Clinical Biochemistry 49 (2016) 90–99

Contents lists available at ScienceDirect

Clinical Biochemistry

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

Analytical

Performance of chemically modified plastic blood collection tubes

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ARTICLE INFO

Article history:
Received 31 July 2015
Received in revised form 8 September 2015
Accepted 9 September 2015
Available online 14 September 2015

Keywords:
Blood collection tube
Quality control
Surface modification
Thyroxine
Triiodothyronine

ABSTRACT

Objective: The objective of this study was to compare newly-modified and aged chemoPET tubes, which contain no problematic surfactants, with commercially available serum blood collection tubes (BCTs) for use in analysis of cortisol, total triiodothyronine (TT_3), total thyroxine (TT_4), and routine clinical chemistry analytes in serum from apparently healthy volunteers and pooled quality control (QC) specimens.

Materials and methods: Blood specimens collected from 60 apparently healthy volunteers (18 males, 42 females) and pooled QC specimens poured into seven different BCTs were analyzed by a trained phlebotomist. Cortisol, TT_3, and TT_4 levels were measured on an Immulite 1000 instrument and routine chemistry tests were analyzed on a Siemens RxL instrument. The significance of differences between chemoPET and other BCT types compared to glass tubes were assessed by Student's paired t-test or repeated measures ANOVA or their non-parametric equivalents. The BCT-related biases (deviation from glass tubes) in analyte concentrations were compared with the current desirable allowable bias, derived from biological variation. Serum analyte concentrations in the different BCTs that exceeded their respective significant change limits were considered clinically significant.

Results: No statistically and/or clinically significant differences were noted in the analyte concentrations from serum specimens and pooled QC material when our newly modified and aged chemoPET tubes were compared to glass and other BCTs.

Conclusions: The chemoPET tubes described here may be a suitable alternative to serum BCTs that contain problematic surfactants known to interfere with some clinical assays on the Immulite 1000 and RxL instruments.

1. Introduction

Health care providers rely on clinical test results to inform their decisions about diagnosis and treatment of patients. Estimates indicate that 70–85% of clinical decisions are based upon information derived from lab test results, with the caveat that the magnitude of error depends on the capacity of the system of error detection and reporting [1,2]. About 32–75% of all laboratory errors occur during the pre-analytical phase and this arises from the complex, labor-intensive work at this stage [1,2]. The pre-analytical phase remains time-consuming, even in light of technological advancements [1,2]. As such, strict monitoring during the pre-analytical phase is necessary for laboratories to maintain adequate performance levels.

Opportunities for improving clinical assays lie in the quality of blood specimens obtained. Blood collection and processing are two major steps involved in pre-analytical testing. Test reliability encompasses proper blood collection and timely processing by well-trained staff who use suitable devices [1,2]. Unfortunately, blood collection devices are typically regarded as inert specimen carriers, with no role to play in the accuracy of clinical tests. Consequently, laboratories have had little interest in investigating existing blood collection device components for their potential effects on test results.

The use of glass versus plastic tubes for blood collection is problematic for different reasons. Glass blood collection tubes (BCTs) have been used traditionally in clinical laboratories; however, they present a risk of exposing clinicians to blood-borne pathogens due to broken glass during handling or centrifugation [3,4]. This has led to the advent and preferred use of plastic tubes. Polyethylene terephthalate (PET) is a polymer (polyester) that is commonly used to manufacture plastic BCTs by way of injection molding [5,6]. Generally, however, plastic tubes have hydrophobic surfaces that interfere with the coagulation process [5,6]. Clots formed on the surfaces of plastic BCTs are more gelatinous when compared to those formed in glass tubes [5,6]. Furthermore, blood does not flow smoothly over hydrophobic plastic surfaces, which can result in the adherence of platelets, fibrin, or clotted

Abbreviations: BCT, blood collection tube; BD, Becton-Dickinson; ChemoPET, chemically-modified polyethylene terephthalate; EG, ethylene glycol; IQR, interquartile range; PET, polyethylene terephthalate; PRT, plain red-top; PT, proficiency testing; QC, quality control; RST, rapid serum tube; SCL, significant change limit; SD, standard deviation; SF, surfactant; SST, serum separator tube; TT_3, total triiodothyronine; TT_4, total thyroxine; USD, usual standard deviation.

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http://dx.doi.org/10.1016/j.clinbiochem.2015.09.003
0009-9120/

blood onto the interior walls of the tubes [5,6]. This clotting and adherence of blood to the walls of plastic BCTs can create difficulties when trying to obtain a clean separation of serum from blood during centrifugation, especially when using micro-collection tubes and during centrifugation of vacuum tubes [5,6].

The hydrophilicity of plastic surfaces can be increased using various surface modification techniques, such as plasma enhanced chemical vapor deposition, corona discharge, ion beam and laser treatment, graft polymerization, or melt blending to introduce polar functional groups [6–14]. However, implementation of these techniques on an industrial scale is challenging because they require expensive equipment and high vacuum systems, they alter the bulk properties of plastic, or the necessary functional polar groups are not well defined [6–14]. Furthermore, many of these techniques are not very practical for surface modification of small diameter tubes because penetration along the entire length of the inside (luminal surface) of plastic tubes is often not uniform [15]. Placing of small diameter tubes in large-volume reactors can result in treatment of only small portions of the tubes; thus, the uniformity and degree of the modification along the length of the tube will be inconsistent [15]. Alternatively, the interior plastic tube wall surface can be coated (via spraying, dipping, filling and aspirating, brushing, wiping) with surfactants (SFs), water-soluble polymers (e.g., hydrogels), or hydrophilic–hydrophobic block copolymers [5,6]. Under relatively static application conditions, the use of polymeric SFs is quite common and is fairly effective in reducing surface-mediated hemolysis and/or protein adsorption [5,6]. Unfortunately, SFs have the potential for desorption (leaching) into the surrounding medium (like blood) and this type of contamination has led to inaccuracies in clinical immunological assays performed on exposed serum [16,17].

The development of BCTs that minimize adsorption of cells, fibrin, and platelets and that are also devoid of substances that can interfere with assays and ultimately lead to erroneous test results is essential to patient care. Recently, the authors described a chemical treatment process of the interior wall surface of plastic (PET) tubes via a transesterification reaction with polyols (e.g., ethylene glycol), catalyzed by a guanidine base, to produce chemically modified PET (chemoPET) tubes [18]. We propose this chemical reaction as a simple, inexpensive, and effective way to modify PET surfaces to make them hydrophilic, thereby minimizing or eliminating inaccuracies in test results using natural PET on blood specimens. Our chemical modification of the BCT tube wall may improve accuracies in clinical assays by reducing re-testing costs and increasing the reliability of tests that health professionals and their patients rely on for timely and effective treatment. The objective of this study was to compare newly-modified and aged chemoPET tubes, which contain no problematic SFs, with commercially available serum BCTs for use in analysis of cortisol, total triiodothyronine (TT_3), total thyroxine (TT_4), and routine clinical chemistry analytes in serum from apparently healthy volunteers and pooled quality control (QC) specimens.

2. Materials and methods

2.1. Sample size

The present study compares the performance of our recently developed chemoPET BCT with that of other commercially available serum BCTs by measuring cortisol, TT_3 and TT_4 concentrations. Cortisol, TT_3, and TT_4 were chosen because their concentrations are greatly affected by changes in the constituents of the interior surfaces of plastic tubes, causing clinically significant errors [16,17]. Serum TT_3 levels were chosen for the sample size calculation because an 80% power to detect a clinically significant difference in TT_3 levels among tube types has been previously described [19,20]. Blood specimens collected from apparently healthy volunteers and QC materials were poured and thoroughly mixed into a range of plastic and glass tube types. Routine clinical chemistry analytes were also measured in these blood specimens.

2.2. Study participants

The study was conducted between July 2014 and January 2015 at the Stanford University Medical Center core clinical laboratory. The study obtained institutional ethics approval (#30855) and informed consent from all participants. A total of 60 apparently healthy volunteers participated in this study. Volunteers were selected based on the following inclusion criteria: 1) subjects must be over 18 years of age; 2) subjects must not be pregnant; 3) subjects have consented to having up to 50 mL of whole blood collected at one time; 4) subjects should be in good health; 5) subjects must be able to communicate effectively with study personnel; 6) subjects must be able to understand and be willing to comply with study procedures and requirements. Our study population consisted of 18 males and 42 females, who ranged in age from 18 to 70 years.

2.3. Blood collection tube types

We examined seven types of evacuated BCTs in this study: (1) plastic Vacuette™ (Greiner Bio-One™, gold-top tube with gel separator; 13 × 75 mm, cat. no. 454228; lot B041406, Monroe, NC); (2) glass tube (Becton Dickinson (BD), Franklin Lakes, NJ); 13 × 100 mm, cat. no. 366431; lot 4034472; (3) plastic SST™ tube (BD, gold-top Vacutainer™ tube with gel separator; 13 × 75 mm, cat. no. 367983; lot 4030600); (4) plastic RST™ tube (BD, orange-top Vacutainer™ tube with gel separator; 13 × 100 mm, cat. no. 368774; lot 140708); (5) plastic plain red-top (PRT) tube (BD, Vacutainer™ tube with no gel separator; 13 × 100 mm, cat. no. 367814; lot 4079576). (6) plastic discard tube (BD, clear-top Vacutainer™ tube with no gel separator; 13 × 75 mm, cat. no. 366703; lot 4023168); and (7) chemically modified tubes made from unmodified (discard) PET tubes (BD, 3-mL Vacutainer™ tubes with no interior coating; 3 mL, cat. no. 366703; lot 2160209). The discard BCTs used to make chemoPET tubes in this study are typically used to avoid potential tissue thromboplastin contamination of the first tube during venipuncture, which may produce inaccurate coagulation test results [21]. Although plastic tubes are preferred in contemporary blood specimen collection, glass tubes were used as the controls in this study because they have been the standard device for collecting serum samples for over the past five decades and glass tubes contain no clot activator, internal tube coating, or separator gel [16,17]. The composition and additives for the glass, Vacuette™, PRT, RST, and SST™ tubes have been previously described [20,22]. All BCTs were stored under conditions recommended by the tube manufacturer and used before their expiration dates.

2.4. Preparation of chemoPET tubes

The chemically modified PET tubes used here were prepared following the protocol outlined in a previous study [18]. Briefly, 5 mL of 40% (v/v) 1,1,3,3-tetramethylguanidine (TMG) solution in ethylene glycol (EG) was poured into unmodified PET tubes (BD, 3 mL Vacutainer tubes with no additives; cat. no. 366703; lot 2160209) and incubated at room temperature (22 °C) for 30 min. After incubation, the TMG/EG solution was collected for the next batches of reactions and the plastic tubes were rinsed with deionized water and dried with a stream of filtered air. The prepared chemoPET tubes did not contain any detectable contaminants (e.g. volatiles) from the chemical reaction as previously described [18].

2.5. Blood collection, serum indices, and clot detection

In the present study, blood from the 60 apparently healthy volunteers was collected from the antecubital vein with the help of a light

tourniquet (<1 min on arm used for collecting blood) to avoid hemoconcentration of blood. All collections were performed by a trained and certified phlebotomist using a 21-gauge butterfly needle connected to a vacuum tube holder. All volunteers were in a seated position for 15 min prior to venipuncture in order to prevent possible interferences due to posture on test results [23]. All specimens were processed within 1 h of blood collection. All BCTs, except the chemoPET tubes, were filled to their tube draw volume through the same venipuncture for each volunteer. The BCTs were then placed in a randomized order to minimize the effects of draw order. Blood was collected last for the chemoPET tubes and drawn into 10 mL plastic syringes that contained no anticoagulant and the syringe components (e.g. lubricants) were determined by the authors not to significantly alter the clinical assays examined in this study (data not shown). Whole blood (3 mL) was slowly dripped from a syringe into the chemoPET tubes. All tubes were mixed thoroughly by 6 to 8 end-over-end tube inversions to ensure proper mixing of the tube additives with blood specimens. Tubes were placed in a rack in an upright position and transported vertically from the collection room to the laboratory located on the same floor (50 ft away) and left in an upright position in a rack at room temperature (22 °C) and allowed to clot for 60 min. No manipulation of the tubes took place during this time period. The tubes were then centrifuged at 1300 ×*g* at 4 °C for 10 min using a swinging-bucket centrifuge, according to the manufacturer's instructions. Following centrifugation, some tubes were inspected visually for complete barrier formation (except for the glass, chemoPET, discard, and PRT tubes), fibrin, and red blood cell film. Each specimen from the different tube types underwent a hemolysis, icterus, lipemia (HIL) index measurement on the Siemens Dimension RxL general chemistry analyzer, as described by Fliser et al. [24]. Briefly, the RxL instrument pipettes a 20 μL aliquot of serum specimen and measures the absorbance at individual wavelengths defined for hemolysis (405 nm), icterus (452 nm); and lipemia (700 nm) [24]. The Siemens Dimension RxL general chemistry analyzer measures the absorbances from each serum specimen and automatically converts them to concentrations (mg/dL) of hemoglobin, bilirubin, and triglycerides [24]. These concentrations are then ranked from 1 to 6 [24]. A higher the number for each category of HIL indicates a greater interference effect on the analyte tested [24]. The serum samples were visually inspected for microclots by holding each sample up to light. Small opaque clots that were detected in some samples were removed with a wooden applicator stick.

The serum drawn in Vacuette, BD SST, and RST tubes remained on the separator gel. In contrast, the sera drawn in the BD glass, discard, PRT, and chemoPET tubes were carefully transferred into non-evacuated 12 × 75 mm polystyrene tubes with no additives (Cardinal Health, CA) in order to minimize the metabolism of the serum analytes by cellular elements in the blood tube, as these four tube types contain no separator gel. The non-evacuated tubes were capped and stored at −80 °C until their analysis, which occurred within 3 days of their collection. Serum samples were thawed only once for 1 h before analysis of chemistry analytes. No statistically or clinically significant differences were observed among the different BCTs used in this study when the analyte concentrations underwent one freeze–thaw cycle (data not shown).

2.6. QC material specimens

As an alternative to test for possible interference from tube additives, pooled QC materials [Bio-Rad Liquichek Immunoassay Plus Control 1 (lot 40861), 2 (lot 40862) and 3 (lot 40863)] from vials (equally volumes of each QC level mixed together to create the QC pool) were pipetted into each of the different BCT types (2 mL per tube). Each tube was inverted eight times (end-to-end) to ensure that the QC materials were exposed to the internal tube walls with additives and stopper lubricants.

2.7. Biochemical analysis

2.7.1. Determination of cortisol, TT_3, and TT_4 concentrations in serum and pooled QC material

After collection in seven different types of BCTs, sera from 60 apparently healthy volunteers were measured in random order for cortisol, TT_3, and TT_4 concentrations using an Immulite™ 1000 analyzer (Siemens Healthcare Global, Malvern, PA). 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. Calibrators (cortisol, lot 131; TT_3, lot 130; TT_4, lot 129) and reagents (cortisol, lot 395; TT_3, lot 367; TT_4, lot 389) were used for the Immulite™ 1000 analyzer. Cortisol, TT_3, and TT_4 levels were measured by competitive immunoassays using limited immobilized antibodies and labeled hormones [20]. All immunoassay tests were performed in triplicate to reduce the analytical coefficient of variation. The intra- and inter-assay precisions of these assays are shown on Tables 1 and 2. Internal QC results and external quality assurance data (College of American Pathologists) were within acceptable limits.

2.7.2. Routine clinical chemistry analytes

We tested the routine clinical chemistry analytes specified in the American Medical Association's comprehensive metabolic panel from 60 randomly selected samples from apparently healthy volunteers. These analytes included sodium, potassium, chloride, carbon dioxide, glucose, urea nitrogen, creatinine, calcium, albumin, total protein, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Testing was performed on the Siemens Dimension RxL™ general chemistry analyzer, according to the manufacturer's specifications (Siemens, Munich, Germany). The comprehensive metabolic panel from pooled QC material was obtained by pouring the material into the seven tube types and conducting measurements on the general chemistry analyzer. The methodology and range of assay imprecision obtained with the serum samples from apparently healthy volunteers and three QCs (low, normal, and high concentrations) for each analyte examined are shown in Supplemental data Tables 1 and 2. All serum aliquots from the different BCTs and QC material were thawed at the same time and analyzed singly in random order and in the same analytical run. All QC samples for analytes were within acceptable limits and all assays yielded satisfactory external proficiency results (College of American Pathologists) during this investigation.

2.7.3. Aged chemoPET tubes

We determined whether our chemoPET tubes maintained their surface modifications by comparing cortisol, TT_3, and TT_4 concentrations and comprehensive metabolic panel test results (from ten volunteers) with other BCT tube types. The chemoPET tubes were stored (capped) at room temperature for 20 months prior to blood collection via syringe.

2.8. Statistical analysis

Exploratory data analysis, frequency histograms, and the Shapiro–Wilk test ($p > 0.05$) for normality were performed to determine if parametric or non-parametric statistical tests would be used for analyte concentrations from the different BCT types [25]. The analyte concentrations from the different BCT types were expressed as mean and standard error of the mean for parametric data or median and interquartile range for non-parametric data. The paired Student's t-test was used for parametric data and Wilcoxon signed rank test for non-parametric data to test for statistical difference between analytes measured from the different BCTs and compared to glass tubes. The repeated measures analysis of variance (ANOVA) and Kruskal–Wallis tests were used for parametric and non-parametric data to test for statistical differences in routine clinical chemistry analyte concentrations among the different

Table 1
Comparison of serum cortisol, TT_3, and TT_4 concentrations from apparently healthy volunteers processed in different tube types.

	Tube type									
	Glass	ChemoPET	Unmodified PET	Vacuette™	PRT	RST™	SST™	USD	SCL[d]	Desirable bias[e] (%)
Cortisol (nmol/L) (N = 50)										
Range of assay imprecision (CV%)[a]: 4.2–7.9										
Mean (SEM)	262.1 (9.5)	263.2 (10.6)	259.4 (9.4)	263.3 (9.6)	258.7 (9.7)	271.9 (9.8)	268.4 (9.7)	52.4	115.9–408.3	
Absolute difference (%)[b]		1.1 (0.42)	−2.7 (−1.03)	1.2 (0.46)	−3.4 (−1.30)	9.8 (3.74)	6.3 (2.40)			10.26
p[c] (vs. glass tubes)		0.629	0.190	0.534	0.272	< **0.0001**	**0.0005**			
TT_3 (nmol/L) (N = 50)										
Range of assay imprecision (CV%)[a]: 6.4–13.5										
Mean (SEM)	1.39 (0.02)	1.43 (0.02)	1.41 (0.03)	1.37 (0.03)	1.40 (0.02)	1.38 (0.02)	1.42 (0.02)	0.18	0.89–1.89	
Absolute difference (%)[b]		0.04 (2.88)	0.02 (1.44)	−0.02 (−1.44)	0.01 (0.72)	−0.01 (−0.72)	0.03 (2.16)			3.53
p[c] (vs. glass tubes)		0.085	0.231	0.307	0.392	0.489	0.029			
TT_4 (nmol/L) (N = 50)										
Range of assay imprecision (CV%)[a]: 5.0–7.0										
Mean (SEM)	90.7 (1.3)	91.2 (1.4)	90.3 (1.4)	90.3 (1.3)	90.0 (1.3)	92.1 (1.4)	91.6 (1.5)	10.3	61.8–118.4	
Absolute difference (%)[b]		0.5 (0.55)	−0.2 (−0.22)	−0.2 (−0.22)	−0.7 (−0.77)	1.4 (1.54)	0.9 (0.99)			3.00
p[c] (vs. glass tubes)		0.545	0.523	0.586	0.247	**0.011**	0.188			

USD, usual standard deviation; SCL, significant change limit.
[a] Inter-assay imprecision across three levels of control materials.
[b] Absolute difference and % change from glass tubes.
[c] Probability paired Student's t-test (two-sided) for mean difference between ChemoPET, unmodified PET, Vacuette™, PRT, RST™, and SST™ compared to glass tubes. $p < 0.017$ is considered statistically significant and indicated in bold.
[d] Mean of quality material from plastic sample cup ±2.8 USD.
[e] Maximum desirable bias (%) based on biological variation [26].

BCT types, respectively. The Bonferroni method was used for conservative adjustment of the significance level to $p < 0.017$ (0.05/3 hormone tests) for hormones and $p < 0.0036$ (0.05/14 chemistry tests) for chemistry analytes to account for multiple comparisons from the tests analyzed. Statistical analyses were performed with Analyze-It™ for Microsoft Excel (version 1.71; Analyze-It Software, Leeds, UK). The clinical relevance of the statistically significant differences in analyte concentrations among tube types was determined using the significant change limit method, as described by Boyanton and Blick [26]. Briefly, the mean for each analyte in the glass tubes represented the initial value. The usual standard deviation (USD) employed for calculating the significant change limit was based on the mean and SD of the quality-control data for the previous six months for each respective analyte [26]. The target means of the QC material that most clearly matched the initial value of glass tubes for each analyte was used to determine the USD [26]. The significant change limit was calculated for each analyte by determining the range (±2.8 USD) from the mean of the glass tubes [26]. Serum analyte concentrations in the different collection tubes that exceeded their respective significant change limits were considered clinically significant [26]. The BCT-related biases

Table 2
Comparison of serum cortisol, TT_3, and TT_4 concentrations from quality control material processed in different tube types.

	Tube type									
	Glass	ChemoPET	Unmodified PET	Vacuette™	PRT	RST™	SST™	USD	SCL[d]	Desirable bias[e] (%)
Cortisol (nmol/L) (N = 3)										
Range of assay imprecision (CV%)[a]: 4.2–7.9										
Mean (SEM)	675.3 (15.1)	675.9 (15.9)	670.7 (11.5)	708.5 (15.8)	696.5 (16.1)	702.0 (17.9)	664.0 (13.8)	52.4	530.2–820.4	
Absolute difference (%)[b]		0.6 (0.09)	−4.6 (−0.68)	33.2 (4.92)	21.2 (3.14)	26.7 (3.95)	−11.3 (−1.67)			10.26
p[c] (vs. glass tubes)		0.978	0.812	0.149	0.352	0.271	0.587			
TT_3 (nmol/L) (N = 3)										
Range of Assay Imprecision (CV%)[a]: 6.4–13.5										
Mean (SEM)	2.60 (0.05)	2.62 (0.08)	2.59 (0.05)	2.60 (0.06)	2.72 (0.04)	2.85 (0.09)	2.69 (0.08)	0.18	2.10–3.10	
Absolute difference (%)[b]		0.02 (0.77)	−0.01 (−0.38)	0.00 (0.0)	0.12 (4.62)[f]	0.25 (9.62)[f]	0.09 (3.46)			3.53
p[c] (vs. glass tubes)		0.836	0.945	0.983	0.0693	0.0241	0.354			
TT_4 (nmol/L) (N = 3)										
Range of Assay Imprecision (CV%)[a]: 5.0–7.0										
Mean (SEM)	115.5 (2.4)	117.4 (3.3)	116.3 (2.3)	114.6 (2.3)	118.4 (1.9)	119.3 (3.1)	117.9 (2.4)	10.3	87.0–144.0	
Absolute difference (%)[b]		1.9 (1.64)	0.8 (0.69)	−0.9 (−0.78)	2.9 (2.51)	3.8 (3.29)[f]	2.4 (2.08)			3.00
p[c] (vs. glass tubes)		0.655	0.805	0.792	0.365	0.347	0.491			

USD, usual standard deviation; SCL, significant change limit.
[a] Inter-assay imprecision across three levels of control materials.
[b] Absolute difference and % change from glass tubes.
[c] Probability paired Student's t-test (two-sided) for mean difference between ChemoPET, unmodified PET, Vacuette™, PRT, RST™, and SST™ compared to glass tubes. $p < 0.017$ is considered statistically significant and indicated in bold.
[d] Mean of quality material from plastic sample cup ±2.8 USD.
[e] Maximum desirable bias (%) based on biological variation [26].
[f] Exceeded maximum desirable bias.

(mean percent differences from glass tubes) were compared with the current desirable allowable bias (B), derived from biological variation according to the formula $B < 0.25\ (CV_w^2 + CV_g^2)^{1/2}$ where CV_w and CV_g are, respectively, within- and between-individual CVs, derived from biological variation for each analyte that was examined [26,27]. The desirable allowable bias for the different tests based on biological variation represents the magnitude of error in bias that can be tolerated without invalidating the medical usefulness of the result [26,27].

3. Results

3.1. Serum indices

Visual inspection revealed no signs of hemolysis in the serum samples. The serum hemolysis index, which was determined by spectrophotometry, for all the different BCTs examined in this study was "1" for hemolysis. The plastic BCTs from different tube manufacturers examined did not demonstrate significant differences when compared with glass tubes in terms of specimen integrity regarding icterus and lipemia serum indices, which both gave values of "1" on the RxL chemistry instrument. Thus, the magnitude of hemolysis, icterus, and lipemia from the different BCTs appears comparable and is unlikely to influence the determination of analyte concentrations examined in this study.

3.2. Clots on the interior tube wall surface

Blood clots appeared on the interior tube wall surfaces of unmodified (discard) PET (~90%) and chemoPET (~20%) BCTs and needed gentle removal with a wooden applicator stick before sera could be transferred to secondary plastic tubes for storage at −80 °C. We did not observe any clot adhesion on the interior tube wall surfaces, nor did we detect any micro-clots or latent clotting by the analyzer among the other BCT types.

3.3. Tube comparisons (versus glass tubes) of cortisol, TT_3, and TT_4 concentrations in serum specimens from apparently healthy volunteers

The differences between chemoPET and other commercially available BCTs with respect to serum cortisol, TT_3, and TT_4 concentrations were determined by placing blood collected from 50 apparently healthy volunteers into seven different tube types, as described. The means (SEM) of the cortisol, TT_3, and TT_4 concentrations determined in 50 serum samples obtained from each type of BCT and measured on the Immulite™ 1000 analyzer are shown in Table 1. When RST and SST tubes were compared to glass tubes, statistically significant but not clinically differences were found in cortisol concentrations ($p < 0.0001$ for RST and $p = 0.0005$ for SST tubes) in serum samples collected from apparently healthy volunteers. No statistically ($p > 0.017$) or clinically significant (results within significant change limit) differences in serum TT_3 concentrations were found among the seven tube types measured on the Immulite™ 1000 analyzer (Table 1) for the serum specimens. When RST were compared to glass tubes, statistically but not clinically significant differences were found in TT_4 concentrations in serum samples collected from apparently healthy volunteers ($p = 0.011$). The ranges for the biases among the BCTs for analyte concentrations were as follows: cortisol, −1.30% to 3.74%; TT_3, −1.44% to 2.88%; and TT_4, −0.22% to 1.54% (Table 1). The observed biases among the BCTs, when compared to glass tubes, for the three hormone concentrations did not exceed the maximum desirable biases (Table 1).

3.4. Tube comparisons (versus glass tubes) of cortisol, TT_3, and TT_4 concentrations in QC material

No statistically ($p > 0.017$) or clinically significant differences (values within significant change limit) were noted in cortisol concentrations of pooled QC material among the BCT types examined in this study (Table 2). The range for biases among the BCTs for cortisol was −1.67% to 3.95% when compared to glass tubes (Table 2). The biases in cortisol among the BCTS did not exceed the maximum desirable bias for cortisol (10.26%). The TT_3 and TT_4 concentrations of the QC material did not show statistically significant differences ($p > 0.017$) among the seven tube types (Table 2). The ranges for the biases among the BCTs for TT_3 and TT_4 concentrations were as follows: TT_3, −0.38% to 4.62% and TT_4, −0.78% to 3.29% (Table 2). In contrast, the bias in TT_3 concentrations in PRT (4.62%) and RST (9.62%) tubes, and the bias in TT_4 concentrations in RST (3.29%) tubes, exceeded the maximum desirable bias for TT_3 (3.53%) and TT_4 (3.00%) when compared with glass tubes (Table 2). Yet, none of the QC material TT_3, and TT_4 concentrations from the different tube types exceeded the significant change limit; consequently, the differences observed in TT_3 and TT_4 among the BCTs would not be considered clinically significant when compared to glass tubes (Table 2).

3.5. Tube comparisons of clinical chemistry analytes (comprehensive metabolic panel) in serum specimens from apparently healthy volunteers

The biases in routine clinical chemistry analytes from the 50 apparently healthy volunteers—collected in different BCTs and then tested on the Siemens RxL Dimension analyzer and then compared to glass tubes were not statistically significant ($p > 0.0036$; Supplemental data Table 1). However, the biases in some tube types did exceed the current quality specification for desirable bias derived from biological variation for some chemistry analytes (albumin, aspartate aminotransferase, blood urea nitrogen, total calcium, chloride, glucose, potassium, sodium, and total bilirubin; Supplemental data Table 1). The magnitudes of the differences among BCTs for these chemistry analytes ranges from −9.9% to 16.7% (Supplemental data Table 1). None of the tube types with sera collected from volunteers exceeded the significant change limit in the routine clinical chemistry analytes examined; therefore, these biases were not considered to be clinically important when compared to glass tubes (Supplemental data Table 1).

3.6. Tube comparisons of clinical chemistry analytes (comprehensive metabolic panel) from QC material

We tested the effect of pouring pooled QC material into seven different BCTs on general clinical chemistry analytes, specifically, a comprehensive metabolic panel (Supplemental data Table 2). The differences among the tube types were statistically significant ($p < 0.0036$) for alkaline phosphatase, total carbon dioxide, and sodium in pooled QC material (Supplemental data Table 2). The magnitudes of the differences among BCTs for these three analytes were as follows: alkaline phosphatase, −2.2% to 4.6%; total carbon dioxide, −1.7% to 2.3%; and sodium, 0.7% to 2.0% (Supplemental data Table 2). The magnitude of these differences for alkaline phosphatase, total carbon dioxide, and sodium in pooled QC material did not exceed the significant change limit for each respective analyte; accordingly, these were not considered as clinically important (Supplemental data Table 2). In contrast, the biases did exceed the maximum desirable bias in some BCTs for albumin, total calcium, chloride, and sodium (ranging from −4.9% to 3.3%; Supplemental data Table 2) but none exceeded the significant change limit and were not considered clinically relevant when compared to glass tubes (Supplemental data Table 2).

3.7. Comparison of aged chemoPET tubes and other BCTs regarding cortisol, TT_3, and TT_4 concentrations in serum specimens

In this study, ten chemoPET tubes that were modified and stored at room temperature with their respective rubber stoppers were visually assessed for any red cell film or clot adhesion to their interior tube wall surfaces. Aged chemoPET tubes were also tested for serum indices, cortisol, TT_3 and TT_4 concentrations, as well as routine clinical chemistry

Table 3
Compa'rison of serum TT_3, TT_4, and cortisol concentrations collected from apparently healthy volunteers via syringe (2 mL of whole blood in each tube) and processed in different tube types.[a]

	Tube type										
	Glass	ChemoPET	Aged ChemoPET	Unmodified PET	Vacuette™	PRT	RST™	SST™	USD	SCL[d]	Desirable bias[e] (%)
Cortisol (nmol/L) (N = 10)											
Range of assay imprecision (CV%)[a]: 4.2–7.9											
Mean (SEM)	253.2 (17.5)	250.5 (17.2)	239.9 (17.4)	243.0 (18.5)	243.4 (16.3)	250.7 (18.1)	260.0 (17.5)	254.0 (16.7)	52.4	121.9–412.2	
Absolute difference (%)[b]		−2.7 (−1.07)	−13.3 (−5.25)	−10.2 (−4.03)	−9.8 (−3.87)	−2.5 (−0.99)	6.8 (2.69)	0.8 (0.32)			10.26
p[c] (vs. Glass tubes)		0.594	0.029	0.042	0.039	0.524	0.258	0.863			
TT_3 (nmol/L) (N = 10)											
Range of assay imprecision (CV%)[a]: 6.4–13.5											
Mean (SEM)	1.49 (0.05)	1.45 (0.05)	1.50 (0.06)	1.48 (0.07)	1.37 (0.05)	1.51 (0.05)	1.47 (0.05)	1.50 (0.05)	0.18	0.99–1.99	
Absolute difference (%)[b]		−0.04 (−2.68)	0.01 (0.67)	−0.01 (−0.67)[f]	−0.12 (−8.05)[f]	0.02 (1.34)	−0.02 (−1.34)	0.01 (0.67)			3.53
p[c] (vs. Glass tubes)		0.221	0.813	0.847	**0.007**	0.622	0.499	0.685			
TT_4 (nmol/L) (N = 10)											
Range of assay imprecision (CV%)[a]: 5.0–7.0											
Mean (SEM)	91.9 (2.3)	91.4 (2.5)	91.8 (2.3)	90.7 (2.3)	90.5 (2.2)	94.0 (2.8)	91.9 (2.5)	91.7 (2.4)	10.3	63.3–120.4	
Absolute difference (%)[b]		−0.5 (−0.52)	−0.1 (−0.11)	−1.2(−1.31)	−1.4 (−1.52)	2.1 (2.29)	0.0 (0.00)	−0.2 (−0.22)			3.00
p[c] (vs. Glass tubes)		0.766	0.907	0.266	0.114	0.095	0.955	0.883			

USD, usual standard deviation; SCL, significant change limit.
[a] Inter-assay imprecision across three levels of control materials.
[b] Absolute difference and % change from glass tubes.
[c] Probability paired Student's t-test (two-sided) for mean difference between ChemoPET, aged ChemoPET, unmodified PET, Vacuette™, PRT, RST™, and SST™ compared to glass tubes. $p < 0.017$ is considered statistically significant and indicated in bold.
[d] Mean of quality material from plastic sample cup ±2.8 USD.
[e] Maximum desirable bias (%) based on biological variation [26].
[f] Exceeded maximum desirable bias.

analytes. These tests were repeated among chemoPET, glass, and other commercially available BCTs. Similarly, no red cell film was seen on any of the BCTs studied and the serum indices yielded the same results for all the tube types. Clot adhesion occurred on the interior tube wall surfaces of the newly prepared chemoPET tubes and some aged chemoPET tubes (2 of the 10 for each newly prepared and aged chemoPET tubes). No statistically ($p > 0.017$) or clinically significant differences were observed for cortisol, TT_3, and TT_4 when aged (~20 months) chemoPET tubes were compared to the other tube types currently on the market (Supplemental data Table 2). The ranges for biases among the BCTs for cortisol when compared to glass tubes were −5.25% to 2.69%, for TT_3 −8.05% to 0.67%; and for TT_4 −1.52% to 2.29% (Table 3). Interestingly, a comparison of Vacuette tubes with glass tubes revealed a statistically significant difference for TT_3 ($p = 0.007$) (Table 3). The bias for TT_3 in Vacuette tubes (−8.05%) exceeded the maximal desirable bias of 3.53% (Table 3). The TT_3 concentrations in this tube type, however, did not exceed the significant change limit; therefore, the TT_3 results from Vacuette tubes were not considered clinically relevant when compared to glass tubes (Table 3). Ultimately, the interior tube wall surfaces of chemoPET tubes were remarkably stable (at least 20 months), as illustrated by the lack of significant differences in the three hormone analyte concentrations.

3.8. Comparison of aged chemoPET tubes and other BCTs regarding routine clinical chemistry analytes in serum specimens

The biases in routine clinical chemistry analytes from 10 apparently healthy volunteers collected in aged chemoPET tubes and other BCT types and then compared to glass tubes were not statistically significant ($p > 0.0036$; Supplemental data Table 3). However, the biases in some tube types when compared to glass tubes did exceed the current quality specification for desirable bias derived from biological variation for some chemistry analytes (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total calcium, chloride, creatinine, glucose, potassium, sodium, and total bilirubin; Supplemental data Table 3). The magnitudes of the differences among BCTs for these chemistry analytes ranges from −23.1% to 13.6% (Supplemental data Table 3). None of the tube types with sera collected from volunteers exceeded the significant change limit in the routine clinical chemistry analytes examined; therefore, these biases were not considered to be clinically important when compared to glass tubes (Supplemental data Table 3).

4. Discussion

This study examined the performance of chemoPET, our recently developed chemical modification of the interior walls of plastic tubes, by comparing our chemically treated tubes with a variety of commercially available plastic (PET) BCTs. This chemical modification process has a fast reaction rate, provides chemically well-defined surface functionality (hydroxyl groups), is scalable for high volume BCT production, and is cost-effective because of the much lower reagent costs and catalyst recycling [18]. Furthermore, the chemical modification method used in this study induces structural changes within a depth of a few molecular layers, while maintaining the bulk mechanical and optical properties of the BCT, as evidenced by the absence of any significant changes in tube dimensions (chemical reaction occurs at room temperature) and gravimetric analysis (data not shown) [18].

Sera for this analysis were provided by 60 apparently healthy volunteers. Biochemical analyses included QC material cortisol, TT_3, and TT_4 assays performed on the DPC Immulite 1000 and general clinical chemistry analyte concentrations (comprehensive metabolic panel) measured on Siemens Dimension RxL platform. This study also investigated the stability of the interior tube wall surface modification in chemically treated tubes aged for 20 months at room temperature when compared with other serum BCTs for the same chemistry analytes.

4.1. Serum indices and red blood cell adherence to interior tube walls

We did not observe any red blood cell hang-up or red cell film on the interior surfaces of the BCTs. Visual assessment of the degree of hemolysis in BCTs was conducted by a lab technologist. This approach is sometimes deemed unreliable, so a spectrophotometry assay was also conducted to obtain hemolysis indices from the RxL instrument [24]. The hemolysis index of "1" reported for all the BCTs correlated with a concentration of hemoglobin less than 50 mg/dL, rendering hemolysis an unlikely source of the differences in test results obtained with the immunoassay, since this index was the same for all specimens collected in all the different BCTs [24]. No BCTs were excluded in this study due to red blood cell adherence to tube walls. These findings additionally support our suggestion that chemoPET tube wall surfaces perform as well as glass and other commercially available BCTs in preventing red blood cell adherence to interior tube wall surfaces [18].

4.2. Clot adherence to interior tube wall surfaces

Glass and plastic tubes can both be problematic with respect to blood clotting and tube adherence. The surfaces of glass tubes are hydrophilic, whereas those of plastic tubes are hydrophobic [6,28]. This difference is important for specimen collection, since glass tube surfaces tend to interact with plasma proteins, causing an increased rate of blood coagulation [6,28]. In contrast, plastic tube surfaces absorb plasma proteins and because of their hydrophobic nature, they tend to hold onto the proteins more readily, thereby decreasing the number of available binding sites for activators of the intrinsic pathway [5,6,28–30]. Clot activators (e.g., silica and thrombin) and other additives (e.g., polyvinylpyrrolidone and SFs), are sprayed onto the interior surfaces of plastic BCTs help to accelerate the clotting cascade [5,6,28–30]. In our study, some gelatinous clots adhered to some discard, chemoPET, and aged chemoPET tubes and were loosened with a wooden applicator stick. This clotting and adherence occurred because the discard and chemoPET tubes used contained no clot activators or any other tube additives that assist in clot formation and inhibit the attachment of clotted blood, fibrin, and other cellular material to interior tube wall surfaces [18,21]. Notably, a smaller number of chemoPET tubes (~20%) required the removal of the adhering gelatinous clots when compared with discard tubes (~90%). We suspect that the hydrophilic surface of the chemoPET tubes reduced the adherence of clots to the inner tube wall surfaces that were chemically modified through the use of hydroxyl groups. This process allows the inner wall surfaces of the chemoPET tubes to mimic the functional group observed on glass tube wall surfaces [18]. We speculate that the clot adhesion that did occur on a few chemoPET tubes may have been due to the incomplete chemical transformation of interior tube wall surfaces with hydroxyl groups and failure to make them hydrophilic throughout. Future studies may require the optimization of the ethylene glycol or other polyols and catalyst concentrations, temperature, and reaction time to ensure that the interior PET tube wall surfaces are completely transformed.

4.3. Blood collection tube comparisons of hormone concentrations in serum specimens

The accuracy of immunoassay results is compromised by many endogenous and exogenous substances [31]. SST tubes used for collecting serum have several constituents, such as tube wall materials, SFs, separator gels, and clot activators, in the tubes or in/applied to rubber stoppers, which have the potential to interfere with immunoassays [16,17,22]. With respect to the three hormone assays performed in this study, our recently developed chemoPET tubes, which that do not

contain SF, were clinically equivalent to commercially available plastic and glass tubes (Table 1, Supplemental data Table 1).

With exception of cortisol collected in RST and SST tubes and TT_4 in RST tubes (Table 1), this study deviates from previous studies on this topic by demonstrating no statistically and clinically significant differences in cortisol, TT_3, and TT_4 concentrations in BD compared to glass tubes from apparently healthy volunteers [16,17,32]. This variance between present and previous studies can be explained by the fact that the tube manufacturer (BD) has considerably reduced the amount of SF (e.g., Silwet L-720) and possibly other tube additives in their BCTs [16,17]. These substances are known sources of the interferences with hormone assays evaluated on the Immulite platform because they may cause desorption and/or denaturation of antibodies on the surface of the polystyrene beads [17]. The Vacuette tubes used in this study do not contain this type of problematic SF, while the chemoPET, glass and discard tubes do not contain any SF, which explains the similar hormone results reported for these tube types with exception of TT3 in Vacuette tubes (Table 3) [16,17].

4.4. Blood collection tube comparison of hormone concentrations in QC material specimens

QC materials are used with test systems to monitor the analytical performance of systems and ensure that test results meet quality requirements [33,34]. We found higher TT_3 concentrations in QC material poured (2 mL per tube) and mixed in PRT and RST tubes, and higher TT_4 concentrations in RST tubes, which exceeded the maximum desirable bias but were not statistically or clinically significant (Table 2). Although these differences in hormone concentrations could be attributed to variations in volume ratios of tube additives to QC material, our findings show that when chemoPET tubes were compared to glass tubes in relation to these hormones, no significant difference in QC material analyte concentrations was observed (Table 2). Overall, our findings demonstrate that pouring QC material into chemoPET tubes for QC testing (as should be done with commercial BCTs [34]) will not contaminate QC specimens as the tubes contain no SF or other tube additives that may potentially alter clinical assays.

4.5. Matrix effects of QC and serum specimens on clinical chemistry test results

Some differences were noted in the three hormone test results between QC material and serum samples from apparently healthy volunteers among the different tube types examined (Tables 1 and 2). The QC material used in this study was serum-based, whereas the specimens from our volunteers were sera isolated from whole blood specimens [20,33]. The cellular material from these whole blood specimens may have adsorbed some of the tube additives, particularly SFs and/or clot activators [20,33]. This would decrease the additive concentration in the serum layer and could result in less interference with components of the immunoassays being studied [20]. Additionally, the higher volumes of whole blood from volunteers collected in the tubes (from 3.5 mL to 7 mL per tube) as compared to QC specimens (2 mL per tube), may have diluted out the interfering substance(s), resulting in minimal alterations in the cortisol, TT_3, and TT_4 concentrations [20,34]. Furthermore, the additives used in commercially available BCTs are likely titrated from whole blood rather than QC specimens [20,33]. Since QC materials are made from artificial sources, QC and patient specimens will quite commonly produce different test results, like those observed in this study [20,33,24].

4.6. Blood collection tube comparison with QC and serum specimens on routine clinical chemistry analytes

Some QC material, but not serum specimens, from the different BCTs exhibited statistically significant differences in routine clinical chemistry concentrations (alkaline phosphatase, total carbon dioxide, and sodium) when compared to glass tubes (Supplemental data Tables 1 and 2). The routine clinical chemistry analytes in some BCTs exceeded the maximal desirable bias for both QC material and serum specimens (Supplemental data Tables 1 and 2). Therefore, the magnitude of the biases of test results compared to glass tubes depends on the BCTs used; however, the differences appear to be relatively small and not clinically significant. This is consistent with previous studies that found that the silicone SF, Silwet™ L-720, did not have a clinically significant effect on routine chemistry analyte concentrations [16,17]. We did not observe any statistically or clinically significant difference in routine clinical chemistry analyte concentrations when comparing chemoPET tubes to glass tubes (Supplemental data Tables 1 and 2). This result demonstrates that chemoPET tubes can be used interchangeably for the routine clinical chemistry analytes studied on Siemens RxL analyzers.

4.7. Effects of aging of chemoPET tubes on clinical chemistry analytes

Many techniques have been utilized to transform tube surfaces from hydrophobic to hydrophilic; however, some techniques create hydrophilic surfaces that deteriorate with time and/or are dependent upon environmental conditions (i.e., indoor and outdoor temperature, sunlight, humidity, oxygen, and ozone), which may limit functionality [35–38]. Partial or complete hydrophobic recovery of tube wall surfaces is due to a combination of surface reorientation effect; migration of mobile species from the bulk of the polymer toward the surface; and external contamination [35–38]. This is why some medical devices with modified surfaces need to be stored under special conditions and used in a timely manner [35–38]. In this study, the chemoPET and discard tubes that were securely stoppered with a rubber stoppers and left at room temperature for 20 months did not show any red blood cell film, and the serum indices were the same as those found for commercially available tubes. With the exception of TT_3 concentrations in Vacuette tubes, the cortisol, TT_3, TT_4 concentrations were not statistically or clinically different among the tube types examined, including aged chemoPET tubes (Table 3). We did not expect to find a statistically significant difference in TT_3 concentrations of serum collected in Vacuette tubes when comparing them to glass tubes ($p = 0.007$ Table 3). Moreover, the bias in TT_3 concentrations in this tube type when compared to glass tubes (−8.05%) exceeded the maximal allowable desirable bias (3.53%); however, when applying the significant change limit, the difference in TT_3 concentrations was not clinically significant. These findings raise concerns and highlight the need for consistency in the quality, quantity, and distribution of any tube additives in BCTs, as they can significantly affect clinical assay results. We analyzed Vacuette tubes twice to exclude sporadic errors that can be attributed to a small clots, bubbles, or misidentification.

Conceivably, variations in tube additives with the lot of Vacuette tubes used in this tube aging experiment only had an effect on the TT_3 results measured on the Immulite 1000 analyzer. Previous studies have shown BCT lot-to-lot differences in assay results [16,17]. In fact, based on the authors' experiences, uneven spraying of tube additives on the interior tube wall surface can be observed in many BCTs from different tube manufacturers, thus reinforcing the fact that BCT additives and lot-to-lot differences in BCTs can have a significant effect on test results [16,17]. BCT manufacturers typically claim a shelf-life of 6–18 months for plastic (PET) tubes and, due to superior gas and moisture barrier performance, 24–36 months for glass tubes [39]. Comparably, our interior tube wall surface modification method is stable for at least 20 months, a duration expected for chemical modification based on covalent bond formation [40]. ChemoPET tubes have a shelf-life (interior tube wall surface) and performance level equivalent to that of currently available BCTs [39].

Serum specimens collected in aged chemoPET tubes and compared to other tube types did not show any statistically significant differences

in routine clinical chemistry concentrations (Supplemental data Table 3). The routine clinical chemistry analytes in some BCTs exceeded the maximal desirable bias for both QC material and serum specimens (Supplemental data Table 3). Therefore, the magnitude of the biases of test results compared to glass tubes depends on the BCTs used; however, the differences appear to be relatively small and not clinically significant. As stated above, these findings are in are in agreement with previous studies demonstrating that the tube surfactants did not have a clinically significant effect on routine chemistry analyte concentrations performed on the Siemens RxL chemistry instrument [16,17]. Thus, aged chemoPET tubes (20 months) did not alter the routine clinical chemistry test results when compared to newly produced BCTs.

4.8. Study limitations

The present study has some limitations. First, this study only examined cortisol, TT_3, TT_4, and comprehensive metabolic analyte concentrations in serum and QC materials. Many other immunology and chemistry analytes evaluated in a typical clinical chemistry laboratory were not examined here. As such, the effects of tube types, including chemoPET tubes, on other serum and QC material analytes are unknown. Second, the cortisol, TT_3, and TT_4 concentrations processed in chemoPET tubes were examined only on the Siemens Immulite 1000 platform, whereas the routine clinical chemistry analytes were examined on the Siemens Dimension RxL platform. The alterations in serum and QC material analyte concentrations processed in chemoPET and other tube types on different immunoassay and chemistry platforms is also unknown. Future work should examine chemoPETs together with other commercially available BCTs for any effects on various clinical assays and on a wide array of platforms. Third, we compared chemoPET tubes to five other types of serum tubes that are commonly utilized by clinical laboratories in North America. We did not examine other commercially available serum tube types made by different blood collection manufacturers, such as Sarstedt™ (Numbrecht, Germany) and Terumo™ (Leuven, Belgium). Future studies comparing other brands of serum BCTs to chemoPET and glass tubes are warranted. Fourth, this study has relied upon blood products from apparently healthy volunteers alone. Future studies looking at hospitalized patients with significantly altered biochemistry values (abnormally low and high analyte concentrations) would be desirable to ascertain the performance of chemoPET tubes in these diverse patient populations. Fifth, the suitability of chemoPET tubes with other tube additives like silica and polyvinylpyrrolidone used to enhance clot activation should be investigated to demonstrate versatility [41]. Last, in the present study, chemoPET and other serum BCTs examined were not tested under stressed conditions that may better simulate real-life situations. Extreme temperatures, transportation conditions, extended contact time between blood and tube additives, and very low blood draw volumes may all affect the clinical assays [42,43]. Testing these newly developed chemoPETs under laboratory and real life conditions would help establish their viability with respect to stability, durability, safety, and compliance to regulatory standards for medical devices [42,43]. Notwithstanding future studies and feasibility testing, our findings support the preferential use of chemoPET tubes for clinical and research purposes.

5. Conclusions

This study shows that the newly developed chemically modified plastic interior tube wall surface gives comparable results to commercially available plastic serum BCTs for the tested analytes and platforms. Our hope is that these new chemoPET tubes, which contain no proprietary SFs, can be used instead of commercially available plastic BCTs that contain problematic SFs, in order to minimize the unpredictable interference of SFs on some clinical assays. In conclusion, the quality of test results depends on the quality of the sample analyzed. The chemoPET tubes developed and evaluated in this study may present a suitable alternative to plastic serum BCTs with spray-coated SFs, as they will lead to more accurate and precise test results and reduce the turnaround time and costs associated with recollection and retesting, thus, improving quality, safety and value to patient care.

Disclosures

The authors (Bowen, Kim, and Zare) have a patent pending on the chemoPET tubes.

Acknowledgments

We would like to thank Andrew J. Ingram and Robert M. Waymouth (Stanford University) for their helpful comments about utilizing organic bases. The authors would also like to thank Phil Cheng for material management and Krista Tanquary for financial management of the study. We would also like to thank the Stanford University School of Medicine Department of Pathology and the Stanford University Department of Chemistry for funding this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clinbiochem.2015.09.003.

References

[1] M. Plebani, Clinical laboratories: production industry or medical services? Clin. Chem. Lab. Med. 53 (2015) 995–1004.
[2] P. Bonini, M. Plebani, F. Ceriotti, et al., Errors in laboratory medicine, Clin. Chem. 48 (2002) 691–698.
[3] D.J. Ernst, Plastic collection tubes decrease risk of employee injury, MLO Med. Lab. Obs. 33 (2001) 48–50.
[4] M.M. Flanders, R. Crist, G.M. Rodgers, A comparison of blood collection in glass versus plastic vacutainers on results of esoteric coagulation assays, Lab. Med. 34 (2003) 732–735.
[5] Anraku H, Shoji Y, Inventors. Vacuum blood collection tubes. U.S. Patent No. 8,565,334, August 1989.
[6] Vogler EA, Harper GR. Surface modified blood collection tubes, U.S. Patent No. 5,257,633, June 1992.
[7] C.M. Chan, T.M. Ko, H. Hiraoka, Polymer surface modification by plasmas and photons, Surf. Sci. Rep. 24 (1996) 1–54.
[8] E.M. Liston, L. Martinu, M.R. Wertheimer, Plasma surface modification of polymers for improved adhesion: a critical review, J. Adhes. Sci. Technol. 7 (1993) 1091–1127.
[9] J. Chai, F. Lu, B. Li, D.Y. Kwok, Wettability interpretation of oxygen plasma modified poly(methyl methacrylate), Langmuir 20 (2004) 10919–10927.
[10] T. Govindarajan, R. Shandas, A survey of surface modification techniques for next-generation shape memory polymer stent devices, Polymers 6 (2014) 2309–2331.
[11] A. Hozumi, T. Masuda, K. Hayashi, H. Sugimura, O. Takai, T. Kameyama, Spatially defined surface modification of poly(methyl methacrylate) using 172 nm vacuum ultraviolet light, Langmuir 18 (2002) 9022–9027.
[12] H. Ye, Z. Gu, D.H. Gracias, Kinetics of ultraviolet and plasma surface modification of poly(dimethylsiloxane) probed by sum frequency vibrational spectroscopy, Langmuir 22 (2006) 1863–1868.
[13] Y. Ikada, Surface modification of polymers for medical applications, Biomaterials 15 (1994) 725–736.
[14] S. Hu, X. Ren, M. Bachman, C.E. Sims, G.P. Li, N. Allbritton, Surface modification of poly(dimethylsiloxane) microfluidic devices by ultraviolet polymer grafting, Anal. Chem. 74 (2002) 4117–4123.
[15] J.L. Lauer, J.L. Shohet, R.M. Albrecht, S. Esnault, J.S. Malter, U.H. von Andrian, et al., Control of uniformity of plasma-surface modification inside of small-diameter polyethylene tubing using microplasma diagnostics, IEEE Trans. Plasma Sci. 33 (2005) 791–798.
[16] R.A.R. Bowen, Y. Chan, J. Cohen, et al., Effect of blood collection tubes on total triiodothyronine and other laboratory assay, Clin. Chem. 51 (2005) 424–433.
[17] R.A.R. Bowen, R.M.E. Chan, G.L. Hortin, G. Csako, S.J. Demosky, et al., Immunoassay interference by a commonly used blood collection tube additive, the organosilicone surfactant Silwet L-720, Clin. Chem. 51 (2005) 1874–1882.
[18] S. Kim, R.A.R. Bowen, R.N. Zare, Transforming plastic surfaces with electrophilic backbones from hydrophobic to hydrophilic, ACS Appl. Mater. Interfaces 7 (2015) 1925–1931.
[19] J.M. Lachin, Introduction to sample size determination and power analysis for clinical trials: controlled clinical trials. 2 (1981) 93–113.
[20] R.A. Bowen, A. Sattayapiwat, V. Gounden, A.T. Remaley, Blood collection tube-related alterations in analyte concentrations in quality control material and serum specimens, Clin. Biochem. 47 (2014) 150–157.

[21] M. Masih, N. Kakkar, Routine coagulation testing: do we need a discard tube? Indian J. Hematol. Blood Transfus. 30 (2014) 347–350.
[22] R.A. Bowen, A.T. Remaley, Interferences from blood collection tube components on clinical chemistry assays, Biochem. Med. 24 (2014) 31–44.
[23] G. Lippi, G.L. Salvagno, G. Lima-Oliveira, G. Brocco, E. Danese, G.C. Guidi, Postural change during venous blood collection is a major source of bias in clinical chemistry testing, Clin. Chim. Acta 440 (2015) 164–168.
[24] E. Fliser, K. Jerkovic, T. Vidovic, M. Gorenjak, Investigation of unusual high serum indices for lipemia in clear serum samples on siemens analysers dimension, Biochem. Med. 22 (2012) 352–362.
[25] R. Yan, A. Lou, G. Watts, H. Tarr, H. Smith, L. Kinney, et al., Comparison of Becton Dickinson vacutainer rapid serum tube with the serum separator tube for routine chemistry and immunoassay tests, J. Clin. Pathol. 67 (2014) 599–604.
[26] B.L. Boyanton Jr., K.E. Blick, Stability studies of twenty-four analytes in human plasma and serum, Clin. Chem. 48 (2002) 2242–2247.
[27] C. Perich, J. Minchinela, C. Ricós, P. Fernández-Calle, V. Alvarez, M.V. Doménech, et al., Biological variation database: structure and criteria used for generation and update, Clin. Chem. Lab. Med. 53 (2015) 299–305.
[28] Vogler EA, Harper GR, Graper JC. Vacuum actuated blood collection assembly including tube of clot-accelerating plastic, U.S. Patent No. 5,344,611, September 1994.
[29] R.A.R. Bowen, G.L. Hortin, G. Csako, O. Otanez, A.T. Remaley, Impact of blood collection devices on clinical chemistry assays, Clin. Biochem. 43 (2010) 4–25.
[30] Montgomery DB, Vogler EA. Tube having regions of different surface chemistry and method therefore, U.S. Patent No. 5,318,806, June 1994.
[31] A.A. Ismail, Identifying and reducing potentially wrong immunoassay results even when plausible and "not-unreasonable", Adv. Clin. Chem. 66 (2014) 241–294.
[32] S. Wang, V. Ho, A. Roquemore-Goins, F.A. Smith, Effects of blood collection tubes, including pediatric devices, on 16 common immunoassays, Clin. Chem. 52 (2006) 892–893.
[33] W.G. Miller, A. Erek, T.D. Cunningham, O. Oladipo, M.G. Scott, R.E. Johnson, Commutability limitations influence quality control results with different reagent lots, Clin. Chem. 57 (2011) 76–83.
[34] L.J. Kricka, J.Y. Park, M.B. Senior, R. Fontanilla, Processing controls in blood collection tubes reveals interference, Clin. Chem. 51 (2005) 2422–2423.
[35] Y.L. Hsieh, D.A. Timm, M. Wu, Solvent- and glow-discharge-induced surface wetting and morphological changes of poly(ethylene terephthalate) (PET), J. Appl. Polym. Sci. 38 (1989) 1719–1737.
[36] B. Gupta, J. Hilborn, C. Hollenstein, C.J.G. Plummer, R. Houriet, N. Xanthopoulos, Surface modification of polyester films by RF plasma, J. Appl. Polym. Sci. 78 (2000) 1083–1091.
[37] A. Vesel, M. Mozetic, A. Zalar, XPS study of oxygen plasma activated PET, Vacuum 82 (2007) 248–251.
[38] K. Gotoh, A. Yasukawa, Y. Kobayashi, Wettability characteristics of poly(ethylene terephthalate) films treated by atmospheric pressure plasma and ultraviolet excimer light, Polym. J. 43 (2011) 545–551.
[39] V. Bush, R. Cohen, The evolution of evacuated blood collection tubes, Lab. Med. 34 (2003) 304–310.
[40] R. Wolf, A.C. Sparavigna, Role of plasma surface treatments on wetting and adhesion, Engineering 2 (2010) 397–402.
[41] A.K. Stankovic, G. Parmar, Assay interferences from blood collection tubes: a cautionary note, Clin. Chem. 52 (2006) 1627–1628.
[42] G. Da Rin, G. Lippi, The quality of diagnostic testing may be impaired during shipment of lithium-heparin gel tubes, Clin. Chem. Lab. Med. 52 (2014) 1633–1637.
[43] R.A.R. Bowen, C. Vu, A.T. Remaley, G.L. Hortin, G. Csako, 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. Chem. Acta 378 (2007) 181–193.