Evaluation of BD Vacutainer SST™ II Plus Tubes for Common Tumor Marker Tests by Roche Diagnostics Modular E 170 Analyzer

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Serum separator tubes were introduced 35 years ago and were widely used in the clinical laboratory in China for routine collection of blood because of providing a closed system that allowed for collection, transport, processing, sampling, and storage of specimens. This type of tubes facilitated rapid separation of serum from cellular constituents of blood and also prevented hemolysis upon prolonged storage. However, there were some limitations associated with gel tubes (i.e., gel and analyte stability). In order to circumvent these problems, BD released a new serum separator tube containing a new gel (BD SST™ II Plus). We investigated the

performance of BD SST™ II Plus tubes for tumor marker tests using BD Serum Glass tubes as controls. Equivalence between the BD SST™ II Plus and BD Serum Glass tubes was demonstrated for all analytes at initial time. Also, all analytes remained stable when stored in BD SST™ II Plus tubes up to 72 hr. Concentration of neuron-specific enolase tended to increase with preservation time up to 72 hr in BD Serum Glass tubes. We conclude that BD SST™ II Plus was suitable for collection of blood and storage of serum for tumor marker tests. J. Clin. Lab. Anal. 24:418–421, 2010.

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Key words: BD SST™ II Plus tubes; serum separator tube; stability; barrier gel; tumor marker

INTRODUCTION

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 owing 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, serum separator tubes gained widespread acceptance gradually in China because of providing a closed system that allowed for collection, transport, processing, sampling, and storage of specimens. This type of tubes contained a gel barrier that moved to the cell/serum interface during centrifugation based on a density gradient and facilitated rapid separation of serum from cellular constituents of blood, and also prevented hemolysis upon prolonged storage. However, there were some limitations associated with gel tubes (i.e., 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), which is superior to the existing gel (4). In this study, we investigated the comparability of tumor markers concentrations using BD Vacutainer® SST™ II Plus and BD Vacutainer® Serum Glass Tubes (all by Beckton Dickinson and company, Franklin Lakes, NJ) for specimens' collection, and evaluated the stability of these analytes when specimens were stored in these two types of tubes.

MATERIALS AND METHODS

Samples

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. Whole blood samples from 30 outpatients who consented to participate in this study were obtained from a single puncture to the two tube types, processed in parallel, and analyzed in adjacent sample positions on the analyzers. All patients were more than 18 years old, with a mix of males and females.

Specimens were centrifuged at $1,300 \times g$ 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 retested at 8 hr (t_8), 24 hr (t_{24}), 48 hr (t_{48}), and 72 hr (t_{72}) (from the time of centrifugation).

Reagents and Apparatus

Tumor markers, including alpha-fetoprotein (AFP), carcino-embryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), carbohydrate antigen 125 (CA125), carbohydrate antigen 15-3 (CA15-3), total prostate specific antigen (TPSA), free prostate-specific antigen (FPSA), neuron-specific enolase (NSE), cytokeratin fragment antigen 21-1 (CYFRA21-1), and carbohydrate antigen 72-4 (CA72-4), were measured by the fully automated electrochemiluminescent immunoassay, run on the Modular E 170 analyzer (Roche Diagnostics GmbH, Mannheim, Germany), 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. Two 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 were 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 of <0.05 .

RESULTS

Comparison of Tumor Markers Concentrations Using the Two Types of Tubes for Specimens Collection

There were no statistical differences between serum levels of tumor markers using BD SST™ II Plus or BD Serum Glass for specimens collection ($P > 0.05$). Regression equation for AFP was [BD Serum Glass (Y)–BD

SST™ II Plus (X)]: $Y = 0.0233 + 0.9967X$ with a Spearman correlation coefficient (*r*) of 0.9956 ($P < 0.0001$). The average bias of AFP using the Bland–Altman analysis was -0.8% , and the 95% confidence interval (CI) of the difference bias was -9.8 to 8.3% , which was considered to be clinically acceptable. Regression equation for CEA was: $Y = -0.0478 + 1.0089X$, $r = 0.9999$ ($P < 0.0001$) and the average bias was 1.3% . Regression equation for CA19-9 was: $Y = -0.0132 + 1.0102X$, $r = 0.9999$ ($P < 0.0001$) and the average bias was -1.4% . Regression equation for CA125 was: $Y = 0.0146 + 1.0087X$, $r = 0.9997$ ($P < 0.0001$) and the average bias was -0.7% . Regression equation for CA15-3 was: $Y = -0.0011 + 1.0097X$, $r = 0.9965$ ($P < 0.0001$) and the average bias was -1.2% . Regression equation for TPSA was: $Y = 0.0231 + 0.9914X$, $r = 0.9999$ ($P < 0.0001$) and the average bias was -0.8% . Regression equation for FPSA was: $Y = 0.0176 + 0.9853X$, $r = 0.9998$ ($P < 0.0001$) and the average bias was -2.7% . Regression equation for NSE was: $Y = 0.4847 + 0.9484X$, $r = 0.9933$ ($P < 0.0001$) and the average bias was 0.7% . Regression equation for CYFRA21-1 was: $Y = -0.0081 + 0.9992X$, $r = 0.9999$ ($P < 0.0001$) and the average bias was 0.6% . Regression equation for CA72-4 was: $Y = -0.0366 + 0.9983X$, $r = 0.9999$ ($P < 0.0001$) and the average bias was 1.4% .

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 NSE indicated comparable stability over time in BD SST™ II Plus and BD Serum Glass tubes. The concentration of NSE tended to increase with preservation time up to 72 hr in BD Serum Glass tubes. The average within-tube bias for NSE concentration after 8 hr vs. instant levels was determined to be 4.6% , then 9.2 , 12.5 , and 19.7% for 24, 48, and 72 hr. The stability of NSE was better in BD SST™ II Plus than in BD Serum Glass tubes. The average within-tube bias after 6, 24, 48, and 72 hr vs. instant levels was determined to be -0.9 , 0.2 , 1.6 , and 1.2% .

DISCUSSION

Glass evacuated blood-drawing tubes have been the standard device for obtaining blood from patients for clinical laboratory testing since the 1950s. Plastic blood collection tubes reduced the potential for tube breakage and specimen spillage compared with glass tubes, thereby reducing the potential for exposure to blood-borne pathogens. Serum separator tubes, which were first introduced to laboratories approximately 35 years

TABLE 1. The Average Within-Tube Bias of Tumor Markers 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_8-t_0	$t_{24}-t_0$	$t_{48}-t_0$	$t_{72}-t_0$	t_8-t_0	$t_{24}-t_0$	$t_{48}-t_0$	$t_{72}-t_0$
AFP (ng/ml)	30	-0.5 (-1.1, 0.1)	0.1 (-0.3, 0.6)	0.2 (-0.8, 1.1)	-0.3 (-1.0, 0.4)	-0.4 (-0.9, 0.1)	-0.2 (-0.7, 0.3)	0.2 (-0.7, 1.2)	-0.1 (-0.8, 0.7)
CEA (ng/ml)	30	0.0 (-0.4, 0.4)	0.1 (-0.6, 0.8)	0.1 (0.0, 0.2)	-0.1 (-1.5, 1.3)	0.2 (-0.4, 0.7)	0.1 (-0.2, 0.3)	-0.1 (-0.4, 0.2)	0.1 (-1.1, 1.3)
CA19-9 (U/ml)	30	0.3 (-0.1, 0.7)	0.2 (-1.0, 1.3)	-0.1 (-0.8, 0.6)	-0.4 (-1.2, 0.4)	0.2 (0.1, 0.3)	-0.3 (-0.7, 0.1)	-0.7 (-1.2, -0.2)	0.0 (-0.7, 0.6)
CA125 (U/ml)	30	0.8 (-0.2, 1.8)	1.1 (0.2, 2.0)	0.0 (-1.0, 1.0)	-0.3 (-1.2, 0.7)	-0.1 (-1.4, 1.2)	0.9 (-1.2, 2.9)	0.5 (-1.4, 2.3)	0.7 (-0.1, 1.4)
CA15-3 (U/ml)	30	1.0 (-0.3, 2.2)	0.4 (-0.4, 1.2)	-0.2 (-1.5, 1.1)	0.7 (-0.5, 1.9)	0.2 (0.1, 0.3)	-0.3 (-0.7, 0.1)	-0.7 (-1.2, -0.2)	0.6 (-0.3, 1.5)
TPSA (ng/ml)	30	1.3 (-0.1, 2.7)	0.2 (-0.4, 0.9)	0.5 (-0.8, 1.8)	-0.1 (-0.2, 0.1)	0.6 (-0.3, 1.6)	1.5 (0.4, 2.6)	0.7 (-0.3, 1.7)	0.8 (-0.5, 2.1)
FPSA (ng/ml)	30	0.3 (-0.2, 0.8)	0.9 (-0.4, 2.1)	0.1 (-0.5, 0.7)	1.4 (-0.2, 3.0)	0.7 (0.1, 1.3)	-0.2 (-0.6, 0.2)	0.8 (-1.0, 2.6)	1.1 (-0.5, 2.7)
NSE (ng/ml)	30	4.6 (2.1, 7.0)	9.2 (2.8, 15.6)	12.5 (4.6, 20.5)	19.7 (8.8, 30.6)	-0.9 (-2.2, 0.4)	0.2 (-0.6, 1.0)	1.6 (0.3, 2.9)	1.2 (-0.2, 2.6)
CYFRA21-1 (ng/ml)	30	0.6 (-0.4, 1.7)	1.0 (-0.2, 2.2)	0.4 (-0.8, 1.6)	0.0 (-1.2, 1.2)	1.2 (0.2, 2.2)	0.3 (-0.4, 1.0)	-0.2 (-0.7, 0.3)	0.6 (-0.2, 1.4)
CA72-4 (U/ml)	30	0.1 (-1.1, 1.3)	0.7 (-0.5, 1.9)	-0.2 (-0.6, 0.2)	-0.4 (-1.5, 0.7)	1.4 (0.3, 2.5)	0.5 (-1.2, 2.2)	0.7 (-1.5, 2.9)	0.2 (-0.4, 0.8)

ago by Becton Dickinson and Company (BD), facilitated rapid separation of serum from cellular constituents of blood 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. Laessig et al. (6) tested the initial serum-separator tube for 81 analytes and methods, primarily using serum from apparently healthy individuals. Also, analyte stability in serum separator tubes had been documented in the past (7,8). One disadvantage of gel separator tubes was that the gel contained within such tubes had the potential for adsorbing analytes of interest (9,10) or releasing material that may interfere with assay chemistries (11). Another disadvantage of gel separator tubes was the instability of certain analytes when serum was stored for long periods of time on the barrier (2,3), such as therapeutic drugs and steroid hormones. Moreover, the gel itself was instable under extreme temperature conditions, commonly recognized as the presence of gel globules or an oily film in the serum. These may cause probe clogged and then instrument down time. In order to circumvent these problems, BD released a new serum separator tube containing a new gel (BD SST™ II Plus) with respect to analyte and gel stability (4). This type of tube was available in Europe, Latin America, and Asia Pacific, and 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.

In this study, the performance of BD SST™ II Plus tubes for tumor marker tests 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, all analytes remained stable when stored in BD SST™ II Plus tubes up to 72 hr. The concentration of NSE tended to increase with preservation time up to 72 hr in BD Serum Glass tubes. The main reason for this change was that, during the storage intervals, hemolysis may occur in BD Serum Glass tubes and NSE contained in erythrocytes released into serum (12).

Depending on the specimen collecting, handling, and processing conditions, clinically significant changes in analyte concentrations may occur in serum separator tubes. It was recommended that specimens collected and stored in serum separator tubes be evaluated in the laboratory's own environment. Through this study, we concluded that BD SST™ II Plus was suitable for collection of blood and storage of serum for common tumor marker tests in a hospital setting.

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