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ABSTRACT

BD Vacutainer® SST™ II *Advance* Tubes were evaluated for performance at alternate centrifugation conditions consisting of higher g force (RCF) and shorter spin time than current recommendations for general use. Tubes collected from 40 subjects were centrifuged at four evaluation conditions consisting of a balanced matrix of two RCF settings (1800, 3000 x g) and two time settings (4 min, 5 min). Performance at these conditions was compared vs. tubes centrifuged at a control condition of 1300 x g for 10 minutes. All tubes used in this study were 13x100 mm and were centrifuged in swing bucket centrifuges at room temperature. After centrifugation, all tubes were evaluated for gel barrier thickness and percent yield of serum, and were tested for a panel of 23 routine chemistry analytes. Clinical equivalence was demonstrated for all routine chemistry analytes between each of the four evaluation conditions and the control condition. Some variation was observed for barrier thickness and percent yield of serum; both were slightly lower at the 1800 x g conditions and were considered equivalent or were greater at the 3000 x g conditions, relative to the control. The design of the study allows for data interpolation to estimate barrier thickness and percent yield of serum for centrifugation conditions spanning 4–5 minutes and 1800–3000 x g. Selection of specific centrifugation settings will depend on both centrifuge capability and the laboratory's requirements for turn-around time and gel tube performance. Centrifugation of BD Vacutainer® SST™ II *Advance* Tubes at alternate conditions may allow for substantial improvement in laboratory turn-around time and process efficiency.

INTRODUCTION

BD Diagnostics – Preanalytical Systems has sold evacuated tubes (BD Vacutainer® Tubes) for blood collection for over 50 years and serum separator tubes (BD Vacutainer® SST™) for over 25 years. BD Vacutainer® SST™ Tubes contain a thixotropic gel, which upon centrifugation moves to the serum/clot interface based on a density gradient and forms an impermeable barrier between the serum and the clot. In 2000, BD introduced a serum separator tube with a new gel barrier material (BD Vacutainer® SST™ II Plus) designed to increase compatibility with certain therapeutic drugs. To offer superior reliability of gel barrier formation under varying centrifugation conditions, in 2004 BD implemented a further improvement to the BD Vacutainer® SST™ II Plus Tube, consisting of an increase in the slope of gel in the tube prior to centrifugation. The improved product is called the BD Vacutainer® SST™ II *Advance* Tube (BD SST™ II *Advance*).

Turn–around time (TAT) and process efficiency are important performance improvement initiatives in the clinical laboratory setting. In recent years, many laboratories have implemented preanalytical or *front–end* automation in order to improve workflow and reduce test result TAT. Instrumentation is now available from several manufacturers for automating one or more of the specimen handling steps prior to analysis, including specimen sorting, centrifugation, decapping and aliquoting. Some laboratories have also combined the use of preanalytical automation with a reduction in the centrifugation time used, in order to provide further improvements in TAT and reduce the bottleneck that the centrifugation step often introduces in automated systems. In order to assist customers wishing to reduce TAT and improve laboratory process efficiency, BD conducted a study to evaluate the clinical performance of BD Vacutainer® SST™ II *Advance* Tubes at alternate centrifugation conditions involving higher g force and shortened centrifugation time. In this paper, the term "alternate" refers to centrifugation settings different from current recommendations for general use of BD Vacutainer® SST™ II *Advanc*e Tubes, which remain at 1300 – 2000 x g for 10 minutes.

OBJECTIVE

To evaluate the clinical performance of BD Vacutainer® SST™ II *Advance* Tubes at alternate centrifugation conditions involving higher g force (RCF) and shortened centrifugation time.

MATERIALS AND METHODS

Blood was collected from 40 apparently healthy adult subjects into five BD Vacutainer® SST™ II *Advance* Tubes (BD SST™ II *Advance*), 13x100 mm, 5 mL draw with BD Hemogard™ Closure. The order of tube draw (i.e., order of tubes assigned to each centrifugation condition) was randomized, and all tubes were inverted six complete times immediately after collection. Tubes were allowed to clot for 30 – 60 minutes, and then centrifuged at five different centrifugation conditions as shown in Table 1 (one tube at each condition). The control condition was 1300 x g for 10 minutes in a conventional floor model swing bucket centrifuge at room temperature. The four evaluation conditions involved centrifugation at room temperature in a Hettich Rotanta 46R centrifuge, which is a swing bucket centrifuge with a faster acceleration (ramp-up time) than the conventional centrifuge used (Table 1). Since only one condition could be processed at a time in the Hettich centrifuge, a randomized clot time with 10 minute interval spacing was used with the four evaluation conditions to allow these four tubes to be centrifuged in sequence. Thus, each evaluation condition had 10 tubes each with a clot time of 30, 40, 50 and 60 minutes, for a total of n=40 for each condition. To balance this distribution of clot times, the control condition had 20 tubes with a clot time of 30 minutes and 20 tubes with a clot time of 60 minutes, for a total n=40 for the control condition. After centrifugation, serum from all five tubes was assayed for 23 routine chemistry analytes using an Olympus® AU5400 chemistry analyzer. Analytes evaluated are listed in Table 2.

In addition to clinical chemistry testing, each tube was evaluated for barrier thickness and serum yield. Gel barrier thickness measurements were performed shortly after centrifugation using digital calipers. Calculation of serum yield required weighing each tube three times: (i) prior to blood collection, (ii) after blood collection, and (iii) after aliquoting of all the serum above the gel after centrifugation. The mass of blood (ii – i) and mass of serum (ii – iii) were then calculated and converted to volumes using densities of 1.06 g/mL and 1.025 g/mL for whole blood and serum, respectively. The percent yield of serum (percent volume of blood specimen expressed as serum after centrifugation) was then calculated as the ratio [volume of serum]/[volume of blood] multiplied by 100.

Table 1. Centrifugation Conditions				
Condition	**RCF (x g)**	**Time* (minutes)**	**Ramp-Up Time (min:sec)**	**Time at RCF (min:sec)**
Control	1300	10	1:05	8:55
Evaluation 1	1800	5	0:38	3:22
Evaluation 2	1800	4	0:38	4:22
Evaluation 3	3000	4	0:55	3:05
Evaluation 4	3000	5	0:55	4:05

* Total of ramp-up time (to reach desired RCF) and time spent at RCF. Does not include ramp-down time.

Table 2. Analytes	
Glucose (GLU)	Total Protein (TP)
Sodium (NA)	Albumin (ALB)
Potassium (K)	Total Bilirubin (TBIL)
Chloride (CL)	Direct Bilirubin (DBIL)
Carbon Dioxide (CO_2)	Alkaline Phosphatase (ALKP)
Urea Nitrogen (BUN)	Gamma Glutamyltransferase (GGT)
Creatinine (CREAT)	Aspartate Aminotransferase (AST)
Uric Acid (UA)	Alanine Aminotransferase (ALT)
Phosphorus (PHOS)	Lactate Dehydrogenase (LDH)
Calcium (CA)	Iron (IRON)
Cholesterol (CHOL)	Magnesium (MG)
Triglycerides (TRIG)	

DATA ANALYSIS

Data for each analyte were obtained from 40 subjects. The mean and standard deviation (which includes a between subject component) were calculated for each centrifugation condition. An analysis of variance (ANOVA) followed by multiple comparisons was also used to analyze the data. Biases representing the average of the within subject differences and intervals corresponding to 95% equivalence tests were calculated. When the clinical acceptance limit was in units, the analysis was performed on the data in original units. When the clinical acceptance limit was in percent, the analysis was performed on the log-transformed data and the bias results back-transformed into percent biases. These data are summarized in Table 3 and Table 4. Barrier thickness and serum yield at intermediate RCF and spin time (i.e., at settings between those used in the study) were estimated by linear interpolation.

RESULTS AND DISCUSSION

Table 3 shows the units and study range for each analyte, which is the range of test results obtained for each analyte in the control tube (C) for all 40 subjects. Also shown for each analyte is the mean and standard deviation at each centrifugation condition, which are listed to one more decimal place than that reported by the analyzer. Examination of Table 3 shows that for each analyte, the means are in close agreement between all centrifugation conditions. Further discussion of the data is provided below in connection with the between–tube comparisons which are shown in Table 4.

Table 3. Summary Results for BD SST™ II *Advance* Tubes (n=40)

Analyte	Units	Study Range in Control Tube	Mean ± SD				
			Control 1300 x g / 10 min	Evaluation 1 1800 x g / 5 min	Evaluation 2 1800 x g / 4 min	Evaluation 3 3000 x g / 4 min	Evaluation 4 3000 x g / 5 min
GLU	mg/dL	72 – 316	100.6 ± 38.2	101.1 ± 38.4	101.2 ± 39.0	101.1 ± 38.0	100.6 ± 38.6
NA	mmol/L	136 – 144	139.8 ± 1.8	139.7 ± 1.6	139.6 ± 2.0	140.0 ± 1.8	139.6 ± 2.0
K	mmol/L	3.6 – 4.8	4.23 ± 0.23	4.25 ± 0.24	4.27 ± 0.34	4.22 ± 0.25	4.23 ± 0.25
CL	mmol/L	100 – 108	103.6 ± 1.9	103.7 ± 2.2	103.6 ± 1.9	103.7 ± 2.0	103.6 ± 2.2
CO_2	mmol/L	18 – 27	22.5 ± 2.1	22.1 ± 2.2	22.2 ± 2.2	22.0 ± 2.2	22.2 ± 2.2
BUN	mg/dL	10 – 32	16.5 ± 4.5	16.5 ± 4.5	16.4 ± 4.6	16.6 ± 4.6	16.5 ± 4.4
CREAT	mg/dL	0.6 – 1.5	0.99 ± 0.18	1.00 ± 0.16	1.00 ± 0.17	1.00 ± 0.17	0.98 ± 0.18
UA	mg/dL	2.5 – 7.8	5.18 ± 1.30	5.18 ± 1.33	5.15 ± 1.31	5.19 ± 1.36	5.16 ± 1.36
PHOS	mg/dL	2.2 – 4.7	3.41 ± 0.61	3.36 ± 0.60	3.40 ± 0.59	3.42 ± 0.61	3.40 ± 0.62
CA	mg/dL	8.7 – 10.2	9.57 ± 0.35	9.57 ± 0.33	9.62 ± 0.34	9.59 ± 0.33	9.57 ± 0.36
CHOL	mg/dL	138 – 278	207.8 ± 32.8	207.6 ± 32.3	208.6 ± 33.5	208.6 ± 32.7	208.7 ± 32.3
TRIG	mg/dL	46 – 494	169.8 ± 104.3	169.2 ± 103.8	169.8 ± 105.1	169.4 ± 104.0	169.6 ± 104.3
TP	g/dL	6.4 – 8.0	7.26 ± 0.32	7.25 ± 0.33	7.26 ± 0.33	7.29 ± 0.33	7.28 ± 0.29
ALB	g/dL	3.9 – 5.2	4.44 ± 0.31	4.45 ± 0.29	4.45 ± 0.31	4.47 ± 0.26	4.43 ± 0.29
TBIL	mg/dL	0.21 – 1.51	0.536 ± 0.261	0.525 ± 0.246	0.521 ± 0.252	0.537 ± 0.244	0.513 ± 0.237
DBIL	mg/dL	0.04 – 0.24	0.107 ± 0.043	0.105 ± 0.042	0.106 ± 0.039	0.104 ± 0.040	0.103 ± 0.037
ALKP	U/L	34 – 141	71.6 ± 24.0	71.9 ± 24.0	72.5 ± 24.5	72.0 ± 24.5	72.6 ± 24.1
GGT	U/L	10 – 83	27.6 ± 13.8	27.6 ± 13.9	27.8 ± 13.5	27.5 ± 13.4	27.7 ± 13.5
AST	U/L	13 – 43	20.9 ± 6.4	21.3 ± 6.5	21.0 ± 6.6	21.4 ± 6.5	21.4 ± 6.7
ALT	U/L	9 – 54	24.1 ± 10.8	24.0 ± 11.0	24.2 ± 10.7	24.2 ± 10.8	24.4 ± 10.6
LDH	U/L	108 – 215	149.2 ± 22.8	151.1 ± 22.1	151.0 ± 22.0	155.0 ± 21.0	153.5 ± 21.6
IRON	µg/dL	38 – 332	95.1 ± 45.9	95.2 ± 45.4	95.1 ± 45.2	95.6 ± 46.1	95.3 ± 45.9
MG	mg/dL	1.78 – 2.36	2.070 ± 0.142	2.077 ±0.149	2.085 ± 0.144	2.078 ± 0.147	2.064 ± 0.143

Table 4 shows the mean between–tube biases and the 95% limit for the mean biases for each analyte. Also shown for each analyte is a clinical acceptance limit (CAL), which represents an estimate of the maximum allowable difference in test results between two tube types. Clinical acceptance limits are used by BD to help identify differences which may be considered clinically significant. These values may reflect the opinion of investigators from BD-sponsored clinical trials and therefore may not be constant from one study to another. The laboratory should evaluate the data presented and make its own determination concerning the acceptability of results. For analytes where the CAL is defined in percent, mean biases and 95% limits are shown in percent to one decimal place. For analytes where the CAL is defined in the units of the analyte, mean biases and 95% limits are shown in the analyte units and to one more decimal place than that reported by the analyzer.

Based on the CAL values used as shown in Table 4, clinical equivalence was demonstrated for BD SST™ II *Advance* Tubes between the control condition and all four evaluation conditions for all analytes. The mean bias plots in the Appendix contain the same information in Table 4 in graphical form.

Table 4. Evaluation – Control Mean Biases (n=40)

Analyte	Units	Clinical Acceptance Limit	Mean Bias (95% Limit) in Units or %				Clinical Equiv-alence
			Evaluation 1 – Control	Evaluation 2 – Control	Evaluation 3 – Control	Evaluation 4 – Control	
GLU	mg/dL	10%	0.6 (-2.1, 3.3)	0.5 (-2.2, 3.2)	0.5 (-2.1, 3.3)	0.0 (-2.7, 2.7)	yes
NA	mmol/L	3 mmol/L	-0.1 (-0.8, 0.6)	-0.1 (-0.8, 0.5)	0.2 (-0.5, 0.8)	-0.1 (-0.8, 0.5)	yes
K	mmol/L	0.3 mmol/L	0.03 (-0.07, 0.12)	0.04 (-0.06, 0.14)	0.00 (-0.10, 0.10)	0.00 (-0.10, 0.10)	yes
CL	mmol/L	3 mmol/L	0.0 (-0.6, 0.6)	-0.1 (-0.7, 0.5)	0.0 (-0.5, 0.6)	-0.1 (-0.7, 0.5)	yes
CO_2	mmol/L	15%	-1.8 (-4.8, 1.3)	-1.5 (-4.5, 1.6)	-2.2 (-5.2, 0.9)	-1.5 (-4.5, 1.6)	yes
BUN	mg/dL	3 mg/dL	0.0 (-0.4, 0.4)	-0.1 (-0.5, 0.3)	0.1 (-0.3, 0.5)	0.0 (-0.4, 0.4)	yes
CREA	mg/dL	0.3 mg/dL	0.01 (-0.02, 0.05)	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)	-0.01 (-0.05, 0.03)	yes
UA	mg/dL	10%	0.0 (-1.8, 1.7)	-0.6 (-2.3, 1.2)	0.0 (-1.7, 1.7)	-0.6 (-2.4, 1.1)	yes
PHOS	mg/dL	0.4 mg/dL	-0.05 (-0.11, 0.02)	-0.01 (-0.07, 0.06)	0.01 (-0.05, 0.07)	0.00 (-0.07, 0.06)	yes
CA	mg/dL	0.4 mg/dL	0.00 (-0.09, 0.09)	0.05 (-0.04, 0.14)	0.02 (-0.07, 0.11)	0.00 (-0.09, 0.09)	yes
CHOL	mg/dL	5%	-0.1 (-1.4, 1.3)	0.3 (-1.0, 1.7)	0.4 (-0.9, 1.7)	0.5 (-0.8, 1.8)	yes
TRIG	mg/dL	5%	-0.3 (-2.0, 1.5)	-0.1 (-1.8, 1.7)	-0.1 (-1.8, 1.6)	-0.1 (-1.8, 1.7)	yes
TP	g/dL	5%	-0.2 (-1.4, 0.9)	-0.1 (-1.3, 1.0)	0.3 (-0.9, 1.4)	0.2 (-0.9, 1.4)	yes
ALB	g/dL	5%	0.1 (-1.6, 1.9)	0.1 (-1.7, 1.9)	0.7 (-1.1, 2.5)	-0.4 (-2.2, 1.4)	yes
TBIL	mg/dL	0.10 mg/dL	-0.011 (-0.040, 0.018)	-0.015 (-0.045, 0.014)	0.001 (-0.028, 0.031)	-0.023 (-0.052, 0.006)	yes
DBIL	mg/dL	0.05 mg/dL	-0.002 (-0.016, 0.013)	0.000 (-0.015, 0.014)	-0.003 (-0.018, 0.012)	-0.004 (-0.018, 0.011)	yes
ALKP	U/L	10%	0.5 (-1.2, 2.1)	1.2 (-0.5, 2.9)	0.4 (-1.3, 2.0)	1.5 (-0.1, 3.2)	yes
GGT	U/L	10%	-0.4 (-3.9, 3.2)	0.9 (-2.7, 4.5)	-0.1 (-3.6, 3.5)	0.4 (-3.1, 4.0)	yes
AST	U/L	10%	2.0 (-1.9, 6.0)	0.5 (-3.3, 4.4)	2.1 (-1.8, 6.1)	1.9 (-1.9, 6.0)	yes
ALT	U/L	10%	-1.2 (-4.6, 2.3)	0.7 (-2.8, 4.3)	0.4 (-3.1, 3.9)	2.1 (-1.4, 5.7)	yes
LDH	U/L	20%	1.4 (-0.7, 3.6)	1.3 (-0.8, 3.5)	4.2 (2.0, 6.4)	3.1 (0.9, 5.3)	yes
IRON	µg/dL	10%	0.0 (-2.1, 2.1)	-0.2 (-2.3, 2.0)	0.3 (-1.8, 2.4)	0.0 (-2.1, 2.1)	yes
MG	mg/dL	0.30 mg/dL	0.007 (-0.025, 0.040)	0.016 (-0.017, 0.048)	0.008 (-0.024, 0.041)	-0.006 (-0.038, 0.027)	yes

Results for visual observations of BD SST™ II *Advance* Tubes are shown in Table 5. Relative to the control condition, mean barrier thickness decreased slightly at both 1800 x g conditions, and increased at the 3000 x g conditions. Mean barrier thickness at the control condition was 8.24 mm; this decreased to 7.91 mm and 7.42 mm at the five and four minute 1800 x g conditions, respectively. While all gel barriers in the study were considered to be complete, the routine use of alternate centrifugation conditions similar to Evaluation 1 and especially Evaluation 2 should be carefully evaluated to confirm that gel barrier formation rates remain acceptable. Conversely, mean barrier thickness increased to 9.54 mm and 9.83 mm at the four and five minute 3000 x g conditions, respectively. Similar trends were observed for median barrier thickness. Barrier thickness variability was also reduced at the 3000 x g conditions (indicated by the lower SD values). Thus, gel barriers at 3000 x g were both thicker and more reproducible (less variable) compared with the control condition. As expected, gel barrier thickness also increased when using a five minute centrifugation time relative to a four minute centrifugation time at the same RCF.

Trends in serum yield were also similar to those observed for barrier thickness. Results in Table 5 are expressed as percent yield of serum, which is the percent volume of the blood volume drawn into the tube that is expressed as serum after centrifugation. Since percent yield of serum accounts for the draw volume, it is considered to be a more meaningful parameter for communicating the effect of centrifugation on serum expression than the absolute volume of serum obtained. As shown in Table 5, mean percent yield of serum at the control condition was 44.3%; this decreased to 42.1% and 40.6% at the five and four minute 1800 x g conditions, respectively. Mean percent yield of serum remained within 0.5% of the control at the four minute 3000 x g condition (43.9%), and increased to 45.2% at the five minute 3000 x g condition. As expected, mean percent yield of serum also increased when using a five minute centrifugation time relative to a four minute centrifugation time at the same RCF. For the 13x100 mm tubes (5 mL draw) used in this study, each 1% change in percent yield of serum corresponds to approximately 0.05 mL serum.

Table 5. Visual Observations (n=40)

Observation		Centrifugation Condition				
		Control 1300 x g 10 min	Evaluation 1 1800 x g 5 min	Evaluation 2 1800 x g 4 min	Evaluation 3 3000 x g 4 min	Evaluation 4 3000 x g 5 min
Barrier Thickness (mm)	Mean	8.24	7.91	7.42	9.54	9.83
	SD	0.75	0.72	0.86	0.41	0.58
	Median	8.3	8.1	7.6	9.5	9.9
	Min	6.4	6.3	5.0	8.8	7.8
	Max	10.0	9.5	8.9	10.6	10.9
% Yield Serum[1]	Mean	44.3	42.1	40.6	43.9	45.2

[1] Percent volume of blood specimen expressed as serum after centrifugation.

This study was performed using specific conditions of centrifugation, and the results obtained correspond to the discrete settings of RCF, ramp up time and total centrifugation time listed in Table 1. However, a wide variety of centrifuges are used in clinical laboratories, and not all centrifuges will be capable of reproducing the specific settings used in this study. Laboratories may also wish to use different centrifugation settings than those used in this study, to better align with their specific requirements for both turnaround time and gel tube performance. Therefore, it is desireable to generalize the results obtained in this study. This can be done by first recognizing that the four evaluation conditions used represent a balanced 2x2 matrix of RCF and time (Figure 1). Based on the data in Table 3 and Table 4, assumptions can then be made about the expected clinical performance of analytes at conditions within the matrix. Interpolation of the data in Table 5 can also allow for prediction of gel barrier thickness and percent yield of serum at conditions within the matrix.

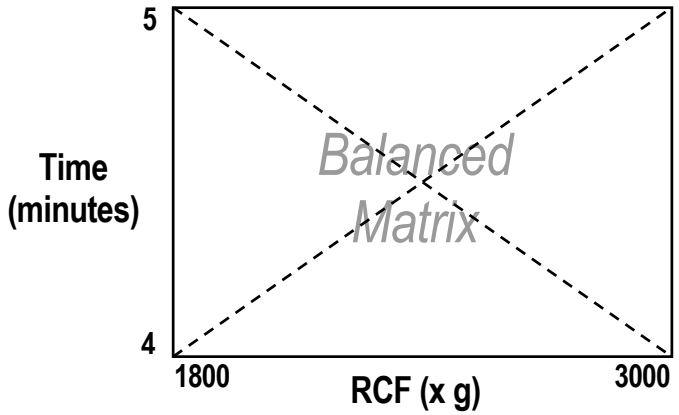


Figure 1. Matrix of RCF and Centrifuge Time used in study.

As shown in Table 4, all four evaluation conditions provided analyte test results which were clinically equivalent to the control condition. Since the four evaluation conditions represent a balanced matrix of two RCF settings (1800, 3000 x g) and two time settings (4 min, 5 min), it is expected that analyte test results will also remain clinically equivalent to the control condition for all centrifuge settings where the RCF is between 1800–3000 x g and the total centrifuge time is between 4–5 min. The one exception to this assumption concerns the ramp-up time. It is possible that very short ramp-up times could result in an increase in hemolysis of the specimen during centrifugation. Therefore, the use of shorter ramp-up times to the final RCF (faster centrifuge acceleration) than those used in Table 1 must be evaluated to ensure serum quality remains acceptable.

To provide more detailed information on barrier thickness and serum yield, two linear interpolations can be applied to the data obtained at the four evaluation conditions. The first is an interpolation across RCF and is shown in Table 6. The RCF range is first divided into intervals of 100 x g, and the 1800 and 3000 x g rows of the table are completed based on the data in Table 1 and Table 5. The ramp-up time, barrier thickness and percent yield of serum at each intermediate RCF are then estimated by interpolation between the values at 1800 and 3000 x g (note: all ramp-up times in the study were obtained using the same acceleration setting [setting "9"] on the Hettich Rotanta 46R centrifuge). The time spent at the final RCF ("Time at RCF") is obtained by subtraction of the ramp-up time from the total centrifuge time.

Table 6. Interpolated Data for Barrier Thickness and % Yield Serum[1]							
RCF (x g)	Ramp Up Time (min:sec)	Centrifuge Time: 4 min			Centrifuge Time: 5 min		
		Time at RCF (min:sec)	Barrier Thickness (mm)	% Yield Serum (%)	Time at RCF (min:sec)	Barrier Thickness (mm)	% Yield Serum (%)
1800	0:38	3:22	7.42	40.6	4:22	7.91	42.1
1900	0:39	3:21	7.60	40.9	4:21	8.07	42.4
2000	0:41	3:19	7.77	41.2	4:19	8.23	42.6
2100	0:42	3:18	7.95	41.4	4:18	8.39	42.9
2200	0:44	3:16	8.13	41.7	4:16	8.55	43.1
2300	0:45	3:15	8.30	42.0	4:15	8.71	43.4
2400	0:47	3:13	8.48	42.3	4:13	8.87	43.6
2500	0:48	3:12	8.66	42.5	4:12	9.03	43.9
2600	0:49	3:11	8.83	42.8	4:11	9.19	44.2
2700	0:51	3:09	9.01	43.1	4:09	9.35	44.4
2800	0:52	3:08	9.19	43.4	4:08	9.51	44.7
2900	0:54	3:06	9.36	43.6	4:06	9.67	44.9
3000	0:55	3:05	9.54	43.9	4:05	9.83	45.2

[1] Data at 1800 x g and 3000 x g are from study. Values between 1900-2900 x g are interpolated.

Using the data in Table 6, a second interpolation across Time at RCF is then performed as shown in Table 7 and Table 8. As noted earlier, it is recognized that a wide variety of centrifuges are used in clinical laboratories. While not all centrifuges will be capable of replicating the ramp-up times used in this study, adjustment of the time spent at the final RCF should be an adjustable feature on most centrifuges. Therefore, the data has been generalized based on the Time at RCF parameter. In Table 7 and Table 8, the RCF range is shown on the vertical axis, and the Time at RCF is shown on the horizontal axis in 10 second intervals. The values for barrier thickness and percent yield of serum from Table 6 are placed into Table 7 and Table 8, respectively, with the Time at RCF rounded up in all cases to the nearest 10 second interval. A linear interpolation across Time at RCF is then performed between the two values on each RCF row. Values for barrier thickness and percent yield of serum were then rounded down to the nearest 0.1 mm or 0.1%, respectively. All rounding conventions employed in Table 7 and Table 8 provide a conservative estimate of the barrier thickness and percent yield of serum at each setting of RCF and Time at RCF. As a visual aid to help differentiate performance, cells corresponding to conditions where the barrier thickness or percent yield of serum are less than the control have been shaded. Cells with no values are shaded based on expected values from trends in adjacent cells.

Table 7. Performance Matrix for Barrier Thickness[1]										
RCF	Time at RCF (not including ramp up/down time)									
	3:00	3:10	3:20	3:30	3:40	3:50	4:00	4:10	4:20	4:30
1800				7.4	7.5	7.5	7.6	7.7	7.8	7.9
1900				7.6	7.6	7.7	7.8	7.9	7.9	8.0
2000			7.7	7.8	7.9	8.0	8.0	8.1	8.2	
2100			7.9	8.0	8.1	8.1	8.2	8.3	8.3	
2200			8.1	8.2	8.2	8.3	8.4	8.4	8.5	
2300			8.3	8.3	8.4	8.5	8.5	8.6	8.7	
2400			8.4	8.5	8.6	8.6	8.7	8.8	8.8	
2500			8.6	8.7	8.7	8.8	8.9	8.9	9.0	
2600			8.8	8.8	8.9	9.0	9.0	9.1	9.1	
2700		9.0	9.0	9.1	9.1	9.2	9.2	9.3		
2800		9.1	9.2	9.3	9.3	9.4	9.4	9.5		
2900		9.3	9.4	9.4	9.5	9.5	9.6	9.6		
3000		9.5	9.5	9.6	9.6	9.7	9.7	9.8		

[1] Data in cells is estimated gel barrier thickness in mm, based on interpolated data in Table 6 and additional interpolation across Time at RCF. Shaded cells indicate expected barrier thickness less than control (< 8.3 mm). Non-shaded cells indicate expected barrier thickess equal to or greater than control. Shading determined for cells without data based on trend across row.

Table 8. Performance Matrix for % Yield Serum[1]										
RCF	Time at RCF (not including ramp up/down time)									
	3:00	3:10	3:20	3:30	3:40	3:50	4:00	4:10	4:20	4:30
1800				40.6	40.8	41.1	41.3	41.6	41.8	42.1
1900				40.9	41.1	41.4	41.6	41.9	42.1	42.4
2000			41.2	41.4	41.6	41.9	42.1	42.3	42.6	
2100			41.4	41.6	41.9	42.1	42.4	42.6	42.9	
2200			41.7	41.9	42.1	42.4	42.6	42.8	43.1	
2300			42.0	42.2	42.4	42.7	42.9	43.1	43.4	
2400			42.3	42.5	42.7	42.9	43.1	43.3	43.6	
2500			42.5	42.7	42.9	43.2	43.4	43.6	43.9	
2600			42.8	43.0	43.2	43.5	43.7	43.9	44.2	
2700		43.1	43.3	43.5	43.7	43.9	44.1	44.4		
2800		43.4	43.6	43.8	44.0	44.2	44.4	44.7		
2900		43.6	43.8	44.0	44.2	44.4	44.6	44.9		
3000		43.9	44.1	44.3	44.5	44.7	44.9	45.2		

[1] Data in cells is estimated yield of serum in %, based on interpolated data in Table 6 and additional interpolation across Time at RCF. Shaded cells indicate expected % yield of serum less than control. Non-shaded cells indicate expected % yield of serum equal to or greater than control. Shading determined for cells without data based on trend across row. % Yield Serum considered equivalent to control (44.3%) if within 0.5% (i.e., ≥ 43.8%). Each 1% yield of serum corresponds to approximately 0.05 mL.

The shaded regions from Table 7 and Table 8 have been combined in Table 9 to provide a simple guide to overall performance. Each of the three regions in Table 9 correspond to combinations of RCF and Time at RCF where the gel barrier thickness and percent yield of serum are expected to be less than, or equal to or greater than, that obtained using the control centrifugation condition of 1300 x g for 10 minutes.

Table 9. Overall Performance Matrix

RCF	Time at RCF (not including ramp up/down time)									
	3:00	3:10	3:20	3:30	3:40	3:50	4:00	4:10	4:20	4:30
1800	lower barrier thickness lower serum yield									
1900										
2000										
2100										
2200										
2300										
2400			equal or greater barrier thickness lower serum yield							
2500										
2600										
2700										
2800							equal or greater barrier thickness equal or greater serum yield			
2900										
3000										

This paper has presented data obtained from the centrifugation of BD Vacutainer® SST™ II *Advance* Tubes in swing bucket centrifuges at one control condition and four evaluation conditions each consisting of higher RCF and shorter centrifuge time relative to the control. Since the four evaluation conditions represent a balanced 2x2 matrix of RCF and centrifuge time, the data obtained at these conditions was interpolated to obtain estimates of barrier thickness and percent yield of serum at intermediate centrifugation conditions spanning 1800–3000 x g and centrifuge times between 4–5 minutes (expressed as time spent at final RCF). In determining the values in Tables 6-9, certain assumptions have been made. One assumption is that the data obtained at the four evaluation conditions can be interpolated in a linear fashion to estimate the barrier thickness and percent yield of serum at intermediate conditions. Another assumption is that the Time at RCF parameter can be used independantly of the ramp-up time to accurately predict barrier thickness and percent yield of serum. Although these assumptions have not been validated, it is believed that as long as extremes of ramp-up time are not used, that the values provided in Tables 6-9 represent reasonable estimates of expected barrier thickness and percent yield of serum for 13x100 mm BD Vacutainer® SST™ II *Advance* Tubes centrifuged in a swing bucket centrifuge. This information is provided only as a tool, to assist with the selection of alternate centrifugation conditions. 1300 – 2000 x g for 10 minutes at room temperature remain the optimal centrifugation recommendations for general use. The use of a specific alternate centrifugation condition should be evaluated by the laboratory to ensure performance expectations are still met.

In addition, this study examined only one size – 13x100 mm – of the BD Vacutainer® SST™ II *Advance* Tube. In swing bucket centrifuges, it is expected that comparable performance would be obtained with 13x75 mm and 16x100 mm BD Vacutainer® SST™ II *Advance* Tubes. Again, it is recommended that the use of alternate centrifuge settings with all sizes of BD Vacutainer® SST™ II *Advance* Tubes be evaluated by the laboratory to ensure performance remains acceptable.

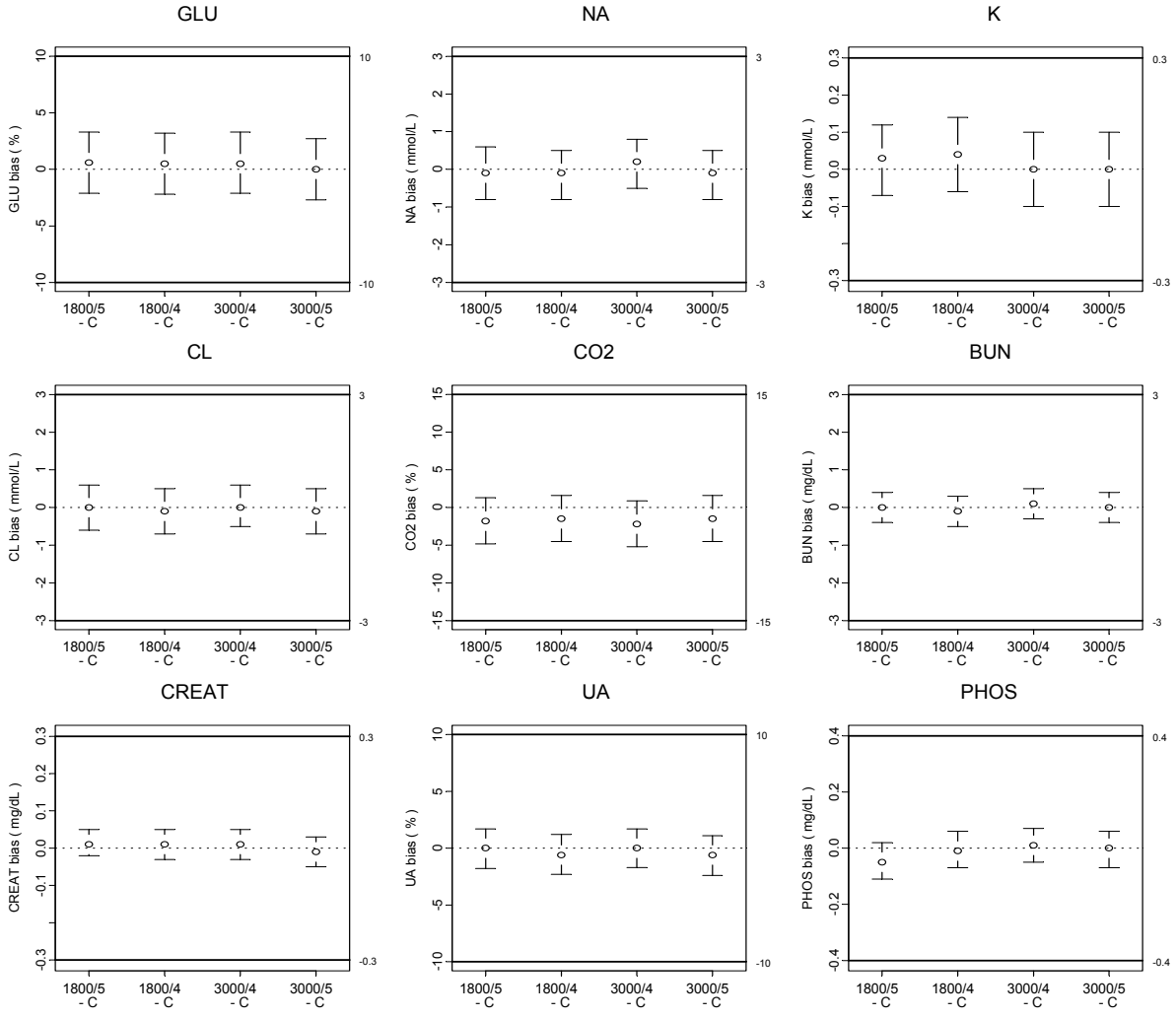
CONCLUSION

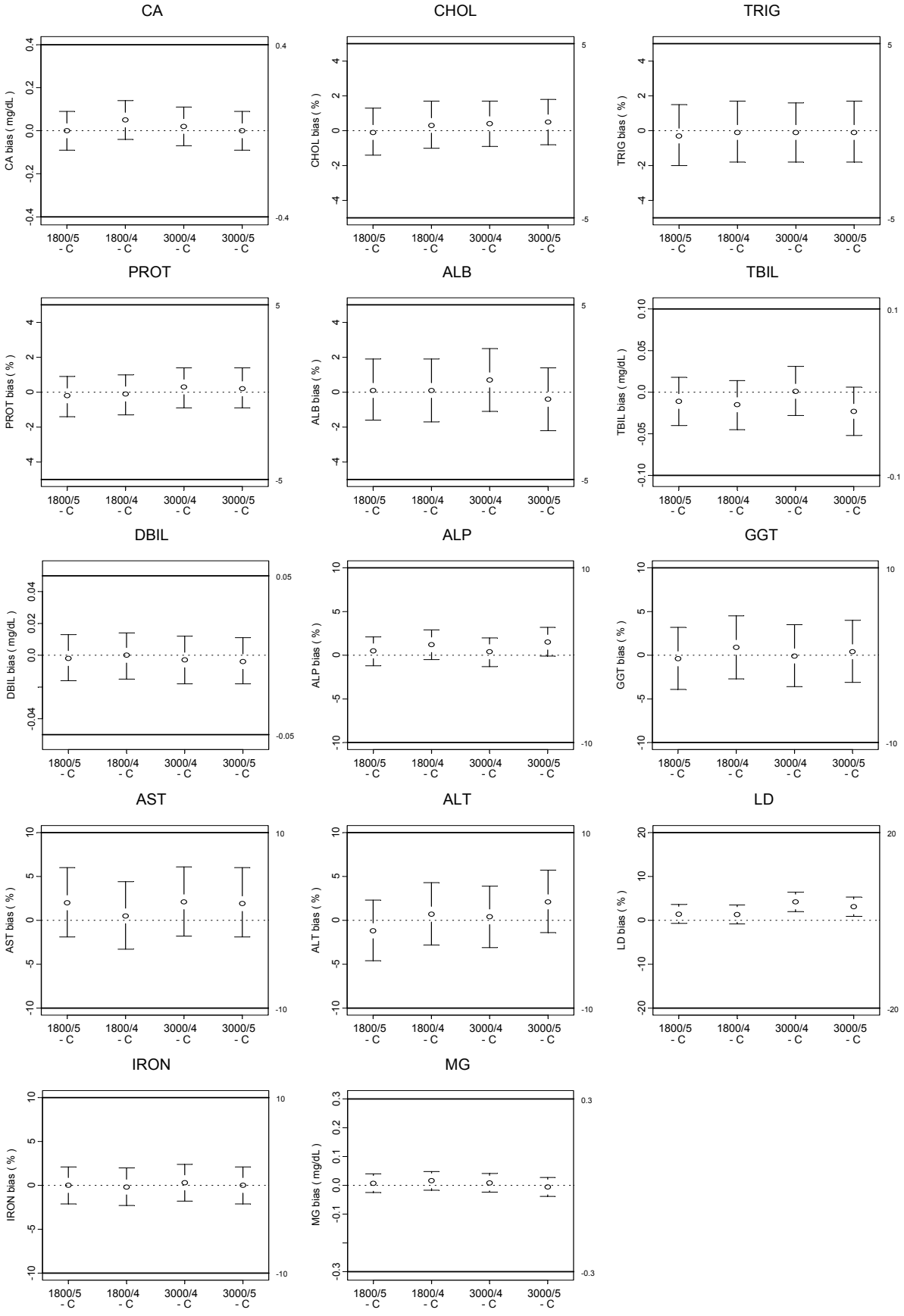
BD Vacutainer® SST™ II *Advance* Tubes were centrifuged in swing bucket centrifuges at one control condition (1300 x g for 10 minutes) and four evaluation conditions consisting of two RCF settings (1800, 3000 x g) and two time settings (4 min, 5 min). Clinical equivalance was demonstrated for a panel of 23 routine chemistry analytes between each evaluation condition and the control condition. Gel barrier thickness and percent yield of serum were slightly lower at the 1800 x g conditions and were considered equivalent or were greater at the 3000 x g conditions, relative to the control. The design of the study allows for data interpolation to estimate barrier thickness and percent yield of serum for centrifugation conditions spanning 4–5 minutes and 1800–3000 x g.

Whenever changing any manufacturer's blood collection tube type, size, or storage condition for a particular laboratory assay, the laboratory personnel should review the tube manufacturer's data and their own data to establish/verify the reference range for a specific instrument/reagent system. Based on such information, the laboratory can then decide if a change is appropriate.

APPENDIX A
Mean Bias Plots

BD Vacutainer® SST™ II *Advance* Tubes centrifuged at evaluation and control conditions in swing bucket centrifuges were compared for equivalence. The four evaluation conditions were 1800 x g and 3000 x g, each for four and five minutes. The control (C) condition was 1300 x g for 10 minutes. The mean bias plots below are a graphical representation of the data in Table 4. There is one plot for each analyte. In each plot, the mean between – tube bias (difference) as well as a 95% limit for the mean bias (shown by the vertical bars) is plotted. These biases are estimated based on an average of the within subject differences. The two horizontal bars at the top and bottom of each plot depict a clinical acceptance limit for each analyte. For 95% confidence in clinical equivalence between evaluation and control centrifugation conditions, the entire 95% limit of the mean bias must lie within the clinical acceptance limit bars.




CA
CA bias (mg/dL)
0.4
0.2
0.0
-0.2
-0.4
0.4
-0.4
CHOL
CHOL bias (%)
4
2
0
-2
-4
5
-5
TRIG
TRIG bias (%)
4
2
0
-2
-4
5
-5
PROT
PROT bias (%)
4
2
0
-2
-4
5
-5
ALB
ALB bias (%)
4
2
0
-2
-4
5
-5
TBIL
TBIL bias (mg/dL)
0.10
0.05
0.0
-0.05
-0.10
0.1
-0.1
DBIL
DBIL bias (mg/dL)
0.04
0.02
0.0
-0.04
0.05
-0.05
ALP
ALP bias (%)
10
5
0
-5
-10
10
-10
GGT
GGT bias (%)
10
5
0
-5
-10
10
-10
AST
AST bias (%)
10
5
0
-5
-10
10
-10
ALT
ALT bias (%)
10
5
0
-5
-10
10
-10
LD
LD bias (%)
20
10
0
-10
-20
20
-20
IRON
IRON bias (%)
10
5
0
-5
-10
10
-10
MG
MG bias (mg/dL)
0.3
0.2
0.1
0.0
-0.1
-0.3
0.3
-0.3
1800/5 - C
1800/4 - C
3000/4 - C
3000/5 - C

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