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Research Article

The evaluation of rapid serum tubes for various immunoassay tests

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Abstract

Objectives: One of the most crucial steps on the preanalytical process that affect laboratory test results is proper and timely blood collection. In this study, we aimed to compare Rapid Serum Tubes (RST) and Serum Separator Tubes (SST) with no additive Z tubes concerning various immunoassay tests.

Methods: Blood samples were collected from 50 healthy volunteers into three blood collection tubes. Sera from Z tube, SST and RST were analysed simultaneously for thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), folate, vitamin B12, 25-OH vitamin D (25-OHD), parathyroid hormone (PTH), cortisol, ferritin, human chorionic gonadotropin (hCG) on DXI800 autoanalyser (Beckman Coulter, USA). The results were evaluated by comparing each tube pairs (Z tube-SST, Z tube-RST) according to desirable bias.

Results: There was no statistically significant difference between the two tube pairs for most analytes. There was not any significant clinical difference according to a desirable bias for the analytes tested.

Conclusion: RST offers acceptable clinical performance for the immunoassay tests performed in this study on DXI platform. The laboratory turn-around-times for immunoassay tests could be shortened approximately 15-20 minutes by the usage of RSTs.

Keywords: Preanalytical phase, rapid serum tube, Z tube

Accurate results in medical laboratories play significant roles in the clinical decision-making process and also have influences on medical diagnoses and therapies. One of the most significant steps in preanalytical process that affect laboratory test results is proper and timely blood collection. However, the effects of blood collection tubes on laboratory test results are often overpassed [1]. Clinical Laboratory Standards Institute (CLSI) recommends performing analyses within two hours following blood collection to avoid prolonged contact of serum with cells and minimize errors in the preanalytical phase [2]. Preanalytical errors constitute 40-70% of total test errors [3-5]. Also, shorter turn-around-time (TAT) is significant for clinicians [6]. Most of the serum tubes used in clinical laboratories require a minimum 30 minutes of clotting time before centrifugation [2]. Using plasma specimens instead of serum to shorten the turnaround time of the test results can be an alternative choice [7]. However, there are some limitations of using plasma specimens as a different concentration of analytes and anticoagulants may reduce analyte stability. If serum is separated from the clot, it remains stable for a longer period [6].

Serum separator tubes and Z tubes (with no additive) need a minimum of 30 minutes for clotting before centrifugation. Rapid serum tubes containing thrombin additive and provides a clotting time of five minutes to improve and shorten TAT.

In this study, we aimed to compare the immunoassay test parameters as follows: thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), folate, vita-

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min B12, 25-OH vitamin D (25-OHD), parathyroid hormone (PTH), cortisol, ferritin, human chorionic gonadotropin (hCG) in BD vacutainer RST, BD vacutainer SST II and reference BD vacutainer Z serum tubes.

Materials and Methods

Subjects

This study was conducted for two months period. Blood samples were collected from 50 apparently healthy volunteers for immunoassay tests. The subjects with any acute or chronic illness and taking medicine were excluded from this study. Informed consent was taken from each volunteer. This study was approved by the local Ethical Committee.

Study design

Blood samples were collected according to the Clinical Laboratory Standard Institute (CLSI) recommendations by an expert phlebotomist in the morning between 8 and 9 am after 12-hours fasting. The volunteers seated for 10 minutes before phlebotomy. Blood was collected using a standardized draw order according to CLSI document-GP41 [8].Tube 1: 7 mL BD vacutainer Z tube (Becton, Dickinson and Company Franklin Lakes, NJ, USA) contains no additive, no coating, Tube 2: 5 mL BD vacutainer serum separator tube II (SST) was coated with micronized silicone particles clot activator and contains gel barrier at the tube bottom, Tube 3: 5 mL BD vacutainer Rapid Serum Tube (RST) contains gel barrier polymer at the tube bottom and tube was coated with thrombin. Tubes were filled to capacity and gently inverted five or six times immediately after collection according to the manufacturer's recommendations. All of the tubes were left in an upright position at room temperature, RSTs were visually inspected for five minutes, Z tubes and SSTs were allowed 30 minutes for clot formation. After clot formation, tubes were centrifuged at 1500×g for 15 minutes according to the manufacturer's recommendations. Sera were separated, aliquoted and stored at –80°C until analysis.

Methods

Sera from Z tube, SST II and RST were analysed randomly for thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), folate, vitamin B12, 25-OH vitamin D (25-OHD), parathyroid hormone (PTH), cortisol, ferritin, human chorionic gonadotropin (hCG) by paramagnetic particle, chemiluminescent immunoassay on DXI800 autoanalyser (Beckman Coulter, USA). All analyses were conducted in two level quality controlled the same run.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) program version 18.0 for Windows (SPSS Inc., Chicago, IL). The conformity of continuous variables to normal distribution was tested using the Kolmogorov-Smirnov test. The descriptive statistics of continuous variables were expressed as mean±standard deviation with normal distribution and median (min-max) with non-normal distribution. One way ANOVA test or Kruskal Wallis test was used to determine the presence of a statistically significant difference for three groups. If there was a statistically significant difference, Student's t-test for parametric and Mann Whitney-U test for non-parametric variables were performed for each paired tube groups. P-value <0.05 was considered statistically significant.

Bias was assessed using the formula: mean difference (%)=[(test tube mean - reference tube mean)/(reference tube mean)]x100 which should be less than desirable bias calculated from within-subject biological variation (CVw) and between-subject biological variation (CVG) (desirable bias <0.25 $(CVw^2+CV_G^2)½$).

Results

Comparison of routine immunoassay test results between RST, SST and Z tubes for ten analytes is shown in Table 1. There was not any statistically significant difference for all analytes.

Table 1. Comparison of the results of three tubes (Z Tube-SST and RST)

Analyte	Units	n	Z tube	SST	RST	p
TSH	mUL/L	50	1.25 (0.05-4.93)	1.29 (0.05-4.60)	1.29 (0.05-4.74)	0.91
T3	pg/mL	50	3.07±0.33	3.02±031	2.96±0.30	0.11
T4	ng/dL	50	0.89±0.13	0.88 (0.68-3.56)	0.88±0.13	0.60
Folate	ng/mL	50	8.25±.48	8.24±3.40	8.13±3.48	0.92
Vitamin B12	pg/mL	50	223 (68-1061)	219 (61-1100)	224 (76-1194)	0.88
25 OH Vitamin D	ng/mL	50	13.15±6.05	13.3±6.23	13.28±6.34	0.95
Parathyroid hormone (PTH)	pg/mL	50	46.4 (9.40-165.6)	46.5 (9.20-179.7)	45.9 (9.20-173.7)	0.97
Cortisol	nmol/L	50	10.05 (5.10-23.27)	10.12 (4.71-23.18)	10.71±3.46	0.98
Ferritin	µg/L	50	28.1 (2.40-241)	26.7 (2.60-250.5)	28.1 (2.30-251.10)	0.86
hCG	mIU/mL	50	0.31 (0.0-8.96)	0.31 (0.0-8.7)	0.27 (0.0-8.28)	0.573

SST: Serum separator tubes; RST: Rapid serum tubes

We also compared the results of the analytes for tube pairs (Z tube-SST, Z tube-RST) (Table 2). All of the calculated bias values of the analytes were below desirable bias.

Discussion

This study was designed to compare two different types of serum separator tubes (SST and RST) for the results of frequently performed immunoassay tests in the clinical laboratory. It was observed that the tubes showed no difference concerning TSH, fT4, folate, vitamin B12, 25-OH vitamin D, PTH and cortisol levels comparing with the reference tube (Z tube) on the Beckman Coulter DXI800 platform.

Turn-around time (TAT) is a significant quality indicator, particularly in the evaluation of emergency laboratory testing. Performing tests in plasma samples obtained from PSTs instead of using serum from standard serum tubes is a well-known strategy to reduce TATs. On the other hand, enough mixing of the specimen with anticoagulant immediately after blood drawing to ensure effective distribution of anticoagulant within the whole blood to prevent clotting is a very important and open to error step and emerges as a drawback of plasma over serum. The RSTs evaluated in the study are proposed to obtain serum samples faster than SSTs owing to shorter clotting time. As a result, reductions in TAT will improve the efficiency of laboratories and contribute to patient care. However, the effects of new equipment on test results should be evaluated before using in the routine clinical practice.

In a study conducted by Yan et al. RST was compared with the widely used serum separator tube (SST) for immunoassay tests on three common analyser platforms (Roche Modular, Abbott Architect, and Siemens Centaur analysers). A significant difference between the tubes according to both statistics and desirable bias (bias: -15.3%, $p<0.01$) was observed for PTH on Abbott Architect System. According to the researchers, short half-life and reduced stability of serum PTH may be the reasons for the difference, and a detailed stability study on PTH for RST was suggested [9]. In our study, we also found no statistically significant difference and calculated bias was below the determined desirable bias limits. The difference in the results of the studies may be due to usage of the different types of kits for different analytical platforms, as antibodies from different origins (e.g., sheep and mouse) are used by manufacturers. In a study with Unical DxI platforms, comparing plasma separator tube (PST) and SST for many analytes, there was a significant difference concerning TSH between two tubes [10]. It is suggested that the difference may be related to the physiological difference between plasma and serum.

In the study of Yan et al., including 24 subjects, no significant difference between RST and SST for hCG on Roche Modular System was reported [9]. In the present study, including 50 subjects, the difference between RST and reference tube was statistically significant but without any accompanying significant clinical difference according to cut-off of hCG.

In our study, we used the Z tube to exclude the effects of the additives. Also, all of the serum samples were transferred to the secondary tube, frozen, thawed and analysed.

Finally, data in this study show that RST and SST can provide comparable results with the Z tube. There was not any significant clinical difference according to the desirable bias for the analytes tested.

One of the limitations of our study was that we only included healthy volunteers; we could not evaluate the performance of the tubes for pathological values. Also, we could not evaluate the stability of the tubes on different temperatures and time.

Conclusion

In a conclusion, RST offers acceptable clinical performance for the immunoassay tests performed in this study on the DXI

Table 2. Comparison of the tests for tube pairs (Z tube-SST and Z tube-RST)

Analyte	n	Z tube	SST	RST	Bias (%) (Z Tube-SST)	Bias (%) (Z Tube-RST)	Desirable bias (%)
TSH (mUL/L)	50	1.25 (0.05-4.93)*	1.29 (0.05-4.60)*	1.29 (0.05-4.74)*	3.2	3.2	7.8***
fT3 (pg/mL)	50	3.07±0.33**	3.02±031**	2.96±0.30**	-1.62	-3.58	4.8***
fT4 (ng/dL)	50	0.89±0.13**	0.88(0.68-3.56)*	0.88±0.13**	-1.12	-1.12	3.3***
Folate (ng/mL)	50	8.25±0.48**	8.24±3.40**	8.13±3.48**	-0.12	-1.45	19.2***
Vitamin B12 (pg/mL)	50	223 (68-1061)*	219 (61-1100)*	224 (76-1194)*	-1.79	0.44	17.7 [11]
25OH Vitamin D (ng/mL)	50	13.15±6.05**	13.3±6.23**	13.28±6.34**	1.14	0.98	10.5 [12]
PTH (pg/mL)	50	46.4 (9.40-165.6)*	46.5 (9.20-179.7)*	45.9 (9.20-173.7)*	0.21	-1.07	8.8***
Cortisol (nmol/L)	50	10.05 (5.10-23.27)*	10.12 (4.71-23.18)*	10.71±3.46**	0.69	6.56	10.26***
Ferritin (µg/L)	50	28.1 (2.40-241)*	26.7 (2.60-250.5)*	28.1 (2.30-251.10)*	-4.98	0.36	5.2***
βhCG (mIU/mL)	50	0.31 (0.0-8.96)*	0.31 (0.0-8.7)*	0.27 (0.0-8.28)*	1.69	-11.47	-

*: median (min-max). **: mean±SD. ***: desirable bias data is based on https://www.westgard.com/biodatabase1.htm (exception of 25OH vitamin D and vitamin B12). Z Tube: 7 mL BD Vacutainer Z; SST: 5 mL BD Vacutainer SST II advance; RST: 5 mL BD vacutainer Rapid Serum Tube

platform. The laboratory turns around time for immunoassay tests could be shortened approximately 15-20 minutes using RSTs. Further studies with a larger number of tests and samples are needed to provide more relevant information.

Conflict of Interest: None declared.

Ethics Committee Approval: This study was approved by the local Ethical Committee.

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