Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/mjafi

Original Article

Quantitative assessment of prevalence of pre-analytical variables and their effect on coagulation assay. Can intervention improve patient safety?

Lt Col Ravi Bhushan^a, Gp Capt Arijit Sen^{b,*}^a Graded Specialist (Pathology), Military Hospital Bhopal, MP, India^b Associate Professor, Department of Pathology, Armed Forces Medical College, Pune 411040, India

ARTICLE INFO

Article history:

Received 27 February 2016

Accepted 4 December 2016

Available online 5 January 2017

Keywords:

Pre-analytical variables

Coagulation assay

Intervention

Indian study

ABSTRACT

Background: Very few Indian studies exist on evaluation of pre-analytical variables affecting “Prothrombin Time” the commonest coagulation assay performed. The study was performed in an Indian tertiary care setting with an aim to assess quantitatively the prevalence of pre-analytical variables and their effects on the results (patient safety), for Prothrombin time test. The study also evaluated their effects on the result and whether intervention, did correct the results. **Methods:** The firstly evaluated the prevalence for various pre-analytical variables detected in samples sent for Prothrombin Time testing. These samples with the detected variables wherever possible were tested and result noted. The samples from the same patients were repeated and retested ensuring that no pre-analytical variable is present. The results were again noted to check for difference the intervention produced.

Results: The study evaluated 9989 samples received for PT/INR over a period of 18 months. The prevalence of different pre-analytical variables was found to be 862 (8.63%). The proportion of various pre-analytical variables detected were haemolysed samples 515 (5.16%), over filled vacutainers 62 (0.62%), under filled vacutainers 39 (0.39%), low values 205 (2.05%), clotted samples 11 (0.11%), wrong labeling 4 (0.04%), wrong vacutainer use 2 (0.02%), chylous samples 7 (0.07%) and samples with more than one variable 17 (0.17%). The comparison of percentage of samples showing errors were noted for the first variables since they could be tested with and without the variable in place. The reduction in error percentage was 91.5%, 69.2%, 81.5% and 95.4% post intervention for haemolysed, overfilled, under filled and samples collected with excess pressure at phlebotomy respectively.

Conclusion: Correcting the variables did reduce the error percentage to a great extent in these four variables and hence the variables are found to affect “Prothrombin Time” testing and can hamper patient safety.

© 2016 Published by Elsevier B.V. on behalf of Director General, Armed Forces Medical Services.

* Corresponding author.

E-mail address: aseniaf@gmail.com (A. Sen).<http://dx.doi.org/10.1016/j.mjafi.2016.12.005>

0377-1237/© 2016 Published by Elsevier B.V. on behalf of Director General, Armed Forces Medical Services.

Introduction

In an era of evidenced-based medicine, therapy is mostly guided by results of laboratory investigation^{1,2} and thus quality assurance of a laboratory is of immense importance.³ Any laboratory test protocol passes through pre-analytical, analytical and post-analytical phases and ends with the delivery of test result. Each phase influences the final test result. Identifying the errors contributed by each phase, prevalent in a laboratory helps in reducing reporting errors. In many previous studies, pre-analytical errors have been identified as the major contributor (3/4th) of all errors in laboratory reporting.³⁻⁵ Identification of pre-analytical errors and efforts directed towards improving sample quality can significantly improve quality assurance and enhance patient safety. Reports generated by the coagulation laboratory to a greater extent influences the clinician due to the critical nature of the therapeutic decisions. Coagulation assays are highly vulnerable to pre-analytical errors because of the complexity of the biochemical and cellular reactions measured. Prothrombin time test is evaluating the same haemostatic pathway which often gets activated during phlebotomy. Errors in coagulation testing often do not allow clinico-pathological correlation, cause patient inconvenience and significantly increase workload due to repeat testing. This may also jeopardize patient safety due to incorrect dosage of anti-coagulant therapy (AC). With this in mind, the present study was designed to identify and quantify the prevalence of various pre-analytical variables affecting the results of the most commonly indented coagulation assay the 'prothrombin time' (PT)/international normalized ratio (INR) in the haematological laboratory of a tertiary care centre. It also intended to check upon the efficacy of the suggested interventions to reduce the respective pre-analytical errors. Very few Indian studies exist, which have quantitatively evaluated pre-analytical variables in coagulation assays.

Materials and methods

All the samples received in the coagulation laboratory of the tertiary care hospital for PT/INR assay from 01 January 2012 to 30 June 2013 were included in the present study. The samples received from patients with a haematocrit of 55% were excluded from the study since; if the haematocrit exceeds 0.55 (55%), the reduced plasma volume requires a decrease in the volume of anticoagulant used to maintain the ratio of 9:1. This would have affected interpretation of results. The samples were collected with standard phlebotomy instructions using citrate vacutainer (3.8% buffered sodium citrate) and needle with barrel. Instruction was given at sample collection centre for not putting excess pressure and tying a tourniquet. In all cases, age, sex, and clinical indication for testing were noted. On receipt, the samples were registered and validated for correct identification, amount, presence of haemolysis, presence of clots, and use of correct vacutainer. The registered samples were categorized into samples without any visible pre-analytical variables and presence of pre-analytical variables. The latter were further categorized into

Fig. 1 – Overview of sequence in study methodology.

presence of haemolysis and clots, overfilling or underfilling of vacutainer, use of wrong vacutainer and chylous plasma. The proportion of various pre-analytical variables was noted. The samples where there were no apparent pre-analytical variable and where the result of test correlated with the clinical status were not evaluated further. The result was noted for all the samples including for samples which had apparent pre-analytical variables. In all cases where samples had apparent pre-analytical variables detected at the time of sample validation, the sampling was repeated. Also, in cases where the test values were <2SD of control, the sampling was repeated with instruction of precaution of not placing pressure while sampling. All these repeat samples were re-tested and their result noted. The effects of the various variables on the test value were noted. The change in the test value in the repeat sample of the same patient, after eliminating the variable was also noted. The results attained on the 1st sample with apparent pre-analytical variable were compared with the values in the repeat sample without the pre-analytical variable.

Testing protocol

The samples were tested using the Transasia Semi-Automated Coagulation Analyzer Sysmex CA-50. The equipment was undergoing annual calibration by the original equipment manufacturer. Daily internal quality control protocols using Thromborel®S, Actin and Ci-Trol®1 were being conducted. Tests were taken up only if the results for PT were within the reference range for the LOT No. of the internal quality control kit. The values attained for the control from the set of pooled plasma for PT were programmed into the equipment on daily basis after taking an average of normal result of 10 healthy adults (among selected blood donors) of both sexes. The international sensitivity index of the kit in use too was updated in the programme on as and when the kit was changed. The effect on the pre-analytical variable on the result of the prothrombin time test was calculated using the percentage reduction in error method, since all samples which clinically correlated in the initial samples in spite of presence of the pre-analytical variable again showed results which were clinically correlating in the repeat sample (without the variable). Thus, inferential statistical test could not be used. Percentage reduction in error was thus noted in each case on eliminating the variable post intervention. Institutional ethics clearance was taken for the study. Overview of

the methodology adopted for the study is tabulated in flow chart format as in Fig. 1.

Results

A total of nine thousand nine hundred and eighty-nine (9989) samples were received in the laboratory of a tertiary care centre from 01 January 2012 to 30 June 2013 for PT/INR. The age group to which these samples belonged ranged from 4 to 80 years. Out of the total samples received, 657 samples showed presence of visible pre-analytical variables at point of sample validation. Remaining 9332 were found to be satisfactory for evaluation at this point. The results obtained on testing these 9332 samples could be grouped into three categories. In the first category were 4063 samples, where the results were found to be within the expected normal range and correlated with routine pre-anaesthetic check (PAC) up for which the test had been indented in with expected normal coagulation profile. In the second category were 5064 samples where the test had been indented for patients who were either on anticoagulant therapy or had a clinical situation where the values were expected to be elevated. In all these cases, the test values found were within the expected elevated range. The first two categories added up to 9127 in which the test results correlated with the clinical status of the patient. In the third category were 205 samples where the test values obtained were less than 2SD of the control though they were from cases who had undergone testing for routine PAC or were on anti-coagulant therapy. In these cases, the sampling was repeated to exclude influence of pre-analytical activation of coagulation pathway at the time of collection of sample. Thus, these 205 samples and the 657 mentioned above in which there was presence of visible pre-analytical variables at point of sample validation added up to 862 (8.63%) samples out of a total of 9989 which were included in this study. Thus, prevalence of pre-analytical variables was 8.63% (862/9989). These samples underwent a repeat sampling and testing. The breakup of these 862 samples for quantitative prevalence of various pre-analytical variables is tabulated in Table 1 (Refer footnote 'a' in Table 1, the confidence interval of variables is for total sample strength of

9989). Prototype of a sample without visible pre-analytical variables received in citrate vacutainer and with various pre-analytical variables is depicted in Fig. 2. Samples appended under serial Nos. 5, 6, 7, 8 and 9 in Table 1 added up to 41 samples. These were excluded from further evaluation in the present study as they could not be tested to arrive at a result and the result compared in repeat testing in case performed. So this left 821 samples tabulated from serial Nos. 1 to 4 of Table 1 which were evaluated further in the present study for effect of pre-analytical variables on the result and change in result once the pre-analytical variable is corrected. 515 samples were haemolysed. This constituted 5.16% of total samples received and the commonest (59.74% – 515/862) pre-analytical variable (refer Serial No. 1 Table 1). 410 gave grossly deranged (test values < 2 s of control) readings for PT in healthy individuals undergoing PAC testing and in patients on anticoagulants, values were within normal range (much lower than the expected derangement due to anti-coagulant therapy). However, in 105 cases, the readings were in range as expected for the case. The samples in all these 515 cases were redrawn and the test repeated. The redrawn samples did not reveal any evidence of visible pre-analytical variables. Fig. 3a summarizes the results of these samples before and after repeat testing. On repeat testing of all these 515 patients' samples, 105 cases which had shown results as expected to the clinical status initially again showed similar results. Out of 410 samples which had shown deranged values earlier, 375 samples now showed results as expected for the clinical status, though 35/410 continued to show low values. Thus, in the repeat testing, 480 samples showed results which correlated with the clinical status. All the 105 samples which had shown clinically correlating results in the initial sample again showed similar results and were included in the 375. As depicted in Fig. 3a, the reduction in error percentage was 91.5% post intervention and removal of haemolysis. Thus, haemolysis as a pre-analytical variable was found to have contributed significantly to errors in PT testing. The 62 (0.62% of total samples) samples in serial no. 2 of Table 1 that had excess volume of blood (overfilling) in relation to the mark provided on the citrate vacutainer were tested. This constituted 7.19% (third most common variable – 62/862) of the pre-analytical

Table 1 – Prevalence of various pre-analytical variables affecting results (n = 9989).

Sl No.	Pre-analytical error/variable identified	Number (%)	CI (95%)
1.	Haemolysed (Fig. 1b)	515 (5.16)	4.74–5.62
2.	Overfilling of the vacutainer resulting in the disproportionate blood to anticoagulant ratio (Fig. 1c)	62 (0.62)	0.48–0.80
3.	Under filling of the vacutainer resulting in the disproportionate blood to anticoagulant ratio (Fig. 1d)	39 (0.39)	0.28–0.54
4.	Values < 2SD from control in patients undergoing testing as a part of PAC or on anticoagulant therapy	205 (2.05)	1.79–2.35
5.	Clotting	11 (0.11)	0.06–0.20
6.	Wrong labelling	04 (0.04)	0.01–0.11
7.	Wrong vacutainer use	02 (0.02)	0–0.08
8.	Chylous	07 (0.07)	0.03–0.15
9.	Sample with more than one factor	17 (0.17)	0.10–0.28
	Total	862 (8.63)	8.09–9.02

^aConfidence Interval of variable in total sample strength of 9989.

Fig. 2 – Clockwise (a) citrate vacutainer without any visible pre-analytical variable. (b) Citrate vacutainer with a haemolysed sample. (c) Underfilled citrate vacutainer. (d) Overfilled citrate vacutainer.

variables. 39 out of these 62 samples (refer Fig. 3b) showed low values than expected for the case. It was <2 s from the control in patients who were clinically not expected to have a derangement and within ± 2 s of the control in patients who were on anticoagulant therapy. However, 23/62 samples showed results, which correlated clinically. All the 62 samples

were redrawn. Care was taken to use exactly the laid down protocol for phlebotomy using the vacutainer barrel with needle. All 62 redrawn samples did not have any visible pre-analytical variables. The test was repeated on all these 62 redrawn samples. Now 50/62 showed results as expected for the clinical status of the case, whereas 12/62 continued to show low values. All the 23 samples which had shown clinically correlating results in the initial sample again showed similar results and were included in the 50. Fig. 3b summarizes the results of these samples before and after repeating the sample. As depicted in Fig. 3b, the reduction in error percentage was 69.2% post intervention and testing on correctly filled samples. Thus, overfilled samples as a pre-analytical variable were found to have contributed significantly to errors in PT testing. The 39 (0.39% of total samples) samples in Table 1 (refer serial No. 3) that had less volume of blood (under filling) in relation to the mark provided on the citrate vacutainer were tested. These constituted the fourth most common (4.52% – 39/862) pre-analytical variable. 27 out of these 39 showed elevated values than expected for the case (refer Fig. 3c). The result was >2 s from the control in patients who were clinically not expected to have a derangement and grossly elevated beyond the expected levels for correlation, in patients who were on anticoagulant therapy. 12/39 samples, however, showed results as expected for the clinical status of the patient. All these 39 samples were redrawn and tested again. Now 34/39 samples showed results as expected for the clinical status, but still five samples out of the 39 mentioned above continued to show elevated results which could not be correlated with the clinical status. All the 12 samples which had shown clinically correlating results in the initial sample again showed similar results and were included in the 34. As depicted in Fig. 3c, the reduction in error percentage was 81.5% post intervention and testing on correctly filled samples. Thus, underfilled samples as a pre-analytical variable were found to have contributed significantly to errors in PT testing. The test results in 205 (2.05% of samples) samples reflected in Serial. No. 4 of Table 1 showed values <2 SD of the control for both patients (expected normal coagulation profile) being tested as a part of PAC and those on anticoagulant therapy. This constituted the second most common variable (23.78% – 205/862). The samples in all these cases were redrawn and tested. Strict instructions were given to the phlebotomist for not putting excess pressure around the point of collection to avoid expression of tissue thromboplastin and to prolong the phlebotomy process. The initial results for these 205 samples were compared with the results of the test on the redrawn sample. The same is appended in Fig. 3d. 196/205 samples now showed result in range as expected for the clinical status of the patient. However, 9 samples still continued to show low values. As depicted in Fig. 3d, the reduction in error percentage was 95.4% post intervention. Thus, it was seen that ensuring strict phlebotomy protocols did drastically reduce errors in PT testing and hence is an important pre-analytical variable which was found to have contributed significantly to errors in PT testing.

Discussion

The majority of all errors in the total testing process are of pre-analytical origin, that is, they occur before the sample arrives

Fig. 3 – (a,b,c,d) Tabulation of data for the four variables evaluated for error % on initial samples and on samples post intervention.

in the laboratory.³⁻⁵ In a study⁶ by Lippi et al., the proportion of errors associated with pre- and post-analytical phases of testing were found to be 4-5 times higher than that seen in analytical phase with pre-analytical phase representing over half of the errors. In another study,⁷ pre-analytical factors were found to be 46-68.2% of total errors while a high error rate 18.5-47% of total errors was found in post-analytical phase. The various pre-analytical errors/variables significantly affecting the test results have been identified and mentioned in the literature. The spectrum of these errors includes patient identification and test tube labelling, errors in sample collection and test requests, defective transport of blood samples, use of wrong coagulation tubes, haemolysis, lipemia and high haematocrit.^{1,2,8-11} In our study, out of total 9989 samples, 862 (8.63%) showed presence of various forms of pre-analytical variables. Though most studies in the literature have not mentioned the exact figures regarding the prevalence of the various variables/errors, they have

suggested that 0.01-0.5% of all test results are erroneous due to pre-analytical variables.^{2,12} An Indian study¹³ identified 1% pre-analytical errors in a haematology laboratory. This study evaluated 135,808 samples out of which they identified presence of pre-analytical errors in 1339 samples, a rate of 1%. The highest number was for misidentification 0.35% and lowest for dilution of samples 0.04%. The overall prevalence of pre-analytical variables in our study (8.63%) was high in comparison. The present study was performed in a large tertiary care government health care institution with large patient load and ever changing phlebotomy staff on daily basis and use of trainees. This was likely the cause for unintentionally non-adherence to phlebotomy guidelines. The results reflect the actual scenario in any busy government Indian health care institution. Most common variable of haemolysed sample and also under/overfilling was likely due to pushing the sample after collection with a needle and syringe into the vacutainer instead using the vacutainer-needle barrel set. Also,

the higher prevalence is likely related to the limited sample size, since it restricts to PT assay. The complex nature of the physiological process the PT reaction involves predisposes it to numerous pre-analytical variables which may affect the reporting. The patient identification and wrong labelling, which have been shown to be responsible for up to 25% of all pre-analytical errors¹⁴ and 50% of all identification errors¹⁵ respectively, accounted for meagre 0.46% (4 out of 862 samples – refer Serial No. 6, Table 1), of various pre-analytical variables identified in our study. The adoption of computerized bar coding of the samples likely led to the dramatic decrease in this error. The test requests have been found to be the source of many pre-analytical errors^{16–19} and use of a secure information system thus leads to a high reduction in the error rates related to this aspect of pre-analytical phase. The drawing of sample from indwelling catheters a common pre-analytical variable could not be identified as the present study was restricted to identification during sample validation at the laboratory. This was a limitation of the study. Haemolysis was the single predominant pre-analytical error/variable found in our study, constituting approximately 60% (515 out of 862 samples–refer serial. No. 1, Table 1) of all errors identified, significantly affecting the coagulation results. Haemolysis has been found as a common pre-analytical error resulting from multiple conditions^{20,21} as mentioned in various studies. A fall in PT values in most haemolysed samples was revealed in our study in conjunction with a previous study.²² In case of patients on oral anticoagulant therapy, it may have a critical impact on the management. The haemolysis basically activates the coagulation cascade through the release of the tissue factor and hence a fall in PT value in line with decreasing fibrinogen occurs in the samples showing haemolysis. However, contrasting results have been found in few previous studies. The study²³ done by Laga et al. showed no statistically significant change in PT and aPTT results raising questions regarding specimen rejection and discard. In contrast to our results, a study²⁴ by Lippi et al. showed prolongation of PT values in haemolysed samples. In view of varied effect of haemolysis on coagulation parameters, more studies emphasizing on the factors interacting to produce such alterations in the results are required to explore this effect of haemolysis on coagulation. In most studies, haemolysis has been found to significantly affect the results. Hence, it is an important pre-analytical variable which requires repeat phlebotomy and testing. In our study, the overfilled vacutainers showed significant shortening of PT results. In contrast to the study²⁵ carried out by Peterson et al. who found no significant change in the coagulation parameter of overfilled samples. The underfilled vacutainers led to significant prolongation of the coagulation parameters in our study in concordance with previous studies explained by raised anticoagulant to blood ratio.^{25–27} Reduced values of PT in the 205 samples which got reversed on adopting strict guidelines at phlebotomy can be explained by attributing it to release of tissue factors, intravascular haemolysis and local hypoxia which can initiate the coagulation cascade and release of various other cellular substances. Applying the tourniquet for more than a minute (causing haemo-concentration) and slow vene-puncture (encourages clotting and activates sample) have been attributed for the same. These

processes can increase concentration of large molecules, cells and coagulation factors.⁶ Thus, it is important to minimize the effects of venous stasis.

Conclusion

Pre-analytical variables inducing error in reporting is an integral part of laboratory testing. The present study done in a busy government tertiary care hospital quantitatively identified haemolysed samples, samples showing abnormally low values (due to excess pressure at phlebotomy), overfilled vacutainers and underfilled vacutainers in descending order of frequency as the main prevalent pre-analytical variables in samples taken for prothrombin time testing. These were found to alter test results (as there was drastic reduction in error on removal of the variable) and could lead to erroneous reporting. Thus, these variables should be carefully looked for during sample validation or while correlating results with clinical status. This will prevent erroneous reporting of PT/INR leading to incorrect therapeutic decision and thus jeopardizing patient safety. The Do's and Dont's of sample collection in coagulation assays is tabulated in Fig. 3. The higher prevalence of pre-analytical variables in a large busy government tertiary care centre stresses on the need to have an intense training and educational programme on phlebotomy, sample collection and transportation guidelines for the ever changing workforce of paramedical staff and nurses. This will go a long way in reducing errors arising out of pre-analytical variables and providing quality patient care.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Plebani M, Carraro P. Mistakes in a stat laboratory: types and frequency. *Clin Chem*. 1997;43(8):1348–1351.
2. Carraro P, Plebani M. Errors in a stat laboratory types and frequencies 10 years later. *Clin Chem*. 2007;53(7):1338–1342.
3. Aakre KM, Langlois MR, Watine J, et al. Critical review of laboratory investigations in clinical practice guidelines: proposals for the description of investigation. *Clin Chem Lab Med*. 2013;51(6):1217–1226.
4. Abdollahi A, Saffar H, Saffar H. Types and frequency of errors during different phases of testing at a clinical medical laboratory of a teaching hospital in Tehran: Iran. *N Am J Med Sci*. 2014;6(5):224–228.
5. Romero A, Cobos A, Gómez J, Muñoz M. Role of training activities for the reduction of pre-analytical errors in laboratory samples from primary care. *Clin Chim Acta*. 2012;413(1–2):166–169.
6. Lippi G, Guidi GC, Mattiuzzi C, Plebani M. Preanalytical variability: the dark side of the moon in laboratory testing. *Clin Chem Lab Med*. 2006;44(4):358–365.
7. Hawkins R. Managing the pre and postanalytical phases of the total testing process. *Ann Lab Med*. 2012;32(1):5–16.

8. Nutting PA, Main DS, Fischer PM, et al. Toward optimal laboratory use. Problems in laboratory testing in primary care. *JAMA*. 1996;275(5):635–639.
9. Bonini P, Plebani M, Ceriotti F, Rubboli F. Errors in laboratory medicine. *Clin Chem*. 2002;48(5):691–698.
10. Galloway M, Woods R, Whitehead S, Baird G, Stainsby D. An audit of error rates in a UK district hospital transfusion laboratory. *Transfus Med*. 1999;9(3):199–203.
11. Astion ML, Shojania KG, Hamill TR, Kim S, Ng VL. Classifying laboratory incident reports to identify problems that jeopardize patient safety. *Am J Clin Pathol*. 2003;120(1):18–26.
12. Plebani M. Errors in clinical laboratories or errors in laboratory medicine? *Clin Chem Lab Med*. 2006;44(6):750–759.
13. Upreti S, Upreti S, Bansal R, Jeelani N, Bharat V. Types and frequency of preanalytical errors in haematology lab. *J Clin Diagn Res*. 2013;7(11):2491–2493.
14. Wiwanitkit V. Types and frequency of preanalytical mistakes in the first Thai ISO 9002: 1994 certified clinical laboratory: a 6 month monitoring. *BMC Clin Pathol*. 2001;1(1):5.
15. Valenstein PN, Raab SS, Walsh MK. Identification errors involving clinical laboratories: a College of American Pathologists Q-probes study of patient and specimen identification errors at 120 institutions. *Arch Pathol Lab Med*. 2006;130:1106–1113.
16. Wagar EA, Tamashiro L, Yasin B, Hilborne L, Bruckner DA. Patient safety in the clinical laboratory: a longitudinal analysis of specimen identification errors. *Arch Pathol Lab Med*. 2006;130(11):1662–1668.
17. Dale JC, Novis DA. Outpatient phlebotomy success and reasons for specimen rejection. *Arch Pathol Lab Med*. 2002;126(4):416–419.
18. Cummins D, Sharp S, Vartanian M, Dawson D, Amin S, Halil O. The BCSH guideline on addressograph labels: experience at a cardiothoracic unit and findings of a telephone survey. *Transfus Med*. 2000;10(2):117–120.
19. Valenstein P, Meier F. Outpatient order accuracy: A College of American Pathologists Q-Probes study of requisition order entry accuracy in 660 institutions. *Arch Pathol Lab Med*. 1999;123(12):1145–1150.
20. Nigam PK. Preanalytical errors: some common errors in blood specimen collection for routine investigations in hospital patients. *J Clin Diagn Res*. 2011;5(June (3)):659–661.
21. Lippi G, Blanckaert N, Bonini P, et al. Haemolysis: an overview of the leading cause of unsuitable specimens in clinical laboratories. *Clin Chem Lab Med*. 2008;46(6):764–772.
22. Emmanuel JF, Dorothy MF, Lippi G. Preanalytical Variables in Coagulation Testing Associated With Diagnostic Errors in Homeostasis. 2012 February; 43: 1–10. <http://dx.doi.org/10.1309/LM749BQETKYPYPVM>. Available from: <http://labmed.ascpjournals.org/content/43/2/1.2.full.pdf+html>.
23. Laga AC, Cheves TA, Sweeney JD. The effect of specimen hemolysis on coagulation test results. *Am J Clin Pathol*. 2006;126(5):748–755.
24. Lippi G, Montagnana M, Salvano GL, Guidi GC. Interference of blood cell lysis of routine coagulation testing. *Arch Pathol Lab Med*. 2006;130(2):181–184.
25. Peterson P, Gottfried EL. The effects of inaccurate blood sample volume on prothrombin time (PT) and activated partial thromboplastin time (aPTT). *Thromb Haemost*. 1982;47(2):101–103.
26. Reneke J, Etzell J, Leserialie S, Ng VL, Gottfried EL. Prolonged prothrombin time and activated partial thromboplastin time due to under filled specimen tubes with 109 mmol/L (3.2%) citrate anticoagulant. *Am J Clin Pathol*. 1998;109(6):754–757.
27. Chuang J, Sadler MA, Witt DM. Impact of evacuated collection tube fill volume and mixing on routine coagulation testing using 2.5-mL (pediatric) tubes. *Chest*. 2004;126(4):1262–1266.