

Three Different Analytical Techniques for Measuring Serum Sodium as Well as Potassium Levels in Yemeni Patients with Kidney Disease: A Comparative Study

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Abstract: The current study used three distinct laboratory methods to determine serum sodium and potassium values among Yemeni patients with renal disease: an ion-selective electrode, a flame photometer, and a spectrophotometer. Between January 1 and 30, 2021, 70 renal patients aged 20 to 70 were selected at the Health of the Public Labs National Center. The data were analyzed with SPSS 25. Mean serum sodium concentrations differed significantly between analytical techniques (ion-selective electrode: 134.63 ± 3.008 mEq/L, flame photometer: 136.86 ± 3.432 mEq/L, and spectrophotometer: 139.23 ± 3.519 mEq/L; $P \leq 0.0001$). Significant differences were found in mean serum potassium concentration (5.190 ± 0.8866) mEq/L, (5.037 ± 0.6836) mEq/L, and (4.521 ± 0.7028) mEq/L assessed by ISE, flame photometer, and spectrophotometer, respectively ($P \leq 0.0001$). The sodium and potassium values determined using all methods fell between the upper and lower limits with a 95% confidence interval and a 95% limit of agreement. Additionally, a strong correlation was observed using correlation coefficient analysis between ISE, flame photometry, and spectrophotometry methods for serum sodium and potassium concentration results.

Keywords: Spectroscopy, metals, ion selective electrode, flame photometer, lipids

1. Introduction:

Electrolytes are found in the blood, tissues, and fluids of the body, as well as in urine, which is consumed with meals, beverages, and supplements [1]. They are critical for several physiologic activities [2, 3], including fluid regulation and neuronal signal transmission [4]. If they are not balanced, essential biological systems might be impacted [5]. Furthermore, electrolyte imbalances can arise from certain medicines and are commonly caused by a loss of body fluids [6]. The extracellular fluid is where sodium, a basic ion, is most commonly found. [7, 8]. While the total quantity of sodium within cells is just 5 mEq/L, the sodium concentration rate in plasma fluctuates between 135 and 145 mEq/L. [1]. In contrast, potassium, which regulates heart function, is the body's third-greatest ion [9]. It is the predominant intracellular ion, accounting for 98% of its concentration within cells. Its plasma concentration ranges from 3.5 to 5.5 mEq/L [10, 11]. Hyponatremia causes water to enter the cells, resulting in personality changes, headaches, and disorientation. This occurs in substantial quantities in brain cells [6]. While hypernatremia causes severe dehydration and diabetic coma following insulin treatment, hyperadrenalism, and other diseases [12, 13],

hypokalemia may not elicit symptoms but may modify how the body stores glucagon or cause abnormal heart rhythms [14]. A level of less than 3 mEq/L might cause muscle weakness, paralysis, and breathing problems [15]. While potassium levels are principally controlled by the steroid hormone aldosterone, which is generated by the adrenal gland when potassium levels rise, other factors influence sodium levels, including the steroid hormone aldosterone, which lowers sodium loss in the urine [16]. Quality control solutions are essential to ensuring the accuracy of laboratory results. Among the different methods for analyzing potassium and sodium readings, we selected three key common approaches [17, 18]. In the 1980s, most laboratories used ion-selective electrodes instead of flame emission spectrophotometry (FES) [19]. This method uses particular filters for Na^+ and K^+ , resulting in concentration discrepancies on both sides of the membrane as well as variances in the electrical potentials of the measuring and reference electrodes. The concentrations of these ions in solution are then determined using the Nernst equation [20], which connects the reaction's reduction potential to the standard electrode potential and the target ion activity at a particular temperature [21]. To prevent making incorrect

therapeutic decisions and treatments when dealing with ISEs, it is critical to identify the kind and degree of interventions [22, 23]. The electrolyte concentration is determined using two separate ISE methods. The first, an unadulterated sample, is fed into the Direct ISE measuring electrode. Indirect ISE requires a diluted sample [22, 24]. The most often used approach is indirect ISE [25, 26]. The contrast between the two approaches is crucial because some scenarios, particularly with salt, provide inconsistent findings and may lead to inappropriate treatment decisions [27, 28]. Flame photometry was one of the first techniques for determining sodium and potassium levels in blood and urine samples. This technique produces faster and more sensitive findings with fewer samples [29]. Atoms of alkali and alkaline earth metals are excited at low temperatures, and their distinctive wavelengths are easily distinguished from those of most other elements due to their large spectral separation [30]. Despite the aforementioned advantages, traditional flames can only directly detect a small number of fluid sample constituents [10]. One of the most significant universal methods is spectrophotometry. The transmittance (T) of a sample is determined by comparing the intensity of light exiting (I_t) and the intensity of light entering (I_0) at a certain wavelength using a light beam [31, 32]. The electromagnetic spectrum in this scenario varies from 190 to 800 nanometers [33, 34]. It is distinguished by its precision, rapidity, and sensitivity, allowing for the detection of an analyte's concentration in solution at extremely low levels [35]. It has a wide range of applications, including research, medicine, food production and analysis, and medicine [36]. However, this is the most common mechanism by which lipemia and hemolysis influence laboratory test results [37]. Many studies compare electrolyte estimations between direct and indirect ISE or between flame photometers and indirect ISE, but few compare electrolyte estimation approaches between flame photometers and direct ISE [30]. In some circumstances, the analytical results obtained by detecting plasma or serum sodium and potassium levels by flame photometry versus direct ISE are therapeutically important. To obtain the best results without producing a false negative mistake in lipaemia or lipaemic serum, compare the spectrophotometry values before and after correction with the ISE technique [38].

Experimental Work:

The current investigation employed empirical assessment to analyze multiple ISEs, flame photometry, and spectrophotometric methods for measuring sodium and potassium levels in human blood samples. Seventy (70) samples were gathered from renal patients of both genders who came to the laboratory in January 2021, ranging in age from 20 to 70. The laboratory experiments took place at the National Center of Public Health Laboratories—Hadramout Branch, which is located in Al-Mukalla. In our experiment, we created a number of conventional sodium and potassium solutions using pure sodium chloride and potassium chloride salts. Five milliliters of venous blood were collected in a clot vial and extracted using a vacutainer syringe. The samples were incubated for a few minutes to allow the blood to coagulate and produce serum. The blood samples were centrifuged at 5000 rpm for 5 minutes. The supernatant sera were collected in sterile vials and stored at 0–8 oC for electrolyte assays.

2.2. Instruments:

The present research employs three main tools. 1- A recently available Beckman Coulter instrument (Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821). This system works automatically and uses a number of methods. The system can do up to 480 tests per hour, employing a combination of slow and rapid testing. 2- The flame photometers PFP7 and PFP7/C are low-temperature, single-channel emission flame photometers that are frequently used to measure sodium (Na) and potassium (K). There are additional filters available for detecting lithium (Li), calcium (Ca), and barium (Ba). Both types offer automatic flame failure detection for user security, making them appropriate for use in clinical, industrial, or educational contexts. The built-in linearizer circuitry displays values of Na and K at average clinical blood concentrations in mEq/L. Serum samples must be diluted 1:100 before being tested with a flame photometer. 3- The V-730 UV visible spectrophotometer (Japan) is a general-purpose twin beam spectrophotometer with a compact design. The system's advantages include simplicity, ease of use, and compact design; high-speed scanning up to 8000 nm/min; a wide range of sampling accessories such as cell holders, flow cells, and controllers for temperature to optimize the V-730 for specific applications; an instrument validation routine (standard) to verify instrument efficiency; and a wavelength range of 190–1100 nm.

2.3. Techniques for measuring both the Na⁺ and K⁺ scales:

It is known that numerous procedures are utilized to determine the electrolyte content of human serum. And our study examines three typical techniques, which include:

2.3.1. The analysis by ISE:

As previously stated, an electrochemical cell acts as the structural foundation of an ISE analyzer. So, in the Beckman-Coulter analyzer, both sodium and potassium levels are assessed using a glass electrode surface [23], and serum was employed as the test material. The sample probe aspirates the sample and transports it to the sample pot, where the mix bar mixes it with the ISE buffer solution. The mixed suction roller motor then transfers the reduced sample into the flow cell, where measurements are taken. After processing a blood sample, the mixture suction roller motor rinses the flow cell by forcing the ISE buffer solution through the flow cell and into the sample pot using the ISE buffer syringe. After each sample has been processed, the MID standard roller motor moves the ISE MID normal solution from the bottle to the sample pot, while the mixed aspiration roller pump suctions the ISE MID characteristic solution through the flow cell to condition the electrodes and gather data. Ultimately, the solution is rejected. In addition, the analyzer automatically calculated the quantities of these salts. The findings were displayed digitally, and the resulting potentials were measured with a voltmeter. This has been carefully confirmed, with two layers of assayed quality check samples done every day prior to processing patient samples to verify that the quality control findings are within the reference range.

2.3.2. The analysis by flame photometry:

Here, 100 ml of 150 mEq/L sodium standard (stock) solution (Eq. 1) and 100 ml of 5 mEq/L potassium standard (stock) solution (Eq. 2) were prepared using dry and pure "Analar" quality NaCl and KCl. Additionally, these initial

solutions were reduced to 1:100 to provide the standard solutions that would be used with the flame photometer.

(Na) weight by(mg)=(150 (mmol)/L X 58.44 X 100)/1000
(K) weight by(mg)=(5 (mmol)/L X 74.54 X 100)/1000

We combined 9.9 milliliters of filtered water with 100 microliters of serum (1% V/V) until well combined. All solutions were kept cold and dark, out of direct sunlight, and at or below 25°C. After calibrating the flame on the photometer, the test solution was sprayed through it, and the reading was recorded. Then, after every spray, the apparatus is required to be thoroughly cleaned. To find the potassium or sodium concentration in the unknown solution, utilize the following equations:

$$S_{STD} = KC_{STD} \quad (1)$$

$$K = \frac{S_{STD}}{C_{STD}} \quad (2)$$

$$\text{From (1) and (2)} \quad C_{UN} = \frac{S_{UN}}{K}$$

Where: K; constant; SSTD; signal of standard solution; CSTD; concentration of standard solution; SUN; signal of unknown sample; and CUN; concentration of unknown sample [19]. The accuracy and precision of the flame photometer were assessed using reference samples.

2.3.3.The analysis by UV visible spectrophotometer:

The sodium and potassium levels in the samples were determined using an experiment performed at room temperature (RT). The wavelengths for sodium and potassium were 630 and 578 nm, respectively. The instrument was set to zero using the reagent blank. The blank, standard, and sample were all placed in three plastic cuvettes with a 1cm light path each. The volumes were placed in a plastic cuvette, as shown in Table 1. After thoroughly mixing the mixture, let it stand for 5 minutes at room temperature. Finally, we properly mixed and measured the absorbance of the samples and standards against the reagent blank.

Table 1. Volumes of Reagent, Standard, and Sample in Cuvettes.

	sodium			potassium		
	A blank	The standard	The sample	A blank	The standard	The sample
Standard (μL)	--	10	--	--	20	--
Sample (μL)	--	--	10	--	--	20
Reagent (mL)	1.0	1.0	1.0	1.0	1.0	1.0

Table 2 displays the spectrum-diagnostic potassium and sodium agents intended for in-vitro quantitative diagnostic detection of sodium and potassium in human serum utilizing manual procedures.

Following the incubation period for analytical preparation, the instrument's wavelength control was set to the desired wavelengths, 630 and 578 nm for sodium and potassium, respectively. And we adjusted the 0 control until the readout

showed 0 with the reagent blank. Next, fill a test tube halfway with reference solution, insert it in the sample holder, and close the lid. The absorbance type was recorded. Following that, we filled another test tube, half full, with an unknown sample liquid. Finally, we assessed the absorbance mode of an unknown sample liquid. The spectrophotometer was calibrated using a reference tube that is identical to the standards and samples in every way except for the electrolyte of interest (potassium or sodium).

Table 2. Reagents for sodium and potassium.

sodium		potassium	
reagent	concentration	reagent	concentration
Chromogen	0.03 gm/L	Sodium hydroxide	0.50 mol/L
EDETA	25 mmol/L	Tetraphenylborate	240 mmol/L
DMSO	75 mmol/L	Potassium as standard	5 mEq/L
Preservatives	0.05%		
Antifoam	0.01%		
Sodium as standard	150 mEq/L		

he amounts of potassium and sodium in each sample were then estimated using the methods [39, 40]:

$$\text{serum sodium conc.} \left(\frac{\text{mEq}}{\text{L}} \right) = \frac{A \text{ of sample } \times 150}{A \text{ of standard}}$$

$$\text{serum potassium conc.} \left(\frac{\text{mEq}}{\text{L}} \right) = \frac{A \text{ of sample } \times 5}{A \text{ of standard}}$$

Where (A) represents the absorbance of the sample and the standard separately.

2.4.Data analysis and Quality Control (QC):

The data was evaluated using IBM's Statistical Tool for the Social Sciences (SPSS) version 25, which determined the sampling mean, standard deviation, and correlation. To confirm the precision and correctness of the techniques used,

the produced control samples were included in each batch assessed in the current investigation.

3.Results & discussion:

The sodium and potassium levels in the blood were evaluated by comparing ISE, flame photometry, and spectrophotometry in 70 individuals with renal disease. Serum samples were obtained and submitted to various analytical procedures. Because hemolysis, jaundice, or lipemia can have a considerable impact on flame photometry and spectrophotometry findings, 10 samples were removed from this experiment [41, 42]. Patients with jaundice had

significantly higher blood salt levels [43]. Tables 3 and 4 indicate the sodium and potassium levels (in mEq/L) obtained by each of the three devices.

Table 3. shows the sodium concentration (mEq/L) measured using ISE, flame photometry, and spectrophotometry.

No.	ISE ^a	f. ph. ^b	spect. ^c	No.	ISE	f. ph.	spect.
1	137	140	141	36	137	142	146
2	137	140	141	37	135	139	145
3	135	139	140	38	133	137	139
4	136	134	140	39	133	136	139
5	138	142	144	40	131	133	136
6	136	137	140	41	131	133	137
7	135	136	140	42	135	138	139
8	133	134	140	43	133	133	139
9	135	134	136	44	136	140	141
10	134	136	140	45	134	133	139
11	136	136	138	46	133	135	137
12	136	135	139	47	129	132	138
13	134	137	137	48	137	140	142
14	136	135	139	49	138	140	141
15	139	140	139	50	136	140	142
16	130	133	135	51	134	135	137
17	128	130	133	52	139	140	143
18	128	130	135	53	134	135	139
19	131	134	135	54	135	139	140
20	134	138	140	55	140	144	145
21	136	135	136	56	137	140	141
22	135	137	140	57	136	139	141
23	136	140	141	58	121	123	126
24	135	137	141	59	136	140	141
25	136	136	138	60	136	139	140
26	134	135	140	61	136	140	141
27	134	137	141	62	136	137	136
28	136	140	142	63	134	136	130
29	135	140	141	64	135	138	132
30	135	139	140	65	138	142	140
31	133	136	143	66	129	131	131
32	139	135	145	67	134	136	139
33	137	140	141	68	131	135	137
34	135	139	145	69	136	139	139
35	137	139	142	70	135	136	140

^a Ion selective electrode. ^b flame photometry. ^c spectrophotometry

Table 4. displays the potassium level (in mEq/L) measured using ISE, flame photometry, and spectrophotometry.

No.	ISE ^a	f. ph. ^b	spect. ^c	No.	ISE	f. ph.	spect.
1	5.3	5.0	4.4	36	3.4	4.2	3.2
2	5.4	5.0	4.4	37	6.6	5.9	5.8
3	5.4	5.7	4.2	38	6.4	6.1	5.1
4	4.0	3.8	4.2	39	6.1	5.8	4.8
5	4.6	3.6	4.4	40	4.9	4.6	4.0
6	5.6	4.9	4.6	41	5.8	5.6	4.8
7	6.6	5.1	6.2	42	4.7	4.6	4.0
8	5.2	5.8	4.2	43	5.8	5.7	4.5
9	5.5	4.9	5.4	44	4.9	4.7	4.5
10	5.4	5.8	5.1	45	5.7	5.6	4.7
11	3.5	5.3	5.8	46	6.6	6.5	5.4
12	5.8	4.1	5.3	47	6.4	5.8	4.9
13	5.4	4.7	4.1	48	5.4	4.9	4.4
14	4.2	4.9	4.2	49	5.4	5.0	4.0
15	4.5	4.6	4.3	50	4.8	4.5	4.3
16	6.0	4.1	4.4	51	4.8	4.7	3.9
17	5.6	5.0	4.6	52	5.5	4.9	4.5
18	4.9	4.9	4.0	53	4.4	4.9	3.7
19	4.7	4.7	4.3	54	5.4	5.7	4.2
20	4.8	4.6	4.4	55	3.8	4.2	3.9
21	5.1	4.9	4.5	56	4.4	5.1	3.7
22	5.3	5.7	4.7	57	5.9	4.9	4.5
23	5.1	5.2	4.4	58	5.3	4.9	4.4
24	5.6	5.7	5.3	59	7.4	6.8	6.2
25	4.9	5.3	4.4	60	5.2	4.8	4.1
26	3.9	4.3	3.6	61	7.4	6.8	5.7
27	5.2	5.0	4.3	62	5.2	4.9	4.9
28	4.5	4.5	4.0	63	4.5	4.3	4.2
29	5.8	5.7	5.0	64	5.0	4.8	4.7
30	3.9	4.1	3.4	65	4.3	4.9	4.2
31	3.9	4.3	3.3	66	4.7	4.8	4.5
32	3.5	4.3	3.0	67	3.7	4.1	4.0
33	4.7	4.3	4.0	68	5.2	5.0	4.7
34	6.0	5.6	4.7	69	6.4	6.1	6.1
35	5.3	4.9	4.4	70	6.8	6.2	6.5

^a Ion selective electrode. ^b flame photometry. ^c spectrophotometry

Table 5 compares the mean differences (in mEq/L) in blood salt and potassium concentrations across the three approaches. Decreased or increasing blood sodium or potassium concentrations are common in both healthy and ill people; hence, it is vital to evaluate the numerous analytical methods used to quantify serum sodium and potassium concentrations, and descriptive statistics were used to describe the findings. Furthermore, as lipemia progressed, electrolyte concentrations

declined due to interference from light scattering, volume displacement, or turbidity [39]. Errors in measurements can also cause pseudohyponatremia, which is caused by the migration of serum water due to high protein or serum lipid levels [44]. The data was presented as mean $\pm$ SD values, and a one-way ANOVA was performed to compare the values of the three analytical processes to normalcy ranges.

Table 5. indicates the mean differences in serum salt and potassium concentrations (mEq/L) between ISE, flame photometry, and spectrophotometers.

	No.	A minimum	A maximum	Mean	Std. Deviation	Standard Error Mean
Na ⁺ conc ^a . in ISE ^b	70	121	140	134.6	3.008	0.360
K ⁺ conc. in ISE	70	3.4	7.4	5.190	0.8866	0.1060
Na ⁺ conc. in flame photometry	70	123	144	136.8	3.432	0.410
K ⁺ conc. in flame photometry	70	3.6	6.8	5.037	0.6836	0.0817
Na ⁺ conc. in spectrophotometry	70	126	146	139.2	3.519	0.421
K ⁺ conc. in spectrophotometry	70	3.0	6.5	4.521	0.7028	0.0840

^a Concentration

^b Ion selective electrode

As shown in tables 6 and 7, the connection between sodium and potassium concentration values obtained using the three approaches was investigated. Pearson's correlation coefficient was calculated using the statistical software for social science

(SPSS) version 25 to determine the presence and degree of correlation between variables. $P < 0.01$ was considered statistically significant. Also, a 95% confidence interval was determined.

Table 6. Correlation analysis of sodium concentration data collected using the three techniques (mEq/L).

		sodium conc. in ISE	sodium conc. in flame photometry	sodium conc. in spectrophotometry
sodium conc. in ISE	Pearson's correlation	1	0.941**	0.905**
	Sig. (2-tailed)		0.0001	0.0001
	No.	70	70	70
sodium conc. in flame photometry	Pearson Correlation	0.941**	1	0.898**
	Sig. (2-tailed)	0.0001		0.0001
	No.	70	70	70
sodium conc. in spectrophotometry	Pearson Correlation	0.905**	0.898**	1
	Sig. (2-tailed)	0.0001	0.0001	
	No.	70	70	70

**There is a significant correlation at the 0.01 level (2-tailed).

It is crucial to recognize that a precise measurement of serum sodium and potassium levels is necessary for the diagnosis and safe management of dysnatremia and dyskalemia. Table 5 illustrates the variability of data in proportion to the mean (coefficient of variation/CV) or relative standard deviation as an extremely small variance between the sodium rate by ISE and flame photometry (0.0028), ISE and spectrophotometry (0.0029), and flame photometry and spectrophotometry (0.0001). Furthermore, the same data revealed a little

difference in potassium rates between ISE and photometry with flames (0.035), ISE and spectrophotometry (0.016), and flame photometry and spectrophotometry (0.019). In this study, Figs. 1 and 2 describe the relationship between the three techniques by comparing them to one another. photometer. According to Garcia [1], there was no significant difference in serum salt concentration measured using the ISE and flame photometer.

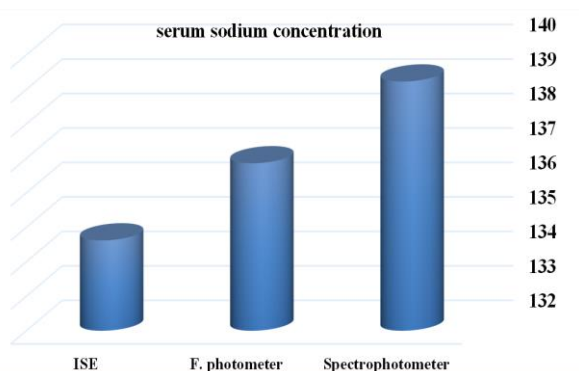


Fig. 1. demonstrates the relationship between ISE, flame photometry, and spectrophotometry for serum sodium level.

Serum sodium concentrations were not significantly different between the ISE (134.63 ± 3.008), flame photometry (136.86 ± 3.432), and spectrophotometry (139.23 ± 3.519) techniques (p value < 0.0001). Worth [45] observed comparable results and concluded that sodium readings by ISE and flame photometry differed very little. According to Garcia [1], there was no significant difference in serum salt concentration measured using the ISE and flame Table 7

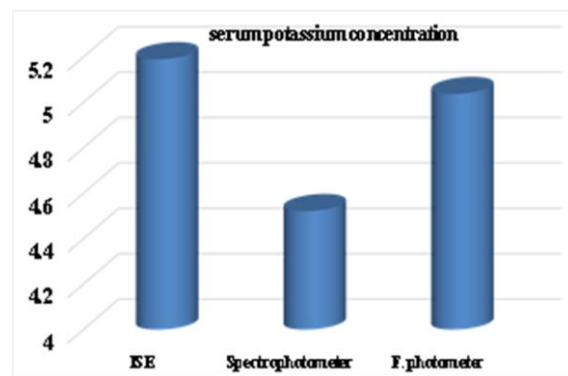


Fig. 2. depicts the association between serum potassium concentration and ISE, flame photometry, and spectrophotometry.

shows significant differences in serum potassium concentration average mean (5.190 ± 0.8866) mEq/L, (5.037 ± 0.6836) mEq/L, and (4.521 ± 0.7028) mEq/L assessed by ISE, flame photometer, and spectrophotometer (p value < 0.0001). Garcia [1] observed a similar result, suggesting that serum potassium concentrations measured by ISE and enzymatic colorimetric approaches varied considerably. The study's data were analyzed to determine whether there was a relationship between these tactics.

Table 7. illustrates a correlation study of data generated by the concentration of potassium (in mEq/L) using three methodologies.

		potassium conc. in ISE	Potassium conc. in flame photometry	potassium conc. in spectrophotometry
potassium conc. in ISE	The pearson correlation	1	0.914**	0.901**
	Sig. (2-tailed)		0.0001	0.0001
	No.	70	70	70
potassium conc. in flame photometry	Pearson Correlation	0.914**	1	0.840**
	Sig. (2-tailed)	0.0001		0.0001
	No.	70	70	70
potassium conc. in spectrophotometry	Pearson Correlation	0.901**	0.840**	1
	Sig. (2-tailed)	0.0001	0.0001	
	No.	70	70	70

** . Correlation is significant at the 0.01 level (2-tailed).

Serum sodium measurement results from ISE and flame photometry, ISE and spectrophotometry, and flame photometry and spectrophotometry showed a strong positive association ($r^2 = 0.8861$, $r^2 = 0.8194$, and $r^2 = 0.8060$, respectively). ISE and flame photometry, ISE and spectrophotometry, and flame photometry and spectrophotometry all show a significant correlation (r^2) in serum potassium measurement.

4. Conclusion and Recommendation:

(1) Serum sodium and potassium values were evaluated using ISE, flame photometry, and spectrophotometry, resulting in substantial differences ($p < 0.0001$).

(2) The sodium and potassium values obtained by ISE, flame photometer, and spectrophotometer were in agreement with reference values, showing 95% confidence, as shown in Tables above, and they did not differ enough to cause clinical complications, as variations were small within clinical

tolerance and did not change the classification of natremias and calemias.

(3) Electrolyte measurement is an essential clinical procedure, and the need for quick results has led to the emergence of automated techniques, including ISE. However, the results showed that the three methods are safe and can be used in clinical laboratories due to the strong correlation among the obtained results. According to the findings of the current study and conclusion, the researchers recommend that:

1. Researchers advocate avoiding hemolytic and jaundice-related samples.
2. Before measuring electrolytes, it's advised to determine lipid and protein concentrations.
3. To corroborate the results of these analytical procedures, use a healthy person's serum as a control sample.

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