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Immunoassay Interference by a Commonly Used Blood Collection Tube Additive, the Organosilicone Surfactant Silwet L-720

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Background: A small number of immunoassays on several different types of analyzers were recently adversely affected by tube additives in Becton Dickinson (BD) Vacutainer® SST™, SST II, and Microtainer™ blood collection tubes. We examined the effect of a commonly used tube surfactant, Silwet™ L-720, on immunoassays and the mechanism for the interference.
Methods: Immunoassays were performed on serum supplemented with Silwet L-720 on the IMMULITE™ 2500 and AxSYM™ analyzers. Direct effects of the surfactant on the chemiluminescent detection step of immunoassays and on antibody immobilization on the solid phase were examined.
Results: Increasing the final surfactant concentration from 0 to 400 mg/L in serum significantly increased (~51%) the apparent total triiodothyronine (TT_3) concentrations measured on the IMMULITE 2500 but not the AxSYM analyzer. Several other competitive, but not noncompetitive, assays were also significantly affected by the surfactant on the IMMULITE 2500 analyzer. The effect was independent of serum components, and the surfactant had no direct effect on chemiluminescence reactions. The capture antibody, however, was displaced from the solid phase by incubation with solutions containing surfactant under conditions similar to the IMMULITE TT_3 assay.
Conclusions: The Silwet L-720 surfactant, which is used to coat the inner surfaces of tubes, appears to account for previously reported immunoassay interference by BD Vacutainer SST blood collection tubes. One of the mechanisms for the interference is the desorption of antibodies from the solid phase by the surfactant. The results identify an important factor in the selection of suitable blood collection tube surfactants and provide an approach for solving similar tube-assay interference problems in the future.
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Blood collection tubes have multiple components that contribute to the optimal formation of serum or plasma for laboratory analysis. For example, in glass blood collection tubes, the glass interior surface itself plays a key role in the activation of blood coagulation. Recent use of plastic as the main component of collection tubes has necessitated the addition of silica particles or other clot activators *(1–3)*. These particles may be coated with compounds such as polyvinylpyrrolidone to assist the adherence of the particles to tube walls and facilitate the rapid dissolution of silica into the blood specimen *(1–3)*. The interior surface of blood collection tubes is also usually coated with a surfactant to improve blood flow into tubes and to minimize the adherence of blood cells to the tube wall, which helps prevent hemolysis and better distributes the clot activator (i.e., silica) along the tube wall *(1, 3, 4)*. Without a surfactant, the hemolysis of erythrocytes and their incomplete separation from serum will alter the serum composition over time *(1, 3)*. Stoppers of tubes also require coating with a lubricant to improve their ease of removal and to maintain the lower pressure within the tube *(2)*. Serum separator gels are also a common component of tubes, and they serve as a barrier between serum and the clot after centrifugation of tubes *(5)*. Serum separator gels are commonly known to interfere with several drug assays *(6)*, but other types of assays

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have also been reported to be impacted by various components of blood collection tubes *(4, 7–13)*. In a recent study, we found a significant positive bias for total triiodothyronine (TT_3)[3] and other analytes when measured in serum collected from Becton Dickinson (BD) blood collection tubes with the IMMULITE™ 2000 analyzer *(8)*. On the basis of experiments involving its removal from tubes, the silicone surfactant tube additive was proposed as the source of the interference *(8)*. Recently, Terumo® VENOSAFE™ SP (plain serum tubes) and VENOSAFE SAS (serum gel tubes), compared with VENOJECT glass tubes, also showed significant positive bias for cortisol (approximately +20%) and negative biases for luteinizing (approximately −20%) and follicle-stimulating (approximately −17%) hormone when measured on the IMMULITE 2000 analyzer, which was attributed to the surfactant (polypropylene glycol) present in the Terumo tubes *(14)*. Other reports have shown that silicone-coated blood collection tubes can interfere with ion-specific electrode determinations of both ionized magnesium *(9–12)* and lithium *(4)*, causing falsely increased analyte concentrations. In addition, the water-soluble silicone polymer coating the interior of serum separator tubes has also been shown to negatively interfere with avidin-biotin binding in an immunoradiometric assay for thyrotropin, prolactin, and human chorionic gonadotropin *(13)*. Recently, Sheffield et al. *(15)* reported a significant increase in false-positive hepatitis B surface antigen results for the AUSZYME™ monoclonal test (Abbott Diagnostics), when using BD Vacutainer® SST™ Plus tubes. In response to immunoassay problems resulting from BD Vacutainer SST, SST II, and Microtainer™ tube interferences, BD has reformulated their tubes by reducing the concentration of the organosilicone surfactant Silwet™ L-720 to help reduce any assay interference *(8, 16, 17)*.

In the present study, we performed a series of experiments to determine the specific mechanism underlying the positive interference with serum collected in BD blood collection tubes for TT_3 measured on the IMMULITE 2500 analyzer. Silwet L-720 was positively identified as the source of assay interference from the BD collection tubes, and the mechanism appears to be a result of the desorption of capture antibodies from the polystyrene (PS) solid-support beads by the surfactant.

Materials and Methods

PREPARATION OF SERUM POOLS

Serum pools were obtained from plain glass blood collection tubes (red-top glass Vacutainer, no-additive blood tube; 10.25 × 64 mm; cat. no. 366397; lots 3253846 and 3029306; BD). The plain glass collection tubes were used because these tubes contain no clot activator, internal tube coating, or separator gel.

Blood samples were drawn after written informed consent was obtained from apparently healthy volunteers (age range, 30–60 years; 10 males and 10 females) by trained technologists using a butterfly connected to a evacuated tube holder. The glass tubes were filled to capacity and inverted 5 times after blood draw to promote clotting. Serum was obtained after clotting for 30 min at room temperature, followed by centrifugation at 2000*g* for 5 min, which is a deviation from the manufacturer's recommendations of <1300*g* for 10 min. All specimens were processed within 2 h of collection. Serum was transferred with disposable plastic Pasteur pipettes into a 500-mL plastic container and kept covered at room temperature until testing within 3 h, or was stored at 4 °C between testing intervals for up to 3 days. All experiments involving the surfactant were done by adding the surfactant to a serum pool.

LABORATORY ANALYSIS

Surfactant solutions. The polydimethylsiloxane surfactant Silwet L-720 (GE Silicone/OSI Specialties) was diluted in methanol to concentrations of 0, 1, 2, 4, 8, 12, 16, and 20 g/L. The Silwet L-720 concentrations used in this study are similar to the polydimethylsiloxane surfactant concentrations described by Anraku and Shoji *(2)* in the original evacuated blood collection tube patent.

Immunoassays. Serum pool TT_3 concentrations from glass tubes, with different concentrations of surfactant, were measured in duplicate in random order on an IMMULITE 2500 analyzer (Diagnostic Products Corporation) and an AxSYM™ analyzer (Abbott Laboratories). Three different reagent lots and one calibrator lot were used for the IMMULITE 2500 analyzer during the study, and two reagent lots and one calibrator lot were used for the AxSYM analyzer. Both the IMMULITE 2500 and AxSYM TT_3 assays gave satisfactory internal and external quality-control results during the study and showed similar intra- and interassay precision, as described previously *(8)*.

The serum pool with different concentrations of surfactant was also analyzed on the IMMULITE 2500 analyzer for thyroid-stimulating hormone [thyrotropin (TSH)], and sex hormone-binding globulin (SHBG) by an immunometric assay, with labeled detection antibody and capture antibodies immobilized on PS beads. Total thyroxine (TT_4), cortisol, progesterone, and thyroxine-binding globulin (TBG) were also measured on the IMMULITE 2500 analyzer but by competitive immunoassays using limiting amounts of immobilized antibodies and labeled hormones. The serum pool samples were analyzed in duplicate in random order. All of the noncompetitive and

[3] Nonstandard abbreviations: TT_3, total triiodothyronine; BD, Becton-Dickinson; PS, polystyrene; TSH, thyroid-stimulating hormone (thyrotropin); SHBG, sex hormone-binding globulin; TT_4, total thyroxine; TBG, thyroxine-binding globulin; PBS, phosphate-buffered saline; ALP, alkaline phosphatase; and SDS, sodium dodecyl sulfate.

competitive immunoassays described above were from the same reagent and calibrator lots.

TESTING OF VARIOUS SURFACTANT CONCENTRATIONS ON MEASUREMENTS WITH IMMUNOASSAYS

To test the effects of various surfactant concentrations on TT_3, progesterone, cortisol, TT_4, TSH, SHBG, and TBG results, we added 40 μL of surfactant solutions (0, 1, 2, 4, 8, 12, 16, and 20 g/L) to nonevacuated plain plastic test tubes with no additives (BD Labware) and left them overnight at room temperature to evaporate the methanol (n = 6 per surfactant concentration). We then added 2 mL of a serum pool from glass blood collection tubes to each test tube and vortex-mixed the tubes to give final surfactant concentrations of 0, 20, 40, 80, 160, 240, 320, and 400 mg/L, which span the concentration of surfactant used in the patent description of evacuated blood collection tubes *(2)*. The serum pool samples were assayed in duplicate for TT_3 on both the IMMULITE 2500 and AxSYM analyzers, whereas progesterone, cortisol, TT_4, TSH, SHBG, and TBG were assayed in singleton on the IMMULITE 2500 analyzer. Various concentrations of surfactant, as described above, were also added to phosphate-buffered saline (PBS) to determine the effect of surfactant concentrations, in the absence of serum components, on the chemiluminescent signal generation with the IMMULITE 2500 TT_3 assay. It is important to note that the addition of surfactant to the tubes does not necessarily reflect the silicone coating procedure used by BD.

STUDY OF SURFACTANT ON T_3-ALKALINE PHOSPHATASE CONJUGATE ACTIVITY

To study potential T_3-alkaline phosphatase (ALP) activity changes with increasing surfactant concentrations, 0–10 μL of a 2 g/L surfactant solutions were added to each plain plastic test tube and left overnight at room temperature to evaporate the methanol (n = 6 for each surfactant concentration). After the tubes had dried, 50 μL of T_3-ALP conjugate from the reagent wedge (lot L2T3A2211) of an IMMULITE 2500 TT_3 assay reagent set was added to each test tube, vortex-mixed, and incubated at 37 °C for 30 min with intermittent agitation. The final surfactant concentration ranged from 0 to 400 mg/L. ALP activity was measured by the addition of 200 μL of a chemiluminescent substrate, a phosphate ester of adamantyl dioxetane from the IMMULITE 2500 TT_3 assay *(18, 19)*. The sustained emission of light from the hydrolysis of the chemiluminescent substrate was photomultiplied and quantified for 8 s at 477 nm with continuous tracing on a LUMAT™ LB 9507 luminometer (EG and G Berthold). Changes in relative light units were measured and compared with that exhibited by the blank tube containing no surfactant after subtraction from background emissions.

EFFECT OF PRETREATMENT OF PS BEADS WITH SURFACTANT ON SERUM TT_3 MEASUREMENTS

To determine whether surfactant directly interferes with the PS beads and/or the murine anti-T_3 antibodies on the PS beads, we added 40 μL of surfactant solutions (0, 2, 8, and 20 g/L) to each plain plastic test tube and left it overnight at room temperature to evaporate the methanol. One PS bead coated with anti-T_3 antibodies (lot L2T31221) from an IMMULITE TT_3 assay reagent set and 2 mL of multidiluent 1 (lot L2M1Z0109) were added to each test tube, vortex-mixed, and incubated at room temperature for 30 min (n = 6 per surfactant concentration). The final concentrations of surfactant incubated with the PS beads were 0, 40, 160, and 400 mg/L. The beads were separated from the liquid phase by decantation, air-dried for 30 min, and transferred to a clean plastic test tube. These beads were then carefully added to an empty TT_3 bead pack and used to assay a serum pool from glass tubes for TT_3 in duplicate on the IMMULITE 2500 analyzer. For a negative control, a sham experiment was also performed in which the PS beads underwent the same conditions as described above but with no pretreatment with surfactant or methanol solutions (n = 6). The serum pool was also analyzed as usual for TT_3 in duplicate on the IMMULITE 2500 analyzer (n = 6).

SODIUM DODECYL SULFATE–POLYACRYLAMIDE GEL ELECTROPHORESIS AND IMMUNOBLOTTING OF SURFACTANT SOLUTIONS AFTER INCUBATION WITH PS BEADS

To determine whether increasing concentration of surfactant desorbs anti-T_3 antibodies from the surface of the PS bead, we suspended the PS beads in 300 μL of PBS containing various concentrations of surfactant and incubated the solutions for 30 min at 37 °C to mimic the IMMULITE TT_3 assay conditions. Three different bead solutions were pooled for each surfactant concentration. PS beads incubated with 300 μL of a 10 g/L sodium dodecyl sulfate (SDS) solution in PBS for 5 min at 95 °C were used as positive control for the Western blot analysis. Before loading on gels, we concentrated the samples by collecting the retentate from a Micron™ 10 concentrator (Millipore). We then mixed 15-μL aliquots of the controls, PBS-surfactant solutions, and molecular markers (6–250 kDa; NOVEX™) with 5 μL of 4× NuPAGE™ lithium dodecyl sulfate sample buffer and 1 μL of 2-β-mercaptoethanol and heated the mixture at 70 °C for 5 min. These aliquots were then loaded on a NuPAGE precast Tris-glycine gradient gel (4%–20% acrylamide; Invitrogen) and electrophoresed in NuPAGE MOPS buffer at 90 V for 1.5 h. Proteins in the gel were electrophoretically transferred to a nitrocellulose membrane in a transfer buffer (12 mmol/L Tris and 96 mmol/L glycine in 200 mL/L methanol) in a NOVEX XCell II transfer apparatus. The transfer was conducted at 25 V for 2.5 h. The efficiency of the protein transfer was confirmed by staining with GelCode Blue reagent (Pierce Biotechnology).

The membrane was then blocked with 50 g/L nonfat dried milk in PBS containing 1 mL/L Tween 20 for 1 h at room temperature. After the membrane was washed 3 times in PBS-Tween 20 (5 min each wash at room temperature), the antibodies desorbed from the PS beads by surfactant were detected by an enhanced chemiluminescent Western blot detection system, with a 1:250 dilution of sheep anti-mouse IgG antibodies conjugated with horseradish peroxidase and luminol as a substrate (Amersham Biosciences). The membrane was enclosed in a piece of Saran Wrap and exposed to Kodak X-OMAT™ AR x-ray film in an x-ray film cassette. Detection of positive signals on the x-ray film occurred in 7 min. The exposed x-ray film was developed in a Kodak RPX-OMAT automated photo image detection system.

STATISTICAL ANALYSES

The means of duplicates from each specimen for TT_3 results were used for statistical analysis. All other serum analytes from each specimen were analyzed in singletons. The results are reported as mean (SD). Results for all measured analytes obtained were compared by the 2-tailed Student *t*-test or ANOVA. Statistical significance was defined as $P < 0.05$. Statistical analyses were performed with StatView™ software (Ver. 5.0; SAS Institute).

Results

EFFECT OF SURFACTANT ON SERUM TT_3 MEASUREMENTS

For the IMMULITE 2500 analyzer, increasing the final Silwet L-720 surfactant concentration from 0 to 400 mg/L in serum significantly increased the apparent TT_3 concentration when measured by a competitive immunoassay, from 1.57 to 2.37 nmol/L, which corresponded to a decrease in chemiluminescent signal from 7.0 to 4.9 × 10^6 relative light units ($P < 0.0001$; Fig. 1A). This change is similar to the bias observed previously when TT_3 was measured in blood collected from BD SST tubes vs glass tubes *(8)*. The exact concentration of Silwet L-720 surfactant used in the BD collection tubes is proprietary, but the concentration range tested is similar to the surfactant concentration described in the original patent description of evacuated blood collection tubes *(2)*. In contrast to what was observed with the IMMULITE 2500, Silwet L-720 did not significantly increase TT_3 results when measured on the AxSYM analyzer (P = 0.57; Fig. 1B), which is consistent with the lack of interference for this assay on blood collected in the BD collection tubes *(8)*.

EFFECT OF SURFACTANT ON OTHER IMMUNOASSAYS

To determine whether other assays on the IMMULITE 2500 analyzer were also affected by the surfactant, we selected assays that were either unaffected or affected by BD blood collection tubes from our previous study *(8)*. As shown in panels A through D of Fig. 2, the competitive assays for progesterone (P = 0.04), cortisol (P = 0.0003), TT_4 ($P < 0.0001$), and TBG (P = 0.0002), when measured

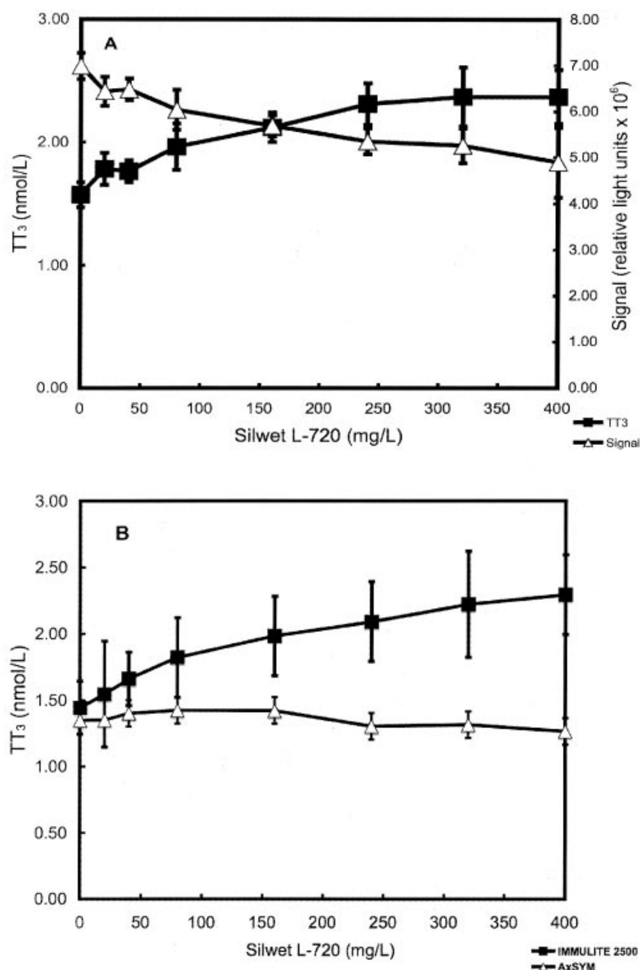

Fig. 1. Effect of Silwet L-720 on serum TT_3 concentrations and chemiluminescent signal from the IMMULITE 2500 analyzer (*A*) and on TT_3 concentrations measured on both the IMMULITE 2500 and AxSYM analyzer (*B*).

(*A* and *B*), n = 6 at each surfactant concentration. Results are presented as the mean of duplicates and SD (*error bars*).

on the IMMULITE 2500 analyzer, were affected by increasing concentration of surfactant. In contrast, as shown in panels E and F of Fig. 2, the noncompetitive assays for SHBG and TSH were not affected by the surfactant ($P > 0.05$).

EFFECT OF SURFACTANT ON CHEMILUMINESCENT SIGNAL

To rule out a possible interaction of the surfactant with a component of the serum as the source of the IMMULITE TT_3 assay interference, we mixed various concentrations of surfactant with PBS. As shown in Fig. 3, increasing the concentration of surfactant from 0 to 400 mg/L in PBS produced a significant decrease in the binding of the tracer, and therefore, the chemiluminescent signal (relative light units) decreased from 3.1 × 10^7 at 0 mg/L (maximum binding of T_3-ALP conjugate to PS bead) to 2.5 × 10^7 at 400 mg/L Silwet L-720 surfactant in the

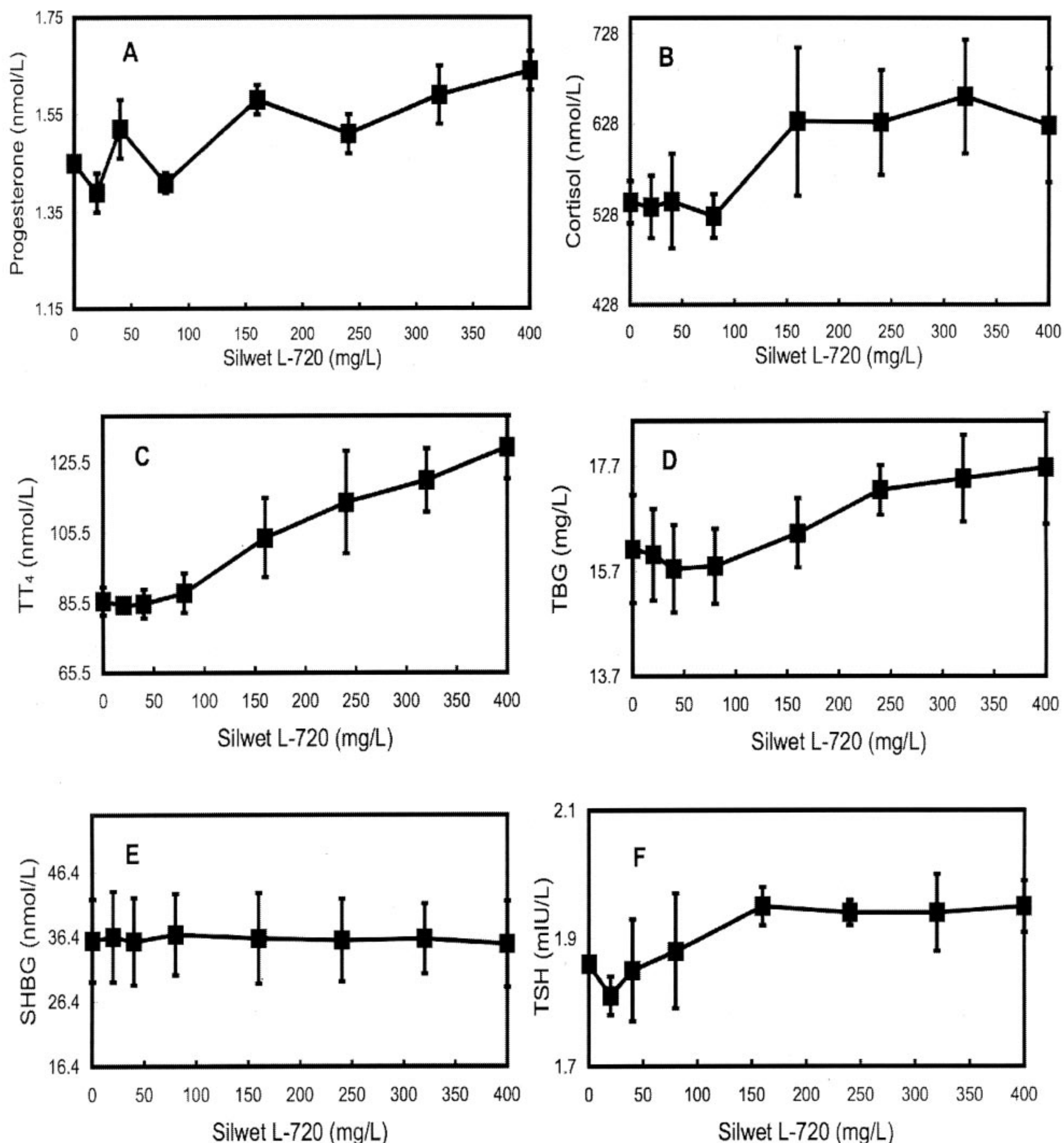

Fig. 2. Effect of Silwet L-720 concentrations on competitive and noncompetitive immunoassays performed on the IMMULITE 2500 analyzer.
n = 6 at each surfactant concentration. Results are presented as the mean and SD (*error bars*). The mean ± 3 SD (*A*, *B*, *D*, and *E*) and mean ± 5 SD (*C* and *F*) are plotted for the *y axis*.

IMMULITE TT_3 assay (P <0.0001). Thus, the interference with the surfactant happens in the absence of serum and appears to be attributable to a direct effect on the assay.

EFFECT OF SURFACTANT ON T_3-ALP CONJUGATE ACTIVITY

To determine whether the observed decrease in the chemiluminescent signal in the presence of the surfactant was attributable to alterations in signal generation, we incubated increasing concentrations of Silwet L-720 with T_3-ALP conjugate for 30 min at 37 °C and measured the chemiluminescent signal intensity on a luminometer. As illustrated in Fig. 4, increasing surfactant concentrations did not significantly alter the kinetics or magnitude of chemiluminescent responses from hydrolysis of adamantyl 1,2-dioxetane phosphate by ALP (P = 0.89). Thus,

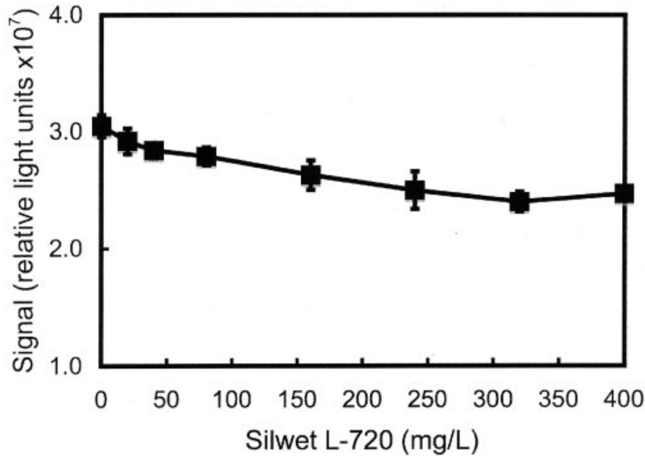

Fig. 3. Effect of various Silwet L-720 concentrations added to PBS on the chemiluminescent signal from the IMMULITE 2500 TT_3 assay.

n = 6 at each surfactant concentration. Results are presented as the mean of duplicates and SD (*error bars*). Silwet L-720 significantly decreased the chemiluminescent signal in a dose-dependent manner.

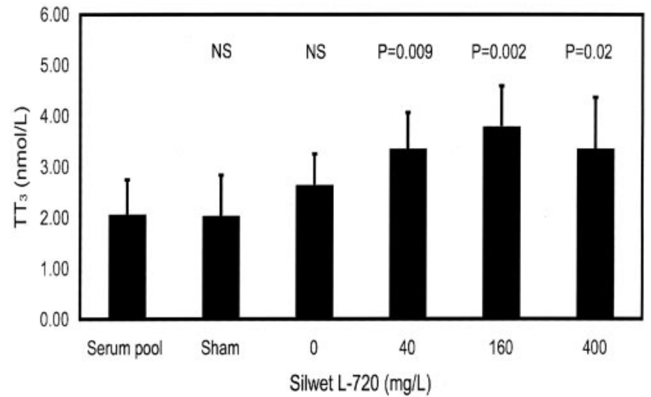

Fig. 5. Effect of pretreatment of PS beads from the IMMULITE 2500 TT_3 assay with Silwet L-720 surfactant on serum TT_3 measurements.

n = 6 at each surfactant concentration. Results are the mean of duplicates and SD (*error bars*). *NS*, not significant.

these findings demonstrate that the surfactant does not directly affect the activity of the T_3-ALP conjugate or the generation of the chemiluminescent signal.

Effect of pretreatment of PS beads with surfactant on serum TT_3 measurements

We next examined the effect of Silwet L-720 on the PS solid support used in the IMMULITE 2500 analyzer. Pretreatment of PS beads with increasing concentrations of Silwet L-720 surfactant for 30 min at room temperature markedly increased the apparent TT_3 concentration in a serum pool from 2.65 nmol/L at 0 mg/L to a peak of 3.79 nmol/L at 160 mg/L and 3.36 nmol/L at 400 mg/L surfactant (Fig. 5). By unpaired *t*-test, the apparent mean serum TT_3 concentrations from PS beads incubated with 40, 160, and 400 mg/L Silwet L-720 surfactant were all significantly higher than that of a serum pool from glass tubes (Fig. 5). The results obtained with untreated beads (sham) and 0 mg/L Silwet L-720 surfactant were not significantly different from the results obtained with the serum pool ($P > 0.05$; Fig. 5). Hence, pretreatment of PS beads with Silwet L-720 surfactant falsely increased the TT_3 values compared with nonpretreated beads, which suggests that the surfactant may be interfering with the assay either by binding to the PS beads and/or interfering with the anti-T_3 capture antibody.

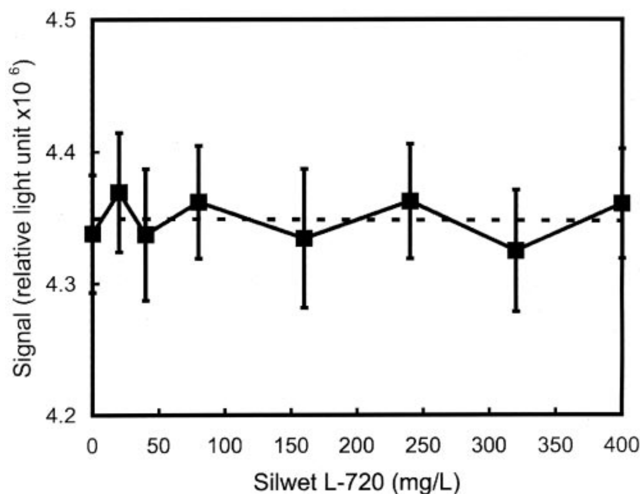

Fig. 4. Effect of Silwet L-720 surfactant on the T_3-ALP conjugate activity from the IMMULITE 2500 TT_3 assay.

n = 6 at each surfactant concentration. Results are presented as the mean of duplicates and SD (*error bars*). *Dashed line*, regression line.

Effect of surfactant on desorption of capture antibody from PS solid-support beads

To determine whether Silwet L-720 surfactant desorbs anti-T_3 antibodies from PS beads, we performed SDS–polyacrylamide gel electrophoresis and immunoblotting on solutions that were incubated at 37 °C for 30 min with PS beads and different concentrations of surfactant. As expected, a 50-kDa mouse IgG heavy chain band was detected when the beads were treated with 10 g/L SDS (Fig. 6, lane 1) but not in the empty control lane (Fig. 6, lane 2). No bands were evident when the PS beads were incubated with the PBS negative control solution (Fig. 6, lane 3). Bands corresponding to the heavy chain of mouse

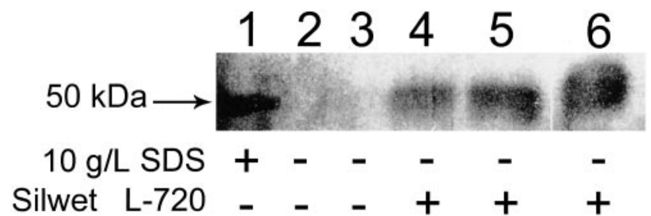

Fig. 6. SDS–polyacrylamide gel electrophoresis and Western blot analysis of antibodies from IMMULITE 2500 TT_3 assay PS beads with various concentrations of Silwet L-720 surfactant.

Blots were revealed with sheep anti-mouse antibodies. *Lane 1*, positive control (10 g/L SDS treatment of PS beads); *lane 2*, empty lane (no solutions); *lane 3*, negative control (PBS); *lanes 4–6*, 20, 40, and 240 mg/L, respectively, of Silwet L-720 incubated with the PS beads at 37 °C for 30 min.

IgG were, however, detected after treatment of the beads with 20 (lane 4), 40 (lane 5), or 240 (lane 6) mg/L Silwet L-720 surfactant, respectively. These results indicate that Silwet L-720 surfactant can desorb capture antibodies from the surface of the PS beads supplied with the IMMULITE 2500 TT_3 assay, which would be predicted to lead to a falsely increased result for competitive-type assays.

Discussion

Although evacuated blood collection tubes have been used for the collection of blood specimens for many years, it is important to recognize that tube additives, such as Silwet L-720, can potentially interact with the specimen and alter test results. Silwet L-720, the silicone surfactant used by BD for coating the interior of their blood collection tubes *(17, 20)*, is a member of a family of nonionic silicone surfactants that contain hydrophilic polyoxyalkylene chains. Typically, they exist as homopolymers or copolymers of polyoxyethylene and polyoxypropylene and are attached to a hydrophobic polydimethylsiloxane backbone *(1, 21–23)*. The molecular structure of Silwet L-720 can be either comb-like, with the polyoxyalkylene chain side-grafted on a polydimethylsiloxane backbone, or linear, arranged with either the AB or ABA type configuration, with A representing a polyoxyalkylene hydrophile and B a polydimethylsiloxane hydrophobe *(1, 21–23)*. The polydimethylsiloxane moiety of Silwet L-720 adsorbs to hydrophobic surfaces such as the plastic (i.e., polyethylene terephthalate) in blood collection tube walls, whereas the hydrophilic, polyalkylene oxide moiety faces outward toward the specimen and prevents erythrocyte adherence *(1, 3, 21–23)*. The findings from this study demonstrate that the addition of increasing concentrations of Silwet L-720 (0 to 400 mg/L) to a serum pool from glass tubes significantly increased the apparent TT_3 concentration when measured on the IMMULITE 2500 analyzer (Fig. 1), with a magnitude similar to that reported for the bias with the BD blood collection tubes *(8)*. Interestingly, the TT_3 concentration did not increase in the presence of the surfactant when measured on the AxSYM analyzer (Fig. 1B), which is consistent with our previous study showing a nonsignificant effect of BD collection tubes on the AxSYM analyzer *(8)*. There are several possible explanations for the above observations. It is conceivable that the anti-T_3 antibodies on the surface of the AxSYM microparticles are more resistant to desorption by the surfactant because of differences in solid support and/or conjugation. Differences in the competitive immunoassay format between the two analyzers could also explain the different responses of the two assays to the surfactant. The IMMULITE 2500 immunoassay for TT_3 is a simultaneous 1-step assay *(18)*, whereas the AxSYM uses a sequential 2-step assay *(24)*. The additional washing step with the AxSYM immunoassay format may more effectively remove the surfactant and prevent its binding to the microparticles and/or antibodies on the surface of the microparticle. The incubation time of the serum with surfactant from blood collection tubes and the microparticles coated with anti-T_3 antibodies from the AxSYM assay is considerably shorter than the 30-min incubation in the IMMULITE 2500 TT_3 assay, thus providing less opportunity for the surfactant to interfere with the microparticle and/or the antibodies on the surface of the microparticle. Finally, differences in the amounts or types of antibodies used for the TT_3 assays could account for the differences in the two assays.

ALP, the reporter enzyme used in the IMMULITE 2500 analyzer, is a metalloenzyme that requires for activity the binding of zinc to the active site as well as other divalent cations, such as magnesium *(25)*. In the case of IMMULITE 2500, a chemiluminescent substrate is used for ALP. It is well known that various components of serum or urine, even in minute amounts, can interfere significantly with photon emission in chemiluminescent reactions *(26)*. Results from this study (Fig. 4) reveal, however, that the surfactant had no effect on the ALP reporter enzyme, which was not completely unexpected because our previous study had shown that only a small number of IMMULITE 2000 assays (progesterone, TT_3, cortisol, TBG, TT_4, insulin) gave a positive bias ranging from 11.9% to 38.6% with BD SST compared with glass blood collection tubes *(8)*. In addition, ALP is frequently used in many other immunoassay systems, such as the AxSYM, which was not affected by the interference with the BD blood collection tubes *(8)*.

The adsorption of proteins on PS surfaces has been studied extensively *(27)*. The findings from these studies have revealed that the PS surface characteristics such as hydrophobicity, surface charge, and coadsorption of or exchange with surfactants, copolymers, and other proteins are important factors for the strength of the noncovalent binding of proteins. These factors are also well known to be particularly important to the stability and specificity of antibodies in immunoassays *(28)*. It is also evident that the amount adsorbed, the orientation, and the conformation of antibodies on the PS beads is very important to the performance of immunoassays *(28)*. It is conceivable that the surfactant may also affect the assay by one of the other potential mechanisms, such as by denaturation of the anti-T_3 antibodies on the surface of the PS bead *(29)*. Previous studies have shown that polydimethylsiloxanes, with their lipophilic qualities, can attract and denature or induce conformational changes in proteins *(30–33)*. Thus, any component in the serum that interferes with the interaction of antibodies with the surface of the PS bead may affect the performance of immunoassays. Because of the competitive format for the TT_3 assay, any of the above-hypothesized mechanisms assay will lead to a decrease in T_3-ALP conjugate binding and chemiluminescent signal and, subsequently, falsely increase the measured serum TT_3 concentration.

As shown in Fig. 6, increasing the concentration of surfactant was found to desorb mouse anti-T_3 IgG on the

surface of the PS beads. Surfactants are known to adsorb strongly to hydrophobic surfaces such as the PS beads, and they potentially can displace larger, less hydrophobic molecules, such as antibodies *(29, 34, 35)*. In addition to Silwet L-720, we also tested the detergents Tween 20 and SDS on the TT_3 assay on the IMMULITE 2500 analyzer and found no effect with Tween 20 but a marked increase in TT_3 with SDS. (data not shown). This is consistent with the common use of Tween 20 as a mild surfactant in the wash solutions of ELISAs and with the dissociation of the capture antibody observed with SDS in Fig. 6. Several competitive and noncompetitive immunoassays that were either unaffected or affected by collection of serum into BD tubes from our previous study *(8)* were examined for the effect of the Silwet L-720 surfactant (Fig. 2). The immunoassays that showed a significant positive bias with BD blood collection tubes (progesterone, cortisol, TT_4, and TBG) in our previous study demonstrated a significant increase with increasing concentrations of surfactant added to a serum pool from glass tubes (Fig. 2, A, B, C, and D, respectively). The immunoassays that were unaffected by the BD blood collection tubes (SHBG and TSH) in our previous study did not show a significant increase with increasing concentrations of surfactant added to serum pools (Fig. 2, E and F, respectively). The reason for the difference in the response to the surfactant by the different assays is most likely related to their different formats. Noncompetitive assays use excess capture and detector antibodies; therefore, they would be expected to be less affected by the partial desorption from the solid support or possible denaturation of antibodies. The reason for the various degrees of interference at the same surfactant concentration in the competitive assays may be attributable to differences in the strength of noncovalent binding of the antibodies to the different types of solid phase used for each type of assay. In addition, the sample volumes differ considerably among assays, which would change the final concentration of surfactant for the different immunoassays.

In conclusion, the present study shows a significant effect of the Silwet L-720 surfactant in BD blood collection tubes on the IMMULITE 2500 TT_3 and other immunoassays. The surfactant present in the serum promoted the desorption of anti-T_3 antibodies from the surface of the PS beads, thus lowering the chemiluminescent signal and falsely increasing the TT_3 concentration. Although they are potentially an important source of preanalytical errors, detection and prevention of interferences from blood collection tube additives is a challenging problem for most clinical laboratories. In addition to performing a careful comparison study in which they change tubes, clinical laboratorians should have a heightened awareness of this problem and should be vigilant for any possible changes in assay performance with new lots of tubes and/or any other types of tube changes. Results from this study also reveal that the identification of suitable tube surfactants should involve assessment of the ability of the surfactant to cause desorption and/or possible denaturation of antibodies from the various solid supports used for immunoassays. Improving the affinity of antibodies to solid supports, or perhaps covalent attachment of antibodies, could also minimize this type of problem in the future and may make immunoassays more robust to other types of interferences.

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