Comparison of 30 Biochemical Analytes in 3 Different Blood Collection Tubes

Üç Farklı Kan Alma Tüpünde 30 Biyokimyasal Analitin Karşılaştırılması

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ABSTRACT

Aim: In this study, we compared 3 different clot-activator gel tubes to a glass reference tube and evaluated the effect of storage time on 30 different biochemical analytes.

Material and Methods: Blood samples were collected in 4 types of tubes: an additive- and gel-free glass tube and three different clot-activator tubes containing gel (Samplix, Vacuette, and Vacutainer). In addition to comparison with the glass tube, stability analyses were performed in Samplix, Vacuette, and Vacutainer tubes after storage for 48 hours at +4°C.

Results: Clinically important differences were found for sodium (-0.29, bias), potassium (2.35) and magnesium (2.78) in Samplix; for sodium (-0.27), potassium (2.82), lactate dehydrogenase (4.47) and magnesium (2.46) in Vacuette; and for calcium (-1.56), chloride (0.66), potassium (3.54), lactate dehydrogenase (9.11) and sodium (0.38) in Vacutainer. At the end of the 48 hours, analytes that demonstrated instability were chloride (1.01), potassium (2.69), sodium (0.54), and total protein (1.95) in Samplix; chloride (1.11), potassium (2.06), and sodium (0.84) in Vacuette; and calcium (1.28), chloride (0.64), free T3 (-8.87), glucose (2.76), potassium (2.19), sodium (0.65), and total protein (2.15) in Vacutainer.

Conclusion: Various blood collection tubes (BCTs) with different contents may cause clinically important differences in test results. Therefore, each laboratory should verify the reference range transfer or create its own reference range before using a new BCT. It should also be noted that all clinical chemistry or immunological test analytes may not remain stable in BCTs up to 48 hours.

Keywords: blood specimen collection; clinical chemistry; immunoassay; serum

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ÖZET

Amaç: Bu çalışmada, 3 farklı pıhtı aktivatörlü-jelli tüpü bir cam referans tüp ile karşılaştırdık ve saklama süresinin 30 farklı biyokimyasal analit üzerindeki etkisini değerlendirdik.

Gereç ve Yöntem: Kan örnekleri 4 tüpte toplandı: katkı maddesi ve jel içermeyen cam tüp ve üç farklı jelli-pıhtı aktivatörlü tüp (Samplix, Vacuette ve Vacutainer). Cam tüp ile karşılaştırmanın yanı sıra, + 4°C'de 48 saat saklandıktan sonra Samplix, Vacuette ve Vacutainer tüplerinde stabilite analizleri yapıldı.

Bulgular: Samplix'te sodyum (-0,29,bias), potasyum (2.35) ve magnezyum (2.78) için; Vacuette'de sodyum (-0.27), potasyum (2.82), laktat dehidrojenaz (4.47) ve magnezyum (2.46) için; ve Vacutainer'da kalsiyum (-1.56), klorür (0.66), potasyum (3.54), laktat dehidrojenaz (9.11) ve sodyum (0.38) için klinik olarak önemli farklılıklar bulundu. 48 saatin sonunda, Samplix'te klorür (1.01), potasyum (2.69), sodyum (0.54) ve toplam protein (1.95); Vacuette'de klorür (1.11), potasyum (2.06) ve sodyum (0.84); ve Vacutainer'da kalsiyum (1.28), klorür (0.64), serbest T3 (-8.87), glukoz (2.76), potasyum (2.19), sodyum (0.65) ve toplam protein (2.15) stabilitesi bozulan analitlerdi.

Sonuç: Farklı içeriğe sahip çeşitli kan toplama tüpleri (BCT'ler) test sonuçlarında klinik olarak önemli farklılıklara neden olabilir. Bu nedenle, her laboratuvar yeni bir BCT kullanmadan önce referans aralığı transferini doğrulamalı veya kendi referans aralığını oluşturmalıdır. Ayrıca, tüm klinik kimya veya immünolojik test analitlerinin, BCT'lerde 48 saate kadar stabil kalmayabileceği unutulmamalıdır.

Anahtar Kelimeler: kan örneği alma; klinik kimya; immunoassay; serum

INTRODUCTION

According to the concept of the "brain-to-brain turnaround time loop", the total testing process begins when a test is ordered and ends when the test result is reported and action is taken by the physician based on that result (1,2). In this comprehensive concept, the laboratory testing process can be evaluated in three phases: pre-analytical, analytical, and post-analytical. Although various errors can take place in each of these phases, up to 70% of all errors in the testing process occur in the pre-analytical phase (3,4). A rarely considered potential source of error in the pre-analytical phase is choice of blood collection tube (BCT). Unfortunately, BCTs are typically regarded as inert specimen carriers with a negligible role in test result accuracy. Therefore, laboratories have shown little interest in investigating the potential impact of BCTs on test results.

Serum is one of the most frequently used sample matrices in the analysis of biochemical parameters (5), and evacuated plastic serum separator tubes (SSTs) are most commonly used to obtain these samples. Despite their similarities, SSTs produced by different vendors can show variation. Differences in BCT contents such as tube surfactant, separator gel, and clot activator may be sources of error that can affect test results (6).

Specimen collection devices are classified as in vitro diagnostics (IVDs); therefore, the Clinical and Laboratory Standards Institute's (CLSI) GP-34A guideline recommends verification of specimen tubes prior to routine use (7). Due to discrepancies between manufacturer validation and clinical laboratory practices, the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE) released a consensus statement to provide criteria for laboratory professionals to verify whether new BCTs meet the basic technical and clinical acceptability criteria (8).

A new SST called Samplix was recently introduced for clinical use. As laboratory specialists are responsible for the evaluation of any laboratory equipment, including BCTs, we conducted the present study for local clinical validation of this novel IVD product. To our knowledge, there are no previous studies in the literature about the evaluation of Samplix clot-activator and gel tubes in clinical chemistry or immunoassays. In this study, we compared three different clot-

activator gel tubes, including Samplix, and evaluated the effect of storage time on numerous biochemical analytes.

MATERIAL AND METHODS

Subjects

The study was conducted in the Department of Medical Biochemistry of a tertiary hospital in January 2019. Blood samples were taken from two groups of volunteers to obtain a wide range of test results. The first group consisted of 21 randomly selected individuals who were being treated in the palliative care unit and had widely varying biochemical profiles. The second group consisted of 28 healthy individuals who had no known diseases (e.g., diabetes mellitus, cancer, cirrhosis, chronic kidney disease) and were not receiving any treatment that were randomly selected from different outpatient clinics. All volunteers were 18–65 years old and none were pregnant. The only exclusion criteria were related to the venipuncture procedure; patients for whom a suitable vein could not be found, those who experienced deterioration of vascular integrity, and those who had a tourniquet applied longer than 1 minute during blood collection were excluded. The local ethics committee approved the study protocol and written informed consent was obtained from each subject in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice.

Blood sampling was conducted according to the CLSI guideline-GP41 (9). For all participants, venous blood samples were collected from the antecubital vein into BCTs using a 21-gauge needle (Becton Dickinson and Company Vacutainer® Eclipse blood collection needle, 21Gx 1–1/4 (0.8x32 mm), lot number 8290672, NJ, USA) between the hours of 08:00 and 10:00. The outpatients fasted overnight (8–10 hours) and were instructed to refrain from smoking or consuming tea or coffee starting on midnight of the day of blood collection, and to avoid alcohol consumption for 3 days before blood collection. The outpatients were seated for 1 min before venipuncture and during blood collection. For the inpatients, no fasting was required and venous blood samples were collected with patients in the supine position from the arm not receiving treatment. All venipunctures in both inpatients and outpatients were done by the same phlebotomist and the samples were immediately transported to the laboratory.

Methods

Blood samples were collected from each of the participants into four different tubes: an additive- and gel-free glass tube (Becton Dickinson and Company [BD] Vacutainer® Z tube, 7 mL, 13x100 mm, lot number 6130558, NJ, USA) (*Z tube*), and three different clot-activator gel tubes: Greiner Bio-One GmbH, Samplix® Blood Collection Tube, 5 mL, 13x100 mm, lot number A18054BE, Thailand (*Samplix*); Greiner Bio-One GmbH, Vacuette® Blood Collection Tube, 5 mL, 13x100 mm, lot number A1902398, Austria (*Vacuette*); and BD Vacutainer® SST II Advance Tube, 5 mL, 13x100 mm, lot number 8162882, USA (*Vacutainer*).

The tubes were kept upright at room temperature for a minimum of 30 minutes to allow complete blood clotting, then centrifuged for 10 min at 1800 × g. For Z tubes, the serum samples were transferred to secondary tubes to prevent contact between the serum and cell pellet. Transfer to secondary tubes was not done for the Samplix, Vacuette, and Vacutainer; primary tubes were used for 0-hour (hr) and 48-hr analyses. No visible hemolysis, lipemia, or icterus was detected in any serum samples. For each participant, all serum analytes were measured within a total of 2 hrs. In order to assess analyte stability in the different tubes, serum samples in the Samplix, Vacuette, and Vacutainer tubes were reanalyzed after storage for 48 hrs at +4° C. The order of tubes was randomized during analysis.

A total of 31 parameters were measured. Routine clinical chemistry analytes were measured using an AU5800 autoanalyzer

(Beckman Coulter Inc., CA, USA): alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase, aspartate aminotransferase (AST), calcium (Ca), creatine kinase (CK), creatinine (Crea), chloride (Cl), C-reactive protein (CRP), direct bilirubin (DBil), iron (Fe), gamma glutamyl transferase (GGT), glucose (Glc), high density cholesterol (HDL), lactate dehydrogenase (LDH), lipase, magnesium (Mg), sodium (Na), phosphate (P), potassium (K), total cholesterol (Chol), total bilirubin (TBil), triglyceride (Tg), total protein (TPro), uric acid (UA) and urea. Immunochemical analytes were measured using a DxI 800 immunoanalyzer (Beckman Coulter Inc., CA, USA): folate, free triiodothyronine (fT_3), free thyroxine (fT_4), and thyroid stimulating hormone (TSH). Internal quality control (QC) materials (Control Serum, Beckman Coulter Ireland Inc., lot numbers 1039F-1040I, Clare, Ireland; MAS Liquimmune, Microgenics Corporation, lot numbers LIA20041- LIA20042, CA, USA) at two different levels per day were used as part of routine laboratory practice.

Statistical analysis

The Z tube was identified as the reference tube because it is an additive- and gel-free glass tube and has been a standard device for collecting serum for over five decades. This study protocol was designed according to CLSI guide-GP34 and related literature (5,7,10). All statistical analyses were done using SPSS 20.0 program (SPSS Inc., Chicago, USA). The Shapiro–Wilk test was used to determine whether the variables showed normal distribution. Continuous variables were presented as mean and standard deviation (SD). For normally distributed data, statistical differences between the results of the samples were evaluated with paired samples t-test; data not normally distributed were evaluated with the Wilcoxon test. When comparing the three evaluated tubes to the reference tube, Bonferroni correction was applied to the level of significance; accordingly, a p value <0.017 (0.05/3) was considered statistically significant for three comparisons (11).

The secondary objective of our study was to investigate the stability of analytes stored for 48 hrs. P <0.05 was considered statistically significant for comparisons of analyte concentrations in each tube at 0 and 48 hrs to evaluate the effect of storage time.

In addition to statistical significance, the clinical significance of the differences was evaluated in terms of bias. Bias between results for the reference Z tube and the evaluated tubes or between 0- and 48-hr values of each SST was calculated as [(mean value of evaluated tube – mean value of reference tube)/ mean value of reference tube] × 100 or [mean value of 48-hr result – mean value of 0-hr result)/ mean value of 0-hr result] × 100. Biological variation data were used to determine the quality tolerance for bias (12). Bias exceeding the desirable specifications for bias based on biological variation was considered a clinically important difference.

RESULTS

Although comparisons between each evaluated SST and the Z tube at 0 hrs revealed statistically significant differences for many analytes, clinically important differences were found for six analytes in total for all the SSTs (Table 1). These differences were detected in three parameters in Samplix, four parameters in Vacuette, and five parameters in Vacutainer.

There was a negative bias for Na (-0.29) in the Samplix, and a positive bias for K (2.35) and Mg (2.78). Vacuette had a negative bias for Na (-0.27), and a positive bias for K (2.82), LDH (4.47), and Mg (2.46). Most of the analytes that showing bias in these two tubes of the same manufacturer were the same (Na, K and Mg) and the bias was in the same direction. There was negative bias for Ca (-1.56) in the Vacutainer and positive bias for Cl (0.66), K (3.54), LDH (9.11), and Na (0.38). The bias determined for Ca and Cl in Vacutainer were not present in other two SSTs. On the other hand, a bias was not detected in Vacutainer for Mg that was

present in the other two tubes. Finally, the bias for K was in the same direction in all three tubes, whereas the bias for Na in the Vacutainer was in a different direction from the other two tubes.

Comparisons of analyte levels in Samplix, Vacuette, and Vacutainer at 0 and 48 hrs revealed statistically significant differences for many analytes, but clinically important differences were found for seven analytes in total for all the SSTs (Table 2). Of the clinically significant differences, four were in Samplix, three in Vacuette, and seven in Vacutainer.

After 48 hrs of storage, we found that Cl, K, Na, and Tpro levels were increased in Samplix (1.01, 2.69, 0.54, and 1.95, respectively). In Vacuette Cl, K, and Na levels were increased after 48 hrs of storage (1.11, 2.06, and 0.84, respectively). In these two tubes, instability in the same direction was observed in the same analytes (Cl, K, and Na), except for Tpro. In Vacutainer, we found that fT_3 levels were decreased (-8.87) at 48 hrs, while Ca, Cl, Glc, K, Na, and Tpro levels were increased (1.28, 0.64, 2.76, 2.19, 0.65, and 2.15, respectively). In addition to the analytes that showed instability in other SSTs (Cl, K, Na, Tpro), we found that Ca, fT_3, and Glc were also unstable in Vacutainer. Direction of instability in Cl, K, Na, and Tpro were consistent between Vacutainer and the other two SSTs.

DISCUSSION

Necessary improvements and potential sources of nonconformity should be identified and the entire laboratory process must be validated, including BCTs. However, some IVD devices such as BCTs are not validated before laboratory personnel decide to start using them or to change the brand used (13). The detailed analysis conducted in the present study attempts to address this issue and serve as a guide for users regarding the effects of newly introduced SSTs. To do this, we compared different brands of BCT in terms of the initial test results of selected biochemical analytes and their stability after storage. Our results demonstrated that all evaluated SSTs had clinically similar results compared to the Z tube. At the initial time point, clinically important differences were found only for Ca, Cl, K, LDH, Mg, and Na (Table 3). Other important findings from our study were the clinically important differences in analytes such as Ca, Cl, fT_3, Glc, K, Na, Tpro after 48 hrs of storage (Table 3).

In the literature, various results have been reported concerning the evaluation of SSTs. Lima-Oliveira et al. compared five SSTs from different manufacturers and contrary to our results, detected no bias for Ca, Cl, K, LDH, or Na in Vacuette and Vacutainer. Clinically important differences were detected for amylase, P, and Mg in Vacuette and Vacutainer, though only their result for Mg was similar to ours. However, it is important to note that there was no comparison with a reference tube in their study (13). Ercan et al. compared Vacutainer with a reference tube and evaluated only thyroid function tests. Consistent with the results of our study, they did not observe any bias for TSH, fT_3, and fT_4 in Vacutainer (14).

Table 1. Clinical and statistical evaluation of analytes in different types of blood collection tubes (Samplix, Vacuette, and Vacutainer) in comparison with the reference tube (Z tube).

Analytes	n	GBO Samplix		GBO Vacuette		BD Vacutainer		Z tube		Bias (%)			Allowable quality specifications (%)	P value		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Z tube-Samplix	Z tube-Vacuette	Z tube-Vacutainer		Z tube-Samplix	Z tube-Vacuette	Z tube-Vacutainer
ALP (U/L)	48	102.4	111.3	102.6	110.8	100.7	109.5	102.4	111.5	0.06	0.24	-1.65	6.72	0.714	0.238	**0.004**
ALT (U/L)	48	24.2	23.3	23.7	23.0	23.4	22.4	23.5	22.8	3.11	0.98	-0.09	11.48	**0.001**	0.162	0.905
Amylase (U/L)	48	53.4	28.1	53.0	28.0	52.2	28.4	52.5	27.9	1.59	0.83	-0.71	7.40	**<0.001**	0.028	0.243
AST (U/L)	48	25.4	20.8	25.4	20.4	25.7	21.2	24.5	20.3	3.57	3.83	5.02	6.54	**<0.001**	**<0.001**	**<0.001**
Ca (mg/dL)	48	9.24	0.73	9.26	0.72	9.07	0.75	9.21	0.69	0.27	0.45	**-1.56**	0.82	0.141	**0.005**	**<0.001**
CK (U/L)	48	73.0	53.7	73.5	53.1	71.8	52.3	72.5	53.0	0.75	1.38	-0.95	11.50	0.206	0.029	0.206
Cl (mmol/L)	47	103.2	6.4	103.3	6.4	104.2	6.4	103.6	6.3	-0.39	-0.27	**0.66**	0.50	0.019	0.031	**<0.001**
CRP (mg/L)	47	31.8	60.6	31.5	60.1	31.3	60.6	31.4	59.7	1.40	0.18	-0.46	21.80	**0.003**	0.332	0.028
DBil (mg/dL)	48	0.14	0.10	0.14	0.10	0.13	0.09	0.14	0.10	-1.16	-0.43	-3.77	14.20	0.313	1.000	0.034
Fe (μg/dL)	48	60.8	38.8	59.7	39.3	59.3	38.0	59.5	39.4	2.14	0.35	-0.39	8.80	**<0.001**	0.304	0.550
Folate (μg/L)	36	8.72	4.54	8.94	4.85	8.62	4.66	8.58	4.56	1.61	4.13	0.39	19.20	0.126	0.018	0.503
fT_3 (ng/L)	37	3.36	0.72	3.33	0.70	3.34	0.74	3.40	0.77	-1.26	-2.28	-1.86	4.80	0.258	0.082	0.159
fT_4 (ng/dL)	37	0.95	0.27	0.95	0.27	0.96	0.26	0.97	0.26	-1.62	-1.77	-0.67	3.30	0.163	0.063	0.503
GGT (U/L)	48	57.2	95.6	55.2	92.9	54.9	91.9	55.0	93.0	3.98	0.34	-0.19	6.70	**<0.001**	0.125	0.677
Glc (mg/dL)	48	113.6	48.7	113.7	48.6	110.3	46.8	112.5	48.8	0.98	1.02	-1.93	2.34	**0.010**	**0.005**	**0.014**
HDL (mg/dL)	48	47.0	18.1	47.0	17.9	46.0	17.6	46.5	17.6	1.07	0.98	-1.21	5.61	**0.001**	**0.011**	0.034
K (mmol/L)	48	4.25	0.51	4.27	0.49	4.30	0.53	4.16	0.50	**2.35**	**2.82**	**3.54**	1.81	**<0.001**	**<0.001**	**<0.001**
Chol (mg/dL)	48	189.3	49.7	190.5	49.0	186.1	48.1	188.4	48.6	0.46	1.13	-1.21	4.10	0.066	**<0.001**	**0.002**
Cre (mg/dL)	47	0.97	0.54	0.97	0.54	0.94	0.55	0.96	0.53	1.44	1.28	-2.29	3.96	**0.002**	**0.004**	0.032
LDH (U/L)	48	188.1	108.0	191.9	106.3	200.5	110.9	183.7	107.4	2.37	**4.47**	**9.11**	4.30	**0.012**	**<0.001**	**<0.001**
Lipase (U/L)	47	30.8	37.8	31.6	37.7	31.1	38.6	31.3	37.2	-1.63	0.95	-0.61	11.31	0.029	0.047	0.111
Mg (mg/dL)	48	2.00	0.20	2.00	0.21	1.92	0.22	1.95	0.21	**2.78**	**2.46**	-1.39	1.80	**<0.001**	**<0.001**	0.069
Na (mmol/L)	47	140.4	5.1	140.4	4.9	141.3	4.9	140.8	4.9	**-0.29**	**-0.27**	**0.38**	0.23	**0.013**	0.023	**<0.001**
P (mg/dL)	47	3.34	0.64	3.47	0.61	3.38	0.64	3.40	0.60	-1.56	2.13	-0.50	3.38	**0.008**	**<0.001**	0.293
TBil (mg/dL)	48	0.72	0.45	0.72	0.45	0.72	0.45	0.71	0.45	1.77	2.12	1.65	8.95	**0.001**	**<0.001**	0.027
Tg (mg/dL)	48	131.9	75.4	134.3	77.4	128.8	72.7	133.7	77.4	-3.07	0.44	-3.68	9.57	**0.002**	**0.010**	**<0.001**
TPro (g/dL)	48	6.68	0.83	6.72	0.85	6.61	0.78	6.69	0.82	-0.16	0.50	-1.18	1.36	0.545	0.027	**0.008**
TSH (U/L)	37	1.38	1.01	1.38	1.01	1.38	0.99	1.39	1.00	-0.71	-0.92	-0.92	9.70	0.055	0.078	0.401
UA (mg/dL)	47	4.77	1.59	4.80	1.58	4.77	1.58	4.79	1.58	-0.58	0.04	-0.53	4.87	0.036	0.837	0.083
Urea (mg/dL)	48	40.7	24.7	41.5	24.8	40.9	24.9	41.2	24.9	-1.37	0.76	-0.71	5.57	**0.007**	0.056	0.342

If values exceeded the allowable quality specifications or p value was <0.017, it was written as bold. Abbr: n: number; SD: standard deviation.

Table 2. Clinical and statistical evaluation of 30 analytes at 0 and 48-hr for each blood collection tube (Samplix, Vacuette, and Vacutainer).

Analytes	GBO Samplix				GBO Vacuette				BD Vacutainer				Allowable quality specifications (%)	Statically difference between 0 and 48-hr / (p value)		
	n	0-hr mean	48-hr mean	Bias (%)	n	0-hr mean	48-hr mean	Bias (%)	n	0-hr mean	48-hr mean	Bias (%)		GBO Samplix	GBO Vacuette	BD Vacutainer
ALP (U/L)	49	102.4	103.2	0.76	49	102.6	103.4	0.76	49	102.9	105.9	2.96	6.72	0.052	0.224	**<0.001**
ALT (U/L)	49	24.0	23.2	-2.98	49	23.4	23.4	-0.35	49	23.0	22.6	-1.48	11.48	**0.002**	0.940	0.353
Amylase (U/L)	49	53.5	53.6	0.19	49	53.1	53.6	1.00	49	52.2	53.3	2.20	7.40	0.563	0.051	**<0.001**
AST (U/L)	49	25.1	25.7	2.28	49	25.2	25.4	0.97	49	25.5	26.1	2.17	6.54	0.056	0.456	0.064
Ca (mg/dL)	49	9.24	9.24	0.02	49	9.25	9.24	-0.11	49	9.08	9.20	**1.28**	0.82	0.934	0.743	**<0.001**
CK (U/L)	49	72.7	72.4	-0.51	49	73.2	72.4	-1.09	49	73.7	73.9	0.29	11.50	0.819	0.135	0.808
Cl (mmol/L)	49	103.1	104.1	**1.01**	49	103.2	104.4	**1.11**	49	104.2	104.8	**0.64**	0.50	**<0.001**	**<0.001**	**<0.001**
CRP (mg/L)	49	31.2	30.8	-1.34	49	30.9	30.7	-0.59	49	31.1	31.3	0.33	21.80	0.419	0.146	**<0.001**
Fe (µg/dL)	49	60.2	58.6	-2.65	49	59.1	57.0	-3.59	49	57.7	57.7	-0.04	8.80	**0.009**	**0.001**	0.705
Folate (µg/L)	38	8.41	8.48	0.75	34	8.44	8.10	-4.05	34	9.23	8.96	-2.91	19.20	0.723	**0.012**	**0.004**
fT_3 (ng/L)	39	3.31	3.20	-3.32	35	3.14	3.12	-0.75	35	3.35	3.05	**-8.87**	4.80	**0.004**	0.609	**<0.001**
fT_4 (ng/dL)	39	0.95	0.95	0.85	34	0.98	1.00	3.02	34	0.97	0.95	-2.32	3.30	0.691	0.088	0.052
GGT (U/L)	49	56.5	54.1	-4.12	49	54.5	54.0	-0.86	49	54.5	53.9	-1.09	6.70	**<0.001**	**0.001**	**0.002**
Glc (mg/dL)	49	113.1	113.9	0.69	49	113.1	113.5	0.31	49	110.2	113.3	**2.76**	2.34	**0.004**	**0.035**	**<0.001**
HDL (mg/dL)	49	47.1	45.3	-3.77	48	47.4	45.7	-3.60	48	46.5	45.4	-2.38	5.61	**<0.001**	**<0.001**	**<0.001**
K (mmol/L)	49	4.26	4.38	**2.69**	49	4.28	4.37	**2.06**	49	4.30	4.39	**2.19**	1.81	**<0.001**	**<0.001**	**<0.001**
Chol (mg/dL)	49	189.2	191.0	0.96	49	190.4	191.6	0.63	49	185.8	189.5	1.98	4.10	**0.016**	**0.035**	**<0.001**
Cre (mg/dL)	48	0.97	0.96	-1.38	48	0.98	0.97	-1.18	48	0.94	0.95	1.36	3.96	0.204	0.248	0.155
LDH (U/L)	49	188.3	192.6	2.30	49	191.9	189.0	-1.53	49	198.7	201.1	1.23	4.30	**0.005**	**0.016**	**0.012**
Lipase (U/L)	48	30.7	33.7	9.99	48	31.4	33.3	5.90	48	31.8	34.9	9.74	11.31	**<0.001**	**<0.001**	**<0.001**
Mg (mg/dL)	49	2.01	2.02	0.51	49	2.00	2.01	0.20	49	1.93	1.95	0.97	1.80	0.225	0.707	0.178
Na (mmol/L)	49	140.3	141.1	**0.54**	49	140.3	141.5	**0.84**	49	141.3	142.2	**0.65**	0.23	**0.001**	**<0.001**	**<0.001**
P (mg/dL)	49	3.34	3.43	2.94	48	3.49	3.47	-0.48	48	3.44	3.52	2.16	3.38	**<0.001**	0.173	**0.001**
Tg (mg/dL)	49	133.4	136.2	2.10	49	135.8	136.4	0.47	49	126.7	132.4	4.54	9.57	**0.017**	0.398	**<0.001**
TPro (g/dL)	49	6.68	6.81	**1.95**	49	6.72	6.81	1.34	49	6.61	6.76	**2.15**	1.36	**<0.001**	**<0.001**	**<0.001**
TSH (U/L)	37	1.37	1.39	1.63	35	1.16	1.19	2.38	35	1.57	1.58	0.70	9.70	**0.027**	**0.001**	0.210
UA (mg/dL)	48	4.75	4.63	-2.54	48	4.81	4.73	-1.82	48	4.68	4.65	-0.65	4.87	**0.004**	**<0.001**	0.108
Urea (mg/dL)	49	40.2	40.4	0.46	49	41.1	40.7	-0.94	49	40.6	40.9	0.82	5.57	0.188	0.056	**0.023**

If values exceeded the allowable quality specifications or p value was <0.05, it was written as bold. Abbr: n: number; SD: standard deviation.

Table 3. Summary of the parameters that showing bias.

	Variations of the analytes in evaluated tubes in comparison with reference tube			Variations of the analytes at 48-hr in comparison with the 0-hr for each evaluated tube		
	GBO Samplix	GBO Vacuette	BD Vacutainer	GBO Samplix	GBO Vacuette	BD Vacutainer
Ca			⇩			⇧
Cl			⇧	⇧	⇧	⇧
fT3						⇩
Glc						⇧
K	⇧	⇧	⇧	⇧	⇧	⇧
LDH		⇧	⇧			
Mg	⇧	⇧				
Na	⇩	⇩	⇧	⇧	⇧	⇧
Tpro				⇧		⇧

While the Z tube is a glass tube with no additives such as surfactant, the plastic tubes evaluated in our study have an internal coating material. Coating the plastic interior with surfactants makes the blood components less adherent to the BCTs. It has been shown that problems with the surfactant concentrations increases the possibility of red blood cell, protein, and platelet adherence to tube walls with subsequent release of intracellular K (15). Like K, LDH and Mg are also intracellular materials and may be released at the same time. This may be a possible mechanism for higher levels of K, LDH and Mg in the evaluated tubes. Another approach to this issue from a mechanistic point of view is that in glass tubes, the coagulation process is slower than in clot-activator tubes. Therefore, it can be expected that levels of K, which is released during the coagulation process, will be higher in clot-activator tubes. The same is also valid for LDH and Mg. No hypothesis could be asserted for the detection of bias in different directions for Na in different manufacturers' tubes, or the mechanisms by which Ca and Cl showed clinically significant differences only in Vacutainer. Although BCTs produced by different manufacturers have generally similar components, they may differ in composition. For example, while some manufacturers use olefin oligomers as serum separator gels, some use polymer gel. Furthermore, some manufacturers use silicon as a surfactant, while others use different materials for the same purpose (10). The variations we observed may be attributed to differences in the materials used by manufacturers. The fact that the materials used by the manufacturers are trade secrets prevents exact comments on this matter. However, parameters for which we detected bias are clinically important in various pathologies, such as, Na and K in patients receiving dialysis and Ca in parathyroid diseases. Therefore, each laboratory should verify reference range transfer or create its own reference range before using a new BCT to ensure that the parameters showing bias in the SST used will accurately guide physicians in treatment.

Discrepant data have been reported from stability studies in immunoassays and clinical chemistry. Kilinc et al. reported that fT_3 levels were significantly increased at 72-hr in Vacutainer (16). In contrast, Ercan et al. did not find any significant differences for thyroid function tests over 48 hrs in Vacuette (14). Schouwers et al. found that there was no clinically significant difference in immunoassay parameters up to 48 hrs in Sarstedt S-Monovette SST, which we did not evaluate in our study (17). Boyanton et al. emphasized that no instability was detected in any parameter up to 56 hrs in Vacutainer, which also included clinical chemistry parameters that deteriorated in our study (18). On the other hand, contradictory results in both comparison and stability studies may be related to methodological differences (pre-analytical, statistical, allowable quality specifications, comparison to a reference tube).

Since the stability of Na, Cl, and K in all tubes evaluated in our study deteriorated after 48 hrs and bias was in the same direction, we believe that the deterioration is independent of the tube brand or content. On the other hand, we can assert that stability problems for Tpro in Samplix and for Tpro, Ca, fT_3, and Glc in Vacutainer are probably related to the tubes. The analyte levels that increased after 48 hrs may be related with hemoconcentration resulting from the absorption of serum water content through interaction with the gel. In addition, it was difficult to explain the increases of the concentration by evaporation, considering that all tubes were kept closed during storage. These findings lead us to question the reliability of the results of repeated tests of the same sample stored for 48 hrs or if sample transport to the central laboratory and analysis is not completed within 48 hrs.
There are some limitations to this work. Because our study involved the analysis of many parameters in small volumes of serum,

we were unable to evaluate hemolysis, icterus, or lipemia using the autoanalyzer indexes. Therefore, these parameters were assessed by visual inspection.

In conclusion, various BCTs are available in different brands and different contents by various manufacturers in routine use. Differences in BCT content, regardless of the manufacturer and brand, may affect test results. In this regard, each laboratory should verify reference range transfer or create its own reference range before using a new BCT. Furthermore, it should be borne in mind by laboratory personnel that in cases where samples cannot be analyzed immediately after blood sampling, all clinical chemistry or immunoassay analytes may not remain stable for up to 48 hrs of storage in SSTs, even at 4°C.

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