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ORIGINAL ARTICLE

Do rapid serum tubes provide comparable test results or improved stability when compared with serum separator tubes?

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ABSTRACT

Objective: For some analytes test results can be affected by choice of blood collection tubes. Several studies comparing the new BD Vacutainer® Rapid Serum Tubes with the Serum Separator Tubes for routine chemistry and immunoassay tests have been published. However, published studies have not investigated Insulin, C-peptide and High Sensitivity C-Reactive Protein (hsCRP) tests. The aim of this study was to evaluate the comparability of some laboratory tests and rates of hemolysis using Rapid Serum Tubes and Serum Separator Tubes and to also investigate the stability on serum storage time.

Materials and Methods: Blood specimens were collected into the two tube types from 97 participants. All tubes were centrifuged (1500×g,10 minutes). Serum from each tube was tested for Insulin, C-peptide, hsCRP, parathyroid hormone, 25-OH Vitamin D, glucose, potassium, phosphorus, AST, magnesium and LDH measurements. Detection of hemolysis was carried out by a spectrophotometric method. Paired blood specimens (n=15) were stored at 4°C and retested at 4 and 24 hour for the evaluation of specimen stability. The significance of the differences between samples was assessed by paired-t test or Wilcoxon test. The mean percentage difference was calculated. Desirable analytical quality specifications and total change limit were used for the interpretation of the test results and the stability of analytes, respectively.

Results: Although the results for measured analytes showed statistically significant differences between the two tube types ($p<0.05$), the mean percentage differences (except for LDH) were within the current desirable allowable bias. No serum samples in the study were hemolysed (≥0.5 g/L) and most of the analytes investigated remained stable (24 hour at 4°C) for both tube types.

Conclusion: This study suggests that BD Rapid Serum Tubes are suitable for collection of blood and storage of serum up to 24 hour at 4°C for some laboratory analytes including Insulin, C-peptide, hsCRP.

Key words: Rapid Serum Tubes, Serum Separator Tubes, Stability, Hemolysis

Introduction

Preanalytical errors account for approximately 40-70% of all mistakes made in laboratory diagnostics [1-3]. An important component of preanalytical errors is associated with the specimen quality. Several factors related to the preanalytical process can affect the accuracy of test results [4,5]. Proper technique of sample collection, sample handling and preparing for analysis can have a major effect on the quality of the specimens. Lack of adherence to proper technique can result in hemolysis, or in incomplete clotting leading to interference from fibrin [5]. According to the National Committee for Clinical Laboratories Standards (NCCLS) document, the blood collection tubes should be processed within 2 hour to reduce preanalytical errors in the serum owing to prolonged contact with cells [6]. Standard serum tubes required a blood clotting time of minimum 30 minutes. Although serum separator tubes are widely used in the clinical laboratories, plasma can be used an alternative specimen type for chemistry analytes to improve test result turnaround time (TAT) [7]. However, there are some limitations with respect to using plasma, such as differences between plasma and serum test results, a problem of

adequate mixing of blood with anticoagulants and reduced analyte stability [8,9].

Preanalytical system manufacturers have introduced a range of blood collection tubes. For some analytes test results can be affected by choice of blood collection tubes [10]. In recent years a new tube from Becton Dickinson (BD) Diagnostics, the BD Vacutainer® Rapid Serum Tube (RST) has been introduced to overcome the clotting problems for serum samples. The tubes' wall is coated with thrombin-based clot activator providing rapid clotting [11]. Previous studies investigating the performance of RST tubes for common analytes did not evaluate Insulin, C-peptide and High Sensitivity C-Reactive Protein [12,13]. The purpose of this study was to evaluate the comparability of some laboratory tests and rates of hemolysis using BD Vacutainer® RST tubes and BD Vacutainer® Serum Separator Tubes (SST) and to additionally investigate the stability on serum storage.

Materials and Methods

Blood specimens were collected into RST and SST from 97 participants (58 female, 39 male, 41.08±9.84 years). Participants were randomly selected from the outpatient clinics. All tubes were inverted gently for 5 to 6 times. RST tubes were centrifuged after 5 minutes and SST tubes were centrifuged after 30 minutes (1500xg for 10 minutes). Serum from each tube was tested for Insulin, C-peptide, High Sensitivity C-Reactive Protein (hsCRP), parathyroid hormone (PTH), 25-OH Vitamin D, glucose, potassium (K), phosphorus, aspartate aminotransferase (AST), magnesium (Mg) and lactate dehydrogenase (LDH) measurements. Glucose, K, phosphorus, AST, Mg, LDH and hsCRP measurements were performed by ADVIA 2400 (Siemens Healthcare Diagnostics Inc., Tarrytown, USA) autoanalyzer. PTH and Insulin measurements were performed by ADVIA Centaur XP (Siemens Healthcare Diagnostics Inc., Tarrytown, USA). C-peptide and 25-OH Vitamin D measurements were performed by Immulite 2000 (Siemens Healthcare Diagnostics Inc., Tarrytown, USA) and Liaison (DiaSorin,Italy), respectively. Hemolysis index was carried out by using absorbance measurements of samples at multiple wavelengths [14]. Paired blood specimens (n=15) were stored at 4°C and retested at 4 hour and 24 hour for the evaluation of specimen stability. This study was approved by the ethics review committee at Diskapi Yildirim Beyazit Training and Research Hospital, Turkey. Informed consent was obtained from each participant.

Statistical Analyses

All statistical analyses were carried out using the SPSS statistical package software version 15.0 (SPSS Inc., Chicago, Illinois, USA). The Kolmogorov-Smirnov test was used to determine the normality of distribution for all variables. The significance of the differences between samples was assessed by paired-t test or Wilcoxon test. Data were described by the mean±standart deviation (SD) for normally distributed data and median and interquantile range (IOR) for non-normally distributed data. For all statistical comparisons, $p<0.05$ values were considered significant.

The mean percentage difference was calculated according to the formula: Mean difference(%)=[(RST mean-SST mean/SST mean)×100]. For the interpretation of the test results, clinical significance was assessed by bias compared with the current desirable allowable bias based on biological variation [15]. The mean percentage difference due to the instability of an analyte was compared with total change limit (TCL) which was calculated by the estimation of the square root of the sum of the squared analytical (CVa) and intra-individual imprecisions (CVb) ($TCL=[(2.77\times CVa)^2 + (0.5\times CVb)^2]^{1/2}$) [16].

Results

The comparison of routine chemistry assay and immunoassay test results between RST and SST tubes for seven routine chemistry assays and four immunoassays are shown in Table 1. Although the results for measured analytes showed statistically significant differences between the two tube types ($p<0.05$), the mean percentage differences (except for LDH) were within the current desirable allowable bias. No serum samples in the study were hemolysed (≥0.5 g/L). Analyte stability studies showed that most of the analytes investigated remained stable (24 hours at 4°C) for both tube types (Table 2 and Table 3). However, PTH remained stable up to 4 hour at 4°C for both tube types. Some similarities and differences between the RST and SST tubes were summarized in Table 4.

Discussion

In the present study we evaluated; (i) the performance of BD RST tubes for some laboratory tests, (ii) the rates of hemolysis, (iii) the effects of BD RST tubes on serum stability in reference with BD SST tubes. There was a statistically significant difference between the two tube types. However, the

Table 1. Comparison of routine chemistry assay and immunoassay test results between RST and SST tubes

Analytes (units)	**RST** (n=97)	**SST** (n=97)	**Mean Bias** (%)	**Desirable Bias** (%)	**P value**
Glucose (mg/dL)	95(16.5)	93(17)	1.27	2.34	0.008
AST (U/L)	22.29±6.24	22.73±6.29	–2.40	6.54	0.001
LDH* (U/L)	175 (36)	181(38.5)	–4.47	4.3	<0.001
K (mmol/L)	4.6 (0.45)	4.6(0.4)	0.70	1.81	0.041
Mg (mg/dL)	2.07 (0.31)	2.09 (0.26)	0.41	1.8	0.033
Phosphorus (mg/dL)	3.3 (0.7)	3.5 (0.65)	–2.31	3.38	<0.001
hsCRP (mg/L)	2.54(4.86)	2.51(4.87)	2.08	25.53	<0.001
25-OHVitamin D (ng/mL)	10.7 (9.33)	9.86 (9.96)	3.94	5	<0.001
PTH (pg/mL)	65.50±29.90	64.22±29.29	1.95	8.8	0.02
Insulin (mU/L)	10.6 (9.13)	10.17(8.28)	9.80	15.5	<0.001
C-peptide (ng/mL)	2.3 (1.82)	2.15(1.72)	3.25	7.1	0.001

Data are presented as mean±SD or median (IQR). *Mean Bias>Desirable Bias for LDH

differences in all except LDH were small enough and within the current desirable allowable bias.

Several studies investigated the performance of RST tubes for some laboratory analytes. In general, the results of these studies show that RST tubes provide acceptable performance for routine chemistry and immunoassay tests and a short clotting time [4,8,13,14,17-21]. To our knowledge, this is the first study that investigates the performance of RST tubes for insulin, C-peptide and HsCRP analytes. Neither the Food Drug Administration (FDA) 510(k) decision summary nor other published studies mention the evaluation of RST tubes for insulin, C-peptide and HsCRP analytes [12]. In our study,

Table 2. Stability analysis of some routine chemistry and immunoassay analytes in RST tubes

Analytes (units)	**RST_0** (n=15)	**RST_4** (n=15)	**RST_{24}** (n=15)	**RST_{0-4} Mean Bias** (%)	**RST_{0-24} Mean Bias** (%)	**Total Change Limit** (TCL)	**Acceptable Delays**
Glucose (mg/dL)	109.66±24.20	112.26±24.30	112.80±24.84	2.37	2.85	5.5	24h
AST (U/L)	19.86±5.69	20.06±5.79	20.20±5.55	1	1.67	8.3	24h
LDH (U/L)	180(28)	180(30)	172(34)	0.96	-3.68	5.3	24h
K (mmol/L)	4.68±0.33	4.72±0.35	4.62±0.34	0.85	-1.28	5.0	24h
Mg (mg/dL)	2.12±0.33	2.38±0.54	2.24±0.37	11.94	5.61	22.0	24h
Phosphorus (mg/dL)	3.38±0.40	3.43±0.55	3.63±0.46	1.39	7.3	6.7	4h
hsCRP (mg/L)	5.41±3.49	5.54±3.58	5.61±3.61	2.35	3.58	25.9	24h
25-OH Vitamin D (ng/mL)	12.57±9.76	13.72±10.62	13.36±10.29	9.16	6.34	15.3	24h
PTH (pg/mL)	73.68±18.66	62.88±15.74	53.65±12.83	–14.64	–27.18	19.5	4h
Insulin (mU/L)	16.48±18.08	13.71±14.21	14.10±14.72	–16.79	–14.46	20.6	24h
C-peptide (ng/mL)	3.19±2.40	3.03±2.14	2.96±2.15	–4.90	–7.09	22.1	24h

Data are presented as mean±SD or median (IQR).

Table 3. Stability analysis of some routine chemistry and immunoassay analytes in SST tubes

Analytes (units)	SST_0 (n=15)	SST_4 (n=15)	SST_{24} (n=15)	SST_{0-4} **Mean Bias** (%)	SST_{0-24} **Mean Bias** (%)	**Total Change Limit** (TCL)	**Acceptable Delays**
Glucose (mg/dL)	110.53±25.65	110.93±25.56	114.40±5.75	0.36	3.49	5.5	24h
AST (U/L)	21±5.50	22.33±6.85	20.66±5.75	6.34	–1.58	8.3	24h
LDH (U/L)	189(30)	194(37)	182(37)	6.08	–3.51	5.3	24h
K (mmol/L)	4.65±0.33	4.74±0.38	4.64±0.31	1.86	–0.14	5.0	24h
Mg (mg/dL)	2.08±0.19	2.18±0.44	2.35±0.52	14.41	12.94	22.0	24h
Phosphorus (mg/dL)	3.44±0.46	3.32±0.66	3.66±0.47	–3.48	6.58	6.7	24h
hsCRP (mg/L)	5.52±3.59	5.53±3.77	5.76±3.73	0.21	4.27	25.9	24h
25-OH Vitamin D (ng/mL)	13.25±10.12	12.79±9.91	12.72±9.98	–3.48	–3.98	15.3	24h
PTH (pg/mL)	71.57±18.66	59.16±16.16	52.28±14.55	–17.34	–26.95	19.5	4h
Insulin (mU/L)	15.50±16.20	13.01±14.05	13.51±14.62	–16.04	–12.82	20.6	24h
C-peptide (ng/mL)	3.26±2.55	3.05±2.28	3.05±2.44	–6.25	–6.31	22.1	24h

Data are presented as mean±SD or median (IQR).

the mean bias was smaller than the desirable bias for these analytes.

In a recent study, Yan et al [17] analyzed for 54, 50 and 10 chemistry and/or immunoassay tests on different analyzer. They found that the RST tubes were comparable with those SST tubes for most analytes, only for PTH on Abbott Architect, the RST tube showed clinically significant bias in opposition to the SST tube (–15.3%). However we found 1.95% bias for PTH. In another study Wai-Yoong et al observed that means of the RST tubes were in close agreement with that of the SST tubes for 22 analytes [8].

The BD RST tubes contain a thrombin-based clotting agent that can provide a fast sample clotting time within 5 minutes following collection of blood, compared with the usual 30 minutes clotting in the SST (Table 4). This property has enabled a shorter TAT [11,20]. Nevertheless, the drawback of BD RST tubes is that it is not recommended for patients on heparin therapy, direct thrombin inhibitor therapy or those with Factor I deficiency [12]. According to visual inspection, the clotting time was up to 5 minutes after blood sampling. However we didn't evaluate the clotting time and its effects on TAT in this study. Budak et al [20] investigated the influence of RST use on cardiac troponin T (hs-cTnT) and creatine kinase-MB (CK-MB) test results. They found that the hs-cTnT and CK-MB test results did not significantly differ when RST or SST tubes were used.

Similar to our findings, Dimeski et al [18] observed that no specimens showed significant hemolysis (≤0.5 g/L free hemoglobin) irrespective of the tube type. This magnitude of hemolysis is very low and unlikely to influence clinically any of the analyte determinations. A larger study compared RST to plasma tubes for hemolysis markers in an emergency department setting in 347 patients and found that RST specimens showed small increases in several hemolysis markers, but the proportion of elevated hemolysis markers was similar to plasma [14].

In addition we investigated the analyte stability

Table 4. Comparison of some characteristics between the RST and SST tubes

	RST	**SST**
Separator Gel	Polymer gel	Polymer gel
Tube Dimension (mm)	13 × 100	13 × 75 13 × 100 16 × 100
Draw Volume (mL)	4	3–10
Clot Activator	Thrombin	Silica
Clotting Time (min)	5	30
Closure	Conventional rubber closure	Conventional rubber closure and BD Hemogard™ closure

by retesting the same analytes after 4 hour and 24 hour storage at 4°C. La'ulu et al [21] designed a study to investigate PTH degradation in RST tubes. In contrast to our study, they found that PTH degradation was faster for RST than SST. However, in the present study PTH remained stable up to 4 hour at 4°C for both tube types. There was no difference between the two tube types in terms of stability for all investigated analytes.

As observed in other studies, using RST tubes have some advantages for clinicians and laboratory practitioners; RST tubes potentially allow time gains in terms of reduced turnaround times with rapid clotting time, which is valuable particularly in an emergency setting and provides an alternative option for the use of plasma instead of serum. From the results of this study, it can be concluded that the BD RST tubes are suitable for collection of blood and storage of serum up to 24 hour at 4°C for some laboratory analytes including insulin, C-peptide, hsCRP and it provides acceptable performance for common analytes.

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