

Medical

The Application of a Dried Balanced Heparin Syringe Can Attenuate the Dilution Effect in Rapid Point-of-care Potassium Testing for the Early Identification of Hyperkalemia in the Emergency Department

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Purpose: Current methods to evaluate the blood concentration of potassium (K) on point-of-care (POC) are influenced by the relative volumes of heparin and arterial blood. Blood potassium concentration may be underestimated with a high volume of heparin or low volume of blood. This dilution effect can produce false negative results that negatively affect decision-making of clinicians and throw critical patients into crisis. We hypothesized that the application of a dried balanced heparin syringe in rapid POC-K⁺ could attenuate the dilution effect and would more accurately and consistently measure the concentration of potassium compared with reference testing in emergency situations.

Methods: This retrospective study was conducted between January, 1, 2008 and September, 30, 2013 at an urban hospital affiliated with our institution. To attenuate the dilution effect, dried balanced heparin syringes (HS) were also used between October, 1, 2011 and September, 30, 2013. Concentrations of potassium were compared between the dried balanced HS group and the liquid HS group. The reliability of each of these outcome measures was assessed using intra-class correlation coefficient analysis.

Results: Application of dried balanced HS improved the degree of concordance for potassium using two different assays. The false negative rate was significantly improved from 9.1% (95% CI 7.3-11.0) to 5.7% (95% CI 3.5-8.0) in the dried balanced HS group compared with the liquid HS liquid group ($p=0.037$).

Conclusion: This study suggests that the usage of dried balanced HS could attenuate the dilution effect in rapid POC-K⁺ and predict potassium levels more accurately for

identification of patients at risk of hyperkalemia in emergency situations.

Key Words: Point-of-care testing, Hyperkalemia, Chronic Chronic kidney failure, Resuscitation

Article Summary

What is already known in the previous study

Current methods to evaluate the blood concentration of potassium on point-of-care (POC) are influenced by the relative volumes of heparin and arterial blood.

What is new in the current study

The application of dried balanced HS improved degree of concordance for potassium using two different assays.

Introduction

Generally, hyperkalemia represents with non-specific symptoms such as nausea, palpitation, muscle pain, and general weakness^{1,2}. It is difficult to detect hyperkalemia early and accurately in emergency situations. The delay caused by waiting for confirmation of potassium (K⁺) results is not critical in most cases of hyperkalemia. However, if hyperkalemia is not immediately recognized and treated, hyperkalemia can lead to fatality by changes of cardiac electrophysiology¹⁻³. Fatal hyperkalemia can be occur frequently in critical patients, such as those in septic shock, with severe trauma, and in a case of sudden cardiac arrest in the emergency department (ED) or intensive care unit (ICU)^{4,5}.

One option, called rapid point of care-potassium (POC-K⁺) can be applied in these situations. POC-K⁺ can decrease the time needed to achieve an accurate diagnosis and administer prompt treatment; however, the accu-

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racy of potassium concentrations measured by POC-K⁺ compared with standard assay is controversial^{6,7}. Some studies have suggested that the hemolysis of blood samples and the dilution error caused by collection of blood sample into a syringe with liquid heparin might affect the observed bias in POC-K⁺ results⁷⁻⁹. In general, arterial blood samples should be collected in heparinized syringes. Under the emergency circumstances, clinicians may unavoidably collect irregular volumes of arterial blood or liquid heparin in these syringes. The concentration of potassium as determined by POC-K⁺ may be underestimated by a relatively high volume of heparin or low volume of arterial blood^{8,10}. Consequentially, this dilution effect causes false negative results that adversely affect the decision making of clinicians and throw critical patients into crisis.

We hypothesized that the application of commercialized dried balanced heparin syringe in rapid POC-K⁺ could attenuate the dilution effect seen in rapid POC-K⁺. This technique would more accurately measure concentration of potassium can more closely approach the results produced by standard reference testing, leading to a rapid, accurate option for potassium concentration measurement in the ED.

Materials and Methods

This retrospective study was approved by the institutional review board and performed between January 1, 2008 and September 30, 2013 at an urban hospital affiliated with our institution. This hospital has an ED census of 65,000 patients per year. In this hospital, an effective screening program using POC has been conducted for the rapid evaluation and treatment of hyperkalemia since January 1, 2008. Upon the arrival of a patient with chronic renal failure (CRF) or end stage renal failure (ESRD) using patient-declared or health database information at the ED, emergency physicians identified patients at risk of hyperkalemia according to the predetermined protocol E-DASH: Emergency-Detection and Acquisition of Suspicious Hyperkalemia. The protocol directed diagnosis of hyperkalemia using known warning symptoms (palpitation, nausea, vomiting, diarrhea, weakness, and muscle pain) and signs (shock, dehydration, and EKG abnormality- peaked T, QRS widening,

and disturbance of cardiac rhythm)^{4,5}.

When a patient with CRF or ESRD had at least one warning symptom or sign listed above, the emergency physician simultaneously ordered both a POC-K⁺ test and routine laboratory tests including the reference laboratory serum- K⁺ test⁵. Arterial blood samples for POC-K⁺ were collected in heparinized syringes by clinicians. The syringes using liquid heparin (23 G) (KOVAX-SYRINGE[®], Korea vaccine, Seoul, Korea) were used to collect arterial blood between January 1, 2008 and September 13, 2011. To attenuate the dilution effect, dried balanced heparin syringes (Dried balanced HS) were also used between October, 1, 2011 and September, 30, 2013. Either the liquid heparin syringes (Liquid HS) or the dried balanced heparin syringes (22 G) (Eclipse[™], Beckton, Dickinson and company, Plymouth, UK) was used between September 14, 2011 and September 30, 2011 and the data during this period were excluded from this study. Although arterial samples in POC may not induce hemolysis and interfere with POC, venous samples in reference assay may cause hemolysis and influence differences between two assays. Samples with hemolysis were excluded from data for the reference assay to obtain more direct comparison between these two assays. POC-K⁺ was performed on whole blood with the NOVA Stat Profile CCX (Nova Biomedical, Waltham MA, USA). The auto-function in equipment and trained technicians of laboratory medicine have been assessed the quality of assay on eight-hour shifts⁵. Reference laboratory tests usually used in the clinical setting were performed on serum obtained from whole blood in the central laboratory with the Hidachi 7600 (Hidachi, Tokyo, Japan)⁵.

1. Statistical analysis

The cut-off concentration for hyperkalemia is usually defined as a serum K⁺ level of 5.5 mmol/L. This cut-off was applied to the population to explore the accuracy of the POC-K⁺ to detect hyperkalemia accurately at this concentration. The proportion of hyperkalemia between POC-K⁺ and reference assays was compared by McNemar test. Sensitivity, specificity, false positive (FP) rate, and false negative (FN) rates between two groups were compared by Chi-square test (Fisher's exact test). Comparisons of AUCs between the dried balanced

HS and the liquid HS were performed using the Delong method. Serum K⁺ levels are maintained within a very narrow range. Usually, serum K⁺ levels have been shown to positively correlate with deteriorating renal function because excretion is a major contributor to K⁺ regulation^{2,5}.

Concentrations of potassium between POC-K⁺ and reference assays were compared using paired *t*-test. Concentrations of potassium and ICC between the dried balanced HS group and the liquid HS group were also compared using the independent two-sample *t*-test and the two-way mixed model. A Bland-Altman plot was constructed for K⁺ levels; this scatter plot reveals the differences between the measurements obtained by the two tests and the mean of the measurements for each subject in the study. The mean difference of zero indicates perfect agreement, while the 95% limits of agreement are the intervals within which 95% of the data lie. Analysis by Passing-Bablok regression was performed for demonstrating the relationship of Y (results obtained using the reference test) and X (results obtained using POC-K⁺) in liquid HS (C) and in dried HS (D).

The reliability of each of the outcome measures was assessed by intra-class correlation coefficient (ICC) analysis. The ICC ranges from 0 to 1, with 0 indicating no agreement and 1 indicating perfect agreement. The ICCs were interpreted as follows; excellent: 0.75-1, modest: 0.40-0.74, and poor: 0-0.39^{11,12}.

The results of serum creatinine (Cr) were divided into five categories and the reliability of POC-K⁺ as a function of serum Cr levels was assessed by ICC analysis using absolute agreement with a two-way mixed model. All analyses were performed on SPSS software package (version 20; SPSS Inc., Chicago, IL, USA), R version 2.13.1 (R foundation for statistical Computing, Vienna, Austria), or SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

A total of 1705 patients with suspected hyperkalemia were included in this study. Of these, 517 patient samples were analyzed with dried balanced HS and 1188 patients with liquid HS. There were no significant differences in age (62.7 ± 12.2 and 62.2 ± 13.9 years, $p=0.486$) and sex (male 690 (58.1%) and 299 (57.8%), $p=0.924$)

Table 1. Sensitivity and specificity analysis of POC-K⁺ and reference tests of K⁺ levels in both liquid and dried balanced HS groups. A cut-off of 5.5 mmol/L was applied to the population, and the sensitivity and specificity was estimated in POC-K⁺.

Samples	K ⁺ level ≥ 5.5	POCT	REFERENCE	<i>p</i> -value	Sensitivity	Specificity	FP rate	FN rate	AUC
Total samples	Liquid HS	986 (83.00)	899 (75.67)	<0.0001*	66.44	98.89	4.95	9.84	82.66
	Hyperkalemia	202 (17.00)	289 (24.33)		(60.99-71.88)	(98.20-99.57)	(1.96-7.94)	(7.98-11.70)	(79.91-85.41)
	Dried balanced HS	423 (81.82)	397 (76.79)	<0.0001*	75.83	99.24	3.19	6.86	87.54
Samples without hemolysis	Liquid HS	94 (18.18)	120 (23.21)	-	(68.17-83.49)	(98.39-100.00)	(0-6.74)	(4.45-9.26)	(87.49-91.86)
	Hyperkalemia	0.5548	0.6199		0.0609	0.7647	0.7614	0.0722	0.0435*
	Dried balanced HS	919 (82.50)	845 (75.85)	<0.0001*	68.77	98.82	5.13	9.14	83.79
Samples with hemolysis	Liquid HS	195 (17.50)	269 (24.15)		(63.24-74.31)	(98.09-99.55)	(2.03-8.22)	(7.28-11.00)	(81.00-86.59)
	Hyperkalemia	401 (81.67)	381 (77.60)	<0.0001*	79.09	99.21	3.33	5.74	89.15
	Dried balanced HS	90 (18.33)	110 (22.40)		(71.49-86.69)	(98.33-100.00)	(0-0)	(3.46-8.01)	(85.31-92.99)
Samples with hemolysis	Liquid HS	0.6901	0.4484	-	0.0428	0.7647	0.7609	0.0371*	0.0272*
	Hyperkalemia								

HS: heparinized syringe, K⁺: potassium, FP: false positive, FN: false negative, AUC: area under curve

* $p < 0.05$

between liquid and dried HS groups. First, the sensitivity and specificity of the POC-K⁺ test was evaluated. When the 5.5 mmol/L threshold of hyperkalemia was applied to the population, the liquid HS group had a sensitivity of 66.4% (95% CI 61.0-71.9) and specificity of 98.9% (95% CI 98.2-99.6). In the dried balanced HS group, the sensitivity was 75.8% (95% CI 68.2-83.5) and specificity was 99.2% (95% CI 98.4-100.0). The sensitivity was improved significantly by using dried balanced HS in all cases except for the samples with hemolysis ($p=0.043$). The sensitivity was also evaluated for patients that

received actually emergency treatment of hyperkalemia (emergency hemodialysis, peritoneal dialysis and usage of medications) based on results of the POC-K⁺ assay. In liquid groups, the sensitivities were 92.9 (95% CI 87.1-96.2) between 5.5 mmol/L and 6.4 mmol/L, 100.0 (95% CI 93.5-100) between 6.5 mmol/L and 7.4 mmol/L, and 100.0 (95% CI 83.9-100) for more than 7.5 mmol/L. In dried groups, the sensitivities were 93.8 (95% CI 85.0-97.5) between 5.5 mmol/L and 6.4 mmol/L ($p=0.821$), 100.0 (95% CI 80.6-100) between 6.5 mmol/L and 7.4 mmol/L ($p=1.000$), and 100.0 (95% CI 75.8-100) for

Table 2. Comparison with differences of mean values for two assays between liquid HS group and dried balanced HS group.

		N	POC-K ⁺	REFERENCE	DIFFERENCE	<i>p</i> -value [†]	<i>p</i> -value [‡]
Total Samples	Liquid HS	1188	4.452 ± 1.166	4.842 ± 1.183	-0.390 ± 0.491	<0.0001	<0.0001
	Dried balanced HS	517	4.587 ± 1.118	4.812 ± 1.132	-0.225 ± 0.435	<0.0001	
Sample without hemolysis	Liquid HS	1114	4.467 ± 1.166	4.835 ± 1.169	-0.368 ± 0.474	<0.0001	<0.0001
	Dried balanced HS	491	4.602 ± 1.123	4.790 ± 1.115	-0.188 ± 0.387	<0.0001	

HS: heparin syringe

[†]: paired *t*-test

[‡]: independent two-sample *t*-test

Table 3. Comparison with the intra-class correlation coefficients (ICC) between liquid HS group and dried balanced HS group.

ICC (95% CI)	Liquid HS	Dried balanced HS	<i>p</i> -value
Total	0.913 (0.903-0.922)	0.925 (0.912-0.937)	0.1428
Samples without hemolysis	0.918 (0.908-0.926)	0.940 (0.929-0.950)	0.0028

CI: confidence interval, ICC: intra-class correlation coefficient, HS: heparinized syringe

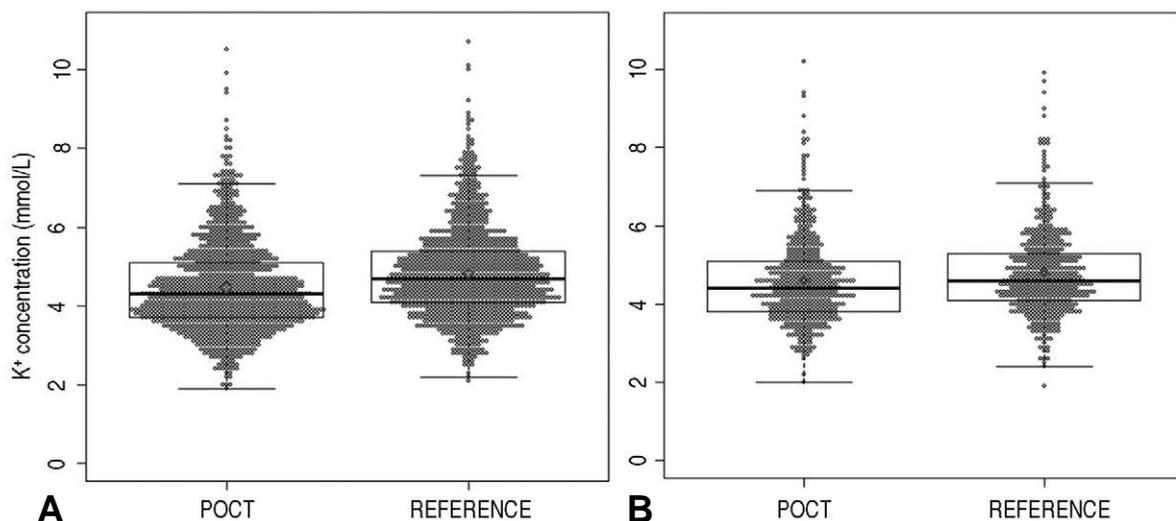


Fig. 1. Potassium concentrations measured by two methods of POC-K⁺. Box and whisker plots depict the performance of liquid HS (A) and dried balanced HS (B) compared to values acquired from the standard reference assay. The propensity of data for K⁺ showed a high degree of concordance between liquid HS and dried balanced HS except for hemolysis samples in reference assay.

more than 7.5 mmol/L ($p=1.000$). Additionally, the area under the curve (AUC) was compared for each test. Compared with the liquid HS group, the AUC of the dried balanced HS group improved from 83.8 (95% CI 81.0-86.6) to 89.2 (95% CI 85.3-93.0) and this improvement was statistically significant ($p<0.027$, Table 3). Importantly, the false negative rate was significantly improved from 9.1% (95% CI 7.3-11.0) to 5.7% (95% CI 3.5-8.0) in the dried balanced HS group compared with liquid group ($p=0.037$) (Table 1). Each POC method was compared to the reference assay by calculating the difference in mean values for each group. Relative to the reference assay, the difference in the mean value was -0.368 mmol/l in the liquid HS group and -0.118 mmol/l in dried balanced HS group, and this difference was significant ($p<0.001$). Additionally, the

difference between the mean values of the liquid HS group and dried balanced HS group was significant ($p<0.001$) (Table 2). The difference in the mean values of the two groups was prominently decreased when dried balanced HS were used. The data showed a high degree of concordance between K^+ levels measured by two assays. These results demonstrated that the application of dried balanced HS improved degree of concordance with the reference assay for potassium concentration. These data are summarized in dot plot and box-and-whisker plots in Fig. 1. Analysis by Passing-Bablok regression demonstrated the relationship of $Y=1.00 X - 0.30$ in liquid HS (X (95% CI) 1.0~1.0, intercept (95% CI) -0.3~-0.3) and $Y=1.00 X - 0.20$ (X (95% CI) 1.0~1.0, intercept (95% CI) -0.2~-0.2) in dried HS (Y: results obtained using the reference test, and X: results obtained using

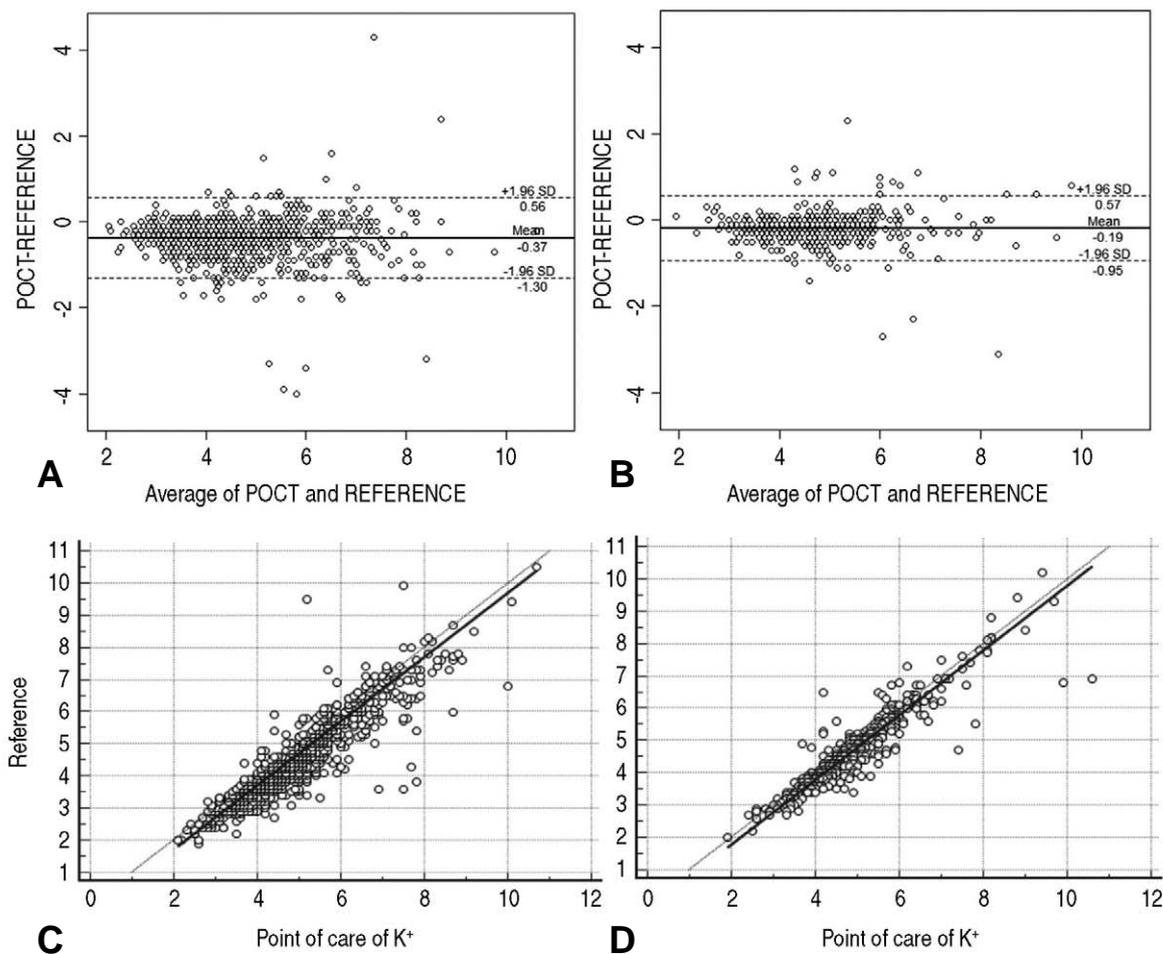


Fig. 2. Residual analysis of POC-K⁺ tested against the reference assay. Bland-Altman plot showing the mean differences (solid line) and 95% limits of agreement (dotted line) for K⁺ levels in liquid HS group (A) and dried balanced HS group (B). Samples in which hemolysis occurred were excluded in reference assay. Analysis by Passing-Bablok regression demonstrated the relationship of Y(results obtained using the reference test) and X (results obtained using POC-K⁺) in liquid HS (C) and in dried HS (D).

Table 4. Comparison with both liquid and dried balanced HS groups for correlations of creatinine levels with K⁺ levels resulting from POC-K⁺ and reference tests. The correction factor resulted in an overall increase in agreement between the two groups by ICC and narrowed 95% CIs.

Samples	Groups	ICC (95% CI)				
		0~20% (X<3.40)	20~40% (3.40≤X<5.48)	40~60% (5.48≤X<7.38)	60~80% (7.38≤X<10.1)	80~100% (X≥10.1)
Total samples	Liquid HS	0.829 (0.586-0.912)	0.838 (0.496-0.927)	0.877 (0.635-0.942)	0.897 (0.520-0.960)	0.877 (0.558-0.947)
	Dried balanced HS	0.874 (0.772-0.925)	0.868 (0.750-0.924)	0.846 (0.745-0.903)	0.912 (0.743-0.959)	0.964 (0.880-0.984)
Samples without hemolysis	Liquid HS	0.821 (0.615-0.902)	0.855 (0.531-0.935)	0.887 (0.665-0.947)	0.917 (0.560-0.969)	0.875 (0.559-0.946)
	Dried balanced HS	0.884 (0.804-0.929)	0.933 (0.855-0.964)	0.884 (0.819-0.925)	0.921 (0.787-0.961)	0.966 (0.887-0.984)

CI: confidence interval, ICC: intra-class correlation coefficient, HS: heparinized syringe

POC-K⁺) (Fig. 2). At the threshold value of 5.5 mmol/L, the total allowable error of potassium analysis was 5.8%, as derived from recent biological variation data⁵⁾. The intervals from arrival at the ED to beginning of test and from beginning of test to the reporting of the results were 18 [10, 31] and 4 [3, 6] minutes ($p=0.221$) for liquid HS and 19 [11, 33] and 4 [3, 5] minutes (<0.001) for dried HS.

Intra-class correlation coefficients (ICC) were used to determine the level of the agreement between the two methods. Overall, high levels of reliability (the consistency agreement) were found between POC and the laboratory reference tests for K⁺ in same patients by ICC (Table 3). Additionally, the agreement between the two tests was evaluated using the Bland-Altman method. The Bland-Altman plots demonstrate the mean differences and 95% limits of agreement between the two methods for K⁺ serum concentrations (Fig. 2).

Finally, the level of reliability was compared between the two POC-K⁺ testing methods. Reliability was determined by comparing each K⁺ test result with the Cr levels measured for each sample. Table 4 shows that high levels of reliability were found between reference test and the each test according to change in serum Cr. The correction factor resulted in an overall increase in agreement between the two groups by ICC and narrowed 95% CIs.

Discussion

Several studies have sought to find a rapid-detection test for hyperkalemia. However, conflicting results regarding the agreement of potassium concentrations to reference testing methods has led to some controversy. The US Clinical Laboratory Improvement Amendment recommended that the acceptable criteria for potassium performance is the target value ± 0.5 mmol/L. Ten Our study also demonstrated that calculated values were within total allowable error recommendations. Several studies have reported underestimation in potassium values measured by POC-K⁺^{5,10)}. There are a number of possible reasons for the observed bias in the POC-K⁺ results, including the hemolysis of blood samples and the dilution effect of liquid heparin^{7,8)}. Dilution error can affect significantly the results of POC-K⁺ and critical decision making. Additionally, medical errors in the pre-analytic

phase frequently affect results of laboratory test because of difficulty in achieving standardization for sample collection⁹. The magnitude of this difference may be even more marked where syringes are less meticulously prepared, or a time delay occurs in sample analysis causing a dilution effect a situation which occurs in everyday practice away from the rigorous conduct of a research trial. An earlier published study showed that the final heparin concentration in a blood gas sample could vary from 100 to 482 IU/mL and produced significant changes in the estimation of pCO₂, HCO₃⁻, Na⁺, K⁺ and ionic calcium varying from - 12% to 12%⁹. In a multi-step experimental study, Küme et al⁹. demonstrated that the results of the PCO₂ and electrolytes are lower than acceptable limits when the sample volume is lower or heparin concentration is higher by non-standardized sampling. To prevent serious medical errors, Küme et al⁹. recommended usage of electrolyte balanced dry heparin.

While hemolysis of samples can cause false positive readings, the underestimation of potassium values measured by POC-K⁺ can cause false negative results and lead to life threatening events in emergency situations. If necessary, the occurrence of hemolysis can be confirmed by obtaining plasma through centrifugation. However, our study demonstrated that the usage of the dried balanced HS could attenuate false negative rate compared with the liquid HS. The limitations of POC-K⁺ in arterial blood samples are observed the potassium regardless of hemolysis. However, our study revealed that the occurrence rate of hemolysis in venous samples from a reference study were as low as 6.23% in liquid HS and 5.03% in dried balanced HS. The results of POC-K⁺ by arterial blood samples for POC-K⁺ were underestimated compared to reference assay by venous blood samples. In rare cases, hemolysis may affect potassium levels measured by POC-K⁺ because arterial samples are collected with larger-bore needles and without tourniquets, and decision making of clinicians because abnormal results for POC-K⁺ showed false positive in most cases⁶.

Clinically, hyperkalemia must be immediately recognized and treated because hyperkalemia can lead to mortality and morbidity. Rapid POC-K⁺ testing has several clear advantages for critical patients in the ED or ICU. An assay using whole blood can be conducted anywhere outside a central laboratory in a hospital¹³. The reference laboratory test usually takes at least 40 minutes, includ-

ing a 30-minute centrifugation step to obtain plasma⁶. In contrast, POC-K⁺ uses whole blood and takes only 2~3 minutes. One previous report revealed that the median interval of time from door to report of results is faster by POC-K⁺ (24 minutes) than the serum reference test (61 minutes) ($p < 0.001$)⁵.

Hyperkalemia is more relevant in emergency situations. For example, rapid changes of K⁺ are associated with end stage renal failure, as well as acute and chronic kidney diseases. To date, there have been no studies investigating the reliability of POC-K⁺ and traditional laboratory K⁺ tests as a function of changes in serum Cr. However, You et al⁹. demonstrated that the high levels of reliability were found between POC-K⁺ and the reference test. This study also revealed that reliability between the two tests was higher when dried balanced HS were used compared with liquid HS for potassium, according to changes in serum Cr levels.

Electrolyte and blood gas analyses can be simultaneously performed in the ED and ICU. These critical values can be useful for making clinical decisions in critical patients, such as those with septic shock, severe trauma, or sudden cardiac arrest⁵. The integration of a POC-K⁺ testing method that uses dried balanced HS to this set of rapid diagnostic tests would help to accurately determine hyperkalemia by reducing the false negative rate. This method has the potential to reduce morbidity and fatality from hyperkalemia, reduce the time to achieve an accurate diagnosis and treatment, and consequently, reduce turnaround time in ED.

This study has several limitations. First, the nature of the retrospective study results in selection bias. Second, the POC-K⁺ assay cannot recognize hemolysis and reports K⁺ values regardless of hemolysis unlike reference assay⁶. In one report, José and Preller⁶. showed that arterial samples may have lower hemolysis than venous samples because these samples were collected with larger-bore needles without tourniquets⁶. However, our retrospective study cannot evaluate the occurrence of hemolysis in arterial blood used for POC-K⁺ testing. Third, unlike samples used for the reference assay, different sized needles were used between liquid HS (23 G) and dried balanced HS (22 G) to collect arterial blood samples for POC-K⁺ and differences for hemolysis between the two needle sizes cannot be evaluated. However, despite the fact that the liquid HS used a

smaller needle than dried balanced HS, the K⁺ concentrations reported for liquid HS group were more underestimated than POC-K⁺. For this study, we assumed that different sized needles for arterial samples had no effect on hemolysis during samplings. Finally, differences in the cost between dried balanced HS and liquid HS should be examined for cost effectiveness. Cost will remain a significant factor in the choice between the two types of syringes, but should not compromise the accuracy of measurement, calculation and clinical decisions. In addition erroneous results will translate into inappropriate interventions and avoidable costs. A future, prospective study with a larger number of patients would be useful to evaluate the dilution effect of liquid heparin and the effect of hemolysis on the accuracy of POC-K⁺ testing methods. This prospective study could also compare the diagnostic utility and cost effectiveness of dried heparinized syringes in POC-K⁺ tests for rapid and accurate screening of life-threatening hyperkalemia.

Conclusion

This study suggests that the usage of dried balanced HS could attenuate the dilution effect observed in rapid POC-K⁺ testing for the early identification of hyperkalemia. Dried balanced HS provided more accurate values than did liquid HS relative to standard laboratory tests, regardless of the change for serum Cr of patients with chronic kidney disease.

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