Clinical Biochemistry 47 (2014) 150–157

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Blood collection tube-related alterations in analyte concentrations in quality control material and serum specimens

Raffick A.R. Bowen [a,*], Annie Sattayapiwat [a], Verena Gounden [b], Alan T. Remaley [b]

[a] Department of Pathology, Stanford University, Stanford, CA 94305, USA
[b] Dept. of Laboratory Medicine, National Institutes of Health, Bethesda, MD 20892, USA

ARTICLE INFO

Article history:
Received 8 September 2013
Received in revised form 5 November 2013
Accepted 6 November 2013
Available online 14 November 2013

Keywords:
Blood collection tube
Quality control material
Thyroxine
Triiodothyronine

ABSTRACT

Objectives: Several previous studies have described the effects of interfering substances on clinical assay results; however, the effects of exogenous substances, particularly additives from blood collection tubes on quality control (QC) specimens and serum specimens have not been well examined. This study examines the effects of blood-collection tube additives on total triiodothyronine (TT_3), and thyroxine (TT_4), cortisol, and routine clinical chemistry tests in QC and serum specimens from apparently healthy volunteers.

Methods: QC and serum specimens were poured or collected into different blood collection tubes. TT_3 and TT_4, cortisol, and routine chemistry tests were analyzed from the different blood-collection tube types.

Results: The findings of this study demonstrate statistically and/or clinically significant blood collection tube-related alterations in the TT_3, TT_4, and cortisol concentrations of QC specimens and TT_4 concentrations from serum specimens.

Conclusions: These findings have important implications for clinical laboratories, demonstrating that QC specimens should ideally, like patients' specimens, be poured into blood collection tubes. This strategy would reveal any adverse effects caused by blood collection tubes, which otherwise would not likely be detected by most routine QC practices. The results of this study also show the importance of producing blood collection tubes that contain additives that are truly inert and do not adversely affect clinical laboratory testing.

Introduction

Blood collection and processing are two major steps in pre-analytical laboratory testing [1]. Proper blood collection and timely processing, by well-trained staff using appropriate devices, are needed to ensure test reliability. Blood collection devices have typically been regarded as inert specimen carriers; many laboratories have thus invested relatively little in evaluating new blood collection devices, and do not routinely monitor their performance. Previous studies have reported statistically and clinically significant differences in some immunoassay test results from blood collected in some types of serum evacuated blood collection tubes manufactured by Becton Dickinson (BD) because of tube additives, particularly surfactants [2–4]. To solve immunoassay problems with the BD Vacutainer serum separator (SST), SST II, and Microtainer tubes that surfaced in 2004, BD reformulated the serum tubes to reduce the amount of surfactant in them in order to eliminate assay interference [2,5]. No clinically significant differences were observed with use of the reformulated tube types; it thus appeared that the reformulated BD tubes had been successfully adjusted to reduce assay interference and yield results that were similar to those of glass and Vacuette tubes for the total triiodothyronine (TT_3), total thyroxine (TT_4), and cortisol assays tested [2,5]. However, further studies by Wang et al. [6] and Lima-Oliveira et al. [7], as well as recent TT_3, TT_4, and cortisol results from patients' specimens in the author's clinical laboratory have indicated that blood collection tube related interference in some clinical assays may not be fully resolved.

One of the central tenets of quality control (QC) and quality assurance is that a) control materials should be handled by well-trained and competent laboratory personnel, and b) these control materials should be treated in exactly the same way as patients' specimens [8]. Unfortunately, this is not always adhered to in routine practice, and previously published studies with blood collection tubes have underscored this point [8].

To the author's knowledge, only one study has investigated the impact of QC material poured into blood collection tubes on TT_3, TT_4, and cortisol concentrations; that single study examined only one tube type, SST [8]. The effects of other BD serum tube types and serum tubes from a different tube manufacturer commonly used in clinical laboratories in North America on QC material analyte concentrations are not known. There is thus little information about the potential impact of blood collection tubes on QC specimen analyte concentrations. It was hypothesized that adverse effects of additives in blood collection tubes would be apparent if the QC specimens were poured into blood

Abbreviations: BD, Becton-Dickinson; PT, proficiency testing; PRT, plain red-top; QC, quality control; RST, rapid serum tube; SCL, significant change limit; SD, standard deviation; SST, serum separator tube; TT_3, total triiodothyronine; TT_4, total thyroxine; USD, usual standard deviation.

* Corresponding author at: 300 Pasteur Drive, Room H-1507B, Stanford, CA 94305, USA. Fax: +1 650 736 1473.
E-mail address: rbowen@stanfordmed.org (R.A.R. Bowen).

0009-9120/$ – see front matter © 2013 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.clinbiochem.2013.11.003

collection tubes and processed in the same way as patients' specimens are processed.

The purpose of this study is to evaluate the QC specimens poured into BD PRT, RST, and SST tubes and in Greiner Vacuette tubes and compare them to BD glass blood collection tubes on the Siemens Immulite™ 1000 analyzer for TT_3, TT_4, and cortisol, which are immunoassay analytes shown to be significantly affected by tube surfactant [2,3]. In addition, routine clinical chemistry assays from QC material poured in the five different blood collection tubes will be evaluated on a Siemens Dimension RxL™ analyzer.

Materials and methods

Collection tube types and QC specimens

Five types of evacuated blood collection tubes were examined in this study as shown in Table 1. Glass collection tubes are considered the control tubes in this study because this tube type has been the standard device for collecting serum samples for over five decades and these tubes contain no clot activator, internal tube coating, or separator gel [2,3]. All blood collection tubes were used before their expiration dates. QC specimens (Bio-Rad Liquichek Immunoassay Plus Control) 1 (lot 40781), 2 (lot 40782), and 3 (lot 40783) were poured (2 mL per tube) and mixed end-over-end into BD and Greiner blood collection tubes and processed as described by Kricka et al. [8].

Blood samples were drawn after written informed consent from 20 apparently healthy volunteers (ages 18 and over) by trained phlebotomists, using a butterfly connected to a vacuum tube holder. Blood samples were collected into Greiner and BD collection tubes in a randomized drawing order, and the tubes were filled to capacity. The blood collection tubes were inverted eight times after the blood was drawn to ensure proper mixing of the blood with tube additives. Serum samples from the BD glass tubes were obtained after clotting for 30 min at room temperature followed by centrifugation at 1300 *g* for 10 min. Following centrifugation, all tubes were inspected visually for complete barrier formation (except the glass and PRT tubes), fibrin, and hemolysis. All serum samples were processed within 2 h of blood collection. The serum drawn in Vacuette and BD SST and RST tubes remained on the separator gel. In contrast, the serum drawn in the BD glass and PRT tubes was transferred into 13 × 75 mm plastic test tubes in order to minimize the metabolism of the serum analytes by cellular elements in the blood tube because these two tube types have no separator gel. These samples were capped at room temperature if they were tested within 4 h. Alternatively, they were stored between testing intervals at 4 °C for up to 7 days. TT_3, TT_4, and cortisol were shown in our laboratory to be stable for 7 days at 4 °C in the different blood collection tube (data not shown). The volunteers were contacted if critical values (based on the clinical laboratory critical values list) were obtained from specimens collected from either the Greiner or the BD plastic tubes. This study was approved by an institutional review board of the National Institute of Diabetes and Digestive and Kidney Disease.

Clinical laboratory analysis

1 Determination of QC TT_3,TT_4, and cortisol concentrations.
Total thyroxine and triiodothyronine and cortisol levels in QC specimens poured into the five different types of blood collection tube were measured in random order on an Immulite™ 1000 analyzer, according to the manufacturer's instructions (n = 18) [2,3]. Multiple reagent and calibrator lots were used for the Immulite™ 1000 analyzer, but the data represent a single lot.
2 Routine chemistry analytes.
A routine chemistry panel (as shown in Supplemental Data Figs. 4–8) was performed on QC materials poured into the five tube types on a RxL™ analyzer (n = 4). The QC specimens were analyzed singly in random order and in the same analytical run.

Table 1
Sources and characteristics of the blood collection tubes examined in this study.

Tube	Catalog number	Lot number	Tube dimensions (mm)	Draw volume (mL)	Wall material	Separator gel	Surfactant	Clot activator	Stopper lubricant	Anticoagulant
Glass[a] (red-top)	366441	2219385	16 × 100	10.0	Glass (borosilicate)	None	None	None	Glycerin	None
Vacuette[b] (gold-top)	454228	B091209	13 × 75	4.0	Plastic (PET)	Olefin oligomer[b] (white-opaque)	Unknown	Silica	Silicone	None
Plain red-top[a] (red-top)	367814	2200653	13 × 100	5.0	Plastic (PET)	None	Unknown	Silica	Silicone	None
Rapid serum tube[c,d] (orange-top)	368774	120804	13 × 100	5.0	Plastic (PET)	Polymer gel[c]	Polyalkylene oxide modified poly-dimethylsiloxane[c]	Thrombin[c,d]	Unknown	None
SST[a] (gold-top)	367983	2258708	13 × 75	3.5	Plastic (PET)	Polymer gel[e,f] (yellow opaque)	Silwet L-720[g]	Silica	Silicone	None

PET, polyethylene terephthalate.
[a] From BD [11].
[b] Greiner Bio-One [23].
[c] From Dubrowny and Harrop [12].
[d] From http://www.bd.com/vacutainer/labnotes/Volume20Number1/serum_tube.asp (accessed January 23, 2013) [24].
[e] From Landt et al. [25].
[f] From Bush et al. [26].
[g] FDA enforcement report [27].

Removal of blood collection tube coatings

To determine whether immunoassay analytes in the QC material measured on the Immulite™ 1000 analyzer were affected by the clot activator, internal tube coating, or lubricant from the rubber stopper, we followed our previously described procedure [2].

Statistical analysis

Serum TT_3 levels were chosen for the sample size calculation for *t*-test because, they are known to be greatly affected by changes in the constituents of the interior surface of the tube [2,3], which caused clinically significant errors. The sample size needed to achieve an 80% power to detect a clinically significant difference in TT_3 levels among tube types (beta = 0.20; $Z_\beta = 0.84$) with a significance level (2-sided) (alpha = 0.01; $Z_\alpha = 2.58$) [alpha = 0.01 is derived from (alpha = 0.05 ÷ 5 tube means compared; Bonferroni adjustment for multiple mean comparisons)] is given by $n = (2.58 + 0.84)^2 \times 2 \times (0.18\ \text{nmol/L})^2 \div (0.54\ \text{nmol/L})^{2=} = 1.40$ (~2) specimens per tube type, where 0.18 nmol/L is the SD of TT_3 from the usual SD of QC material at a mean TT_3 concentration of 1.20 nmol/L determined in our laboratory over a six-month period, and 0.54 nmol/L is the clinically significant difference in TT_3 results based on analytical and biological variation [2,9,10]

For the removal of tube coating experiments, the means of triplicate results for TT_3, TT_4, and cortisol measurements were used for statistical analysis. For all other experiments, the analytes were analyzed in singleton. The results were reported as the mean (SD). A two-tailed Student *t*-test was used to compare the results of all measured immunoassay analyte concentrations obtained from the different plastic collection tubes compared to glass tubes [2,3]. A repeated-measures ANOVA was used to compare unadulterated and cleaned tubes TT_3, TT_4, and cortisol concentrations and for the QC material chemistry analytes among the tube types.

All P values were adjusted using the Bonferroni correction for the multiple comparisons inherent in the pairwise testing procedures using Analyze-It™ for Microsoft Excel (version 1.71; Analyze-It Software) software and significant change limits were calculated as described by Boyanton and Blick [10]. QC analyte concentrations for the different blood collection tubes that exceeded their respective significant change limits were considered to be clinically significant. The bias for TT_3, TT_4, and cortisol in the Vacuette, PRT, RST, and SST tubes were compared with the current desirable quality specifications for bias derived from biological variation as described by Wang et al. [6].

Results

Tube comparisons (versus glass tubes) with QC material for TT_3, TT_4, and cortisol concentrations

Compared to the glass tubes, there were no significant differences in TT_3 and TT_4 concentrations from the Vacuette tubes across the three levels of QC material (Tables 2 and 3). In contrast, the TT_3 and TT_4 concentrations in PRT, RST, and SST tubes were all significantly higher, (~7.8% to 24.9%) than those in the glass tubes across the three levels of QC material (Tables 2 and 3). Compared to the glass tubes, the maximum desirable bias for TT_3 (4.8%) and TT_4 (3.0%) in the QC material was exceeded by the three QC levels in the PRT, RST, and SST tubes (Tables 2 and 3). The maximum desirable bias for TT_4 in the QC material was also exceeded in the Vacuette tubes for QC level 2. The higher TT_3 and TT_4 concentrations in the SST tubes compared to glass tubes exceeded the significant change limit for QC levels 2 and 3 for TT_3 (Table 2) and QC level 3 for TT_4 (Table 3).

Compared to the glass tubes, Vacuette had significantly higher (~9.2%) cortisol concentrations for QC material level 1, but not for QC material levels 2 and 3 (Table 4). The comparison of the PRT tubes to the glass tubes revealed that the cortisol concentrations were significantly higher (~12.2%) in level 1, but not in levels 2 and 3 of the QC material (Table 4). Both RST and SST tubes had significantly higher cortisol concentrations compared to the glass tubes across the three QC levels (Table 4). The maximum desirable bias for cortisol (~12.5%) was exceeded only in the RST and SST tubes for QC level 1 (Table 4). None of the tube types exceeded the significant change limit for cortisol concentrations among the three levels of QC material (Table 4).

Effect of blood collection tube components on QC material TT_3, TT_4, and cortisol concentrations

To determine whether TT_3, TT_4, and cortisol concentrations in QC materials were affected by the clot activator, internal tube coating, or lubricant on the stopper, we poured QC materials into unadulterated tubes and into tubes that were cleansed with a gauze sponge to remove coating in the tube and on the rubber stopper. No significant difference in TT_3 concentrations was observed for unadulterated and cleaned glass

Table 2
Comparison of TT_3 (nmol/L) concentrations from QC material processed in glass, Vacuette™, PRT, RST™, and SST™ tubes and measured on the Immulite™ 1000 analyzer.

Range of assay imprecision (CV%)[a]: 8.1–13.0%								
	Tube type							
	Glass	Vacuette™	PRT	RST™	SST™	USD	SCL[d]	Desirable bias (%)[e]
QC level 1 (N = 18)								
Mean (SD)	1.21 (0.16)	1.22 (0.11)	1.33 (0.15)	1.49 (0.16)	1.51 (0.14)	0.18	0.89–1.59	
Absolute difference (%)[b]		0.01 (1.1)	0.12 (10.7)[g]	0.28 (23.2)[g]	0.30 (24.9)[g]			4.8
P[c] (vs. glass tubes)		0.781	**0.01**	<**0.0001**	<**0.0001**			
QC level 2 (N = 18)								
Mean (SD)	2.45 (0.15)	2.45 (0.12)	2.64 (0.13)	2.75 (0.26)	2.89 (0.14)[f]	0.14	2.04–2.80	
Absolute difference, %		0.00 (0.0)	0.19 (7.8)[g]	0.30 (12.2)[g]	0.44 (18.0)[g]			4.8
P[c] (vs. glass tubes)		0.848	**0.0004**	**0.0002**	**0.0001**			
QC Level 3(N = 18)								
Mean (SD)	3.99 (0.30)	4.09 (0.24)	4.53 (0.18)	4.69 (0.42)	4.81 (0.32)[f]	0.24	3.46–4.78	
Absolute difference, %		0.10 (2.5)	0.54 (13.5)[g]	0.70 (17.5)[g]	0.82 (20.6)[g]			4.8
P[c] (vs. glass tubes)		0.276	<**0.0001**	<**0.0001**	<**0.0001**			

P < 0.01 is considered statistically significant and indicated in bold. USD, usual standard deviation from QC data over a six-month period; and SCL, significant change limit.
[a] Inter-assay imprecision across three levels of control materials.
[b] Absolute difference and % change from glass tubes.
[c] Probability Student paired *t*-test (two-sided) for mean difference between Vacuette™, PRT, RST™, and SST™ compared to glass tubes.
[d] Mean of quality material from plastic sample cup ±2.8 USD.
[e] Maximum desirable bias (%) based on biological variation [6].
[f] Exceeded SCL.
[g] Exceeded maximum desirable bias.

Table 3
Comparison of TT_4 (nmol/L) concentrations from QC material processed in glass, Vacuette™, PRT, RST™, and SST™ tubes and measured on the Immulite ™1000 analyzer.

Range of assay imprecision (CV%)[a]: 5.0–7.8%								
	Tube type							
	Glass	Vacuette™	PRT	RST™	SST™	USD	SCL[d]	Desirable bias (%)[e]
QC level 1 (N = 18)								
Mean (SD)	105 (9.0)	108 (6.4)	115 (14.2)	117 (6.8)	117 (11.6)	7.3	83–124	
Absolute difference (%)[b]		3.0 (2.9)	10.0 (9.5)[g]	12.0 (11.4)[g]	12.0 (11.4)[g]			3.0
P[c] (vs. glass tubes)		0.227	**0.01**	<**0.0001**	<**0.0007**			
QC level 2 (N = 18)								
Mean (SD)	139 (9.0)	143 (14.2)	150 (6.4)	160 (10.3)	163 (12.9)	10.3	111–168	
Absolute difference, %		4.0 (2.9)[#]	11.0 (7.9)[g]	21.0 (15.1)[g]	24.0(17.3)[g]			3.0
P[c] (vs. glass tubes)		0.242	**0.0002**	<**0.0001**	<**0.0001**			
QC Level 3(N = 18)								
Mean (SD)	194 (12.9)	199 (18.7)	218 (7.6)	220 (17.1)	223 (15.1)[f]	9.1	170–222	
Absolute difference, %		5.0 (2.6)	24.0 (12.4)[g]	26.0 (13.4)[g]	29.0 (14.9)[g]			3.0
P[c] (vs. glass tubes)		0.3633	<**0.0001**	<**0.0001**	<**0.0001**			

P < 0.01 is considered statistically significant and indicated in bold. USD, usual standard deviation from QC data over a six-month period; and SCL, significant change limit.
[a] Inter-assay imprecision across three levels of control materials.
[b] Absolute difference and % change from glass tubes.
[c] Probability Student paired *t*-test (two-sided) for mean difference between Vacuette™, PRT, RST™, and SST™ compared to glass tubes.
[d] Mean of quality material from plastic sample cup ±2.8 USD.
[e] Maximum desirable bias (%) based on biological variation [6].
[f] Exceeded SCL.
[g] Exceeded maximum desirable bias.

and Vacuette tubes across the three levels of QC material (Supplemental Data Fig. 1). Unadulterated PRT tubes were significantly higher than cleaned PRT tubes in TT_3 concentrations for QC levels 2 and 3 (Supplemental Data Fig. 1). The TT_3 concentrations in unadulterated RST tubes were higher than in the cleaned PRT tubes only in QC level 3 materials (Supplemental Data Fig. 1). The TT_3 concentrations were significantly higher in unadulterated SST tubes than in cleaned SST tubes for all three QC levels (Supplemental Data Fig. 1).

Similar to TT_3, no significant differences in TT_4 concentrations were observed in unadulterated and cleaned glass and Vacuette tubes across the three levels of QC material (Supplemental Data Fig. 2). The TT_4 concentrations were significantly higher in unadulterated PRT tubes compared to cleaned PRT tubes for QC levels 1 and 3. In contrast, TT_4 concentrations were higher in unadulterated RST tubes compared to cleaned RST tubes for QC level 2 (Supplemental Data Fig. 2). The TT_4 concentrations were significantly higher in unadulterated SST tubes compared to cleaned SST tubes for QC levels 2 and 3 (Supplemental Data Fig. 2).

No significant differences in cortisol concentrations were observed for unadulterated and cleaned glass tubes for QC levels 1, 2, and 3 (Supplemental Data Fig. 3). Unlike TT_3 and TT_4, the cortisol concentrations were significantly higher in unadulterated Vacuette tubes compared to cleaned Vacuette tubes for QC material level 1 (Supplemental Data Fig. 3). Unadulterated PRT tubes were also significantly higher than cleaned PRT tubes in cortisol concentrations in QC level 1 (Supplemental Data Fig. 3). The cortisol concentrations in unadulterated RST tubes were higher compared to cleaned RST tubes for QC material levels 1 and 3 (Supplemental Data Fig. 3). No significant differences in cortisol concentrations were observed between unadulterated SST tubes and cleaned SST tubes across the three levels of QC material (Supplemental Data Fig. 3).

Tube comparisons of chemistry analytes from QC material

We tested the effect of pouring QC material in glass, Vacuette, PRT, RST, and SST tubes on general chemistry analytes (Supplemental Data

Table 4
Comparison of Cortisol (nmol/L) concentrations from QC material processed in glass, Vacuette™, PRT, RST™, and SST™ tubes and measured on the Immulite™ 1000 analyzer.

Range of assay imprecision (CV%)[a]: 5.1–7.8%								
	Tube type							
	Glass	Vacuette™	PRT	RST™	SST™	USD	SCL[d]	Desirable bias (%)[e]
QC level 1 (N = 18)								
Mean (SD)	131 (8.3)	143 (8.3)	147 (30.4)	158 (16.3)	154 (11.6)	10.2	111–168	
Absolute difference (%)[b]		12.0 (9.2)	16.0 (12.2)[f]	27.0 (20.6)[g]	23.0 (17.6)[g]			12.5
P[c] (vs. glass tubes)		**0.0002**	<**0.0001**	<**0.0001**	**0.0001**			
QC level 2 (N = 18)								
Mean (SD)	635 (44.1)	651 (39.7)	650 (30.4)	707 (42.5)	706 (53.0)	29.5	548–713	
Absolute difference, %		16.0 (2.5)	15.0 (2.4)	72.0 (11.3)	71.0 (11.2)			12.5
P[c] (vs. glass tubes)		0.260	0.234	<**0.0001**	<**0.0001**			
QC level 3(N = 18)								
Mean (SD)	968 (47.2)	995 (56.6)	988 (66.2)	1056 (79.5)	1045 (93.8)	52.7	789–1084	
Absolute difference, %		27.0 (2.8)	20.0 (2.1)	88.0 (9.0)	77.0 (8.0)			12.5
P[c] (vs. glass tubes)		0.130	0.310	<**0.0003**	<**0.0036**			

P < 0.01 is considered statistically significant and indicated in bold. USD, usual standard deviation from QC data over a six-month period; and SCL, significant change limit.
[a] Inter-assay imprecision across three levels of control materials.
[b] Absolute difference and % change from glass tubes.
[c] Probability Student paired *t*-test (two-sided) for mean difference between Vacuette™, PRT, RST™, and SST™ compared to glass tubes.
[d] Mean of quality material from plastic sample cup ±2.8 USD.
[e] Maximum desirable bias (%) based on biological variation [6].
[f] Exceeded SCL.
[g] Exceeded maximum desirable bias.

Tables 1 and 2). The difference among the tubes types was not statistically significant (Supplemental Data Tables 1 and 2). None of the tube types exceeded the significant change limit in the chemistry analytes examined across the two levels of QC material (Supplemental Data Tables 1 and 2).

The bias in chemistry analytes from QC materials processed in Vacuette, PRT, RST, and SST tubes compared to glass tubes exceeded the desirable bias in many chemistry analytes (Supplemental Data Tables 1 and 2). The concentrations of all chemistry analytes examined in QC specimens were similar and not statistically different in the unadulterated and cleaned tubes in every type of collection tube (Supplemental Data Figs. 4–8.).

Tube comparisons with serum specimens from apparently healthy volunteers for TT_3, TT_4, and cortisol

The effects of blood collection tube types on serum TT_3 and cortisol concentrations collected from 20 apparently healthy volunteers are shown in Table 5. No statistically or clinically significant differences in serum TT_3 and cortisol concentrations were found among the five tube types measured in the Immulite™ 1000 analyzer (Table 5). The maximum desirable bias for TT_3 and cortisol concentrations were not exceeded by the serum specimens collected from the healthy volunteers.

For TT_4, compared to glass tubes, statistically significant differences in serum TT_4 concentrations were found in Vacuette™ ($p = 0.0037$) and SST ($p = 0.0001$) tubes (Table 5). However, no clinically significant differences in serum TT_4 concentrations were observed among the tubes types (Table 5). The maximum desirable bias for TT_4 was exceeded for the serum specimens collected in both the Vacuette and SST tubes from the apparently healthy volunteers.

Tube comparisons of chemistry analytes in serum specimens from apparently healthy volunteers

To determine the effect of the blood collection tubes on serum clinical chemistry analyte concentration, we collected blood in the five different tube types from 20 apparently healthy volunteers, and then ran the serum specimens on the Siemens RxL Dimension analyzer for the chemistry assays shown in Supplemental Data Fig. 9. We observed no statistically and clinically significant differences among the tube types for the 14 serum analytes examined.

Discussion

The overall purpose of this study was to compare the effect of QC material poured into different blood collection tubes on a variety of routine clinical laboratory assays. As discussed below, the individual components of blood collection tubes were individually examined for their ability to affect the measurement of TT_3, TT_4, cortisol, and general chemistry analytes. We also discuss how potential changes in QC testing practices can be used to monitor blood collection tube problems.

Tube wall

Based on this study, materials on the wall of the collection tube can be excluded as a possible cause of the significant increase in QC material TT_3 and TT_4 concentrations poured into PRT, RST, and SST tubes, as compared to glass tubes (Tables 2 and 3). The Vacuette, PRT, RST, and SST tubes are made of similar tube-wall material, polyethylene terephthalate (PET), and there was a significant increase only in QC material TT_3 and TT_4 concentrations in the PRT, RST, and SST tubes but not in the Vacuette tubes (Tables 2 and 3). In addition, the cortisol concentrations in the QC material were variable and unlikely because of the tube wall material (Table 4). For example, neither Vacuette nor PRT tubes showed significantly elevated cortisol concentrations when compared to glass tubes across all QC levels. Hence, it is not likely that the tube wall material was responsible for the elevation in these analyte concentrations compared to those in the glass tubes since all the tube types examined were made of PET.

Stoppers

The results of this study showed that the rubber stoppers in the different collection tubes examined are not likely the cause for a significant increase in the QC material TT_3 and TT_4 concentrations (Tables 2 and 3)

Table 5
Comparison of serum TT_3, TT_4, and cortisol concentration from 20 apparently healthy volunteers processed in glass, Vacuette™, PRT, RST™, and SST™ tubes and measured on the Immulite™ 1000 analyzer.

Tube type	Glass	Vacuette™	PRT	RST™	SST™	USD	SCL[d]	Desirable bias (%)[e]
TT_3 (nmol/L)								
Range of assay imprecision (CV%)[a]: 6.36–13.5								
Mean (SD)	1.83 (0.35)	1.75 (0.41)	1.79 (0.35)	1.83 (0.45)	1.91 (0.44)	0.15	1.41–2.25	
Absolute difference (%)[b]		−0.08 (−4.37)	−0.04 (−2.19)	0.00 (0.0)	0.08 (4.37)			4.8
P[c] (vs. Glass tubes)		0.02	0.262	0.889	0.154			
TT_4 (nmol/L)								
Range of assay imprecision (CV%)[a]: 5.0–7.0								
Mean (SD)	120.8 (18.0)[f]	126.1 (20.6)	122.7 (18.0)	119.7 (18.0)	126.6 (18.0)	6.82	101.8–139.9	
Absolute difference (%)[b]		**5.30** (**4.39**)[g]	1.90 (1.57)	−1.10 (−0.91)	**5.80** (**4.80**)[g]			3.0
P[c] (vs. glass tubes)		**0.0037**	0.342	0.456	**0.0001**			
Cortisol (nmol/L)								
Range of assay imprecision (CV%)[a]: 4.2–7.9								
Mean (SD)	303.6(74.5)	312.4 (85.6)	310.2 (77.3)	311.1 (88.3)	320.2 (80.0)	43.6	181.9–425.9	
Absolute difference (%)[b]		8.80 (2.89)	6.60 (2.17)	7.50 (2.47)	16.6 (5.47)			12.5
P[c] (vs. glass tubes)		0.563	0.624	0.733	0.145			

P < 0.01 is considered statistically significant and indicated in bold. USD, usual standard deviation from QC data over a six-month period; and SCL, significant change limit.
[a] Inter-assay imprecision across three levels of control materials.
[b] Absolute difference and % change from glass tubes.
[c] Probability Student paired *t*-test (two-sided) for mean difference between Vacuette™, PRT, RST™, and SST™ compared to glass tubes.
[d] Mean of quality material from plastic sample cup ±2.8 USD.
[e] Maximum desirable bias (%) based on biological variation [6].
[f] Exceeded SCL.
[g] Exceeded maximum desirable bias.

because the rubber stoppers in the BD glass tubes were presumably made of the same material as that of the PRT, RST, and SST tubes [11]. Moreover, although the rubber stoppers in the PRT tubes were presumably made of the same material as those in the RST and SST tubes, the PRT tubes showed significantly elevated cortisol concentrations only in the level 1 QC material (Table 4). Furthermore, incubation of rubber stoppers from the different blood-collection tubes for 30 min in level 1 and 2 QC materials did not significantly alter the TT_3, TT_4, and cortisol concentrations (data not shown), which is consistent with our previous publications [2,3]. Thus, compared to glass tubes, the rubber stopper can be excluded as the cause of the significantly higher concentrations of TT_3, TT_4, and cortisol in the QC material in the different tube types.

Clot activators

Blood collected in evacuated tubes often must be clotted prior to clinical examination. To achieve this, blood collection tubes often employ a contact (e.g. inorganic silicates) or biochemical (e.g. thrombin) clot activators with polyvinylpyrrolidone as a carrier [12–15]. The clot activators and/or polyvinylpyrrolidone used in the BD tubes can be excluded as a potential cause of higher QC material TT_3 and TT_4 concentrations in the PRT, RST, and SST tubes compared to those in glass tubes, because the PRT and SST tubes use inorganic silicates and the RST tubes use thrombin as clot activators (Table 1). Moreover, the Vacuette tubes also use inorganic silicates clot activators presumably the same as PRT and SST tubes, but there was no significant difference compared to the glass tubes in concentrations of TT_3 and TT_4 (except level 2) in the QC material (Tables 2 and 3). There was a significantly higher cortisol concentration in the QC material compared to that found in the glass tubes, particularly in the RST and SST tubes, although the clot activators are different in these two tubes (Table 4). Therefore, the results showed that the clot activators in the blood collection tubes most likely did not cause the higher concentrations of TT_3, TT_4, and cortisol in the QC material observed in the different tube types.

Separator gels

The separator gel contains viscous liquid, fillers, and/or tackifiers [16–18]. The main difference among the Vacuette, RST, and SST tubes examined in this study is the kind of separator gel used in them. The separator gel used in Vacuette tubes is an unhalogenated olefin oligomer [19], whereas the RST and SST tubes use a polymer gel (Table 1) [12]. The separator gel in the RST tube is composed of a different polymeric material than that in the SST tube (Table 1) [11]. The glass and PRT tubes used in this study do not use separator gel. It is conceivable that the higher TT_3 and TT_4 concentrations of QC material poured into the RST and SST tubes, compared to the Vacuette tubes, might be caused by the release or increased release of liquid polymer, inorganic filler, or tackifier from the separator gel of RST and SST tubes, but not Vacuette tubes. The release of separator gel component(s) may interfere with TT_3 and TT_4 assays. However, increases in TT_3 and TT_4 concentrations in the QC material were observed in the PRT, RST, and SST tubes, although the PRT tubes had no separator gel. The cortisol concentrations in the QC material were variable and unlikely to have been caused by the separator gel because, in addition to the RST and SST tubes having separator gels, the PRT tubes without separator gel also produced a significant increase in cortisol concentrations, compared to those found in glass tubes. It thus appears unlikely that the separator gel caused statistically and/or clinically significant increases in the concentrations of TT_3, TT_4, and cortisol in the QC material poured into the different blood collection tubes.

Surfactants

We previously identified a silicone surfactant, Silwet L-720, in BD plastic serum-collection tubes that was associated with falsely elevated concentrations of some analytes, most notably TT_3, when measured on the Immulite 2000 and 2500 analyzers [2,3]. In the present study, the variable increases in TT_3, TT_4, and cortisol concentrations in QC material among the tube types, particularly PRT, RST, and SST, were likely caused by surfactant interference. There were some relationships among certain tube types with and without Silwet L-720 and measured concentrations of TT_3, TT_4, and cortisol in the QC material (Tables 2–4). The Silwet L-720 causes the desorption from and/or possible denaturation of antibodies on the polystyrene beads in the Immulite TT_3 assay [3]. The same mechanism of action by the Silwet L-720 surfactant on the Immulite TT_4 and cortisol assays might account for the variable increase in the concentrations of TT_3, TT_4, and cortisol in the QC material among the PRT, RST, and SST tubes, compared to glass tubes in this study [2,3]. However, tube additives other than the silicone used to coat the interior of blood collection tubes (i.e., polyvinylpyrrolidone, polyethylene oxide, and polyvinyl alcohol) might also be responsible for the significantly elevated TT_3, TT_4, and cortisol concentrations in QC material among the different tube types when compared to glass tubes, as measured on the Immulite™ 1000 analyzer [2–4]. The removal of the clot activator, internal tube coating, and stopper lubricant did not alter the QC material concentrations of routine chemistry analytes in the blood collection tubes examined (Supplemental Data Figs. 4–8). These results are in agreement with a previous study that found that the silicone surfactant, Silwet L-720, did not significantly alter the concentrations of routine chemistry analytes [2,3].

The significantly higher TT_3, TT_4, and cortisol concentrations in the QC material specimens that were poured into the Vacuette, PRT, RST, and SST tubes and compared to the glass tubes measured on the Immulite™ 1000 analyzer might also be dependent not only on the presence of silicone but also on its quantity in the collection tubes. This point is supported by the observation that 2 mL of QC exposed to different tubes with varying volumes and presumably different amounts of surfactants had variable TT_3, TT_4, and cortisol concentrations compared to the glass tubes with no internal tube coating. It is also conceivable that during production, not all collection tubes were coated with a homogeneous layer of the silicone coating. Hence, this possible variation in the quantity of silicone in the collection tubes might explain the differences among the tube types in the concentrations of TT_3, TT_4, cortisol, and clinical chemistry analytes in the QC material. The exact mechanism of the interference caused by the components of the blood collection tubes in different assays is not very clear. Further studies are warranted to elucidate the exact mechanism by which tube components interfere with different chemistry assays.

Tube-related alterations in TT_3, TT_4, and cortisol concentrations were observed in serum specimens collected from apparently healthy volunteers (Table 5). Yet, there were no statistically and clinically significant differences in serum TT_3 and cortisol concentrations among tube types from apparently healthy volunteers (Table 5). Compared to glass tubes, TT_4 concentrations were statistically and clinically significant for Vacuette and SST tubes (Table 5). The reason for the differences in findings between TT_3 and cortisol and TT_4 among the tube types is not known and further work is needed to fully understand the immunoassay analyte and tube type effects. No statistically and clinically significant differences among tube types were observed for the 14 general chemistry analytes (Supplemental Data Fig. 9). This is consistent with previous studies showing that these five tube types did not significantly alter chemistry test results [2].

The discordance between QC material and specimens from apparently healthy volunteers in TT_3, TT_4, and cortisol concentrations among tube types may be attributable to matrix effects. The QC material used in this study is serum-based, whereas the specimens from apparently healthy adults are serum isolated from whole blood specimens. The cellular material in the whole blood specimens from volunteers may adsorb some of the tube additives, particularly surfactants and/or clot activators, and, therefore, decrease its concentration in the serum layer, producing possibly less interference with components of the

immunoassays studied. It is also conceivable that the higher volumes of whole blood from volunteers collected in the tubes (from 3.5 mL to 10 mL per tube) compared to QC specimens (2 mL per tube) may have diluted out the interferent(s), resulting in minimal alterations in the TT_3, TT_4, and cortisol concentrations. These findings suggests that the effect of tube additives on TT_3, TT_4, and cortisol assays may be greater if specimens from volunteers were partially compared to completely filled to their designated tube volumes with blood. Furthermore, the additives used in commercially available blood collection tubes are likely titrated for whole blood rather than QC specimens. Because QC materials are made from artificial sources that are different from authentic clinical samples, it is not uncommon that these two specimens may give different test results and interpretations as seen in this study. However, even with the differences in matrix between QC and serum specimens from healthy volunteers, tube-related alterations in immunoassay test results were observed in each specimen type. Future work should determine the concentration of surfactants and other tube components in both the serum and cellular phase of blood specimens. Hence, it appears that tube-related changes in immunoassay analyte concentrations of QC materials and serum specimens from apparently healthy volunteers are affected by multiple processes and it is unpredictable in magnitude for any particular blood collection tube type and assay combination. Thus, reinforcing the importance of having tube additives that are truly inert to clinical assays.

Study limitations

The present study has some limitations. First, this study examined only TT_3, TT_4, cortisol and a panel consisting of 14 routine chemistry analyte concentrations in QC materials. Therefore, many immunology and chemistry analytes performed in a typical clinical chemistry laboratory were not examined in this study. Thus, the effects on QC material for these other analytes poured into different blood collection tubes are unknown. Second, the TT_3, TT_4, and cortisol concentrations processed in different blood collection tubes were examined only on the Immulite 1000 platform. The general chemistry analytes were examined on the RxL platform. The effects of QC material processed in different blood collection tubes and examined on other immunoassay and chemistry platforms are also unknown. Third, we examined five types of serum tubes that are commonly used by clinical laboratories in North America; however, we did not examine other commercially available tube types made by different manufacturers. Fourth, QC specimens are typically noncommutable with native patient specimens because the QC specimen matrix is usually altered by manufacturing processes from that of native patient specimens [20]. Hence, the variation in the matrix of QC material due to blood collection tube constituents as demonstrated in this study may not exactly mimic patient specimens. Nonetheless, pouring QC material into blood collection tubes may be good for showing collection tube additive interference on test results. If the QC material analyte concentrations are not significantly altered by different tube additives, the blood collection tube may be considered acceptable to use for patient testing. However, if the QC material analyte concentrations are significantly different among tube types used in the clinical laboratory, this should prompt a further investigation by laboratorians as to the root cause of the tube-related differences in QC material analyte concentrations and action should be taken to fix this problem. This strategy will have a significant impact on the laboratory resources and costs, so it may be more appropriate to test blood collection tubes on a lot-by-lot basis or with each new shipment of tubes.

It is noteworthy that the participation in external quality assurance programs would not reveal the tube-related problems described in this study. Proficiency samples received by the clinical laboratory for evaluation are contained in the same type of sealed vials or tubes as QC specimens; therefore, any effect of variations in the components of the collection tubes on the analyte(s) test result(s) would have been excluded from proficiency testing as shown in Supplemental Data Table 3. This is of particular importance for clinical laboratories that receive serum or plasma specimens collected in tubes that are made by different manufacturers. Routine monitoring of moving averages based on patient data may be potentially useful for identifying future tube-related problems, but this may be difficult to track if a wide variety of tubes from different phlebotomy locations are used [21,22]. Furthermore, the routine evaluation of blood collection tubes by clinical laboratories should be incorporated into QC plans based on risk management to help prevent or detect tube-related errors and enhance the quality of test results [28].

Conclusions

Although it is a potentially important source of pre-analytical errors, the detection and prevention of interference from additives in blood collection tubes is a challenging problem for most clinical laboratories. The findings of this study demonstrate statistically and/or clinically significant blood collection tube-related alterations in the TT_3, TT_4, and cortisol concentrations of QC specimens. The data also show that the alterations in analyte concentrations in QC specimens from blood collection tubes are manufacturer-dependent. Other immunoassays from different manufacturers that use a similar approach of adsorbing antibodies to the solid phase may also be affected by blood collection tube components, such as surfactants. These findings have important implications for clinical laboratories, demonstrating that QC materials should routinely be poured into blood collection tubes in current use by laboratories before performing test analysis. This strategy should reveal any adverse effects caused by blood collection tube components. The results of this study also show the importance of producing blood collection tubes that contain additives that do not adversely affect clinical laboratory testing. Blood collection tube-related interferences observed in this study may potentially influence patient outcomes, decrease laboratory efficiency, delay test results, and increase cost per test due to recollection and retesting. Thus, optimization and standardization of blood collection tubes are crucial for the reliable test analysis. All stakeholders should increase their vigilance regarding the effect of blood collection tube components on laboratory assays, in addition to working together to prevent and minimize these problems.

Acknowledgments

The authors would like to thank Dr. Glen L. Hortin for his helpful comments of the manuscript. We also wish to thank Ms. Krista Tanquary for reviewing the parts of the manuscript prior to submission and Mr. Phil Cheng for the material management. Research by one of the authors (AR) was supported by intramural research funds from NIH Clinical Center.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clinbiochem.2013.11.003.

References

[1] Lippi G, Becan-McBride K, Behúlová D, Bowen RAR, Church S, Delanghe J, et al. Preanalytical quality improvement: in quality we trust. Clin Chem Lab Med 2012;16:1–13.
[2] Bowen RAR, Chan Y, Cohen J, Rehak NN, Hortin GL, G. Csako, et al. Effect of blood collection tubes on total triidothyronine and other laboratory assay. Clin Chem 2005;51:424–33.
[3] Bowen RAR, Chan Y, Ruddel ME, Hortin GL, Csako G, Demosky SJ, et al. Immunoassay interference by a commonly used blood collection tube additive, the organosilicone surfactant silwet L-720. Clin Chem 2005;51:1874–82.
[4] Bowen RAR, Vu C, Remaley AT, Hortin GL, Csako G. Differential effect of blood collection tubes on total free fatty acids (FFA) and total triiodothyronine (TT3) concentration: a model for studying interference from tube constituents. Clin Chim Acta 2007;378:181–93.

[5] Stankovic AK, Parmar G. Assay interferences from blood collection tubes: a cautionary note. Clin Chem 2006;52:1627–8.
[6] Wang S, Ho V, Roquemore-Goins A, Smith FA. Effects of blood collection tubes, including pediatric devices, on 16 common immunoassays. Clin Chem 2006;52:892–3.
[7] Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Picheth G, Guidi GC. Pre-analytical management: serum vacuum tubes validation for routine clinical chemistry. Biochem Med 2012;22:180–6.
[8] Kricka LJ, Park JY, Senior MB, Fontanilla R. Processing controls in blood collection tubes reveals interference. Clin Chem 2005;51:2422–3.
[9] Lachin JM. Introduction to sample size determination and power analysis for clinical trials. Control Clin Trials 1981;2:93–113.
[10] Boyanton Jr BL, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem 2002;48:2242–7.
[11] Becton Dickinson website. www.bd.com. [Accessed April 2013].
[12] Dubrowny NE, Harrop AJ. "Collection device", U.S. Patent No 6,686,204, February 2004.
[13] Anraku H, Shoji Y, inventors. Vacuum blood collection tubes. U.S. Patent No. 8,565,334, August 1989.
[14] Ali K. "Methods for improving uniformity of silica films on substrates", U.S. Patent No. 4,420,517, May 1982.
[15] Kessler SB. "Method for collection blood", U.S. Patent No. 4,153,739, June 1977.
[16] Bush VJ, Janu MR, Bathur F, Wells A, Dasgupta A. Comparison of BD Vacutainer SST plus tubes and BD SST II plus tubes for common analytes. Clin Chem 2001;306:139–43.
[17] Lin FC, Cohen R, Losada R, Bush V. Cellular sedimentation and barrier formation under centrifugal force in blood collection tubes. Lab Med 2001;32:588–92.
[18] Gigliello JF, Kragle HA. "Method and apparatus for multiphase fluid collection and separation", U.S. Patent No. 3,920,549, November 1975.
[19] Karppi J, Akerman KK, Parviainen M. Suitability of collection tubes with separator gel for collecting and storing blood samples for therapeutic drug monitoring. Clin Chem Lab Med 2000;38:313–20.
[20] Miller WG. Specimen materials, target values and commutability for external quality assessment (proficiency testing) schemes. Clin Chim Acta 2003;327:25–7.
[21] Cembrowski GS. Thoughts on quality-control systems: a laboratorian's perspective. Clin Chem 1997;43:886–92.
[22] Kazmierczak SC. Laboratory quality control: using patient data to assess analytical performance. Clin Chem Lab Med 2003;41:617–27.
[23] http://www.vacuette.com/documents/980102_Handhabungsempfehlunge10_e_small.pdf. [accessed January 20, 2013].
[24] http://www.bd.com/vacutainer/labnotes/Volume20Number1/serum_tube.asp. [accessed January 23, 2013].
[25] Landt M, Smith CH, Hortin GL. Evaluation of evacuated blood-collection tubes: effects of three types of polymeric separators on therapeutic drug-monitoring specimens. Clin Chem 1993;39:1712–7.
[26] Bush VJ, Leonard L, Szamosi DI. Advancements in blood collection devices. Lab Med 1998;29:616–22.
[27] FDA enforcement report. http://www.fda.gov./bbs/topics/ENFORCE/ENF00484.html. [accessed February and March 2005].
[28] Nichols JH. Laboratory quality control based on risk management. Ann Saudi Med 2011;31:223–8.