

Research Article



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Comparison of some biochemical tests in different blood collection tubes in hemodialysis patients

Hemodiyaliz hastalarında farklı kan alma tüplerinde bazı biyokimya testlerinin karşılaştırılması

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Abstract

Objective: Blood collection tubes (BCTs) related interferences in test results can adversely influence on patient outcomes. We compared test results of samples in BD (Becton-Dickinson, Franklin Lakes, NJ, USA) Vacutainer Serum Separator Tubes (SST), BD Vacutainer® Barricor™ Plasma BCTs (Barricor™) and BD Vacutainer® Rapid Serum Tube (RST).

Materials and methods: Thirty-two samples were obtained from patients after the hemodialysis were included in this study. Eight routine clinical chemistry parameters (AST, creatinin, urea, PTH, glucose, LDH, K, calcium) were measured on Roche Cobas Analyzer (Roche Diagnostics, North America). The results of samples obtained from RST and Barricor™ were compared with SST as reference tubes.

Results: Results of Glucose, K, Urea, PTH from the SST and Barricor™ were statistically significantly different ($p=0.017$, $p<0.001$, $p=0.011$, $p<0.001$, respectively). In

addition, results of PTH, LDH from SST and RST were significantly different ($p<0.001$, $p=0.019$). However, statistical significance of test results was not clinically significant for the biochemical parameters.

Conclusion: Working with Barricor™ may provide not just a fast, clean, high-quality plasma samples, safety results, but also time and cost-effectivity. Therefore, these types of tubes, which are less costly than other BCTs, may be preferred to obtain plasma.

Keywords: Plasma; Serum; Blood specimen collections; Analysis; Biochemistry.

Öz

Amaç: Kan Alma Tüpleri (KAT) ile ilişkili test sonuçlarında oluşan interferanslar hasta sonuçlarını olumsuz yönde etkileyebilir. Biz çalışmamızda BD (Becton-Dickinson, Franklin Lakes, ABD) Vacutainer Serum Separator Tüpleri (SST), BD Vacutainer® Barricor™ Plazma Kan Alma Tüpleri ve BD Vacutainer® RST (Hızlı Serum Tüpü) test sonuçlarını karşılaştırdık.

Gereç ve Yöntem: Çalışmaya diyaliz sonrası hastalardan alınan 32 örnek dahil edildi. 8 rutin klinik kimya parametresi (AST, Kreatinin, Üre, PTH, Glukoz, LDH, K, Kalسيوم) Roche Cobas analizöründe (Roche Diagnostics, Kuzey Amerika) ölçüldü. RST ve Barricor™ tüplerinden elde edilen test sonuçları referans tüp olarak SST ile karşılaştırıldı.

Bulgular: Glukoz, K, Üre, PTH sonuçları, SST ve Barricor™ tüpleri arasında istatistiksel olarak anlamlı farklılık gösterdi (sırasıyla; $p=0.017$, $p<0.001$, $p=0.011$, $p<0.001$). Ayrıca PTH ve LDH sonuçları SST ve RST

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arasında önemli düzeyde farklılık vardı ($p < 0.001$, $p = 0.019$). Ancak bu biyokimyasal parametreler için istatistiksel farklılıklar klinik olarak değerlendirildiğinde anlamlı değildi.

Sonuç: Barricor™ ile hızlı, temiz, yüksek kaliteli plazma numuneleri ile güvenli sonuçlar elde edilir iken aynı zamanda maliyet ve zaman avantajı sağlayabilir. Bu nedenle, plazma elde etmek için diğer kan alma tüplerinden daha az maliyetli olan bu tüpler tercih edilebilir.

Anahtar Kelimeler: Plazma; serum; kan örneği toplama; analiz; biyokimya.

Introduction

Sample collection, transportation and its preparation by centrifugation is also among the most important steps in the laboratory process. During centrifugation, Serum Separator Tubes (SST) are observed to include a gel barrier moving to the serum/clot interface [1, 2]. During the laboratory analysis the sample material, duration of centrifugation, its speed and temperature are crucial in order to get best results. To obtain plasma or serum, centrifugation performed at low speed for a short period of time may cause insufficient separation of blood cellular blood components or prolonged centrifugation at high speed may lead to hemolysis and cellular damage.

Optimal centrifuge conditions contribute to the decrease of the Turnaround Time (TAT) [3]. Considering the recommendations for sample centrifugation, a large variation can be observed in centrifugation temperature, time and rate, ranging from 15 to 25°C for 10–20 min of sample preparation, ranging from 1500 to 3000 g. However, a great number of laboratories try to decrease the total test duration.

Fibrin formation, which has the potential to interfere with the test results, can be caused by latent clotting in samples collected from patients who are taking anticoagulants. Hemodialysis patients receive anticoagulants, therefore it is too difficult to obtain serum samples with high quality from these patients. Those who are under anticoagulation therapy may have a longer blood clotting time [1].

In order to overcome these problems, the companies have released various Blood Collection Tubes (BCTs). BD (Becton Dickinson) Vacutainer® Barricor™ plasma blood collection tube (Barricor™ Tube) is a new type of tube designed to get stable plasma which includes a barrel-shaped polymer as the mechanical separator [4]. The Barricor™ Tube has a mechanical separator that contributes

to decrease the number of cells and platelets in the plasma samples. In Barricor™ tube the optimal centrifugal rotation time is 3 min at 4000 g, which is quite compatible with STAT procedures. After the barrier is opened during centrifugation, the cells migrate to the base of the tube. When the centrifugation is stopped, the barrier creates a seal between the plasma and cells. Therefore, fewer cells (including platelets) can be obtained in the plasma. The cells and platelets in the plasma affect the durability of high-sensitivity assays because of cell-mediated metabolism. However, in the Barricor™ tube, the number of platelets in plasma specimens decrease, therefore the stability of these analytes enhance.

The BD Vacutainer Rapid Serum Tube (RST) overcomes the clotting problems with occurring in current commercial serum tubes. It has a thrombin-based coagulation agent that can give a clotted sample within 5 min after blood collection [5, 6].

The biological half-life of intact PTH is approximately 2–5 min. Detection of the decrease in PTH level after parathyroidectomy is significant to confirm successful surgical removal. Therefore, it is important to measure the PTH levels quickly and reliably [6–8].

In this study we compared the results of various routine biochemical tests which required to be with short turnaround time (TAT) and PTH results between RST and Barricor™ tube, with reference to SST using blood samples from hemodialysis patients with to determine whether the BCT preference had any effect on biochemical test results especially for PTH levels.

Materials and methods

This study was conducted in the Clinical Biochemistry Laboratory of Ankara Numune Training and Research Hospital in June 2017. This comparative analytical study was performed in accordance with the Helsinki Declaration and with the approval of the local ethics committee (Ankara Numune Training and Research Hospital Ethics Committee with Protocol Number: E 17-1513.)

Blood samples were taken into SST (Lot No: 6179605; BD Diagnostics, Plymouth, UK), RST (Lot No: 161207; BD Diagnostics, Franklin Lakes, NJ, USA) and BD Barricor™ (Lot No: 5323984; BD Diagnostics, Franklin Lakes, NJ, USA) in a randomized drawing order from 32 chronic hemodialysis patients at the end of the session on the same day. Three blood samples from each volunteer were obtained from a single puncture, then they were collected in a randomized drawing order and the tubes

were filled to capacity by the same phlebotomist. Subsequently, BCTs were centrifuged in NF 800/NF 800R centrifuges (Nuve, Ankara, Turkey) (in SST, 1300 g for 10 min at 22°C; in RST, 1200 g for 10 min at 4°C and in Barricor™ tube, 2400 g for 10 min at 22°C) after the incubation period (for SST, 30 min; for RST 5 min and for Barricor™ Tube, no incubation period). Seven biochemistry parameters [creatinine, urea, potassium (K), aspartate transaminase (AST), calcium, glucose, LDH] were measured on Roche Cobas C501 Analyzer (Roche Diagnostics, North America), while PTH levels were measured by Roche Cobas E601 Analyzer.

Statistics and clinical significance

All statistical calculations were performed with SPSS® for Windows 14.0 (SPSS Inc. Headquarters, Chicago, IL, USA) and MedCalc 18.5 (Medcalc, Ostend, Belgium) statistical software program. The results of biochemical tests from the RST and Barricor™ were compared with SST as reference tube. After normality of test results was checked by Kolmogorov-Smirnov test, the significance of difference between the samples was assessed by paired t-test or Wilcoxon Rank test. The Bland-Altman and Passing-Bablok regression analysis were used for association and differences between analytes measured from the different tubes. Values are put forward as mean ± standard deviation (SD) and median ± interquartile range (IOR). Bonferroni correction was used to

adjust the value of the significance level in the binary comparison of groups, and p value <0.025 was considered as statistically significant. The results with statistical significance were further analyzed to evaluate in terms of clinical significance. The evaluation of clinical significance was based on the desirable bias [9]. Bias was determined for each analyte on each tubes as follows: Bias% = (Average absolute deviation from the target value/Target) × 100 [10].

Results

LDH activities and PTH levels which had been measured in serum in RST tubes were lower than serum from SST tubes ($p=0.019$, $p<0.001$, respectively). Mean glucose values in Barricor™ Tubes were higher than SST tubes ($p=0.017$). The mean levels of K, urea, LDH, AST in Barricor tubes were lower than those in SST Tubes ($p<0.001$, $p=0.011$, $p=0.006$, $p=0.004$, respectively) (Table 1).

The Bland-Altman plot denoted that the different tubes had the influence on the mean values of K, creatinine, calcium, AST, PTH, LDH, glucose, urea as seen in our results (Figure 1). However, the statistical significance was not compatible with clinical significant in terms of all parameters analyzed in all three tubes according to desirable bias. Bias% values of K (5.2%), AST (8.8%) were lower in BD Barricor™ tubes than same parameters in reference SST Tubes and RST tubes (Table 2). Bias% value for PTH

Table 1: Comparison of biochemical parameters between BD Barricor™, RST and SST.

	BD Barricor™ (mean ± SD)	Median ± IQR	BD SST (mean ± SD)	Median ± IQR	RST (mean ± SD)	Median ± IQR	p-Value
Creatinine (mg/dL)	2.92 ± 1.24	2.68 (1.46–6.44)	2.95 ± 1.22	2.67 (1.47–6.32)	2.92 ± 1.26	2.49 (1.51–6.70)	0.181 ^a 0.408 ^b
Calcium (mg/dL)	9.6 ± 0.72	9.45 (8.67–11.40)	9.53 ± 0.76	9.47 (8.05–11.34)	9.53 ± 0.89	9.42 (6.84–11.54)	0.342 ^a 0.710 ^b
AST (U/L)	13.30 ± 6.31	12.30 (1–28)	14.13 ± 5.639	13.35 (5–29)	14.03 ± 5.96	11.85 (4–29)	0.004 ^{a,c} 0.967 ^{b,c}
PTH (pg/mL)	648.70 ± 503.65	578.5 (30–1961)	612.01 ± 474.17	552.30 (23–1806)	538.74 ± 413.12	485.9 (21.2–1647)	0.000 ^{a,c} 0.000 ^{b,c}
Glucose (mg/dL)	127.13 ± 39.76	126 (72–280)	125.86 ± 39.07	124.80 (73–276)	125.52 ± 39.38	124.15 (68–275)	0.017 ^{a,c} 0.530 ^b
LDH (U/L)	223.10 ± 70.12	204.5 (125–449)	224 ± 64.88	216.5 (123–451)	219.27 ± 66.42	205.5 (120–464)	0.006 ^{a,c} 0.019 ^{b,c}
Urea (mg/dL)	36.50 ± 14.58	33.5 (13–66)	36.89 ± 14.24	34 (15–67)	37.20 ± 14.34	33.75 (15–67)	0.011 ^{a,c} 0.247 ^b
K (mmol/L)	3.16 ± 0.36	3.20 (2.51–3.85)	3.34 ± 0.38	3.35 (2.60–4.25)	3.36 ± 0.39	3.42 (2.56–4.42)	0.000 ^{a,c} 0.166 ^b

^ap-Value for SST and BD Barricor™. ^bp-Value for SST and RST. ^c $p<0.025$.

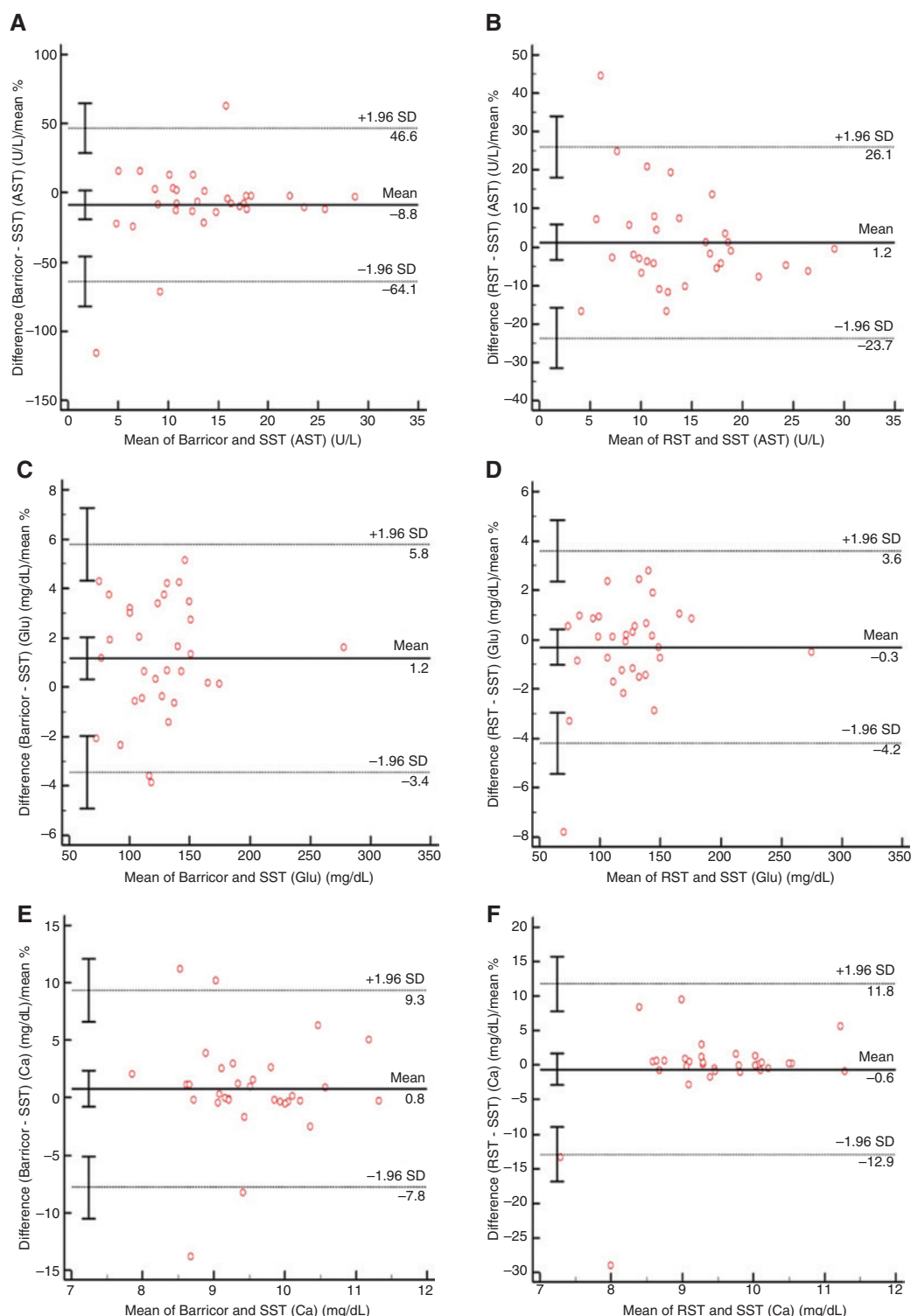


Figure 1: Bland-Altman difference plots for the eight biochemical analytes obtained with RST, Barricor TM and SST Tubes. (A, B) aspartate aminotransferase; (C, D) glucose; (E, F) calcium; (G, H) creatinine; (I, J) potassium; (K, L) LDH; (M, N) PTH; (O, P) urea.

was detected as 11.2% which was lower in RST and SST tubes than its mean value in Barricor™ tubes (Table 3).

Passing Bablok regression analysis (Figure 2) was performed for all parameters. Regression analysis

[regression equation PTH (RST)] $y = 4.57 + 0.87x$ showed an intercept of 4.57 [95% CI, (0.0001–10.5)] and a slope of 0.87 [95% CI, (0.85–0.89)]. The correlation coefficient was 0.997 with $p < 0.001$. K (Barricor™ tube) $y = 0.14 + 0.91x$

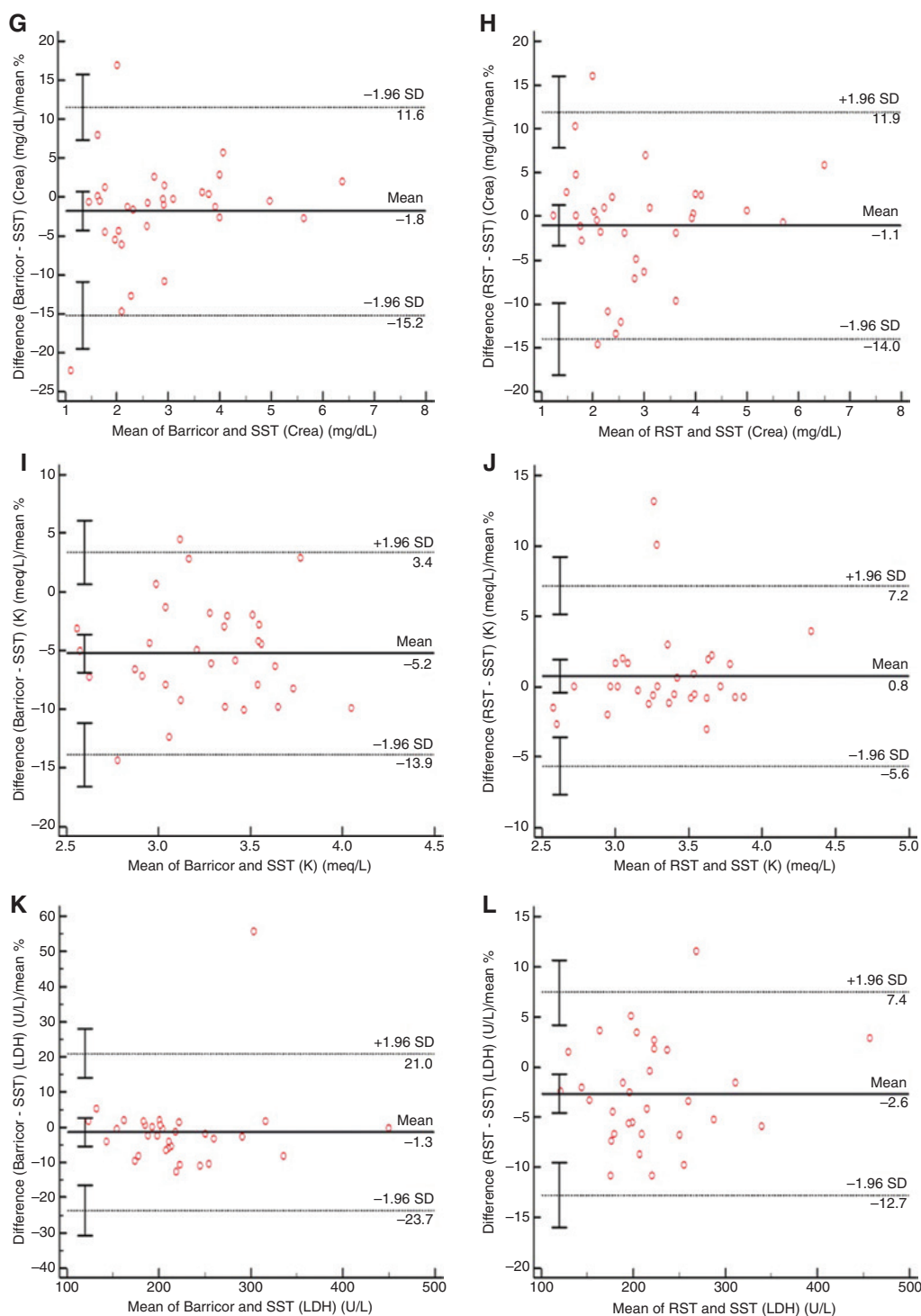


Figure 1 (continued)

showed an intercept of 0.14 [95% CI, (-0.41 to 0.56)] and a slope of 0.91 [95% CI, (0.77–1.07)]. The correlation coefficient was 0.915 with $p < 0.001$. AST (Barricor™ tube) $y = -0.26 + 0.94x$ showed an intercept of -0.26 [95% CI, (-1.57 to 0.73)] and a slope of 0.94 [95% CI, (0.87–1.05)].

The correlation coefficient was 0.911 with $p = 0.004$. Results of Passing and Bablok regression consist of several parts and each has its role in interpreting tubes comparison data and concluding on tubes agreement. The first result is scatter diagram with regression line

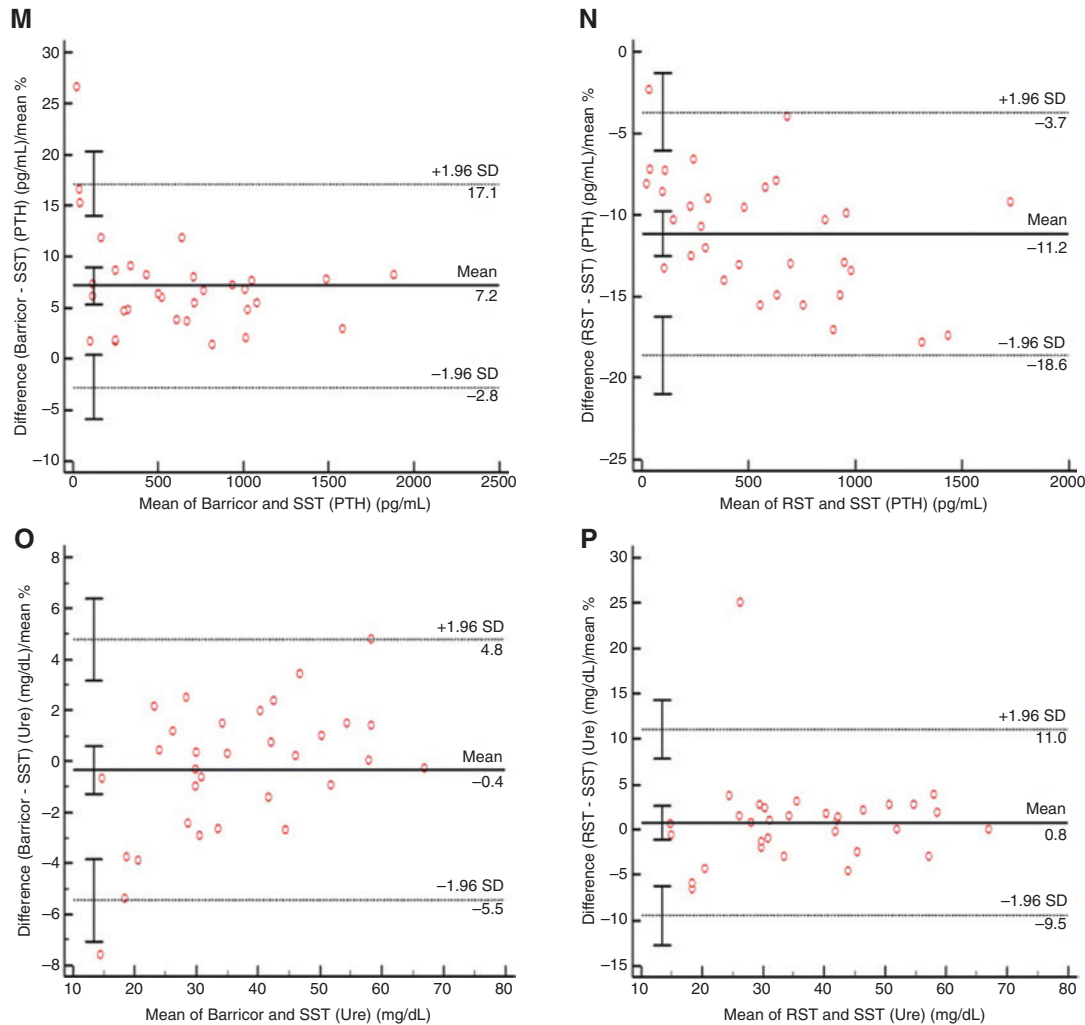


Figure 1 (continued)

Table 2: Comparison of biochemical parameters between Barricor™ and SST Tubes.

	Unit	BD Barricor™ (mean ± SD)	BD SST (mean ± SD)	Bias (%)	Desirable bias (%)
Creatinine	mg/dL	2.92 ± 1.24	2.95 ± 1.22	-1.8	8.87
Calcium	mg/dL	9.6 ± 0.72	9.53 ± 0.76	0.8	0.82
AST	U/L	13.30 ± 6.31	14.13 ± 5.6.39	-8.8	6.54
PTH	pg/mL	648.70 ± 503.65	612.01 ± 474.17	7.2	12.56
Glucose	mg/dL	127.13 ± 39.76	125.86 ± 39.07	1.2	2.34
LDH	U/L	223.10 ± 70.12	224 ± 64.88	-1.3	4.3
Urea	mg/dL	36.50 ± 14.58	36.89 ± 14.24	-0.4	5.57
K	mmol/L	3.16 ± 0.36	3.34 ± 0.38	-5.2	1.81

Bold values are exceeding the desirable bias (%).

that enables visual inspection of measured data and obvious agreement of fitted regression line and identity line (Figure 2). Regression equation ($y = a + bx$) revealed constant [regression lines intercept (a)] and proportional [regression lines slope (b)] error with their confidence intervals of 95% (95% CI).

Discussion

Manufacturers have produced various tubes which provide high-quality separation between serum/plasma and cells in order to reduce TAT and obtain less cellular content. After our literature evaluation, this was the first study including

Table 3: Comparison of biochemical parameters between RST and SST Tubes.

	Unit	RST (mean $\pm$ SD)	BD SST (mean $\pm$ SD)	Bias(%)	Desirable bias (%)
Creatinine	mg/dL	2.92 $\pm$ 1.26	2.95 $\pm$ 1.22	-1.1	8.87
Calcium	mg/dL	9.53 $\pm$ 0.89	9.53 $\pm$ 0.76	-0.6	0.82
AST	U/L	14.03 $\pm$ 5.96	14.13 $\pm$ 5.6.39	1.2	6.54
PTH	pg/mL	612.01 $\pm$ 474.17	538.74 $\pm$ 413.12	-11.2	8.8
Glucose	mg/dL	125.86 $\pm$ 39.07	125.52 $\pm$ 39.38	-0.3	2.34
LDH	U/L	224 $\pm$ 64.88	219.27 $\pm$ 66.42	-2.6	4.3
Urea	mg/dL	37.20 $\pm$ 14.34	36.89 $\pm$ 14.24	0.8	5.57
K	mmol/L	3.36 $\pm$ 0.39	3.34 $\pm$ 0.38	0.8	1.81

Bold values are exceeding the desirable bias (%).

newly developed Barricor™ tube studied with hemodialysis patients. In this study, we compared results of various routine biochemistry parameters between RST and Barricor™ tube, with reference to SST using blood samples from hemodialysis patients. The selection of the tube was performed after the effect of BCTs on biochemistry tests in hemodialysis patients.

Mean values of K, AST were lower in Barricor™ tube than the mean in reference SST tubes according to Bias%. We supposed that decreasing levels of AST, K in Barricor™ tube is due to the analysis from cell-free of plasma. Arslan et al. stated that K levels is higher in serum due to the excretion from erythrocytes and thrombocytes during the coagulation as also mentioned in the literature [11].

RST tube was previously evaluated against SST through only healthy subjects and non-immunoassay analytes [5, 10]. The study with RST offers rapid clotting time by allowing rapid serum separation. In RST samples visible clotting was obtained rapidly; particularly in healthy subjects. It was observed that patients receiving anticoagulants had a slightly longer coagulation time ~10 min [5, 12]. Dimeski et al. stated that patients receiving high-dose heparin had a clotting time in RST up to 20 min or longer [13]. In this study, we found that the use of this tube represented a development over the existing SST serum tube when certain parameters were taken into account. The main findings are as followed: a significant statistical difference between RST tubes and SST tubes for the levels of PTH, urea, AST, creatinine, glucose, LDH and K. RST ensures fast clotting time by making quick serum separation possible. Visible clotting was achieved rapidly in RST tubes. The similarities and differences in the concentration of analytes measured in RST have been reported beforehand. In the Ng and Yeo study, no differences were observed in the concentrations of the test analytes [14]. However, Dimeski et al. indicated the difference in LDH activity [13]. This difference was attributed to a faster coagulation process that reduced cell lysis to the lowest level. In addition, Huyghe et al. found that

the measured serum LDH activity was significantly lower among samples collected in RST than those collected in SST [12]. In our study, LDH results in the RST tube were lower than those in SST tubes. As Dimeski reported that this situation could be explained by a decrease in cell lysis and hence AST, LDH and K release of fast clotting in the RST tubes [13]. The statistical significance we detected for LDH levels was not significant clinically. Latent clotting in RST samples continued to occur in the RST specimens collected from participants receiving high doses of anticoagulants (heparin or warfarin/heparin), even when samples were allowed to stand for 30 min prior to centrifugation. Such covert clotting may put the accuracy of the results in jeopardy.

This study also assessed the effect of serum samples by using different tubes on PTH results. We found statistically significant differences for PTH and LDH parameters between RST and SST tubes in the Cobas E 601 analyzer. There was a statistically significant difference between RST and SST tubes in PTH levels. Its Bias% value was below % desirable bias. Serum samples were incubated with exogenous bovine thrombin, direct thrombin inhibitor hirudin. Subsequently, both of them in a study in which La'ulu et al. investigated why PTH results were lower than SST in RST. It was reported that the low PTH detected in RST was caused by bovine thrombin and caused lower levels of PTH due to fragmentation. However, the same study also showed minimal reduction in PTH levels for SST and it was suggested that this might be due to endogenous thrombin during active coagulation [7]. Interestingly, in this study, there was a decrease in PTH levels despite the addition of hirudin, which suggests that additional mechanisms such as non-thrombin proteolytic enzymes may be responsible for PTH destruction [7]. Other differences among tubes apart from thrombin content can also be taken into account; one of which is a separate chemical structure of the gelatin they contain. All of these may be related to the difference we observed in PTH levels, however we do not have sufficient data to explain this finding. Our results

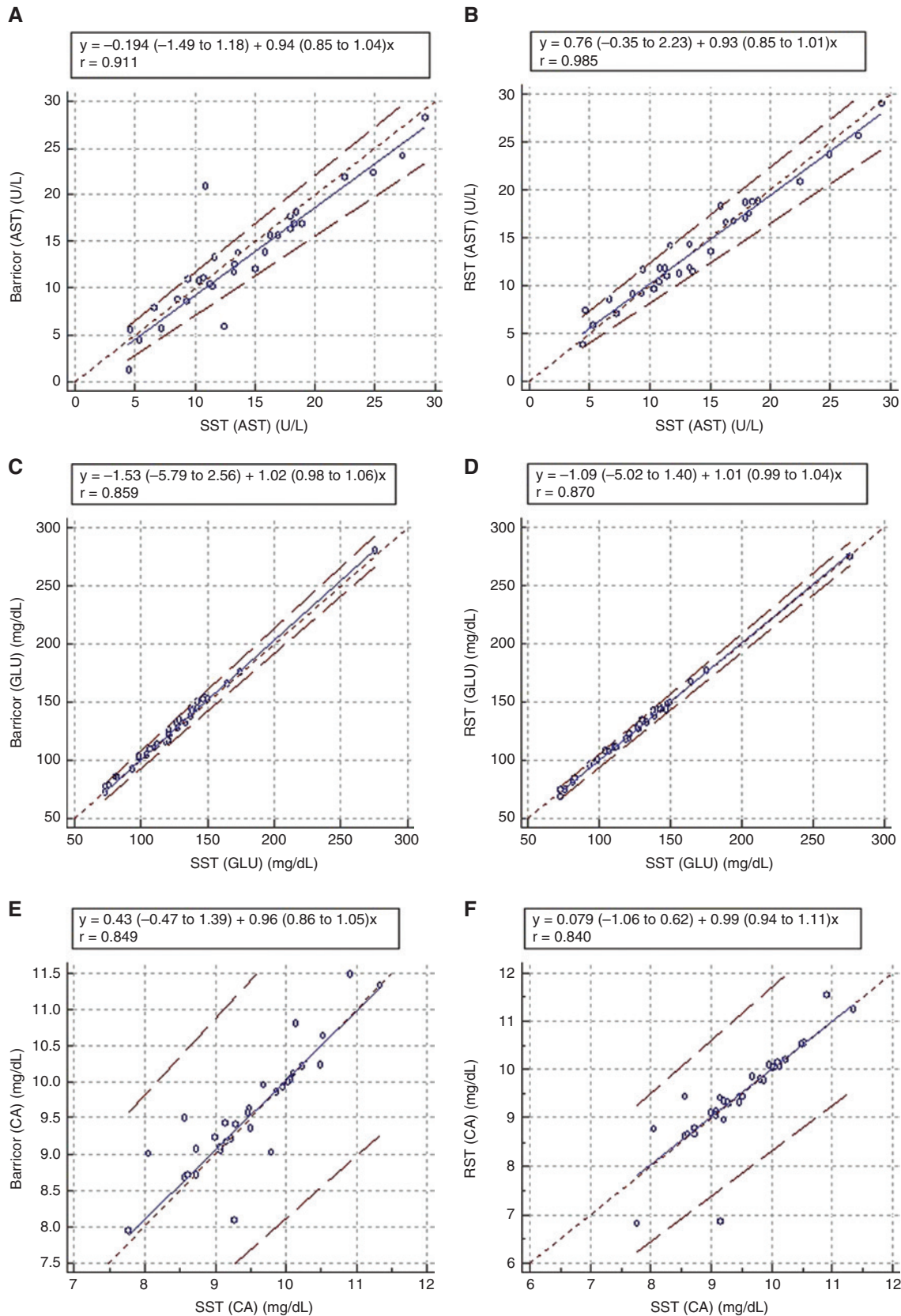


Figure 2: Passing-Bablok regression analysis.

Comparison of results for RST, Barricor TM and SST tubes for the biochemical eight analytes. (A, B) aspartate aminotransferase; (C, D) glucose; (E, F) calcium; (G, H) creatinine; (I, J) potassium; (K, L) LDH; (M, N) urea; (O, P) PTH.

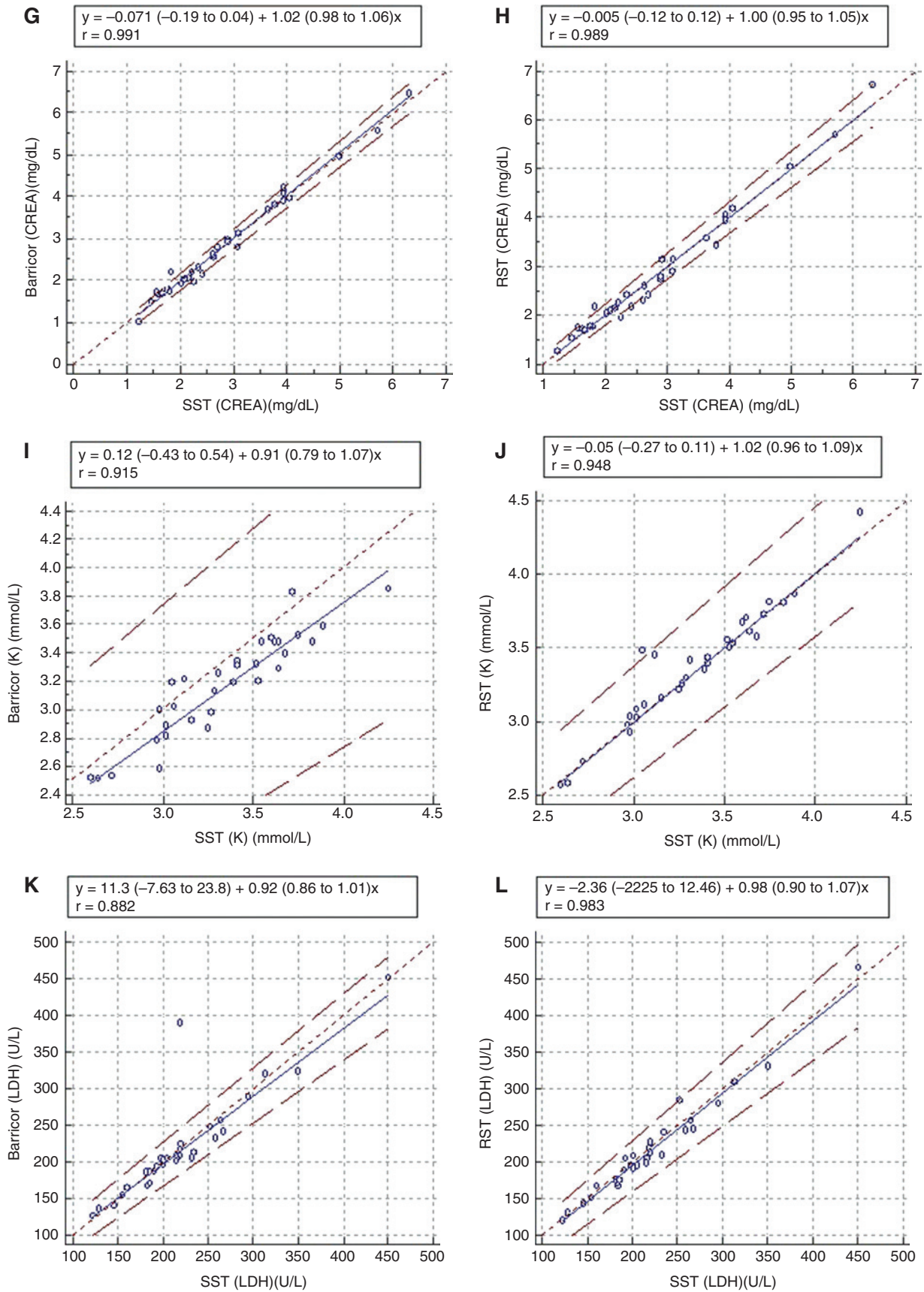


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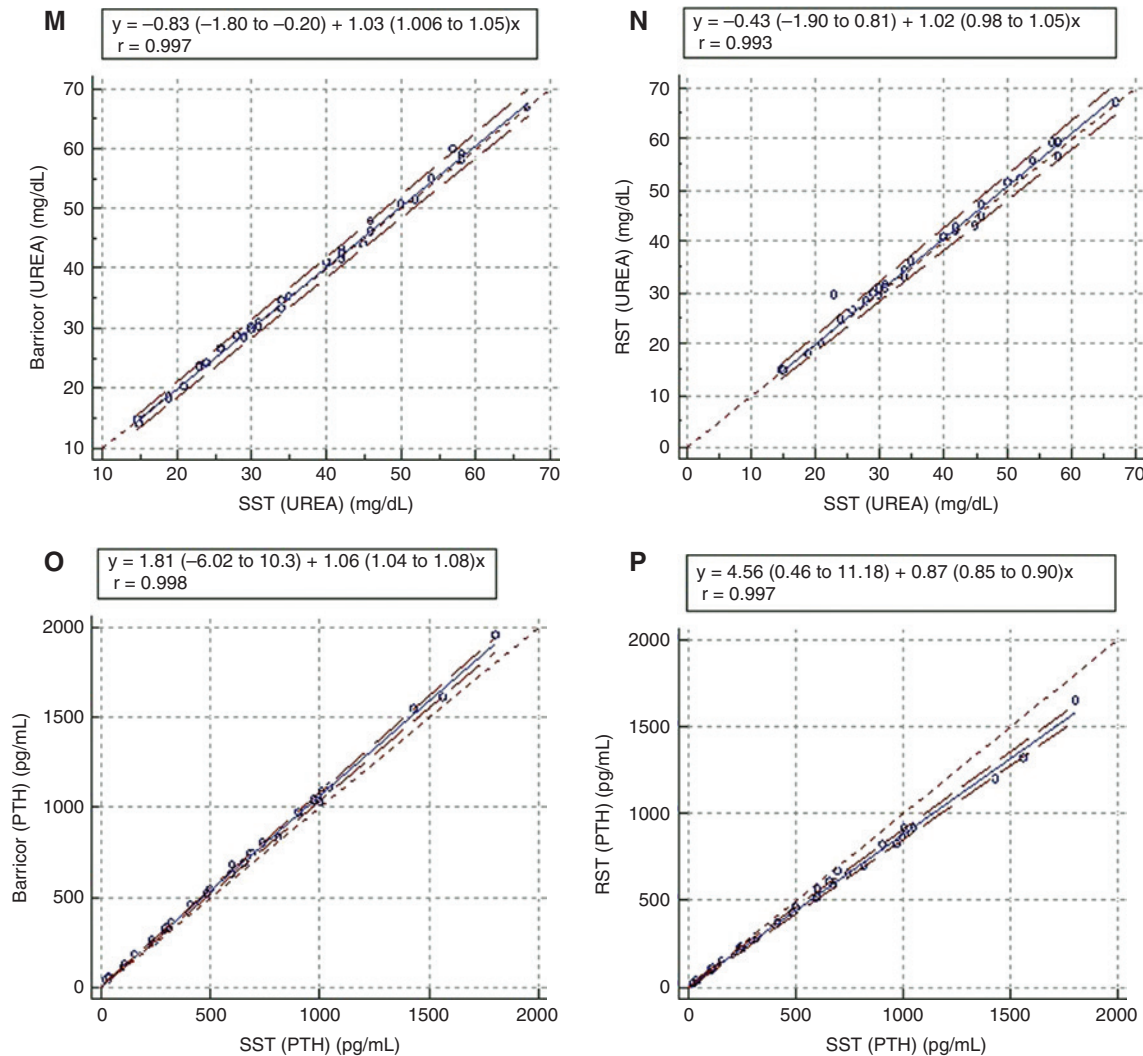


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also similarly indicated that PTH levels were lower in RST than in SST. However, in Ucar et al. study of routine chemistry and immunoassay parameters with 97 healthy volunteers in 2015, they found that PTH levels were significantly higher in RST in comparison to other studies and 1.95% of the difference between the two tubes was within the acceptable range [15]. In non-separated blood samples, Glucose is still metabolized at approximately 5%–7% per hour at room temperature because upstream enzymes continue to metabolize it to glucose-6-phosphate [11]. In Barricor™ tubes, fewer cells (including platelets) can be obtained in the plasma. The cells and platelets present in the plasma affect the stability of high-sensitivity assays because of cell-mediated metabolism, particularly glucose. Thus, we found that glucose values in Barricor™ tube were higher than SST. However, Arslan et al. showed that there was clinical significance in lower concentrations of Glucose only in Barricor™ plasma, whereas there

was not differences those in lithium heparin (LiH) plasma not. They explained that the preservation of glucose in LiH plasma might be explained with the transfer of plasma into a secondary tube. However, there was not difference for glucose values between plasma and serum samples in some studies [16–18].

All samples were taken as part of routine clinical practice in a daily clinical situation. The results in this study reflect normal routine process, which we suppose that it limits the influence of potential selection bias on our results.

There were some limitations in our study. Firstly, we did not perform precision and stability evaluation. Secondly, we did not include healthy volunteers in this study. Cadamura et al. were collected plasma and serum samples into BD Vacutainer PST II-heparin-gel, SST and Barricor™ from healthy volunteers and measured 81 clinical chemistry analytes using a Roche COBAS Analyzer. They found that test except LDH did not have significant differences between

tubes in Barricor™ tube [19]. Arslan et al. also found that LDH levels in Barricor™ tubes were higher than other tubes [11]. They thought that LDH values may have increased although there was a mechanical separator in these tubes due to the prolonged contact time or the continuation of cellular shift. We also found that LDH results were statistically different in all tubes from hemodialysis patients.

As a result, this study has demonstrated that there was not difference between all three tubes when they were used correctly. Significant differences observed among the measured analyte concentrations were small and not clinically significant.

It can also be used safely particularly in patients receiving hemodialysis or using anticoagulant.

Owing to the shorter turnaround time, plasma samples are used in many laboratories in comparison to serum [20]. Working with plasma samples can improve laboratory workflow efficiency and may reduce the cost.

In conclusion, the Barricor™ tube, which is a less costly tube may be preferred to obtain plasma instead of RST.

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Conflict of interest: The authors have no conflict of interest.

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