Routine Coagulation Testing: Do We Need a Discard Tube?

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Abstract When coagulation tests are performed, the recommended guideline is that a discard tube be used and the coagulation testing should be done only on the second tube. This guideline is however inconsistently enforced and most laboratories follow a single tube draw for routine coagulation testing. Few studies have however, challenged this guideline and have shown that comparable results can be obtained in both tubes when a two tube draw is used. This prospective study was done over a 3 months period in the hematology laboratory under the Clinical Hematology unit of a tertiary care teaching institution in North India. Fifty-six paired specimens were drawn from healthy volunteers following the prescribed “two tube draw” method. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were performed within 1 h of sample collection on a fully automated photo-optical coagulation instrument (Ceveron-Alpha). Paired results for PT and APTT were compared using Bland–Altman plots for method comparison. There was good correlation between the PT, INR and APTT of the first and second tubes with bias of 0.09, −0.05 and 0.3 respectively). Bland–Altman plots showed acceptable agreement between the two values with 95 % confidence interval ranging from −0.62 to 0.79 for PT, −0.05 to 0.06 for INR and −3.9 to 4.6 for APTT. Our study has shown no significant difference between PT

and APTT values for the first and second tubes. Hence the use of a discard tube is not required.

Keywords Coagulation · Prothrombin time · Activated partial thromboplastin time · Discard tube

Introduction

When coagulation tests are performed, the recommended guideline is that a discard tube be used and the coagulation testing should be done only on the second tube. This is done as the contamination in the first tube by tissue thromboplastin released during the venipuncture may affect the test result. If the venipuncture is not clean or when it takes a long time, chances of this interference increase [1–3].

This guideline is however inconsistently enforced and most laboratories follow a single tube draw for routine coagulation testing. Few studies have challenged this guideline and have shown that comparable results can be obtained in both tubes when a two tube draw is used, thus negating the use of such a practice [4–7].

This study was designed to determine whether a significant difference exists between the coagulation results obtained by the first and second tubes.

Materials and Methods

This prospective study was done over a 3 months period in the hematology laboratory under the Clinical Hematology Unit of a tertiary care teaching institution in North India. Fifty-six paired specimens were drawn from healthy volunteers for coagulation testing (prothrombin time (PT) and activated partial thromboplastin time (APTT)) after written informed

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consent. Sample size was calculated using OpenEpi software considering the hypothesis that the test results could be shorter in the first tube for PT and APTT as compared to the second tube (to a proportion of 70:30 for the two tubes. A power of 80 % was taken for the study. Phlebotomy was performed by standard procedure, using disposable needles, adapters and 5 ml evacuated specimen tubes. Following the prescribed “two tube draw” procedure, 4.5 ml of blood was drawn into each of the two specimen tubes. No other sample was drawn apart from coagulation testing. A blood to citrate ratio of blood of 9:1 (v/v) was maintained. The tubes were spun at 2,500 rpm ($1,500\times g$) for 20 min to prepare the platelet poor plasma for testing. The specimens were not refrigerated and PT and APTT were performed within 1 h of sample collection. PT and APTT were performed on a fully automated photo-optical coagulation instrument (Ceveron-Alpha). PT was done using a rabbit brain thromboplastin (Technoplastin) with an International Sensitivity Index (ISI) of 1.2. APTT was done using a phospholipid based reagent (Daptin). No samples were rejected for over or under-filling of tubes.

Paired results for PT and APTT were compared using Bland–Altman plots for method comparison [8].

Results

Fifty-six paired specimens from healthy volunteers were taken using the two tube draw. PT and APTT were performed on both tubes. INR was calculated for all PT estimations.

Prothrombin Time (PT)

The PT for tube one ranged from 12.0 to 17.0 s (mean: 13.9 s). For tube two, the range for PT was 11.6–17.3 s (mean: 13.8 s). PT in the second tube was longer in 18 (32.1 %) estimations. The INR for the first tube ranged from 0.79 to 1.22 while that for the second tube ranged from 0.73 to 1.19. There was good correlation between the PTs of the first and second tubes (Fig. 1). Bland–Altman plots showed acceptable agreement between the two values. There was good correlation between the PT of the first and second tubes with bias of 0.09 and 95 % confidence interval ranging from -0.62 to 0.79 (Fig. 2).

There was good correlation between the INRs of the first and second coagulation tubes (Fig. 3). Bland–Altman plots showed acceptable agreement between the two values (INR 1 and INR 2) with bias of -0.05 and 95 % confidence interval ranging from -0.05 to 0.06 (Fig. 4).

Activated Partial Thromboplastin Time (APTT)

The APTT for tube one ranged from 29.3 to 41.4 s (mean: 36.1 s). For tube two, the range for (APTT) was 28.9–44.0 s

Fig. 1 Good correlation between the PT of the first and second tubes

Fig. 2 The Bland–Altman plot for PT of the first and second tubes

(mean: 35.7 s). APTT in the second tube was longer in 20 (35.7 %) estimations. There was good correlation between the APTTs of the first and second tubes (Fig. 5). Bland–Altman plots showed acceptable agreement between the two values. There was good correlation between the APTT of the first and second tubes with bias of 0.3 and 95 % confidence interval ranging from -3.9 to 4.6 (Fig. 6).

Discussion

Our study has shown no significant difference between PT and APTT values for the first and second tubes.

During venipuncture, trauma induced can release tissue thromboplastin which can contaminate the first tube sample. This can lead to shorter clotting times and hence can affect the accuracy of coagulation testing. To circumvent

Fig. 3 Good correlation between the International Normalised Ratio (INR) of the first and second tubes

Fig. 4 The Bland–Altman plot for INR of the first and second tubes

Fig. 5 Good correlation between the APTT of the first and second tubes

Fig. 6 The Bland–Altman plot for APTT of the first and second tubes

this phenomenon, most text books, laboratory manuals and guidelines recommend the use of a discard tube for coagulation tests [1–3]. However, many studies have challenged this practice as the discard tube was initially recommended due to the use of glass syringes with reusable needles that became blunt with repeated use and caused significant trauma to the vein, thus leading to shorter clotting times in the first tube. In present times, the use of disposable syringes and needles has minimized tissue trauma and avoided this problem [7].

Bamberg et al. [4] compared results obtained for the PT and the APTT using 35 specimens drawn with and without a discard tube in healthy adults. They reported no difference in mean PT and APTT results between specimens drawn with a discard tube and those drawn without a discard tube.

McGlasson et al. [5] studied PT and APTT in the first and second tubes in 95 subjects (15 normal patients and 80 patients on coumadin therapy) on three different reagent and instrument systems. No clinical or statistically significant difference was seen between the first or second tubes on any of the three reagent/instrument combinations in the PT, international normalized ratio reporting, or APTT results. The authors recommended elimination of the need of a discard tube.

Gottfried and Adachi [7] studied 175 paired coagulation specimens for PT and APTT done on the first and second tubes. They found the values in both tubes to be comparable for PT ($r = 0.995$) and APTT ($r = 0.993$).

Our study has also shown good agreement between the clotting times of the first and second tube for PT and APTT with a narrow bias and acceptable 95 % confidence interval.

Two other studies that recommend abolition of the use of a discard tube were done on blood samples for PT only. In a study by Yawn et al. [9], first and second evacuation blood

tube was drawn from 343 outpatients who had a physician-ordered PT test. There was no statistically significant difference in the paired PT or calculated INR from any of the first and second tubes. The average difference in the INR from tube one to tube two was 2 % (standard deviation [SD] 1.1 %). Another study on PT among 241 patients on oral anticoagulant therapy concluded that the use of a discard tube was unnecessary [10]. Two studies performed specialized coagulation testing (without PT and APTT) in two tubes and showed no statistically significant differences between tube 1 and tube 2 for any of the tests performed [6, 11].

The recommendation of the use of a discard tube was dropped by the Clinical and Laboratory Standards Institute (CLSI) after several studies [12]. However, most of the studies which have verified these conclusions and refuted the need for a discard tube have been done when drawing samples for coagulation tests in healthy volunteers.

A recent study from Turkey analysed 376 blood samples drawn for PT/INR and APTT testing. The authors found statistically significant differences between tube one and tube two ($P < 0.05$), and satisfactory correlation coefficients were obtained by linear regression analysis (0.86 or greater in all cases). The authors recommended that drawing a discard tube is still necessary for coagulation testing and suggested a relook of international guidelines related to the necessity of a discard tube for repeated evaluation of coagulation tests especially receiving long term oral anticoagulant treatment [13]. However, other authors have questioned the findings of this study as it mentions statistically significant difference between the test times obtained by the first and second tubes, as based on a paired t test. However there was no evidence provided of any clinically significant difference, a very important yardstick in the interpretation of coagulation results. The authors suggested that the CLSI recommendation for abolition of the two tube draw was still valid [14].

Although most studies have used the mean and Pearson's correlation coefficient to compare the results between the clotting times for PT and APTT, we have used the Bland–Altman method [8] for comparing these values as this method is recommended for paired estimations for single variable where one is not dependent on the other.

The limitation of our study is that it has been done on healthy volunteers. More studies in patients on long term anticoagulation therapy need to be done to assess the degree of variability of results between the first and the second tubes in real life situations.

Conclusion

Our study has shown that use of a discard tube is not necessary for routine coagulation testing.

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