ORIGINAL ARTICLE

Investigation of D-dimer stability with INNOVANCE D-dimer assay

Yan Fu, Yii Sen Wee and Rhonda Lucas

ABSTRACT

Objectives: The present study aims to examine whether the D-dimer stability (using INNOVANCE® D-dimer assay) time can be extended from 4 hours to 6 or 8 hours at room temperature to account for late arrival samples and test add requests received after 4 hours.

Methods: Three citrate blood tubes were collected from each of the 25 patients. For each patient, a baseline tube was centrifuged and tested for D-dimer upon arrival (within 4 hours). This baseline tube was then repeatedly tested at 6 hours and 8 hours post-sample collection. The other two tubes were stored horizontally at room temperature until 6 hours and 8 hours post-sample collection, at which they were centrifuged and tested, respectively. All tests were analysed with the INNOVANCE® D-dimer assay.

Results: The D-dimer levels at baseline ranged from less than 190 µg/L FEU to 10512 µg/L FEU. The mean percentage changes at 6 hours and 8 hours were all below the clinically acceptable cut-off of 10% compared with the baseline. Specifically, for centrifuged samples, a decrease of 2.4% and 2.2% were found after 6 and 8 hours, respectively. For uncentrifuged samples, a decrease of 2.8% and 1.9% were observed at 6 and 8 hours, respectively. Results from each tested condition all met the allowed limit of performance in EFLM (European Federation of Clinical Chemistry and Laboratory Medicine). Further analysis using Passing-Bablock regression showed excellent agreement between D-dimer levels at baseline and D-dimer levels after 6- and 8-hours storage.

Conclusion: Our study demonstrated that the stability of D-dimer using INNOVANCE® D-dimer assay at room temperature can be reliably extended to 8 hours for both centrifuged and uncentrifuged whole blood samples.

Keywords: D-dimer, stability, INNOVANCE® D-dimer assay

NZ J Med Lab Sci 2023; 77(1): 19-23

INTRODUCTION

D-dimer is a small protein fragment derived from plasmin-mediated degradation of cross-linked fibrin (1). The generation of D-dimer requires the activity of thrombin, activated factor XIII, and plasmin. First, thrombin generated by the coagulation system converts soluble fibrinogen to fibrin monomers. Then, the fibrin monomers are covalently crosslinked into insoluble stable fibrin polymers by factor XIIIa. This stabilising crosslink occurs between the D domains of adjacent fibrin monomers and the E domain of a third fibrin monomer. The adjacent dimeric D domains are referred to as D-dimers. Lastly, plasmin digests fibrin clots and results in the D-dimer molecules and other fibrin degradation products (FDPs). Therefore, the presence of D-dimer is an indication of the activation of intravascular coagulation and subsequent fibrinolysis (1). The measurement of D-dimer is mainly using immunoassays with monoclonal antibodies specific for plasmin-degraded fibrin (2).

D-dimer test is one of the most frequently requested coagulation tests, which is mainly employed for the exclusion of venous thromboembolism (3) such as deep venous thrombosis (DVT), pulmonary embolism (PE), disseminated intravascular coagulation (DIC) and stroke (4). A negative D-dimer result, along with a low clinical pre-test probability, can be used to exclude venous thromboembolism in suspected patients (5). However, a positive D-dimer value is not conclusive of thrombosis, given that various diseases and conditions can increase D-dimer levels.

Many pre-analytical variables can affect the performance of a specific D-dimer assay (6,7), such as sample collection device (butterfly devices, needle bore size), sample transportation, processing method, storage time, storage temperature, and the status of the specimens (whole blood or separated platelet poor plasma). In Dunedin, Southern Community Laboratory (SCL), the blood is collected into a 0.109M buffered sodium citrate tube with 21G BD vacutainer flashback needles to the required level. The sample is immediately carefully mixed, the tourniquet released, stored and transported at room temperature (15–25 °C). The whole blood must arrive in the laboratory within 3 hours and testing performed in <4 hours. This short time frame can be challenging when samples are collected in satellite centres outside of the major testing centre.

In addition, clinicians often request that D-dimer testing be performed on samples already drawn for other coagulation tests after the 4-hour window. While many studies have examined the stability of centrifuged and refrigerated D-dimer, very few studies have investigated room-temperature D-dimer stability. Therefore, it would be useful to explore whether the stability of the D-dimer assay can be extended to a wider time window at room temperature for whole blood samples.

The current study aims to examine: first, whether the D-dimer stability time on whole, uncentrifuged sodium citrate blood can be extended from 4 hours to 6 or 8 hours. This accounts for late arrival samples. Secondly, whether the D-dimer stability time on whole centrifuged samples can be used after 4 to 6 or 8 hours, given that add-on test requests are frequently received on tested samples after 4 hours.

MATERIAL AND METHODS

Blood sample collection and processing

Twenty-five sets of patient laboratory samples participated in this study. Three whole blood samples were collected from each patient in 0.109M buffered sodium citrated tubes by venepuncture staff during routine blood tests. Consent forms or consent stickers were obtained from each patient before sample collection.

After samples arrived, one tube was randomly assigned as the baseline tube, which was centrifuged (10 min, 2000 g) and tested for D-dimer immediately, within 4 hours. Any sample that has HIL level ≥2 (equivalent to 40 mg/dl free haemoglobin in the plasma) is deemed as haemolysed and therefore unsuitable for this study. After analysis, the baseline sample tube was stored on a rack at room temperature. Repeated tests were conducted on the tested centrifuged tube at 6 hours and 8 hours post-sample collection. The other two uncentrifuged whole blood sodium citrate tubes were stored in a bag horizontally after arrival at RT; they were centrifuged and tested respectively at 6 and 8 hours post-sample collection.

Of all the 25 samples, four patients' data were incomplete due to sampling collection error (3 patients only had two sample collected) or testing error (1 patient missed a repeated test at 6 hr and one patient missed a repeated test at 8 hr).

Principle of the INNOVANCE® D-dimer assay

INNOVANCE® D-dimer assay is a particle-enhanced immunoturbidimetric assay (8) for quantitatively determining cross-linked fibrin degradation products (D-dimer) in plasma. The assay relies on the 8D3 monoclonal antibody, which is covalently coupled to polystyrene particles that aggregate when mixed with samples containing D-dimer. The degree of agglutination was directly proportional to the concentration of D-dimer in the sample and detected turbidimetrically via the increase in turbidity. Results were expressed in µg/L fibrinogen equivalent units (FEU) with report limits between 190 µg /L FEU to 35200 µg /L FEU and clinical cut-off at 500 µg /L FEU.

In this study, D-dimer testing was performed using the Sysmex CS2500 automated coagulation analyser with the INNOVANCE® D-Dimer. INNOVANCE® D-Dimer is a particle-enhanced, immunoturbidimetric assay for the quantitative determination of cross-linked fibrin degradation products (D-Dimers). Daily QCs were performed on the analyser, and good diagnostic capability was maintained.

Statistical analysis

To assess stability, the percentage changes were calculated to compare with the clinically relevant difference, which was defined as a percentage change greater than 10% (9). This was done by taking the result obtained from 6 hr, and the 8 hr sample minus the baseline result and then divide that result by the baseline result and multipled by 100. If the percentage change is greater than 10% at 6 hr but corrected back to values less than 10% at 8 hr, it was assumed that deviation was not entirely caused by the storage-dependent change of D-dimer levels (10). Results were further assessed against the Allowed Limit of Performance (ALP) guideline set by EFLM (European Federation of Clinical Chemistry and Laboratory Medicine), which has the Imprecision% at 11.6%, Bias % at 8.8 % and Total Error % at 28 % (11).

Bland-Altman plots (12) were used to display the relative difference of D-dimer levels between baseline and different storage conditions (see Figure 2 and 3) by Analyse-it add-in of Microsoft Excel. Statistical analysis were conducted using Passing-Bablok regression (13) to estimate the agreement between the baseline test and each storage condition test, which is a robust, non-parametric statistical analysis that is not sensitive to the distribution of errors and data outliers. For this analysis, a slope between 0.9 and 1.1, an intercept lower than 50µg/L FEU and a Pearson correlation coefficient higher than 0.99 were considered to indicate substantial agreement (10).

RESULTS

The D-dimer level at baseline ranged from <190µg/L FEU to >4000µg/L FEU (Figure 1). The line charts for each patient showed an overall stable pattern at 6 hr and 8 hr post-sample collection for both centrifuged and uncentrifuged whole blood samples (Figure 2). The relative difference between D-dimer levels at baseline and D-dimer levels at different storage conditions were plotted in Bland-Altman plots for centrifuged (Figure 3.) and uncentrifuged whole blood samples (Figure 4). The mean percentage changes were all below the clinically acceptable cut-off of 10% (11) at 6 hours and 8 hours for both centrifuged and uncentrifuged samples. For centrifuged samples, a median decrease of 2.4% and 2.2% were found after 6 and 8 hours, respectively. For uncentrifuged samples, a median decrease of 2.8% and 1.9% were observed at 6 and 8 hours, respectively. Samples with results below the measuring range of 190µg/L FEU were excluded from the line charts and absolute number for percentage change calculations were not calculated.

The Bias%, Imprecision%, and Total Error% for each storage condition were calculated and compared against the allowed limit of performance in EFLM. Results from each conditions all met the guideline (Table 1). Passing-Bablock regression analysis showed an excellent agreement between D-dimer levels at baseline and D-dimer levels in different storage conditions by showing regression slopes between 0.9–1.10, intercepts <50µg/L and r > 0.99 (Table 2 & Figure 5), which suggested that the D-Dimer levels were stable after 6 hours and 8 hours for both centrifuged and uncentrifuged sample. Therefore, the stability time for D-dimer can be confidently extended to 8 hours.

DISCUSSION

The present study aimed to examine whether the D-dimer stability time on centrifuged and uncentrifuged whole blood can be extended from the current 4 hours to 6 or 8 hours to account for the test add requests and late samples received after 4 hours. Twenty-five patients with a wide range of D-dimer levels were tested with INNOVANCE® D-Dimer Assay. For each patient, three 0.109M buffered citrated tubes were collected. One baseline tube was centrifuged and tested after arrival and repeatedly tested at 6- and 8-hours post-sample collection. The other two tubes were centrifuged and tested at 6 and 8 hours post-sample collection, respectively. The results showed that the mean percentages change was all less than the clinically acceptable age related cut-off of 10% (17) after 6 hours and 8 hours storage for both centrifuged and uncentrifuged samples. In addition, all results met the criteria set in EFLM guideline and excellent agreements were shown between tests at baseline and tests at 6 and 8 hours.

Of all 25 patients, a decrease of percentage change over 10% occurred only in two patients. For one patient, the baseline D-dimer value was at 2716µg/L FEU and the deviation over 10% only occurred at 6 hours (-20.8% and -21.5% for centrifuged and uncentrifuged), However, the percentage changes were lower than 10% at the 8 hours measurement (3.1% and -3.2% for centrifuged and uncentrifuged). Therefore, the latter correction suggested that the storage-dependent change of D-dimer levels did not fully cause the deviation. So 8 hours stability is still acceptable in this case. For the second patient, the baseline D-dimer level was around the borderline (605 µg/L FEU, with age adjusted cut-off of 600µg/L), the percentage changes were above 10% for the centrifuged sample at 6 hours (-27.3%) but not for uncentrifuged samples at 6 hours (-9.09%). They were also above 10% at 8 hours for both centrifuged (-18.7%) and uncentrifuged samples (-16.7%). Therefore, this result suggests that it is potentially possible to have false negative results when measuring the D-dimer at 8 hours, especially when the patient's D-dimer level is around the borderline. However, these two outliers do not significantly change the overall statistical results. Future studies with larger sample sizes can be useful to further clarify this problem.

According to the guideline from the Clinical and Laboratory Standards Institute (CLSI), D-dimer is a stable measurand and may tolerate storage times of up to 24 hours at room temperature or in a refrigerator (14). However, the manufacturer's package insert for INNOVANCE® D-Dimer assay suggests that the stability time for D-Dimer is 24 hours at 2-8°C and only 4 hours at room temperature (15). Therefore, it is necessary to do local validations on INNOVANCE® D-Dimer assay to extend the stability time.

Previous studies using the same INNOVANCE® D-Dimer assay have demonstrated supportive evidence for 24 hours D-dimer stability at room temperature. For example, Böhm-Weigert et al.(10) used a different sample collection tube (0.106 mol/L trisodium citrate), a different analyser system (BCS system), and different sample processing methods (separated plasma) with a larger sample size (n=147) showed the D-dimer were stable after 8 and 24h storage, which suggested that the acceptable storage time can be extended to 24 hours. Same findings were also reported in another study (16), using the Sysmex CA7000 system and fasting samples (n=80). The current study further confirmed the D-dimer stability at 8 hours, which extended the acceptability age of D-dimer samples from the previous 4 hours to 8 hours. This allowed the late arrival samples and test add requests to be processed in a wider time range and saved time and money for rebleeding patients.

There are some limitations in this study. First, compared with the Böhm-Weigert et al (10) and Zhao Y, Lv G. (16) studies, the

sample size in the present study is relatively small. Secondly, D -dimer levels over 500µg/L FEU are routinely checked for clots before reporting the result as per Dunedin SCL Protocol - Routine Coagulation manual. Clotted samples can have falsely elevated D dimer levels. However, the clot check was not conducted on all patients in this study. This might affect the accuracy of D dimer results. Lastly, some patients (n=3) only have two tubes collected, so the 8-hour test cannot be undertaken. Despite these limitations, the overall result was not significantly affected.

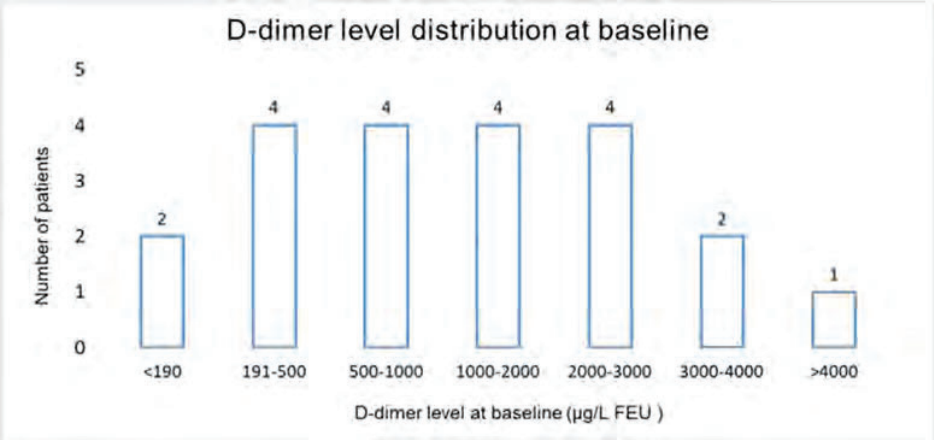

Figure 1. 21 D-dimer level distribution at baseline test.

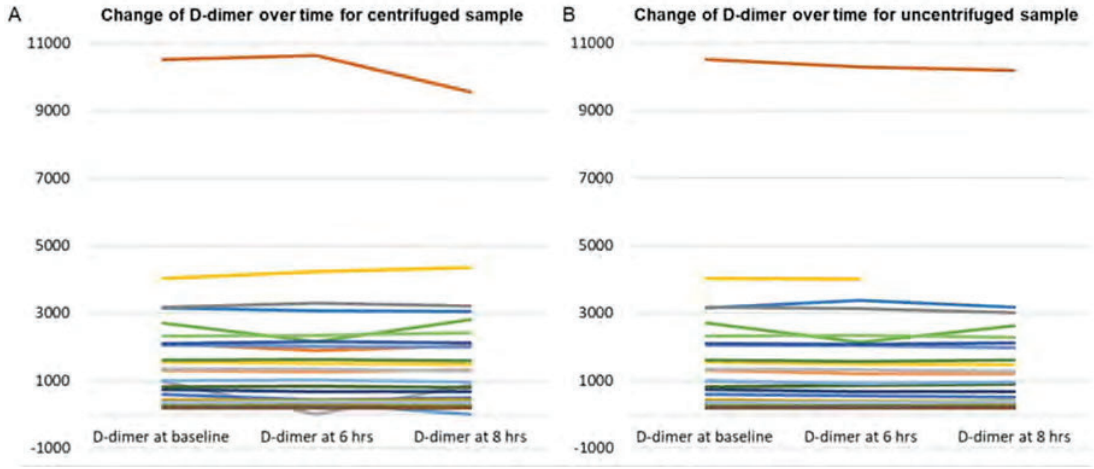

Figure 2. Line charts of D-dimer variation over time for 24 centrifuged samples (2A) and 25 uncentrifuged whole blood samples (2B). The median change for centrifuged sample is -2.4% for 6hr and -2.2% for 8hr. The median change for uncentrifuged sample is -2.8% in 6hr and -1.9 in 8hr.

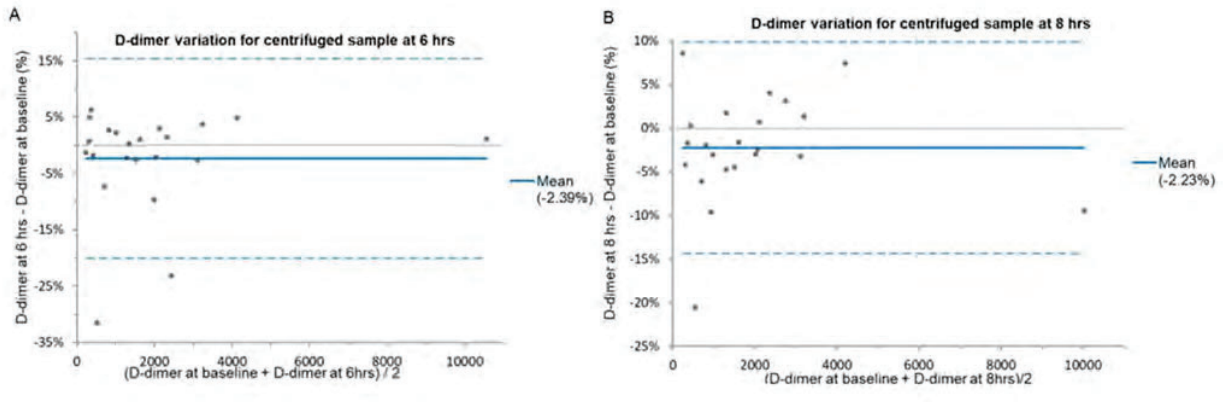

Figure 3. Bland-Altman plots of D-dimer variation for all individuals between baseline and after 6hrs (3A) and 8 hrs (3B) for 24 centrifuged samples.

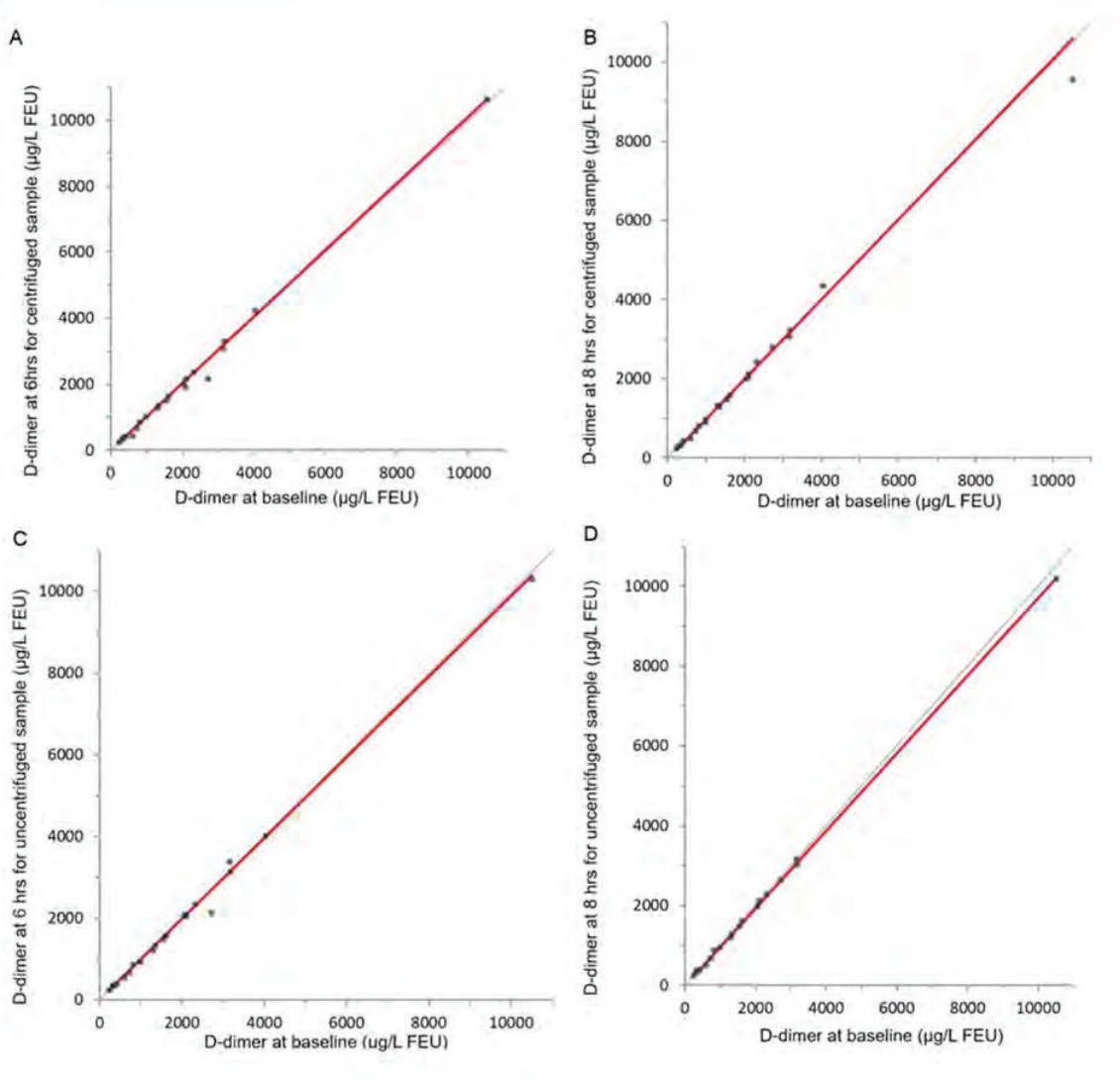

Figure 5. Comparison of D-dimer values at baseline versus D-dimer values at 6 hours (5A) and 8 hours (5B) for 24 centrifuged samples and 22 uncentrifuged whole blood samples (5C and 5D). The results of the regression analysis are given in Table 2.

Table 1. Summary of tested samples compared against the allowed limit of performance

Storage Condition	Sample Tested	% change versus baseline				Test Pass/Fail
		Mean (B%)	SD	CV (I%)	TE%	
6 hours centrifuged	24	-2.39	9.03	-3.78	12.51	PASS
8 hours centrifuged	24	-2.23	6.17	-2.77	7.95	PASS
6 hours uncentrifuged	25	-2.83	6.69	-2.36	8.21	PASS
8 hours uncentrifuged	22	-1.93	6.36	-3.29	8.56	PASS

Table 2. Passing-Bablock regression result for different storage conditions

Storage condition	Slope	Intercept	Pearson's r
6 hours centrifuged	1.011	-3.488	0.998
8 hours centrifuged	1.010	-32.16	0.997
6 hours uncentrifuged	0.988	-0.896	0.998
8 hours uncentrifuged	0.970	-5.596	1

CONCLUSIONS

In summary, our study demonstrates that INNOVANCE® D-dimer assay can be reliably tested at 8 hours post-sample collection and the acceptability age of D-dimer samples can be extended to 8 hours for both centrifuged and uncentrifuged samples at room temperature.

ACKNOWLEDGEMENTS

I would like to acknowledge and thank the staff in Haematology Department at SCL Dunedin, especially, Katherine Christie for organising the experiments and Lauren Eddington for conducting the late sample testing. My thanks also go to the venepuncture staff in the SCL collection centres of South Dunedin, Filleul Street and Dunedin Hospital for collecting samples.

AUTHOR INFORMATION

Yan FU, 4th year BMLSc Student[1]
Yii Sen Wee, BMLSc, Haematology Head of Department[2]
Rhonda Lucas, ANZIMLS, Coagulation Technical Specialist[2]

[1] University of Otago, Dunedin
[2] Southern Community Laboratories, Dunedin

Correspondence: Yii Sen Wee, Haematology Head of Department, Southern Community Laboratories, Dunedin
Email: yiisen.wee@sclabs.co.nz

REFERENCES

1. Francis CW, Marder VJ, Barlow GH. Plasmic Degradation of Crosslinked Fibrin. *J Clin Invest* 1980; 66(5): 1033–1043.
2. Korte W, Riesen W. Latex-enhanced immunoturbidimetry allows D-dimer determination in plasma and serum samples. *Clin Chem* 2000; 46(6): 871–872.
3. Soomro AY, Guerchicoff A, Nichols DJ, et al. The current role and future prospects of D-dimer biomarker. *Eur Heart J Cardiovasc Pharmacother* 2016; 2(3): 175–184.
4. Righini M, Perrier A, De Moerloose P, Bounameaux H. D-dimer for venous thromboembolism diagnosis: 20 years later. *J Thromb Haemost* 2008; 6(7): 1059–1071.
5. Elf JL, Strandberg K, Nilsson C, Svensson PJ. Clinical probability assessment and D-dimer determination in patients with suspected deep vein thrombosis, a prospective multicenter management study. *Thromb Res* 2009; 123(4): 612–616.
6. Favresse J, Lippi G, Roy PM, et al. D-dimer: Preanalytical, analytical, postanalytical variables, and clinical applications. *Crit Rev Clin Lab Sci* 2018; 55(8): 548–577.
7. Magnette A, Chatelain M, Chatelain B, et al. Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories. *Thromb J* 2016; 14(1): 49.
8. Arai N, Collins A. Innovance® D-DIMER. *Sysmex J Int* 2008; 18(1):15–22.
9. Zürcher M, Sulzer I, Barizzi G, et al. Stability of coagulation assays performed in plasma from citrated whole blood transported at ambient temperature. *Thromb Haemost* 2008; 99(02): 416–426.
10. Böhm-Weigert M, Wissel T, Muth H, et al. Long-and short-term in vitro D-dimer stability measured with INNOVANCE D-Dimer. *Thromb Haemost* 2010; 103(02):461–465.
11. Aarsand AK, Fernandez-Calle P, Webster C, Coskun A, Gonzales-Lao E, Diaz-Garzon J, et al. The EFLM biological variation database. https:biologicalvariation.eu. Accessed Dec 20th. 2020
12. Giavarina D. Understanding bland altman analysis. *Biochem Medica* 2015; 25(2): 141–151.
13. Bilic-Zulle L. Comparison of methods: Passing and Bablok regression. *Biochem Medica* 2011; 21(1): 49–52.
14. JD O, Adcock DM, Ambrose Bush T, et al. Quantitative D-dimer for the Exclusion of Venous Thromboembolic Disease. *Approved Guideline H59-A. Clinical and Laboratory Standards Institute;* 2011.
15. INNOVANCE® D-Dimer [package insert]. Siemens Healthcare Diagnostics Products GmbH; 2018.
16. Zhao Y, Lv G. Influence of temperature and storage duration on measurement of activated partial thromboplastin time, D-dimers, fibrinogen, prothrombin time and thrombin time, in citrate-anticoagulated whole blood specimens. *Int J Lab Hematol* 2013; 35(5): 566–570.
17. Schouten H, Geersing J, Koek H, et al. Diagnostic accuracy of conventional or age adjusted D-dimer cut-off values in older patients with suspected venous thromboembolism: systematic review and meta-analysis. *BMJ* 2013; 346: f2492 doi: 10.1136/bmj.f2492.