Original Article

Revisiting coagulation centrifugation protocol for integration into total laboratory automation workflow

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Background: The process of centrifugation is one of the most crucial stages in the coagulation pre-analytical phase but can be a major source of bottleneck in total laboratory automation (TLA) workflow. In an effort to harmonise centrifugation protocol and therefore improve turnaround times (TAT), this study examined the effect of adopting a 4,000 g centrifugal force of 5-minute duration on commonly requested coagulation assays.

Methods: Duplicate sodium citrate 9 NC/2.9 mL specimens were centrifuged at 2,000 g for 10 minutes and 4,000 g for 5 minutes with supernatant plasma measured for prothrombin time (PT), routine activated partial thromboplastin time (APTT), unfractionated heparin (UFH) APTT, Fibrinogen, D-Dimer, Factor V (extrinsic), Factor VIII (intrinsic), Thrombin time and platelet count.

Results: This study indicates acceptable levels of correlation and agreement between both protocols for coagulation parameters tested. UFH APTT exhibited a mean bias of –3.31 seconds but was determined to have no impact on therapeutic intervention or patient management. The D-Dimer assay demonstrated the highest incidence of >10% paired result deviation (30%) but may be explained by the high CV% (15–20%) of the assay at the lower end of the analytical range. There was no indication that result deviation observed would have an impact on clinical diagnosis or patient management. Less than half of the specimens on the higher centrifugal force setting yielded a platelet count of less than 10×10^{9}/L.

Conclusions: Our study indicates that centrifugation at 4,000 g for 5 minutes duration does not significantly alter the results and interpretation of commonly requested coagulation assays and hence can be considered for revising the standard recommendation for coagulation plasma preparation protocol. The existing laboratory protocol of double centrifugation of thrombophilia or lupus like anticoagulant specimens must be maintained to ensure platelet counts conform to CLSI guidelines.

Keywords: Automation; laboratory; pre-analytical phase; centrifugation; blood coagulation

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Introduction

Laboratory investigations are expected to provide timely, accurate and precise results in order to assist the clinician in proper diagnosis, management and prognostication. It has been reported that laboratory data influence up to 70% of medical decisions (1). Laboratory testing protocol encompasses the pre-analytical, analytical and post-analytical phases before the final test result is issued. In many instances, the analytical and post-analytical processes are now covered adequately by using various blood sciences TLA and information technology systems. Specimen

preparation is often deemed as the most vulnerable step of the overall testing process (2-5). Coagulation assays can highly susceptible to pre-analytical variables due to the complexity of the biochemical and cellular reactions and the unique specimen matrix required for haemostasis testing. The pre-analytical phase is also an acknowledged contributor of protracted TAT which can consist up to 80% of complaints received by the laboratory (6). One aspect which could help improve an assay result TAT is reducing the centrifugation time without infringement on standards (7).

The process of centrifugation is a crucial step in the coagulation pre-analytical phase to separate cells and plasma. Aside from manufacturer suggestions, there is no internationally standardized centrifugation protocol required to generate suitable plasma for coagulation assays. The establishment of centrifugation protocol in verifying specimen quality is usually decided locally by individual laboratories which may be complicated by various centrifugation setting requirements of different specimen types.

CLSI guidelines for coagulation assays (H21-A5) advocate reducing platelet counts for routine coagulation screening tests (prothrombin time (PT); activated partial thromboplastin time (APTT)) to less than 200×10^9/L while other coagulation assays require lower than 10×10^9/L (8). Numerous studies have investigated a higher centrifugal force for a reduced duration in order to achieve platelet poor plasma. When these settings are adopted, it has been shown that there is no significant impact on routine coagulation assay results and interpretation (9-11). The presence of residual platelets in plasma can affect phospholipid-dependent coagulation tests (12,13). As a precaution, some laboratories use double centrifugation to minimise this potential effect. The potential release of platelet factor 4 (PF4), through higher centrifugal force, can possibly neutralize the action of UFH, thereby reducing clotting times in such patients (14). However, we are not aware of any coagulation centrifugation study that investigates the potential release and impact of PF4 on APTT results of UFH patients.

Methods

This prospective study was performed in the month of May 2019 in University Hospital Limerick (UHL), Rep. of Ireland. Phlebotomies were performed in a single day on 30 patients including volunteer normal donors, coronary care unit, UFH patients and patients attending the thrombosis review clinic in UHL. All patients gave informed consent and duplicate coagulation and single EDTA specimens were anonymised (labelled 1A/B 2A/B or EDTA1, EDTA 2, etc.) immediately post-phlebotomy before being forwarded to the laboratory.

To control pre-analytical variables and ensure process consistency, venous blood collection techniques adhered to CLSI recommendations (8). All collected Sarstedt® S-Monovette Sodium Citrate 9 NC/2.9 mL specimens were visually checked for complete filling, lipaemia, haemolysis and the presence of clots. The 10 minutes centrifugation at 2,000 g with swing-out bucket rotor centrifuges is the routine centrifugation setting in use in the UHL laboratory for coagulation assays. Duplicate citrate tubes were centrifuged at the 2,000 g × 10 minutes (A) and 4,000 g × 5 minutes (B) setting.

EDTA specimen haematocrit values from volunteers ranged from 0.36 to 0.44 L/L (within the reference range of the test parameter). All paired platelet poor specimens were processed and analysed for platelet count in less than 30 minutes of collection using an automated cell counter, Siemens Advia® 2120i (Siemens Healthcare Diagnostics, Erlangen, Germany).

All measurements from each centrifuge setting were performed on the same analyzer within 15 minutes of centrifugation using the Stago STA-R Evolution® coagulometer and associated reagents (Diagnostica Stago, France). Coagulation based assays PT, routine APTT, UFH APTT, Fibrinogen, D-Dimer, Factor V (extrinsic), Factor VIII (intrinsic) and Thrombin time were performed using Stago® STA-reagents. In the haematology laboratory UHL setting, the UFH therapeutic range of 0.3–0.7 IU/mL, at the time of testing, corresponded to an APTT range of 64–108 seconds.

Level of agreement and concordance between the 5- and 10-minute centrifugation protocol was evaluated by calculating the Lin concordance correlation coefficient, Blant-Altman statistics and associated plots and Passing-Bablock regression analysis. Bland-Altman plots with 95% confidence intervals (CI) (mean ± 1.96 standard deviation) were generated to determine the level of agreement in paired results between both centrifugation protocols. The cusum test of the linearity assumption ($P>0.05$) was applied. Statistical software MedCalc version 19.1 and Stata version 16.0 were used to analyse the data generated. A 10% deviation in paired results was applied to assess result reliability and interpretation.

Table 1 Calculated means and analytical range of coagulation parameters studied.

Coagulation assay (n=30)	10 min, 2,000 g		5 min, 4,000 g	
	Mean	Range	Mean	Range
PT (s)	20.1	12.4–43.4	20.1	12.7–42.7
Routine APTT (s)	33.6	27.4–47.2	33.4	27.4–46.2
UFH APTT (s)	74.2	40.1–148.0	70.9	40.6–144.1
Fibrinogen (g/L)	3.49	2.32–5.97	3.47	2.31–5.88
D-dimer (µg/mL FEU)	0.34	0.04–1.49	0.37	0.11–1.56
Thrombin time (s)	17.2	16.3–20.1	17.3	16.3–20.2
Factor V (%)	91	43–131	93	46–154
Factor VIII (%)	164	105–282	164	100–300
Platelet count ($\times10^9$/L)*	18	2–36	11	4–22

*, mean platelet count difference –7 ($\times10^9$/L). Twelve of thirty specimens yielded a platelet count of less than 10×10^9/L for the 5-minute setting. PT, prothrombin time; APTT, activated partial thromboplastin time; UFH APTT, unfractionated heparin activated partial thromboplastin time; TT, thrombin time.

Table 2 Statistical level of agreement, mean bias and deviation in paired coagulation results between the 10 minutes at 2,000 g and 5 minutes at 4,000 g centrifugation protocols.

Parameters	LIN analysis		Bland-Altman analysis			Passings-Bablock Regression analysis					Result deviation >10%
	CCC	95% CI	Mean Bias	LLA	ULA	Intercept	95% CI	Slope	95% CI	P	
PT	0.9987	0.9973–0.9993	–0.01	–0.73	0.76	–0.10	–0.43–0.36	1.01	0.98–1.03	0.91	0 (0)
APTT	0.9879	0.9764–0.9939	0.11	–1.80	1.60	0.54	-0.85–2.60	0.98	0.93–1.02	0.76	0 (0)
UFH APTT	0.9777	0.956–0.9888	–3.31	–5.00	11.70	–2.59	–8.24–1.66	1.08	1.01–1.18	1.00	3 (10%)
Fibrinogen	0.9851	0.9692–0.9929	–0.02	–0.20	0.30	–0.04	–0.28–0.12	1.01	0.97–1.09	0.89	1 (3.3%)
D-dimer	0.9617	0.9182–0.9823	0.02	–0.21	0.16	–0.01	–0.05–0.03	1.00	0.90–1.16	0.84	9 (30%)
Factor VIII	0.9802	0.9583–0.9907	0.04	–19.90	20.00	11.55	–3.48–26.05	0.93	0.84–1.04	0.80	1 (3.3%)
TT	0.9028	0.7491–0.9643	0.07	–0.84	0.70	0.91	–5.70–7.30	0.94	0.57–1.33	0.95	0 (0)
Factor V	0.9105	0.8032–0.9606	1.40	–24.10	21.20	–3.73	–10.30–6.64	1.03	0.93–1.13	0.99	1 (3.3%)

PT, prothrombin time; APTT, activated partial thromboplastin time; UFH APTT, unfractionated heparin activated partial thromboplastin time; TT, thrombin time; CCC, concordance correlation coefficient; LLA, lower limits of agreement; ULA, upper limits of agreement.

Results

Table 1 illustrates the coagulation parameter means and analytical result range examined in this study. Lin's concordance correlation coefficient analysis demonstrated acceptable levels of correlation between both centrifugation protocols for first line and subsequent coagulation assays tested. The Lin concordance correlation coefficient ranged from 0.9028 to 0.9987. Using Passing-Bablock analysis each parameter's linear model was validated with the Cusum test for linearity which verified no significant deviation from linearity (*Table 2*). Blant-Altman plots using 95% CI comparing the two centrifugation protocols showed acceptable levels of agreement (*Figure 1*). Blant-Altman analysis indicated PT produced a mean bias of –0.01 seconds (95% CI, –0.73–0.76), APTT 0.11 seconds (95% CI, –1.8–1.6), UFH APTT –3.31 seconds (95% CI, –5–11.7),

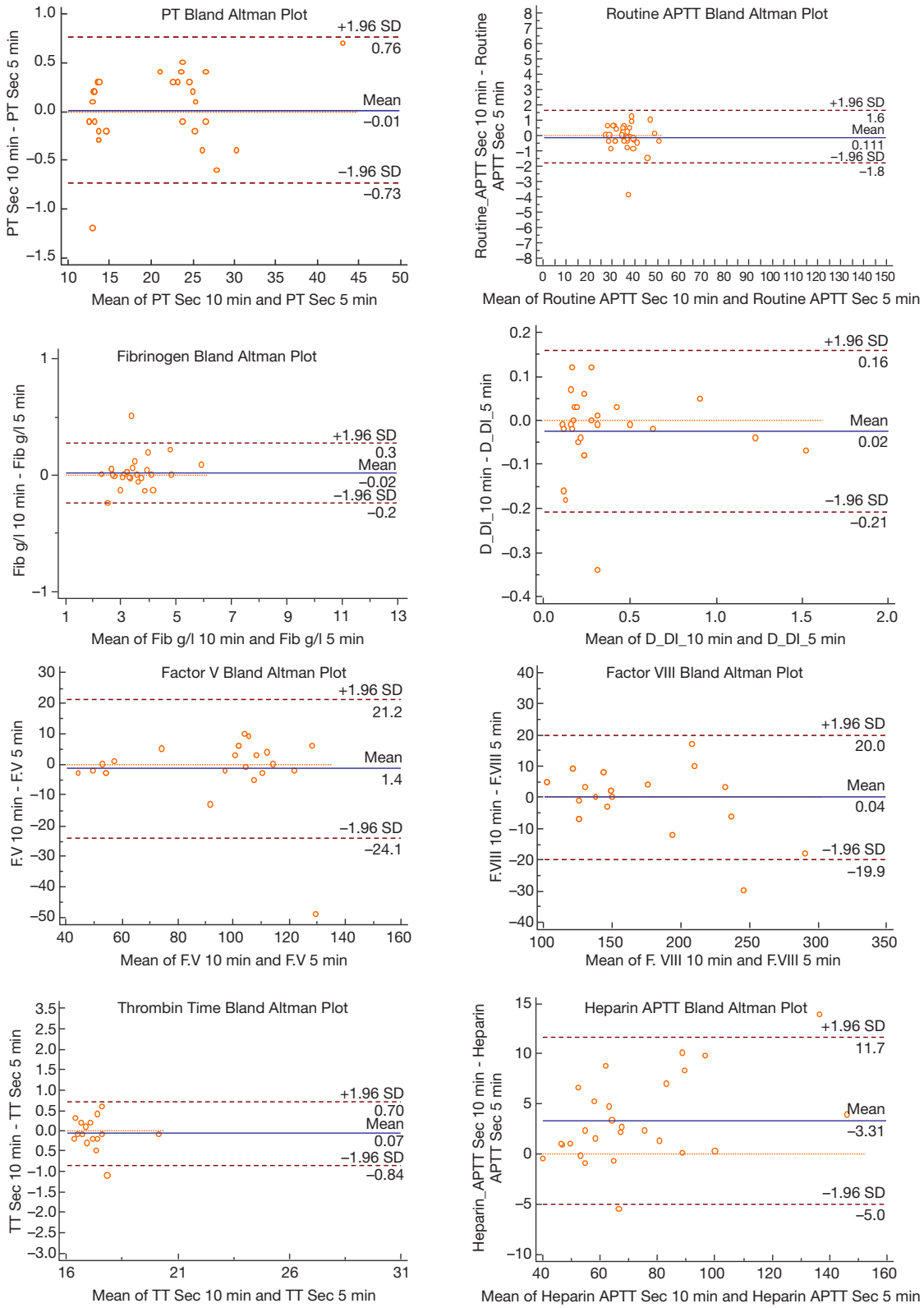

Figure 1 Blant-Altman plots for each coagulation parameter measured. The 3 horizontal lines represent the mean bias for each parameter and the accepted levels of agreement (1.96 standard deviation).

Fibrinogen –0.02 g/L (95% CI, –0.2–0.3 g/L), D-Dimer 0.02 µg/mL FEU (95% CI, –0.21–0.16 µg/mL FEU), Factor VIII 0.04% (95% CI, –19.9–20%), Thrombin time 0.07 seconds (95% CI, –0.84–0.7 seconds), Factor V 1.4% (95% CI –24.1–21.2%). The D-Dimer assay demonstrated the highest incidence of paired result deviation (>10% unit difference in 30% of cases). Examination of residual platelet counts of plasma obtained by both centrifugation protocols revealed consistently lower (-7×10^9/L) platelet counts in the 4,000 g for 5-minute setting. However, only twelve of thirty specimens yielded a platelet count of less than 10×10^9/L with the 5-minute setting.

Discussion

Centrifugation of coagulations specimens can be a major source of bottleneck in the TLA workflow resulting in increased TATs. Current laboratory practice is heterogenous and often based on manufacturer recommendations (15). Reported centrifuge settings used for coagulation testing range from 1,500 g for 10 minutes to 11,000 g for 1 minute (16,17). Choice of optimum centrifuge settings for blood specimens plays a pivotal role for specimen quality and accuracy in addition to potential improvements in TAT and user satisfaction with the laboratory service.

This study demonstrated acceptable levels of correlation in first line and subsequent coagulation tests when a higher centrifugal force of 4,000 g for a reduced time of 5 minutes was applied. The Lin concordance correlation coefficient ranged from 0.9028 to 0.9987. Blant-Altman and Passing-Bablock analysis revealed strong agreement between both centrifugation protocols with minimal mean bias. CLSI guidelines advocate for as low a platelet count as possible in coagulation specimen preparation. Platelets can potentially release PF4 and provide the phospholipid surface for activating clotting factors and hence interfere with the laboratory results (18). The mean difference between the 2,000 g for 10 minutes and 4,000 g for 5 minutes setting for UFH APTT was determined to be –3.31 seconds. This difference which covered UFH patient results spanning 40.6–144.1 seconds was not deemed to be clinically relevant in terms of its effect on their therapeutic management. The therapeutic UFH range of 0.3–0.7 IU/mL corresponds to an APTT range of 64–108 seconds in our laboratory setting. There appears to be minimal platelet contamination, release of PF4 and detrimental effect on the UFH APTT when this centrifugation setting is used. We believe our study is one of the first to demonstrate the minimal impact of a higher centrifugal force on UFH APTT results extending the therapeutic range. In a French study, no difference in heparin anti-Xa activity (IU/mL) for a 2 minutes 4,500 g centrifugation was found (19). However, this involved the use of CTAD tubes which are not commonly used for routine coagulation assays. The D-Dimer assay demonstrated the highest incidence of >10% result deviation (30% of cases) which can be explained by the high CV% (15–20%) of the assay at the lower end of the analytical range (below the cut-off value of 0.5 µg/mL FEU). Examination of residual platelet counts of plasma obtained by both centrifugation protocols revealed consistently lower (-7×10^9/L) platelet counts in the 4,000 g for 5-minute setting. Although this protocol demonstrated a reduction in platelet count with the 5-minute centrifugation protocol, only twelve of thirty specimens yielded a platelet count of less than 10×10^9/L. It is therefore recommended to continue with current laboratory protocol of double centrifugation for thrombophilia or lupus like anticoagulant assays. Similar to other studies, we found no evidence of sufficient deviation in results of coagulation tests which could have implications in clinical decision making (16,17,20). However, this study examined a wider set of coagulation parameters, considered the use of UFH, and analysed sodium citrate specimens, which is the preferred anticoagulant for routine coagulation measurements. This study recognizes its limitations and further studies with a larger group number could be more informative. Expansion of the analytical range investigated may be of benefit. Our findings cannot be extended to coagulation parameters or assay principles (e.g., chromogenic) not examined in this study. Laboratories should recognize their responsibility in validating and achieving the most efficient coagulation centrifugation protocol without infringement of standards.

This study shows that centrifugation at a 4,000 g RCF for 5 minute duration has a negligible impact on commonly requested coagulation test results and interpretation, and therefore can be considered for revising the guidelines for plasma preparation protocol for coagulation tests.

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Footnote

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm.2020.02.01). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was performed in accordance with the Declaration of Helsinki (as revised in 2013) and in accordance with the respective local Institutional Ethical Committee guidelines in the University Hospital of Limerick. Written informed consent was obtained from participants.

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