

# Effect of Drawing a Discard Tube on PT and APTT Results in Healthy Adults

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**OBJECTIVE:** The purpose of this study was to compare results obtained for the prothrombin time (PT) and the activated partial thromboplastin time (APTT) using specimens drawn with and without a discard tube in healthy adults.

**DESIGN:** A specimen of blood in a 3.2% sodium citrate, 5.0 mL tube was drawn from one arm with a discard tube and from the other arm without a discard tube on 35 healthy adults. A PT and APTT were performed on each specimen using a fibrometer.

**SETTING:** The PT and APTT tests were all performed in the student laboratory of the Clinical Laboratory Science Program at East Carolina University.

**PATIENTS OR OTHER PARTICIPANTS:** Study subjects included technician, technologist, and phlebotomist employees of the clinical laboratory at Pitt County Memorial Hospital, and faculty and junior and senior students in the Clinical Laboratory Science Program at East Carolina University, Greenville, North Carolina. All participants signed an informed consent prior to venipuncture.

**INTERVENTIONS:** Two coagulation specimens were drawn from each subject. Specimens in a discard (no anticoagulant) tube and then an anticoagulated tube were drawn from one arm, and a specimen in a single anticoagulant tube was drawn from the other arm. The PT and APTT were performed using standardized procedures with Pacific Hemostasis reagents and controls and a BBL FibroSystem fibrometer.

**MAIN OUTCOME MEASURES:** The degree of difference between PT results and between APTT results for specimens drawn with and without a discard tube.

**RESULTS:** Based on paired t-test analyses, no difference in mean PT results and no difference in mean APTT results were found between specimens drawn with a discard tube and those drawn without a discard tube at an alpha of 0.05. Paired-samples correlation coefficients were significant for both the PT and the APTT at an alpha of 0.05, showing precision between results with and without a discard tube for both coagulation tests.

**CONCLUSIONS:** Relative to sampling from a population of healthy adults, drawing a discard tube before a sodium citrate tube for coagulation testing appears to make an insignificant difference. Replication of these results with patients receiving anticoagulant therapy and/or patients with abnormal coagulation results, would offer cost savings by justifying elimination of discard tubes for blood draws for coagulation testing only. Such a change in protocol would also reduce the likelihood of nosocomial blood loss in vulnerable patient populations.

**ABBREVIATIONS USED:** APTT = activated partial thromboplastin time; NCCLS = National Committee for Clinical Laboratory Standards; PT = prothrombin time.

**INDEX TERMS:** APTT; coagulation testing; discard tube; PT.

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## BACKGROUND

The National Committee for Clinical Laboratory Standards (NCCLS) currently recommends that a specimen in a discard tube be drawn prior to drawing specimens to be used for coagulation testing. If specimens in multiple tubes are drawn during a venipuncture, it is recommended that the non-anticoagulated specimens be drawn first. If only a coagulation specimen is to be drawn, then a specimen in a non-anticoagulated tube is drawn first and discarded. This first tube is considered the discard tube. Tubes with 3.2% sodium citrate are recommended over tubes with 3.8% sodium citrate.<sup>1-3</sup>

Research supports the NCCLS recommendation for adopting tubes with 3.2% sodium citrate as opposed to 3.8%, though the research on whether to draw a specimen in a discard tube is less supportive of the NCCLS recommendation.<sup>3-10</sup> The justification for the NCCLS standard is that using the first tube specimen or drawing a single sodium citrate specimen for coagulation testing can introduce tissue thromboplastin and affect the accuracy of results for the PT and APTT. The concerns about tissue thromboplastin originated when the whole blood clotting time was a primary coagulation lab test and it was known to be shortened with poor venipuncture technique.<sup>6,7,10,11</sup>

Recent studies have investigated the PT relative to test results and/or international normalized ratio (INR) values. In a study of 241 adult outpatients receiving consistent dosages of oral anticoagulant, results were analyzed by the INR ranges of 1.2-2.0, 2.1-3.5, 3.6-5.9, and  $\geq 6.0$ . In comparing results by paired t-test analyses for specimens drawn with a discard tube versus without, no statistically or clinically significant differences were found for any INR range at  $p < .05$ .<sup>6</sup>

In another study of 343 adult outpatients including persons receiving oral anticoagulant and those not receiving such medication, PT results and INRs were compared for specimens drawn with and without a discard tube. PT results ranged from 10.9 to 79.6 seconds and INRs from 0.81 to 37.6. There were no significant differences in the mean PT values or the mean INRs based on the paired t-test at  $p < .05$ , for specimens drawn with versus without a discard tube. The mean difference in PT values between specimens drawn with versus without a discard tube was 0.05 seconds and the mean difference in INRs was 0.021.<sup>11</sup> In a study of 31 outpatients receiving warfarin, a mean difference of 0.007 was found in INRs from specimens drawn with versus without a discard tube which was not statistically significant by paired t-test analysis at  $p < .05$ .<sup>8</sup>

A study of 175 outpatients with the majority receiving oral anticoagulant therapy, found a mean difference in the PT of 0.10 seconds and in the APTT of 0.48 seconds for specimens drawn with a discard tube as compared to those drawn without a discard tube. Paired t-tests indicated no significant differences in either the PT ( $p=.995$ ) or APTT ( $p=.993$ ) by the two methods of collection.<sup>7</sup>

Differences in PT and APTT results by drawing method have also been evaluated with the added variables of differing sodium citrate concentrations and reagents of varying sensitivities in a study of 380 outpatients receiving oral anti-

coagulant therapy. No significant differences between drawing a coagulation testing specimen with versus without a discard tube were found for 3.2% sodium citrate tubes and for 3.8% sodium citrate tubes for the PT, for the INR, and for the APTT based on the paired t-test at  $p < .05$ . When comparing the use of a less sensitive thromboplastin reagent (Thromboplastin C Plus) and a more sensitive reagent (Innovin) in performance of the PT, no significant differences were found by paired t-tests for either reagent in PT and in INR results for specimens drawn with versus without a discard tube.<sup>5</sup>

In another study with 15 healthy subjects and 80 subjects receiving coumadin therapy, no significant differences in PT, in INR, and in APTT results were found for specimens drawn with versus without a discard tube. The specimens in this study were each analyzed on three different instruments and reagent systems including photo-optic, mechanical, and nephelometric clot detection instruments, and no significant differences between the drawing methods were found for all three automated systems.<sup>9</sup>

All the studies cited above with the exception of a 1974 study by McPhedran utilized automated coagulation instruments other than the fibrometer.<sup>4-11</sup> For the studies cited that used only one concentration of sodium citrate in the tubes, four used 3.8% sodium citrate,<sup>7,9-11</sup> while two used 3.2%.<sup>6,8</sup> All the studies reported here utilized 5.0 mL anticoagulated tubes and the drawing of specimens in two consecutive anticoagulated tubes from each patient with the first tube being labeled as the specimen drawn without a discard tube and the second tube being the specimen drawn with a discard tube. All reviewed studies using automated coagulation testing other than a fibrometer, found no statistically significant differences in test results by performing PTs and APTTs on specimens drawn with versus without a discard tube.<sup>4-10</sup>

Based on the literature reviewed, the researchers believed that their study would demonstrate no difference in results between specimens drawn with a discard tube and those drawn without a discard tube for both the PT and the APTT.

## METHOD

### Purpose and subjects

The research study described was conducted by the second and third author under the guidance of the first author, as a required component of the senior curriculum in the clinical laboratory science (CLS) BS degree program at East Carolina University (ECU). Within the constraints of conducting a research project of minimal costs but clinical relevance to the

practice of CLS, the second and third authors as senior CLS students at the time, decided to investigate the effect of drawing versus not drawing a specimen in a discard tube on results for the PT and the APTT. The study design and informed consent form were approved by the institutional review board for East Carolina University and Pitt County Memorial Hospital prior to implementation of the project.

Due to the difficulty of accessing subjects receiving anticoagulant therapy in the primary clinical affiliate, the study was conducted on 35 volunteer subjects who were all healthy adults and included persons employed in the clinical laboratory at Pitt County Memorial Hospital (n = 24) and persons who were students or faculty in the CLSC program at ECU (n = 11). All subjects were over 18 years of age, and signed an informed consent form for voluntary participation. All subjects indicated they were not receiving any anticoagulant medication, had not ingested aspirin or aspirin-containing products in the past 72 hours, and did not have any known bleeding or other coagulation disorder.

**Specimen collection**

The initial intent was to have all blood specimens drawn by the student researchers. But some subjects felt uncomfortable with this approach due to the limited phlebotomy experience, i.e., 6 days, of the students at the time of conducting the study. Consequently all specimens were drawn either by an experienced CLS at Pitt County Memorial Hospital or by one of the CLSC faculty. This also eliminated the venipuncturist being experienced or inexperienced as an additional variable.

Each subject had two venipunctures performed on him/her with universal

precautions used at all times. In one arm, a specimen was drawn in a non-anticoagulated, discard tube and then a specimen was drawn in an anticoagulated tube, i.e., specimen with a discard tube. In the other arm, a specimen in a single, anticoagulated tube was drawn, i.e., specimen without a discard tube. All anticoagulated tubes were 5.0 mL and contained 3.2% sodium citrate, while all non-anticoagulated were 5.0 mL. All specimens were drawn during the second half of January 2001. Specimens were centrifuged in a Baxter STAT-SPIN table-top centrifuge at 13,000g for five minutes, plasma separated, and the plasma specimens stored at -80 °C until testing during the first week of February 2001. No specimens were hemolyzed, icteric, or lipemic.

Each subject was given a unique identifier number. The two specimens from each subject were labeled with the subject number and whether the specimen was 'with discard' or 'without discard'.

A two-arm approach was incorporated into the original study design to give the students more venipuncture experience, and was approved by the institutional review board in this format. Consequently, this approach was implemented even though the preference of the study subjects resulted in the venipunctures being performed by an experienced CLS.

**SPECIMEN TESTING AND DATA ANALYSIS**

A PT and APTT were performed in duplicate on each of the 70 specimens. For both the PT and APTT assays, Pacific Hemostasis reagents and Levels I, II, and III controls were used with a BBL FibroSystem fibrometer. All specimens were tested over a two-day period. Levels I, II, and III controls were analyzed each day. All subject specimens had duplicate results that agreed within one second for the PT and within 10% for the APTT.

The data generated were analyzed by descriptive statistics, paired-samples correlation coefficients, and paired t-tests using SPSS-PC+ version 9.0 statistical software. All data were analyzed and reviewed in consultation with a biostatistician faculty at ECU.

**RESULTS**

Initially the data were plotted for both the PT and APTT using a Stem and Leaf Plot. Three outlier data points for the PT and one outlier for the APTT were discovered and eliminated from further data analyses leaving a final sample size of 32 for the PT and 34 for the APTT.

The descriptive statistics for the PT and APTT are displayed in Table 1. The average paired-difference between values with and without a discard tube was 0.08 seconds for the PT and 0.43

**Table 1.** Coagulation test results in seconds with and without a discard tube

	Prothrombin time n = 32		Activated partial thrombo- plastin time, n = 34	
	With*	Without†	With*	Without†
Mean	11.80	11.88	21.69	22.11
Median	11.80	11.85	21.40	21.60
Standard deviation	0.35	0.46	2.11	2.19
Range	2.00	1.80	9.00	8.00

\* Specimen drawn after a discard tube  
 † Specimen drawn without a prior discard tube

seconds for the APTT. For the PT, 20 (63%) of the subjects had higher results for the specimens drawn without discard tubes as compared to the specimens drawn with discard tubes. Of the 34 subjects, 21 (62%) had higher APTT results for the specimens drawn without discard tubes.

Based on paired t-tests comparing the mean values for specimens drawn with and without discard tubes, no statistically significant differences for the PT, i.e.,  $t(30) = 1.41, p = .17$ , and for the APTT, i.e.,  $t(32) = 1.23, p = .23$ , were found. When paired-samples correlation coefficients, i.e.,  $r$ , were calculated, statistically significant correlations for results with and without a discard tube were found for the PT ( $r = 0.75, p = .0001$ ) and for the APTT ( $r = 0.55, p = .001$ ).

### CONCLUSIONS

The results of this study are in agreement with the majority of other studies evaluating the effect of drawing coagulation testing specimens with and without a discard tube. Though most studies have been conducted exclusively with subjects receiving oral anticoagulant or with a combination of subjects receiving and not receiving anticoagulant therapy, this study obtained findings similar to other studies but with a homogenous sample of healthy adults.

This study as well as previous studies reviewed,<sup>5-11</sup> has found that the differences in PT and APTT results by comparing specimens drawn with versus without a discard tube are not statistically significant at  $p < .05$ . The applicability and utility of the study results reported here, though, are limited by having a small sample size, including only results found in most normal ranges for the PT and APTT, i.e., only healthy subjects, and using the fibrometer which is not routinely used in clinical laboratories today for testing. Though the correlation coefficients were statistically significant, the  $r$  values for both the PT and the APTT were still relatively low, i.e.,  $r < 0.8$ . The statistical significance of the correlation coefficients may simply be a consequence of the small sample size and possibly not a true effect. The fact that over 60% of the results for the PT and the APTT were longer for the specimens drawn without a discard tube as compared to the paired specimens drawn with a discard tube supports this questioning of the true statistical significance of the correlation coefficients.

Overall, based on the consistency in research findings with healthy subjects and those receiving anticoagulant therapy and with varying reagent and instrument systems, it appears that the current continued practice of drawing a specimen in a discard tube before drawing a specimen in a sodium citrate tube for only a PT and/or APTT test should continue to be evalu-

ated for possible obsolescence. Considering the prevalence of thromboses and patients receiving anticoagulant therapy to remedy and prevent this morbidity, the number of single anticoagulated tube draws for therapy monitoring is fairly substantial in most hospitals and in some outpatient clinics.<sup>12,13</sup> Elimination of the discard tube in such draws could assist clinical laboratories in cost containment by decreasing the associated costs for discarded tubes, phlebotomy personnel time, and waste processing. Policy revision in this aspect of phlebotomy services would also help reduce the level of nosocomial blood loss and anemia, particularly in elderly patients. Replication of this study with a substantially larger sample and with both subjects receiving and not receiving anticoagulant therapy, may provide a clearer evaluation of the need for using a discard tube in drawing specimens for coagulation testing.

The results of this study were presented at Carolinas Clinical Connections 2001: Joint Meeting of the North Carolina and South Carolina Societies of Clinical Laboratory Science.

### REFERENCES

1. Calam RR, Cooper MH. Recommended "order of draw" for collecting blood specimens into additive-containing tubes. *Clin Chem* 1982;28(6):1398-9.
2. Levens JK. How reliable are your coagulation results? *Adv Med Lab Professionals* 2001;22:12-5.
3. NCCLS. Collection, transport, and processing of blood specimens for coagulation testing and general performance of coagulation assays: approved guideline, 3rd ed. Wayne, PA, NCCLS, 1998.
4. Adcock DM, Kressin DC, Marlar RA. Effect of 3.2% vs 3.8% sodium citrate concentration on routine coagulation testing. *AJCP* 1997;107:105-10.
5. Adcock DM, Kressin DC, Marlar RA. Are discard tubes necessary in coagulation studies? *Lab Med* 1997;28:530-3.
6. Brigden ML, Graydon C, McLeod B, Lesperance M. Prothrombin time determination: the lack of need for a discard tube and 24-hour stability. *AJCP* 1997;108:422-6.
7. Gottfried EL, Adachi MM. Prothrombin time and activated partial thromboplastin time can be performed on the first tube. *AJCP* 1997;107:681-3.
8. Hendryx RJ, Mead CE, Macklin LR. Letters to the editor: effect of collection sequence on the international normalized ratio. *Lab Med* 1998;29:135-6.
9. McGlasson DL, More L, Best HA, and others. Drawing specimens for coagulation testing: is a second tube necessary? *Clin Lab Sci* 1999;12:137-9.
10. Yawn BJ, Loge C, Dale J. Prothrombin time: one tube or two. *AJCP* 1996;105:794-7.
11. McPhedran P, Clyne LP, Ortoli NA, and others. Prolongation of the activated partial thromboplastin time associated with poor venipuncture technic. *AJCP* 1974;62:16-20.
12. Fritsma G. Laboratory results that predict arterial thrombosis. *Clin Lab Sci* 2001;14:262-8.
13. Quinn C, Hill J, Hassouna H. A guide for diagnosis of patients with arterial and venous thrombosis. *Clin Lab Sci* 2000;13:229-37.