Evaluation of BD Vacutainer SST™ II Plus Tubes for Special Proteins Testing

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In the past few years, because of providing a closed system that allowed for collection, transport, processing, sampling, and storage of specimens, serum separator tubes gained widespread acceptance gradually in China. However, some limitations associated with gel tubes had been observed, for example, gel and analyte stability. In order to circumvent these problems, a new tube (BD Vacutainer® SST™ II Plus (BD SST™ II Plus)) containing a new gel was released by BD with respect to analyte and gel stability. We investigated the performance of BD SST™ II Plus tubes for special proteins testing using BD Vacutainer® Serum Glass Tubes (BD Serum Glass) as controls.

Key words: BD SST™ II Plus Tubes; serum separator tube; stability; barrier gel; special proteins

Equivalence between these two types of tubes was demonstrated for all analytes at initial time, and data for all analytes except complement 3 (C3) and complement 4 (C4) indicated comparable stability over time in these two types of tubes. Concentration of C3 and C4 tended to increase with preservation time up to 72 hr in BD Serum Glass tubes. The stability of C3 and C4 was better in BD SST™ II Plus, which was demonstrated at timepoints up to 48 hr. We conclude that BD SST™ II Plus was suitable for collection and storage of samples for special proteins testing. *J. Clin. Lab. Anal.* 25:203–206, 2011. © 2011 Wiley-Liss, Inc.

INTRODUCTION

Standard laboratory practice for serum analyte testing was to collect blood into the tubes and centrifuge these tubes after sufficient clotting has occurred. The National Committee for Clinical Laboratory Standards (NCCLS) recommended processing of the tube to take place within 2 hr of blood collection to minimize preanalytical errors due to prolonged contact of serum with cells (1). Plain blood collection tubes suffered from many limitations. A complete separation between serum and blood cells may not be achieved; moreover, hemolysis of specimens upon prolonged storage was a serious problem. In the past few years, because of providing a closed system that allowed for collection, transport, processing, sampling, and storage of specimens, serum separator tubes gained widespread acceptance gradually in China. This type of tubes contained a gel barrier that moved to the cell/serum interface during centrifugation based on a density gradient and formed an impermeable barrier between the serum and the clot, and then facilitated rapid separation of serum from cellular constituents of blood and also prevented hemolysis upon prolonged storage. However, some limitations associated with gel tubes had been observed,

for example, gel and analyte stability (2,3). In order to circumvent these problems, BD released a new serum separator tube containing a new gel (BD Vacutainer® SST™ II Plus (BD SST™ II Plus)) which was superior to the existing gel (4). In this study, we investigated the comparability of special proteins concentrations using BD Vacutainer® SST™ II Plus and BD Vacutainer® Serum Glass Tubes (BD Serum Glass) for specimens' collection, and evaluated the stability of these analytes when specimens were stored in these two types of tubes.

MATERIALS AND METHODS

Samples

Whole blood samples from 30 outpatients who consent to participate in this study were taken routinely. BD Vacutainer® SST™ II Plus Tubes (BD SST™ II Plus), which contain a thixotropic gel barrier, and BD

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Vacutainer® Serum Glass Tubes (BD Serum Glass) were used for specimen collection. Samples were obtained from a single puncture to the two tube types, processed in parallel, and analyzed in adjacent sample positions on the analyzer. All patients were over 18 years old, with a mix of males and females.

Specimens were centrifuged at 1,300g for 10 min after clotting for 30 min. Serum was then tested for selected analytes at initial time (t_0 , within 2 hr of collection). Specimens were recapped and stored at 4°C, and then re-tested at 6 hr (t_6), 24 hr (t_{24}), 48 hr (t_{48}), and 72 hr (t_{72}) (from the time of centrifugation).

Reagents and Apparatus

Special proteins, including immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), complement 3 (C3), complement 4 (C4), anti-Streptolysin O (ASO), rheumatoid factor (RF), and C-reactive protein (CRP), were measured by the fully automated immunoassay, run on the IMMAGE 800 immunochemistry analyzer (Beckman Coulter Inc., Fullerton, CA), using the accessory reagent kits.

According to the manufacturer, calibration of the assay system should be performed once a month and a new lot of reagents used each time. Three levels of quality control were performed each day. The calibration was repeated and controls were retested whenever controls were outside the expected range.

Statistical Analysis

Statistical calculation was performed using MedCalc ver 6 (Medcalc software, Mariakerke, Belgium). The results was compared using paired t test and tested for correlation with linear regression analysis and with the Bland–Altman analysis for concordance (5). The P values were two-sided, and the term statistically significantly implies a P value <0.05 .

RESULTS

Comparison of Special Proteins Concentrations Using the Two Types of Tubes for Specimens Collection

The results of RF assay were not analyzed because there were 27 subjects who had RF concentration below the detection limit (RF <20 IU/ml). For ASO, the number of valid data was 23 (seven subjects had ASO <25 IU/ml).

There were no statistical differences between serum levels of special proteins using BD SST™ II Plus or BD Serum Glass for specimens collection ($P>0.05$). Regression equation for IgA was [BD SST™ II Plus (Y)–BD Serum Glass (X): $Y = -0.0061 + 0.9965X$ with

a Spearman correlation coefficient (r) of 0.9992 ($P<0.0001$). The average bias of IgA using the Bland–Altman analysis was -0.6% , and the 95% confidence interval (CI) of the difference bias was -3.9 to 2.7% , which was considered to be clinically acceptable. Regression equation for IgG was $Y = 0.1808 + 0.9864X$, $r = 0.9946$ ($P<0.0001$) and the average bias was -0.1% . Regression equation for IgM was $Y = 0.0245 + 0.9719X$, $r = 0.9960$ ($P<0.0001$) and the average bias was 0.6% . Regression equation for C3 was $Y = -0.0197 + 1.0092X$, $r = 0.9949$ ($P<0.0001$) and the average bias was -0.9% . Regression equation for C4 was $Y = 0.0144 + 0.9269X$, $r = 0.9866$ ($P<0.0001$) and the average bias was -1.2% . Regression equation for ASO was $Y = 2.5167 + 0.9414X$, $r = 0.9930$ ($P<0.0001$) and the average bias was -2.1% . Regression equation for CRP was $Y = -0.1024 + 0.9989X$, $r = 0.9985$ ($P<0.0001$) and the average bias was -2.9% .

Comparison of Serum Analytes Stability Using the Two Types of Tubes for Specimens Collection

Table 1 showed the average within-tube bias and 95% CI for the evaluation of storage stability for each tube. Data for all analytes except C3 and C4 indicated comparable stability over time in BD SST™ II Plus and BD Serum Glass tubes. The concentrations of C3 and C4 tended to increase with preservation time up to 72 hr in BD Serum Glass tubes. The average within-tube bias for C3 concentration after 6 hr vs. instant levels was determined to be 3.2% , then 6.5% , 17.7% and 17.1% for 24 hr, 48 hr, and 72 hr. The average within-tube bias for C4 concentration after 6 hr vs. instant levels was determined to be 2.7% , then 6.8% , 16.2% and 16.5% for 24 hr, 48 hr, and 72 hr. The stability of C3 and C4 was better in BD SST™ II Plus than in BD Serum Glass tubes, which was demonstrated at timepoints up to 48 hr in BD SST™ II Plus. The average within-tube bias for C3 after 6 hr, 24 hr, and 48 hr vs. instant levels was determined to be 0.8% , -0.1% , and 1.6% . The average within-tube bias for C4 after 6 hr, 24 hr, and 48 hr vs. instant levels was determined to be 0.5% , 0.2% , and 1.3% . The average within-tube bias for C3 and C4 concentrations after 72 hr vs. instant levels were all over 10.0% .

DISCUSSION

Recognizing and controlling factors that contribute to preanalytical variability was crucial for the interpretation of analyte testing results. It was strongly recommended that analysis must occur within 2 hr of blood collection to minimize preanalytical errors due to prolonged contact of serum with cells (1). Since the 1950s, glass tubes have been the standard device for blood collection for clinical laboratory testing.

TABLE 1. The Average Within-Tube Bias of Special Proteins Concentrations After Storage

Analytes	n	Average within-tube bias (%) for BD Serum Glass (95% confidence interval)					Average within-tube bias (%) for BD SST™ II Plus (95% confidence interval)				
		t_0-t_0	$t_{24}-t_0$	$t_{48}-t_0$	$t_{72}-t_0$	$t_{72}-t_0$	t_0-t_0	$t_{24}-t_0$	$t_{48}-t_0$	$t_{72}-t_0$	$t_{72}-t_0$
IgA (g/l)	30	0.5 (-0.4, 1.4)	1.0 (-0.3, 2.3)	0.3 (-0.8, 1.4)	0.1 (-1.1, 1.3)	0.1 (-1.1, 1.3)	0.2 (-0.5, 0.9)	0.4 (-0.4, 1.2)	-0.1 (-0.7, 0.5)	0.7 (-0.3, 1.7)	0.7 (-0.3, 1.7)
IgG (g/l)	30	0.2 (-1.1, 1.5)	-0.3 (-1.5, 0.9)	-0.1 (-0.9, 0.7)	-0.4 (-1.8, 1.0)	-0.4 (-1.8, 1.0)	-0.2 (-1.3, 0.9)	-0.3 (-1.7, 1.1)	-0.5 (-2.2, 1.2)	0.0 (-1.0, 1.0)	0.0 (-1.0, 1.0)
IgM (g/l)	30	0.3 (-1.1, 1.7)	0.5 (-1.5, 2.5)	-0.2 (-1.6, 1.2)	-0.1 (-1.4, 1.2)	-0.1 (-1.4, 1.2)	0.4 (-0.3, 1.1)	-0.5 (-2.2, 1.2)	0.8 (-0.5, 2.1)	0.3 (-1.4, 2.0)	0.3 (-1.4, 2.0)
C3 (g/l)	30	3.2 (1.1, 5.3)	6.5 (1.8, 11.2)	17.7 (6.2, 29.2)	17.1 (5.6, 28.6)	17.1 (5.6, 28.6)	0.8 (-1.4, 3.0)	-0.1 (-4.2, 4.0)	1.6 (-0.2, 3.4)	13.8 (3.2, 24.4)	13.8 (3.2, 24.4)
C4 (g/l)	30	2.7 (1.0, 4.4)	6.8 (1.4, 12.2)	16.2 (5.6, 26.8)	16.5 (5.4, 27.6)	16.5 (5.4, 27.6)	0.5 (-1.1, 2.1)	0.2 (-2.0, 2.4)	1.3 (-0.5, 3.1)	12.2 (2.6, 21.8)	12.2 (2.6, 21.8)
ASO (IU/ml)	23	-1.0 (-7.6, 5.6)	-0.6 (-5.9, 4.7)	-0.7 (-6.7, 5.3)	0.1 (-5.2, 5.4)	0.1 (-5.2, 5.4)	-0.4 (-4.9, 4.1)	-0.7 (-6.2, 4.8)	0.6 (-2.3, 3.5)	0.3 (-3.1, 3.7)	0.3 (-3.1, 3.7)
CRP (mg/dl)	30	1.2 (-4.1, 6.5)	0.7 (-2.5, 3.9)	1.1 (-3.6, 5.8)	0.9 (-3.0, 4.8)	0.9 (-3.0, 4.8)	0.1 (-1.8, 2.0)	0.5 (-2.2, 3.2)	-0.1 (-3.5, 3.3)	0.7 (-3.2, 4.6)	0.7 (-3.2, 4.6)

Compared to glass tubes, plastic blood collection tubes reduced the potential of tube breakage and specimen spillage, thereby reducing the potential of exposure to blood borne pathogens. Approximately 35 years ago, serum separator tubes were first introduced to laboratories by Becton Dickinson and Company (BD), which facilitated rapid separation of serum from blood cellular constituents and also prevented hemolysis on prolonged storage. Other advantages of a serum separator tubes included analyte stability, primary tube sampling and storage, and reduced need for aliquot tubes. The analyte compatibility of serum separator tubes and analyte stability in these tubes had been well documented in the past (6,7). However, some limitations associated with gel tubes. One limitation was that the gel contained within serum separator tubes had the potential for adsorbing analytes of interest (8,9) or releasing material that may interfere with analytes (10). Another limitation of serum separator tubes was that certain analytes, such as therapeutic drugs and steroid hormones, may be instable when serum were stored on the barrier for long periods of time (2,3). Moreover, the instability of the gel itself and the presence of gel globules or an oily film in the serum were observed under extreme temperature conditions, which may cause probe clogged and then instrument down time. In order to circumvent these problems, a new serum separator tube (BD SST™ II Plus) containing a new gel was released by BD with respect to analyte and gel stability (4). The new gel was composed of a different polymeric material and provided a better barrier between serum and cellular constituents of blood as well as a wider centrifugation range. This type of tube was available in Europe, Latin America, and Asia Pacific.

In this study, the performance of BD SST™ II Plus tubes for special proteins testing was evaluated using BD Serum Glass tubes as controls. Testing was performed at initial time and also after various storage time intervals up to 72 hr in order to assess analyte stability within the tubes. Equivalence between the BD SST™ II Plus and BD Serum Glass tubes was demonstrated for all analytes at t_0 . Also, data for all analytes except C3 and C4 indicated comparable stability over time in BD SST™ II Plus and BD Serum Glass tubes. The concentrations of C3 and C4 tended to increase with preservation time up to 72 hr in BD Serum Glass tubes. The main reason of this increase was that in vitro activation of C3 and C4 may take place in the serum during the storage intervals, and an overestimation of total C3 and C4 was observed (11,12). The stability of C3 and C4 was better in BD SST™ II Plus than in BD Serum Glass tubes, which was demonstrated at timepoints up to 48 hr in BD SST™ II Plus. However, after 72 hr, the average

within-tube bias for C3 and C4 concentrations vs. instant levels were all over 10.0%. So, conditions of collection and storage of samples must be optimized for the accurate definition of activation of the complement cascade. If C3 and C4 concentrations cannot be analyzed within 48 hr, serum should be stored at -20 or -70°C .

It was strongly recommended that specimens collected and stored in serum separator tubes be evaluated in the laboratory's own environment because depending on the specimen collecting, handling, and processing conditions, clinically significant changes in analyte concentrations may occur in serum separator tubes. According to the results observed in this study, we conclude that BD SST^{TM} II Plus was suitable for collection of blood and storage of serum for special proteins testing in a hospital setting.

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