

Effect of Blood Collection Tubes on Total Triiodothyronine and Other Laboratory Assays

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Background: Increased total triiodothyronine (TT₃) assay results in apparently euthyroid patients triggered an investigation of the effect of blood collection tubes on serum TT₃ and other laboratory assays.

Methods: We examined potential assay interference for three types of tubes: plastic Greiner Bio-One™ Vacuette™; glass Becton Dickinson (BD) Vacutainer™; and plastic BD Vacutainer SST™ tubes. Serum samples from apparently healthy volunteers (age range, 30–60 years; 15 males and 34 females) were collected in different tube types and analyzed in 17 immunoassays (n = 49), 30 clinical chemistry tests (n = 20), and 33 immunology assays (n = 15). Tube effects were also examined by adding pooled serum to different tube types.

Results: TT₃ values, when measured by the IMMULITE™ 2000 but not the AxSYM™ analyzer, were significantly higher ($P < 0.0001$) for SST (2.81 nmol/L) than either glass (2.15 nmol/L) or Vacuette (2.24 nmol/L) tubes. The effect was large enough to substantially shift the distribution of patient values, increasing the percentage of values above the reference interval from 11.3% to 35.8%. The degree of interference from SST tubes on TT₃ differed among various tube lots and could be attributed to a tube additive shared by other plastic tubes. Results from several other tests statistically differed among tube types, but differences were not considered to be clinically significant.

Conclusions: Assay interferences from blood collection tubes represent challenges to clinical laboratories because they are not detected by the usual quality-control or proficiency testing programs. Laboratories can, how-

ever, address this problem by monitoring distribution of patients' results.

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Over the past two decades, there have been substantial changes in the tubes that are used for collection of blood for most laboratory tests. Two of the most obvious changes that have been widely adopted are (a) the use of serum separator tubes, which include a gel that serves as a barrier between serum and the clot containing cellular elements, and (b) substitution of plastic for glass as the primary tube component. These changes in tubes provided several practical operational advantages, such as reduced centrifugation time, ability to use primary collection tubes for testing, increased sample stability in collection tubes, decreased breakage hazard, decreased weight, and suitability for disposal by incineration (1–10). These practical advantages generally are achieved without affecting the quality of laboratory results. Plastic blood collection tubes have been widely shown to be suitable for routine clinical chemistry analytes, hormone analysis, and therapeutic drug monitoring (1, 2, 5–8).

Blood collection tubes, however, represent much more complex devices than is commonly appreciated by laboratory workers. There are multiple components (Table 1) that may influence clot formation, cause interaction with the tube and stopper surface, and either shed materials into the samples or adsorb components from the sample. Previous studies have suggested that serum separator tubes may have small analytical effects on many assays, but apart from the problem with therapeutic drugs, none of these effects was considered to be clinically significant (1, 3, 5–13). Mass spectrometry recently has shown that many types of tubes shed polymeric materials into samples and that these may interfere with some types of analyses, such as protein profiling (14). In the present study, evidence of a substantial effect of blood collection tubes on serum total triiodothyronine (TT₃) results prompted an investigation of the effects of collection tubes on TT₃ and other laboratory assays.

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Table 1. Characteristics of the blood collection tubes examined in this study for obtaining serum.

	Glass tube ^a	Vacurette tube ^b	SST tube ^c
Draw volume, mL	3.0	4.0	3.5
Separator gel	None	Olefin oligomer	Polymer gel
Internal coating	None	Not Silicone	Silicone
Wall material	Glass (borosilicate)	Plastic (polyethylene terephthalate)	Plastic (polyethylene terephthalate)
Clot activator	None	Silica	Silica
Stopper lubricant	Glycerin	Silicone	Silicone

^a From BD (26).^b From Karppi et al. (7) and Greiner Bio-One (27).^c From Landt et al. (9) and BD (26).

Materials and Methods

SAMPLE COLLECTION

Three types of evacuated blood collection tubes were examined in this study: (a) the VacuretteTM tube (a red and yellow top plastic tube with gel separator; 13 × 75 mm; cat. no. 454067; lots B120307 and B04040L); (b) the glass tube (a red-top glass VacutainerTM no-additive blood tube; 10.25 × 64 mm; cat. no. 366397; lots 3253846 and 3029306); and (c) the SSTTM tube (a gold-top Vacutainer plastic tube with gel separator; 13 × 75 mm; cat. no. 367983; lots 4034512 and 3308587). The glass collection tubes were 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 (Table 1). The first tube type was purchased from Greiner Bio-OneTM, and the latter two tube types were obtained from Becton-Dickinson (BD). The composition and additives for the glass, Vacurette, and SST tubes are shown in Table 1. For serum TT₃ only, plastic red-top (cat. no. 367820; lots 3178911, 4033349, and 3328498) and tiger-top Vacutainer tubes (speckled; cat. no. 367975; lots 4042780, 3251239, and 3211254) from BD were also examined. All blood collection tubes were used before their expiration dates.

Blood samples were drawn after written informed consent from apparently healthy volunteers (age range, 30–60 years; 15 males and 34 females) by trained technologists using a butterfly connected to a vacuum tube holder. Blood samples were collected into glass, Vacurette, and SST collection tubes in a randomized drawing order, and the tubes were filled to capacity. The blood collection tubes were inverted eight times after blood draw to ensure proper mixing of the blood with clot activators. Serum was obtained after clotting for 30 min at room temperature followed by centrifugation at 2000g for 5 min. All samples were processed within 2 h of blood collection. Serum drawn in Vacurette and SST tubes remained on the separator gel, whereas serum drawn in glass tubes was transferred into 13 × 75 mm test tubes. These samples were kept capped at room temperature until testing within 3 h or were stored between testing intervals at 4 °C for up to 2 days. As an alternative approach to test for possible tube interference from plastic

tube additives, pooled serum collected from glass collection tubes was pipetted into each of the three tube types and placed on a roller mixer for 30 min before analysis.

LABORATORY ANALYSIS

Immunoassay analytes. Serum TT₃ concentrations in samples from 49 apparently healthy volunteers were measured in random order after collection in three different tube types on an IMMULITE 2000 analyzer (Diagnostic Products Corporation). Serum samples were analyzed in the same run in duplicate. Three reagent lots and one calibrator lot were used for the IMMULITE 2000 analyzer during the study. The reportable range of the IMMULITE 2000 TT₃ assay is 0.62–9.24 nmol/L. The intra- (n = 21) and interassay (n = 21) CVs for the IMMULITE TT₃ assay were, respectively, 6.7% and 8.1% at 1.36 nmol/L, 9.4% and 15% at 3.19 nmol/L, and 16% and 16% at 5.56 nmol/L. Serum samples from healthy volunteers (n = 23) were also assayed for TT₃ concentrations by an AxSYM automated fluorescent analyzer (Abbott Laboratories). The reportable range of the AxSYM TT₃ assay is 0.46–12.32 nmol/L. The intra- (n = 80) and interassay (n = 80) CVs for the AxSYM TT₃ assay were, respectively, 3.8% and 8.7% at 0.97 nmol/L, 0.7% and 5.5% at 2.40 nmol/L, and 1.2% and 4.2% at 5.82 nmol/L. All assays examined, including TT₃, gave satisfactory internal and external quality-control results during the study.

The same serum samples were also analyzed on an IMMULITE 2000, according to the manufacturer's instructions, for thyroid-stimulating hormone, sex hormone-binding globulin, growth hormone, ferritin, insulin, and β₂-microglobulin by immunometric principles with labeled detection antibodies and binding antibodies immobilized to polystyrene beads. Total and free thyroxine, folate, vitamin B₁₂, cortisol, C-peptide, thyroid-binding globulin, dehydroepiandrosterone sulfate, and testosterone were measured by competitive immunoassays using limited immobilized antibodies and labeled hormones. Serum samples from each volunteer were analyzed singly in the same batch.

Routine chemistry analytes. Routine chemistry analytes were measured in the sera collected in SST and Vacurette tubes from apparently healthy volunteers (n = 20) with a

Synchron LXTM 20 Clinical System analyzer (Beckman Coulter). The serum samples were analyzed singly in random order and in the same analytical run. The methodology and range of assay imprecision obtained with the three quality controls (low, normal, and high concentration) for each analyte examined are shown in Table 1 of the Data Supplement that accompanies the online version of this article at <http://www.clinchem.org/content/vol51/issue2/>. Serum osmolality was determined with a FiskeTM 2400 osmometer (Fiske Associates).

Immunology tests. Serum samples collected in SST and Vacuette tubes from 15 volunteers were tested for plasma proteins, including apolipoproteins A-I and B, albumin, α_1 -antitrypsin, ceruloplasmin, C-reactive protein, high-sensitivity C-reactive protein, rheumatoid factor, haptoglobin, C3 and C4, IgG, IgM, IgA, and IgE on an IMAGETM nephelometer (Beckman-Coulter). Anti-cytomegalovirus IgM and IgG, anti-rubeola IgG, anti-thyroglobulin, anti-thyroid peroxidase, anti-cardiolipin IgG and IgM, anti-cyclic citrullinated peptide, and anti-*Varicella zoster* virus antibodies were assayed with the LabotechTM ELISA test system (Biochem ImmunoSystems). Anti-nuclear, anti-double stranded-DNA, anti-extractable nuclear antigen, and anti-Jo-1 antibodies were assayed by ELISA on the PhDTM System (Helix Diagnostics). Anti-Epstein-Barr virus-viral capsid antigen IgG antibodies were determined by a slide immunostaining method using horseradish peroxidase-conjugated anti-IgG and peroxide for detection. Serum samples from each volunteer were analyzed in random order in the same run in singleton. The methodologies and assay imprecision for the quality-control materials are shown in Table 2 of the online Data Supplement. All immunology assays were from the same reagent and calibrator lots.

STUDIES OF INTERFERENCE FROM TUBE COMPONENTS

To determine whether serum TT₃ measured on the IMMULITE 2000 analyzer is affected by the clot activator, internal tube coating, or the lubricant from the rubber stopper, we used a dry gauze sponge to remove the clot activator, internal coating, and rubber stopper lubricant from each tube type, including plastic red- and tiger-top Vacutainer tubes. The separator gels were not disturbed during the process. A 2-mL aliquot of a serum pool from glass tubes was then added to the unaltered tubes and the cleaned glass, Vacuette, SST, and red- and tiger-top tubes ($n = 5$). Serum from each tube type was mixed on a roller mixer for 30 min at room temperature before analysis to assure contact of the serum with the entire tube surface. To examine the effect of serum contact with rubber stoppers, serum samples from each tube type were exposed to the respective rubber stoppers by inversion of the collection tube for 30 min, and the results were compared with serum samples from the same tube type that were not inverted ($n = 5$). Serum TT₃ concentrations

in these studies were analyzed in the same analytical run in duplicate.

STATISTICAL ANALYSIS

The means of the duplicate results for each TT₃ sample were used for statistical analysis. All other serum analytes from each sample were analyzed in singleton. The results are reported as the mean (SD). Results for all measured analytes obtained from the three different collection tubes were compared by the two-tailed Student *t*-test or Wilcoxon signed-rank test for paired samples and by repeated-measures ANOVA. For TT₃, Passing-Bablok regression analysis was used to compare the results among collection tube types; 95% confidence intervals for the slope and intercept were calculated from the standard error of regression and used to determine concordance with the target values of 1.00 and 0.00, respectively (15). Spearman rank correlation coefficients and Bland-Altman plots were used to assess the difference between TT₃ results obtained with different types of collection tubes (16). All *P* values were adjusted by use of Bonferroni correction for the multiple comparisons inherent in the pairwise testing procedures. Statistical analyses were performed with StatViewTM (Ver. 5.0; SAS Institute Inc.) and Analyze-ItTM for Microsoft Excel (Ver. 1.71; Analyze-It Software) software.

To determine whether statistically significant differences in analyte concentrations among tube types were clinically relevant, we used the significant change limit method, as described by Boyanton and Blick (17). Briefly, the mean for each analyte in the glass or Vacuette tube represented the initial value. The usual SD used for calculating the significant change limit was based on the mean SD of the quality-control data for the previous 6 months for each respective analyte. The quality-control material whose target mean most clearly matched the initial value from either glass or Vacuette tubes for each analyte was used to determine the usual SD. The significant change limit was calculated for each analyte by determining the range (± 2.8 usual SD) from the mean of glass or Vacuette tubes. Serum analyte concentrations from the different collection tubes that exceeded their respective significant change limits were considered to be clinically significant.

Results

APPARENT SHIFT IN PATIENT TT₃ RESULTS

The laboratory was contacted by a clinician in February 2004 about suspected falsely increased TT₃ results for patients who were clinically euthyroid and had otherwise normal thyroid function tests. Repeat analysis of the samples in question yielded similar TT₃ values, and there was no evidence of analytical assay problems, as assessed by an examination of recent quality-control test results, proficiency test results, and calibration curves. Inspection of the distribution of TT₃ results for all patients, however, did reveal an apparent shift (Fig. 1A). A comparison of the

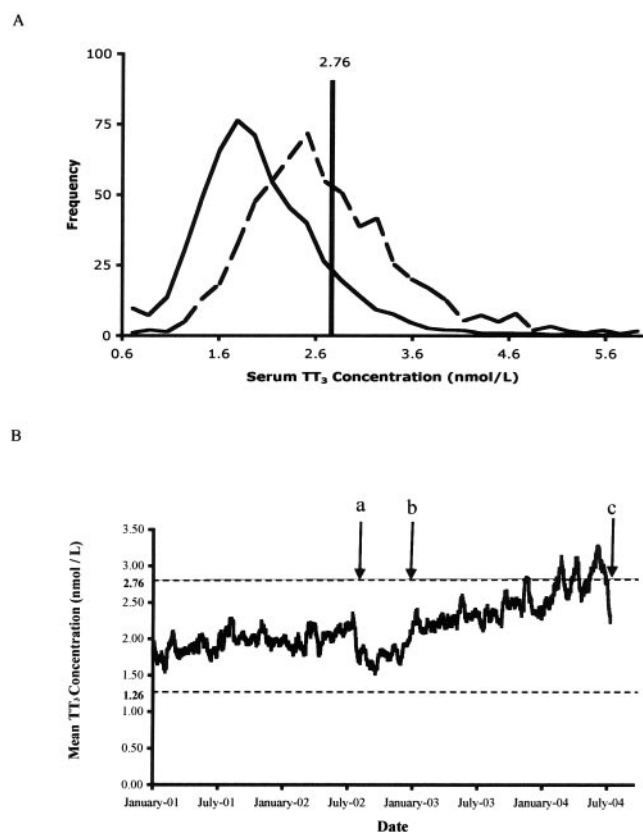


Fig. 1. Distribution of serum TT₃ results measured on an IMMULITE 2000 analyzer in our laboratory from January 2004 to July 2004 (A), and the weekly moving average analysis of serum TT₃ concentrations measured on an IMMULITE 2000 analyzer from January 2001 to July 2004 (B).

(A), the solid line indicates the distribution of serum TT₃ results from January 2001 to December 2003 ($n = 9139$). The dashed line indicates the distribution of TT₃ results from January 2004 to July 2004 ($n = 1855$). The vertical solid line indicates the upper reference interval limit (2.76 nmol/L) for serum TT₃ in our laboratory. (B), $n = 10\,994$. The dashed lines indicate the upper (2.76 nmol/L) and lower (1.26 nmol/L) limits of the reference interval for serum TT₃ in our laboratory. Arrow a indicates the negative bias reported with the IMMULITE 2000 TT₃ assay (17). Arrow b indicates a change in calibration of the IMMULITE 2000 TT₃ assay (December 11, 2002). Arrow c indicates when our laboratory began using Vacuette blood collection tubes for serum TT₃ analysis (July 17, 2004).

results for the period from January 2001 to December 2003 with the results for the period January 2004 to July 2004 indicated that the mean serum TT₃ results shifted by ~33% (Fig. 1A). The percentage of patient results above the reference interval increased from 11.3% (1031 of 9139) to 35.8% (664 of 1855). During this time interval, the same IMMULITE 2000 TT₃ assay and SST collection tubes were used, and there was no obvious change in patient demographics. Inspection of weekly means of patient serum TT₃ results measured on the IMMULITE 2000 analyzer also revealed several apparent shifts in the previous 3-year period (Fig. 1B).

In July 2002, there was a downward shift in serum TT₃ results, which was apparently observed by other users of the IMMULITE 2000 TT₃ assay as well (18) (Fig. 1B, arrow a). In response, the manufacturer reformulated and reca-

librated the assay beginning with lot no. 144 of the L2KT3 reagent set (18), and when the new assay was implemented by our laboratory in December 2002, we observed an upward shift of the serum TT₃ results (Fig. 1B, arrow b). No change in the reference interval of the assay was recommended by the manufacturer during this time period. However, an unexplained gradual upward shift in the serum TT₃ results appeared, starting around January 2004. A reference interval study was performed on apparently healthy volunteers, and the reference interval was changed from 1.26–2.76 nmol/L to 1.54–3.31 nmol/L. The laboratory then began an investigation of the source of the shift in assay values. After identification of assay interference from blood collection tubes in July 2004, the serum TT₃ results shifted back to the previous range after the laboratory switched from plastic SST tubes to Vacuette plastic collection tubes (Fig. 1B, arrow c).

BLOOD COLLECTION TUBE COMPARISON OF SERUM TT₃

To assess the possible contribution of blood collection tubes to problems with the TT₃ assay, serum samples were collected in three tube types: glass tubes (a red-top glass Vacutainer, no-additive blood tube; 10.25 × 64 mm); SST tubes (a gold-top Vacutainer plastic tube with gel separator; 13 × 75 mm); and Greiner Bio-One Vacuette tubes (a red and yellow-top plastic tube with gel separator; 13 × 75 mm). The means of the TT₃ concentrations measured in 49 serum samples from each type of blood collection tube measured on the IMMULITE 2000 analyzer are shown in Table 2. The results of Passing-Bablok regression analysis of serum TT₃ concentrations among tube types are shown in Fig. 2, A, C, and E. The serum TT₃ concentrations obtained with the SST tubes showed a significant positive proportional difference compared with glass and Vacuette tubes (Fig. 2, A and E, respectively). Serum TT₃ concentrations from glass and Vacuette tubes did not demonstrate significant differences (Fig. 2C).

For the Bland-Altman plots of the 49 serum samples (Fig. 2, B, D, and F), the mean (SD) differences 0.66 (0.061) nmol/L for SST vs glass tubes, 0.094 (0.028) nmol/L for Vacuette vs glass tubes, and 0.57 (0.049) nmol/L for SST vs Vacuette tubes, respectively.

EFFECT OF TUBE LOT-TO-LOT VARIATION ON SERUM TT₃ RESULTS

We investigated a defective lot of SST tubes as a possible source of the increase in TT₃ concentrations by comparing the predominant lot of SST tubes in use at the time (lot no. 3308587) with a new lot of SST tubes (lot no. 4034512). We also tested multiple lots of the other tube types. We observed a significant lot-to-lot variation for serum TT₃ concentrations with the SST tubes (lot nos. 3308587 vs 4034512; 2.97 and 2.59 nmol/L, respectively; $n = 10$; $P = 0.003$) but not with the glass collection tubes (lot nos. 3253846 vs 3029306; 2.32 and 2.34 nmol/L, respectively;

Table 2. Comparison of serum analytes in samples obtained from 49 apparently healthy volunteers collected in glass, Vacuette, and SST tubes and measured on an IMMULITE 2000 analyzer.

Analyte	n	CV, %	Mean (SD)			Mean (SD)			Change, absolute (%) ^b	P ^c	USD ^d	SCL ^e
			Glass	SST	Glass	Vacuette	Glass	Vacuette				
Progesterone, nmol/L	49	9.2–9.8	3.37 (8.62)	4.67 (9.54)	3.37 (8.62)	3.34 (8.30)	3.37 (8.62)	3.34 (8.30)	–0.03 (–0.9)	0.446	0.6	1.7–5.1
TT ₃ , nmol/L	49	6.8–8.1	2.15 (0.62)	2.81 (0.90) ^f	2.15 (0.62)	2.24 (0.70)	2.15 (0.62)	2.24 (0.70)	0.09 (–4.2)	0.006	0.1	1.8–2.5
Cortisol, nmol/L	49	11–12	341.70 (142.4)	389.40 (146.8)	341.70 (142.4)	364.90 (144.3)	341.70 (142.4)	364.90 (144.3)	23.2 (6.8)	0.004	17.7	292.1–391.3
TBG, mg/L	49	11–11	20.96 (4.92)	23.57 (5.68)	20.96 (4.92)	23.40 (5.74)	20.96 (4.92)	23.40 (5.74)	2.44 (11.6)	<0.0001	3.5	11.0–30.9
TT ₄ , nmol/L	49	7.6–8.3	102.80 (22.8)	115.10 (22.1)	102.80 (22.8)	105.10 (22.1)	102.80 (22.8)	105.10 (22.1)	2.3 (2.2)	0.113	7.4	82.2–123.4
Insulin, pmol/L	49	4.8–13	57.00 (48.0)	63.80 (48.3)	57.00 (48.0)	62.50 (50.7)	57.00 (48.0)	62.50 (50.7)	5.5 (9.6)	<0.0001	4.4	44.7–69.3
B2M, mg/L	49	8.9–12	1.18 (0.23)	1.27 (0.27)	1.18 (0.23)	1.26 (0.27)	1.18 (0.23)	1.26 (0.27)	0.08 (6.8)	<0.0001	0.1	0.8–1.5
DHEA-SO ₄ , μmol/L	48	9.4–10	3.21 (2.19)	3.43 (2.21)	3.21 (2.19)	3.43 (2.32)	3.21 (2.19)	3.43 (2.32)	0.22 (6.9)	<0.0001	0.4	2.0–4.4
GH, mg/L	49	3.6–4.8	3.26 (5.29)	3.43 (5.44)	3.26 (5.29)	3.38 (5.48)	3.26 (5.29)	3.38 (5.48)	0.12 (3.7)	0.727	0.2	2.6–3.9
Ferritin, μg/L	49	4.6–6.1	52.73 (54.2)	54.28 (55.3)	52.73 (54.2)	53.96 (56.2)	52.73 (54.2)	53.96 (56.2)	1.23 (2.3)	0.035	1.7	47.9–57.5
C-Peptide, nmol/L	48	12–13	0.64 (0.34)	0.65 (0.37)	0.64 (0.34)	0.65 (0.37)	0.64 (0.34)	0.65 (0.37)	0.01 (1.6)	0.586	0.04	0.52–0.76
SHBG, nmol/L	49	4.5–6.0	54.24 (32.0)	54.95 (32.4)	54.24 (32.0)	55.09 (34.0)	54.24 (32.0)	55.09 (34.0)	0.85 (1.6)	0.244	4.5	41.8–66.7
TSH, mIU/L	49	6.6–9.2	1.82 (1.25)	1.84 (1.29)	1.82 (1.25)	1.87 (1.31)	1.82 (1.25)	1.87 (1.31)	0.05 (2.7)	0.013	0.1	1.7–2.0
FT ₄ , pmol/L	49	5.6–12	16.90 (2.45)	17.03 (2.32)	16.90 (2.45)	16.90 (2.58)	16.90 (2.45)	16.90 (2.58)	0.00 (0.0)	0.846	1.2	13.6–20.2
Testosterone, nmol/L	49	8.2–8.5	8.68 (7.64)	8.54 (9.04)	8.68 (7.64)	9.39 (7.85)	8.68 (7.64)	9.39 (7.85)	0.71 (8.2)	0.016	0.6	7.0–10.3
Vitamin B ₁₂ , pmol/L	48	10–13	388.90 (160.8)	374.50 (148.0)	388.90 (160.8)	385.40 (153.9)	388.90 (160.8)	385.40 (153.9)	–3.5 (–0.9)	0.548	40.0	276.9–500.7
Folate, nmol/L	34	11–15	32.43 (9.3)	30.96 (8.7)	32.43 (9.3)	32.19 (9.0)	32.43 (9.3)	32.19 (9.0)	–0.24 (–0.7)	0.438	3.1	23.7–41.1

^a Interassay imprecision across three concentrations of control materials.

^b Values in parentheses indicate percentage change from glass tubes.

^c Probability calculated by use of Wilcoxon signed-rank test. $P < 0.003$ is considered statistically significant and indicated in bold.

^d USD, usual standard deviation; SCL, significant change limit; TBG, thyroid-binding globulin; TT₄, total thyroxine; B2M, β₂-microglobulin; DHEA-SO₄, dihydroepiandrosterone sulfate; GH, growth hormone; SHBG, sex hormone-binding globulin; TSH, thyroid-stimulating hormone; FT₄, free thyroxine.

^e Mean for glass ± 2.8 USD.

^f Exceeded significant change limit.

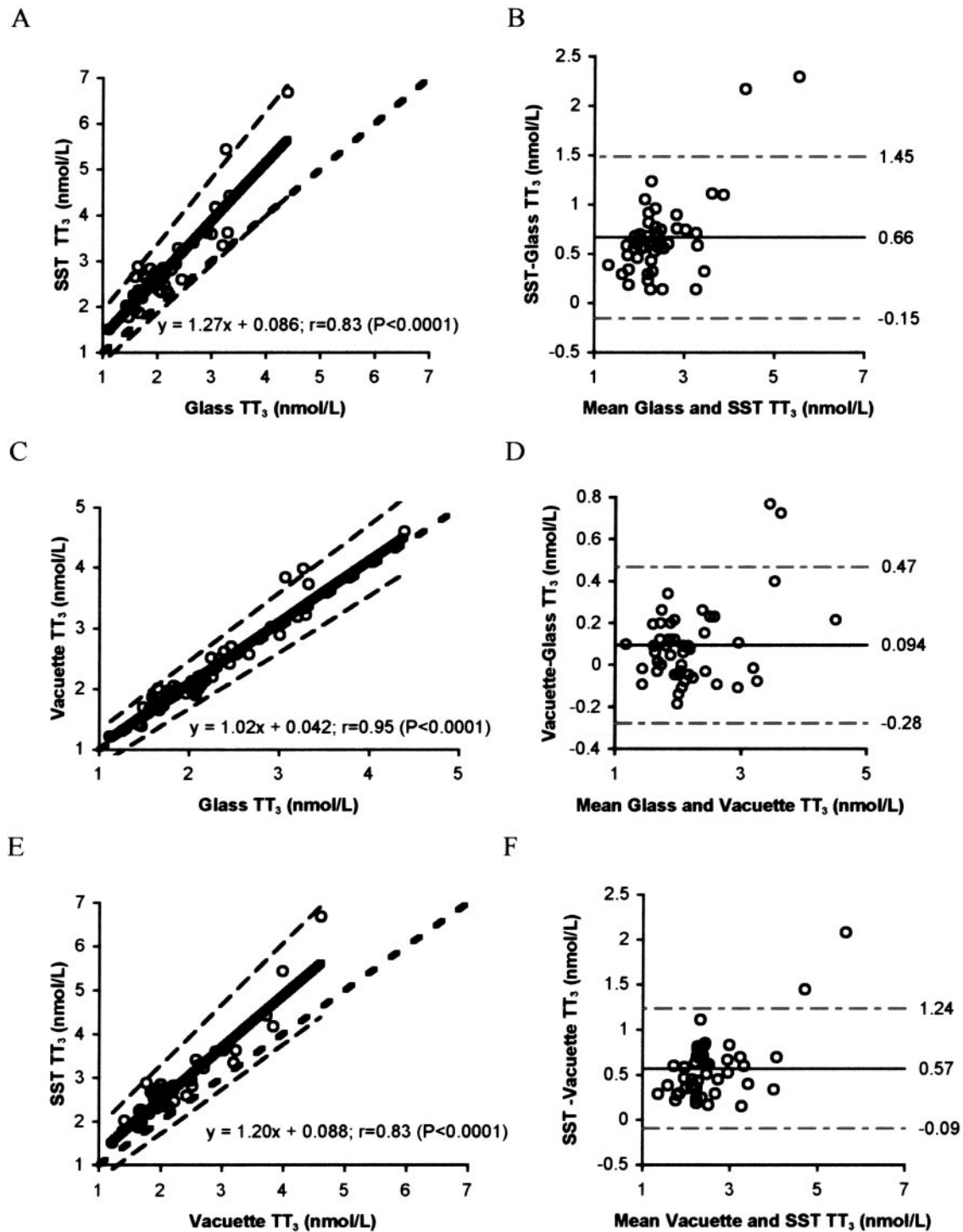


Fig. 2. Comparisons of measured serum TT₃ concentrations in samples collected from 49 apparently healthy volunteers into different blood collection tubes.

(A, C, and E), Passing-Bablok regression scatterplots. *Solid line*, regression line; *dashed lines*, 95% confidence intervals; *bold dashed line*, line of identity. The equation and Spearman rank correlation coefficient for the regression lines are shown. (B, D, and F), Bland-Altman difference plots. The *solid lines* indicate the mean difference among tube types; *dashed lines* indicate the 95% confidence intervals for the differences. Mean (SD) differences for the Bland-Altman plots are 0.66 (0.061) nmol/L (B), 0.094 (0.028) nmol/L (D), and 0.57 (0.049) nmol/L (F).

$n = 10; P = 0.80$) or Vacurette (lot nos. B120307 vs B04040L; 2.33 and 2.34 nmol/L, respectively; $n = 10; P = 0.22$). Although the new lot of SST tubes appeared to yield

lower TT₃ results than the old lot, TT₃ results from serum collected in the new lot of tubes were still higher than those with the other two tube types ($P = 0.03$).

TUBE TYPES AND DIFFERENT IMMUNOASSAY METHODS

To determine whether tube types had similar effects on different TT₃ methods, we measured serum TT₃ in samples collected with the different collection tubes on both the IMMULITE 2000 and AxSYM analyzers (n = 23). We observed no significant differences in serum TT₃ concentrations among different tube types on the AxSYM analyzer ($P = 0.98$). The serum TT₃ concentrations in glass, Vacuette, and SST tubes measured on the AxSYM were 1.24, 1.23, and 1.25 nmol/L, respectively. In contrast, we found significant differences in serum TT₃ concentrations from the different tube types on the IMMULITE 2000 analyzer ($P = 0.02$). The serum TT₃ concentration in SST tubes measured on the IMMULITE was significantly higher (2.20 nmol/L) than in glass (1.68 nmol/L) or Vacuette (1.70 nmol/L) tubes. We found no significant difference between glass and Vacuette tubes, as measured on the IMMULITE 2000 analyzer.

DILUTION STUDIES

We evaluated the effect of dilution on the SST tube interference in the TT₃ assay by performing 1:1 to 1:3 dilution studies using human serum albumin (40 g/L) in phosphate-buffered saline as the diluent. The regression equations for observed (y) vs expected (x) serum TT₃ values on the diluted samples were $y = 0.94x + 10.0$ ($r = 0.99$) for glass, $y = 1.05x - 10.0$ ($r = 0.99$) for Vacuette, and $y = 0.98x + 0.5$ ($r = 0.99$) for SST tubes, demonstrating that the interferent diluted linearly from 1:1 to 1:3 with an albumin solution. Sample dilution, therefore, was not a potential solution to the interference problem.

Addition of saline (8.5 g/L NaCl) to SST tubes for up to 48 h, however, did not extract any interfering substance, as determined by use of the saline incubated in the tubes as a diluent for the TT₃ assay.

EFFECTS OF TUBE COMPONENTS ON TT₃ ASSAYS

Incubation of serum originally collected in glass tubes and then transferred to the SST tubes revealed the presence of an interfering substance in the SST tubes. A serum pool obtained from glass collection tubes with a TT₃ concentration of 2.05 nmol/L on repeat analysis after incubation in SST tubes for 30 min had a concentration of 3.25 nmol/L when measured on the IMMULITE 2000 analyzer (n = 4; $P = 0.003$). Analysis of the same serum samples on the AxSYM analyzer revealed no significant change in TT₃ concentrations (glass tube, 1.25 nmol/L; SST tube, 1.19 nmol/L; $P = 0.46$). We observed no significant difference in the TT₃ concentration when the serum pool obtained from glass tubes was compared with the same serum pool from glass tubes that was incubated in Vacuette tubes and measured on the IMMULITE 2000 (glass tube, 2.05 nmol/L; Vacuette tube, 2.07 nmol/L; $P = 0.49$) or the AxSYM (glass tube, 1.25 nmol/L; Vacuette tube, 1.29 nmol/L; $P = 0.80$) analyzer.

To determine whether TT₃ measured on the IMMULITE 2000 analyzer was affected by the clot activator,

internal tube coating, or lubricant on the rubber stopper, we transferred serum samples from glass tubes to tubes that were cleansed with a gauze sponge to remove coatings on the tube and rubber stopper, as described in the *Materials and Methods*. The results indicated that the TT₃ concentrations were significantly higher in unaltered compared with cleaned tubes for SST (2.48 vs 1.99 nmol/L, respectively; n = 5, $P = 0.01$) but not glass (1.48 vs 1.57 nmol/L, respectively; n = 5; $P = 0.43$) or Vacuette tubes (1.45 vs 1.51 nmol/L, respectively; n = 5; $P = 0.30$). Although not statistically significant, measured TT₃ concentrations were higher in unaltered compared with cleaned tubes for red-top (2.22 vs 1.97 nmol/L, respectively; n = 5; $P = 0.13$) and tiger-top (2.48 vs 2.13 nmol/L, respectively; n = 5; $P = 0.14$) Vacutainer collection tubes. These findings support the hypothesis that the coating in the collection tubes and the lubricant on the rubber stopper are the interferent from SST tubes.

To further establish whether the increased TT₃ concentrations found in the SST tubes were attributable to contact with its rubber stopper or the materials applied to the stopper, we exposed serum samples from each tube type to the respective rubber stoppers by inversion of collection tubes and compared the results with serum samples from the same tube type that were not inverted (n = 5). TT₃ concentrations were higher in samples exposed to the rubber stoppers compared with those that were not exposed to rubber stoppers for SST (2.74 vs 2.36 nmol/L, respectively; $P = 0.03$) but not the glass (1.98 vs 1.97 nmol/L, respectively; $P = 0.96$) and Vacuette collection tubes (1.96 vs 2.00 nmol/L, respectively; $P = 0.45$).

Recently, SST tubes with a new separator gel formulation, Vacutainer SST II (plastic tube; 13 × 100 mm; cat. no. 367955; lot no. 3293573), have been developed and marketed by BD. We examined whether TT₃ concentrations obtained from SST and SST II tubes differed (n = 10). We found significant differences between the SST and SST II tubes (2.98 and 2.77 nmol/L, respectively; $P = 0.02$), but both the SST and SST II tubes gave higher TT₃ results than glass and Vacuette tubes (2.34 and 2.33 nmol/L, respectively; $P = 0.03$). Therefore, the positive interference observed with the SST and SST II tubes on the IMMULITE 2000 TT₃ assay is independent of the type of separator gel.

EFFECT OF TUBE TYPES ON OTHER ASSAYS

In view of the effect of collection tubes on TT₃ results, we tested other serum assays in the laboratory with samples collected in the three tube types. Serum samples from 49 apparently healthy volunteers were tested on the IMMULITE 2000 analyzer for the assays shown in Table 2. We observed statistical differences ($P < 0.003$) between tube types for 10 of the 17 serum analytes examined. The difference between glass and SST tubes was >10% for progesterone, TT₃, cortisol, thyroid-binding globulin, total thyroxine, and insulin, whereas only thyroid-binding globulin differed by >10% for glass vs Vacuette tubes

(Table 2). Apart from TT_3 , none of the other test differences would be regarded as clinically important (Table 2) based on the calculated significant change limit for each test (17). Similarly, we tested the effect of collecting serum in SST and Vacuette tubes on general chemistry analytes and immunology tests (Tables 1 and 2, respectively, in the online Data Supplement). The difference between the two types of tubes ($\text{mean}_{\text{SST}} - \text{mean}_{\text{Vacuette}}$) was statistically significant ($P < 0.002$) for 6 of 30 measured general chemistry analytes, but the magnitude of these differences was small (range, -3.15% to 5.18%) and not considered to be clinically relevant (17). For all of the immunology analytes examined, we observed no significant differences in results between the Vacuette and SST collection tubes ($\text{mean}_{\text{SST}} - \text{mean}_{\text{Vacuette}}$; Table 2 in the online Data Supplement).

Discussion

In the present study, TT_3 concentrations in serum samples collected in SST tubes were significantly higher than those collected in glass and Vacuette tubes (Table 2 and Fig. 2), and interference by tube additives is the most likely explanation (Table 1). The magnitude of the difference in TT_3 concentrations between SST and glass and Vacuette tubes was determined to be clinically relevant based on its significant change limit [Table 2 and Ref. (17)]. We also observed a higher serum TT_3 concentrations in Vacuette compared with glass tubes (Table 2 and Fig. 2), but the magnitude of the difference in serum TT_3 concentrations between Vacuette and glass tubes was not clinically significant (Table 2). According to a National Academy of Clinical Biochemistry guideline (19), which takes into account analytical variability together with between- and within-person biological variability for serum TT_3 , an absolute serum TT_3 difference > 0.54 nmol/L constitutes a clinically significant change. The clinical significance of the tube effects on measured TT_3 concentrations was further supported by a substantial change in the fraction of patients classified as having increased TT_3 (Fig. 1A), which could cause possible misinterpretation, misdiagnosis, or even incorrect treatment of patients.

The substantial lot-to-lot variation of SST tube effects on TT_3 suggests that the positive bias between TT_3 results cannot be adequately compensated by an adjustment in the reference interval. We also observed higher measured serum TT_3 concentrations in samples from plastic red-top (2.19 nmol/L) and tiger-top Vacutainer tubes (2.53 nmol/L) compared with glass tubes (1.80 nmol/L) and Vacuette (1.83 nmol/L) tubes ($n = 25$ for each tube type). Overall, these results suggest that the SST tubes and other plastic serum blood collection tubes from this manufacturer are not suitable for performing the TT_3 assays on the IMMULITE 2000 analyzer, although the tubes appear to be suitable for TT_3 analysis on the AxSYM analyzer. The SST tubes, however, appeared to function acceptably for all other laboratory tests that were examined (Table 2 and Tables 1 and 2 in the online Data Supplement). The effect

of the blood collection tubes on any assay, however, should be considered in the context of the total allowable error; thus, the overall diagnostic utility of some of the other assays that showed a blood collection tube bias may still be adversely affected by the problem with the BD collection tubes.

The identity of the interfering substance in the SST tube is not known, but as shown in Table 1, there are only a few possible candidates. The main difference among the glass, Vacuette, and SST tubes is the interior coating. A silicone polymer was used to coat the interior of the SST but not the glass or Vacuette tubes (Table 1). Silicone is used to fix the silica powder (clot activator) to the plastic tubes and as a stopper lubricant (20). The silicone coating in the tubes is also used to reduce adherence of erythrocytes to the walls of the collection tubes (20). Previous reports have shown that silicone-coated collection tubes can interfere with ion-specific electrode determinations of both ionized magnesium (21–24) and lithium (20), causing falsely increased concentrations. In addition, the water-soluble silicone polymer coating the interior of serum separator tubes has been shown to interfere negatively with avidin-biotin binding in an IRMA for thyrotropin, prolactin, and human chorionic gonadotropin (25). Interestingly, the higher TT_3 results obtained in the red- and tiger-top compared with glass collection tubes may also be attributable to the silicone polymer because both of these tubes have silicone as an internal tube coating and stopper lubricant (26). It is important to note, however, that Vacuette tubes also contain silicone as a lubricant on the rubber stopper but not as a coating on the interior of the tube [Table 1; personal communication with Greiner Bio-One technical services; Ref. (27)]. Hence, the presumably smaller amount of silicone present on the rubber stopper of Vacuette tubes may explain the small mean difference in measured TT_3 in these tubes compared with the glass tubes, which have glycerin as the stopper lubricant with no internal tube coating [Tables 1 and 2 and Fig. 2D; Ref. (26)].

In our study, removing the clot activator, internal tube coating, and lubricant from the rubber stopper with a gauze sponge significantly decreased the measured serum TT_3 concentration in SST and other BD plastic serum tubes, thus supporting the hypothesis that an interferent, such as silicone, coating the tubes and as a stopper lubricant falsely increases the serum TT_3 concentration measured by the IMMULITE TT_3 assay. However, tube additives other than silicone used to coat the interiors of SST tubes (i.e., polyvinylpyrrolidone, polyethylene oxide, and polyvinyl alcohol) may also be responsible for the falsely increased serum TT_3 concentrations in SST compared with glass or Vacuette tubes when measured on the IMMULITE 2000 analyzer (28).

The falsely increased serum TT_3 concentrations obtained from the SST and other plastic BD serum tubes measured on the IMMULITE 2000 analyzer may also be dependent not only on the presence of silicone, but also

on the quantity in the collection tubes. This point is supported by the observation that the measured TT₃ in serum exposed to SST tubes with rubber stoppers was significantly higher than TT₃ values for serum exposed to SST tubes without rubber stoppers. It is also conceivable that not all collection tubes were coated during production with a homogeneous layer of the silicone coating in the tubes and/or on the rubber stoppers. Hence, this possible variation in quantity of silicone in the collection tubes may explain the differences in measured serum TT₃ concentrations among the tube types and among the different SST tube lots (Table 2 and Fig. 2). The mechanism of interference of tube components with the IMMULITE 2000 TT₃ assay is not clear. Further studies are warranted to elucidate the exact mechanism by which tube components interfere with the TT₃ assay.

It is noteworthy that participation in internal and external quality assurance programs would not reveal the type of preanalytical problem described in this study. Proficiency samples received by the clinical laboratory for evaluation are contained in the same type of sealed vials or tubes; therefore, any effect of variations in collection tube components on the analyte test result(s) would have been excluded from these proficiency samples. This is of particular importance for clinical laboratories that receive serum or plasma samples collected in different types of tubes from different manufacturers. Although it was done only retrospectively, in this case the routine monitoring of moving averages based on patient data may be potentially useful for identifying future tube-related problems (Fig. 1B). Moving average procedures are often criticized for (a) being time-consuming and insensitive compared with common quality-control rules (29, 30); (b) being complex, thus requiring sophisticated computer programs to smooth the patient data and to produce meaningful graphic summaries (29, 30); and (c) being unduly sensitive to outlying groups of patient results (29, 30). Despite these limitations, the moving average data would have alerted, in this case, the laboratory to an increase in TT₃ concentrations in patient samples that conventional internal and external quality-control material missed.

Other than increased vigilance when inspecting laboratory results and improving the feedback between the clinical laboratory and clinicians, there is not much that clinical laboratories can do to readily detect blood collection tube problems. It is impractical for clinical laboratories to repeat a tube evaluation study with each new lot of tubes, but laboratories should consider comparing results from separate lots of the same tube type to detect any lot-to-lot variations when first evaluating a new tube. Similarly, it is impractical for tube manufacturers to test their tubes on all the various assay platforms, but they should ensure during the manufacturing process consistency in the amount and quality of any tube additives. Diagnostics companies could also help in the identification of future tube problems by providing detailed infor-

mation not only on the tube type but the commercial source of the tubes that they use when determining the reference interval for any new assay. Any reference interval study done by diagnostics companies on previously developed assays with older tubes that are no longer widely used, such as glass tubes, should ideally be repeated with tube types that are currently used by their customers. Finally, all stakeholders in this issue should be more vigilant about the effect of blood collection tubes on laboratory assays and work together to prevent and minimize the problem.

Since submission of this manuscript, BD has sent out a technical bulletin (VS7313; www.bd.com/vacutainer/techbulletins/) describing the SST tube interference on various instrument platforms other than the Diagnostics Products Corporation [ADVIATM CENTAUR and ACS 180TM (Bayer Healthcare Diagnostics Division) and ACCESSTM, ACCESS 2, UNICELTM DXI800, SYNCHRON LX I 725 (Beckman Coulter Inc)] and on a variety of different assays (TT₃, TT₄, folate, vitamin B₁₂, follicle-stimulating hormone, hepatitis B surface antigen, cancer antigen 27.29, and cortisol). BD has recommended alternative blood collection tubes that can be used in the interim until the SST tube interference problem is resolved.

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NOTE ADDED IN PROOF. In response to immunoassay problems with the BD Vacutainer SST, SST II, and Microtainer tubes, BD has recently reformulated the tubes to reduce the amount of surfactant in the tubes to eliminate the assay interference (www.bd.com/vacutainer/techbulletins/). We have evaluated serum samples collected in the adjusted BD Vacutainer SST (13 × 100 mm and 13 × 75 mm) and the adjusted tiger-top (16 × 100 mm) tubes and have compared the results to BD glass (10.25 × 64 mm) and Greiner Vacuette (13 × 75 mm) blood collection tubes on the IMMULITE 2000 analyzer for the immunoassay analytes shown in Table 2 (n = 10 for each tube type in duplicate). No clinically significant differences (see Table 2 for significant change limit criteria) among the tube types were observed (data not shown); therefore, it appears that the new BD tubes have been successfully adjusted to reduce the assay interference and now yield results that are similar to glass and Vacuette tubes for the assays tested.

References

1. Hill BM, Laessig RH, Koch DD, Hassemer DJ. Comparison of plastic versus glass evacuated serum-separator (SST™) blood-drawing tubes for common clinical chemistry determinations. *Clin Chem* 1992;38:1474–8.
2. Landt M, Wilhite TR, Smith CH. A new plastic evacuated tube with plasma separator. *J Clin Lab Anal* 1995;9:101–6.
3. Smets EML, Dijkstra-Lagemaat JE, Blankenstein MA. Influence of blood collection in plastic vs glass evacuated serum-separator tubes on hormone and tumor marker levels. *Clin Chem Lab Med* 2004;42:435–9.
4. Van Cott EM, Lewandrowski KB, Patel S, Grzybek DY, Patel HS, Fletcher SR, et al. Comparison of glass K₃EDTA versus plastic K₂EDTA blood-drawing tubes for complete blood counts, reticulocyte counts, and white blood cell differentials. *Lab Hematol* 2003;9:10–4.
5. Reinartz JJ, Ramey ML, Fowler MC, Killeen AA. Plastic vs glass SST™ evacuated serum-separator blood-drawing tubes for endocrinologic analytes [Letter]. *Clin Chem* 1993;39:2535–6.
6. Dasgupta A, Blackwell W, Bard D. Stability of therapeutic drug measurement in specimens collected in Vacutainer plastic blood collection tubes. *Ther Drug Monit* 1996;18:306–9.
7. Karppi J, Ackerman KK, Parviainen M. Suitability of collection tubes with separator gels for collecting and storing blood samples for therapeutic drug monitoring. *Clin Chem Lab Med* 2000;38:313–20.
8. Boeynaems JM, DeLeener A, Dessars B, Villa-Lobos HR, Aubry JC, Cotton F, et al. Evaluation of a new generation of plastic evacuated blood-collection tubes in clinical chemistry, therapeutic drug monitoring, hormone and trace metal analysis. *Clin Chem Lab Med* 2004;42:67–71.
9. 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.
10. Koch TR, Platoff G. Suitability of collection tubes with separator gels for therapeutic drug monitoring. *Ther Drug Monit* 1990;12:277–80.
11. Dasgupta A, Dean R, Saldana S, Kinnaman G, McLawhon RW. Absorption of therapeutical drugs by barrier gels in serum separator blood collection tubes. *Am J Clin Pathol* 1994;101:456–71.
12. Bush VJ, Janu MR, Bathur F, Wells A, Dasgupta A. Comparison of BD Vacutainer SST™ Plus Tubes with BD SST™ II. Plus Tubes for common analytes. *Clin Chim Acta* 2001;306:139–43.
13. Bush V, Blennerhasset J, Wells A, Dasgupta A. Stability of therapeutic drugs in serum collected in Vacutainer serum separator tubes containing a new gel (SST™ II). *Ther Drug Monit* 2001;23:259–62.
14. Drake SK, Bowen RAR, Remaley AT, Hortin GL. Potential interferences from blood collection tubes in mass spectrometric analyses of serum polypeptides [Technical Brief]. *Clin Chem* 2004;50:2398–401.
15. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, part I. *J Clin Chem Clin Biochem* 1983;21:709–20.
16. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1:307–10.
17. Boyanton BL, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. *Clin Chem* 2002;48:2242–7.
18. Diagnostic Products Corporation. Immulite™ 2000. Technical Bulletin 1112. Los Angeles, CA: Diagnostic Products Corporation, September 25, 2002.
19. Demers LM, Spencer CA. National Academy of Clinical Biochemistry (NACB). NACB: laboratory support for the diagnosis and monitoring of thyroid disease. <http://nacb.org> (accessed May and June 2004).
20. Sampson M, Ruddle M, Albright S, Elin RJ. Positive interference in lithium determinations from clot activator in collection container. *Clin Chem* 1997;43:675–9.
21. Marsoner HJ, Spichiger UE, Ritter C, Sachs C, Ghahramani M, Offenbacher H, et al. Measurement of ionized magnesium with neutral carrier based ISE's. Progress and results with the AVL 988-4 magnesium analyzer. *Scand J Clin Lab Invest* 1994;54:45–51.
22. Zoppi F, De Gasperi A, Guagnellini E, Marocchi A, Mineo E, Pazzucconi F, et al. Measurement of ionized magnesium with AVL 988/4 electrolyte analyzer: preliminary analytical and clinical results. *Scand J Clin Lab Invest Suppl* 1996;224:259–74.
23. Ritter C, Ghahramani M, Marsoner HJ. More on the measurement of ionized magnesium in whole blood. *Scand J Clin Lab Invest Suppl* 1996;224:275–80.
24. Cao Z, Tongate C, Elin RJ. Evaluation of AVL 988/4 analyzer for measurement of ionized magnesium and ionized calcium. *Scand J Clin Lab Invest* 2001;61:389–94.
25. Wickus GG, Mordan RJ, Matheus EA. Interference in the Allegro immunoassay system when blood is collected in silicone-coated tubes [Letter]. *Clin Chem* 1992;38:2347–8.
26. Becton Dickinson™ Vacutainer™ blood collection tubes. http://www.bd.com/vacutainer/products/venous/ordering_info_tubes.asp (accessed May and June 2004).
27. Greiner Bio-One™ Vacuette™ tubes. <http://vacuette.gbo.com/en/products/> (accessed May and June 2004).
28. Anraku H, Shoji Y, inventors. Vacuum blood collection tubes. US Patent No. 4,856,533; 1989.
29. Cembrowski GS. Thoughts on quality-control systems: a laboratorian's perspective. *Clin Chem* 1997;43:886–92.
30. Kazmierczak SC. Laboratory quality control: using patient data to assess analytical performance. *Clin Chem Lab Med* 2003;41:617–27.