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Determination of Sodium and Potassium Electrolytes in Human Serum by Indirect and Direct ISE Methods in Linical and Biochemical Laboratories

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Abstract: The determination of sodium and potassium electrolytes in human serum requires particular attention, because the alternation of different analyzers/methodologies during the day, in a general hospital. This research compares the data produced by 2 different analyzers that applied different methodologies which are commonly used in clinical chemistry laboratories to measure sodium and potassium ions. Olympus AU640 analyzer (Olympus, Japan) uses indirect ISE method while Microlyte 6.0 analyzer (KONELAB, Finland) uses direct ISE. It is shown that for the potassium a linear fitting model was the most appropriate for data transformation from Olympus AU640 to Microlyte 6.0. Also it is shown that a linear transformation model was not the most appropriate for transformation in the case of sodium concentrations. Finally some tentative conclusions are derived concerning the problem of transferability of results exists when laboratories use the above analyzers for the determination of the electrolytes sodium and potassium.

Key words: Sodium, potassium, indirect potentiometry, direct potentiometry, transferability, clinical laboratory

INTRODUCTION

The concentration of sodium and potassium in human blood plays a significant role in a human's biochemical profile.

Excessive urine loss, diarrhea, Addison's disease and renal tubular disease may cause low sodium levels. High sodium concentrations may occur in severe dehydration, some types of brain injury, diabetic coma and excessive intake of sodium salts. Measurement of serum potassium is used for the evaluation of electrolyte imbalance, cardiac arrhythmias, muscular weakness, hepatic encephalopathy, renal failure and for the monitoring of ketoacidosis in diabetes mellitus and intravenous fluid replacement therapy. More than 90% of hypertensive patients with aldosteronism have a low K^+; a low K^+ is also common in vomiting, diarrhea, alcoholism and folic acid deficiency. High K^+ values occur in rapid K^+ infusion, stage renal failure, hemolysis, trauma, Addison's disease, metabolic acidosis, acute starvation, dehydration and acute medical emergency (Titez, 1987).

Therefore, accurate determination of sodium and potassium ions provides the precise clinical diagnosis of the patients. Many methods have been used for the determination of sodium and potassium ions in human blood. Flame photometry is one of the reference methods and has been widely used in many biochemical laboratories and diagnostic center worldwide (Velapoldi, 1978). However, difficulties, several protocols and procedures with differential value and accuracy characterize this method. The need of simplifying the determination of sodium and potassium electrolytes has led to the growth of new methods. The electrochemical methods are acceptable for their precision and have advantages against the reference method for their simplicity and speed of implementation (Siggard, 1986). Recently, the automated instrumental methods for determination of sodium and potassium have dominating position and their virtues have been recognized.

Frequently, different instruments and measurement methods for a particular analyte are used in the same laboratory or in laboratories belonging to the same clinical area. If transferability of results is possible, it allows the laboratories to give the results obtained by different methods in terms of the same rank of reference. This is particularly important in the case of serum electrolytes, as

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they are one of the most frequently requested tests in Clinical laboratories and there is a great variety of instruments and measurement methods for analyzing these ions (Olafsdottir *et al*., 1992; Maas *et al*., 1985). Accordingly, the discrepancies in the results for sodium and potassium between the direct Ion-Selective Electrode (ISE) methods and those needing predilution of the sample (indirect ISE) are well known (NCCLS, 1995; Landerson *et al*., 1981, 1982).

In the current study, the differences of indirect and direct method used for the determination of sodium and potassium ions in human blood samples by potentiometry have been assessed. In particular, the results obtained for the determination of sodium and potassium ions in human blood by Olympus AU640 analyzer using indirect ISE method and Microlyte 6.0 analyzer using direct ISE method, have been compared and analyzed statistically.

MATERIALS AND METHODS

The data used in this study derived from the findings of the elaborated blood samples that were taken in the biochemical laboratory of General Hospital of Drama (Greece). The blood samples were collected in Vacutainer tubes (Becton Dickinson Co., Rutherford, NJ) free of anticoagulant, in biochemical laboratory of General Hospital of Drama (Greece), according to international specifications (Slockbower and Blumenfield, 1983; NCCLS, 1991a,b). The Vacutainer tubes were left for a period of time at ambient temperature in order the blood to be clotted. The blood serum was separated by centrifussion at 1000 g for 20 min and after that the Olympus AU640 and Microlyte 6.0 analyzers determined sodium and potassium concentrations simultaneously.

The samples with protein and/or triglyceride concentrations which have lied out of the ranges of total protein 65-83 g L^{-1} and triglycerides 0.28-1.69 mmol L^{-1} (considered as normal ranges for our laboratory) were eliminated, so bias in the results obtained by direct potentiometry to be avoided.

In order to study the within- and between-day variation samples of normal sera were aliquoted and preserved at -20°C and were analyzed during the determination of sodium and potassium electrolytes.

The instruments used in this elaboration were analyzers of type Olympus AU640 (Olympus, Japan) using indirect ISE method and Microlyte 6.0 (KONELAB, Finland) using direct ISE. Indirect method (Olympus AU640) used ion-selective electrode (type Crown-membrane), in diluted solution of ion. More specifically, 20 μL of sample is diluted with 10 μL of free-ions water and with 618 μL of buffer. Then the measurement is executed in 37°C. Microlyte 6.0 using direct ISE method, at which the serum is not diluted with water, while the determination takes place in room temperature that in our case ranged between 17 and 25248°C. The electrodes are what have been proposed by the corresponding manufactures and for analyzer Olympus AU640 (A) is type-membrane of determined time duration, while for analyzer Microlyte 6.0 (B) is type-capillary.

Before each determination, calibration and internal control of analyzers with calibrators and quality controls of the corresponding manufactures preceded, according to manufacturer's instructions and international literature (NCCLS, 1991c; Kafka, 1988; Burnett *et al*., 2000).

The reagents provided in the commercial kits were used in the two analyzers and the methods were adapted according to the manufacture's instructions. The water, free from metal ions, had a receptivity of 18.2 Mohm.cm at 25°C.

The information was recorded in an MS Excel database and processed using statistical software MedCalc version 6.15.000 and SPSS 12.0 for windows.

RESULTS

The results from examining the within-day variation of sodium and potassium ions are presented in Table 1, by means of the corresponding Coefficient of Variation (CV). The within day precision was determined by using three to four replications for each one of the two runs per day. The within day CV values, measured for 3 levels ranged between 0.54 and 0.96 for sodium and between 0.97 and 3.24% for potassium ion.

In Table 2 the results from examining the between day variation of sodium and potassium ions are presented by means of the corresponding Coefficient of Variation (CV). The between day CV values, measured over a three-month period for 3 levels, ranged between 0.79 and 1.19% for sodium and between 1.40 and 3.52% for potassium ion.

Sodium and potassium determined twenty one times in a 3 month period by the Olympus AU640 and Microlyte 6.0 analyzers, using quality controls of the corresponding manufactures. The results of the inaccuracy study are presented in Table 3.

The study of reliability of the two analyzers is presented in Table 4.

The linearity range for sodium and potassium in the Olympus AU640 and Microlyte 6.0 analyzers is given in Table 5.

Then, 12 different runs of serum were simultaneously measured by the 2 analyzers, ten times each one, for determined sodium and potassium concentrations. Every run of serum was measured at different day in the studied period.

Table 1: Statistical characteristics of sodium and potassium for within day variation

		Sodium		Potassium	
System	No. observ	Mean (mmol L^{-1})	CV (%)	Mean (mmol L^{-1})	CV (%)
Low level					
Olympus AU640	120	119.6	0.82	3.12	1.25
Microlyte 6.0	120	118.8	0.96	3.06	3.24
Medium level					
Olympus AU640	160	141.3	0.54	4.62	1.05
Microlyte 6.0	160	139.4	0.91	4.62	2.05
High level					
Olympus AU640	120	153.8	0.41	6.73	0.97
Microlyte 6.0	120	151.0	0.89	6.76	1.50

Maximum allowable Coefficient of Variation (CV_a): sodium 0.97% and potassium 3.82% (NCCLS, 1995)

Table 2: Statistical characteristics of sodium and potassium for between day variations

		Sodium		Potassium	
System	No. Observ	Mean (mmol L^{-1})	CV (%)	Mean (mmol L^{-1})	CV (%)
Low level					
Olympus AU640	41	125.7	0.90	2.89	2.80
Microlyte 6.0	41	129.0	1.19	3.06	3.52
Medium level					
Olympus AU640	41	141.2	0.82	4.60	1.40
Microlyte 6.0	41	139.4	1.00	4.69	3.03
High level					
Olympus AU640	41	153.4	0.79	6.70	2.51
Microlyte 6.0	41	152.4	1.10	6.05	3.35

Maximum allowable Coefficient of Variation (CV_a): sodium 0.97% and potassium 3.82% (NCCLS, 1995)

Table 3: Study of inaccuracy

		Mean (mmol L^{-1})			
Sodium	n	Theoretical	Observed	Significance	AAE
Low level					
Olympus AU640	21	127.0	126.7	NS	1.4
Microlyte 6.0	21	127.0	126.2	NS	1.4
High level					
Olympus AU640	21	157.0	155.9	NS	1.4
Microlyte 6.0	21	157.0	150.2	p>0.05	1.4
Potassium					
Low level					
Olympus AU640	21	4.59	4.61	NS	0.2
Microlyte 6.0	21	4.59	4.69	N.S	0.2
High level					
Olympus AU640	21	6.72	6.69	NS	0.2
Microlyte 6.0	21	6.72	6.04	p>0.05	0.2

Table 4: Study of reliability (TAE: Total Analytical Error, AAE: Allowable Analytical Error)

	Sodium			Potassium	
	TAE	AAE		TAE	AAE
Low level			Low level		
Olympus AU640	3.1±2.5	1.4	Olympus AU640	0.09±0.08	0.2
Microlyte 6.0	4.5±3.2	1.4	Microlyte 6.0	0.12±0.08	0.2
Medium level			Medium level		
Olympus AU640	2.4±1.8	1.4	Olympus AU640	0.05±0.08	0.2
Microlyte 6.0	2.8±2.5	1.4	Microlyte 6.0	0.10±0.09	0.2
High level			High level		
Olympus AU640	2.9±2.4	1.4	Olympus AU640	0.09±0.08	0.2
Microlyte 6.0	4.6±3.5	1.4	Microlyte 6.0	0.10±0.08	0.2

The results of the study relative to Na and K during the studied period by 2 different methods/analyzers are presented in Fig. 1 and 2 as error bars. In these figure the mean values per analytical run as well as the 95% confidence intervals (95% C.I.) of the above mentioned electrolytes are presented.

The values of sodium and potassium concentrations per run are normally distributed, according to the Kolmogorov-Smirnov criterion. Moreover, statistical analysis for the comparison of sodium and potassium averages per run between the two methods was performed by the Student's t-test; $p<0.05$ was considered statistically significant. The results of this analysis are presented in Table 6.

The determined values of sodium and potassium concentrations for the entire studied period are normally

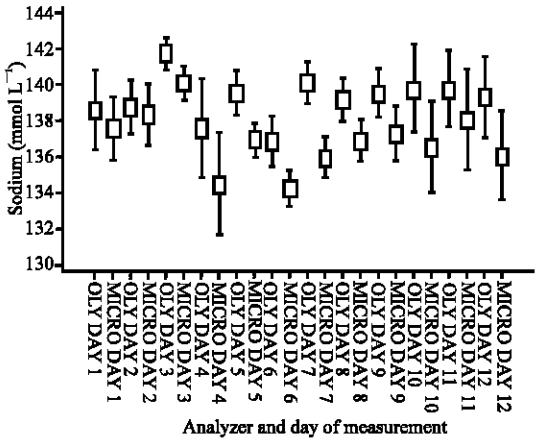

Fig. 1: Sodium means (mmol L^{-1}) per analytical run by the 2 analyzers

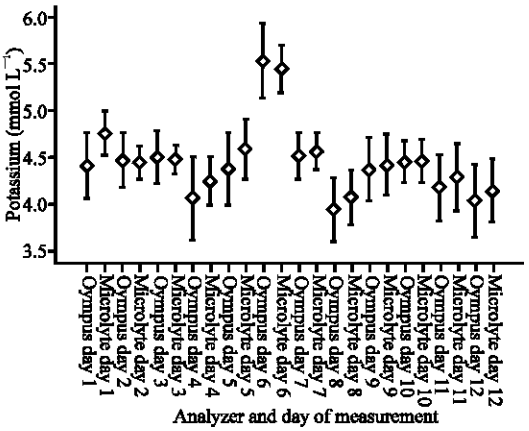

Fig. 2: Potassium means (mmol L^{-1}) per analytical run by the 2 analyzers

Table 5: Study of linearity

	Linearity range (mmol L^{-1})	
Analyzer	Sodium	Potassium
Olympus AU640	48-192	2-8
Microlyte 6.0	48-192	2-8

Table 6: Results from the performed statistical analysis for the comparison of sodium and potassium averages per analytical run between the considered analyzers (Student's t-test; $p<0.05$, OLY: Olympus and Micro: Microlyte)

Run	OLY Na vs Micro Na	OLY K vs Micro k
1	$p<0.05$	$p<0.001$
2	$p<0.0001$	NS
3	$p<0.001$	NS
4	$p<0.0001$	NS
5	$p<0.0001$	NS
6	$p<0.001$	$p<0.05$
7	$p<0.0001$	NS
8	$p<0.001$	$p<0.001$
9	$p<0.001$	$p<0.05$
10	$p<0.001$	NS
11	$p<0.05$	$p<0.001$
12	$p<0.05$	$p<0.05$

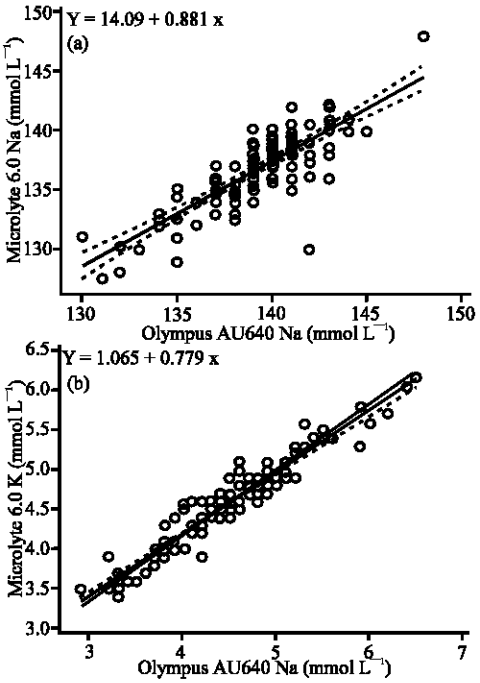

Fig. 3: Scatter diagrams of mean values of Microlyte 6.0 against Olympus AU640 analyzer. The solid line is the best-fit curve expressed by the equation [MICRO] = 14.090 + 0.881 * [OLY] for sodium and [MICRO] = 14.065 + 0.779 * [OLY] for potassium and the dashed line represents the 95% of confidence intervals for all the studied period

distributed, according to the Kolmogorov-Smirnov criterion. Moreover, statistical analysis for the comparison of sodium and potassium averages for all the studied period between the two methods/analyzers was performed by the Student's t-test; Friedman test; Kendall's W test; $p<0.05$ was considered statistically significant. The results show that there are significant differences between the two methods/analyzers (Student's t-test: $p<0.0001$ for sodium and potassium; Friedman test: $p<0.0001$ for sodium and potassium; Kendall's W test: $p<0.0001$ for sodium and potassium, too). Figure 3 presents the plots of Microlyte 6.0 against Olympus AU640 analyzer, for sodium and potassium concentrations respectively, as well as the best-fit lines with 95% of confidence intervals, over the studied period.

The comparison of sodium and potassium median values determined by the 2 different methods analyzers over the studied period are presented in Fig. 4 as Box-and-Whiskers plots. Definition of box-plots: These plots show the median, interquartile range and outliers of individual variables. The box length is the interquartile range. The

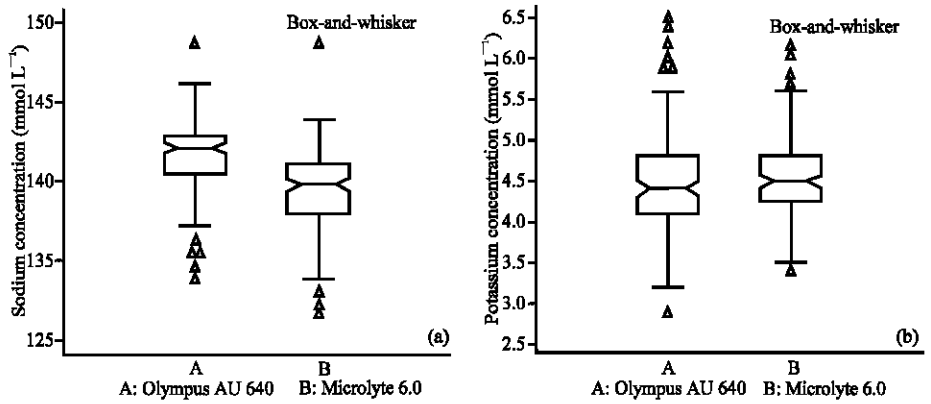

Fig. 4: Box and Whiskers plots for (A): Sodium and (B): Potassium, over the studied period

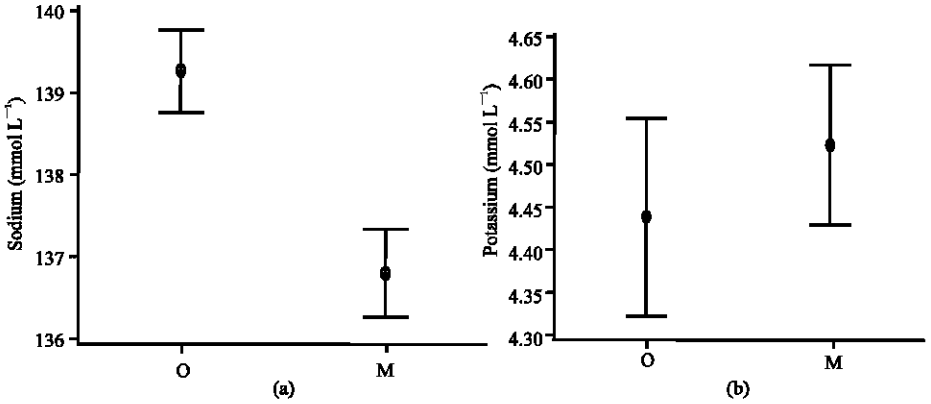

Fig. 5: Interval plots for (A): Sodium and (B): Potassium (O: Olympus AU640 and M: Microlyte 6.0)

median is represented as a horizontal line inside the box. Outside points, plotted as small triangles, are cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box.

The comparison of sodium and potassium mean values determined by the two analyzers for the entire studied period are presented in Fig. 5 as interval plots. In this Fig the mean values as well as the 95% confidence intervals (95% C.I.) of the above mentioned electrolytes are presented.

DISCUSSION

The within precision results analysis for sodium and potassium are presented in Table 1. The within-run CV values, measured for three levels, did not exceed the maximum allowable values of coefficient of variation (CV_a), for sodium and potassium, analyzed in the two analyzers. The between-run CV values, measured for three levels also, did not exceed the maximum CV_a values, for sodium and potassium analyzed in the Olympus Au640 analyzer (Table 2). The medically acceptable CV values (Cv_a) exceeded for the 3 levels of sodium analyzed in the Microlyte 6.0 analyzer, although these values were not significantly different.

The data obtained by testing the results for inaccuracy with protein free solutions are summarized in Table 3. In most cases the difference between means is not higher than the medically Acceptable Error (AAE). For all the cases, of high-level comparison for both sodium and potassium ions determined by Microlyte 6.0 analyzer, it is notable that the difference of averages is lower than the AAE. Inaccuracy was clinically significant for sodium ion (high level) and potassium ion (high level) analyzed by the Microlyte 6.0 analyzer, as in every case the difference between means is higher than the AAE.

The potassium ion is the only ion which has proved to be the most reliable in the two analyzers (Table 4), as the AAE did not exceed in any of the two analyzers by the high estimate limit of the Total Analytical Error (TAE) at a confidence level of 95%. On the contrary the sodium ion was unreliable in the two analyzers because the TAE was found higher than the AAE.

The results from the application of an analysis, in order to provide quantitative criteria for the study of linearity are summarized in Table 5. These results show

that linearity was acceptable for the two ions assessed and with every piece of equipment, as the normal and pathological rank of values was largely covered in all of the cases.

The assess and comparison of mean values and confidence intervals per run for sodium and potassium, obtained by indirect (Olympus AU640 analyzer) and direct ISE method (Microlyte 6.0 analyzer), is shown in Fig. 1 and 2 as error bars, respectively. From these figures it can be concluded that there are significant differences between means and confidence intervals per day concerning the determination of sodium and potassium by the two methods/analyzers.

Table 6 presents the results from the performed statistical analysis for the comparison of sodium and potassium averages per run between the considered methods/analyzers (Student's t-test). It can be seen form Table 6 that the comparison of sodium means between the two analyzers reveals significant differences for all the 12 runs studied. On the contrary, the comparison of potassium means between the two analyzers shows significant differences only for the 50% of the studied runs.

The comparison of sodium and potasium means for all the studied period show that there are significant differences between means determined by the two analyzers (Student's t-test: $p<0.0001$; Friedman test: $p<0.0001$; Kendall's W test: $p<0.0001$, for both sodium and potassium).

In order to provide quantitative relations between sodium and potassium concentrations determined by Microlyte 6.0 (Y) and Olympus AU640 (X) analyzers, scatter diagrams were constructed (Fig. 3 and 4). Figure 3 presents the plot of values of Microlyte 6.0 against, both expressed as mean values over the examined period, as well as the best line of fit given by the Eq. 1 for sodium. Figure 4 presents the corresponding plot for potassium and the corresponding best line of fit given by the Eq. 2. In both figures the dashed lines represents the 95% of confidence intervals for all the studied period.

$$[MICRO] = 14.090 + 0.881 * [OLY], r^2 = 0.664 \quad (1)$$

$$[MICRO] = 14.065 + 0.779 * [OLY], r^2 = 0.914 \quad (2)$$

From these results it appears that, for sodium, by using the linear model, only 66.4% of the variance ($r^2 = 0.664$) of annual values of values of Microlyte 6.0 analyzer can be explained by the variations of values Olympus AU640 analyzer. On the contrary, for potassium, using the linear model, 91.4% of the variance ($r^2 = 0.914$) of values of Microlyte 6.0 analyzer can be explained by the variations of values Olympus AU640 analyzer. Conclusively, the interconvetribility of results per run between the two methods/analyzers is rather problematic due to the fact that the different linear fitting models cannot be used to predict the value on the other instrument.

Box-and-Whisker plots (Fig. 4) reveal that there are significant differences about the sample distribution characteristics between Olympus and Microlyte analyzers concerning sodium. The medians of sodium determined by the two methods are significantly different at a ±95% confidence level. The comparison of potassium concentrations determined by the two methods shows that there are not significant differences between the two analyzers concerning the medians at a ±95% confidence level.

Figure 5 (interval plots), presents the access and comparison of mean values and confidence intervals for all the studied period for sodium and potassium, respectively. From this figure it can be concluded that there is significant difference between mean values and confidence intervals of sodium determined by the two methods at a ±95% confidence level. The interval plot for potassium shows that there is not significant difference between mean values and the confidence intervals of the two methods at a ±95% confidence level.

CONCLUSION

The use of different methods/analyzers in a biochemical laboratory requires the continuous control of measurements' quality and their transferability. This is extremely important in order to obtain results without crucial statistical deviation.

More specifically, concentrations of sodium and potassium ions must have the above characteristics in the highest degree, because these are usually the blood examinations that help doctors to form a first impression of the patient. They also assist the clinical doctor to diagnose and monitor the patient's health.

The differences presented in this study, show the existence of problems with regard to the compatibility of the 2 analyzers. Consequently, the problem is the accurate report of results in regard to the two electrolytes. More specifically, this problem creates difficulties to the clinical doctor in monitoring the values of these electrolytes for one patient, from day-to-day. The results show that there are problems for a linear correlation between the 2 mentioned analyzers, for the determination of the 2 electrolytes concentrations. Thus, when more than one analyzer is used in biochemical laboratories, all the above should be taken into consideration by the technicians, clinical chemists and doctors.

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