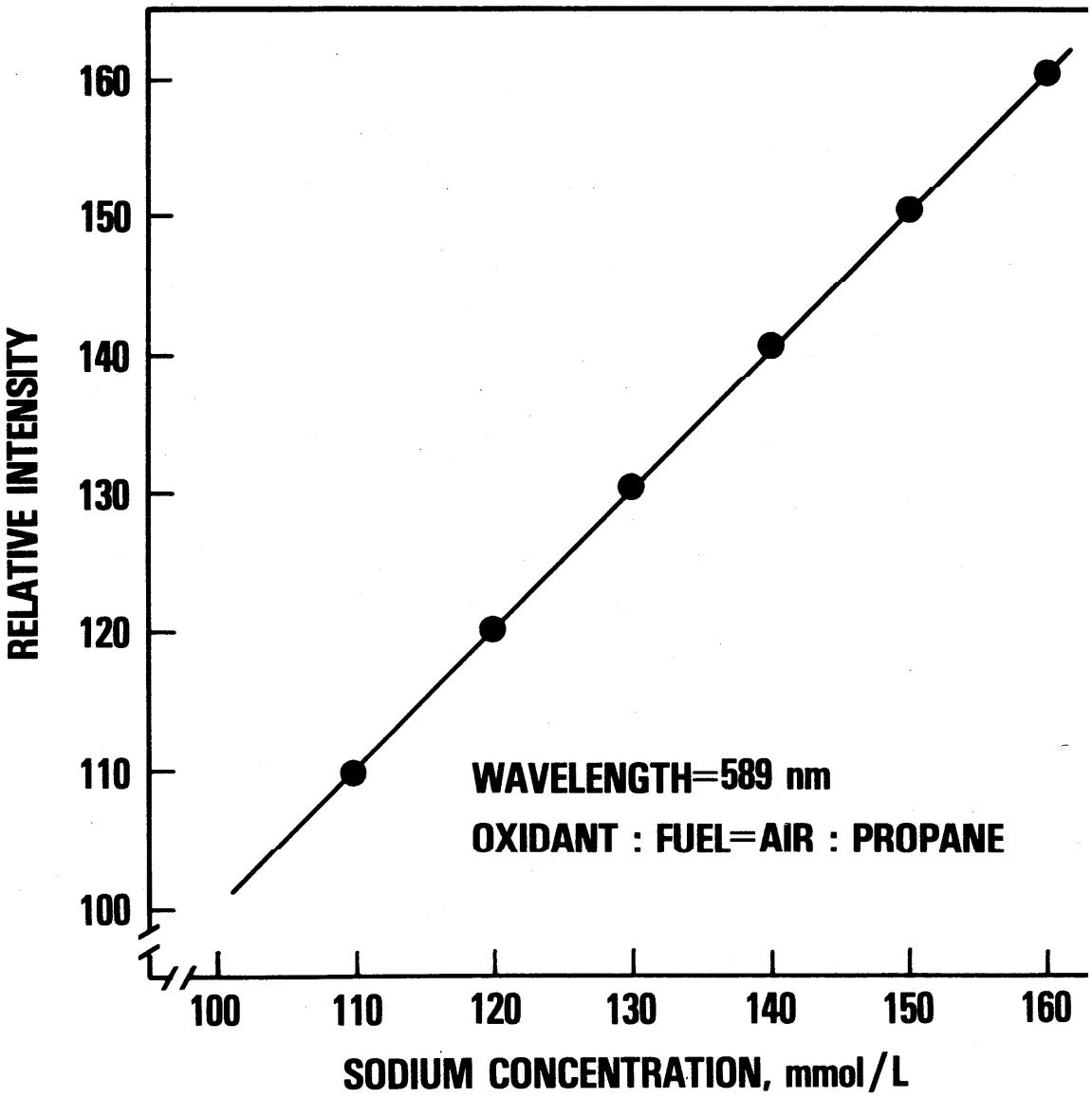

Figure 2. Typical calibration curve for the determination of serum sodium by flame atomic emission spectroscopy.

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

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

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-3.)

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 = -0.3), the Sample (Set_2-Set_1 = -0.6) and the High Standard (Set_2-Set_1 = 0.5) 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 = -2.9), and Sample values (Set_4-Set_3 = -2.8), 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 130.0 mmol/L	Sample	High Standard 140.0 mmol/L
1	130.7	137.4	140.6
2	130.4	136.8	140.1
3	131.0	137.5	140.5
4	128.1	134.7	139.6
5	130.2	136.8	140.2
6	130.4	137.1	140.5

f. Data Recording and Calculations:

(1) On the data sheet, record the concentrations of the standard solutions in mmol/L of sodium 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 sodium 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 sodium in mmol/L,

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

C_2 is the high standard concentration of sodium 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 potassium 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 readings, 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; a detailed statistical analysis was performed on the results from RRII.

A. Round Robin Results

1. Preliminary Round Robin (Dates Run: March-May 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 are within ±1.5 percent of the collective average with a standard deviation of ±0.8 mmol/L. 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 sodium concentration values determined by the definitive method.

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

	- - - - - - - - $[Na^+]$, mmol/L[a] - - - - - - - -			
Laboratory	Vial 1	Vial 2	Vial 3	Laboratory Average
3	147.0	148.9	149.0	148.3
4	147.7	146.7	147.4	147.3
5	148.8	148.9	148.5	148.7
7	148.2	148.0	148.0	148.1
8	147.0	148.2	147.2	147.5
9	150.1	150.1	150.1	150.1
10	148.4	148.1	148.1	148.2
11	148.2	148.4	147.9	148.2
			Collective Average	148.3

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

2. Round Robin I (RRI. Dates Run: June-August 1975).

a. Objectives: To test the full protocol on serum samples having a wider range of sodium values and determine the imprecision and bias of the test results.

b. Samples: RRI was a test series run on 12 samples – four vials of each of three different concentrations (Pools 2, 4, and 5). Each laboratory was to analyze two vials of each pool on one day and the remaining pairs of samples on a subsequent day with the requirement that a minimum of one day and a maximum of seven days should elapse between analyses.

c. Protocol: The manual pipetting protocol was used.

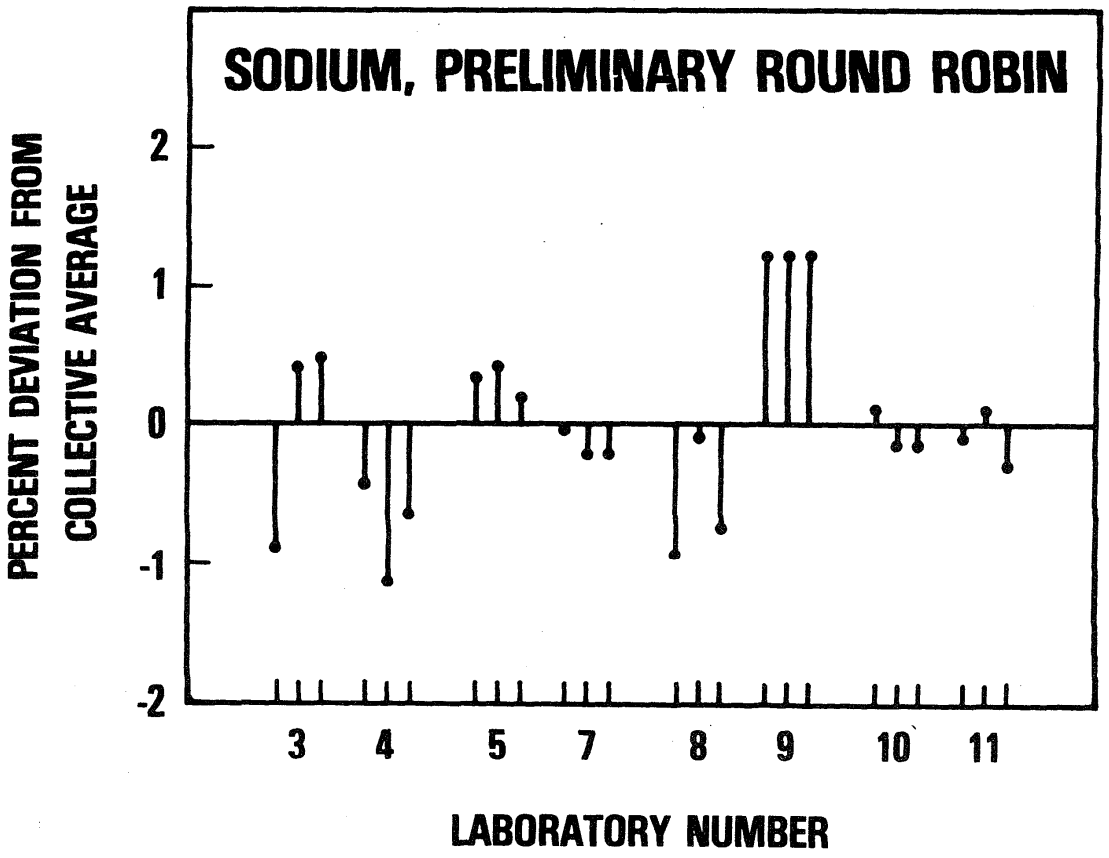

Figure 3. Percent deviations of individual results from the collective average of the measurements obtained in the Preliminary Round Robin test.

d. Data: The single-measurement data reported by the laboratories are summarized in Table 6. The data are presented graphically in figure 4 as percent deviations of each one-day 'single measurement' average from the definitive method value. Except for laboratory 8 which showed a large error in a single determination on one sample from pool 5 on the subsequent day, all the values reported by the laboratories were within ±1.5 percent of the definitive method sodium values. Most of the laboratories reported values that fell both above and below the definitive method value.

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

(1) Lab 3: Used 1-mL samples and standards and diluted to 200 mL—not 5 mL diluted to 1000 mL as required by the protocol;

(2) Lab 4: Encountered instrument problems; consequently, working samples and standards had been prepared 18 h before being measured.

(3) Lab 5: Encountered instrument problems; Suggested linearity requirement be set for calibration curve.

(4) Lab 8: Lost one sample. Thus on last day, the value for pool 5 is for a single sample.

(5) Labs 4 and 15: Recommended that a semi-automated pipetting version of the protocol be evaluated.

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

Laboratory	[Na], mmol/L[a]					
	Pool 2		Pool 4		Pool 6	
	Day 1	Day 2	Day 1	Day 3	Day 1	Day 2
3	120.28	120.94	135.66	136.86	147.54	147.08
	120.78	120.12	135.54	136.72	146.98	147.52
4	121.12	122.14	136.80	137.60	146.92	147.88
	121.28	122.16	136.80	138.16	146.90	147.74
5	120.68	119.84	135.56	135.76	147.30	147.44
	119.90	120.86	135.50	135.06	147.02	147.28
7	120.85	120.44	136.31	135.97	147.59	147.17
	122.19	120.94	136.13	136.02	147.27	147.00
8	120.08	119.28	136.36	134.92	147.42	153.7
	------[b]	120.04	136.04	134.78	147.22	-----[b]
9	121.00	120.60	137.32	136.50	147.44	147.02
	121.00	120.90	137.00	136.54	147.50	147.22
10	121.44	121.14	136.80	136.08	148.00	147.34
	121.20	120.84	135.20	136.30	147.34	147.68
11	120.90	120.48	136.54	136.08	147.44	147.28
	120.94	120.80	136.00	136.00	147.42	147.24
13	120.42	120.88	136.52	136.34	147.36	147.32
	120.22	120.64	136.32	137.00	147.60	147.16
Definitive Method Values	121.0		136.6		146.3	

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

[b] Values not reported.

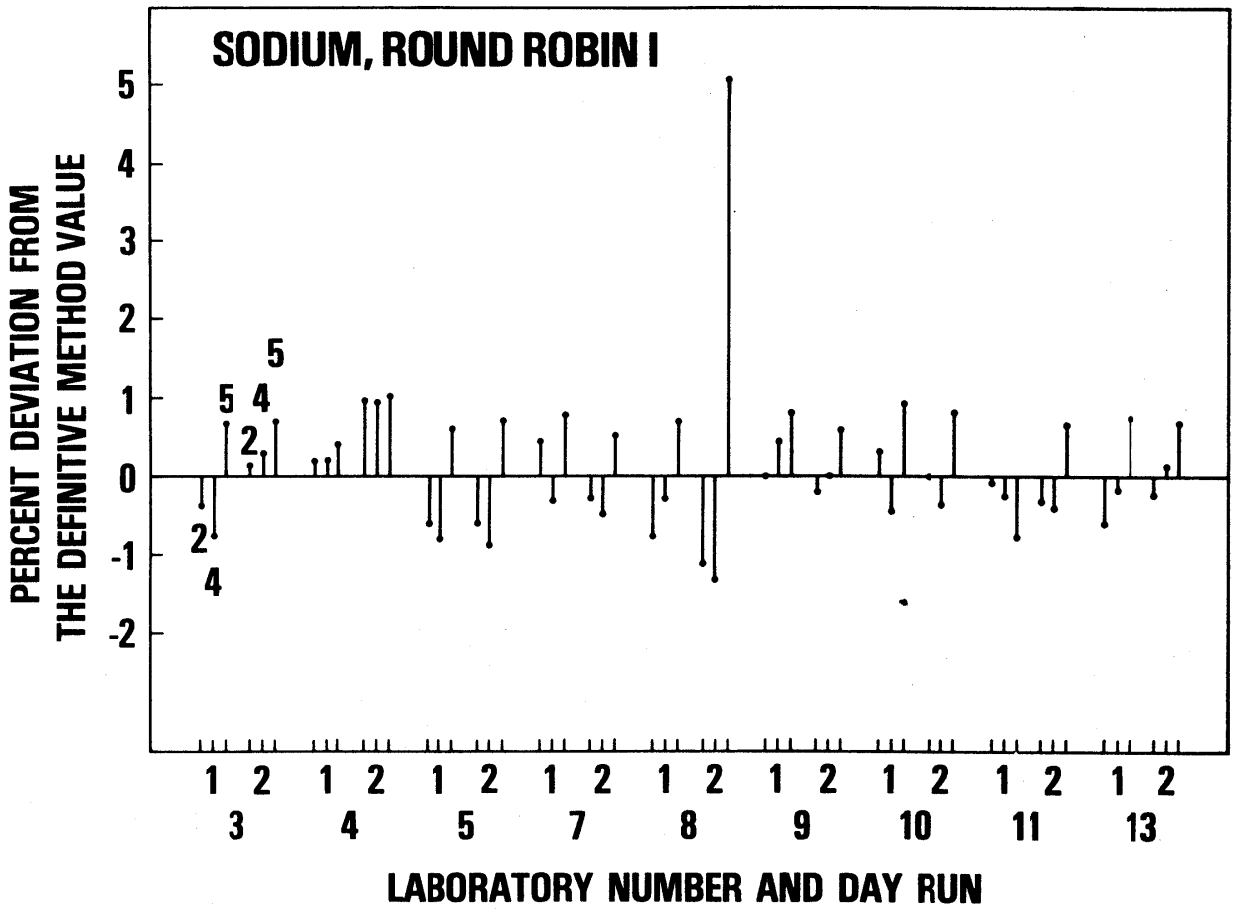

Figure 4. Percent deviations of the Round Robin I measurements from the definitive method values. The analyzed pools are identified by the numbers 2, 4, and 5 next to the data from laboratory 3. The designations are similar for the remaining results. The numbers 1 and 2, placed directly above the laboratory number, designate the first day and subsequent day test results, respectively.

f. Direction: A semiautomated pipetting alternative was written into the protocol. 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 sodium concentrations.

3. Round Robin II: (RRII. Dates Run: November 1975-February 1976).

a. General: The addition of the semiautomated pipetting alternative to RRII 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 semiautomated sampling device.

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

b. Objective: To test both the manual and semiautomated pipetting alternatives of the protocol on samples with sodium concentrations over the nominal range of 110 to 160 mmol/L.

c. Samples: RRII was a test series run on a total of 20 samples – four vials of each of five different concentrations (Pools 1, 3, 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.

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

e. Data and Statistical Analysis: Results from RRII are given in Tables 7-8 and illustrated in figures 5-6. 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 110 to 160 millimoles of sodium 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 sodium concentrations in the sample pools are listed at the bottom of Tables 7-8.

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. This procedure showed that the manual procedure results of Laboratory 15 differed greatly from the laboratory averaged pool results. [During this RR, Laboratory 15 encountered instrumental stability problems. It was reluctant to provide this data, but could not reschedule a rerun of the RR. Laboratory 15

Table 7. Concentrations of serum sodium reported by the participating laboratories for Round Robin II, manual pipetting protocol.

Laboratory[a]	[Na], mmol/L				
	Pool 1	Pool 3	Pool 4	Pool 5	Pool 7
4-1	113.68	129.36	136.44	146.94	157.86
	113.46	129.38	136.26	146.98	156.96
4-2	113.14	129.12	135.60	146.78	157.72
	113.50	128.92	135.34	146.70	157.42
5-1	113.90	129.90	136.48	152.86	157.58
	113.90	129.60	136.22	146.76	157.66
5-2	113.78	129.58	136.28	147.96	157.94
	114.00	129.76	136.58	148.06	157.80
7-1	113.64	128.58	135.86	146.74	158.26
	114.22	129.26	135.86	147.06	157.68
7-2	113.38	129.46	135.48	146.44	157.88
	113.50	129.06	135.88	146.52	157.56
8-1	113.66	130.64	134.76	146.92	159.32
	113.72	128.82	137.96	147.74	157.10
8-2	112.08	129.66	134.40	146.06	158.24
	114.30	128.38	134.92	144.92	156.68
9-1	113.02	128.66	135.92	146.82	157.94
	112.94	128.22	136.44	147.30	158.48
9-2	112.66	128.22	136.22	146.46	157.06
	113.36	128.42	137.18	146.72	156.88
11-1	113.86	129.43	136.56	147.29	158.73
	113.92	129.45	136.12	147.43	156.88
11-2	113.62	129.71	137.46	145.26	157.47
	113.74	129.44	136.54	146.32	157.97
13-1	113.50	130.62	136.94	148.20	158.46
	113.58	129.64	138.43	149.00	158.24
13-2	113.14	129.00	136.20	147.12	158.04
	113.44	129.60	136.80	147.88	158.12

continued

Continuation of Table 7.

Laboratory[a]	[Na], mmol/L				
	Pool 1	Pool 3	Pool 4	Pool 5	Pool 7
15-1[b]	118.46	133.26	140.22	149.54	161.83
	118.94	129.63	139.23	149.43	162.61
15-2[b]	118.33	133.77	140.67	150.00	160.61
	117.57	134.53	142.27	150.89	160.93
Definitive Method Values	113.2	129.9	136.6	146.3	158.6

[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] These results are not included in any pooled values, or final results.

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

Laboratory[a]	[Na], mmol/L				
	Pool 1	Pool 3	Pool 4	Pool 5	Pool 7
1-1	113.56	129.12	135.82	146.88	158.02
	114.12	129.32	136.14	147.52	157.60
1-2	114.00	130.06	136.10	147.14	157.98
	113.94	129.90	136.04	147.28	157.88
2-1	113.80	129.45	135.81	145.76	156.87
	113.61	129.09	134.67	146.57	157.18
2-2	114.68	130.28	137.29	147.78	158.42
	113.51	129.99	136.42	147.76	158.07
4-1	113.14	129.50	135.54	146.32	156.70
	113.16	129.12	135.44	146.48	156.82
4-2	113.12	129.24	136.38	146.82	157.58
	113.30	129.78	135.80	147.80	157.56
9-1	112.76	128.40	136.88	146.36	157.24
	114.42	128.54	138.54	146.54	157.58
9-2	113.10	128.82	136.44	147.34	157.58
	113.62	128.58	136.60	146.82	157.48
10-1	113.72	129.54	136.02	147.62	158.28
	113.70	129.26	135.36	146.96	158.12
10-2	113.22	129.04	135.78	146.54	157.18
	113.08	129.72	136.42	146.60	157.20
11-1	114.77	131.00	137.40	148.19	159.16
	115.69	129.95	137.01	149.10	159.83
11-2	113.86	130.43	137.02	147.83	158.31
	114.20	130.30	137.32	147.61	158.51

continued

Continuation of Table 8.

Laboratory[a]	[Na], mmol/L				
	Pool 1	Pool 3	Pool 4	Pool 5	Pool 7
15-1	113.56	128.59	135.00	146.30	157.31
	113.80	129.55	134.49	146.46	157.66
15-2	114.84	128.98	136.12	146.18	156.11
	114.60	130.70	136.04	145.15	155.95
Definitive Method Values	113.2	129.9	136.6	146.3	158.6

[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.

was persuaded to send in its data. Their data was found to be consistent; however, it showed a large blank for the manual pipetting procedure (the only lab to do so for any procedure). If the blank could be ignored, the results would bracket the definitive method values.] Laboratory 15 data are included for information only [12] but were not used to calculate the manual procedure pool averages. The percent deviation values were recalculated; these percent deviation values for all laboratories are listed in Table 9. The semiautomated procedure percent deviation values are reported in Table 10.

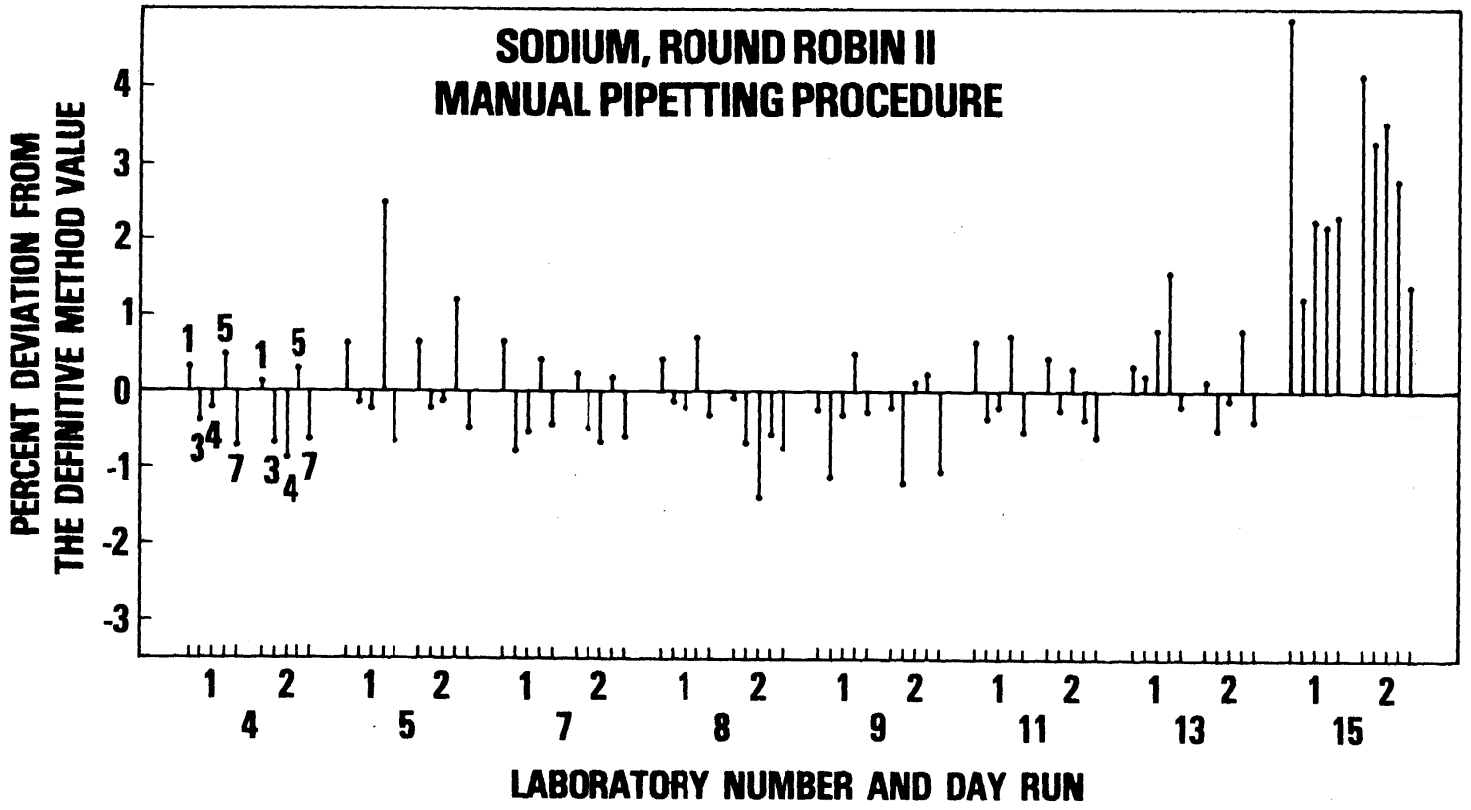

Figure 5. Percent deviations of the Round Robin II measurements using manual pipetting from the definitive method values. The analyzed pools are identified by the numbers 1, 3, 4, 5, and 7 near the results from laboratory 4. The designations are similar for the remaining results. The numbers 1 and 2 placed directly above the laboratory number, designate the first day and subsequent day test results, respectively.

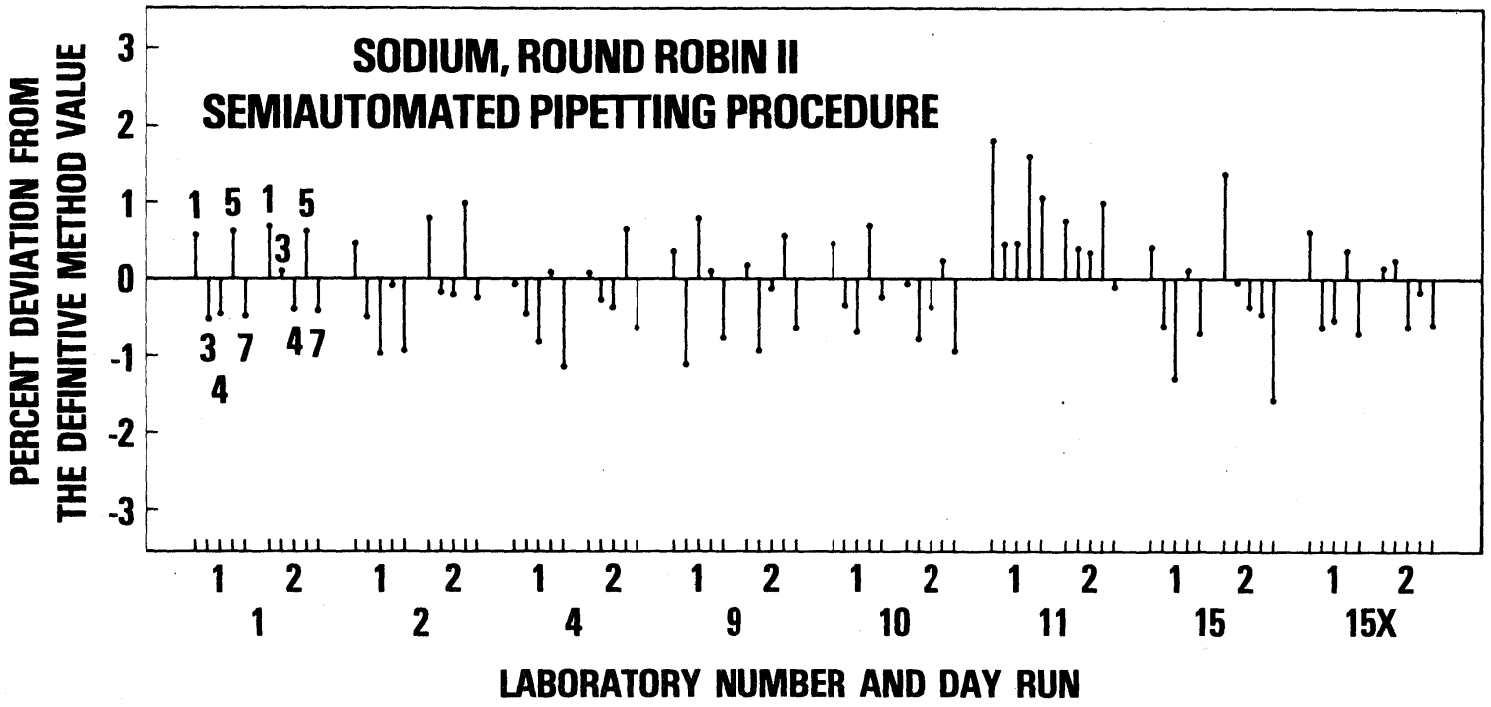

Figure 6. Percent deviations of the Round Robin II measurements using semiautomated pipetting from the definitive method values. The pools are identified by the numbers 1, 3, 4, 5, and 7 near the results from laboratory 1. The designations are the same for the remaining results. The numbers 1 and 2, placed directly above the laboratory number, designate the first day and subsequent day test results, respectively.

Table 9. Percent deviations from averages for sodium in serum from Round Robin II, manual pipetting protocol.

Laboratory[a]	Pool 1	Pool 3	Pool 4	Pool 5	Pool 7
4-1	.04	.07	.07	-.15	-.24
4-2	-.18	-.20	-.58	-.30	-.14
5-1	.33	.36	.07	1.78	-.10
5-2	.32	.30	.13	.56	.06
7-1	.36	-.28	-.29	-.20	.12
7-2	-.07	-.02	-.42	-.48	-.04
8-1	.15	.35	.08	.10	.27
8-2	-.29	-.28	-1.17	-1.15	-.20
9-1	-.48	-.65	-.05	-.09	.27
9-2	-.45	-.74	.33	-.41	-.52
11-1	.32	.12	.06	.12	.01
11-2	.14	.23	.55	-.95	-.04
13-1	.02	.66	1.05	.96	.36
13-2	-.21	.01	.18	.21	.19
15-1	4.56	1.67	2.55	1.56	2.81
15-2	3.90	3.77	3.83	2.21	1.89
Averages used in calculations[b]	113.52	129.28	136.25	147.19	157.78

[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] The averages do not include data from Laboratory 15.

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

Laboratory[a]	Pool 1	Pool 3	Pool 4	Pool 5	Pool 7
1-1	.02	-.20	-.17	.14	.10
1-2	.13	.39	-.10	.15	.18
2-1	-.10	-.16	-.71	-.56	-.40
2-2	.24	.51	.47	.53	.38
4-1	-.59	-.13	-.53	-.40	-.56
4-2	-.53	.03	-.09	.22	-.05
9-1	-.20	-.77	1.10	-.37	-.15
9-2	-.40	-.60	.23	.06	-.08
10-1	-.09	-.06	-.38	.20	.35
10-2	-.59	-.46	-.08	-.29	-.29
11-1	1.24	.77	.73	1.13	1.17
11-2	.19	.69	.70	.50	.48
15-1	-.12	-.31	-1.08	-.41	-.10
15-2	.79	.28	-.10	-.90	-1.03
Averages used in calculations[b]	113.82	129.47	136.21	146.99	157.65

[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] The averages include results obtained only from the semiautomated procedure.

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 11-12). If all of the participating laboratories were of the same population in regard to replication error, the standard deviation ratios reported in Tables 11-12 would be larger than 2.40 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 than 2.40, 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.)

Table 11. Ratios of standard deviations to average standard deviations for sodium in serum from Round Robin II, manual pipetting protocol.

Laboratory[a]	Pool 1	Pool 3	Pool 4	Pool 5	Pool 7
4-1	.60	.04	.25	.05	1.33
4-2	.98	.38	.36	.09	.44
5-1	.00	.57	.36	7.01	.12
5-2	.60	.34	.42	.11	.21
7-1	1.59	1.29	.00	.37	.86
7-2	.33	.76	.56	.09	.47
8-1	.16	3.45	4.46	.94	3.28
8-2	6.07	2.42	.72	1.31	2.31
9-1	.22	.83	.72	.55	.80
9-2	1.91	.38	1.34	.30	.27
11-1	.16	.04	.61	.16	2.73
11-2	.33	.51	1.28	1.22	.74
13-1	.22	1.86	2.08	.92	.33
13-2	.82	1.14	.84	.87	.12
15-1[b]	1.31	6.88	1.38	.13	1.15
15-2[b]	2.07	1.44	2.23	1.02	.47
Average Standard Deviation,[c] mmol/L	.259	.373	.508	.615	.478

[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] The results from this laboratory were not included in any pooled values.

[c] The average standard deviations do not include Laboratory 15 values.

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

Laboratory[a]	Pool 1	Pool 3	Pool 4	Pool 5	Pool 7
1-1	1.25	.41	.60	1.38	1.77
1-2	.13	.33	.11	.30	.42
2-1	.42	.74	2.14	1.75	1.31
2-2	2.62	.60	1.63	.04	1.48
4-1	.04	.79	.19	.35	.51
4-2	.40	1.12	1.09	2.11	.08
9-1	3.71	.29	3.11	.39	1.43
9-2	1.16	.50	.30	1.12	.42
10-1	.04	.58	1.24	1.42	.67
10-2	.31	.66	1.20	.13	.08
11-1	2.06	2.17	.73	1.96	2.83
11-2	.76	.27	.56	.47	.84
15-1	.54	1.99	.96	.35	1.48
15-2	.54	3.56	.15	2.22	.67
Average Standard Deviation,[b] mmol/L	.316	.342	.377	.328	.168

[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] The average standard deviations include results obtained only from the semiautomated procedure.

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 [13]:

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

where:

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

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

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

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

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

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

ε_{ijk} = a random replication error.

The above model is quite general and extensive experience has shown that it is well suited to describe a number of measurement factors in inter-laboratory tests [14].

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 15 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 13.

From the analyses, it was observed that the ranges of values for the $\hat{\sigma}_{\varepsilon}$ and $\hat{\sigma}_{D}$ components of standard deviation were small, and that the values did not depend significantly on the sodium concentration. Because of this, only average $\hat{\sigma}_{\varepsilon}$ and $\hat{\sigma}_{D}$ values are reported. The trend of $\hat{\sigma}_{L}$ as a function of concentration, however, was large enough to justify reporting smoothed $\hat{\sigma}_{L}$ values as a function of concentration.

Table 13. Components of standard deviation in mmol/L for sodium in serum, Round Robin II.

Manual Pipetting Protocol
(Pooled Results from 7 Laboratories)

Sodium Level, mmol/L	$\hat{\sigma}_{\varepsilon(\text{Repl.})}$	$\hat{\sigma}_{\text{Day}}$	$\hat{\sigma}_{\text{Lab}}$
113.5	.73	0	.36
129.3	.73	0	.42
136.3	.73	0	.45
147.2	.73	0	.50
157.8	.73	0	.54

Semiautomated Pipetting Protocol
(Pooled Results from 7 Laboratories)

Sodium Level, mmol/L	$\hat{\sigma}_{\varepsilon(\text{Repl.})}$	$\hat{\sigma}_{\text{Day}}$	$\hat{\sigma}_{\text{Lab}}$
113.8	.41	.51	.20
129.5	.41	.51	.25
136.2	.41	.51	.36
147.0	.41	.51	.55
157.6	.41	.51	.75

Because of the relatively small size of the sodium round robin tests we must consider the individual components of standard deviation to be only advisory in nature. The nature of analysis of variance calculations is such that, if one obtains an overly large component of replication $\hat{\sigma}_{\varepsilon}$ due to restricted rejection of outliers, a low value for $\hat{\sigma}_{Day}$ results. In other words, restricted rejection of values having large deviations may result in some misclassification of the components of variability. We judge, from past experience, that this may have occurred for the ($\hat{\sigma}_{\varepsilon}$, $\hat{\sigma}_{Day}$) pair for the sodium manual procedure. This is not of great concern since the final practical statements of uncertainty involve 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 14, and are calculated as follows:

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

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:

Table 14. Summary of imprecision and bias results in mmol/L for sodium in serum, Round Robin II.

	1σ Precision						
	Manual Pipetting Protocol				Semiautomated Pipetting Protocol		
Na Level	$\hat{\sigma}_{comp}$	$\hat{\sigma}_{within}$	$\hat{\sigma}_{total}$	Goal	$\hat{\sigma}_{total}$	$\hat{\sigma}_{within}$	$\hat{\sigma}_{comp}$
114	.19	.36	.51	1.5	.46	.41	.18
129	.21	.36	.56	1.5	.48	.41	.18
136	.22	.36	.58	1.5	.55	.41	.21
147	.23	.36	.62	1.5	.69	.41	.26
158	.25	.36	.65	1.5	.86	.41	.33

	Accuracy			
	Manual Pipetting Protocol		Semiautomated Pipetting Protocol	
Na Level	Round Robin Composite Bias $(\bar{X}_{obs}-X_{DM})$	Goal	Round Robin Composite Bias $(\bar{X}_{obs}-X_{DM})$	
114	.3	±2.0	.6	
129	-.6	±2.0	-.5	
136	-.3	±2.0	-.4	
147	.9	±2.0	.7	
158	-.8	±2.0	-1.0	

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

Columns four and six in the top section of Table 14 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 goal has been met at the nominal serum sodium value of 140 mmol/L.

The standard errors of the round robin composite values are given in columns two and eight of the top section of Table 14. These standard errors are calculated from the components of standard deviation as follows:

$$\hat{\sigma}_{Comp} = \sqrt{\frac{1}{7}\left(\frac{\hat{\sigma}_{\varepsilon}^2}{4} + \frac{\hat{\sigma}_D^2}{2} + \hat{\sigma}_L^2\right)} \quad . \qquad (6)$$

The bottom section of Table 14 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 15 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 composite round robin values are given to two decimal places as these numbers express the averages of the numerous measurements made in the round robin. The definitive method values are given to

Table 15. Summary of sodium in serum values ±2 standard errors.

Round Robin II - Composite Values (mmol/L)		Definitive Method Values (mmol/L)
Manual	Semiautomated	
113.52 ± 0.38	113.82 ± 0.36	113.2 ± 0.6[a]
129.28 ± 0.42	129.47 ± 0.36	129.9 ± 0.6
136.25 ± 0.44	136.21 ± 0.42	136.6 ± 0.6
147.19 ± 0.46	146.99 ± 0.52	146.3 ± 0.6
157.78 ± 0.50	157.65 ± 0.66	158.6 ± 0.6

[a] 95% confidence limits.

one decimal place since they are less numerous results made in a single laboratory.

The accuracy of the round robin results is within the recommended goal of the reference method. There does not appear to be any significant bias for either the manual or the semiautomated techniques over the range of sodium concentrations studied.

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 16-17. Straight line least square fits were made to these data and the resultant standard deviations of fit are given in Table 18. These standard deviations of fit are expressed in units of sodium concentration (mmol/L). Our analysis indicates that if in the calibration step it is found that any calibration point deviates from the calibration curve by more than 1.0 mmol/L, then the standard solutions and the instrument should be checked for sources of excessive error before proceeding further into the analysis.

Table 16. Calibration curve data for sodium in serum, Round Robin II using manual pipetting.

Lab. No.[a]		Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Std. 6
4-1	X[b]	109.98	119.98	129.98	139.98	149.97	159.97
	Y[c]	109.70	119.60	129.80	140.20	150.10	160.20
4-2	X	109.98	119.98	129.98	139.98	149.97	159.97
	Y	110.10	120.40	130.60	140.80	150.60	160.40
5-1	X	110.00	120.00	130.00	140.00	150.00	160.00
	Y	110.60	120.40	130.40	140.40	150.90	160.00
7-1	X	109.69	120.24	129.97	139.78	150.13	160.07
	Y	110.20	120.20	130.10	139.50	149.60	158.90
7-2	X	109.69	120.24	129.97	139.78	150.13	160.07
	Y	110.10	119.90	130.00	139.20	149.30	158.50
8-1	X	110.00	120.00	130.00	140.00	150.00	160.00
	Y	113.60	122.40	131.70	141.60	151.00	160.00
9-1	X	109.70	120.20	130.00	140.30	150.40	160.10
	Y	67.60	73.80	80.60	87.30	92.80	98.20
9-2	X	109.80	120.00	130.10	140.10	150.00	160.00
	Y	547.00	600.00	653.00	704.00	754.00	795.00
11-1	X	110.00	120.15	130.04	140.08	149.97	160.08
	Y	107.60	118.30	129.10	140.00	150.10	160.20
11-2	X	110.07	120.53	130.06	141.42	150.37	160.74
	Y	108.40	118.60	128.70	140.20	149.50	160.40
13-1	X	110	120	130	140	150	160
	Y	110	120	130	140	150	160
15-1	X	110.00	120.00	130.00	140.00	150.00	160.00
	Y	110.28	118.18	130.02	139.02	149.24	157.66
15-2	X	110.00	120.00	130.00	140.00	150.00	160.00
	Y	109.08	118.96	128.52	137.54	146.74	156.40

[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 17. Calibration curve data for sodium in serum, Round Robin II using semiautomated pipetting.

Lab. No.[a]		Std. 1	Std. 2	Std. 3	Std. 4	Std. 5	Std. 6
1-1	X[b]	110.00	120.00	130.00	140.00	150.00	160.00
	Y[c]	107.70	118.00	128.40	138.70	148.40	160.00
1-2	X	110.00	120.00	130.00	140.00	150.00	160.00
	Y	108.00	118.30	128.70	139.30	149.10	160.00
2-1	X	110.00	120.00	130.00	140.00	150.00	160.00
	Y	108.90	119.70	129.70	140.50	150.50	159.90
2-2	X	110.00	120.00	130.00	140.00	150.00	160.00
	Y	108.50	118.90	129.10	139.10	149.10	159.90
4-1	X	109.98	119.98	129.98	139.98	149.97	159.97
	Y	109.10	119.80	129.80	139.90	149.90	159.50
4-2	X	109.98	119.98	129.98	139.98	149.97	159.97
	Y	109.90	120.10	130.30	140.60	149.90	159.80
9-1	X	109.70	120.20	130.00	140.30	150.40	160.10
	Y	60.80	65.00	71.10	77.50	82.00	87.20
9-2	X	109.80	120.00	130.10	140.10	150.00	160.00
	Y	465.00	509.00	551.00	595.00	637.00	683.00
10-1	X	110.00	120.00	130.00	140.00	150.00	160.00
	Y	109.30	119.60	130.30	140.30	150.60	160.60
10-2	X	110.00	120.00	130.00	140.00	150.00	160.00
	Y	108.20	119.00	129.70	140.20	150.80	161.00
11-1	X	111.99	120.24	130.85	139.98	150.61	160.21
	Y	109.20	117.56	128.20	138.46	149.16	159.13
11-2	X	110.18	120.92	131.87	141.69	150.11	160.46
	Y	109.13	120.40	131.16	141.50	149.56	160.26
15-1	X	110.00	120.00	130.00	140.00	150.00	160.00
	Y	111.44	121.90	131.12	140.76	150.70	160.00
15-2	X	110.00	120.00	130.00	140.00	150.00	160.00
	Y	111.62	122.18	132.16	141.06	151.04	160.00

[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 results for sodium in serum as standard deviation of fit (s_{fit}) in mmol/L.

Manual		Semiautomated	
Laboratory Number[a]	s_{fit}	Laboratory Number[a]	s_{fit}
4-1	.14	1-1	.43
4-2	.23	1-2	.22
5-1	.32	2-1	.45
7-1	.22	2-2	.23
7-2	.30	4-1	.32
8-1	.33	4-2	.33
9-1	.87	9-1	1.24
9-2	.79	9-2	.28
11-1	.34	10-1	.21
11-2	.23	10-2	.18
13-1	--	11-1	.27
15-1	.96	11-2	.22
15-2	.29	15-1	.32
		15-2	.56

[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.

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 sodium, the similarity of results obtained by White and Mavrodineanu using FAES and the similarity of their results with those obtained using the highly specific IEG and NAA methods, 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 [3,6,16], 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 19. Internal and non-internal standard instruments for which nine laboratories used 200-fold dilution 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 bracketing 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 sodium concentrations from 110 to 160 mmol/L. More than 80 percent of the calibration curves showed standard deviations of fit of about 0.5 percent and the remainder about 1 percent. A standard deviation of fit larger than 1 percent would clearly warrant a laboratory's investigation of its operation of the procedure and/or preparation of the standard solutions.

Table 19. Instruments and operating conditions used by the participating laboratories in RRII.

Lab. #	Instrument	Burner	λ, nm	Flow Rates–Pressure		Dilution	Pipetting Alternatives	
				Air	Propane		M[a]	SA[a]
1	IL143	Premix[b]	589	30 psig	110 psig	200	-	X
2	IL143	Premix[b]	589	0.45 L/min	1.42 L/min	200	-	X
4	IL143	Premix[b]	589	NR[c]	NR[c]	200	X	X
5	IL343	Premix[b]	589	0.5 CFM	NR[c]	200	X	-
7	IL343	Premix[b]	589	7.5 SCF	NR[c]	200	X	-
8	IL343	Premix[b]	589	30 psig	25 psig	200	X	-
9	--[d]	--[d]	589	20.6 psi[e]	0.45 psi[e]	200	X	X
10	Beckman, Klina	Total Consumption	591[f]	5.9 L/min	0.35 L/min	100	-	X
11	IL443	Premix[b]	589	30 psig	30 psig	200	X	X
13	Beckman, Klina	Total Consumption	591[f]	NR[c]	NR[c]	100	X	-
15	IL343	Premix[b]	589	0.5 SCF	110 psig	200	X	X

[a] M = manual; SA = semi-automated.
[b] Glass chimney.
[c] Not reported.
[d] Instrument built in laboratory.
[e] Used air-acetylene.
[f] 591 nm reported.

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 the 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.

With the exclusion of results from one laboratory using the manual procedure, the imprecision and bias goals of ±1.5 and 2.0 mmol/L, respectively set at the 140 mmol/L level, 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 either the imprecision or bias values obtained by the two pipetting alternatives as evident in Table 14. In fact, except for the imprecision of the semiautomated procedure ($\hat{\sigma}_{total}$ = 0.86 mmol/L) at the 158.6 mmol/L level, all other imprecision and bias values are at least twice as good as the original goals set for the reference method by the Experts Committee. Thus 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 ±0.75 mmol/L and biases within 1.0 mmol/L can be expected in the performance of this reference method.

VI. CONCLUSIONS

A candidate reference method, specified by a written protocol for the determination of serum sodium by flame atomic emission spectroscopy was evaluated by comparing results of analyses run on serum and aqueous samples in a selected group of laboratories against definitive method values obtained on samples from the same pools. The results for samples having sodium concentrations in the 110 to 160 mmol/L range showed a total imprecision of approximately 0.75 mmol/L or less and a maximum bias of 1.0 mmol/L. Similar imprecisions and biases were found whether manual pipetting, requiring large sample volumes, or semiautomated pipetting, requiring small sample volumes, was used in the FAES procedure. An ion-exchange separation of sodium from the serum samples followed by its conversion into sodium sulfate for determination by gravimetry was used as the definitive method.

Statistical analysis of the results shows that the FAES method can be carried out with the accuracy and precision expected of a reference method for serum sodium. Hence, the candidate method can be considered to be the reference method. This reference method may be used to establish the accuracy of field methods for sodium by comparative testing. It may also be used to determine reference serum sodium 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, Chief, 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 and Mr. Carl Barry 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.

The Bureau of Medical Devices of The Food and Drug Administration is acknowledged for their financial support of this project.

VII. REFERENCES

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[16] Clinical reference methods are also being developed at NBS for potassium, 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. David Hassemer
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 919
Sodium 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 sodium and chloride ions in clinical analyses. The sample consists of highly purified sodium chloride. Chemical assay as well as analyses for specific impurities indicate that the material may be considered essentially pure, except for occluded moisture.

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. At room temperature sodium chloride is hygroscopic above 60 percent relative humidity. The sorbed water can be removed, however, by desiccation over freshly exposed P_2O_5 or $Mg(ClO_4)_2$ for 24 hours. Chloride was determined using the coulometric method of Marinenko and Taylor [J. Res. NBS, 67A, 31(1963)].

Based on 8 independent measurements of chloride content, the sample is considered homogeneous.

When the material is crushed and dried at 200 °C for 18 hours, the loss of moisture is about 0.08 percent. Coulometric determinations of chloride on the dried material indicate 99.995 ± 0.004 percent purity.

The sodium 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, J. R. Baldwin, M. Darr, and T. C. Rains.

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

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 T. W. Mears.

Washington, D. C. 20234
August 6, 1973
Revised November 23, 1973

J. Paul Cali, Chief
Office of Standard Reference Materials

(over)

Supplemental Information

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

Sodium was assayed using a gravimetric procedure in which the sodium chloride was converted to sodium sulfate. Approximately 250 mg of sodium chloride (dried at 500 °C for 4 hours in a platinum crucible) was dissolved in ultrapure sulfuric acid solution (1:1) and evaporated to dryness. Ammonium carbonate was added and the crucible slowly heated to 600 °C, then 900 °. This treatment was repeated until the weight of sodium sulfate remained constant. Based on 6 determinations, the sodium assay is 39.3_2 weight percent or 99.9_6 percent of the amount computed for perfectly pure, stoichiometric NaCl.

A semiquantitative survey for trace elements by emission spectroscopy indicated less than 10 μg/g calcium, copper, iron, and magnesium. A value of less than 3 μg/g magnesium was obtained by atomic absorption spectrometry. Flame emission spectrometry indicated the presence of the following elements: potassium, 11 μg/g; calcium and cesium, less than 2 μg/g; and rubidium and lithium less than 0.5 μg/g.

APPENDIX C

DEFINITIVE METHOD

The Determination of Serum Sodium by Ion Exchange-Gravimetry (IEG)

I. Introduction

One of the classical analytical techniques for the determination of sodium is gravimetry as sodium sulfate [1][1]. The major difficulty in this procedure is obtaining a precipitate of constant weight. This is usually due to the formation of sodium bisulfate which is difficult to decompose into sodium sulfate. Lundell and Hoffman suggest treating the weighed original residue with water, and then evaporating, igniting, and reweighing until constant weight is obtained. Below 700 °C, there is no loss of sodium sulfate due to volatilization of the precipitate. This procedure was adapted for the determination of sodium in serum by the application of ion-exchange chromatography as a quantitative scheme for separating the sodium from serum. The method which follows describes the details of the sodium determination using a chromatographic separation of sodium from serum and its subsequent conversion into sodium sulfate.

To minimize sampling errors, the serum samples were taken by weight. Results were converted from a weight/weight to a weight/volume basis by measuring the density of three serum pools, using a pycnometer. The density of the remaining serum pools were determined by interpolation, Table 1. Following previous practice at this laboratory, all density measurements are corrected to 23 °C which is approximately the mean laboratory temperature. Concentration values reported, therefore, are also at 23 °C.

---

[1]Figures 1 and 2 in brackets denote references at the end of this appendix.

The ion-exchange chromatography is fairly routine but some precautions should be observed. The columns should be prepared by adding a slurry of resin and water to a column largely filled with water, so that relatively few air bubbles are entrained. If a column is stored overnight, it should be tightly capped with clean polyethylene film to prevent drying. The sample should be added to the column in small increments; all of the initial increment should enter the resin bed before adding more sample increments.

With some samples, the serum may be deproteinized by the hydrogen ions that are released during the exchange of sample cations. In severe cases, the column flow-rate will be restricted enough to warrant using a Teflon stirring rod to break up the cake of material at the top of the column. Other than slowing the rate of elution, the deproteinization does not effect the separation of sodium. After the column has been washed with water to remove the non-absorbed components of serum, the cations are eluted with 0.4 mol/L HCl.

The normal order of elution with this reagent is Li^+, Na^+, K^+, Mg^{++}, and Ca^{++}. Fortunately, there is usually little Li in serum so that the separation of sodium from lithium is ordinarily easy. For sera with elevated Li values, the separation may not be complete; however, analyses of the Li and Na fractions by FAAS and FAES permit appropriate corrections to be made. In eluting with the 0.4 mol/L HCl, it is important to add small increments of the reagent without disturbing the resin bed, allowing each addition to enter the resin bed before adding the next. Failure to observe these precautions may produce band-broadening and complicate the separation of sodium from potassium. Because the amount of resin in each column and the efficiency of the chromatography may vary slightly, it is very important to use a flame test to judge when to collect the various fractions. Usually it is only the sodium/potassium separation that poses a problem and, if it is suspected that poor separation has occurred, the

post-sodium fraction can be examined by FAES to determine the presence of sodium and suitable corrections then can be made. Similarly, after the sample has been ignited and weighed as sodium sulfate, the sodium fraction can be checked for contamination by FAES (K, Li) or FAAS (Ca, Mg, Li, etc.) For the analyses reported here, corrections were negligible. Most of the impurities that were observed are believed to have come from the platinum crucible used for the ignition. A reddish-brown to black discoloration of the sodium sulfate, observed after ignition, may be attributed to small amounts of dissolved platinum.

The platinum crucibles were cleaned and ignited at 900 °C to constant weight before they were used in this procedure. Because of the relatively large mass of the crucible (∿15 g) compared to the Na_2SO_4 (∿50 mg) weighing errors following the ignition can be the most troublesome part of the analysis. To improve weighing accuracy, several empty platinum crucibles were used as weighing tares to correct for changes in temperature, barometric pressure, and relative humidity which caused day-to-day variations in the weights of the empty crucibles. By correcting for such day to day changes, it was possible to determine the weights of the ignited samples more accurately. When sucessive ignitions yielded only a loss of approximately 10 µg, a sample was considered to be at constant weight. Some slight loss of mass will occur during each ignition, therefore the number and duration of sample ignitions should be kept to a minimum. Crucibles containing samples, standards, blanks, and tares should be ignited, cooled, and weighed in sets so that corrections or losses will be reflected to the same degree in all samples. A loss of more than 20 µg between ignitions was taken as evidence that the sample had not yet been ignited to constant weight. Usually, two ignitions are sufficient to achieve constant weight. Crucibles were left

only partially uncovered during the ignition to reduce the potential for loss of precipitate.

II. Samples and Solutions

A. Samples: Three samples from each of the seven serum pools, two blanks and seven standards were analyzed by the procedure summarized in Section III.

B. Reagents: All acids and water used were purified by a sub-boiling distillation technique, from quartz stills [2].

C. Sodium Standard Solutions: Standard sodium solutions were prepared from NaCl (SRM 919) and KCl (SRM 918). The concentrations of sodium and potassium were equivalent to those in normal serum.

D. Chromatographic Columns: Fifteen ion-exchange chromatographic columns were prepared (AG 50X8 resin, 100-200 mesh, 0.9x30 cm) and pretreated by washing with 100 mL of 5 mol/L HCl, followed by rinsing with water until the effluent was neutral. [Note: The samples, blanks, and standards were divided into two groups for analysis at different times since a maximum of only 15 columns could be handled efficiently by one person.]

E. Blanks: Water was used as the blank.

III. Procedure

Weighed samples of serum ($\sim$8 g) were taken as follows: The frozen serum samples were allowed to thaw and equilibrate to room temperature. Each serum sample was mixed during this time by inverting the vials at least 20 times. Then, a sample was drawn from a vial into a clean plastic syringe through a platinum needle and the syringe containing the serum was weighed on a semi-microbalance. After the serum

was discharged into a clean Teflon beaker, the emptied syringe was re-weighed. A clean syringe and needle combination was used for each sample.

Each weighed sample was diluted to ∿30 mL and loaded onto a separate column a few milliliters at a time. About 100 mL of distilled water was used to wash non-ionic matter from each column. The effluent from each column was monitored qualitatively by a flame test for sodium. The sodium fraction did not elute exactly between 105 and 260 mL, as described below; however, the exact volumes for fractionating were easily recognized by the flame test. [NOTE: For normal serum, any error due to Li contributing to the Na fraction will be insignificant if the first cut at about 105 mL is made when the first positive flame test for sodium is obtained. The fraction at about 260 mL is determined when the yellow flame test for sodium is barely detectable but before the flame test for potassium is evident when viewed through a cobalt-blue glass. A record of flame test results indicates whether any of the eluted fractions need to be checked by FAES or FAAS to determine appropriate corrections.]

The elution was begun with 0.4 mol/L HCl. Small volumes (<5 mL) were added. Each was allowed to pass into the column before adding the next volume of acid. The first 75 mL of effluent was discarded. The 75-105 mL fraction, the 105-260 mL fraction containing the sodium, and the 260-300 mL fraction containing potassium were saved. If the flame tests gave a positive sodium indication with the 75-105 mL and/or the 260-300 mL fractions, FAES was used to determine the amounts of sodium present in these fractions. The fraction containing the sodium eluted from each column was evaporated to about 5-10 mL in a Teflon beaker on a hot plate. The concentrated samples were transferred quantitatively from the beakers to previously ignited and weighed platinum crucibles. Small volumes of water were used to complete the transfer. The samples were then evaporated to dryness on the hot plate.

When cool, the crucible walls were treated with water to wash residues to the bottoms of the crucibles. Then a few drops of concentrated H_2SO_4 were added and the samples were heated to dryness. When the samples were again cool, the procedure was repeated. Finally, a lump of ammonium carbonate (∿0.5 g) was added to each crucible and the crucibles were partly covered with platinum covers. [NOTE: The ammonium carbonate helps prevent the formation of sodium bisulfate.] The crucibles were heated slowly in a muffle furnace (∿1.5 h) to 900 °C, and held at that temperature for 15-20 minutes. After the furnace had cooled sufficiently to permit retrieval with asbestos gloves and platinum tongs, the crucibles were transferred to a desiccator to cool overnight. The crucibles were weighed the next day. Then a few drops of water were added to each residue, the water was evaporated, and the samples were again ignited, stored in the desiccator, and weighed. This process was continued until constant weight was achieved. Ignite samples no more than necessary. Carry all blanks and standards through the same procedure.

IV. Results

The sodium concentrations and the densities determined for the seven serum pools are summarized in Table 1.

V. Analysis of Estimated Errors

A. Samples: The total uncertainty in sampling by weight (∿8 mL serum, weighing by difference) is ±0.004 percent. The uncertainty in the density determinations are approximately ±0.02 percent. Under the worst conditions the maximum error due to sampling and density determinations would be ±0.05 percent.

Table 1. Sodium concentrations and densities determined for the seven serum pools.

Serum	Density g/mL	- - Sodium Concentration in Serum - - Replicates, mmol/L	Average, mmol/L
1	1.0248	113.98 112.72 112.91	113.2± 0.6[a]
2	1.0255[b]	120.47 120.95 121.54	121.0± 0.6[a]
3	1.0262[b]	129.91 129.70 130.04	129.9± 0.6[a]
4	1.0269	136.15 137.15 136.42	136.6± 0.6[a]
5	1.0276[b]	145.62 146.14 147.20	146.3± 0.6[a]
6	1.0283[b]	153.70 153.92 153.90	153.8± 0.6[a]
7	1.0290	158.29 158.46 158.95	158.6± 0.6[a]

[a] 95% confidence limit for the mean of the serum pool. (A pooled estimate of the standard deviation was used for this limit.)

[b] Extrapolated value of density.

B. Chromatography: The major source of error is incomplete resolution of sodium. This could result in partially compensating errors through loss of sodium to other elemental fractions and contamination of the sodium fraction. Considering these factors and actual analysis for sodium, lithium, potassium, calcium, and magnesium in suspect fractions as shown by the FAES and FAAS tests, the maximum error could be as much as ±0.2 percent.

C. Weighing and Ignition: Errors due to day-to-day weighing imprecision (caused by changes in humidity, barometric pressure, tares) and to the ignition process (volatilization of precipitate, etc.) could approach ±0.2 percent.

D. Recovery of Standards: The quantitative recovery of standards averaged 99.91 percent of theoretical with a standard deviation of ±0.15 percent. The estimated maximum error is thus ∿±0.2 percent.

E. Other Sources: Error due to non-quantitative solution transfer, spills, spatter, etc. are estimated at 0.2 percent.

F. Total Estimated Error: In the worst case, the values estimated for the various sources of error, as listed in Table 2, would be added to give a value for the total estimated error of 0.75 percent, which corresponds to 1.05 mmol/L at the 140 mmol/L sodium level. This estimated error is consistent with the experimentally observed random error.

Table 2. Estimated systematic errors in the various steps for the determination of sodium in serum by ion exchange separation followed by gravimetric determination as sodium sulfate.

	Source	Magnitude, %
1.	Sampling	0.05
2.	Chromatography	0.20
3.	Ignition	0.20
4.	Recovery of Standards	0.20
5.	Other	0.10
	Total	0.75

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

Supplementary Neutron Activation Analysis of Serum Pools

REPORT OF ANALYSIS

Type of Analysis: Neutron Activation, Nondestructive
Irradiation Facility: Californium-252 Facility
Element Reported: Na
Analysts: James E. Suddueth, Russell M. Morris, and Harry L. Rook

Purpose: To ascertain the Na concentration in bovine blood serum within a relative standard deviation of ± 1.0% or better.

Sample Preparation: Samples, as received, were thawed overnight and thoroughly mixed by gentle shaking and tumbling for a minimum of 5 minutes. The thorough mixing was carried out to negate the demonstrated stratification of sodium in the serum caused by freezing and thawing. Three ml of each sample and each standard was pipetted into a cleaned polyethylene rabbit. All rabbits were sealed with Carter's rubber cement.

Standards: Three concentrations of standard solutions were prepared by dissolving NBS Standard Reference Material 919, Sodium Chloride (clinical standard), in high purity water. Five samples were taken from a solution containing 2708 μg Na/g H_2O, two from a solution containing 3401 μg Na/g H_2O, and four from a solution containing 2966 μg Na/g H_2O. Before starting the analysis of the serum, a set containing each of the three standards was irradiated for 2-1/2 hours and counted for 2 hours. After decay, corrections and calculations were made; the relative percent standard deviation among the set was 0.2 percent.

Blanks: Two blanks were run with the same procedure as any other standard or sample except they contained high purity water. The counts from background were subtracted from the counts from the blanks. The total blank was less than 2 μg Na/g.

Procedure: Samples and standards were individually irradiated in the Californium-252 facility for 2-1/2 hours at a source distance of 3 cm. After irradiation, the outside of each capsule was cleaned with conc. HNO_3, rinsed, and wiped dry.

All samples were allowed to decay at least 12-1/2 hours to allow chlorine and argon (if present) to completely decay. The samples were counted 2 hours each in a NaI(Tl) well detector. The maximum decay variations between samples was 112 min and individual corrections were made.

The counting system consisted of the detector, preamp, amplifier, two single channel analyzers, two scalers and a timer. One SCA was set to integrate the 1.368 MeV peak area and the other to integrate the 2.75 MeV peak area. Each SCA was fed to a separate scaler. This allowed a ratio check to be made on the two peak areas to determine if any interferences were present. A sodium citrate sample was run initially to obtain a ratio with only Na present in the sample. One sample was counted on a 25 cc Ge(Li) detector and no interferences to the two ^{24}Na photo-peaks were observed.

Calculation: The two peak's activities were corrected to each separate daily run, added together and the average blank and background activity were subtracted.

The results were calculated by the comparator method. The standard activity used was obtained from a pooled set of all standards data obtained during the analytical period.

Errors: The estimate of analytical error was obtained by the standard deviation among the 14 standards. The standard deviation of the mean was .76% relative. This is a valid estimate on the error of the total analytical system.

Results: Precision for all lots was .5% relative standard deviation or better. The results are shown in Tables I and II. Table III gives the average results for each lot, corrected for density, as millimoles per liter.

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Table I

Standard No.	Counts/μg Na 2 hr.
* 1	134.38
* 2	131.72
* 3	134.08
1	135.15
2	135.58
3	134.57
1	134.53
1	133.46
1	134.54
3	133.23
1	134.88
3	134.75
2	132.94
3	133.34

Mean 134.08

Relative Standard Deviation: 0.76%

*Standards used to check calculated concentrations.

Table II

Lot	Sample No.	Sample Result µg/g	Mean µg/g	Relative Standard Deviation
1	258243	2576		
1	223776	2553	2562	.5%
1	223776	2556		
2	253001	2738		
2	253001	2724	2728	.3%
2	53408	2721		
3	663097	2935		
3	663097	2932	2928	.3%
3	223696	2917		
4	531706	3078		
4	531706	3085	3077	.3%
4	632506	3069		
5	291349	3316		
5	291349	3328	3324	.2%
5	729874	3327		
6	432826	3445		
6	432826	3467	3459	.4%
6	30284	3465		
7	313203	3530		
7	313203	3552	3546	.4%
7	244982	3555		

Table III

Lot	$[Na^+]$, mmol/L
1	114.2
2	121.7
3	130.7
4	137.4
5	148.6
6	154.7
7	158.7

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. 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 changes may necessitate appropriate correction.

Note 2:

Glassware Required:

a) Manual pipetting alternative:

Volumetric Flasks: (for one hundred-fold dilutions): one 2-L; one 1-L; six 100-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 100-mL; eight 1-L plus one additional 1-L volumetric flask for each sample.

Pipets: one 5-mL.

b) Semiautomated pipetting alternative:

Volumetric Flasks: (for one hundred-fold or two hundred-fold dilutions): One 2-L; one 1-L; six 100-mL; and seven 50-mL plus one 50-mL volumetric flask for each sample.

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 Device: The accuracy and precision of the device is determined by weighing fixed volumes of water repetitively delivered by the device.

1. The water is delivered into tared, stoppered flasks that are 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 0.250 mL or 0.500 mL delivery volumes 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 ten filled bottles.
(5) Calculate the weight of each aliquot (water) 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 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 aliquots delivered (from part d).

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

Table 43. — Indicated capacity 100 mL.

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

1) Determine the volume of the nominally 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 LiCl diluent (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 KCl diluent are to be subtracted from the readings for the standards. Additionally, if the LiCl 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 completing a round robin test.

ELECTROLYTES IN SERUM — CLINICAL REFERENCE METHOD

ION Na

LABORATORY 8 ANALYST TN/JK

EXERCISE NO. RRII

DATE SAMPLES RECEIVED 12/10/75 DATES ANALYZED (1) 1/13/76 (2) 1/16/76

INSTRUMENT MANUFACTURER IL MODEL 343

WAVELENGTH 589 NM SCAN _____ SIT ON PEAK MAX _____

SLIT WIDTH _____ µM

BURNER TYPE Glass Chimney, Premix Design

OXIDANT Air FLOW RATE 0.5 St. Feet^3 L/MIN 30 psig

FUEL Propane FLOW RATE _____ L/MIN 25 psig

INSTRUMENT TIME CONSTANT 30-50 S

RECORDER TIME CONSTANT 7 S

READOUT: RECORDER _____ , DIGITAL X , OTHER _____

LABORATORY TEMPERATURE 18 °C TO 22 °C (VARIATION DURING ROUND ROBIN)

COMMENTS: 1. The results were achieved after optimizing the instrument by the adjustments proposed in the maintenance manual.

2. The linearity of the detector is still not satisfactory, but we are working on the problem.

DATA REPORTING SHEET FOR VALID MEASUREMENTS

PROTOCOL USED: MANUAL X SEMI-AUTOMATED _____

LAB 8 ION ____ ROUND ROBIN II DATE ANALYZED 1/13/76 OPERATOR TN

SAMPLE # 943277

STANDARD CONCENTRATIONS MMOL/L (CALCULATED)	VALID SET	RELATIVE INTENSITIES LO STD (X_1)	SAMPLE (Y)	HI STD (X_2)	$\hat{C}$
LO 140.0 (C_1)	1.	143.3	148.9	151.2	147.1
HI 150.0 (C_2)	2.	143.1	148.6	151.3	146.7
	3.	143.0	148.7	151.4	146.8
	4.	143.0	148.9	151.5	146.9
	5.	142.9	148.9	151.4	147.1

SAMPLE # 121276

STANDARD CONCENTRATIONS MMOL/L (CALCULATED)	VALID SET	RELATIVE INTENSITIES LO STD (X_1)	SAMPLE (Y)	HI STD (X_2)	$\hat{C}$
LO 110.0 (C_1)	1.	114.8	118.0	123.6	113.6
HI 120.0 (C_2)	2.	114.6	118.0	123.8	113.7
	3.	114.7	118.1	123.7	113.8
	4.	114.9	118.0	123.7	113.5
	5.	114.6	118.0	123.7	113.7

SAMPLE # 331174X

STANDARD CONCENTRATIONS MMOL/L (CALCULATED)	VALID SET	RELATIVE INTENSITIES LO STD (X_1)	SAMPLE (Y)	HI STD (X_2)	$\hat{C}$
LO 150.0 (C_1)	1.	151.1	158.2	160.0	158.0
HI 160.0 (C_2)	2.	151.3	158.6	160.0	158.4
	3.	151.3	158.5	160.3	158.3
	4.	151.1	158.1	159.3	158.5
	5.	151.4	158.3	160.0	158.0

DATA SHEET: STANDARD CURVE

PROTOCOL USED: MANUAL X SEMI-AUTOMATED _____

STANDARD	CALCULATED ION CONCENTRATION, MMOL/L	RELATIVE INTENSITY VALUES	CORRECTED RELATIVE INTENSITY VALUES
1	110.0	113.6	
2	120.0	122.4	
3	130.0	131.7	
4	140.0	141.6	
5	150.0	151.0	
6	160.0	160.0	
DILUENT BLANK	—		
LITHIUM BLANK	—		