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Stability of 21 Routine Chemistry Tests in the BD Barricor™ Tube and the Sarstedt S-Monovette® LH Tube up to 7 Days After Blood Collection

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Abstract

Objectives: We compared the stability of 21 routine chemistry parameters (albumin, ALP, ALT, AST, bicarbonate, bilirubin, chloride, cholesterol, CK, GGT, glucose, HDL, LDH, creatinine, lipase, potassium, sodium, protein, transferrin, triglycerides, urea) in the BD Barricor™ lithium-heparin plasma tube, recently developed by Becton, Dickinson and Company, versus the S-Monovette® LH tube from Sarstedt, routinely used in the University Hospital Brussels of the Brussels Free University.

Methods: Forty paired plasma samples from healthy volunteers were analyzed on the Ortho VITROS 4600 Chemistry System up to 7 days after collection. Differences between tubes and over time were compared respecting the Ricos criteria.

Results: None of the parameters showed clinical significant differences between the tubes at baseline. For albumin, cholesterol and lipase, difference *vs.* baseline were within the predefined criteria up to 72 h in the BD Barricor™ tube (*vs.* 48 h in the S-Monovette® tube); for AST 7 days (*vs.* 48 h); for LDH 72 h (*vs.* 24 h); for potassium and transferrin 24 h (*vs.* 6 h). Measurement of GGT fulfills the criterion up to 72 h in the BD Barricor™ tube *vs.* 7 days in the S-Monovette® tube.

Conclusions: 13 parameters (ALP, ALT, bicarbonate, total bilirubin, chloride, CK, creatinine, glucose, HDL, sodium, total protein, triglyceride, urea) showed equal stability in both blood collection tubes; 7 parameters (albumin, AST, total cholesterol, LDH, lipase, potassium, transferrin) showed better stability in the BD Barricor™ tube. Only GGT had a longer stability in the S-Monovette® tube. This observation creates some perspectives for use of this new Barricor™ tube in clinical laboratories.

Keywords: Stability, Clinical chemistry, BD Barricor™ tube

Introduction

The total laboratory process for the determination of parameters in a patient sample begins with the creation of an order by the physician, includes the pre-analytical, analytical and post-analytical phases, and ends with the interpretation of the results by the physician [1,2]. Since pre-analytical errors still account for nearly 60-70% of all the problems occurring in the laboratory diagnostics, this phase plays an important role in the whole process [1,3,4]. Better control or standardization of this pre-analytical phase can improve the quality of the process and consequently patient care.

Important pre-analytical components are the specimen type and the time between sampling and obtaining a serum/plasma fraction ready for analysis. It is known that plasma has the advantages of a shorter processing time, but overall analyte stability is better in serum, probably due to the presence of less cellular debris [5,6]. To combine the advantages of both serum and plasma, a new gel-free mechanical separator technology is designed by Becton, Dickinson and Company (BD) to obtain plasma with improved sample quality and stability, shorter centrifugation time, no gel-related assay failures (such as instrument probe clogging) neither test interferences due to gel adsorption [7,8]. This newly developed BD Barricor™ Plasma Blood Collection Tube is a single-use, plastic blood collection tube with a sterile interior and a safety-engineered BD Hemogard™ closure. The interior tube wall surface has a coating of Li-heparin anticoagulant and the non-gel separator contains a surface coating of surfactant. The separator rests at the top of the tube and allows blood to pass through by its orientation. During centrifugation, the separator stretches and is launched into the blood column. The principle of differential buoyancy is used for proper positioning during centrifugation: an upward force exerted by the blood opposes the weight of the immersed separator. Throughout the centrifugation process, channels are created around the stretched separator, allowing blood cells and fragments to sediment out of the plasma. When the centrifuge slows down, the separator returns to its original shape and forms a seal between the plasma (above) and blood cells (below), creating a stable and robust barrier [8,9]. The first studies with this newly developed blood collection tube have been published recently. They showed that the BD Barricor™ tube is an acceptable and robust alternative for measurement of cardiac troponin I and electrolytes in comparison with routinely used blood collection tubes [8,9]. However, only a limited number of parameters have been investigated.

Hospital laboratories are performing an increasing number of tests on blood samples provided by secondary sites, other hospitals and general practitioners. In this context, pre-analytical variations related to storage time are of major importance [1]. Several studies have investigated the stability of routine chemistry parameters, but the results and conclusions often differ depending on the study variables with

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differences in the tested analytes, matrices (whole blood, serum, plasma with different anticoagulants, blood collection tubes), populations (hospitalized patients *vs.* healthy volunteers) and sample size, storage time (h to days), temperature conditions (room temperature, 2-8°C, -20°C, -80°C), analyzers and methods, acceptance limits (analytical and/or clinical) and statistical analysis [10-13].

In our study, we aimed to investigate the stability of 21 routine chemistry parameters in the S-Monovette® Li-heparin Tube, the plasma tube routinely used in our hospital, and the BD Vacutainer® Barricor™ Plasma Blood Collection Tube up to 7 days after the blood collection. We compared differences at 5 time points (6, 24, 48, 72 and 168 h) from baseline measurement using predefined criteria as described by Dr. Carmen Ricos and colleagues in a comprehensive database of biologic variation for more than 300 parameters, which was last updated in 2014 [14,15].

Methods

Subjects and collection tubes

The study was conducted in the University Hospital Brussels (UZ Brussel) of the Brussels Free University (Vrije Universiteit Brussel-VUB) in March-April 2017 and included 40 apparently healthy volunteers. The group consisted of 20 male and 20 female adults (≥ 18 years). There were no exclusion criteria. The study had institutional ethics approval and was conducted according to the Declaration of Helsinki as revised in 2013. Informed consents were obtained from all participants. Venous blood was sampled in the morning using a butterfly needle and collected, following best practice and according to manufacturer's instructions, in the study tubes in duplicate (S-Monovette® LH tube ref 04.1936.001, Sarstedt AG&Co, Nümbrecht, Germany; BD Barricor™ tube ref 365030, Beckton, Dickinson and Company, NJ, USA). Blood was collected by trained staff in order to exclude any pre-analytical variability, in a randomized drawing order and the two type of tubes were sampled alternately [16]. Tubes were manually transported from the collection room to the laboratory by the investigator.

Thirty to ninety minutes after collection, samples were centrifuged at room temperature according to the manufacturer's instructions in a swing bucket centrifuge (S-Monovette® tube 10' at 2000 g, BD Barricor™ tube 5' at 3000 g). The samples were stored in the dark at room temperature (day 1) and at 2-8°C (day 2-8).

Instruments

Immediately after centrifugation, samples were measured for all the parameters (albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), bicarbonate, total bilirubin, chloride, total cholesterol, creatine kinase (CK), gamma-glutamyltransferase (GGT), glucose, high-density lipoprotein (HDL), lactate dehydrogenase (LDH), creatinine, lipase, potassium, sodium, total protein, transferrin, triglycerides, urea) on the Ortho Clinical Diagnostics (Ortho) VITROS 4600 Chemistry System (Ortho-Clinical Diagnostics, Rochester, NY, USA). Analytical methods were colorimetry for all of the investigated parameters with the exception of chloride, potassium and sodium (direct potentiometry) and transferrin (turbidimetry). Plasma measurements were repeated 6, 24, 48, 72 and 168 h after the initial measurement. All 80 samples were measured on the same Ortho VITROS 4600 Chemistry System with the same lot of reagents and slides. Quality control was assured by measurements of commercially available controls (Liquid Unassayed Multiqual Control, Bio-Rad Laboratories, CA, USA) 4 times a day at 2 different levels for the electrolytes, and twice a day at 2 different levels for the other parameters.

Statistics

Data were reported as sample size and median with interquartile range (IQR). Results were analyzed using Passing-Bablok and difference plots for comparison between the tubes. Spearman-rank correlation coefficient (R_s) was used to assess correlations between measurements. To test statistical differences between analytes measured from the different tubes, a Wilcoxon rank test was used. These tests were performed two sided and P<0.05/k for k comparisons (Bonferroni adjustment) was considered statistically significant.

Differences in concentration between tubes at one time point were calculated as: difference (%)=(concentration BD Barricor™ tube–concentration S-Monovette®)/concentration S-Monovette® *100 and the mean % difference was compared with the current desirable total allowable error (TE_a) based on the Ricos Criteria [14,15]. Differences in concentration over time in one type of tube were calculated as: difference (%)=(concentration time point X–concentration time point 0)/concentration time point 0*100 and the mean % difference was compared with the current desirable allowable imprecision (analytical coefficient of variation, (CV_a) based on the Ricos Criteria [14,15]. Stability is not acceptable if the mean % difference exceeds the CV_a for the first time.

Statistical tests were performed with SPSS 24.0 software (IBM SPSS Statistics, Armonk, NY, USA) and the figures were generated with Graph Pad Prism 5.00 software (Graph Pad Software, Inc., San Diego, CA, USA) or MedCalc for Windows, version 17.6 (MedCalc Software, Ostend, Belgium).

Results

Baseline measurements of the levels of the different parameters in the S-Monovette® tube and the BD Barricor™ tube are compared in Table 1. For a few patients, HDL (n=2), GGT (n=4) and CK (n=1) were below (GGT) or above (HDL, CK) the measuring range of the analyzer and no manual dilution was made. For one patient, triglyceride level was above the measuring range and an automated dilution was made. There was a statistically significant difference between the tubes for ALP, AST, chloride, LDH and urea. Passing-Bablok regression analysis showed no bias for most of the analytes. Only for chloride, a systematic difference was found (95% CI of the intercept [-22.2; -0.5] does not include "0" and for potassium, a systematic and proportional difference was observed between the two investigated tubes (95% CI of the intercept [-2.0; -0.4] does not include "0" 95% CI of the slope [1.11; 1.50] does not include "1"). For all the analytes, a strong correlation was found between the measurements in the two tubes, with R_s ranging from 0.797 to 0.998. Importantly, the observed differences were not clinically significant since the mean difference between the two tubes at baseline for none of the parameters exceeded the TE_a.

Figure 1 shows the concentration over time of the different parameters in the S-Monovette® tube (left) and the BD Barricor™ tube (right), expressed as percentage of the initial measurement (t=0 h). The patterns during follow up differed according to the investigated parameter. Glucose and bicarbonate levels showed a decrease over time, more pronounced in the S-Monovette® tube in comparison with the BD Barricor™ tube (Figure 1 panel A). For glucose, respectively 68% and 87% of the initial value was measured after 7 days, for bicarbonate respectively 78% and 86%. For sodium, levels in both tubes remained between 99% and 102% of the initial measurement, for ALT between 97% and 104% (Figure 1 panel A). For AST, LDH and potassium a strong increase in the S-Monovette® tube was seen in comparison with the BD

			S-tube	BD-tube						
Analyte	**Unit**	**n**	**Median baseline value[a] (IQR)**	**Median baseline value[a] (IQR)**	**P[b]**	**PB[c] 95% CI slope**	**PB[c] 95% CI intercept**	**R_s[d]**	**Mean difference (%)[e]**	**TE_a (%)[f]**
Albumin	g/L	40	44 (43-47)	44 (43-46)	0.619	0.74; 1.07	-3.2; 11.3	0.824	-0.2	4.1
ALP	U/L	40	67 (57-81)	66 (55-80)	**0.002**	0.95; 1.05	-4.8; 1.9	0.987	-1.6	12.0
ALT	U/L	40	33 (27-42)	31 (25-42)	0.952	0.97; 1.11	-4.6; 1.5	0.949	-0.1	27.5
AST	U/L	40	24 (22-31)	25 (23-32)	**<0.001**	0.96; 1.05	-0.8; 2.0	0.970	3.2	16.7
Bicarbonate	mmol/L	40	28 (26-29)	28 (26-29)	0.008	0.91; 1.19	-4.9; 2.9	0.914	1.2	5.6
Bilirubin (tot)	mg/dL	40	0.7 (0.6-0.9)	0.7 (0.6-0.9)	0.478	0.98; 1.09	-0.07; 0.01	0.981	-0.6	26.9
Chloride	mmol/L	40	102 (101-103)	101 (100-103)	**<0.001**	1.00; 1.21	**-22.2; -0.5**	0.940	-0.4	1.5
Cholesterol (tot)	mg/dL	40	182 (154-199)	172 (154-201)	0.480	1.00; 1.12	-19.5; 0.8	0.978	0.1	9.0
CK	U/L	39	98 (82-157)	96 (81-160)	0.169	0.96; 1.01	-2.0; 3.0	0.992	-0.8	30.3
Creatinine	mg/dL	40	0.85 (0.76-0.92)	0.85 (0.74-0.90)	0.003	0.95; 1.03	-0.03; 0.03	0.986	-1.0	8.9
GGT	U/L	36	22 (15-35)	22 (16-35)	0.283	0.97; 1.03	-0.6; 0.8	0.994	0.8	22.1
Glucose	mg/dL	40	89 (76-94)	88 (76-93)	0.132	0.99; 1.09	-9.0; 0.5	0.979	-0.6	7.0
HDL	mg/dL	38	60 (44-66)	57 (45-66)	0.442	0.93; 1.05	-2.8; 3.3	0.972	-0.3	11.6
LDH	U/L	40	418 (373-473)	438 (403-512)	**< 0.001**	0.95; 1.19	-48.1; 57.2	0.923	8.0	11.4
Lipase	U/L	40	107 (71-147)	109 (67-148)	0.919	0.97; 1.03	-1.6; 2.8	0.991	0.1	37.9
Potassium	mmol/L	40	4.0 (3.9-4.2)	4.0 (3.8-4.3)	0.819	**1.11; 1.50**	**-2.0; -0.4**	0.867	0.2	5.6
Sodium	mmol/L	40	142 (141-143)	142 (141-144)	0.065	0.84; 1.09	-13.2; 22.1	0.893	-0.1	0.7
Total protein	g/L	40	78 (76-82)	79 (76-81)	0.320	0.70; 1.07	-5.8; 23.2	0.797	-0.4	3.6
Transferrin	g/L	40	2.7 (2.4-3.0)	2.7 (2.4-3.1)	0.252	0.94; 1.15	-0.5; 0.2	0.942	-0.7	3.8
Triglyceride	mg/dL	40	100 (67-127)	100 (67-131)	0.245	1.00; 1.05	-3.9; 0.4	0.994	0.4	26.0
Urea	mg/dL	40	30 (25-35)	30 (25-36)	**<0.001**	0.99; 1.03	-0.7; 0.7	0.998	1.2	15.6

ALP=Alkaline Phosphatase, ALT=Alanine Transaminase, AST=Aspartate Aminotransferase, CK=Creatine Kinase, GGT=Gamma-Glutamyl Transferase, HDL=High-Density Lipoprotein, LDH=Lactate Dehydrogenase

[a]The baseline value is considered the plasma concentration measured immediately after centrifugation (60-90' after blood collection)

[b]Wilcoxon signed-rank test, statistically significant if P<0.05/21=0.0023

[c]Passing-Bablok regression analysis

[d]Spearman correlation coefficient

[e]Differences are calculated as [(concentration time point X–concentration time point 0)/concentration time point 0*100]

[f] Total allowable error based on the Ricos Criteria (14,15)

Table 1: Comparison of routine chemistry tests on plasma obtained from blood collected in the Sarstedt S-Monovette® LH tube and the BD Barricor™ tube at baseline.

Barricor™ tube (Figure 1 panel B). For AST, respectively 119% and 105% of the initial value was measured after 7 days; for LDH 137% and 106%; for potassium 226% and 106%. For CK and lipase, an increase is observed in both tubes, up to 110% for CK and 120% for lipase (Figure 1 panel B). For the parameters shown in Figure 1 panel C and D, changes over time and between tubes were less pronounced and levels remained between 95% and 105% of the initial measurement during follow-up, with the exception of the 7 day measurement of urea in the S-Monovette® tube (106%) and of GGT in the BD Barricor™ tube (107%).

The mean differences (%) over time in comparison with the baseline measurements in the S-Monovette® tube and the BD Barricor™ tube for all the parameters are shown in Table 2. For ALT, total bilirubin, CK, total protein, triglyceride and urea the mean percentage differences *vs.* the initial measurement did not exceed the CV_a at any time point up to 7 days follow-up. For creatinine, differences *vs.* baseline did not exceed the CV_a up to 72 h for both tubes. For ALP, HDL and sodium the criteria were fulfilled up to 24 h. For chloride and glucose, only the measurements at the day of the blood collection, *i.e.* 6 h after the initial measurements, were below the CV_a and this for both blood collection tubes. For bicarbonate levels in both tubes, none of the measurements during follow-up were within the predefined criteria (2.4%). For the other parameters, the accepted stability was different between the two investigated blood collection tubes. Only for GGT, a longer stability was observed in the S-Monovette® tube in comparison with the BD Barricor™ tube (respectively 168 h *vs.* 72 h). For albumin, AST, total cholesterol, LDH, lipase, potassium and transferrin, the stability was better in the BD Barricor™ tube in comparison with the S-Monovette® tube. For albumin, total cholesterol and lipase, the differences versus the initial measurement did not exceed the CV_a up to 48 h in the S-Monovette® tube *vs.* 72 h in the BD Barricor™ tube. For AST, the stability in the S-Monovette® tube was below the CV_a up to 48 h, but measurements in the BD Barricor™ tube were within the predefined criteria up to 7 days. For LDH measurements in the S-Monovette® tube, the differences *vs.* baseline did not exceed the CV_a up to 24 h, and this in comparison with 72 h in the BD Barricor™ tube. Finally, for the parameters potassium and transferrin, it was observed that measurements in the S-Monovette® tube fulfilled the predefined criteria only up to 6 h after the initial measurement *vs.* 24 h in the BD Barricor™ tube. The differences during the 7 days follow-up period were most pronounced for LDH and potassium. For potassium, 130% more than the baseline value was measured 7 days after the blood collection in the S-Monovette® tube *vs.* 7% in the BD Barricor™ tube; for LDH respectively 50% and 5%. Figure 2 shows the ratio of the mean difference (%) and the CV_a for both tubes in function of time for the parameters AST (panel A), LDH (panel B), potassium (panel C), bicarbonate (panel D), glucose (panel E) and sodium (panel F). When values are between -1 and 1, stability is acceptable in comparison with the Ricos criteria. This figure clearly shows that for AST, LDH and potassium the predefined criteria are longer fulfilled in the BD Barricor™ tube, that for bicarbonate none of the investigated time points is acceptable and that for glucose and sodium, a similar pattern is observed in both tubes.

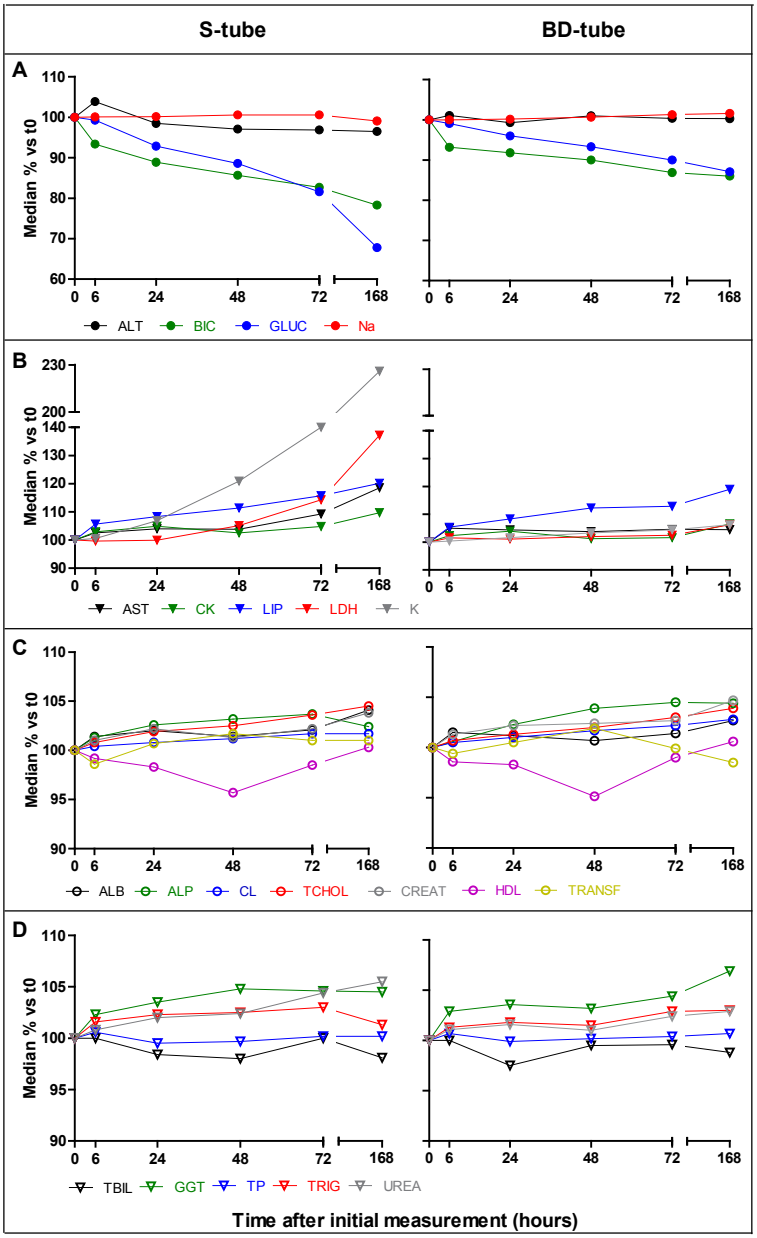


Figure 1: Concentrations over time in the S-Monovette® LH tube (S, left) and the BD Barricor™ tube (BD, right) expressed as % of the baseline measurement for ALT, bicarbonate (BIC), glucose (GLUC) and sodium (Na) (panel A); AST, CK, lipase (LIP), LDH and potassium (K) (panel B), albumin (ALB), ALP, chloride (CL), total cholesterol (TCHOL), creatinine (CREAT), HDL and transferrin (TRANSF) (panel C) and total bilirubin (TBIL), GGT, total protein (TP), triglycerides (TRIG) and urea (panel D).

In Figure 3, difference plots between the S-Monovette® tube and the BD Barricor™ tube for different time points are shown. For AST (panel A), LDH (panel B) and potassium (panel C), the values in the S-Monovette® tube were higher in comparison with values in the BD Barricor™ tube during follow-up, with a shift downwards at time point 72 and 168 h. For LDH and potassium, also a pronounced shift to the right was observed, in line with the observed increases in these levels shown in Figure 1 and Table 2. Bicarbonate, glucose and sodium levels are shown in Figure 2 panels D, E and F respectively. For these parameters, a decrease over time was observed with lower values measured in the S-Monovette® tube *vs.* the BD Barricor™ tube. This is illustrated in the difference plot by a shift upwards over time and for bicarbonate by a left shift.

Conclusion

This study evaluated the stability of 21 routine chemistry parameters in plasma up to 7 days after blood collection in the BD Barricor™ tube, a blood collection tube recently developed by Becton, Dickinson and Company, *vs.* the S-Monovette® tube, the plasma blood collection tube routinely used in the University Hospital Brussels. For 13 parameters (ALP, ALT, bicarbonate, total bilirubin, chloride, CK, creatinine, glucose, HDL, sodium, total protein, triglyceride and urea), stability was equal in both blood collection tubes; 7 parameters (albumin, AST, total cholesterol, LDH, lipase, potassium and transferrin) showed better stability in the BD Barricor™ tube and 1 parameter (GGT) had a

			Mean difference (%)[a]						
Analyte	**n**	**Tube**	**+6 hrs**	**+24 hrs**	**+ 48 hrs**	**+ 72 hrs**	**+ 168 hrs**	**CV_a (%)[b]**	**Acceptable delay[c]**
Albumin	40	S	1.4	1.3	1.3	**2.3**	**4.3**	1.6	**+48 hrs**
		BD	1.6	1.3	0.8	1.6	**3.0**		**+72 hrs**
ALP	40	S	1.5	2.8	**3.7**	**4.0**	2.3	3.2	+ 24 hrs
		BD	1.3	2.7	**4.6**	**5.3**	**5.2**		+ 24 hrs
ALT	40	S	3.8	-1.3	-1.6	-2.7	-6.2	9.7	+ 168 hrs
		BD	1.9	-1.5	1.4	0.9	0.3		+ 168 hrs
AST	40	S	2.6	4.1	4.6	**8.4**	**18.0**	6.2	**+ 48 hrs**
		BD	4.9	4.1	3.3	4.5	4.5		**+ 168 hrs**
Bicarbonate	40	S	**-6.6**	**-11.2**	**-14.6**	**-18.1**	**-21.7**	2.4	+ 0 hrs
		BD	**-6.8**	**-9.1**	**-10.3**	**-12.7**	**-13.9**		+ 0 hrs
Bilirubin (tot)	40	S	-0.4	-1.7	-1.7	-0.2	-2.2	10.9	+ 168 hrs
		BD	0.4	-2.1	0.4	0.0	-0.5		+ 168 hrs
Chloride	40	S	0.4	**0.8**	**1.2**	**1.6**	**1.9**	0.6	+ 6 hrs
		BD	0.5	**1.0**	**1.8**	**2.3**	**2.8**		+ 6 hrs
Cholesterol (tot)	40	S	0.8	1.9	2.6	**3.2**	**4.0**	3.0	**+ 48 hrs**
		BD	0.7	1.6	2.0	2.8	**4.0**		**+ 72 hrs**
CK	39	S	3.3	4.5	3.6	4.1	9.0	11.4	+ 168 hrs
		BD	2.6	4.1	2.2	2.3	5.7		+ 168 hrs
Creatinine	40	S	0.7	2.0	1.6	2.2	**3.6**	3.0	+ 72 hrs
		BD	1.2	2.0	2.1	2.8	**4.6**		+ 72 hrs
GGT	36	S	3.0	5.4	5.2	5.8	6.4	6.7	**+ 168 hrs**
		BD	3.4	4.9	4.0	4.6	**8.1**		**+ 72 hrs**
Glucose	40	S	-0.8	**-6.7**	**-11.8**	**-18.4**	**-32.9**	2.8	+ 6 hrs
		BD	-1.1	**-4.4**	**-7.7**	**-10.8**	**-16.7**		+ 6 hrs
HDL	38	S	-0.8	-1.8	**-4.6**	-1.8	-0.7	3.7	+ 24 hrs
		BD	-0.9	-2.2	**-4.5**	-1.0	-0.2		+ 24 hrs
LDH	40	S	-0.1	0.0	**6.0**	**16.0**	**49.9**	4.3	**+ 24 hrs**
		BD	1.6	1.1	2.5	3.1	**5.3**		**+ 72 hrs**
Lipase	40	S	5.5	8.9	12.6	**16.7**	**20.6**	16.1	**+ 48 hrs**
		BD	5.0	8.7	13.2	14.6	**20.3**		**+ 72 hrs**
Potassium	40	S	0.7	**6.7**	**20.3**	**40.2**	**128.3**	2.3	**+ 6 hrs**
		BD	0.4	1.9	**3.9**	**4.9**	**7.2**		**+ 24 hrs**
Sodium	40	S	0.1	0.2	**0.6**	**0.6**	**-0.8**	0.3	+ 24 hrs
		BD	0.0	0.2	**0.8**	**1.3**	**1.5**		+ 24 hrs
Total protein	40	S	0.5	-0.4	-0.3	0.2	0.2	1.4	+ 168 hrs
		BD	0.7	-0.3	0.2	0.5	0.7		+ 168 hrs
Transferrin	40	S	-0.6	**1.8**	**1.8**	0.8	0.2	1.5	**+ 6 hrs**
		BD	0.1	0.7	**1.9**	-0.3	-1.4		**+ 24 hrs**
Triglyceride	40	S	1.7	2.4	2.7	3.2	1.0	10.0	+ 168 hrs
		BD	1.1	1.8	1.8	3.8	2.5		+ 168 hrs
Urea	40	S	1.1	2.1	2.5	4.2	5.6	6.1	+ 168 hrs
		BD	1.1	1.4	0.5	2.3	2.8		+ 168 hrs

ALP=Alkaline Phosphatase, ALT=Alanine Transaminase, AST=Aspartate Aminotransferase, CK=Creatine Kinase, GGT=Gamma-Glutamyl Transferase, HDL=High-Density Lipoprotein, LDH=Lactate Dehydrogenase

[a]Differences are calculated as [(concentration BD-tube–concentration S-tube)/concentration S-tube*100]

[b]Analytical coefficient of variation based on the Ricos Criteria [14,15]

[c]Boldface values indicate that the acceptable delay is different between the two investigated blood collection tubes

Table 2: Stability of routine chemistry tests on plasma obtained from blood collected in the Sarstedt S-Monovette® LH tube and the BD Barricor™ tube.

longer stability in the S-Monovette® tube. Stability for each parameter was defined as acceptable when the mean % difference *vs.* the initial measurement did not exceed the CV_a defined by the Ricos criteria [14,15]. For all the tested analytes, the initial measurements did not show clinical significant differences between both tubes, as compared to the TE_a defined by the Ricos criteria [14,15].

None of the samples was hemolysed, therefore the increase in the level observed for some parameters might be due from leakage of residuals cells. As it is claimed by BD that there is a more stable and robust barrier after centrifugation, our results suggests that contents or parts of the blood cells are less likely to leak into the plasma in the BD Barricor™ tube in comparison with our routinely used blood collection tube. Especially for LDH and potassium the observed differences between the 2 tubes are pronounced, particularly during follow-up where we found a mean concentration difference *vs.* baseline

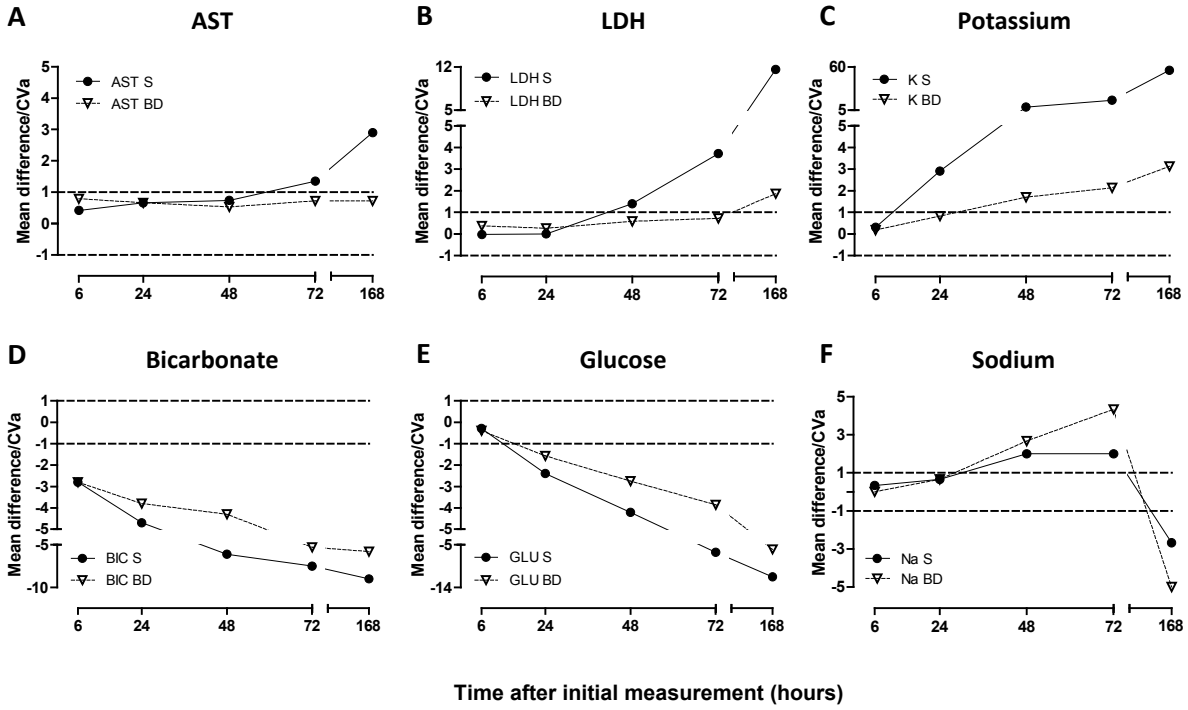
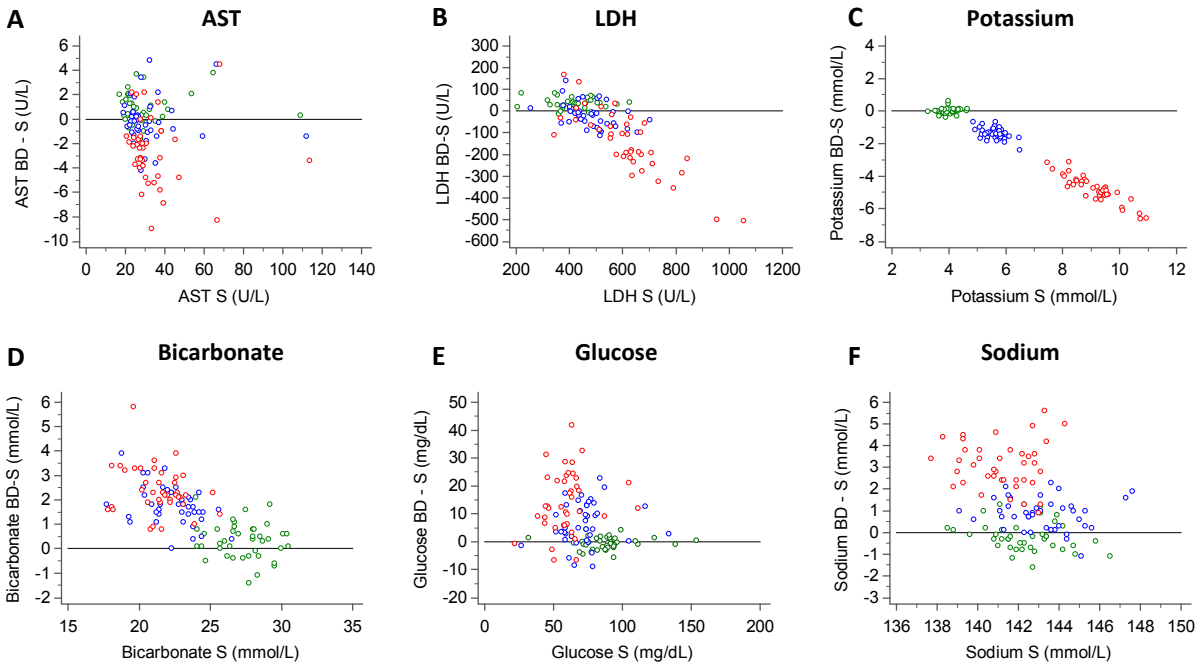


Figure 2: Ratio of the mean difference and the CV_a in function of time for the Sarstedt S-Monovette® LH tube (full line) and the BD Barricor™ tube (broken line) for AST (panel A), LDH (panel B), potassium (panel C), bicarbonate (panel D), glucose (panel E) and sodium (panel F).



Green dots = 0 h, Blue dots = 72 h, Red dots = 168 h

Figure 3: Difference plots between the Sarstedt S-Monovette® LH tube and the BD Barricor™ tube at different time points (green=baseline values, blue=72 h after initial measurement, red=168 h after initial measurement) for AST (panel A), LDH (panel B), potassium (panel C), bicarbonate (panel D), glucose (panel E) and sodium (panel F).

respectively 5 and 10 times higher in the S-Monovette® tube *vs.* the BD Barricor™ tube 72 h after the initial measurement and 10 and 20 times higher in the S-Monovette® tube *vs.* the BD Barricor™ tube 7 days after the initial measurement. In contrast, stability for one parameter, namely GGT, was better in the S-Monovette® tube in comparison with the BD Barricor™ tube. GGT levels are increased in both tubes during follow-up but 7 days after the initial measurement, the levels in the BD Barricor™ tube minimally exceeded the CV_a.

In general, stability studies often differ depending on the studied variables with differences in the tested analytes, matrices, populations and sample size, storage time, temperature conditions, analyzers, acceptance limits and statistical analysis. To our knowledge, this is the first study that directly compared the stability of 21 routine chemistry parameters in a routinely used blood collection tube (S-Monovette® tube) with the new BD Vacutainer® Barricor™ Plasma Blood Collection Tube. Our results are in line with the first and recently published studies of the BD Barricor™ tube. In the study of Balbas et al., stability of the plasma electrolytes (sodium, potassium and chloride) was investigated up to 15 h after centrifugation in open and sealed tubes [9]. Füzery et al. investigated whether the BD Barricor™ tube could be used as an alternative tube for cardiac troponin testing in comparison with routinely used blood collection tubes [8]. Both studies showed that the BD Barricor™ tube is an acceptable and robust alternative for routinely used plasma blood collection tubes. In addition, Balbas et al. showed that the significant increase in potassium was less pronounced in the BD Barricor™ tubes in comparison with the routinely used blood collection tubes which are in line with our observations.

One of the strengths of this study is that the storage conditions chosen are similar to the routine handling of samples in our laboratory. Second, all the measurements over time for all the samples are performed on the same tube, with the exception of one sample. Since for this sample it was necessary to make an (automated) dilution for measurement of triglycerides (concentration at baseline 750 mg/dL [8.48 mmol/L]), measurements 7 days after the baseline measurement are performed on the second tube collected during blood sampling, and this for both types. Third, all measurements were performed on the same Ortho VITROS 4600 Chemistry System with the same lot of reagents and internal quality control for all the parameters was verified thoroughly. Fourth, we investigated more time points over a longer follow-up period (up to one week after blood collection) in comparison with other studies [9-13].

A limitation of the study is the rather small sample size (n=40 for each tube) in comparison with some other studies [10,11,13]. Since we wanted to measure in total 21 parameters at 6 different time points, we decided not to include more samples for practical reasons. Despite the relative low sample number, we are convinced that by including 40 samples per measurement preliminary observations are reliable. Moreover, comparable studies were published with less samples included [12]. Another limitation is the inclusion of apparently healthy subjects. Further studies should investigate whether similar results are obtained in diseased subjects (e.g. hospitalized patients).

The observed longer stability of routine chemistry parameters (with the exception of GGT) in the BD Barricor™ tube, creates some important perspectives for the use of this tube in clinical laboratories. First, additional requests on a blood sample are possible over a longer period. Second, a shorter centrifugation time of this new blood collection tube leads to shorter turnaround times and higher sample throughput. Third, the extended stability enables extend transportation. This could be of interest in the context of hospital networking and in countries with a low population density and/or less clinical laboratories. It is however a prerequisite that the samples are being centrifuged before transportation. Preliminary experiments done by BD demonstrated that the barrier (when the tube is centrifuged) stays in place even after several transportations to different sites. Further investigations are necessary, e.g. in diseased patient populations, but also the effect of pneumatic tube transportation. At the moment, the BD Barricor™ tube is slightly more expensive in comparison with the S-Monovette® LH tube (about 20%). Hereby, one should take into account that by using the BD Barricor™ tube routinely in the clinic, additional financial cost will be reduced. By using this new separator technology the need for secondary aliquots, e.g. when using the S-Monovette® LH tube, can be abandoned. This will lead to a decrease in secondary cost of material, labor, storage and waste management.

In conclusion, our data suggest that the stability of the 21 routine chemistry parameters tested in this study is equal or better in the BD Barricor™ tube in comparison with the S-Monovette® LH tube according to the Ricos criteria [14,15], especially for albumin, AST, total cholesterol, LDH, lipase, potassium and transferrin.

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Conflicts of Interest

No potential conflicts of interest were declared by the co-authors.

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