Evaluation of a High-speed Centrifuge with Rapid Preparation of Plasma for Coagulation Testing to Improve Turnaround Time

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Turnaround time(TAT) is one of the most remarkable signs of laboratory service and is often used as a key performance indicator(KPI) of clinical laboratory. The purpose of this study is to evaluate a new centrifugation method for coagulation testing to provide faster laboratory reports with the aim to improve the TAT thresholds. Six 2mL citrated blood collected from 10 consecutive subjects were centrifuged on a traditional centrifuge at 1500g for 15 minutes and the high-speed centrifuge at 4000g, 5000g, 6000g, 7000g and 8000g for one minute respectively and then supernatant plasma samples were immediately examined for residual platelets. Results revealed consistently achievable equivalence at 7000g for one minute centrifugation in platelet count compared with conventional centrifugation. Additional forty paired samples were tested to determine if any possible interferences after centrifugation at 7000g for one minute affected the results of prothrombin time (PT) and activated partial thromboplastin time (aPTT) tests. We found no significant differences in assay results and conclued that this high-speed technique for preparation of platelet-poor plasma(PPP), shaving about 14 minutes off preanalytical specimen processing time, is a reliable and useful option for minimizing TAT for PT and aPTT tests.

Key words: specimens handle, centrifugation, turnaround time(TAT)

Introduction

Quality can be defined as the ability of a product or service to satisfy the needs and expectations of the customer. Since our clinical customers demand faster results, TAT has become the tool many clinical laboratories use to monitor their ability to meet these demands. Nowadays laboratories are often judged by their ability to meet specified TAT goals, but often have problems meeting their internal goals, particularly in stat analyses. Delays in TAT elicit immediate complaints from clinicians while adequate TAT goes unremarked[1].

Centrifuges are commonly used to separate components of a mixture on the basis of particle density. The most frequent laboratory application is the separation of blood into cells and a serum or plasma supernatant. Each application requires specific centrifugal forces and defined time periods. Laboratories traditionally centrifuge the citrated blood at 1500g for 15 minutes at room temperature to prepare a quality sample according to the instructions given by the coagulation testing reagent manufacturers. Furthermore, Clinical and Laboratory Standards Institute (CLSI) recommends that sodium citrate tubes be spun at 1500g for no less than 15 minutes to achieve PPP (plasma with a platelet count less than 10,000/uL) and accurate coagulation test results[2]. Consequently, specimen centrifugation is the most time-consuming task associated with preanalytical specimen processing in hematology laboratory. Centrifugation of blood specimens for coagulation tests can be the cause of the major bottleneck in hematology laboratory throughout. Because of the complete blood count (CBC) is the most commonly ordered blood tests and these calculations are generally determined by special machines that analyze the different components of blood in less than two minutes, and usually hematology laboratories receive clinical orders and requests for both stat CBC and coagulation tests (PT& aPTT) in Tiawan.

Received: November 16, 2009 Revised: January 4, 2010 Accepted: January 12, 2010
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However, reliable strategies for reducing the time required for specimen processing without affecting quality should be recognized. To achieve this, we identified the need for a high-speed centrifuge to spin samples quickly. The centrifugal speed and duration must be established by the laboratory, higher speed and shorter duration centrifuges, commonly known as "Stat-fuge" may be used[2]. The centrifugation time was inversely associated with residual blood cell elements in plasma, especially platelets. Hypothetically, there is one major solution that may be pursued to reduce the centrifugation time for coagulation testing: increase the speed (relative centrifugal force, RCF). Blood cells are rapidly separated from plasma by centrifugation at increased RCF, in gravity units(g). Besides the manufacturer's suggestions, there is no definitive information on the minimal centrifugation time on a conventional centrifuge required to obtain suitable plasma specimens for coagulation testing. The current laboratory practice is heterogenous and mostly based on local empirical observations[3]. Nevertheless there is little evidence so far on the influence of different RCFs in a rapid centrifugation on sample's residual platelets and coagulation testing. The primary goal of this study is to establish a minimal suitable centrifugation time for coagulation testing to improve the TAT.

Materials and Methods

The evaluational proposal was approved by the Institutional Review Board of the St. Martin De Porres Hospital. Venipunctures were performed in the afternoon of the same day on ten apparently healthy subjects, who had given informed consent, and no hemolyzed or lipemic specimens were encountered. Blood specimens were collected from each of 10 volunteers using a 22-gauge blood collection needle (BD Vacutainer, 0.7mm x 25mm) directly into 6 sequential 2mL primary evacuated tubes containing 3.2% sodium citrate (Greiner VACUETTE, #454322, 13 x 75mm, plastic) were gently inverted 4 times according to the instructions given by the tube manufacturer[4,5]. Excessive mixing can cause hemolysis and/or platelet clumping and activation, leading to erroneous results[2].

The six sample tubes were centrifuged within 30 minutes after blood collection, respectively. Two centrifuges were used in this study including KUBOTA 5420, a conventional horizontal tabletop centrifuge as the reference, and KUBOTA 3700, a high-speed refrigerated centrifuge with fixed-angle rotor. The high-speed centrifuge requires refrigeration chambers to compensate for the considerable heat produced. This centrifuge accelerates rapidly and brakes very fast, decreasing specimen processing time. Tests of the coagulation system are very sensitive to storage time and temperature[2]. The influence of temperature, extreme cooling down or warming up in the centrifuge, can lead to haemolysis. However, preliminary study showed no statistically relevant PT/INR differences among studied centrifugation conditions on temperature 20℃ or no temperature control[6]. In order to avoid high temperatures generated by high-speed centrifugation. The temperature during KUBOTA 3700 centrifugation was set at 21℃, whereas KUBOTA 5420 is a non-refrigerated centrifuge. Six evacuated tubes collected from 10 consecutive subjects were centrifuged in KUBOTA 5420 at 1500g for 15 minutes and KUBOTA 3700 at 4000g, 5000g, 6000g, 7000g and 8000g for one minute respectively, and then plasma samples were immediately tested for residual platelets using the Beckman Coulter LH750 analyzer within 10 minutes after centrifugation. A total of 60 samples were evaluated.

The next study was designed to evaluate if any possible interferences after centrifugation at 7000g for 1 minutes, another duplicate samples were obtained from 40 randomly selected outpatients instead of the usual one, who had given informed consent, were centrifuged in KUBOTA 5420 at 1500g for 15 minutes and KUBOTA 3700 at 7000g for one minute respectively, and tested for PT and aPTT on the Sysmex CA1500 analyzer with the instrument's accompanying reagents (SIEMENS Dade Innovin, Dade Actin FSL Activated PPT Reagent) in order to compare results obtained using two types of centrifugation. For the sake of analytical interferences from hemolysis, we carried out one more experiment: checked supernatant plasma potassium(K) and lactate dehydrogenase (LDH) on the Beckman Coulter Synchron LX20PRO analyzer, as indicators of hemolysis.

All measurements were immediately performed in duplicate on the same analyzer within 10 minutes after centrifugation. Residual platelet results were evaluated based on recommendations of the CLSI for PPP (less than 10,000/μL)[2]. The significance of differences between samples was assessed by paired Student *t* -test, and the level of statistical significance was set at $P < 0.05$.

Results

Results of the present investigation were shown in Table 1 and Table 2. The conventional 1500g for 15 minutes

Table 1. Residual platelet counts (n=10) on 2mL citrated blood tubes centrifuged at 1500g for 15 min and 4000g, 5000g, 6000g, 7000g, 8000g for 1 min

Centrifuge Time	15 min	1 min				
Centrifuge RCFs	1,500g	4,000g	5,000g	6,000g	7,000g	8,000g
Platelet ($x10^3$/uL)	2.0 (0.47)	13.1(6.38)	4.8(1.31)	3.9(1.73)	2.2(0.63)	2.4(0.70)
p – value	-	<0.001	<0.001	0.004	0.443	0.168

Platelet counts are expressed as mean(standard deviation). Differences from the reference 1500g for 15 min centrifuge specimens are evaluated by paired Student *t* –test.

Table 2. K, LDH and Coagulation testing on 2mL citrated blood tubes centrifuged at 1500g for 15 min and 7000g for 1 min

Analyte	N	1500g, 15 min	7000g, 1 min	*p* -Value	*r*
PT (sec)	40	11.05(2.15)	11.09(2.13)	0.192	0.996
PT INR	40	1.05(0.21)	1.06(0.21)	0.224	0.996
aPTT (sec)	40	28.99(3.21)	28.98(3.10)	0.918	0.984
K (m mol/L)	25	3.2(0.32)	3.2(0.34)	0.245	0.939
LDH (U/L)	25	162.76(103.12)	160.84(102.95)	0.326	0.996

Data are given as mean(standard deviation).Differences from the reference 1500g for 15 min centrifuge specimens are evaluated by paired Student *t* –test. *r* is the Pearson correlation coefficient.

centrifuge specimen was considered as a reference, as currently suggested by the manufacturer and CLSI[2]. For one minute centrifugation time, the RCFs was inversely associated with the residual blood cell elements measured in the plasma using 2mL sodium citrate tubes. The amount of platelets in plasma increased significantly in specimens centrifuged at 6000g or less, respectively. Statistically significant variations from the 6000g or less centrifuge specimens were observed for residual platelets, and showed that was statistically different but not clinically significant, residual platelet counts meet the recommendations of the CLSI for PPP, in specimens centrifuged at 6000g and 5000g. In contrast, elevated residual platelet counts that greater than the recommendations of the CLSI for PPP after centrifugation at 4000g for 1 minute was statistically and clinically significant difference. This study demonstrates that 2mL Greiner VACUETTE 3.2% sodium citrate tubes spun in the KUBOTA 3700 at 7000g for 1 minutes showed consistent achievable results, no statistically significant differences, and was able to produce tubes with residual platelets of less than 3000/µL for 100% of samples, well within the acceptable range given by CLSI for PPP. Table 2 shows that compared PT/INR and aPTT after 7000g for 1 minute rapid centrifugation and after 1500g for 15 minutes' conventional centrifugation on 40 randomly selected outpatients, and found no significant differences in assay results between the two centrifugation methods. The sample for each of the analytes had a strong correlation with values above than 0.984 as shown by the respective data. Additionally, according to two paired–sample *t* -tests, no significantly elevated K and LDH after centrifugation by the high-speed centrifuge at 7000g for 1 minute compared with the reference, no haemolysis occurs.

Discussion

Improving TAT is a complex task involving equipment, acquisition, staff training, and planing. All the steps from test ordering to results reporting should be monitored and steps taken to improve the processes[7]. There are three distinct phases in a clinical laboratory: pre-analytical (specimens receipt and preparation), analytical, and post-analytical. The latter two activities are now covered adequately by using automated analyzers and relevant information technology applications. The specimen preparation activities is now arresting attention[8]. Generally, most of the delays attributed to the stat laboratory do not occur in the analytical phase, but rather during those preanalytical processes that have a queue. Consequently, sample preparation, when required, is a well-acknowledged cause of prolonged TAT, which may lead to customer dissatisfaction with the laboratory service[3].

Preanalytical factors are an important source of variation in clinical laboratory measurements. The control of preanalytical variables is critical, particularly for coagulation assays, since this has a direct influence on the quality of results and clinical reliability[6]. Although it has been demonstrated that centrifugation periods shorter than 2 minutes at 11,000 g effectively clear plasma of cellular components[9,10] and some laboratories have currently validated stat centrifuges for routine coagulation testing with an RCF of 6153g in 2 minutes[11], 4440g in 2 minutes[12,13] and 4000g in 3 minutes[14]. RCFs over 2000 g are not recommended as they may be associated with analytical interferences from hemolysis and platelet activation, and fixed-angle centrifuges are also not recommended as if tubes that have been centrifuged are transported upright, even the slightest of shakes during road transportation could disturb buffy coat and cause the sloping layer break down, lead to the platelets drift back into plasma [4].Hemolyzed specimens should not be processed since there could be activation of the clotting factors and interference from blood cell lysis on routine coagulation testing.[2,15,16] Nevertheless, our approach showed no hemolysis occurs.

Another technical issue with platelet activation was if such a high-speed centrifugation would potentially damage the platelets releasing platelet factor 4 (PF4, a small cytokine), thus compromising the plasma. PF4 is a heparin neutralizing factor in platelet alpha-granules, can be released by activated platelets during platelet aggregation or platelet damage, and promotes blood coagulation by moderating the effects of heparin-like molecules[17], thereby shortening aPTT results. To prevent this occurrence *in-vitro*, the specimen should be collected with a minimum of trauma. Cold temperatures are known to induce platelet aggregation and release PF4; therefore, centrifugation at room temperature is recommended. However, we found no study that specifically addresses PF4 release in the literature[18], preliminary studies[9-11] and this study showed that there is no difference in the aPTT result, we can probably conclude no *in vitro* PF4 release.

Finally, preliminary studies suggest that samples should be analyzed or the supernatant plasma removed within 10 minutes of centrifugation to avoid redistribution of platelets at the plasma blood interface. High-speed centrifuges employ fixed-angle rotor, which force a number of platelets to adhere to the side of the tube. When the tube is removed from the centrifuge and placed upright position during transportation, the platelets drift back into the plasma, raising the platelet count to above 10000/uL. This is an important limitation that affects specimen management. Each laboratory should perform its own studies to determine appropriate time limitations between centrifugation and completion of coagulation analysis to assure that residual platelet counts remain within acceptable limits[19]. We also found this phenomenon while supernatant plasma samples were not immediately tested for residual platelets within 10 minutes after centrifugation. Futhermore, if the patient is on both heparin and coumarin-based anticoagulant therapy, the results may vary with time of sample storage, delay in testing samples will result in prolonged PT or aPTT determinations. For these reasons, plasma samples should be analyzed for coagulation testing within 10 minutes of completed centrifugation and care must be take in the transportation of the sample to the analyzer to accurate coagulation test results and minimise TAT, encompasses the adoption of suitable strategies for reducing undue variability throughout the whole testing process. Appropriate preanalytical specimen processing guidelines must be established and standard operation procedures was designed based on the sequence of the process in this study. Poor standardization of preanalytical variables exerts a strong influence on the reliability of coagulation testing. Changes in any procedure should be properly validated and approved by the laboratory director, with date and initials recorded.

Generally, we use a typical large batch conventional centrifuge (up to 80 tubes, in some cases). There is a strong temptation to let a batch accumulate before pressing the "start" button. Even with 24 tubes per batch, the centrifuge is not fully utilized, particularly in performing stat analyses. Tubes wait either for a batch to accumulate or they wait because a batch is already going (which takes 15 minutes for conventional centrifugation). In contrast, the rapid centrifuge that holds only 6~8 tubes, smaller batches means shorter cycle times and no waiting for batches, tubes are processed as they arrive. All measurements were immediately performed within 10 minutes after centrifugation. We are moving in the direction of one piece flow with very little wait time in a highly controlled manner.

This study demonstrated that the KUBOTA3700 is capable of providing improved efficiency in the laboratory due to faster test results and minimise the hemaetology laboratory stat TAT thresholds by 10 minutes. In the“best case”, we can generate PT, aPTT and CBC test results in less than 12 minutes all at once, exclusive of transport to the hemaetology laboratory. Our new TAT goal is to have 90 percent of stats resulted within 20 minutes of receipt in the hemaetology laboratory for emergency department(ED) patients and 30 minutes for outpatients to reflect clinical needs. That would be a new benchmark in Taiwan. With this improvement, meeting the new TAT goals was a easy task. Our daily TAT summary report from laboratory information mamagement systems gives us a representation of performance. At present, the achievement rates of reporting of stat results for ED patients and outpatients were always above 95% and 93% since June 2009.

Although these findings have practical applications in our experimental conditions, they may also serve as a rational basis for additional investigations aimed to establish the most suitable local centrifugation procedures for the preparation of quality specimens, each laboratory should validate its own equipment to confirm acceptable results. Future research should examin not only the centrifugation conditions but also the volume of whole blood sample, and we did not evaluate the second-line coagulation testing, such as fibrinogen level, D-dimer, antithrombin III, and dilute Russell viper venom time. Because they are not common ordered blood tests. Additionally, the final citrate concentration in the blood should be adjusted in patients with hematocrit values above 55%. In samples with an elevated hematocrit, the blood-to-anticoagulant ratio drops below 9:1, causing excess citrate for the volume of plasma present in the tube. This leads to excess binding of the calcium added to the clotting test reaction and possible dilutional effect due to the volume to liquid anticoagulant present, tending to increase the clotting time. For hematocrit values of less than 25%, the citrate concentration does not need to be adjusted [20]. A note should be added to the laboratory record and patient record stating that the hematocrit was elevated and the citrate concentration was adjusted [2]. It might be smart to repeat this study using high hematocrit or heparin therapy specimens.

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以快速離心處理血液凝固檢體縮短血液檢驗報告時間之評估

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檢驗報告完成時間(**TAT**)經常被用來作為臨床實驗室服務品質良窳最顯著的績效指標。本研究的目的是評估一種新的凝血測試檢體快速離心法，以提供更快速的檢驗報告並改善**TAT**。本研究依序從**10**個自願者收集了**6**管**2**毫升血液，分別依序在傳統離心機上以**1,500g**離心**15**分鐘，在高速離心機以**4,000g**、**5,000g**、**6,000g**、**7,000g**、**8,000g**離心**1**分鐘，離心後立即血漿樣本進行殘餘血小板計數檢測。結果顯示，在**7,000g**離心**1**分鐘與傳統離心方式，血漿殘餘血小板數無差異，離心效果相同。附加**40**對樣本測試比較評估以**7,000g 1**分鐘高速離心後是否有產生任何可能的干擾，並影響**PT**和**APTT**凝血試驗結果。我們發現兩種離心方式對凝血試驗結果並無統計上的顯著差異。總之，本研究所評估的快速離心處理凝血試驗檢體方法，約可剔除分析前凝血試驗檢體處理時間**14**分鐘，顯示此高速快速離心是縮短**PT**和**APTT**檢驗報告時間(**TAT**)的一個可靠和有用的方法。

關鍵詞：檢體處理、離心、報告完成時間

收稿日期：98 年 11 月 16 日　修稿日期：99 年 1 月 4 日　接受日期：99 年 1 月 12 日

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