Research Article

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Influence of reduced centrifugation time on clinical chemistry analytes and literature review

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Abstract

Objectives: Centrifugation is a time-consuming step which increases the turnaround time (TAT) in laboratories. A few studies have addressed the effect of altering centrifugation settings on analytical quality for clinical chemistry analytes, and most of these studies have used collection tubes with gel separators. However, gel separator tubes may be unsuitable for some laboratories because they are slightly more expensive than tubes without gel separators and are not appropriate for some special tests. The aim of this study was to investigate the effect of centrifugation conditions on clinical chemistry analytes.

Methods: We compared centrifugation times of 7 min at 2,200×*g* and 5 min at 2,750×*g* with the manufacturer's protocol of 10 min at 1,300×*g* as the reference condition. Twenty general chemistry analytes were studied in lithium heparin plasma tubes without gel separators.

Results: For all analytes except carbon dioxide (CO_2), no significant differences in analyte results were observed when the centrifugation time was reduced. Deming regression and Bland–Altman plots demonstrated an acceptable clinical concordance within the limits of total allowable error for all analytes between the two rapid centrifugation conditions with the reference centrifugation condition.

Conclusions: Our results confirmed that alternate centrifugation conditions for either 7 min at 2,200×*g* or 5 min at 2,750×*g* of samples collected in lithium heparin tubes without gel are acceptable for clinical chemistry analytes. Our data support using centrifugation at higher speeds for shorter times to improve TAT without altering the quality of the analytical results.

Keywords: centrifugation time; clinical chemistry analyte; plasma; relative centrifugal force; turnaround time

Introduction

Reducing intra-laboratory turnaround time (TAT) is a major challenge for clinical laboratories. The reduction in TAT can lead to improved patient care and physician efficiency and to improved satisfaction for both patients and physicians [1]. Delays in reporting and laboratory results would delay the diagnosis and treatment of patients [2]. Laboratory TAT is defined as the time from the reception of a specimen to releasing results in the informatics lab system. This includes specimen preparations, centrifugation steps, analytical measurements, and post-analytical steps. Among these processes involved in the laboratory TAT, centrifugation steps for sample preparation are potential bottlenecks in the laboratory throughput, causing prolonged TAT, which may lead to stakeholder's unsatisfaction with the laboratory service [3], especially in emergency laboratories. Choosing optimum centrifuge conditions for blood samples plays an important role in the pre-analytical procedure for sample quality, accuracy, precision, and TAT for the laboratory results.

The separation efficacy of the centrifugation process depends on five main parameters: centrifugation time, speed or relative centrifugal force (RCF or *g*), temperature, and acceleration and deceleration profiles [4]. Variations in centrifugation temperatures are limited by the stability of the analyte being tested. The acceleration and deceleration rates of centrifugation depend on the type of sample separation usually used according to the manufacturer's instructions. Thus, centrifugation time and speed can be

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easily varied to achieve the desired analytical quality of sample separation for subsequent analyses. High-speed or long-time centrifugation can generate hemolysis, but low-speed centrifugation can lead to insufficient separation of plasma or serum from the cellular blood components [5]. The Clinical and Laboratory Standards Institute (CLSI) recommends using centrifugation time and relative centrifugal force following the manufacturer's guidelines for specific blood collection tubes that typically recommend centrifugation for 10–15 min, depending on the type of tube [6]. The WHO guidelines recommend a centrifugation time of at least 10 min and 1,500×*g* for serum and at least 15 min and 2,000–3,000×*g* for plasma [7, 8].

Centrifugation is an important step in the pre-analytical phase, and the influence of centrifugation on clinical chemistry test results has been reported under varying centrifugation conditions and different blood sample types. A wide variety of centrifugation conditions have been recommended in various guidelines from scientific societies, but most rely either on recommendations by experts or manufacturers rather than on published scientific research. A few studies have investigated the effect of centrifugation times of less than 10 min on serum or plasma chemical laboratory results, but these studies used different centrifugation times, speeds, and types of blood collection tubes [4, 9–12], so there are no consistent results among them. Although most studies on the effect of centrifugation conditions have used collection tubes with gel separators [4, 9, 11, 13–15], previous studies have suggested that the gel separator tubes are not appropriate for some special tests, especially for therapeutic drug monitoring, clinical toxicology procedures, and allergy test [16, 17]. In addition, inappropriate blood separation following centrifugation from gel separator tubes can occur due to collection tube factors, laboratory factors (e.g., speed of centrifugation, temperature, conditions of storage, and acceleration/deceleration), and patient factor [18, 19]. A statistically significant difference in myoglobin and CK-MB levels has been reported between tubes with and without separator gels [20]. The gel separator tubes are slightly more expensive than non-gel separator blood collection tubes. Moreover, collection tubes with gel separators are still unavailable in our country and other low- and middle-income countries due to cost savings strategy.

Therefore, the aim of this study was to compare the manufacturer's recommended centrifugation times with two shorter centrifugation times, 2,200×*g* for 7 min and 2,750×*g* for 5 min, using lithium heparin tubes without gel on clinical chemistry testing.

Materials and methods

Sample collection and centrifugation

Venous blood samples were collected, following informed consent, by experienced phlebotomists from 120 patients at the Emergency Department wards at Songklanagarind Hospital, Thailand. Each blood sample was collected into a BD Vacutainer lithium-heparin tube (Cat No. 368496) (Becton Dickinson, Plymouth, UK). All samples were anonymized immediately after the phlebotomy, and centrifugation was performed within 1 h after blood sampling. Of the three collected tubes of each individual, centrifugation was carried out for one tube according to routine laboratory practice following the BD manufacturer's instruction (10 min at 1,300×*g*) [21]. The other two blood collection tubes were centrifuged in each of these setting conditions based on WHO recommendation [7] and literature review [4, 8–15, 22, 23]: (a) 7 min at 2,200×*g* and (b) 5 min at 2,750×*g*. Each tube was gently inverted 6 to 8 times and then immediately centrifuged using a Beckman Coulter Allegra X-12 refrigerated centrifuge with a swinging bucket rotor at a controlled temperature of 21 ± 1 °C, an acceleration time of 10 s (included in the overall centrifugation time), and a deceleration time of 11 s (after centrifugation). Following centrifugation, all samples were inspected visually for any visible hemolysis, icterus, and lipemia and the quality of plasma separation from the blood cells. They were then analyzed immediately in random order. Blood samples with visible hemolysis after centrifugation using reference condition (10 min at 1,300×*g*) were excluded from the study.

Methods of analysis

After centrifugation, the three tubes of each individual were immediately analyzed using a Cobas 6,000 analyzer (Roche Diagnostics, Rotkreuz, Switzerland) on a c 501 modules for chemistry assays. The following selection of general chemistry analytes was measured in all samples: glucose, blood urea nitrogen (BUN), creatinine, sodium (Na), potassium (K), chloride (Cl), carbon dioxide (CO_2), calcium (Ca), phosphorus (P), direct bilirubin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, and lactate dehydrogenase (LDH). Serum Index Gen.2 (Roche Diagnostics) was used for semi-quantitative determination of the lipemia, hemolysis, and icterus indices. A single analysis was performed for all analytes. As is standard procedure in our laboratory, the Cobas machine underwent a quality control check daily using control samples from the manufacturer (Roche Diagnostics) to ensure all measurements remained within the target ranges.

We also evaluated the effects of different centrifugation conditions on the TAT of results. Of the three collected tubes of each individual, centrifugation was carried out in each condition. The tube for reference condition (10 min at 1,300×*g*) of the routine analysis was first centrifugated. The tubes for rapid centrifugations at 7 and 5 min were subsequently centrifugated. After centrifugation, the tube of each individual was immediately analyzed using a Cobas 6,000 analyzer. The TAT for each specimen was calculated as the time from specimen receipt in the laboratory until the result was verified. However, the waiting time for the centrifugation step was not included.

Statistical analysis

The clinical chemistry analytes from the samples centrifuged following the manufacturer's instructions (10 min at 1,300×*g*) were compared with samples centrifuged for 7 min at 2,200×*g* and 5 min at 2,750×*g* by plotting the data in the EP Evaluator software (Data Innovations, South Burlington, USA) and R program (version 4.0.3). The slopes, intercepts, standard errors of the estimate, and correlation coefficients (R) were depicted in a scatter plot with a Deming regression fit using total allowable error (TEa) as acceptance limits [24]. Total allowable error of each assay was based on the Clinical Laboratory Improvement Amendments (CLIA) and the Royal College of Pathologists of Australasia (RCPA). As some of the parameter values were normally distributed while others were not, differences were considered non-significant when a p-value of >0.05 was obtained using the Mann–Whitney U Test or t-test, depending on the data distribution. Differences in the centrifugation conditions were assessed using a Bland-Alman difference plot with an error index. The error index in the Bland–Alman plot assesses the ratio of the difference between two centrifugation conditions to the total allowable error. An index greater than 1.00 or less than −1.00 means the difference between the two conditions exceeds the total allowable error, and the results are unacceptable. If more than 5 % of the specimens have an unacceptable error index, the experiment fails (EP Evaluator software). The results were also compared with the available analytical quality specifications for total allowable error [25]. Coefficients of variation (CV) were calculated by the Cobas 6,000 instrument software from internal quality control samples of two levels (normal and clinically relevant abnormal concentrations) for each analyte (Table 1). These quality controls were run once a day.

Table 1: Analytical variation and total allowable error of chemistry analytes.

Parameters	Units	CV, %[a]	Total allowable error[b]
Albumin	g/dL	3.16	8 %
Alkaline phosphatase (ALP)	U/L	2.47	20 %
Alanine aminotransferase (ALT)	U/L	3.00	15 %
Aspartate aminotransferase (AST)	U/L	2.23	20 %
Blood urea nitrogen (BUN)	mg/dL	2.49	2 mg/dL or 9 %
Calcium (Ca)	mg/dL	1.10	1 mg/dL
Chloride (Cl)	mmol/L	1.30	5 %
CO_2	mmol/L	1.98	20 %
Creatinine	mg/dL	2.82	0.2 mg/dL or 10 %
Direct bilirubin	mg/dL	4.08	0.4 mg/dL or 20 %
Glucose	mg/dL	1.61	8 %
Lactate dehydrogenase (LDH)	U/L	1.70	15 %
Phosphorous (P)	mg/dL	1.30	0.3 mg/dL or 10 %
Potassium (K)	mmol/L	1.46	0.3 mmol/L
Sodium (Na)	mmol/L	1.22	4 mmol/L
Total bilirubin	mg/dL	3.64	20 %
Total protein	g/dL	3.54	8 %

[a]Total coefficient of variation (CV) is the standard deviation divided by the mean as deduced from internal control measurements of two-level concentrations. [b]Total allowable error of each parameter is evaluated based on CLIA and RCPA.

Results

The results of the routine clinical chemistry analyses in this study are summarized in Table 2. The samples centrifuged for 10 min at 1,300×*g* following the manufacturer's instructions were considered as the reference. The slope and intercept of most measured analytes vary around the ideal targets of 1.00 and 0.00, respectively. Although the slope and intercept of LDH were aberrant from the ideal values, strong correlations were found between the two rapid centrifugation conditions and the reference centrifugation condition. Most results of the clinical chemistry tests centrifuged 7 min at 2,200×*g*, and 5 min at 2,750×*g* were not significantly different from the results of the reference centrifugation condition. Only CO_2 was significantly lower in the specimens centrifuged at both 7 min at 2,200×*g* and 5 min at 2,750×*g* (p=0.019). There was a strong agreement between the values of the chemistry analytes derived from the two rapid centrifugation conditions and the reference centrifugation condition: the correlation coefficient of the Deming regression ranged between 0.982 and 0.999 in comparisons of the analytes centrifuged for 7 min at 2,200×*g*, and 0.984 and 0.999 in comparisons of those centrifuged for 5 min at 2,750×*g* (Table 2, Supplemental Figures S1, S2).

To determine whether the two rapid centrifugation conditions were equivalent within the total allowable error, scatter plots with Deming regression fit and Bland–Altman plots were created and analyzed. In comparisons between the 10 min at 1,300×*g* and 7 min at 2,200×*g* centrifugation trials, four analytes, albumin, direct bilirubin, glucose, and LDH, had specimens that were outside the acceptable limits of total allowable error. Comparing the differences between samples centrifuged for 10 min at 1,300×*g* and samples centrifuged for 7 min at 2,200×*g* revealed a high rate of samples outside the acceptable allowable error limits, notably 2.5 % (3/120 samples) for albumin and 3.3 % (4/120 samples) for LDH. Comparisons between samples centrifuged 10 min at 1,300×*g* and samples centrifuged 5 min at 2,750×*g* revealed that one specimen of albumin, ALT, and direct bilirubin was outside the acceptable limits of total allowable error. However, none of the analytes were outside the limit of unacceptable because the error index for less than 5 % of the specimens was outside the acceptable limits (Figures 1 and 2). The hemolysis index, lipemia index, and icterus index were generally low in most samples of all centrifuge settings.

Table 2: Comparison of chemistry analytes measured in plasma samples at different centrifugation conditions.

Parameters, units	Comparison of centrifugation 1,300×*g* for 10 min and 2,750×*g* for 5 min				Comparison of centrifugation 1,300×*g* for 10 min and 2,200×*g* for 7 min			
	Slope (95 % CI)	Intercept (95 % CI)	Correlation coefficient (AL)	Correlation	Slope (95 % CI)	Intercept (95 % CI)	Correlation coefficient (AL)	Correlation
Albumin, g/dL	0.976 (0.953, 0.999)	0.088 (0.005, 0.172)	0.992 (>0.9)	Acceptable	0.989 (0.964, 1.013)	0.049 (−0.040, 0.137)	0.991 (>0.9)	Acceptable
Alkaline phosphatase (ALP), U/L	0.996 (0.993, 0.999)	−0.10 (−0.60, 0.30)	0.999 (>0.9)	Acceptable	1.004 (1.000, 1.007)	−0.50 (−1.00, 0.00)	0.999 (>0.9)	Acceptable
Alanine aminotransferase (ALT), U/L	0.998 (0.995, 1.000)	−0.13 (−0.25, 0.00)	0.999 (>0.9)	Acceptable	1.007 (1.004, 1.010)	−0.38 (−0.54, −0.21)	0.999 (>0.9)	Acceptable
Aspartate aminotransferase (AST), U/L	0.996 (0.994, 0.998)	−0.22 (−0.40, −0.04)	0.999 (>0.9)	Acceptable	0.994 (0.991, 0.996)	−0.23 (−0.45, −0.02)	0.999 (>0.9)	Acceptable
Blood urea nitrogen (BUN), mg/dL	0.998 (0.995, 1.000)	0.01 (−0.06, 0.08)	0.999 (>0.9)	Acceptable	1.005 (1.002, 1.008)	−0.02 (−0.11, 0.07)	0.999 (>0.9)	Acceptable
Calcium (Ca), mg/dL	0.995 (0.983, 1.006)	0.025 (−0.076, 0.127)	0.998 (>0.9)	Acceptable	0.990 (0.979, 1.001)	0.085 (−0.015, 0.186)	0.998 (>0.9)	Acceptable
Chloride (Cl), mmol/L	0.995 (0.978, 1.013)	0.85 (−0.92, 2.62)	0.995 (>0.9)	Acceptable	1.003 (0.985, 1.020)	0.08 (−1.69, 1.85)	0.995 (>0.9)	Acceptable
CO_2, mmol/L[b]	0.967 (0.936, 0.999)	−0.29 (−1.00, 0.43)	0.984 (>0.9)	Acceptable	0.968 (0.934, 1.001)	−0.40 (−1.16, 0.37)	0.982 (>0.9)	Acceptable
Creatinine, mg/dL	0.995 (0.993, 0.997)	0.005 (−0.001, 0.011)	0.999 (>0.9)	Acceptable	0.998 (0.995, 1.001)	0.005 (−0.004, 0.014)	0.999 (>0.9)	Acceptable
Direct bilirubin, mg/dL	0.991 (0.985, 0.996)	−0.003 (−0.013, 0.007)	0.999 (>0.9)	Acceptable	0.994 (0.984, 1.003)	0.0004 (−0.0168, 0.0177)	0.999 (>0.9)	Acceptable
Glucose, mg/dL	0.987 (0.982, 0.991)	0.70 (0.00, 1.40)	0.999 (>0.9)	Acceptable	0.998 (0.992, 1.004)	−1.27 (−2.12, −0.41)	0.999 (>0.9)	Acceptable
Hemolysis index	1.014 (0.999, 1.030)	0.434 (−0.232, 1.099)	0.993 (>0.9)	Not interpret[a]	1.041 (0.990, 1.091)	0.659 (−0.153, 1.471)	0.992 (>0.9)	Not interpret[a]
Icterus index	0.772 (0.184, 1.360)	0.162 (−0.505, 0.829)	0.804 (>0.9)	Not interpret[a]	0.769 (0.179, 1.359)	0.177 (−0.492, 0.847)	0.805 (>0.9)	Not interpret[a]
Lactate dehydrogenase (LDH), U/L	0.997 (0.987, 1.008)	−5.90 (−9.50, −2.40)	0.998 (>0.9)	Acceptable	1.002 (0.990, 1.015)	−5.70 (−9.90, −1.50)	0.998 (>0.9)	Acceptable
Lipemia index	0.856 (0.575, 1.138)	−1.627 (−4.409, 1.155)	0.742 (>0.9)	Not interpret[a]	0.712 (0.513, 0.912)	−0.557 (−2.572, 1.457)	0.795 (>0.9)	Not interpret[a]
Phosphorous (P), mg/dL	1.003 (0.997, 1.009)	−0.029 (−0.050, −0.008)	0.999 (>0.9)	Acceptable	0.995 (0.988, 1.002)	−0.007 (−0.033, 0.020)	0.999 (>0.9)	Acceptable
Potassium (K), mmol/L	1.000 (0.991, 1.009)	−0.027 (−0.064, 0.010)	0.999 (>0.9)	Acceptable	1.006 (0.997, 1.016)	−0.064 (−0.103, −0.025)	0.999 (>0.9)	Acceptable
Sodium (Na), mmol/L	1.008 (0.985, 1.031)	−1.00 (−4.10, −2.10)	0.992 (>0.9)	Acceptable	1.008 (0.983, 1.033)	−0.90 (−4.30, 2.50)	0.991 (>0.9)	Acceptable

Table 2: (continued)

Parameters, units	Comparison of centrifugation 1,300×*g* for 10 min and 2,750×*g* for 5 min				Comparison of centrifugation 1,300×*g* for 10 min and 2,200×*g* for 7 min			
	Slope (95 % CI)	Intercept (95 % CI)	Correlation coefficient	Correlation (AL)	Slope (95 % CI)	Intercept (95 % CI)	Correlation coefficient (AL)	Correlation
Total bilirubin, mg/dL	0.985 (0.982, 0.988)	0.007 (0.001, 0.013)	0.999 (>0.9)	Acceptable	0.991 (0.988, 0.994)	0.002 (−0.004, 0.008)	0.999 (>0.9)	Acceptable
Total protein, g/dL	1.001 (0.982, 1.020)	−0.017 (−0.153, 0.119)	0.995 (>0.9)	Acceptable	1.016 (0.997, 1.034)	−0.095 (−0.229, 0.039)	0.995 (>0.9)	Acceptable

For the slope and intercept, the 95 % confidence intervals (95 % CI) are depicted in parentheses. For the correlation coefficient, the acceptable limit (AL) is depicted in parentheses. [a]Results cannot be interpreted because most measured values are below the lowest detection limit of the assay. [b]p-value <0.05 is considered a statistically significant difference between the reference centrifugation condition at 1,300×*g* for 10 min and the two rapid centrifugation conditions.

Reduced centrifugation time resulted in improvements in TAT, with a median TAT for stat results decreasing from 30 to 27 min in samples centrifuged for 7 min at 2,200×*g* and 25 min in samples centrifuged 5 min at 2,750×*g*. Using centrifugation at 10 min as the baseline, the number of samples that exceeded the TAT limit >45 min decreased from 11.3 to 9.3 % and 8.2 % in centrifugation time at 7 and 5 min, respectively. Moreover, the rate of outliers with laboratory TAT >30 min decreased from 42.3 to 28.9 % and 23.7 % in centrifugation at 7 and 5 min, respectively (Supplemental Table S1).

Discussions

Timely reporting of patient test results to clinicians is a critical indicator of laboratory performance [26, 27]. Most delays attributed to laboratory testing do not occur in the analytical phase but rather during the preanalytical process. Specimen preparation for centrifugation is a well-recognized cause of prolonged TAT, which may lead to stakeholder's unsatisfaction with the laboratory service, especially in emergency laboratories [3]. Centrifugation settings can be altered to optimize sample flow and TAT in the clinical laboratory, but it is important to ensure that the altered centrifugation settings do not compromise analytical quality. The current CLSI guideline recommends using a centrifugation time and relative centrifugal force following the manufacturer's guidelines for their blood collection tubes [6]. Although shorter centrifugation times have been investigated, the studies used different centrifugation times, speeds, and blood collection tubes [4, 9–12, 15]. Given the large disparity in recommended centrifugation conditions, the results from studies using a particular type of collection tube may not be generalizable to collection tubes from different manufacturers. The blood collected by widely used BD Vacutainer lithium-heparin suggests centrifugation of blood samples at ≤1,300×*g* for 10 min at 25 °C [21]. The BD study suggested that BD blood collection tubes with separators, including BD Vacutainer SST II Advance tubes, BD Vacutainer PST II tubes, and BD Vacutainer Barricor tubes enable faster centrifugation times of 5 min at 3,000×*g* [13]. However, results of the previous studies indicated that some chemistry analytes, especially LDH, are sensitive to high-speed centrifugation at 3,000×*g* [10, 11]. Thus, in this study, we chose centrifugation conditions below 3,000×*g* based on the results of previous studies and WHO guidelines. Other than the manufacturer's instructions, there are no studies in the current literature providing information on the minimal centrifugation time that might be possible while still obtaining suitable plasma specimens for clinical chemistry

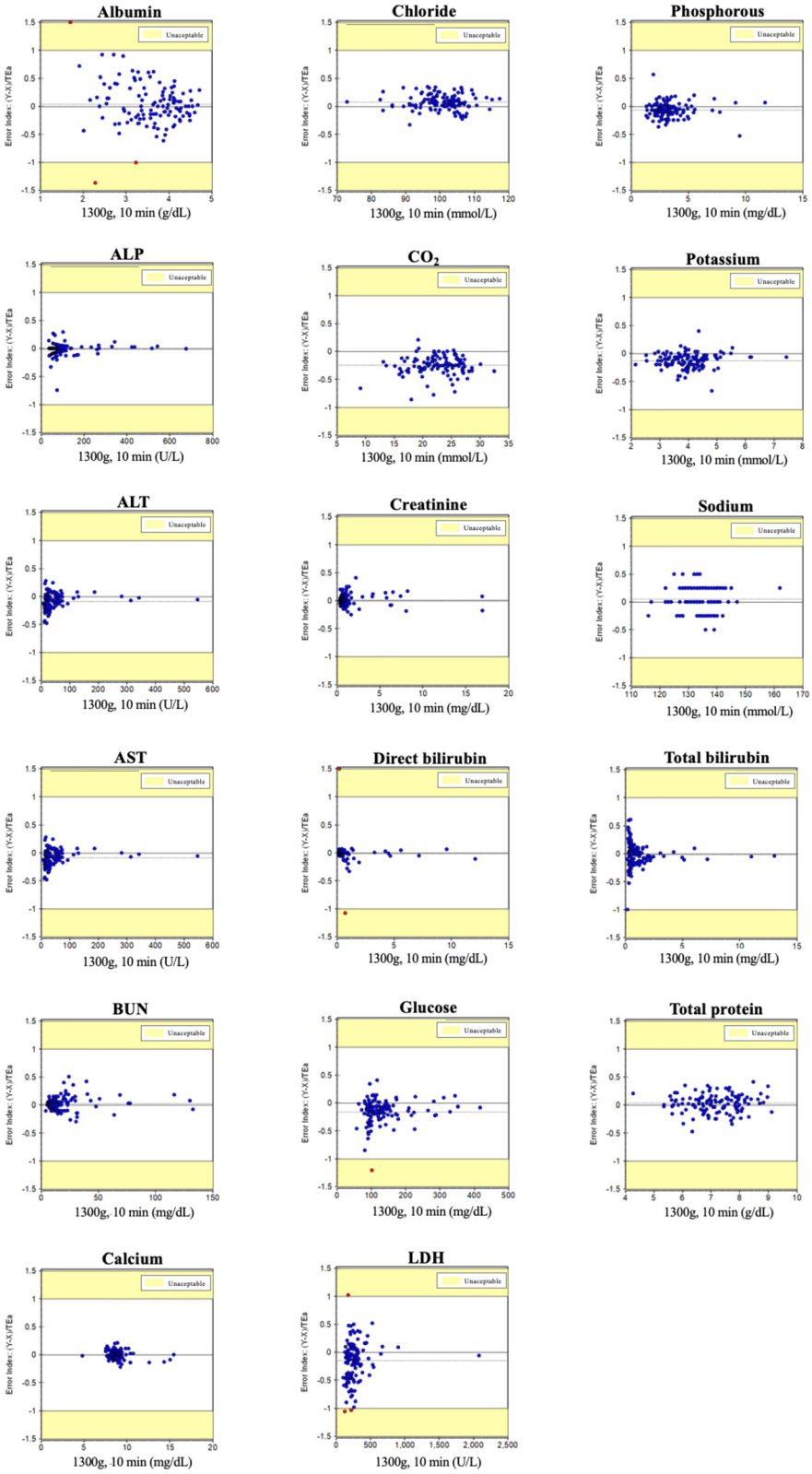

Figure 1: The error index plots for each chemical chemistry test of centrifugation at 1,300×*g* for 10 min and 2,200×*g* for 7 min. The X-axis represents the values of centrifugation at 1,300×*g* for 10 min. The Y-axis represents the error index which is the ratio of the difference of centrifugation at 2,200×*g* for 7 min (Y) and 1,300×*g* for 10 min (X) to the total allowable error (TEa). The error index is measured for each X–Y pair. Points that fall in the shaded areas have an unacceptable error index (>1.00 or <–1.00). The error index for less than 5 % of the specimen is acceptable, indicating that the two conditions are clinically equivalent. The dotted lines represent the average error index. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CO_2, carbon dioxide; LDH, lactate dehydrogenase.

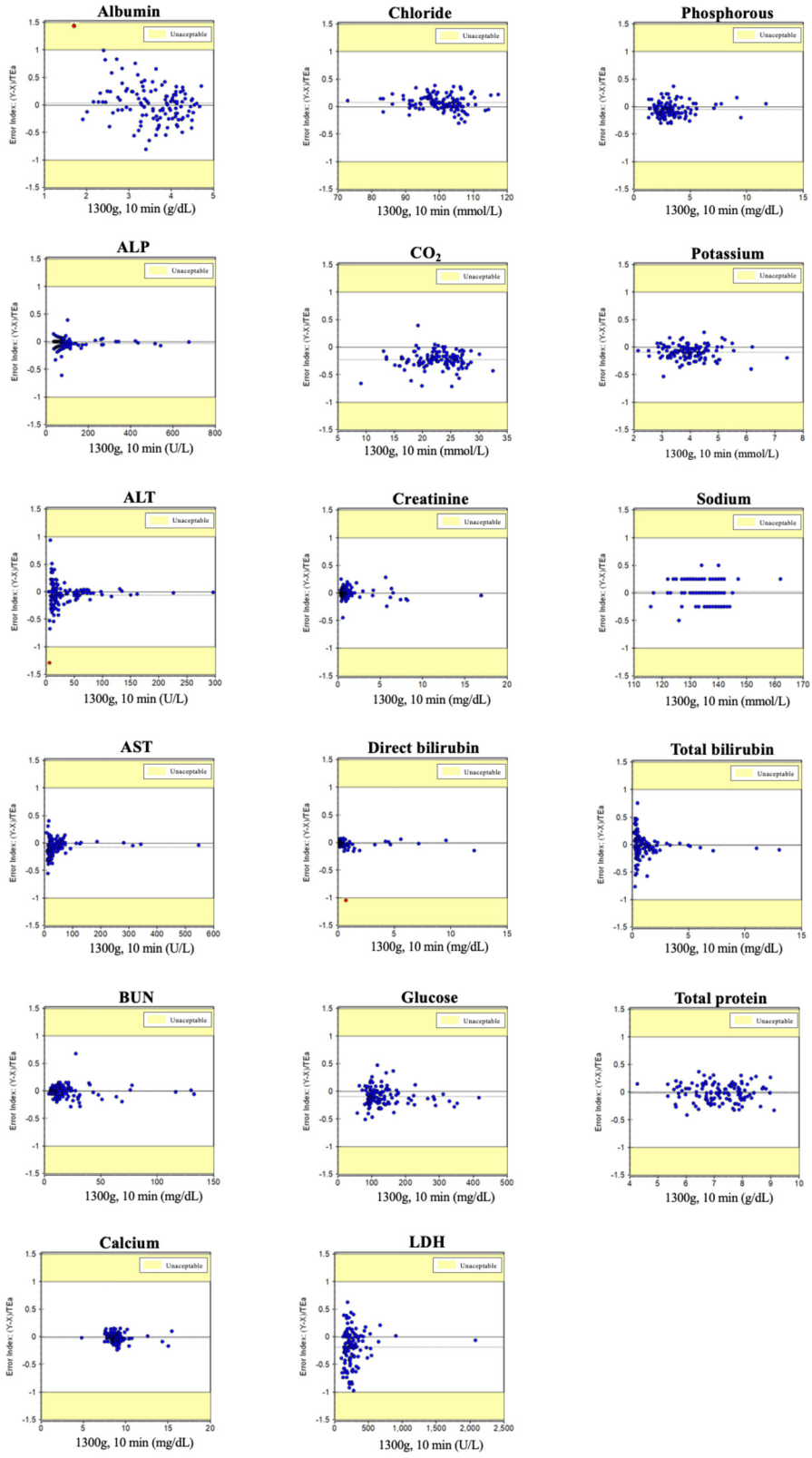

Figure 2: The error index plot for each chemical chemistry test of centrifugation at 1,300×*g* for 10 min and 2,750×*g* for 5 min. The X-axis represents the values of centrifugation at 1,300×*g* for 10 min. The Y-axis represents the error index which is the ratio of the difference of centrifugation at 2,750×*g* for 5 min (Y) and 1,300×*g* for 10 min (X) to the total allowable error (TEa). The error index is measured for each X-Y pair. Points that fall in the shaded areas have an unacceptable error index (>1.00 or <–1.00). The error index for less than 5 % of the specimen is acceptable, indicating that the two conditions are clinically equivalent. The dotted lines represent average error index. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CO_2, carbon dioxide; LDH, lactate dehydrogenase.

analytes using original BD lithium-heparin tubes without gel separators.

The present study was designed to establish lower acceptable centrifugation times for the original BD Vacutainer lithium-heparin plasma preparation tube for clinical chemistry testing to reduce the laboratory TAT. We compared the performance of centrifugation for 10 min at 1,300×*g* as recommended by the manufacturer, with two alternate rapid centrifugation conditions, 7 min at 2,200×*g* and 5 min at 2,750×*g*, which were chosen for optimization following a literature review for evidence-based recommendations [4, 7, 9–15, 22, 23]. We reduced the centrifugation speed from 3,000×*g* to 2,200×*g* and 2,750×*g* because some previous studies revealed that centrifugal force greater than 3,000×*g* might increase the rate of hemolysis [10, 11]. Our results indicate that centrifugation at both 7 min at 2,200×*g* and 5 min at 2,750×*g* using a swing bucket centrifuge for samples collected in BD Vacutainer lithium-heparin tubes without gel separator may still be suitable for clinical chemistry analytes. Although a significant difference in the median concentrations was observed for CO_2 in samples centrifuged in both rapid conditions, both were still within the acceptable limits of total allowable error, much lower than the result of 5 % of samples of each test outside the limits indicating an unacceptable finding. This finding is in contrast to a previous study [14], which found CO_2 was not adversely affected at centrifugation at 1900×*g* for 4 min. However, the specimens in this study were collected into BD Vacutainer PST gel and lithium heparin tubes, unlike the BD Vacutainer lithium-heparin blood collection tubes used in our study. To confirm that higher *g*-forces did not increase *in vitro* hemolysis which might interfere with some analytes, we measured AST, LDH, potassium, and hemolysis index as indicators of hemolysis. There was no difference between the reference and two rapid centrifugation conditions in AST, LDH, and potassium. Most measured hemolysis index values were below the lowest detection limit of the assay in all three centrifugation settings. We can conclude that alternate rapid centrifugation conditions, 7 min at 2,200×*g* and 5 min at 2,750×*g*, did not increase hemolysis.

In order to compare our results to other studies, we reviewed the literature for published scientific investigations and found a few studies regarding the analysis of clinical chemical analytes (Table 3). A few studies investigated the effect of reduced centrifugation time on analytical quality using other instruments and collection tubes, especially lithium heparin gel tubes. Generally, they found evidence that centrifugation time can be decreased to 4–7 min without adversely affecting the analytical quality of the clinical chemistry testing [4, 9–14]. One previous study examined the difference between 3 different centrifugation protocols: 2,180×*g* for 15 min, 2,180×*g* for 10 min, and 1870×*g* for 7 min [4]. In that study, blood samples were collected using lithium heparin with gel tubes (Greiner Bio-One) and tested using a Cobas 6,000 analyzer. The study concluded that there were no differences in results from many general chemistry tests and immunoassays between the centrifugation conditions. Another study examined the effect of even a broader range of centrifugation conditions, including 1, 2, 5, 10, or 15 min with constant centrifugal force at 1,200×*g*. Specimens were collected in lithium heparin with gel tubes (Terumo Europe) and analyzed for general chemistry and immunoassay analytes using a Roche Modular P system and a Roche Elecsys 2010. Statistically significant differences between ALT, calcium, glucose, potassium, urea nitrogen, and creatine kinase-MB results were observed in specimens centrifuged for less than 5 min. They suggested that a 10-min centrifugation time at 1,200×*g* for samples collected in lithium heparin with gel tube was suitable for clinical chemistry testing [9]. To our knowledge, there have been only two investigations using BD Vacutainer lithium-heparin tubes without gel, carried out by Møller et al. [10] and Koenders et al. [12]. In the study by Møller et al., nine selected chemistry and immunochemistry analytes were measured in specimens collected into BD Vacutainer serum tubes and BD Vacutainer lithium-heparin tubes. Each sample pair was centrifuged at 2,200×*g* for 10 min as a standard protocol and at 3,000×*g* for 5 min. The study found that a centrifugation protocol at 3,000×*g* for 5 min gave acceptable results for the general chemistry and immunochemistry analytes, except LDH. LDH is present in large amounts in red blood cells and platelets, and lysis of blood cells due to centrifugation at higher *g*-forces may lead to an overestimation of LDH. However, it is difficult to directly compare the analyte results between their study and ours because the reference centrifugation conditions were different, as we performed analyses using 1,300×*g* for 10 min and Møller et al. used 2,200×*g* for 10 min. In our results, no differences in the median concentrations for LDH between centrifugation settings were detected. In the other study, Koenders et al. [12] analyzed the chemistry and immunochemistry results of BD Vacutainer lithium-heparin samples centrifuged for 5 and 10 min at 1,885×*g*. They concluded that centrifugation could be reduced to 5 min for most routine chemistry and immunochemistry analytes except for the lipemia index. Overall, the findings from previous studies and our study supported the hypothesis that shorter centrifugation times can be used for general chemistry analytes without compromising the quality of the analytical results.

Intralaboratory TATs of up to 60 min for common laboratory tests are suggested as an initial goal for acceptable TAT. However, TAT would differ based on the specificity of the test ordered, the type of test (stat and routine), and the

Table 3: Summary of studies on the influence of different centrifugation conditions for clinical chemistry analytes.

Reference	Blood collection tubes	Instruments	Centrifugation conditions	Analyte	Main results/statistically significant difference
Lippi et al. [9]	– Terumo lithium heparin with gel tubes	– Roche Modular P system – Roche Elecsys 2010	The temperature at 21 °C – 1,200×*g*, 1 min – 1,200×*g*, 2 min – 1,200×*g*, 5 min – 1,200×*g*, 10 min – 1,200×*g*, 15 min	ALT, ALB, AMY, BUN, Ca, CK-MB, Cl, Cr, cTnT, GLU, K, Na, PLT, RBC, TBIL, WBC	– Statistically significant differences from the 15 min centrifuge reference were observed for ALT, Ca, GLU, K, BUN, and CK-MB in specimens centrifugated for less than 5 min.
Mensel et al. [13]	– BD Vacutainer SST™ II advance tubes with gel separator (REF 367955)	– Dimension RxL Max (Siemens Healthcare Diagnostics) – Dade BN II system	The temperature at 21 °C – 1,700×*g*, 13 min – 3,000×*g*, 5 min	AST, fHb, K, LDH	– Two centrifugation conditions showed identical results in all parameters. – Higher centrifugation speed of 3,000×*g* for 5 min did not increase the rate of hemolysis.
Kao et al. [22]	– Vacuette® Coagulation Sodium Citrate 3.2 % tubes (Greiner Bio-One, REF: 454322)	– Beckman Coulter Synchron LX20PRO Analyzer	– 1,500×*g*, 15 min – 7,000×*g*, 1 min	K, LDH, PLT, PT, aPTT	– No significant differences in results between the two centrifugation conditions.
Minder et al. [4]	– Vacuette® lithium heparin separator tubes (Greiner Bio-One, REF: 456083 and 456087)	– Cobas 6,000 system (Roche Diagnostics)	The temperature at 15 °C – 2,180×*g*, 15 min – 2,180×*g*, 10 min – 1870×*g*, 7 min	72 clinical chemistry and immunology tests	– No significant differences in all parameters.
Holland and Dom-Bourian [14]	– BD serum separator tubes – BD plasma separator tubes	– UniCel DxI 800 (Beckman Coulter) – ADVIA Centaur XP (Siemens Healthcare Diagnostics)	– 1,600×*g*, 10 min – 1,900×*g*, 4 min	BUN, Ca, Cl, Cr, CO_2, cortisol, FER, GLU, iron, K, LDH, Mg, Na, P, PSA, Tg, TgAb, transferrin, troponin, T4	– No significant differences in all parameters.
Koenders et al. [12]	– BD Vacutainer SST™ II advance tubes with gel separator (REF 367955) – BD Vacutainer lithium heparin tubes (REF 368886)	– Cobas 6,000 system (Roche Diagnostics)	– 1,885×*g*, 10 min – 1,885×*g*, 5 min	ALT, ALB, AST, BIL, Ca, Cl, CK, DD, FER, FOL, GLU, HEM, ICT, K, LDH, LIP, Mg, Na, P, PS, TP, TSH	– Serum samples: No significant differences – Plasma samples: LIP showed significantly higher results after centrifugation at 1885×*g* for 5 min compared with 1885×*g* for 10 min.
Monneret et al. [15]	– Lithium heparin with gel separator tubes (Greiner Bio-One, REF: 474080) – BD Vacutainer serum tubes (REF 369032)	– Roche Modular P800 analyzer – Roche Modular E170 analyzer	– 1885×*g*, 15 min – 1885×*g*, 10 min	40 clinical chemistry tests	– Centrifugation at 1885×*g* for 10 min slightly increases plasma LDH and LIP – LIP showed significantly higher results after centrifugation at 1885×*g* for 10 min compared with 1885×*g* for 15 min.
Møller et al. [10]	– BD Vacutainer serum tubes without gel separator (REF 369032) – BD Vacutainer lithium-heparin tube without gel separator (REF 368884)	– Abbott Architect c16000 and Abbott architect i2000SR (Abbott Diagnostics)	The temperature at 21 ± 1 °C – 2,200×*g*, 10 min – 3,000×*g*, 5 min	Ca, Cbl, folic acid, FT3, HEM, ICT, IgM, K, LIP, P	– LDH showed significantly higher results after centrifugation at 3,000×*g* for 5 min compared with 2,200×*g* for 10 min.

Table 3: (continued)

Reference	Blood collection tubes	Instruments	Centrifugation conditions	Analyte	Main results/statistically significant difference
Cadamuro et al. [11]	– BD Vacutainer SST^{TM} II serum gel tubes (REF: 367953) – BD Vacutainer PST^{TM} II lithium heparin gel tubes (LiHepGel) (REF:367378) – BD Vacutainer Barricor lithium heparin tubes with a mechanical separator (LiHepBar) (REF: 365039)	Cobas 8,000 (Roche Diagnostics)	The temperature at 22 °C – 2,000×*g*, 10 min – 3,000×*g*, 7 min – 3,000×*g*, 5 min	77 clinical chemistry tests	– Higher centrifugation speed of 3,000×*g* is associated with a higher fHb concentrations. – fHb, LDH, and bicarbonate showed significant differences between LiHepGel and LiHepBar tubes at different centrifugation conditions.
Alllison et al., [23]	Vacutainer heparin tubes	Not available	– 1,000, 2,000, 3,000, 4,000 rpm for 3 min – 4,000 rpm for 3, 6, 9, 12, 15 min	Cl, bicarbonates, K, Na,	– No significant differences in results among different speeds at the same time – No significant differences in results at different times at the same speed
This study	BD Vacutainer lithium-heparin tube without gel separator (REF 368496)	Cobas 6,000 system (Roche Diagnostics)	The temperature at 21 ± 1 °C – 1,300×*g*, 10 min – 2,200×*g*, 7 min – 2,750×*g*, 5 min	ALB, ALP, ALT, AST, BUN, Ca, Cl, CO_2, Cr, DBIL, GLU, HEM, ICT, K, LDH, LIP, Na, P, TBIL, TP	CO_2 showed significantly lower results after centrifugation at 2,200×*g* for 7 min or 2,750×*g* for 5 min compared with 1,300×*g* for 10 min, but they were still within acceptable limits of total allowable error.

aPTT, activated partial thromboplastin time; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMY, amylase; AST, aspartate aminotransferase; BIL, bilirubin; BUN, blood urea nitrogen; DBIL, direct bilirubin; DD, D-dimer; Ca, calcium; Cbl, cobalamin; CK, creatine kinase; CK-MB, creatine kinase-MB; Cl, chloride; Cr, creatinine; cTnT, cardiac troponin T; CO_2, carbon dioxide; FER, ferritin; fHb, free hemoglobin; FOL, folic acid; FT3, free triiodothyronine; GLU; glucose; HEM, hemolysis index; ICT, icterus index; IgM, immunoglobulin M; K, potassium; LIP, lipemia index; LDH, lactate dehydrogenase; Mg, magnesium; Na, sodium; P, phosphorus; PLT, platelet; PS, protein spectrum; PSA, prostate-specific antigen; PT, prothrombin time; RBC, red blood cell; TBIL, total bilirubin; Tg, thyroglobulin; TgAb, thyroglobulin antibody; TP, total protein; TSH, thyrotropin; T4, free thyroxine; WBC, white blood cell.

type of patient. Some studies have suggested that reasonable laboratory TATs are 30 min or less for urgent samples from the emergency department or intensive care unit (ICU) [28, 29]. In our study, median TAT was lower when the time of centrifugation was reduced to 7 or 5 min. Furthermore, reducing the time of centrifugation from 10 to 5 min affected the percentage of laboratory TAT outliers, reducing from 42.3 to 23.7 %. Thus, decreasing centrifugation time resulted in a reduction not only for the median TAT but also for the number of outliers.

Limitations of the study

A limitation of this study is that immunochemistry analytes that are highly sensitive to higher-force centrifugation were not investigated. However, studies using centrifugation protocol at 3,000×*g* for 5 min [10, 11] and 4,400×*g* for 3 min [30] showed no impact on measured immunochemistry parameters. The current laboratory practice recommended that the optimal centrifugation conditions required to complete plasma separation from blood cells for clinical chemistry testing and centrifugation at higher speeds or longer times could be a more effective platelet clearance [6, 21]. Although platelet counts were also not investigated in this study, the visual inspection and optical measurement of blood samples centrifugated with three conditions did not reveal any difference, including visible hemolysis and quality of plasma separation. Centrifugation at higher speeds for shorter times would lead to more platelet remaining in the plasma than centrifugation with lower speeds [31], and centrifugations at high speeds might result in an increase in AST, LDH, hemolysis index, potassium, and phosphorus. However, we did not observe any increased plasma potassium and phosphorus concentrations released from platelets supporting that centrifugation conditions used in our study did not affect plasma separation.

Conclusions

In this study, we provide evidence that blood specimens collected in a tube without a gel separator for the measurement of clinical chemistry analytes may be centrifuged at 2,200×*g* for 7 min or 2,750×*g* for 5 min without affecting the test results compared to centrifugation at 1,300×*g* for 10 min as the reference centrifugation condition. Blood collection tubes without gel separators used in our study could be more advantageous than tubes with gel separators for the laboratory process because of the cost-effectiveness and convenience of transporting specimens from the collection site to the laboratory for routine analysis. A reduced centrifugation time from 10 to 5 min, a 50 % decrease, would lead to significantly faster intra-laboratory TAT. Therefore, it can reduce the pre-analytical turnaround time without significant clinical effects on routine clinical chemistry tests. However, further studies should consider the effect of centrifugation at higher speeds for shorter times on platelet counts and immunochemistry analyses.

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