Eur J Clin Chem Clin Biochem 1997; 35(5):387–392 © 1997 by Walter de Gruyter · Berlin · New York

Intermethod Variability of Sodium and Potassium Results: Patients Sera and Commercially Available Control Sera

Giampaolo Cattozzo[1], *Alessandra Fabi*[2] and *Carlo Franzini*[3]

[1] Laboratorio di Analisi, Ospedale C. Ondoli, Angera (Varese), Italy
[2] Laboratorio di Analisi, Ospedale F. Del Ponte, Varese, Italy
[3] Università di Milano, Istituto di Scienze Biomediche Ospedale L. Sacco, Milano, Italy

Summary: Sodium and potassium were measured in sets of 102 to 107 patients sera, and in 31 commercially available control sera. The results from four routine analytical methods/systems (indirect potentiometry: two; direct potentiometry and enzymatic assay: one each) were compared with those from a flame photometry-based reference method. In the assay of patient sera, substantial agreement was observed in some comparisons, clinically relevant bias in others. The inter-assay changes observed for the control sera differed significantly from those shown by the patients sera (i. e. commercial control sera were non-commutable) in about 12% of the comparisons, as a whole. Recalculation of serum sample results with a single control serum as calibrator lowered or increased the bias originally present according to whether the serum itself was commutable or not. Moreover, the inter-method variability in the assay of commercial control sera was lower with commutable sera, higher with non-commutable sera. With the exception of liquid sera stabilized with ethylene glycol, there was no evident link between any specific characteristic of the commercial control sera (matrix and physical state) and their degree of commutability.

Introduction

Methods based on "indirect" potentiometry (diluted sample), with ion-selective electrodes, are widely used for the clinical measurement of sodium and potassium in serum. Alternative analytical approaches include "direct" potentiometry (undiluted sample), also widely used (1), and enzyme activation-based photometric methods (2, 3). Flame atomic emission photometry is mainly used for reference purposes (4).

The very low intra-individual biological variation of serum sodium and potassium (5) requires accurate measurements (6, 8). On the other hand, methods based on different principles may be expected to give different results, more so if the different methods respond to different, although related, quantities, either substance concentration or ion activity (1). It has been suggested that method-dependent results be equalized by making them consistently traceable to flame photometry, by means of adequate calibration processes, in spite of different analytical principles and different quantities measured (1).

In practice, more or less extensively processed sera are used in pursuing or monitoring trueness. The inter-method behaviour of such commercially available sera may differ from that of fresh patients sera. The term "commutability" was first suggested to refer to *"the ability of an enzyme material to show inter-assay changes comparable to those of patient sera"* and it was later extended to non-enzymic components in control sera (9). Many commercially available control sera have been shown to lack such a property (10, 11), and fresh-frozen human serum proved to be the best material for transferring the trueness from reference to ion-selective electrode measurements of sodium and potassium (1).

We assessed the trueness of some routine measurement procedures by comparison with flame emission photometry, and we compared the inter-method behaviour of a number of commercially available control sera to that of patients sera. The effect of commutability of such sera on the trueness of routine results after recalibration, and on the inter-laboratory (inter-method) variability in their assay, was also evaluated.

Materials and Methods

Sets of 102 to 107 fresh patient serum samples, covering wide ranges of sodium and potassium concentrations, and 31 commercially available control sera were used in each comparison experiment (see tab. 2).

The routine measurements were performed with the following four methods/instruments/systems.

Method 1
Indirect potentiometry, E2A instrument, from Beckman, reagents and calibrators from the same source.

Method 2
Indirect potentiometry, Hitachi 717 analyzer, from Boehringer Mannheim, reagents and calibrators from the same source.

Method 3
Direct potentiometry, Cobas Mira S plus analyzer, from Roche, reagents and calibrators from the same source.

Method 4
Enzymatic-photometric, Cobas Mira S plus analyzer, from Roche, reagents and calibrators from Boehringer Mannheim, based on activation of β-galactosidase[1]) by Na^+ ions (2) and of pyruvate kinase[1]) by K^+ ions (3), respectively.

The reference methods, based on flame atomic emission photometry, were performed essentially as described (4), including bracketing calibration, with the main modification that caesium instead of lithium was used as the internal standard: an IL 943 instrument, from Instrumentation Laboratory, was used.

The control sera were assayed in triplicate.

The within-run analytical imprecision of each method, as CV (%), was calculated from replicate measurements on the control sera. The relationship between the results from each routine method (assigned the y-axis) and the reference method values (assigned the x-axis) was assessed by means of non-parametric linear regression (12, 13). The dispersion around the regression line was estimated as residual standard deviation (S_{yx}); correlation coefficients (r) were also calculated. The regression equations were used to calculate the relative (percent) bias of the routine methods versus the reference method at three clinically important decision values.

For each control serum, in each pair of methods, the residual (distance from the regression line along the y-axis) was computed, and divided by the residual standard deviation, to yield the normalized residual of the control serum, in standard deviation units (SD units): this was taken as the measure of its degree of commutability (9). A normalized residual outside the ± 3.0 SD units interval means a very low probability for a control serum to share the same inter-method behaviour with the patients sera's population, and therefore is taken to mean lack of commutability.

To assess the effect of recalibration with a given control serum, the results for a whole set of patients' sera with a stated routine method were recalculated, taking a control serum, with its conventional true value as assigned by means of the reference method, as the calibrator. The differences [(original routine value) − (reference method value)] and [(recalculated routine value) − (reference method value)] were then computed, and the distributions of such differences were displayed graphically, as empirical cumulative distribution plots (unfolded) (14).

Results

The median within-run coefficients of variation of the routine methods were (sodium/potassium):

method 1: 0.3%/0.2%;
method 2: 0.3%/0.2%;
method 3: 0.2%/0.1%;
method 4: 1.8%/1.4%.

Corresponding figures for the reference method were 0.5%/0.5%.

The statistical assessment of method comparison results is shown in table 1. In three out of four comparisons for sodium, and in one out of four for potassium, the slope values are significantly different from 1 (1 outside the 95% confidence interval of the slope). In such comparisons a y-intercept value significantly different from 0 mmol/l (0 outside the 95% confidence interval of the intercept) is also observed: this partially corrected the

[1]) Enzymes
β-Galactosidase: β-*D*-Galactoside galactohydrolase, EC 3.2.1.12.
Pyruvate kinase: ATP : Pyruvate 2-O-phosphotransferase, EC 2.7.1.40.

Tab. 1 Statistical assessment (non-parametric linear regression) of method-comparison results in the measurement of patient sera. Results from each system (y-axis) are compared with those from the reference method (x-axis).

System/Analytical principle/Analyte	N	Intercept [mmol/l]	Slope	S_{yx} [mmol/l]	r	Relative bias at decision values[a]		
						1st Value [%]	2nd Value [%]	3rd Value [%]
1/Indirect potentiometry/Na	106	15.8 (5.09 ÷ 26.4)	0.88 (0.80 ÷ 0.95)	2.54	0.899	1.7	0.3	−1.5
2/Indirect potentiometry/Na	104	−0.19 (−11.4 ÷ 9.64)	1.01 (0.94 ÷ 1.09)	3.79	0.890	0.9	0.9	0.9
3/Direct potentiometry/Na	102	15.3 (5.19 ÷ 27.6)	0.91 (0.82 ÷ 0.98)	3.26	0.887	4.3	2.4	1.2
4/Enzymatic/Na	104	20.8 (4.52 ÷ 33.8)	0.84 (0.75 ÷ 0.95)	3.40	0.861	2.1	0.6	−2.1
1/Indirect potentiometry/K	107	0.04 (−0.09 ÷ 0.03)	1.00 (0.97 ÷ 1.01)	0.09	0.996	1.3	0.7	0.5
2/Indirect potentiometry/K	104	0.00 (−0.04 ÷ 0.06)	0.99 (0.98 ÷ 1.00)	0.09	0.989	−1.0	−1.0	−1.1
3/Direct potentiometry/K	102	−0.11 (−0.17 ÷ 0.05)	1.06 (1.04 ÷ 1.07)	0.09	0.993	2.3	4.1	4.5
4/Enzymatic/K	105	−0.05 (−0.24 ÷ 0.12)	1.04 (1.00 ÷ 1.08)	0.23	0.981	2.3	3.1	3.3

[a] 1st, 2nd and 3rd decision values [mmol/l] for sodium and potassium: 115; 135; 150 and 3.0; 5.8; 7.5.

effect of the slope, and therefore minimized the bias at clinical decision levels (tab. 1). The dispersion around the line, as measured by the residual standard deviation, is rather constant in the different comparisons, with the exception of potassium measurement with the enzymatic method.

The normalized residuals of the 31 control sera, in the 4 pairs of methods (the reference method values always as the independent variable) are listed in table 2 (sodium and potassium). As mentioned, control sera showing a normalized residual outside the ± 3 SD unit interval were classified as non-commutable. Occasionally, some commercial control sera gave "absurd" values with one or more methods: these too were classified as non-commutable. Considering a total of 124 evaluations for each analyte (31 control sera × 4 pairs of methods), the overall frequency of non-commutability was 14/124 (11.3%) for sodium and 16/124 (12.9%) for potassium. The higher rate of non-commutability of control sera was observed for direct potentiometry (24.2%), followed by indirect potentiometry (8.9%) and by the enzymatic method (6.4%). With the two analytical systems based on indirect potentiometry, the frequency of non-commutability of the control sera was respectively 17.7% (equally divided between sodium and potassium) and 0%.

The inter-method behaviour of the commutable and non-commutable control sera, in comparison with the relevant regression line from patient sera results, is shown in figure 1 (sodium) and figure 2 (potassium).

In order to exemplify the effect of recalibration with commutable and non-commutable control sera, the method showing the highest bias in the assay of patient sera was chosen (direct potentiometry, see tab. 1). The results generated by this method in the assay of patient sera (n = 102) were recalculated, using respectively one commutable and one non-commutable material as calibrators. Materials number 26 and 9 (normalized residuals 0.0 and 3.6) were chosen for sodium, and number 19 and 5 (normalized residual 0.2 and 7.9) were chosen for potassium (tab. 2). Cumulative plots of the differences versus the reference method values, before and after re-

Tab. 2 Main characteristics of the 31 commercially available control sera included in the study, and relevant normalized residuals in 4 pairs of methods.

N	Matrix	Physical state	Normalized residuals [SD units]							
			Method 1		Method 2		Method 3		Method 4	
			Na	K	Na	K	Na	K	Na	K
3	human[a]	liquid	5.4	6.2	0.6	1.2	*	*	1.4	1.7
12	human	lyophilized	5.4	10.8	0.1	0.2	0.7	1.5	2.6	3.5
2	human[a]	liquid	3.9	2.2	0.6	0.7	*	*	1.3	0.5
1	human[a]	liquid	3.8	3.6	0.8	0.5	*	*	0.3	*
10	aqueous	liquid	3.2	6.6	0.1	0.2	1.4	2.8	2.0	1.9
9	aqueous	liquid	3.2	1.8	0.6	0.9	3.6	2.5	0.4	0.4
7	human	lyophilized	2.9	8.4	0.2	0.1	1.7	2.4	0.1	0.1
5	aqueous	liquid	2.8	1.2	0.3	0.4	3.4	7.9	0.8	1.3
8	human	lyophilized	2.6	2.7	0.8	0.0	0.8	3.0	*	0.7
11	human	lyophilized	2.0	0.6	0.2	0.6	0.3	1.1	0.3	0.4
26	human	lyophilized	1.8	0.5	0.1	0.0	0.0	1.5	2.2	3.4
20	human	lyophilized	1.7	0.3	0.0	0.1	0.5	0.5	0.5	1.4
28	human	liquid	1.6	0.3	0.7	0.7	1.7	0.7	1.7	1.7
30	aqueous	liquid	1.5	1.0	0.1	0.0	*	3.4	0.2	*
29	aqueous	liquid	1.3	0.9	1.2	1.4	2.2	0.3	0.8	0.4
14	equine	lyophilized	1.3	0.1	0.3	0.4	0.9	2.2	1.1	1.8
15	human	lyophilized	1.1	0.2	0.3	0.3	1.0	1.0	2.3	0.5
19	human	lyophilized	0.8	0.4	0.0	0.0	0.3	0.2	1.0	1.4
6	human	lyophilized	0.7	2.2	0.1	0.3	1.3	1.3	0.4	0.6
4	aqueous	liquid	0.6	0.8	0.8	0.4	*	3.8	0.6	0.2
21	human	lyophilized	0.6	0.5	0.4	0.5	2.0	2.0	1.5	0.6
24	unknown	lyophilized	0.6	0.3	0.4	0.6	0.3	0.1	2.2	0.9
23	unknown	lyophilized	0.5	0.5	0.0	0.5	0.4	0.4	1.2	0.2
27	human	liquid	0.5	0.2	0.2	0.1	0.1	0.8	2.1	0.1
13	equine	lyophilized	0.5	0.0	0.2	0.3	0.3	0.8	0.6	0.3
17	aqueous	liquid	0.4	0.6	0.6	0.2	1.4	1.8	1.9	0.5
31	bovine	liquid	0.4	0.5	0.4	0.7	1.1	0.7	0.2	0.9
16	equine	lyophilized	0.4	0.0	0.1	0.2	0.4	1.0	0.9	1.7
18	aqueous	liquid	0.4	0.0	0.9	0.3	1.7	4.1	2.0	1.3
22	human	lyophilized	0.2	0.8	0.8	1.9	1.9	3.7	0.2	0.2
25	human	lyophilized	0.0	0.8	0.5	0.9	0.3	1.0	2.1	0.4

[a] stabilized with ethylene glycol
* "absurd" results with the relevant method, or normalized residual higher than 20.0

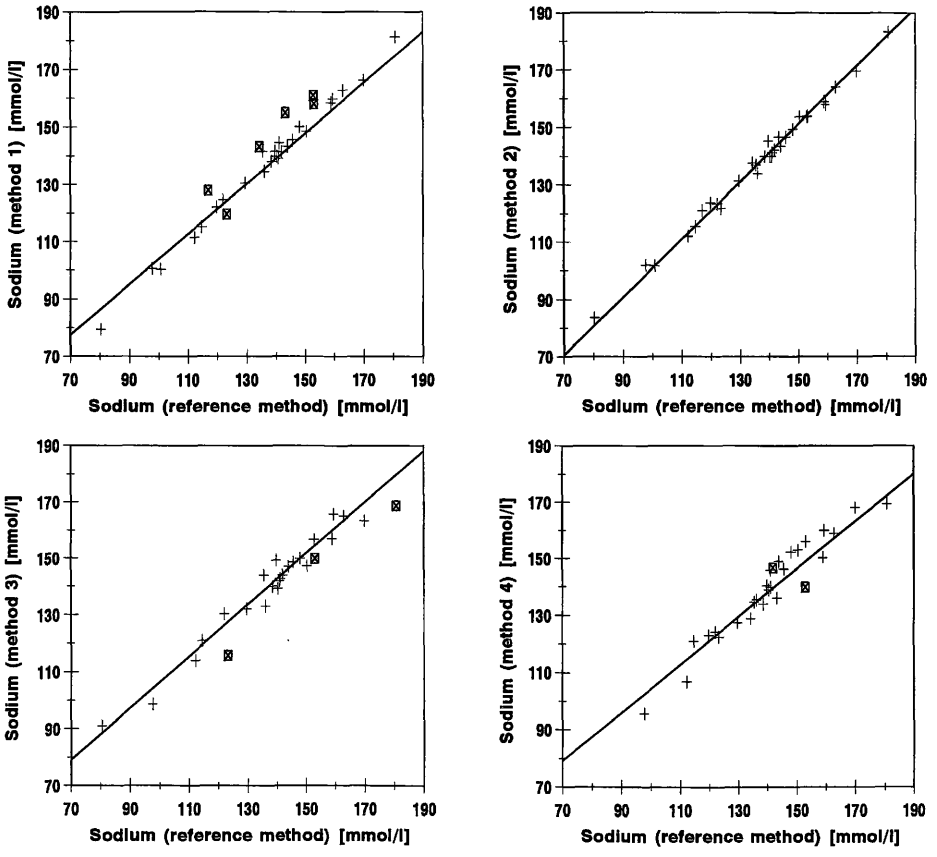

Fig. 1 Intermethod behaviour of commutable (crosses) and non-commutable (squares) control sera, compared with the regression line from patient sera, in the measurement of sodium. Method 1 and method 2: indirect potentiometry; method 3: direct potentiometry; method 4: enzymatic.

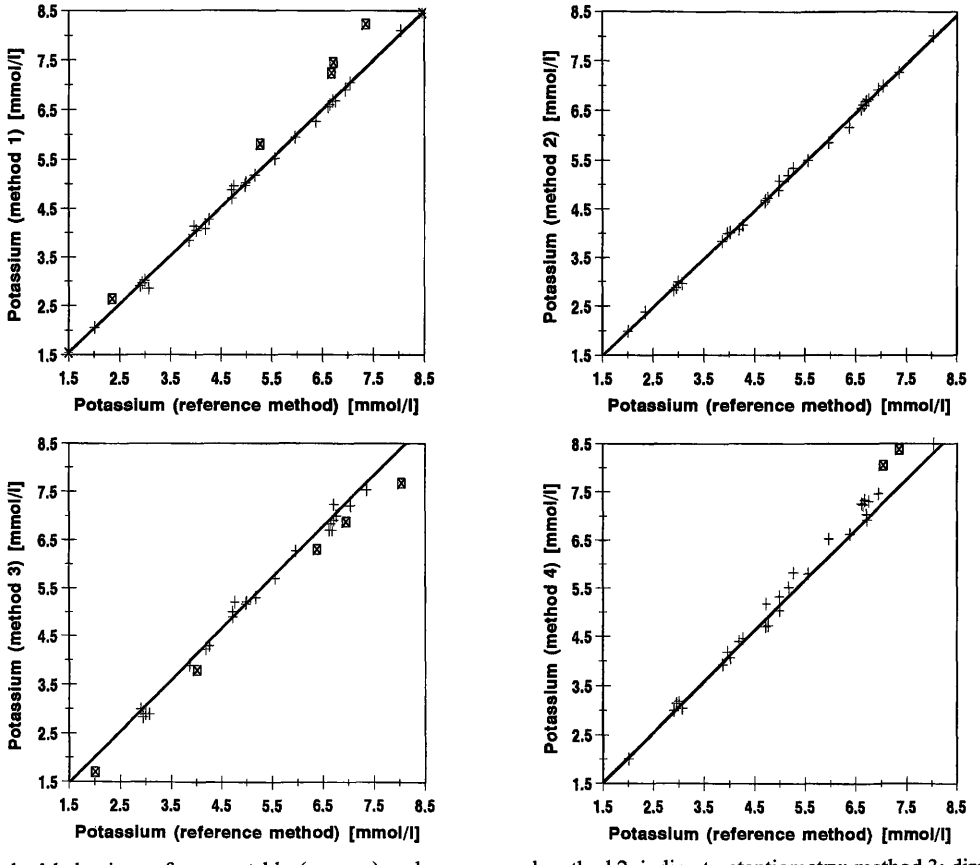

Fig. 2 Intermethod behaviour of commutable (crosses) and non-commutable (squares) control sera, compared with the regression line from patient sera, in the measurement of potassium. Method 1 and method 2: indirect potentiometry; method 3: direct potentiometry; method 4: enzymatic.

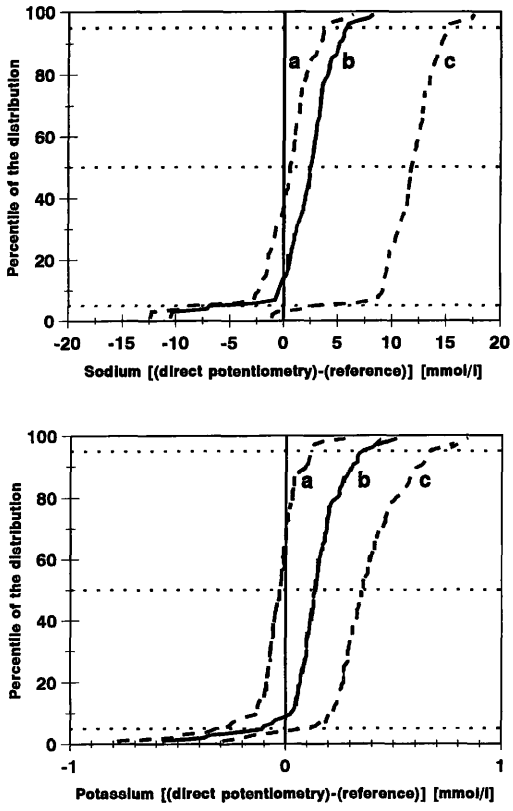

Fig. 3 Cumulative distribution of the differences [(direct potentiometry) − (reference method)] in the measurement of sodium (upper graph) and potassium (lower graph) in 102 samples of fresh patient serum: b: original data; a: recalculated on the basis of a commutable control serum; c: recalculated on the basis of a non-commutable control serum.
Horizontal dotted lines are drawn at the 5th, 50th and 95th percentiles.

calibration, are shown in figure 3. Clearly, the distribution's position (50th percentile) is improved (i. e. brought nearer to zero) or worsened (i. e. made more distant from zero) when either a commutable or a non-commutable material is used for calibration.

The inter-laboratory (inter-methods) variation (as CV %) in the measurement of both analytes in representative sets of commutable and of non-commutable control sera is shown in table 3: such variation appears lower in the assay of the former control sera, higher in the assay of the latter.

Tab. 3 Inter-laboratory (inter-method) variability (as CV %) observed for selective sets of commutable and non-commutable control sera.

Material no.[a]	Analyte	Interval of normalized residuals [SD units]	Interval of inter-laboratory CV [%]
6; 13; 19; 23	Sodium	0.0 ÷ 1.3	0.8 ÷ 2.5
5; 9; 10; 12	Sodium	0.1 ÷ 4.4	1.8 ÷ 4.6
13; 25; 27; 31	Potassium	0.0 ÷ 1.0	0.8 ÷ 2.5
5; 7; 10; 12	Potassium	0.1 ÷ 10.8	3.6 ÷ 6.2

[a] from table 2

Discussion

With the main exception of the enzymatic assay of sodium, within-run imprecision values generally approach or fulfill the very stringent desirable analytical performance specifications derived from the biological variation of the two analytes (6−8).

The bias between enzymatic and indirect potentiometric measurements of sodium and potassium has been reported to be acceptably low, in spite of abnormalities in the serum samples (15). However, in this study, the relative bias at clinically important decision levels of concentration often exceeds the recommended specifications (6, 8), especially in the measurement of sodium (direct potentiometry) and of potassium (direct potentiometry and enzymatic methods). In conjunction with unavoidable imprecision, such as bias may generate total errors higher than the limits currently used in European external quality assessment schemes, based on biological variation (16). This points out the need for improving the trueness of routine measurements, by improving their traceability to flame emission photometry.

The observed bias may be corrected for by recalibration with materials having values assigned by flame photometry (1). For such a procedure to be effective, however, the materials must show an interassay behaviour similar to that of patient sera, i. e. they have to be commutable. Examples given here show how the recalibration with commutable control sera permits an almost complete correction of the original bias of a method (fig. 3). On the other hand, about 12% frequency of non-commutability of commercially available control sera was observed in this study; the perverse effect of recalibrating with non-commutable materials has also been exemplified (fig. 3).

Control sera similar to those included in the present study are used in external quality assessment schemes. Our data show that the inter-laboratory (inter-methods) variability measured with such control sera varies as a function of their degree of commutability (tab. 3), and therefore may be not representative of the variability observed in the assay of patient sera (17).

The reasons for the lack of commutability of the materials are generally ascribed to the matrix, but they are not always evident (17, 18). Modifications of the matrix during the processing of commercial control sera may contribute to non-commutability. The irregular behaviour of the sera stabilized with high concentrations of ethylene glycol in direct potentiometry is a common experience. Also, replacement of bicarbonate by chloride in some commercial sera has been reported to give undesirably high residual liquid junction potential with the electrodes of dry-chemistry systems (Vitros analyzer, formerly Ektachem), thereby producing spuriously elevated values in sodium measurement (19). On the other

hand, from our data (tab. 2), it is not easy to link the irregular behaviour of any material either to the origin of its matrix (human, animal or aqueous), or to its physical state (liquid or lyophilized). Furthermore, irregular behaviour of a stated control serum was shown for either of the two analytes or with either of the four methods tested. With a few exceptions, the lack of commutability seems therefore to represent an unpredictable event, due to the interaction of the properties of the control serum with the characteristics of the analytical system.

It seems reasonable to conclude that the commutability of commercially available control sera in different pairs or sets of methods should be tested if they are to be successfully used either for external quality assessment or for the calibration of methods, in order to make results consistently traceable to a common reference method. Alternatively, target values adjusted for method-material specific matrix effect should be adopted in external quality assessment schemes (20, 21), or fresh patients serum samples should be used. In fact, liquid/frozen pools of non-modified human sera have been satisfactorily used in several such schemes during the last ten years (22).

All automatic systems are reported to show matrix effects for some analytes (20); in spite of some pessimistic attitudes (20), efforts should be made to improve both the robustness of the methods and the quality of the control sera, in order to minimize the frequency of non-commutability. One of the methods tested in the present study (method 2), besides being the best aligned with flame photometry in the assay of patient sera, as previously observed (15), was also sufficiently robust to give consistent results in the assay of all the tested control sera.

References

1. Gunaratna PC, Koch WF, Paule RC, Cormier AD, D'Orazio P, Greenber N, et al. Frozen human serum reference material for standardization of sodium and potassium measurements in serum or plasma by ion-selective electrode analyzers. Clin Chem 1992; 38:1459–65.
2. Berry MN, Mazzacchi RD, Pejakovic M, Peaake MJ. Enzymatic determination of sodium in serum. Clin Chem 1988; 34:2295–8.
3. Berry MN; Mazzacchi RD, Pejakovic M, Peaake MJ. Enzymatic determination of potassium in serum. Clin Chem 1989; 35:817–20.
4. Külpmann WR, Lagemann J, Sander R, Maibaum P. A comparison of reference method values for sodium, potassium and chloride with method-dependent assigned values. J Clin Chem Clin Biochem 1985; 23:865–74.
5. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci 1989; 27:409–37.
6. Fraser CG, Hyltoft Petersen P, Ricos C, Haeckel R. Proposed quality specifications for the imprecision and inaccuracy of analytical systems for clinical chemistry. Eur J Clin Chem Clin Biochem 1992; 30:311–7.
7. Fraser CG, Hyltoft Petersen P. Desirable standards for laboratory tests if they are to fulfill medical needs. Clin Chem 1993; 39:1447–55.
8. Stöckl D, Baadenhuijsen H, Fraser CG, Libeer J-C, Hyltoft Petersen P, Ricos C. Desirable routine analytical goals for quantities assayed in serum. Eur J Clin Chem Clin Biochem 1995; 33:157–69.
9. Van Helden WCH, Visser RWJ, Van Den Bergh FAJ-TM, Souverijn JHM. Comparison of intermethod analytical variability of patient sera and commercial quality control sera. Clin Chim Acta 1979; 93:336–47.
10. Rej R. Accurate enzyme activity measurement: two decades of progress in the commutability of enzyme materials [review]. Arch Pathol Lab Med 1993; 117:352–64.
11. Franzini C. Commutability of reference materials in clinical chemistry. JIFCC 1993; 5:186–93.
12. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Applications of linear regression procedures for method comparison studies in clinical chemistry. J Clin Chem Clin Biochem 1983; 21:709–20.
13. Passing H, Bablok W. Comparison of several comparison procedures for method comparison studies and determination of sample sizes. Applications of linear regression procedures for method comparison studies in clinical chemistry. J Clin Chem Clin Biochem 1984; 22:431–45.
14. Krouwer JS, Monti KL. A simple, graphical method to evaluate laboratory assays. Eur J Clin Chem Clin Biochem 1995; 33:525–7.
15. Hubl W, Wejbora R, Shafti-Keramat I, Haider A, Hajdusich P, Bayer PM. Enzymatic determination of sodium, potassium, and chloride in abnormal (hemolyzed, icteric, lipemic, paraproteinemic, or uremic) serum samples compared with indirect determination with ion-selective electrodes. Clin Chem 1994; 40:1528–31.
16. Ricos C, Baadenhuijsen H, Libeer J-C, Hyltoft Petersen P, Stöckl D, Thienpont L, et al. External quality assessment: currently used criteria for evaluating performance in European countries, and criteria for future harmonization. Eur J Clin Chem Clin Biochem 1996; 34:159–65.
17. Lawson NS, Williams TL, Long T. Matrix effects and accuracy assessment. Identifying matrix-sensitive methods from real-time proficiency testing data. Arch Pathol Lab Med 1993; 117:401–11.
18. Eckfeldt JH, Copeland KR. Accuracy verification and identification of matrix effects. The College of American Pathologists' protocol. Arch Pathol Lab Med 1993; 117:381–6.
19. Uldall A, Blaabjerg O, Elfving S, Elg P, Gerhardt W, Holmberg H, et al. A programme for assigning target values for external quality assessment schemes in countries with no authorized reference laboratories. Annex. Experiences with deviating results on Ektachem 700 XR. Scand J Clin Lab Invest 1993; 53 Suppl 212:31–7.
20. Powers DM. Overcoming the limitations of artificial clinical specimens: linkage to performance of patients' specimens [editorial]. Clin Chem 1995; 41:1689–92.
21. Miller WG, Ross JW, Myers GL. Definitive/reference method target values are inappropriate for lyophilised materials [abstract]. Proceedings of the Sixteenth International Congress of Clinical Chemistry; 1996 Jul 7–12; London (UK). Cambridge, Association of Clinical Biochemists 1996: 516.
22. Uldall A, Glavind-Kristensen S, Bak S. Preparation of fresh frozen human sera for external quality assessment. Scand J Clin Lab Invest 1989; 49:11–4.

Received December 12, 1996/March 7, 1997

Corresponding author: Prof. Carlo Franzini, Università di Milano, Istituto di Scienze Biomediche Ospedale L. Sacco, Via G. B. Grassi, 74, I-20157 Milano, Italy, Fax: +2/38201626