

Five-minute preparation of platelet-poor plasma for routine coagulation testing

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إعداد يستغرق 5 دقائق للبلازما الفقيرة بالصفائح من أجل الاختبار الروتيني للتخثر أحمد حسن سلطان

الخلاصة: تتطلب الدلائل الإرشادية لمؤسسة المعايير الإكلينيكية ومعايير المختبرات أن تجرى الاختبارات الروتينية للتخثر ببلازما فقيرة بالصفائح (أقل من 10 000 صفيحة/مكرو لتر)، وتحضر من كامل الدم المضاف إليه السترات وجرى تنبيذه بسرعة منخفضة لمدة 10 – 30 دقيقة. ومقارنة النتائج التي تم الحصول عليها من البلازما التي خضعت للتنبيد لمدة 5 دقائق بسرعة 3000 جاذبية أو لمدة 10 دقائق بسرعة 2000 جاذبية، أجريت مقارنة شملت 46 عينة مأخوذة من البالغين أصحاء أسوياء بقصد التعرف على زمن البروثرومين، وزمن الثرومبوبلاستين الجزئي المفعل والمعدل الدولي المسوي. لا يوجد اختلافات يعتد بها في نتائج الاختبار، واستنتج أن النبذ لمدة خمس دقائق بمقدار 3000 g يعتبر اختياراً موثقاً فيه ومفيداً للحد من الوقت المستغرق في هذه الاختبارات.

ABSTRACT Clinical and Laboratory Standard Institute guidelines require that routine coagulation tests are performed with platelet-poor plasma (< 10 000 platelets/ μ L) and prepared from whole citrated blood centrifuged at low speed for 10–30 minutes. To compare results obtained from plasma centrifuged for 5 minutes at 3000 g or for 10 minutes at 2000 g, 46 blood samples from normal healthy adults were assayed for prothrombin time, international normalized ratio and activated partial thromboplastin time. No significant differences were found in test results and it was concluded that 5 minutes centrifugation at 3000 g is a reliable and useful option to reduce the turnaround time for these tests.

Préparation en cinq minutes d'un plasma pauvre en plaquettes pour les tests d'hémostase de routine

RÉSUMÉ Les recommandations du CLSI (Clinical and Laboratory Standard Institute) imposent que les tests d'hémostase soient réalisés avec du plasma pauvre en plaquettes (< 10 000 plaquettes/ μ L) et préparés à partir de sang total citraté, centrifugé à vitesse lente pendant 10 à 30 minutes. Afin de comparer les résultats obtenus à partir de plasma centrifugé pendant 5 minutes à 3000 g ou pendant 10 minutes à 2000 g, 46 échantillons de sang prélevés sur des adultes en bonne santé ont été analysés pour évaluer le temps de prothrombine, le rapport international normalisé et le temps de thromboplastine partielle activée. Nous n'avons constaté aucune différence significative dans les résultats des tests et avons conclu que la centrifugation à 3000 g pendant cinq minutes était une possibilité fiable et utile pour réduire le temps nécessaire à la réalisation de ces tests.

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Introduction

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are the 2 most commonly requested routine coagulation tests for the screening of acquired and inherited coagulation disorders and the monitoring of anti-coagulation therapy [1]. Quality care requires reliable test results and prompt turnaround times. Reliable results can be achieved by carefully controlling pre-analytical variables, which are responsible for 64% of all errors in testing [2,3].

PT and APTT tests represent about 90% of coagulation tests ordered, and it takes 40–180 minutes or more for reporting results with standard laboratory methods [4,5]. Such long turnaround times can be very costly in some medical situations associated with critical, surgical and emergency cases, when good care could be denied [6]. Faster turnaround time, while maintaining sample quality and accuracy of results, depends mainly on the time consumed on coagulation sample collection, handling, processing and testing [7,8]. The shortest recommended centrifugation time to produce the recommended platelet-poor plasma (PPP) with platelet counts $< 10\,000/\mu\text{L}$ is 10 minutes at 2000 g using regular centrifuges [9]. High-speed centrifuges have been used for the rapid preparation of PPP in 2–3 minutes but these are unavailable in most laboratories. In addition, most of them are microcentrifuges with limited capacity (up to 12 samples) and require the transfer of aliquots from the collected blood into conical microcentrifuge tubes [10–13].

This study in the United Arab Emirates (UAE) used readily available laboratory centrifuges to compare 5-minute centrifugation at 3000 g with 10-minute centrifugation at 2000 g to prepare PPP in accordance with the approved guidelines of the Clinical and Laboratory Standards Institute (CLSI) [14]. The

prepared PPP obtained at each speed was used to measure platelet count, PT, international normalized ratio (INR) and APTT.

Method

Between 25 February and 28 May 2006, blood samples were taken from 46 healthy volunteers at the University of Sharjah, Sharjah, UAE. The sample consisted of 38 female and 8 male students and staff members who gave informed consent, age range 18–33, with a mean age of 22.3 years. Pre-collection verbal questions to the volunteers revealed that they did not have any coagulation disorders, had not ingested aspirin within the previous 3–7 days and were not on anticoagulant therapy.

Duplicate 2.7 mL plastic vacuum tubes (with 3.2% sodium citrate) and 21-gauge needles [Becton-Dickinson, Rutherford, New Jersey, United States of America (USA)] were used to collect the samples. Capillary haematocrit values ranged from 32 to 42 mg/dL. To control preanalytical variables and protect the quality of the tested samples the venous blood collection techniques and sample handling and processing conformed to the CLSI guidelines [14]. These were: the tourniquet was placed for under 1 min to locate the vein and release it as soon as the blood entered the first tube without any needle probing to ensure nontraumatic venepuncture; second and third tube draws were used following a 3 mL red-top plain tube to avoid contamination with tissue thromboplastin; collected samples were immediately mixed gently 3–5 times by inversion; all collected samples were visually checked for complete filling against a sample tube to ensure 9:1 ratio of blood to anticoagulant; tubes were gently inverted 2–5 times before centrifugation to facilitate residual platelet suspension and to check for the presence of clots; all centrifuged samples were visually checked for

lipaemia and haemolysis (plasma with pink to red colour) in the second and/or third tubes.

A fixed-angle, tabletop centrifuge [Centurion-K40 model, Centurion Scientific, Sussex, United Kingdom (UK)] was used for the preparation of PPP at room temperature within 1 h of collection by centrifuging the second draw tubes at 3000 g for 5 min and the third draw tubes at 2000 g for 10 min. The instrument was checked twice a year (once per academic semester) with a digital photo tachometer (Tenma 72-6633, Springboro, Ohio, USA) and a sports stopwatch to ensure that the obtained speed and time did not exceed 5% of the expected speed [15]. Before the study started the centrifuge was checked for the required speed (3000 g) and time (1, 5 and 10 min) and the results were acceptable. The selection of 3000 g speed was determined empirically after a number of trials of centrifuging duplicate 2.7 mL sodium citrated samples at speeds that ranged from 2500–3000 g and achieving reproducible platelet counts $< 10\,000/\mu\text{L}$ (data not shown).

About 1 mL supernatant plasma was carefully removed with a plastic transfer pipette from the middle of the sample and away from the buffy coat to avoid contamination with excess platelets. All paired PPP samples were prepared and tested for platelet count within 0.5 h of collection using a semi-automated haematology analyser (Medonic CA 620, Stockholm, Sweden). The PT, INR and APTT were tested at room temperature within 2 h of collection using a coagulation analyser with the required controls and reagents (Dade BFT II, Dade Behring, Marburg, Germany).

Data were entered using Microsoft *Excel* and descriptive statistical analyses—mean, range, standard deviation (SD) and paired Student *t*-test—were computed using *SPSS*, version 14.

Results

Platelet counts $< 10\,000/\mu\text{L}$ were produced from all 46 PPP samples centrifuged for 5 minutes and only 1 sample out of the 46 (2%) had a platelet count $> 10\,000/\mu\text{L}$ ($13\,000/\mu\text{L}$) and was excluded as an outlier ($Z\text{-score} > 3.9$) [16].

Descriptive statistics for platelet count, PT, INR and APTT for PPP prepared for 5 min at 3000 g and 10 min at 2000 g centrifugations are shown in Table 1. The mean and SD values of the parameters were very close and were within the normal values for healthy adults. The mean platelet count was also the same for PPP prepared for 10 min and 5 min. Examination of the histograms for each parameter showed that the values fitted within the normal distribution (data not shown). Based on the paired *t*-test comparison of the mean values, no significant difference between 5 min and 10 min centrifugations for platelet count ($P = 0.29$), PT ($P = 0.29$), INR ($P = 0.42$) and APTT ($P = 0.54$) values were found in our study.

Calculation of paired-samples correlation found a high significant correlation coefficient for PT ($r = 0.99$, $P < 0.001$), INR ($r = 0.96$, $P < 0.001$) and APTT ($r = 0.98$, $P < 0.001$).

Discussion

This is the first study to show that PPP with platelet counts $< 10\,000/\mu\text{L}$ can be prepared by using 2.7 mL (3.2% sodium

citrate) vacuum tubes with 5 min centrifugation at 3000 g in a tabletop centrifuge with fixed angle. These residual platelet counts met the approved CLSI guidelines for PPP used for coagulation tests [14] and showed no significant differences in platelet count, PT, INR and APTT values compared with PPP prepared by 10 min centrifugation at 2000 g.

Our platelet counts prepared in 5 min were lower than the range reported by previous studies ($5\text{--}79 \times 10^9/\text{L}$) that used patients' blood collected in 4.5 mL tubes and centrifuged for 5 min and 10 min at regular speeds. These studies reported no significant differences ($P < 0.05$) when their effect was measured on selected routine coagulation tests, particularly PT, APTT and fibrinogen [17–19]. On the other hand, our low residual platelet counts were in agreement with other studies that have also used 4.5 mL tubes to evaluate the effect of 2 min and 3 min centrifugation times at 4400 g and rapid speeds $\geq 11\,000$ g respectively [10,11–13].

As with our study (which used only normal healthy subjects), these 4 earlier studies (on samples from patients) also reported high correlations, with no significant differences between the results for PT, APTT and fibrinogen level with both high and routine centrifugation speeds. It was interesting to note that the PPP prepared from routinely submitted patient samples and with platelet counts $< 15\,000/\mu\text{L}$, which are higher than those produced by our study, reported no significant difference for PT, APTT, fibrinogen level, antithrombin-

III, heparin, D-dimer and Russell dilute venom time values [11].

Conclusion

This study showed that residual platelet counts in PPP prepared with 5 min centrifugation at 3000 g were $< 10\,000/\mu\text{L}$, as recommended by CLSI guidelines for PPP used for coagulation tests [14]. No significant difference was found for healthy individuals between platelet count, PT, INR and APTT values produced by PPP prepared in 5 and 10 min. We conclude that such PPP can be used for routine and STAT test requests on normal patients without compromising the quality and reliability of the results. These findings invite future work to examine other routine, special and sensitive coagulation tests that are performed on fresh and or frozen PPP samples.

The 5 min preparation can be a valuable contribution to reducing the overall turnaround time for routine and STAT results without significant disruption of workflow and therefore can contribute to the better medical care of all patients with coagulation test requests.

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Table 1 Comparison of 5- and 10-minute centrifugation for preparing platelet-poor plasma: platelet count, prothrombin time, international normalized ratio and activated partial thromboplastin time values ($n = 46$)

Test	10 min @ 2000 g		5 min @ 3000 g		<i>t</i> -value	<i>P</i> -value
	Mean (SD)	Range	Mean (SD)	Range		
Platelets ($\times 10^9/\text{L}$)	4 (2)	1–9	4 (2)	1–13	-1.70	0.29
Prothrombin time (s)	11.9 (0.52)	11.0–13.3	11.9 (0.54)	11.0–13.5	-1.06	0.29
International normalized ratio	1.01 (0.05)	0.93–1.10	1.01 (0.05)	0.93–1.20	-1.80	0.42
Activated partial thromboplastin time (s)	23.2 (1.40)	19.9–26.1	23.1 (1.40)	20.1–26.2	0.61	0.54

SD = standard deviation.

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