Clin Chem Lab Med 2010;48(5):651–657 © 2010 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2010.141

Evaluation of the Becton-Dickinson rapid serum tube: does it provide a suitable alternative to lithium heparin plasma tubes?

Goce Dimeski[1,2,*], Paul P. Masci[3], Manuela Trabi[1,4], Martin F. Lavin[4,5] and John de Jersey[1]

[1] School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Australia
[2] Department of Chemical Pathology, Pathology Queensland, Princess Alexandra Hospital Ipswich Road, Woolloongabba, Queensland, Australia
[3] Center for Integrative Clinical and Molecular Medicine, School of Medicine, Southern Region, Faculty of Health Sciences, University of Queensland, Brisbane, Queensland, Australia
[4] Queensland Institute of Medical Research, Radiation Biology and Oncology, Brisbane, Queensland, Australia
[5] The University of Queensland Center for Clinical Research, Brisbane, Queensland, Australia

Abstract

Background: Obtaining a suitable specimen for analysis in a timely manner is pivotal in clinical chemistry service provision. Serum is recognized as the preferred specimen for most assays, but because of time constraints for completion of clotting and an increasing number of patients on anticoagulant therapy, latent clotting or no clotting is an outcome which can lead to errors and delay in delivery of critical results. Although lithium heparin plasma has unique problems, it has become an alternative in hospital-based laboratories.
Methods: The Becton-Dickinson (BD) rapid serum tube (RST) was evaluated in a hospital environment using a total of 53 participants, both healthy and anticoagulated, for 31 analytes against BD PST II and BD SST II tubes measured with Beckman DxC800 and DxI800 analyzers.
Results: Most results from the RST tube were comparable with those from the SST II tube. Potassium results were closer to the PST II plasma concentrations. Incomplete and latent clotting was encountered in the RST specimens from participants (cardiac and dialysis) who had received a total of >7000 units of heparin [activated partial thromboplastin time (APTT) >150 s], warfarin/heparin combination, and specimens from cardiac surgery patients who had received a total of >25,000 units of heparin (APTT >200 s) at the time of collection of specimens.
Conclusions: The RST tube provides a suitable alternative to lithium heparin plasma tubes for most patients in a hospital environment. However, latent clotting continued to occur in specimens collected from participants who had received high concentrations of anticoagulants.
Clin Chem Lab Med 2010;48:651–7.

Keywords: anticoagulants; latent clotting; plasma; rapid serum tube; serum.

Introduction

Service quality encompasses total test error (impression and inaccuracy), availability, cost, relevance and timeliness (1). Delays in turn-around time (TAT) are the most common complaint that laboratories receive from clinicians (2), and as many as 87% of these complaints originate from Emergency Department (ED) clinicians (3). This is understandable because up to 80%–90% of medical decisions are based on laboratory data (4–6). For laboratories to meet these expectations, specimen quality and the time required to obtain a suitable specimen (serum/plasma) for biochemical analysis are key factors. Serum and heparinized plasma specimens are considered equivalent for many assays, and it is not uncommon for hospital-based laboratories to receive serum or plasma specimens interchangeably for general chemistry analysis. It is also well documented that there are significant differences in the values for some analytes when measured in serum or plasma, particularly potassium and total protein (TP) (7–9), and these require different reference intervals. Serum is considered fibrinogen-, fibrin- and cell-free under optimal clotting conditions, and it is the preferred matrix particularly for immunoassays. However, latent clot formation that occurs post-centrifugation can lead to risk of fibrin clot interference on automated analyzers, especially those with a common sample probe and no clot detection capacity (10). The major advantage of lithium heparin plasma is that it enables laboratories to achieve faster TATs. A number of reports discuss some of the other major advantages (7, 8, 11) and disadvantages (8, 12–16) of lithium heparin plasma.

Two recent studies by Chance et al. (17) and Giavarina et al. (11), sponsored by Becton-Dickinson (BD), demonstrated that plasma obtained with the BD Vacutainer® PST™ II tubes can be used interchangeably with serum for most special chemistry analytes and immunoassays performed on dif-

*Corresponding author: Goce Dimeski, School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Australia
Phone: +61-7-3240-5082, Fax: +61-7-3240-7070,
E-mail: goce_dimeski@health.qld.gov.au;
gdi56790@bigpond.net.au
Received August 19, 2009; accepted January 12, 2010;
previously published online March 11, 2010

ferent analytical platforms, permitting further consolidation of test menus and use of single tubes for multiple tests. However, Giavarina et al. (11) found that lithium heparin plasma could not be used for analysis of folate, testosterone, vitamin B12, and progesterone with the Siemens Advia Centaur® analyzer (Siemens Healthcare Diagnostics, Medfield, MA, USA). These findings were in agreement with findings reported by Morovat et al. (18).

A new tube from BD Diagnostics, the BD Vacutainer® rapid serum tube (RST) is claimed to overcome the clotting problems found with current commercial serum tubes. It contains a thrombin-based clotting agent that can provide a clotted specimen within 5 min following collection of blood (19). This tube has been previously evaluated against the serum separator tube (SST) using only healthy participants and non-immunoassay analytes (20). The aim of this study was to evaluate the BD Vacutainer® RST in a tertiary hospital setting against the BD Vacutainer® PST™ II lithium heparin plasma and BD Vacutainer® SST™ II serum tubes (BD Vacutainer Systems, Plymouth, UK) for both general chemistry and immunoassay analytes. In addition, we wished to determine if this is a suitable alternative to lithium heparin plasma for rapid testing. The evaluation was performed by recruiting both healthy individuals and participants receiving different anticoagulants and concentrations of anticoagulants, as routinely encountered in a tertiary referral hospital.

Materials and methods

The study was performed at the Princess Alexandra Hospital, Brisbane, Australia with appropriate ethics approval and with informed consent from all participants. All 53 participants were adults >18 years of age, with a mix of males and females. Of the 53 participants, 24 were healthy participants, three were outpatients on low doses of warfarin, while the remaining 26 were inpatients. Of the 26 inpatients, seven were undergoing cardiac surgery and had received a total of 25–41,000 units of heparin at the time of blood collection, which was performed within 30 min post heparin infusion. The specimens were collected while participants were on bypass. There were nine cardiac care unit participants recruited the night before specimen collection receiving heparin by intravenous (IV) infusion, 950–1450 units of heparin per hour. Seven remained on IV heparin infusion (≥12 h) at the time of specimen collection. Two patients that were to undergo surgery had their infusion stopped ~3 h prior to specimen collection. From information obtained in the patient record, the heparin concentration in the infusate and the infusion rate were unchanged for the participants over this period. The remaining inpatients were undergoing dialysis, eight were on IV heparin infusion, and one on warfarin/heparin (~1750–7000 units of heparin, initial bolus plus hourly top up doses), and one on clexane. The specimens were collected at least 1 h following the start of dialysis.

Blood was collected using a standardized draw order: citrate, serum tubes (BD Vacutainer® SST™ II REF367954, 5.0 mL fill volume and BD Vacutainer® RST REF368771, 4.0 mL fill volume) before the lithium heparin tube which contains 77 IU of heparin (BD Vacutainer® PST™ II REF367375, 4.5 mL fill volume). Blood was collected by venipuncture from healthy individuals and those in the cardiac care unit. Collection was via the bypass port from patients in cardiac surgery and via a blood line from dialysis participants. The SST II tubes were allowed to clot for 30 min from healthy participants and for 60 min for anticoagulated participants. These tubes were visually inspected for clot formation prior to loading in the centrifuge. The RST specimens were visually inspected for clot formation at 5 min for all participants at the point of collection (phlebotomy or clinical unit). The RST specimens from healthy participants and anticoagulated participants that formed a solid clot at 5 min were centrifuged as soon as they were delivered to the laboratory (<20 min). If clotting was incomplete, the specimens were rechecked every 10–15 min for clotting, and allowed to clot for a maximum of 60 min. The lithium heparin specimens were centrifuged immediately following delivery to the laboratory (<20 min from collection).

All tubes were centrifuged at 3000 *g* for 10 min at 20°C in a swing bucket centrifuge, and then stored at ~21°C. The tubes were visually inspected for latent clotting immediately after centrifugation and then again just prior to loading onto the analyzers. The primary tubes were used for analysis except in cases where latent clotting was observed. In these cases, serum was transferred to an aliquot tube, re-centrifuged to remove the clots, and the clean serum transferred to another aliquot tube.

Analysis was performed using the Beckman DxC800 general chemistry analyzer and a DxI800 immunoassay analyzer (Beckman Coulter, Fullerton, CA, USA). Samples were loaded on the same instruments at the same time and within 1–2 h post-centrifugation, except in cases where recurrent latent clotting was encountered.

The upper limit of imprecision of the between-run coefficient of variation (CVs) from two internal quality control samples for the 31 analytes tested on the Beckman DxC800 analyzers were as follows: <2% Na^+, K^+, Cl^-, urate, TP, albumin, creatinine kinase (CK), total calcium (TCa), inorganic phosphorus (Pi); <3% glucose, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), Mg^{2+}, cholesterol, <4% HCO_3^-, urea, aspartate transaminase (AST), triglycerides, high-density lipoprotein cholesterol (HDL-C), transferrin (Trf); <5% creatinine; <6% γ-glutamyltransferase (GGT), Fe^{2+}; <7% total bilirubin, alanine transaminase (ALT), C-reactive protein (CRP), and lipase. A semiquantitative assessment of hemolysis was also performed. For the DxI800 analyzer, the CV% at three concentrations of quality control material was as follows: cortisol and thyroid stimulating hormone (TSH) <8%, free thyroxine (fT4) <9%, and troponin I (TnI) ~16% at 0.052 μg/L, and ~8% at 0.5 μg/L. We also measured the activated partial thromboplastin time (APTT) using an AC^{TOPS} (Instrumentation Laboratory, Lexington MA, USA) as it was difficult to ascertain the exact anticoagulant concentration in the participants' blood at the time of specimen collection in the cardiac care unit and for dialysis participants.

The mean and standard deviation (SD) were calculated for each test for the three tubes, and the percent difference between the means of the three tubes. Exploratory analysis and frequency histograms and tests of normality were performed to determine if parametric or non-parametric statistical tests would be used with the Statistica V 6.0 software package (StatSoft, Tulsa, OH, USA). The paired t-test was used for parametric data and the Wilcoxon Matched-Pairs Rank test for non-parametric data to test for statistical difference between analytes measured from the different tubes. Results were considered statistically significant and analytically important for $p<0.05$, and if the percent difference was greater than the analyzers' CV%. If a measurement was not obtained for an analyte in any of the three tubes as a result of recurrent latent clotting leading to insufficient specimen, insufficient specimen collected, analyte not requested on the analyzer, or insufficient reagent, the result was not included in the calculation. Thus, there was variability in the number of specimens analyzed per assay.

Results

The blood from the 24 healthy and three participants on low dose warfarin therapy clotted within 5 min in the SST and RST tubes, forming a solid immobile clot, with no latent clotting encountered. The SST and RST specimens from the seven cardiac surgery participants that received 25,000–41,000 units of heparin had APPTs >200 s. The SST sample did not clot at all, and no latent clotting was visually observed or detected by the analyzers. With the RST specimens, limited latent clotting was visually observed but not detected by the analyzers. Blood specimens from the nine participants in the cardiac care unit who had received 950–1450 units of heparin per hour took 20 min or longer to form a visibly semi-solid or solid clot in the SST, and up to 20 min in the RST specimens. When the APTT was <60 s, no latent clotting was visually observed or detected by the analyzers. Latent clotting was visually observed in three specimens, but detected by the analyzers in two of the RST specimens with an APTT >70 s. Similarly, the SST specimens from the dialysis participants who received 3250–7000 units of heparin and who were also taking 100–150 mg salicylate daily, took 20 min or longer to form a visibly semi-solid or solid clot. The RST specimens visibly clotted within ~20 min. No latent clotting was visually observed or detected by the analyzers in specimens with <5000 units of heparin and APTT <150 s. Also, the one participant on clexane 40 mg/4 h did not show latent clotting and no clotting was detected by the analyzers. In contrast, latent clotting was visually observed and detected by the analyzers in both SST and RST specimens in the two participants who received 6000–7000 units of heparin and had an APTT >150 s. For the single participant receiving 6×4 mg/day warfarin plus ~5000 units of heparin (APTT >150 s), latent clotting was detected by the analyzers in the RST specimen only. In one dialysis participant who received only 1750 units of heparin, the RST specimen visibly clotted in ~5 min, and the SST in ~30 min. No latent clotting was encountered despite the fact that the APTT was >200 s.

Table 1 lists the analyte results determined in the three different tubes, plasma separator tube (PST), SST and RST. For the 31 analytes tested in the three tubes, there was generally good agreement in the concentrations determined, particularly between the SST and the RST. Differences that were statistically significant and analytically important were: a) RST vs. SST – LDH and TnI; b) RST vs. PST – K^+, Pi, TP, LDH, ALP, HDL-C and TnI; and c) SST vs. PST – K^+, Pi, TP, LDH, ALP, HDL-C and TnI. Results from the healthy and groups receiving anticoagulants were analyzed separately for analytes showing statistical and analytical difference: K^+, TP, LDH, Pi, ALP and HDL-C to demonstrate the effects of incomplete clotting on the concentration of these analytes (Table 2). The data confirmed that the K^+ values were closer to plasma values for the anticoagulated participants because of incomplete clotting, and decreased lysis of cells that occurs during clotting. The means for LDH and K^+ were slightly lower in the RST tube compared with the SST tube, perhaps due to faster clotting which minimizes cell lysis and release of LDH and K^+. As expected, due to removal of fibrinogen during the clotting process, the TP results in the SST and RST were lower by 3.3%–4.7% compared to results from the PST II. The TnI exceptions were due to three false positive TnI results (one in PST plasma and two in SST serum samples) (Table 3). When repeated, the results matched those from the other tubes. No specimens, irrespective of the tube type, showed significant hemolysis, and all the hemolysis results were zero or one (<0.5 g/L free hemoglobin). This magnitude of hemolysis is very low and unlikely to influence clinically any of the analyte determinations.

Discussion

This study evaluated the BD RST tube in a major tertiary hospital with a very large cardiac and renal dialysis population, where anticoagulated patients may be responsible for up to 10% of all specimens received in the clinical chemistry laboratory. The findings indicate that the use of this tube represents an improvement over the current SST serum tube. Visible clotting was achieved rapidly in RST specimens; within ~5 min where participants had received a total of 0 to ~5000 units of heparin (APTT <150 s). However, the RST and SST were ineffective for complete clotting of blood specimens in a suitable time to be used interchangeably with lithium heparin plasma (~15 min) from participants who received a total of >7000 units of heparin (APTT >150 s). This is approximately the time it takes specimens to get to the laboratory and be accessioned for centrifugation in our hospital. Latent clotting problems in the RST serum were also encountered with cardiac and dialysis participants who received a total of >7000 units of heparin (APTT >150 s), and in a dialysis patient who had received 24 mg/day warfarin in combination with heparin. No clotting or very minimal clotting of blood in RST specimens was observed in cardiac surgery participants who received a total of more than 25,000 units of heparin. Further, latent clotting was a problem in some specimens. Therefore, use of the RST tube would not be appropriate for specimens from patients receiving high doses (>7000 units) of heparin. The number of such patients represents 1%–2% of all specimens received in our clinical chemistry laboratory.

Differences in the concentrations of K^+, Pi and TP measured in lithium heparin plasma and serum are well established and have been reported previously (7, 9). The SST tubes showed smaller differences for K^+ compared with heparin plasma (5.4%; Table 1) compared with data reported in literature, 7.1%–9.6% (9). The division of results into normal and anticoagulated participant groups clearly demonstrated that the variations in K^+ concentrations for the normal group is within published limits, 7.42% in the SST tube, but was lower in the RST tube for both groups. This can be explained by the failure of blood to clot in some of the anticoagulated participants. These specimens effectively remained as plasma, since platelets were not activated by the clotting process to release K^+. The higher concentrations of K^+ in serum are

Table 1 Data for each analyte from the three different tubes.

Analyte	Units	n	RST (Mean ± SD)	SST II (Mean ± SD)	PST II (Mean ± SD)	% Difference between means (RST-SST)	p (RST vs. SST)	% Difference between means (RST-PST)	p (RST vs. PST)	% Difference between means (SST-PST)	p (SST vs. PST)
Na^+	mmol/L	53	137.5 ± 2.3	137.7 ± 2.3	137.4 ± 2.3	–0.10	0.375	0.12	0.310	0.22	0.066
K^+	mmol/L	53	4.13 ± 0.60	4.20 ± 0.61	3.99 ± 0.66	–1.84	**< 0.001**	**3.51**	**< 0.001**	**5.44**	**< 0.001**
Cl^-	mmol/L	53	104.4 ± 3.4	104.5 ± 3.4	104.5 ± 3.7	–0.09	0.451	–0.07	0.551	0.00	0.880
HCO_3^-	mmol/L	53	25.3 ± 2.7	25.5 ± 2.7	25.7 ± 2.5	–0.74	0.214	–1.40	**0.043**	–0.66	0.310
Gluc	mmol/L	53	6.04 ± 2.0	5.97 ± 2.03	6.11 ± 2.01	1.23	**0.006**	–1.05	**0.011**	–2.26	**< 0.001**
Urea	mmol/L	53	5.33 ± 2.01	5.33 ± 2.05	5.33 ± 1.99	–0.11	0.587	–0.07	0.553	0.04	0.936
Creat	μmol/L	53	129.2 ± 112.3	127.9 ± 111.9	127.2 ± 109.8	1.00	**0.013**	1.56	**0.001**	0.55	0.192
Urate	mmol/L	53	0.285 ± 0.110	0.287 ± 0.110	0.286 ± 0.110	–0.59	**0.019**	–0.59	0.766	0.52	**0.044**
TP	g/L	53	63.5 ± 11.2	63.7 ± 11.1	66.3 ± 11.8	–0.24	0.424	**–4.13**	**< 0.001**	**–3.90**	**< 0.001**
Alb	g/L	53	36.6 ± 8.0	36.5 ± 8.0	36.2 ± 7.8	0.15	0.497	1.09	**< 0.001**	0.94	**< 0.001**
T Bili	μmol/L	52	13.3 ± 4	13.4 ± 3.9	13.3 ± 3.6	–1.07	0.610	0.00	0.609	1.16	1.000
ALP	U/L	53	73.0 ± 27.0	73.5 ± 27.4	70.8 ± 26.8	–0.62	0.141	**3.14**	**< 0.001**	**3.78**	**< 0.001**
GGT	U/L	53	29.4 ± 16.8	29.8 ± 16.3	29.6 ± 16.4	–1.06	1.000	–0.70	0.657	0.37	0.654
[b]ALT	U/L	53	32.1 ± 20.1	31.2 ± 19.2	30.8 ± 19.1	2.90	**0.005**	4.16	**< 0.001**	1.22	0.145
[b]AST	U/L	53	33.6 ± 41.0	33.3 ± 39.8	33.4 ± 37.8	0.86	0.827	0.46	0.196	–0.40	0.668
[b]LDH	U/L	50	212.0 ± 115.7	224.4 ± 117.2	215.4 ± 105.7	**–5.53**	**< 0.001**	–1.57	**0.005**	**4.20**	**0.001**
[b]CK	U/L	51	142.5 ± 226.9	143.5 ± 231.7	138.8 ± 210.2	–0.73	0.152	2.61	**0.007**	3.36	**< 0.001**
TCa	mmol/L	53	2.221 ± 0.247	2.232 ± 0.247	2.199 ± 0.231	–0.48	**0.023**	1.00	**< 0.001**	1.48	**< 0.001**
Pi	mmol/L	53	1.109 ± 0.296	1.109 ± 0.294	1.050 ± 0.284	–0.03	0.892	**5.57**	**< 0.001**	**5.61**	**< 0.001**
Mg^{2+}	mmol/L	50	0.973 ± 0.242	0.974 ± 0.240	0.976 ± 0.242	–0.12	0.691	–0.33	0.504	–0.20	0.644
Lipase	U/L	50	28.1 ± 8.1	28.4 ± 8.1	28.3 ± 8.0	–1.13	**0.028**	–0.50	0.391	0.64	0.245
Chol	mmol/L	49	4.37 ± 1.43	4.35 ± 1.44	4.25 ± 1.39	0.59	1.000	2.95	**< 0.001**	2.35	**< 0.001**
Trig	mmol/L	46	1.31 ± 1.012	1.31 ± 1.008	1.291 ± 0.993	–0.03	0.950	1.45	0.081	1.48	0.067
HDL-C	mmol/L	50	1.275 ± 0.6	1.277 ± 0.567	1.338 ± 0.587	–0.16	0.820	**–4.68**	**< 0.001**	**–4.53**	**< 0.001**
Fe^{2+}	μmol/L	51	14.8 ± 6.6	15.1 ± 6.6	14.8 ± 6.7	–2.46	**0.001**	–0.26	0.642	2.25	**< 0.001**
Trf	g/L	51	2.25 ± 0.74	2.26 ± 0.69	2.21 ± 0.69	–0.21	0.848	2.09	0.089	2.30	**0.001**
CRP	μg/L	48	±	17.0 ± 56.8	16.6 ± 54.8	–0.67	0.258	1.95	0.206	2.64	0.158
[b]TnI[a]	μg/L	52	1.037 ± 6.170	0.957 ± 5.634	1.047 ± 6.180	**8.31**	**< 0.02**	–1.12	0.700	**–8.61**	**< 0.02**
Cortisol[a]	nmol/L	52	354.2 ± 259.0	350.6 ± 253.2	353.8 ± 261.0	1.03	0.363	0.12	0.888	–0.90	0.386
fT4[a]	pmol/L	51	12.75 ± 3.75	12.65 ± 4.05	12.68 ± 3.63	0.82	0.463	0.54	0.564	–0.28	0.821
[b]TSH[a]	μIU/mL	51	1.679 ± 1.459	1.656 ± 1.370	1.709 ± 1.399	1.39	0.571	–1.74	0.069	–3.09	**0.0185**

[a]Analysis performed with the DxI800 immunoassay analyzer. A p-value < 0.05 is considered statistically significant and indicated in bold. For parametric analysis, the p-value was determined by the paired t-test. [b]For non-parametric analysis, the p-value using the Wilcoxon Matched-Pairs Rank test. Alb, albumin; T Bili, total bilirubin.

Table 2 Data for each analyte from the three different tubes divided into [a]–normal and [b]–anticoagulated participants.

Analyte	Units	n	RST (Mean ± SD)	SST II (Mean ± SD)	PST II (Mean ± SD)	% Difference between means (RST-SST)	p (RST vs. SST)	% Difference between means (RST-PST)	p (RST vs. PST)	% Difference between means (SST-PST)	p (SST vs. PST)
K^+	mmol/L	53	4.13 ± 0.60	4.20 ± 0.61	3.99 ± 0.66	–1.84	**< 0.001**	**3.51**	**< 0.001**	**5.44**	**< 0.001**
[a]K^+	mmol/L	24	4.10 ± 0.28	4.16 ± 0.27	3.88 ± 0.29	–1.50	**0.044**	**5.81**	**< 0.001**	**7.42**	**< 0.001**
[b]K^+	mmol/L	29	4.15 ± 0.78	4.24 ± 0.79	4.08 ± 0.85	**–2.11**	**0.002**	1.70	0.064	**3.89**	**< 0.001**
TP	g/L	53	63.5 ± 11.2	63.7 ± 11.1	66.3 ± 11.8	–0.24	0.424	**–4.13**	**< 0.001**	**–3.90**	**< 0.001**
[a]TP	g/L	24	70.1 ± 3.6	70.4 ± 3.9	72.8 ± 4.2	–0.36	0.388	**–3.61**	**< 0.001**	**–3.29**	**< 0.001**
[b]TP	g/L	29	58.1 ± 12.5	58.1 ± 12.1	60.9 ± 13.3	–0.12	0.787	**–4.64**	**< 0.001**	**–4.54**	**< 0.001**
[c]LDH	U/L	50	212.0 ± 115.7	224.4 ± 117.2	215.4 ± 105.7	**–5.53**	**< 0.001**	–1.57	0.005	**4.20**	**0.001**
[a,c]LDH	U/L	24	188.2 ± 30.5	201.3 ± 32.6	196.4 ± 32.4	–6.54	**< 0.001**	**–4.18**	**< 0.001**	2.52	**0.021**
[b,c]LDH	U/L	27	234.0 ± 155.9	245.7 ± 158.1	232.9 ± 142.4	–4.77	**0.001**	0.46	0.572	**5.50**	**0.008**
Pi	mmol/L	53	1.109 ± 0.296	1.109 ± 0.294	1.050 ± 0.284	–0.03	0.892	**5.57**	**< 0.001**	**5.61**	**< 0.001**
[a]Pi	mmol/L	24	1.267 ± 0.213	1.266 ± 0.217	1.186 ± 0.219	0.10	0.799	**6.85**	**< 0.001**	**6.75**	**< 0.001**
[b]Pi	mmol/L	29	0.978 ± 0.293	0.979 ± 0.289	0.938 ± 0.286	–0.18	0.587	**4.23**	**< 0.001**	**4.41**	**< 0.001**
HDL-C	mmol/L	50	1.275 ± 0.6	1.277 ± 0.567	1.338 ± 0.587	–0.16	0.820	**–4.68**	**< 0.001**	**–4.53**	**< 0.001**
[a]HDL-C	mmol/L	24	1.605 ± 0.500	1.590 ± 0.495	1.675 ± 0.508	0.94	0.277	**–4.15**	**0.011**	**–5.05**	**0.001**
[b]HDL-C	mmol/L	26	0.971 ± 0.500	0.988 ± 0.473	1.027 ± 0.478	–1.79	0.109	**–5.47**	**< 0.001**	–3.75	**0.015**
ALP	U/L	53	73.0 ± 27.0	73.5 ± 27.4	70.8 ± 26.8	–0.62	0.141	**3.14**	**< 0.001**	**3.78**	**< 0.001**
[a]ALP	U/L	24	65.9 ± 188.7	66.3 ± 19.5	63.8 ± 19.9	–0.57	0.443	**3.26**	**< 0.001**	**3.85**	**< 0.001**
[b]ALP	U/L	29	78.9 ± 31.4	79.4 ± 31.6	76.6 ± 30.5	–0.65	0.198	**3.06**	**< 0.001**	**3.74**	**< 0.001**

Percent differences between means considered clinically significant are indicated in bold. A p-value < 0.05 is considered statistically significant and indicated in bold. For parametric analysis, the p-value was determined using the paired t-test. [c]For non-parametric analysis, the p-value was determined using the Wilcoxon Matched-Pairs Rank test.

Table 3 Data from the false positive troponin (TnI) results.

Participant	Original TnI result (repeat TnI result by analyzer reflex mode) μg/L		
	RST	SST II	PST II
18 (healthy participant)	0.000	**0.053** (0.004)	0.000
20 (cardiac surgery participant on heparin)	0.004	0.013	**0.093** (0.015)
38 (healthy participant)	0.004	**1.334** (0.002)	0.006

The values in bold are the erroneous results.

also due to the contribution from lysis of red cells and leukocytes, not just platelets. Serum specimens have slightly higher levels of hemolysis compared with plasma (21). Intermittently higher results can be obtained in plasma with K^+ or LDH or other analytes than in serum. However, repeat results are lower than the original result. This is most likely due to microclots containing cells that are aspirated and release their contents during the analytical process or lead to inaccuracies in analytical sampling volume (16). In this study, not all K^+ concentrations were lower in the plasma specimen.

For TnI results, the specimens were automatically analyzed in duplicate using the analyzers' reflex capability when the first result was ≥ 0.04 μg/L (Table 3). Also, the results were compared to those obtained in the other tubes. The observed difference in the duplicates of the three specimens were considered significant if they exceeded the recommended change between patient samples (>3 SD for results between ≥ 0.03–0.10 μg/L or $>20\%$ for results >0.10 μg/L) (22). This appears to be due to fibrin which may be from latent clots, or incomplete removal during centrifugation. Fibrin has been implicated as the causative agent of false positives in the Beckman AccuTnI assay (23). False positive TnI results have been reported with the Beckman AccuTnI assay for several years (23, 24), and most have been detected by clinical staff rather than at the laboratory level. Unlike our approach, performing analysis in duplicate in order to identify discrepancies before reporting, recentrifugation of samples has been suggested if the reported result is questioned by clinicians and thought to be false positive (23) or recentrifuged automatically if it is above 0.1 μg/L (24). These types of discrepancies clearly support the need for a better specimen.

The results obtained here confirm that the BD RST tube provides a suitable alternative to lithium heparin plasma for most specimens collected in the hospital environment. However, latent clotting continued to occur in the RST specimens when collected from participants receiving high doses of anticoagulants (heparin or warfarin/heparin), even when specimens were allowed to stand for 60 min prior to centrifugation. Such latent clotting can compromise the accuracy of results. It is evident from this and other studies that currently no single tube fulfills all the requirements for biochemistry testing in all patients.

Acknowledgements

The BD RST tubes were the only tubes provided free of charge by BD Diagnostics.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. BD and research funding played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.
Research funding: This project was funded by a Uniquest Pty Ltd Pathfinder grant 2008.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. Watts NB. Reproducibility (precision) in alternative site testing. A clinician's perspective. Arch Pathol Lab Med 1995;119:914–7.
2. Howanitz JH, Howanitz PJ. Laboratory results. Timeliness as a quality attribute and strategy. Am J Clin Pathol 2001;116:311–5.
3. Howanitz PJ, Cembrowski GS, Steindel SJ, Long TA. Physician goals and laboratory test turn-around times. A College of American Pathologists Q-Probes study of 2763 clinicians and 722 institutions. Arch Pathol Lab Med 1993;117:22–8.
4. Boone DJ. Is it safe to have a laboratory test? Accred Qual Assur 2004;10:5–9.
5. Howanitz PJ. Errors in laboratory medicine. Arch Pathol Lab Med 2005;129:1252–61.
6. Apple F, Jesse RL, Newby LK, Wu AH, Christenson RH, Cannon CP, et al for the NACB Writing Group Members; NACB Committee Members; and IFCC Committee on Standardization of Markers of Cardiac Damage (CSMCD). National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine Practice Guidelines: Analytical issues for biochemical markers of acute coronary syndromes. Clin Chem 2007;53:547–51.
7. Hartland AJ, Neary RH. Serum potassium is unreliable as an estimate of in vivo plasma potassium. Clin Chem 1999;45:1091–2.
8. Miles RR, Roberts RF, Putnam AR, Roberts WL. Comparison of serum and heparinized plasma samples for measurement of chemistry analytes. Clin Chem 2004;50:1704–5.
9. O'Keane MP, Cunningham SK. Evaluation of three different specimen types (serum, plasma lithium heparin and serum gel separator) for analysis of certain analytes: clinical significance of differences in results and efficiency in use. Clin Chem Lab Med 2006;44:662–8.
10. Boyanton BL, Blick K. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem 2002;48:2242–7.
11. Giavarina D, Fortunato A, Barzan E, Church S, Berube J, Green S, et al. Evaluation of BD Vacutainer® PST™ II tubes for a

wide range of immunoassays. Clin Chem Lab Med 2009; 47:237–41.
12. Dimeski G, Carter A. Rare IgM interference with Roche/Hitachi Modular glucose and gamma-glutamyltransferase methods in heparin samples. Clin Chem 2005;51:2202–4.
13. Meng QH, Krahn J. Lithium heparinised blood-collection tubes give falsely low albumin results with an automated bromcresol green method in hemodialysis patients. Clin Chem Lab Med 2008;46:396–400.
14. Hermsen D, Apple F, Garcia-Beltràn L, Jaffe A, Karon B, Lewandrowski E, et al. Results from a multicenter evaluation of the 4th generation elecsys troponin T assay. Clin Lab 2007;53:1–9.
15. Davidson AS, Darn SM, Sodi R. Can lithium heparin plasma be used for protein electrophoresis and paraprotein identification? Ann Clin Biochem 2006;43:31–4.
16. Dimeski G, Badrick T, Flatman R, Ormiston B. Roche IFCC methods for lactate dehydrogenase tested for duplicate errors with Greiner and Becton-Dickinson lithium-heparin and Greiner serum samples. Clin Chem 2004;50:2391–2.
17. Chance J, Berube J, Vandersmissen M, Blanckaert N. Evaluation of the BD Vacutainer® PST™ II blood collection tube for special chemistry analytes. Clin Chem Lab Med 2009;47: 358–61.
18. Morovat A, James TS, Cox SD, Norris SG, Rees MC, Gales MA, et al. Comparison of Bayer Advia Centaur® immunoassay results obtained on samples collected in four different Becton-Dickinson Vacutainer® tubes. Ann Clin Biochem 2006;43: 481–7.
19. BD Diagnostics Preanalytical Systems PAS-CAT-2007.
20. Zhang Q, Dubrowny N, Clegg D. A comparison of the BD RST tubes versus BD SSTtm tubes for chemistry performance on the UniCel DxC800 clinical system from Beckman Coulter. Clin Chem 2008, AACC Annual Meeting, E-41.
21. Haemolysis as interference and interference factor. Thomas L, eJIFCC vol 3 no 4: http://www.ifcc.org/ejifcc/vol13no4/130401002.htm
22. Kavsak PA, MacRae AR, Yerna MJ, Jaffe AS. Analytic and clinical utility of a next-generation, highly sensitive cardiac troponin I assay for early detection of myocardial injury. Clin Chem 2009;55:573–7.
23. Fleming SM, O'Byrne L, Finn J, Grimes H, Daly KM. False-positive cardiac troponin I in a routine clinical population. Am J Cardiol 2002;89:1212–5.
24. Hejl CG, Astier HT, Ramirez JM. Prevention of pre-analytical false-positive increases of cardiac troponin I on the Unicel® DxI800 analyser. Clin Chem Lab Med 2008;46:1789–90.