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Analytical

Performance of chemically modified plastic blood collection tubes

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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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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