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Barricor blood collection tubes are equivalent to PST for a variety of chemistry and immunoassay analytes except for lactate dehydrogenase

Joshua E. Raizman[a,b,*,1], Bobbi Lynn Goudreau[b,1], Anna K. Füzéry[a,b,1], George S. Cembrowski[a,b]

[a] Department of Laboratory of Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada
[b] Alberta Public Laboratories Ltd., Edmonton, Alberta, Canada

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ABSTRACT

Introduction: The BD Barricor tube uses a novel mechanical separator designed to eliminate gel artifacts, decrease cellular contamination, and improve stability. Here, we evaluated the Barricor tube as a possible replacement for PST using Beckman Coulter analyzers under both optimal, alternative, and suboptimal centrifugation conditions based on BD recommendations.

Methods: Paired PST and Barricor samples were collected from 4 local hospitals and processed based on site-specific preanalytical systems involving automated or manual centrifugation. Centrifugation conditions ranged from 1912 ×*g* for 10 min (suboptimal), 2060 *g* for 10 min (alternative), and 4000 ×*g* for 3 or 10 min (optimal). Tube volume (4.5 vs. 5.5 ml) was also assessed. Forty-three chemistry and immunochemistry analytes were measured on Beckman Coulter DxC and DxI analyzers.

Results: Using an automated preanlaytical system with suboptimal spin conditions, no bias between PST and Barricor was observed for all analytes tested except lactate dehydrogenase (LD). Further investigation revealed significant increase in LD when Barricor was spun for 10 min at 1912, 2060 and 4000 ×*g*, ranging from +7.4–19.4% vs. PST across the entire measurement interval (87–493 U/l). Smaller tube volume was also associated with higher LD. Differences in LD occurred despite no change in other hemolysis markers such as potassium, phosphate, and AST.

Conclusions: LD is most sensitive to varying centrifugation conditions (time and speed) in Barricor tubes. We recommend that BD centrifugation protocols should be closely evaluated to determine if Barricor is equivalent to PST under local preanalytical configurations.

1. Introduction

The pre-analytical phase is recognized to produce the majority of errors encountered within the total laboratory testing process [1]. Quality of patient results in large part is dependent on the quality and integrity of specimens [2,3]. As such, inadequate specimen quality can produce result and instrument errors (e.g., probe obstructions, dirty flow cells, and interferences from cellular contaminating or fibrin clots) as well as delay reporting, all of which can compromise patient care. Obtaining optimal sample quality is contingent on several factors such as collector technique [2], tube components (anticoagulants and gel separators) [4], and centrifugation conditions [5]. Although the faster turnaround time of plasma separator tubes is an attractive alternative to serum, the presence of residual cellular and fibrin contamination is widely documented in plasma separator tubes [3,4,6]. This has been shown to impact various assays including therapeutic drug measurements [7] and mass spectrometry systems [8] as well as routine chemistries namely potassium [9], glucose [3], lactate dehydrogenase (LD) [3], and troponin [10]. Furthermore, latent clot formation and the presence of fibrin material in the plasma sample post-centrifugation can lead to probe obstruction on automated analyzers. These probe obstruction errors are particularly problematic if they go undetected or if no clot detection system is present, leading to risk of erroneous results and subsequent delay in releasing results.

Becton Dickinson (BD) released the Barricor plasma collection tube in 2016 as a novel alternative to gel-based plasma and serum separator tubes [11]. Unlike gel barriers, which separate cells only within the first 2 centrifugation minutes, channels created from the stretched Barricor

Abbreviations: PST, plasma separator tube

* Corresponding author at: Royal Alexandra Hospital Diagnostic Treatment Center, Room 5047, 10240 Kingsway Avenue NW, Edmonton, Alberta T5H 3V9, Canada.
E-mail address: josh.raizman@albertapubliclabs.ca (J.E. Raizman).

[1] These authors contributed equally to this work.

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separator allow cells to spin out of the plasma throughout the entire centrifugation process [11,12]. BD claims that this novel technology improves plasma quality, eliminates gel artifacts, lengthens sample stability, and enables more efficient laboratory workflow with faster turnaround time. Over the last several years, several independent studies have been published on the analytical performance of Barricor samples showing improved stability and reduced cellular contamination of plasma in these tubes [13–17]. General chemistry analytes appear to show similar, and in some cases improved, stability in Barricor over traditional lithium heparin plasma tubes from BD [15,16]. For the most part, analytical equivalence between Barricor and other types of lithium heparin plasma samples is excellent and this has been confirmed using different chemistry and immunoassay platforms [14,16,18,19]. However, a number of analytes have been noted to show clinically and/or statistically significant differences, including LD [18,19], sodium [18], potassium [18], bicarbonate [19], aspartate aminotransferase (AST) [18], glucose [18], total protein [18], and total and free PSA [16]. These differences have not been uniformly present across all studies but seem to vary based on local experimental design. Despite the reduced cellularity of plasma in Barricor and lack of detectable hemolysis in these studies, a clearly defined mechanism has not been elucidated thus far to explain these inconsistent biases between Barricor and PST. In addition, the use of healthy control samples and/or narrow concentration ranges in these studies limit transference of the majority of these data to different patient and laboratory populations.

Recently, we implemented Barricor tubes only for troponin collections to address non-reproducible spurious false positives troponin results reported by us [10] and others [20,21] using PST tubes on the Beckman AccuTnI + 3 assay. To determine if Barricor could replace PST for a wider variety of chemistry and immunoassay analytes, we sought to evaluate its performance compared to PST on Beckman Coulter DxC and DxI platforms under different centrifugation conditions (time, speed, and tube volume) found among our hospital laboratory network and using wide clinical ranges.

2. Material and methods

2.1. Ethics

This study was part of a quality improvement project and does not constitute clinical research. Review by the University of Alberta Human Research Ethics Board determined that ethics approval was not required.

2.2. Study rationale

Alberta Public Laboratories operates 14 laboratories in Edmonton and its surrounding area. These laboratories range in size from a reference laboratory (full front-end and post-analytic automation, extensive general and special chemistry test menu) to small rapid response laboratories (completely manual sample handling, stat chemistry test menu). The diverse needs of these laboratories have led to a heterogeneous set of instrumentation and sample processing protocols across the city. This study was designed to assess the analytical equivalence of Barricor plasma samples to those currently collected into BD lithium heparin plasma separator tubes (PSTs) under Edmonton's heterogeneous instrumentation and sample processing environments. Four laboratories participated in the study. The University of Alberta Hospital (UAH) is a quaternary care academic centre with a full lab automation system. The Royal Alexandra Hospital (RAH), the Misericordia Community Hospital (MIS) and Grey Nuns Community Hospital are tertiary care hospitals that use manual sample processing. While such a study design restricts the control of confounding variables, it more realistically simulates the heterogeneity in hospital laboratory set-ups that is seen in growing health care organizations

2.3. Blood collection

Blood was collected into Barricor and PST tubes (BD) concurrently from patients who already had to have a PST collected as part of clinical management. Specimens were collected from a variety of hospital locations including inpatient and emergency departments (ED). All tubes were 13 × 100 mm in size with vacuums allowing for either a maximal fill volume of 4.5 or 5.5 ml for Barricor tubes and only 4.5 ml for PSTs.

2.4. Sample processing and analysis

The optimal spin condition recommended by BD in the Barricor package insert is 3 min at 4000 × *g* [12]. If these conditions cannot be met, alternative conditions are recommended based on centrifugation speed, time, and volume configuration. These options allow adoption of Barricor under a wide variety of pre-analytical conditions, from fully automated power processors to manual systems, as well as difference in volume selection that best suites clinical needs.

The data generated in this study was part of a larger evaluation undertaken in our integrated health region to validate the use of the Barricor tube for a multitude of analytes across different sites and instruments. Supplemental Table 1 summarizes the sample processing parameters used for the individual comparisons as well as the corresponding platforms. It also indicates if these parameters met BD's recommendations for handling and processing the Barricor tube. As previously indicated above, the optimal spin time and speed for Barricor is 3 min at 4000 × *g*, but BD also offers alternative parameters dependent on the radius of the centrifuge and Barricor tube configuration (size and vacuum volume).

At all sites, samples were transported to the laboratory either on foot or by a pneumatic tube system. Tubes were handled and processed immediately upon receipt in the laboratory but were processed and spun using centrifuges and speeds dependent on the site. The resulting plasma was analyzed without delay on one of 3 Beckman Coulter analyzers depending on the site location: DxC800 (chemistry), DxC600i (chemistry) or DxI800 (immunoassay).

The DxC800 and DxI800 analyzers at UAH were configured to an automation line with a power processor. The combination of the power processor, centrifuge radius, and maximum centrifugation speed did not meet the recommendations for spin conditions listed in the Barricor package insert for either the 4.5 ml or 5.5 ml tube volumes (Supplemental Table 1). As a result, the 4.5 ml tube volume was chosen for these studies because the centrifugation conditions were closest to the recommendations for this tube configuration.

For studies that compared different centrifugation conditions (spin time, speed, and tube volume) these were performed in laboratories with manual sample processing capabilities using centrifugation conditions that met BD recommendations for both tube volumes (Supplemental Table 1). Since the 5.5 ml tube provided a larger sample volume, we selected this tube for evaluation at all these sites, but not at UAH due to the limitations of the automated power processor as described above.

For the platelet study, paired Barricor and PST tubes were collected via nurse draw from ED, and inpatient settings. Barricor tubes were spun for 3 min at 4000 × *g* and PSTs were spun for 5 min at 2600 × *g*. The corresponding plasma was analyzed for platelet count using the Beckman Coulter LH780 hematology analyzer.

2.5. Data analysis

Data analysis, basic statistics, and graphs were tabulated using Microsoft Excel 2010. Simple linear regression, Bland-Altman plots, *t*-tests, and one-way ANOVA were performed using Analyze-IT for Microsoft Excel ver 5.11. Statistical significance was defined as $p < .05$. For ANOVA, if statistical significance between groups were found, the Tukey post-hoc test was conducted to determine statistical

Table 1
Comparison of 4.5 ml Barricor tubes to PST using a Beckman Coulter DxC800 analyzer configured to an automated power processor*.

Analyte (units)	Sample pairs	Barricor (Mean ± SD)	PST (Mean ± SD)	% Difference	Slope	Y-intercept	R^2	Range	Precision&# (QC mean - %CV)
Albumin (g/l)	59	38.3 ± 6.1	38.2 ± 6.0	0.17	1.005	−0.1025	0.994	23.1–49.2	30.3–1.0% 45.6–1.0%
ALP (U/l)	57	91.2 ± 110.6	90.1 ± 110.2	1.12	1.003	0.8183	1.000	31–881	93.4–2.9% 432.3–8.7%
ALT (U/l)	63	44.8 ± 97.2	44.8 ± 98.5	1.30	0.9861	0.6873	1.000	11–791	29.5–3.3% 93.1–1.5%
Amylase (U/l)	58	63.8 ± 35.1	63.6 ± 35.2	0.46	0.995	0.4534	0.998	10–155	81.7–1.9% 403.9–1.7%
AST (U/l)	56	51.2 ± 65.3	50.7 ± 66.2	1.66	0.9848	1.1964	0.998	14–435	33.4–2.7% 191.8–1.3%
Bicarbonate (mmol/l)	141	25.1 ± 3.7	24.6 ± 3.6	2.51	0.9164	2.5938	0.771	9.9–43.8	16.5–4.6% 30.1–1.5%
Bilirubin, direct (μmol/l)	23	11.6 ± 30.6	11.3 ± 30.3	8.26	1.0024	0.2419	1.000	2–148	3.6–13.8% 12.1–5.2%
Bilirubin, total (μmol/l)	55	18.96 ± 19.49	18.75 ± 19.04	12.58	0.9916	0.3646	0.939	2–107	20.2–9.8% 88.2–2.7%
CRP, high sensitivity (mg/l)	65	42.0 ± 71.5	41.7 ± 71.1	1.04	1.0043	0.1244	1.000	2–365	0.7–7.6% 6.2–3.9%
Calcium, total (mmol/l)	87	2.24 ± 0.12	2.28 ± 0.12	−1.57	0.9865	−0.0048	0.970	2.00–2.61	2.1–1.5% 2.9–1.3%
Chloride (mmol/l)	142	102.6 ± 5.2	102.9 ± 5.0	−0.28	1.0015	0.4484	0.951	83–111	86.2–1.4% 99.8–1.2%
Cholesterol, total (mmol/l)	58	4.1 ± 1.3	4.1 ± 1.3	1.19	0.998	0.0531	0.996	1.1–6.8	2.9–1.2% 6.9–1.3%
Creatinine, alkaline picrate (μmol/l)	144	100.1 ± 62.4	101.5 ± 63.0	−1.38	0.9884	−0.2281	0.996	35–402	73.5–4.7% 528.5–1.5%
Creatinine, enzymatic (μmol/l)	87	84.8 ± 49.2	83.1 ± 48.8	2.34	1.0067	1.1132	0.998	40–415	77.6–2.3% 522.4–1.2%
Creatine kinase (U/l)	124	341.2 ± 675.2	346.4 ± 686.2	−1.28	0.9796	2.7838	1.000	14–3973	156.3–1.5% 535.1–1.1%
C3 (g/l)	54	1.3 ± 0.3	1.3 ± 0.3	1.43	0.9829	0.0388	0.980	0.5–2.1	0.9–2.1% 1.5–2.3%
C4 (g/l)	54	0.3 ± 0.2	0.4 ± 0.2	−6.73	0.9228	0.0037	0.964	0.09–0.94	0.1–6.9% 0.3–4.1%
GGT (U/l)	41	85.2 ± 94.8	86.0 ± 94.9	2.21	0.9989	−0.7823	0.998	6–338	26.7–8.6% 217.5–2.8%
Glucose (mmol/l)	112	6.5 ± 2.0	6.4 ± 2.0	0.23	1.0028	−0.0085	0.968	3.8–15.9	4.7–2.4% 16.3–1.6%
HDL-C (mmol/l)	56	1.2 ± 0.4	1.2 ± 0.4	1.01	0.9993	0.0115	0.996	0.20–2.18	0.75–3.0% 1.9–2.2%
IgA (g/l)	54	2.7 ± 1.6	2.6 ± 1.6	0.66	1.0074	−0.0027	0.998	0.21–8.3	1.2–1.8% 1.9–2.9%
IgG (g/l)	55	12.1 ± 4.6	12.1 ± 4.7	0.10	0.9763	0.2715	0.989	4.7–28.6	6.2–1.5% 10.4–1.5%
IgM (g/l)	54	1.1 ± 0.5	1.1 ± 0.5	1.17	0.9902	0.0199	0.998	0.36–3.0	0.6–2.5% 1.0–2.6%
Iron (μmol/l)	57	11.9 ± 6.9	11.8 ± 6.7	0.39	1.0205	−0.1412	0.998	1.2–29.4	11.0–2.1% 44.7–1.5%
LDH (U/l)	**93**	**215 ± 68.0**	**183.7 ± 66.5**	**19.4&**	**0.9666**	**37.4**	0.837	**87–488**	**121.8–2.4% 3511–1.6%**
Lipase (U/l)	50	46.4 ± 81.7	45.7 ± 81.1	1.73	1.008	0.3862	1.000	10–381	28.1–8.5% 58.4–3.9%
Magnesium (mmol/l)	101	0.82 ± 0.09	0.82 ± 0.09	−0.16	1.0015	−0.0025	0.962	0.58–1.21	0.81–2.0% 1.6–2.7%
Osmolality (mmol/kg)	36	292.6 ± 13.8	292.8 ± 13.7	−0.08	0.9804	5.5036	0.953	255–297	289.1–0.5% 334.0–1.2%
Phosphate (mmol/l)	59	1.1 ± 0.2	1.1 ± 0.2	−0.35	1.0337	−0.0386	0.984	0.46–1.62	1.0–1.9% 2.7–1.3%
Potassium (mmol/l)	141	3.93 ± 0.649	3.96 ± 0.664	−0.57	0.9575	0.1419	0.960	2.8–7.3	3.9–1.2% 6.8–1.0%
Prealbumin (g/l)	57	0.2 ± 0.1	0.2 ± 0.1	0.31	1.0113	−0.0016	0.996	0.231–0.388	0.2–2.3% 0.3–2.5%
Protein, total (g/l)	59	68.3 ± 7.7	67.9 ± 7.7	0.64	0.9823	1.6059	0.958	50–95	42.4–1.5% 69.5–1.6%
Sodium (mmol/l)	142	137.63 ± 4.0714	137.25 ± 4.049	0.28	0.9815	2.9224	0.953	117–144	119.2–1.0% 151.2–0.9%
Triglyceride (mmol/l)	57	1.4 ± 0.1	1.4 ± 0.7	1.75	1.0108	0.0072	0.996	0.4–4.3	1.1–1.8% 2.3–1.8%
UIBC (μmol/l)	56	11.6 ± 30.7	11.3 ± 30.6	0.97	0.9941	0.5576	0.980	14.7–81.8	31.3–8.7% 23.5–15.8%
Urea (mmol/l)	135	7.2 ± 6.6	7.2 ± 6.6	−0.69	0.9973	−0.0018	0.996	0.8–42.4	5.1–4.2% 17.3–2.5%

(*continued on next page*)

Table 1 (*continued*)

Analyte (units)	Sample pairs	Barricor (Mean ± SD)	PST (Mean ± SD)	% Difference	Slope	Y-intercept	R^2	Range	Precision[&#] (QC mean - %CV)
Uric acid (μmol/l)	58	311.5 ± 111.8	312.1 ± 112.5	−0.12	0.9926	1.6931	0.998	134–639	244.3–1.2% 554.9–1.1%

[*] All samples were spun for 10 min at 1912 *g* – suboptimal spin conditions for Barricor.
[&] $p < .001$ versus PST tubes.
[#] Precision data derived from 6 months of routine QC of the same BioRad unassayed chemistry control lots during the study period. QC performed twice per day at each indicated level.

significance between the individual groups. Total allowable error for LD was set at 15% with an allowable bias of 7.5%. These criteria were derived from the Institute of Quality Management in Healthcare (IQMH) [22]. Long term precision at all levels were derived from routine QC runs over a course of 6 months during the period of time Barricor tubes were being evaluated.

3. Results

Forty three chemistry and immunoassay analytes were initially evaluated in 4.5 ml Barricor against PST using Beckman Coulter DxC800 for chemistry anlaytes (Table 1) and DxI800 for immunoassay-based analytes (Table 2) configured to a power processor automated sample handling system. This power processor could not be adjusted and therefore limited spin conditions to 10 min at 1912 × *g*. These conditions are considered suboptimal according to the BD package insert for Barricor. Concentration of samples selected spanned the linear range of all assays. Linear regression analysis of paired Barricor and PST samples across both platforms and comparisons revealed slopes ranging from 0.901 to 1.0337, and coefficient of determination (R^2) from 0.771 to 1.0. Despite using suboptimal spin conditions for Barricor on the power processor, all analytes, except for LD, demonstrated equivalency thereby allowing reference intervals to be transferred to PST. Bias across all the comparisons were acceptable except for LD and ranged from −6.73 to +12.58% across all analytes tested. Precision at all levels tested were also well within acceptable limits. LD demonstrated a statistically significant positive bias of 19.4% under these centrifugation conditions. This bias was not associated with any major analytical variation during the study period with the LD assay demonstrating excellent precision ranging from 1.6 to 2.4% CV. Furthermore, the hemolysis index on the analyzer was negative and there were no difference observed for other hemolysis markers such as potassium, phosphate, and AST. We also confirmed significantly lower platelet count in Barricor plasma compared to PST in a subset of samples (Supplemental Fig. 1), however these samples were spun only using optimal conditions. Therefore, we sought to further investigate and characterize the impact of centrifugation conditions on LD in Barricor.

In our first follow-up experiment, we kept the tube volume and centrifugation time the same but increased the centrifugation speed from 1912 × *g* to 2060 × *g* in order to meet alternative BD centrifugation recommendations for the Barricor tube. Linear regression analysis showed that spinning under either the suboptimal or the alternative condition produced similar large constant biases with y-intercepts ranging from 25.8 to 37.4 U/l (Fig. 1A). Bland-Altman difference plots confirmed these large biases (19.4% at 1912 × *g* and 18.4% at 2060 × *g*) while also demonstrating a large scatter of data points in the concentration range of 100–250 U/l (Fig. 1B). These findings suggest that adjusting the centrifugation speed alone does not impact LD results for samples collected into Barricor tubes.

In our second follow-up experiment, we kept the centrifugation time and speed constant but changed the Barricor tube volume from 4.5 to 5.5 ml. Agreement and correlation with PST was slightly worse for the 4.5 ml Barricor tube compared to 5.5 ml tube, with y-intercepts of 25.8 vs. 8.9 U/l (Fig. 1C). In addition, Bland-Altman plots demonstrated a significant bias of 18.4% using the smaller tube volume, compared to the much lower bias of 7.4% for the larger tube volume (Fig. 1D). These findings suggest that tube volume impacts LD for samples collected into Barricor tubes.

In our last investigation, we examined the effect of varying centrifugation times on LD results. Barricor tubes of 5.5 ml size were spun for either 3 min at 4000 × *g* (optimal condition) or for 5 min at 4000 × *g* (alternative condition) and demonstrated excellent correlations and biases of 2.2% and −1.7%, respectively (Figs. 2A, B and D). However, centrifugation for 10 min at 2060 × *g* (alternative condition) led to a higher bias of 7.4% that nearly exceeded our bias goal of 7.5% (Figs. 2C and D). These findings suggest that centrifugation time impacts to some degree the LD results for samples collected into Barricor tubes.

Table 2
Comparison of 4.5 ml Barricor tubes to PST[*] using a Beckman Coulter DxI800 analyzer configured to an automated power processor. All samples were centrifuged for 10 min at 1912 *g*.

Analyte (units)	Sample pairs	Barricor (Mean ± SD)	PST (Mean ± SD)	% Difference	Slope	Y-intercept	R^2	Range	Precision[#] (QC mean - %CV)
AFP (μg/l)	66	10.9 ± 63.8	11.2 ± 67.0	1.86	0.901	0.7917	0.992	0.8–7.6	27–6.3% 105–6.4%
Ferritin (μg/l)	66	218.1 ± 321.1	217.6 ± 331.3	4.57	0.9634	8.4403	0.988	9–143	318–5.4%
Folate (nmol/l)	52	29.0 ± 10.9	29.2 ± 11.0	−0.21	0.9881	0.4742	0.968	9.5–54.3	7–7.6% 30.2–5.0%
Prolactin (μg/l)	66	12.2 ± 15.0	12.7 ± 16.6	−2.14	0.901	0.7917	0.992	1–139	7–3.8% 14.9–4.5%
TSH (mU/l)	68	2.10 ± 1.66	2.13 ± 1.67	−0.87	0.9934	−0.0097	0.994	0.001–9.38	0.67–3.1% 5.6–3.8% 28.7–4.5%
Vitamin B12 (pmol/l)	64	334.3 ± 181.8	335.5 ± 188.4	1.18	0.944	17.549	0.958	67–1080	129–8.8% 464–6.9%

[*] No statistical significance was determined between any of the means.
[#] Precision data derived from 6 months of routine QC of the same BioRad unassayed chemistry control lots during the study period. QC performed twice per day at each indicated level.

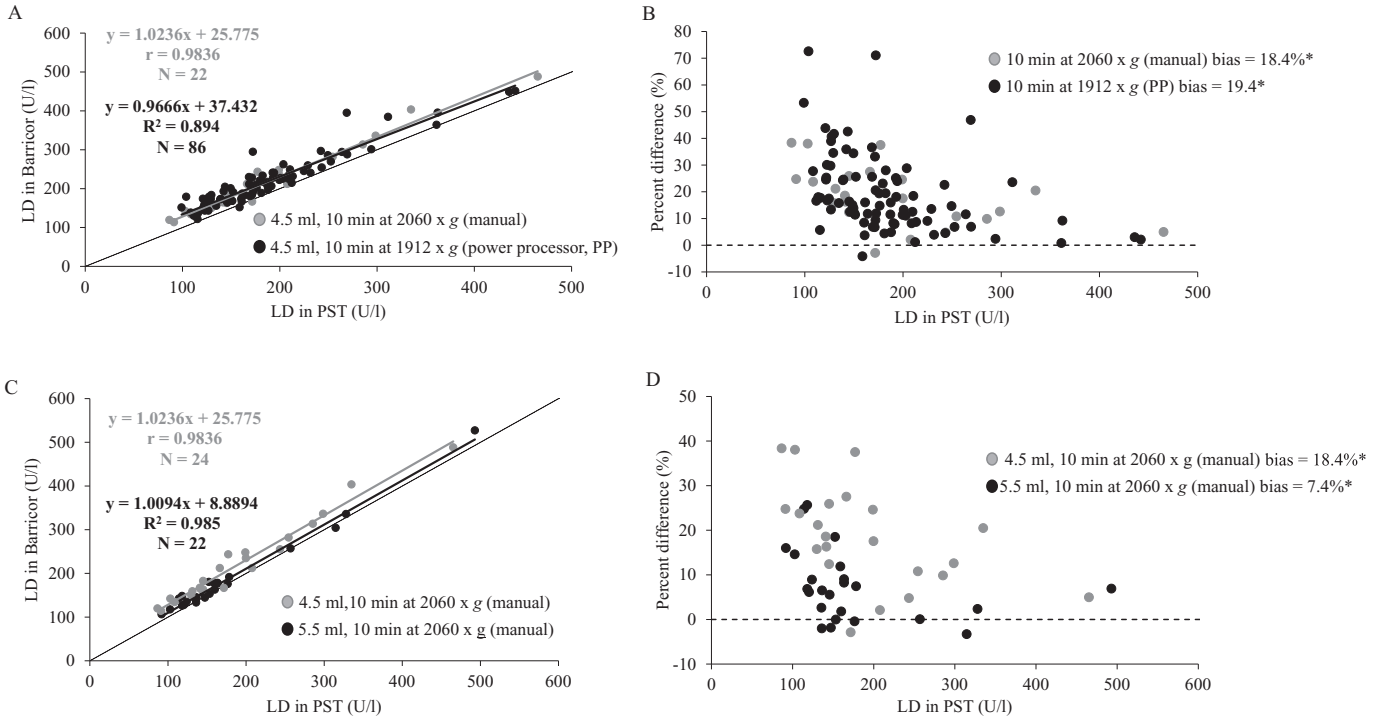

Fig. 1. Effect of centrifuge speed and tube volume on LD. Linear regression (A) and a Bland-Altman difference plot (B) both indicate that spinning 4.5 ml Barricor tubes under either suboptimal (10 min, 1912 ×*g*) or alternative conditions (10 min, 2060 ×*g*) produces large constant biases for LD when compared to PST. Linear regression (C) and a Bland-Altman difference plot (D) both indicate that spinning 4.5 vs. 5.5 ml Barricor tubes under alternative conditions (10 min, 2060 ×*g*) produces a larger bias for the 4.5 vs. 5.5 ml tube compared to PST. *$p < .001$ between Barricor and PST.

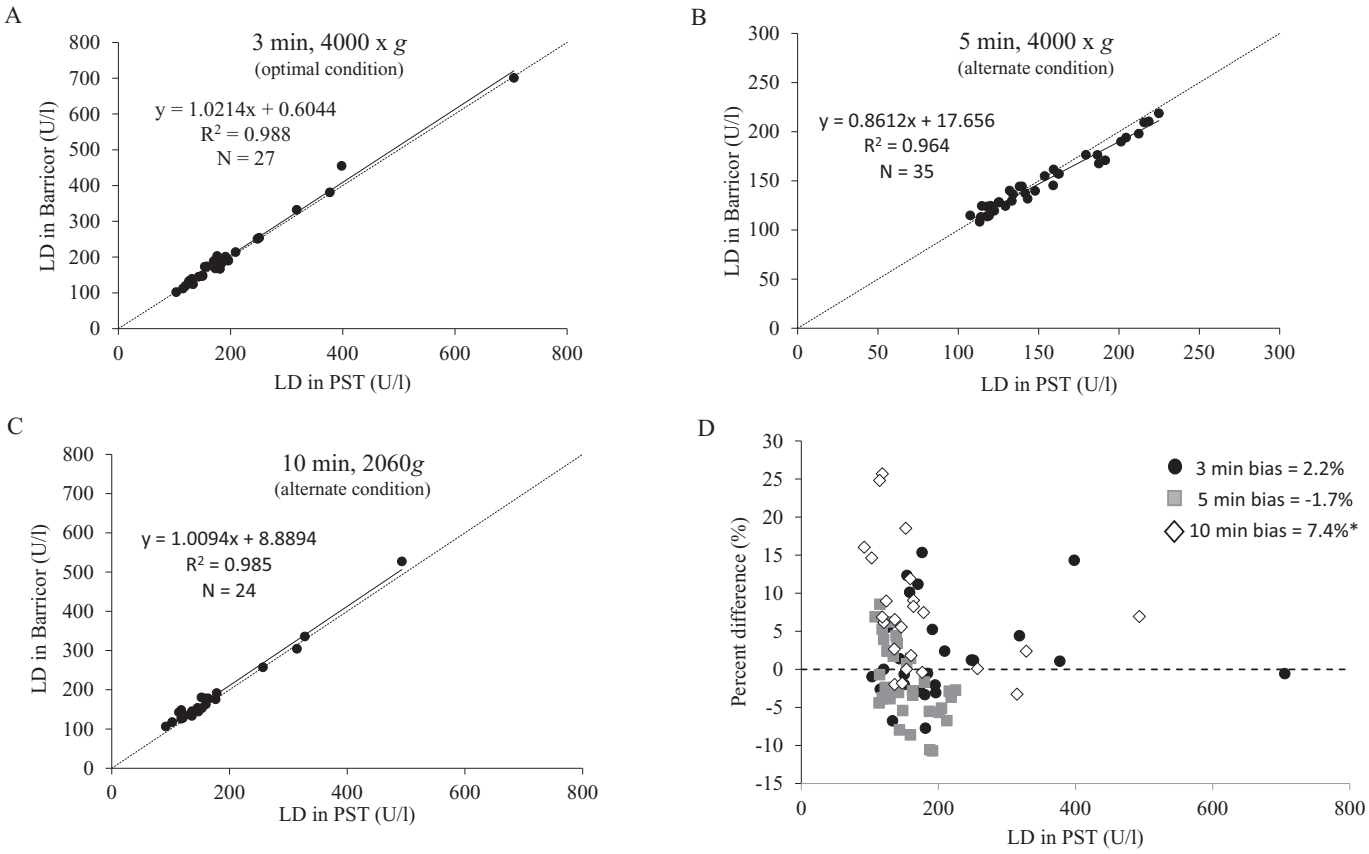

Fig. 2. Effect of centrifuge time on LD. Paired 5.5 ml Barricor and 4.5 ml PST tubes were centrifuged for increasing spin times at either 2060 ×*g* or 4000 ×*g*. Linear regression (A, B, C) indicates good correlation between Barricor and PST under all three conditions. Bland-Altman difference plots (D) show a statistically significant positive bias for Barricor compared to PST when Barricor is spun for 10 min.*$p < .001$ between Barricor and PST.

4. Discussion

We evaluated the Barricor tube on our Beckman Coulter DxC800 and DxI800 analyzers by comparing 43 chemistry and immunoassay analytes to PST across a wide range of concentrations. Despite our automated power processor centrifugation not meeting BD recommendations for alternative or optimal spin conditions, we were still able to demonstrate excellent agreement between these two tubes for the majority of chemistry and immunoassay analytes. Out of the 43 analytes evaluated, LD was the only analyte with poor performance under these conditions, with clinically and statistically significant positive biases compared to PST. To further explore this phenomenon, we compared Barricor against PST under a variety of centrifugation conditions. We found that tube volume and centrifugation time, but not speed, had the greatest effect on LD results.

Several recent studies have validated Barricor for a number of routine chemistry and immunoassay analytes [14,16,18,19]. These studies demonstrated equivalency between Barricor and other tube types thereby enabling reference intervals to be transferred to Barricor. Interestingly, among these studies LD was commonly found to perform differently in Barricor, with one study reporting significant positive bias [18] while another study demonstrating the opposite phenomenon [19]. Our study was most consistent with Arslan et al., who found a 23.5% positive bias in Barricor compared to PST on the Beckman Coulter AU platform when subjected to suboptimal spin conditions (3 min at 2360 $\times g$) [18]. In contrast, Cadamuro et al. found a significant negative LD bias in Barricor compared to PST on the Roche Modular platform [19]. This bias remained consistent at both suboptimal (10 min at 2000 $\times g$, assuming a radius of > 210 mm) and alternative (5 min at 3000 $\times g$) centrifugation conditions. In yet two other more recent studies, LD in Barricor and PST were found to perform equivalently when Barricor was centrifuged under optimal conditions (4000 $\times g$ for 3 min) [14,16]. In contrast to our power processor centrifugation conditions and to those of Arslan et al. and Cadamuro et al., the recent studies by Founier et al. and Dupuy et al. used optimal centrifugation conditions of 3 min at 4000 $\times g$ for Barricor. Even under alternative centrifugation conditions (10 min at 2060 $\times g$), we still observed a significant bias of 18.4% in Barricor. Taken together, all of this data suggests that BD's optimal and alternative centrifugation conditions may not be equivalent after all when it comes to LD. The biological variation for LD based on the Westgard database is 8.6% and using precision data during the study period (Table 1) the real change value (RCV) is calculated as approximately 24–25%. The RCV exceeds the bias observed in Barricor tubes, however, the extend of this positive bias still raises questions about transference of reference intervals to Barricor tubes given the potential to falsely raise LD in patients.

The underlying mechanisms remain unclear. We were careful to include patient samples free of hemolysis based on hemolysis index assessment, ruling out the possibility of artefactual increases in LD due to leakage from cells. Other hemolysis markers such as potassium, phosphate, and AST were also unaffected. When spun under optimal conditions we confirmed the improved quality of Barricor plasma in a separate subset of Barricor patient specimens compared to concurrently collected PST from ED and inpatients by showing 55% fewer platelets in Barricor. However, we did not measure cell counts such as platelets, red blood cells (RBC) and white blood cells (WBC) in the LD comparison studies under different centrifugation conditions, and as a result are unable to correlate centrifugation conditions with plasma cellularity contamination. Furthermore, samples were transported to the laboratory by different conditions (foot and pneumatic tube system). The pneumatic tube system is used at UAH where the positive bias in LD was observed using suboptimal centrifugation conditions. Turbulence created as a result of transport through the system may contribute to cell lysis, in turn, increasing LD in Barricor and further confounding the findings of this study. However, PST tubes were transported at the same time as Barricor thereby controlling this variable and the other markers of hemolysis were also unaffected.

Due to the relative novelty of Barricor tubes, little is known about itsperformance under different pre-analytical systems and patient populations. The discrepancy between Barricor and PST for LD is large enough to have clinical consequences depending on the centrifugation conditions. Surprisingly, only optimal spin conditions (3 min at 4000 $\times g$) but not alternative (10 min at 2060 $\times g$) was acceptable using 4.5 ml tubes, and alternative conditions was only acceptable if 5.5 ml tubes were used. Thus, LD appears to be most sensitive to changes in centrifugation conditions of which can directly influence cellular content of plasma and in turn its quality. For these reasons, serum is recommended as the best sample type for LD [23] and has been shown to improve accuracy of LD measurement vs. collection in lithium heparin plasma [18,24]. However, in a study by Herzum et al., contamination of plasma with thrombocytes did not increase LD activity compared to plasma free from cells [25] and Moller et al. demonstrated that altering centrifugation time and force contributed to slight but insignificant increases in LD (6.3%) [26]. In contrast to PST, we report variable increases in LD in Barricor tubes depending on the centrifugation configuration and tube volume.

Barricor has been considered the new standard for lithium heperan plasma owning to plasma quality that in many regards is equivalent to the quality of serum [13], yet the increases in LD independent from presence of other hemolysis markers suggest other factors need to be considered to explain this discrepancy. We hypothesize that the force of the RBCs against the bottom of the tube during centrifugation (in both PST and Barricor) may potentiate LD leakage out of cells without lysing them. The longer the spin time, the more time for these analytes to leak (or be generated) and released into the plasma. With the PST, the barrier between plasma and cell contents is completely formed at 2 min preventing analytes from crossing into the plasma. In contrast, the Barricor tube separator device remains open during the entire centrifugation. This may explain the correlation between higher LD levels in Barricor vs. PST at longer spin times. Additionally, Arslan et al. observed higher bias for LD collected from hospitalized patients compared to healthy individuals and attributed these differences to cellular fragility between these populations [18].

While additional experiments are needed to confirm our hypothesis, a recent paper [17] may also hint at such a phenomenon. Padoan et al. performed a study in which they compared platelets, RBCs, white blood cells (WBCs) and free hemoglobin in the plasma fraction of paired Barricor and PST samples. The paired samples were spun either (a) for increasing times at constant speed, or (b) for a constant time at increasing speeds. While samples spun for longer times or at higher speeds showed incremental reductions in platelets, RBCs and WBCs within the plasma fraction, they also showed incremental increases in free hemoglobin. Hemoglobin, like LD, is present in very high concentrations within RBCs so if our hypothesis of “leaky” cells is correct then it could also explain the observations of Padoan et al. It is unclear why we did not observe any hemolysis effect nor differences with other intracellular analytes such as potassium, phosphate, and AST. One explanation could be that because LD exists in much higher concentrations within the cellular cytoplasm than these other analytes, it is more susceptible to pre-analytical variation. Cellular contamination of plasma, cellular fragility in hospitalized patients, and differences in chemistry platforms likely further confounded these observations. Lastly, it remains unclear why the larger tube size of 5.5 vs. 4.5 ml reduces the bias with PST when spun under alternative conditions. Bias was reduced from 18.4% to 7.4% but still remained statistically significant and near the upper range of our bias goal of 7.5%. Differences in force exerted on these tubes with different volumes may contribute to variation in cellular lysis during centrifugation. Further studies are required to investigate if there is a link between blood volume, centrifugation speed, and cellular contamination.

5. Conclusions

Using Beckman Coulter analyzers and suboptimal Barricor centrifugation conditions, we demonstrated equivalency between Barricor and PST for 42 different analytes. There was a positive bias for LD. This bias was eliminated completely when using BD's recommendations for optimal spin conditions. However, the bias remained to varying degrees when using alternative spin conditions as specified by the tube manufacturer. Our findings emphasize that centrifugation protocols should be evaluated closely to avoid the introduction of artefactual pre-analytical changes in analyte concentrations, especially for LD. If alternative or suboptimal centrifugation systems are the only options present and depending on the tube size used, either separate reference intervals or serum should be considered for LD. Further studies are warranted to conclusively explain the mechanisms underlying increase in LD activities in Barricor despite lack of hemolysis.

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Appendix A. Supplementary data

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