

Effect of Carryover of Clot Activators on Coagulation Tests During Phlebotomy

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Abstract

We investigated the effect of clot activators carried over from the serum tube on major coagulation tests during phlebotomy. First, blood specimens from 30 normal subjects were mixed with small amounts of fluid containing clot activators, and their effects on various coagulation tests were determined. Only the value of fibrin monomer complex displayed a remarkable change when thrombin-containing fluid was added to the blood specimens. Subsequently, 100 paired blood specimens (taken from 75 healthy volunteers and 25 patients taking warfarin) were collected in coagulation tubes before and after the serum tube using standard phlebotomy procedures. Various coagulation tests were performed to determine the effect of contamination of thrombin-containing blood on coagulation parameters. Differences between the 2 tubes were minimal but significant for some of the coagulation tests. Therefore, we conclude that the effect of clot activators in the serum tube on coagulation tests is minimal when standard phlebotomy procedures are used.

The order of draw is critical when collecting multiple specimens for clinical laboratory testing during a single venipuncture using the evacuated tube method. The most important factor in deciding the order of draw is the carryover of the additives contained in the former tube into the subsequent tube.^{1,2} An additive-containing blood specimen that adheres to a rubber sleeve of the phlebotomy needle can contaminate the blood specimen in the subsequent tube, thus causing erroneous results. This is particularly important when performing coagulation tests, because most of the tubes contain anticoagulants or clot activators which can easily affect the coagulation test results with a minimal amount of carryover. Other factors include the influence of the tissue fluid usually contained in the first-draw blood specimen, which can also contribute to errors in some tests.

Before 2003, the Clinical and Laboratory Standards Institute (CLIS, formerly National Committee for Clinical Laboratory Standards [NCCLS]), in its guidelines for venipuncture (H3-A4), recommended that the coagulation tube be filled after the serum tube (“coagulation after serum”).² This recommendation was based on the possibility that tissue thromboplastin might contaminate the coagulation tube when it is drawn first and cause erroneous results in coagulation tests. However, subsequent studies in which 2 coagulation tubes were drawn successively revealed no significant differences in prothrombin time (PT) and activated partial thromboplastin time (aPTT) between the first and second tubes.^{3,4} Based on these results, NCCLS introduced a “coagulation before serum” policy to the H3-A5 version and all subsequent versions.⁵ This revised order of draw was mainly intended to avoid contaminating the coagulation tube with clot activators contained in the serum tube that might influence coagulation test results.

However, to date, to our knowledge, no information has been published on whether contamination of a coagulation tube with clot activators in the serum tube affects major coagulation tests when the serum tube is drawn before the coagulation tube. Therefore, we investigated the effect of contamination of clot activators from the serum tube on major coagulation test results in studies performed both in vivo and in vitro.

Materials and Methods

Currently, thrombin and silica or glass particles are mainly used in the serum tubes as clot activators. In the present study, the following 3 types of evacuated blood collection tubes with clot activators were examined: (1) Venoject II tubes (Terumo, Tokyo, Japan) containing thrombin and glass particles; (2) Venoject II tubes (Terumo) containing only glass particles; and (3) Vacutainer tubes (Becton-Dickinson, Tokyo, Japan) containing silica particles. These 3 types of collection tubes are referred to as T1, S1, and S2, respectively. The Venoject II tubes containing 0.2 mL of 3.13% sodium citrate were used for coagulation testing. PT, aPTT, fibrinogen, D-dimer, and fibrin monomer complex (FMC) were measured with the STA-R (Diagnostica Stago, Roche, Tokyo, Japan) mechanical automated coagulation analyzer according to the manufacturer's protocol.

To determine the effect of clot activators on coagulation tests, we performed both in vivo and in vitro evaluations. This study was approved by the ethics committee of the institute, and written consent was obtained from all volunteers and patients.

Experiment 1: Study of Clot Activators in Vitro

To reproduce the carryover of clot activators in vitro, various amounts of clot activator fluid were added to the coagulation tubes, and their effects on the coagulation test results were analyzed. Thirty healthy volunteers were enrolled in this study. Phlebotomy was performed by trained medical technologists according to the standard procedure recommended by CLSI document H3-A6.⁶

First, clot activator fluid was prepared by adding appropriate volumes of saline to T1, S1, and S2 tubes. Blood samples were then collected successively into 12 coagulation tubes from each volunteer, with each tube filled with 1.8 mL of blood for a final ratio of blood to citrate of 9:1 (vol/vol). Immediately after blood collection, 2, 5, or 10 μ L of clot activator fluid prepared in the T1, S1, and S2 tubes was added to each of the 9 coagulation tubes. In the other 3 coagulation tubes, 2, 5, or 10 μ L of saline was added to serve as a negative control. After inverting each tube 5 times to ensure adequate mixing, the tubes were centrifuged immediately at 3,000 rpm for 10 minutes and subjected to coagulation testing.

Experiment 2: Order-of-Draw Study in Vivo

To examine the effect of clot activator carryover in the actual phlebotomy setting, 100 paired specimens were collected in coagulation tubes before and after the serum tube. The subjects included 75 healthy volunteers and 25 patients with cardiovascular disease taking warfarin daily to prevent vascular thrombosis. Blood specimens were drawn using butterfly needles into 4 tubes in the following order: a plain tube without additives, a coagulation tube containing sodium citrate, a serum tube containing thrombin and glass particles, and another coagulation tube. The last coagulation tube was used to evaluate the possible effect of contamination with clot activators from the previous serum tube. After the collection of blood, the 2 coagulation tubes were inverted 5 times to ensure adequate mixing followed by centrifugation at 3,000 rpm for 10 minutes. Standard coagulation tests were then performed.

In detail, a plain tube without additives was used first to fill the tubing from the butterfly needle with the blood specimen for each puncture. This was done to avoid underfilling the subsequent coagulation tube. This procedure was also effective in minimizing the interference of tissue fluid contained in the initial blood flow that could possibly initiate the clotting sequence. To enhance the possibility of the carryover of clot activators, the needle inside the serum tube was soaked in the blood while the specimen was drawn by slightly orienting the bottom of the tube in an upward direction. This procedure was performed at least 5 cm below the puncture site to prevent the additive-containing blood specimen from flowing back into the vein. Other venipuncture procedures were performed in accordance with the CLSI document H3-A6.⁶

To determine statistical significance, mean values and standard deviations for each coagulation test were calculated and compared using the paired Student *t* test. Differences were considered significant when *P* values were less than .05.

Results

Experiment 1

The mean \pm standard deviation of each coagulation test is shown in **Table 1**. Differences in PT between the tubes that contained each type of clot activator and the negative controls were statistically significant, but these differences were within 3% of the control value. When thrombin fluid from the T1 tube was added, aPTT shortened in proportion to the amount of solution added, and these differences were statistically significant. In contrast, the addition of silica or glass particle fluid from the S1 or S2 tube had minimal effects on the aPTT values. FMC increased remarkably by the addition of T1 fluid in proportion to the amount of fluid added, and the differences

Table 1
Change of Coagulation Test Results by Adding Various Amounts of Clot Activators (Mean ± Standard Deviation)

Clot Activator Fluid								
Prepared in	Volume (μL)*	PT (%)	PT (Ratio)	PT-INR	aPTT (s)	Fibrinogen (mg/dL)	DD (μg/mL)	FMC (μg/mL)
T1	2	104.8 ± 8.8 [†]	0.980 ± 0.04 [†]	0.975 ± 0.05 [†]	34.0 ± 2.3 [§]	271.4 ± 46.9	0.21 ± 0.11 [†]	48.30 ± 34.13 [§]
	5	105.2 ± 8.1 [†]	0.978 ± 0.04 [†]	0.971 ± 0.04 [†]	32.5 ± 2.3 [§]	269.3 ± 49.2	0.31 ± 0.24	124.19 ± 55.76 [§]
	10	104.7 ± 8.8 [†]	0.981 ± 0.04 [†]	0.975 ± 0.05 [†]	31.2 ± 2.8 [§]	248.9 ± 58.3 [†]	0.41 ± 0.48 [†]	176.22 ± 27.04 [§]
S1	2	105.3 ± 9.2 [§]	0.979 ± 0.04 [§]	0.972 ± 0.05 [§]	35.8 ± 2.5	270.3 ± 51.0	0.20 ± 0.11	3.45 ± 0.96
	5	105.1 ± 8.4 [§]	0.979 ± 0.04 [§]	0.973 ± 0.04 [§]	35.5 ± 2.6	272.6 ± 50.0	0.20 ± 0.12	4.24 ± 1.41
	10	105.0 ± 8.3 [§]	0.979 ± 0.04 [§]	0.973 ± 0.04 [§]	35.7 ± 2.5	271.5 ± 45.6	0.19 ± 0.10	3.60 ± 1.27
S2	2	106.0 ± 9.1 [§]	0.976 ± 0.04 [§]	0.968 ± 0.05 [§]	35.7 ± 2.8	273.5 ± 46.1	0.20 ± 0.10 [†]	3.87 ± 1.61
	5	105.8 ± 8.8 [§]	0.975 ± 0.04 [§]	0.969 ± 0.04 [§]	35.4 ± 2.6 [†]	273.1 ± 48.7	0.19 ± 0.09	3.69 ± 1.11
	10	105.7 ± 8.5 [§]	0.976 ± 0.04 [§]	0.969 ± 0.04 [§]	35.4 ± 2.5 [†]	272.1 ± 46.5	0.21 ± 0.11	3.72 ± 1.34
Control (saline)	2	102.8 ± 8.0	0.988 ± 0.04	0.984 ± 0.04	35.8 ± 2.6	273.5 ± 48.4	0.24 ± 0.12	3.27 ± 1.48
	5	102.8 ± 7.7	0.990 ± 0.04	0.985 ± 0.04	35.7 ± 2.6	270.6 ± 47.9	0.24 ± 0.14	3.73 ± 1.99
	10	102.7 ± 7.80	0.989 ± 0.04	0.985 ± 0.04	35.8 ± 2.5	268.2 ± 44.9	0.20 ± 0.10	3.54 ± 1.63

aPTT, activated partial thromboplastin time; DD, D-dimer; FMC, fibrin monomer complex; PT, prothrombin time; PT-INR, prothrombin time–international normalized ratio;

S1, glass particles only; S2, silica particles only; T1, thrombin + glass particles.

* Volume of clot activator fluid added to each coagulation tube.

[†] $P < .05$.

[‡] $P < .01$.

[§] $P < .001$ (compared with control).

were statistically significant. However, the addition of S1 or S2 fluid had no effect on the value of FMC. Although fibrinogen and D-dimer exhibited statistically significant differences in some experiments, they were not proportional to the amount of clot activators added.

Experiment 2

Table 2 displays the mean ± standard deviation of each coagulation test when the coagulation tube was drawn before or after the serum tube. In healthy volunteers, the mean PT ratio and PT-international normalized ratio (INR) were significantly lower in the second coagulation tube compared with the first tube (PT ratio, 0.96 vs 0.95, $P = .0001$; PT-INR, 0.95 vs 0.94, $P = .0002$). None of the other coagulation tests including FMC exhibited a statistically significant difference between the first and second coagulation tubes.

In patients taking warfarin, the mean PT ratio and PT-INR were significantly lower, whereas fibrinogen was significantly higher in the second tube compared with the first tube (PT ratio, 1.59 vs 1.57, $P = .011$; PT-INR, 1.86 vs 1.84, $P = .024$; fibrinogen, 352.3 mg/dL [10.36 μmol/L] vs 362.4 mg/dL [10.65 μmol/L], $P = .0087$). No other coagulation test exhibited a statistically significant difference between the first and second coagulation tubes.

Discussion

The in vitro study (Experiment 1) revealed that most of the coagulation tests were affected by the addition of 2 to 10 μL of thrombin solution from the T1 serum tube. In particular, aPTT and FMC values showed remarkable changes even

Table 2
Results of Coagulation Tests of the First and Second Tubes (Mean ± Standard Deviation)*

	Healthy Volunteers (n = 75)		Patients Taking Warfarin (n = 25)	
	First Tube	Second Tube	First Tube	Second Tube
PT (%)	99.3 ± 3.4	99.4 ± 3.0	45.5 ± 13.5	45.8 ± 12.7
PT ratio	0.96 ± 0.05	0.95 ± 0.05 [†]	1.59 ± 0.25	1.57 ± 0.24 [†]
PT-INR	0.95 ± 0.06	0.94 ± 0.06 [†]	1.86 ± 0.40	1.84 ± 0.37 [†]
aPTT (s)	36.9 ± 4.3	36.9 ± 4.2	45.5 ± 7.8	45.2 ± 7.6
Fibrinogen (mg/dL)	311.9 ± 68.3	314.0 ± 69.6	352.3 ± 68.4	362.4 ± 75.5 [†]
DD (μg/mL)	0.28 ± 0.18	0.28 ± 0.18	0.90 ± 1.77	0.93 ± 1.76
FMC (μg/mL)	2.90 ± 1.66	3.42 ± 3.35	4.05 ± 6.09	4.09 ± 5.84

aPTT, activated partial thromboplastin time; DD, D-dimer; FMC, fibrin monomer complex; PT, prothrombin time; PT-INR, prothrombin time–international normalized ratio;

* The first and second tubes were drawn before and after the serum tube, respectively.

[†] $P < .01$.

[‡] $P < .05$.

when only 2 μL of thrombin solution was added. Although the addition of silica or glass solution from serum tubes S1 and S2 affected the values of some tests (mostly PT), the changes in the values were not remarkable.

Based on the results of Experiment 1, it appeared that only thrombin may affect the coagulation test results because of a carryover effect. Therefore, we conducted an in vivo study (Experiment 2) using T1 tubes containing thrombin. Experiment 2 revealed a minimal but statistically significant effect of clot activators on the values of several coagulation tests, when the coagulation tube was drawn after the serum tube. The PT of the second coagulation tube was thought to be shortened by contamination of the clot activators in the serum

tube, thus leading to a lower PT ratio and PT-INR. The cause of the change in fibrinogen values observed in patients taking warfarin is unknown.

Taken together, the results of Experiments 1 and 2 indicate that a “coagulation after serum” blood draw sequence could affect the results of standard coagulation tests, though the effect was minimal when applying standard phlebotomy procedures. In other words, a “coagulation after serum” sequence might be acceptable if standard phlebotomy procedures are used. Although several previous studies demonstrated the feasibility of a “coagulation before serum” policy, they analyzed only PT and aPTT. Therefore, it is unclear whether blood specimens for other coagulation tests can also be collected in the first tube. For example, it is well known that thrombin-antithrombin complex (TAT) is easily elevated by the contamination of tissue fluid.⁷ In fact, CLSI recommends the draw of a second tube for coagulation tests other than PT and aPTT. However, it would be convenient if all the coagulation tests including PT and aPTT can be measured using blood specimens collected after the serum tube. The results of the present study suggest the feasibility of such a procedure when phlebotomy is performed using standard procedures. It has also been demonstrated that a poor venipuncture technique could be responsible for abnormal coagulation tests on blood drawn in the first tube.⁷ The “coagulation after serum” policy would be effective in avoiding testing errors when nonstandard phlebotomy procedures are used.

In contrast, a “coagulation before serum” policy might be superior in certain clinical settings. For instance, PT-INR is widely used to ensure an adequate yet safe dose of warfarin for preventing stroke in cardiovascular patients. Even a small decrease in PT-INR value may lead to an inappropriate increase in warfarin dose. Furthermore, it is known that the blood specimen in the tube can flow back into the needle when inappropriate phlebotomy procedures are used.⁸ For instance, the release of a tourniquet before removing a tube from the holder may cause substantial backflow of blood from the tube. In addition, the delay in tube removal after cessation of blood flow from a vein may cause backflow of blood because of unexpected changes in vein pressure. Such an increase in backflow of blood containing clot activators may significantly influence coagulation test results. Taking these factors into consideration, a “coagulation before serum” blood draw sequence might be preferable when the accuracy of the coagulation value is critical, as in the measurement of PT-INR in patients taking warfarin.

This study has several limitations. First, not all coagulation tests were investigated. Other coagulation tests such as TAT may be influenced by the minimal contamination of clot activators in the coagulation tube. Second, the venipuncture procedures used here followed the standard procedures approved by CLSI. However, violation of the standard

procedure can influence coagulation tests, as stated before. Our results can be applied only for coagulation tests performed using standard venipuncture procedures. Furthermore, the number of subjects, particularly the number of patients taking warfarin, was relatively small, and only the patients with cardiovascular diseases were analyzed. A larger number of patients with different underlying diseases is necessary to make the results of the present study applicable to a wider patient population.

In conclusion, the present study suggests that the carry-over effect of the clot activators in the serum tubes on major coagulation tests is minimal in the clinical setting. Therefore, a “coagulation after serum” blood draw sequence may be acceptable when standard phlebotomy procedures are used. Further studies including a large number of patients and the use of other coagulation tests are needed to verify the feasibility of a “coagulation after serum” blood draw sequence.

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