

Minimum Specimen Volume Requirements for Routine Coagulation Testing

Dependence on Citrate Concentration

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We evaluated the effect of sample volume and citrate concentration on results of routine coagulation assays (prothrombin time [PT] and activated partial thromboplastin time [APTT]). The study was performed on samples obtained from healthy persons and patients receiving oral anticoagulant therapy. Standard evacuated tubes (3.2% and 3.8% sodium citrate) were filled to varying total sample volumes ranging from 3.0 to 5.0 mL, and results of routine coagulation tests were compared. Underfilling may significantly affect the APTT and PT, resulting in artifactual prolongation of results. This effect is most pronounced in samples drawn into 3.8% citrate. By using 3.8% citrate, there is a statistically significant difference in the results of PT assays in the samples less than 80% filled compared with those that are 100%

filled. For APTT assays performed on samples drawn into 3.8% citrate, a statistical difference occurred at less than 90% filled. This effect was less pronounced when samples were drawn into 3.2% sodium citrate. We found no statistically significant difference in PT results from a 3.2% citrate tube between fill volumes of 60% and 100% and none for APTT results between fill volumes of 70% and 100%. This study further supports the recommendation to use 3.2% sodium citrate concentration, because 60% of the optimum filled volume for PT and 70% of the optimum filled volume for APTT are acceptable. (Key words: Coagulation testing; Prothrombin time [PT]; Activated partial thromboplastin time [APTT]; Sample volume; Sodium citrate concentration) *Am J Clin Pathol* 1998;109:595-599.

A variety of preanalytic variables may affect the results of routine coagulation assays. Two reported variables, the concentration of sodium citrate and the ratio of blood to liquid anticoagulant, may alter the results of the activated partial thromboplastin time (APTT) and prothrombin time (PT) assays.

Standard evacuated tubes for coagulation studies contain 0.5 mL sodium citrate anticoagulant (3.2% or 3.8%) and are partially evacuated to fill with 4.5 mL of blood. Evacuated tubes filled to the proper volume will contain the recommended ratio of 9 parts blood to 1 part anticoagulant, assuming a hematocrit between 25% (0.25) and 55% (0.55). The National Committee for Clinical Laboratory Standards (NCCLS; Wayne, Pa) recommends that samples for coagulation testing be rejected if an evacuated tube is less than 90% full or if the hematocrit is greater than 55% (0.55).¹ In these situations, the

blood/anticoagulant ratio is altered, resulting in too much anticoagulant for the amount of plasma. The APTT and PT determinations may be prolonged owing to a dilutional effect of the liquid anticoagulant on the plasma or to decreased availability of assay-added calcium. In the last approved NCCLS guideline (1993), a concentration of 3.2% or 3.8% sodium citrate is acceptable, and the aforementioned recommendation for specimen rejection is not specific for either concentration. The new NCCLS guideline proposes that 3.2% sodium citrate be used only.² There is no published literature on evaluating the potential difference in minimal sample volume required based on the citrate concentration. Contrary to the NCCLS guidelines, two previous studies using 3.8% citrate reported minimum acceptable fill volumes of 70% to 90% for APTT and 70% for PT determinations.^{3,4}

The present study was undertaken to determine the minimal fill volume necessary for accurate APTT and PT determinations and whether the fill volume is dependent on citrate concentration.

MATERIALS AND METHODS

After informed consent was obtained, blood was collected by means of standard venipuncture. The study was performed using two different protocols.

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For protocol one, 30 healthy volunteers and 11 patients receiving oral anticoagulant therapy were studied. Thirty milliliters of blood was drawn into a plastic syringe. The blood was distributed among 5-mL glass Vacutainer tubes (Becton Dickinson, San Jose, Calif) for total volumes of 3.0 mL, 3.5 mL, 4.0 mL, 4.5 mL, and 5.0 mL with 3.8% citrate, and 2.0 mL, 2.5 mL, 3.0 mL, 3.5 mL, 4.0 mL, and 5.0 mL with 3.2% citrate tubes. The order in which the tubes were filled was randomized. Plasma was centrifuged at 2,500g for 15 minutes at room temperature (platelet count, $<10 \times 10^9/L$). Samples were maintained at room temperature and evaluated within 2 hours of venipuncture.

Samples from 20 healthy volunteers were evaluated using 3.8% citrate, and samples from 10 were evaluated using 3.2% citrate. The PT reagent was Innovin (Dade, Miami, Fla) and the APTT reagent was Actin FS (Dade). Testing was completed on a model 900 Instrument (Medical Laboratory Automation, Pleasantville, NY). In

addition, samples from 11 patients receiving oral anticoagulant therapy were evaluated using 3.2% sodium citrate in the manner described using Thromboplastin C+ and Actin (Dade). The APTT and PT assays were performed in duplicate, and the results were averaged.

In the second arm of the protocol, samples from 138 patients receiving oral anticoagulant therapy were evaluated. Specimens were drawn into two 3.8% sodium citrate Vacutainer glass tubes; the first tube was purposely underfilled, and the second tube was filled to a total volume of 5.0 mL. Samples from 138 patients were drawn into 3.8% citrate tubes and the other 130 were drawn into 3.2% citrate. Results of a previous study showed no statistical difference in results whether the PT is performed from the first or second tube drawn.⁵ The amount of blood drawn into the first tube was arbitrary, and the total fill volume was determined after the sample was drawn. Samples were evaluated as described using Innovin and Actin FS. Results for clinical use were reported from the second sodium citrate tube drawn, as is the standard procedure.

The mean \pm SEM was calculated for each coagulation test using the Student's *t* test for the groups with a normal distribution or the Mann-Whitney Rank Sum test (Sigma Stat, Jandel Software, San Rafael, Calif).

TABLE 1. EFFECT OF CITRATE CONCENTRATION AND FILL VOLUME ON APTT AND PT RESULTS IN HEALTHY VOLUNTEERS

Citrate/Volume (%)	PT (s)	P	APTT (s)	P
3.8% (n = 20)				
100	10.9 \pm 0.2	NS	27.3 \pm 0.5	NS
90	11.1 \pm 0.2	NS	28.3 \pm 0.6	NS
80	11.4 \pm 0.2	NS	29.8 \pm 0.7	<.05
70	12.5 \pm 0.2	<0.05	34.7 \pm 0.9	<.05
60	14.2 \pm 0.2	<0.05	40.4 \pm 1.2	<.05
3.2% (n = 10)				
100	10.7 \pm 0.6	NS	25.2 \pm 1.5	NS
90	10.6 \pm 0.7	NS	25.7 \pm 1.6	NS
80	10.7 \pm 0.7	NS	26.1 \pm 1.4	NS
70	10.9 \pm 0.8	NS	26.8 \pm 1.8	NS
60	11.2 \pm 1.0	NS	28.2 \pm 1.8	<.05
50	12.7 \pm 1.1	<.05	31.5 \pm 2.5	<.05
40	14.7 \pm 1.8	<.05	36.9 \pm 4.3	<.05

APTT = activated partial thromboplastin time; PT = prothrombin time; NS = not significant.

TABLE 2. EFFECT OF FILL VOLUME ON APTT AND PT VALUES IN PATIENTS RECEIVING ORAL ANTICOAGULANT THERAPY USING 3.2% SODIUM CITRATE IN COLLECTION TUBES

Volume (%)	PT (s)	P	APTT (s)	P
100	19.1 \pm 3.6	NS	31.4 \pm 4.3	NS
90	19.0 \pm 3.5	NS	34.5 \pm 4.6	NS
80	19.1 \pm 3.7	NS	35.3 \pm 4.9	NS
70	20.3 \pm 6.2	NS	37.1 \pm 5.5	NS
60	20.7 \pm 4.8	NS	47.3 \pm 10.4	<.05

APTT = activated partial thromboplastin time; PT = prothrombin time; NS = not significant.

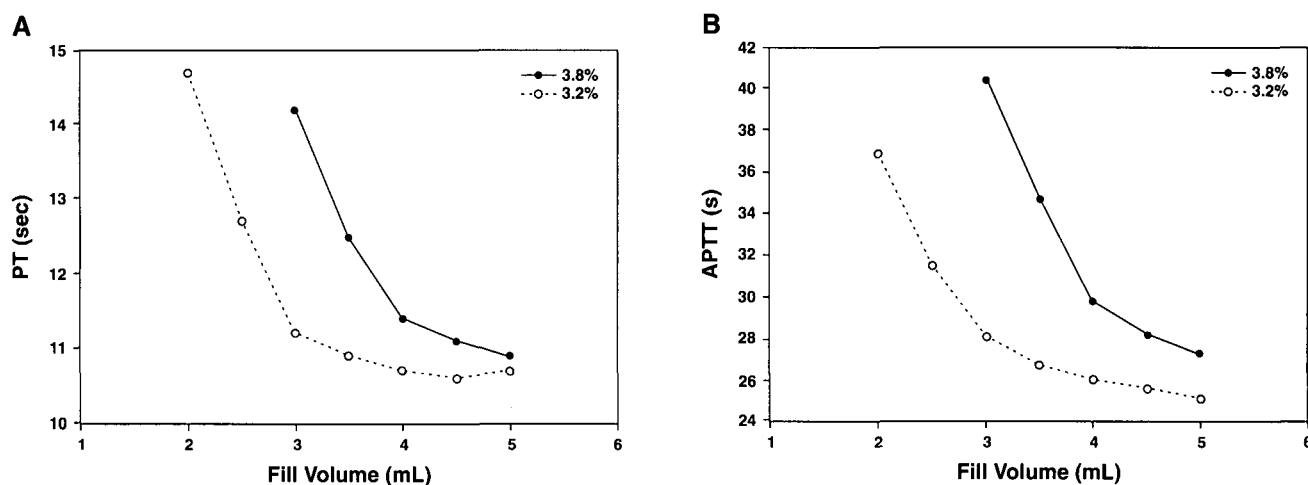
RESULTS

The results for APTT and PT in healthy volunteers at different total fill volumes using different citrate concentrations are given in Table 1. A statistically significant difference ($P < .05$) was found in total filled volumes of 70% or less for PT determinations and in 80% or less for APTT determinations with a 3.8% sodium citrate tube. In comparison, when using 3.2% citrate, no statistically significant difference was found in the fill volume until the volume was 50% or less for PT and 60% or less for APTT.

The results for APTT and PT in the 11 patients receiving oral anticoagulant therapy at 5 different fill volumes in 3.2% citrate are given in Table 2. No statistically significant difference was found in PT determinations between any of the total filled volumes between 60% and 100%. For APTT determinations, a statistically significant difference was found at a total filled volume of 60%.

In the samples studied, the results of APTT and PT were prolonged with decreasing filled volumes. However, in the 3.8% citrate, the coagulation values were even more prolonged than in the 3.2% citrate at the same volume (Fig).

Minimum Specimen Volume Requirements for Routine Coagulation Testing



The effect of fill volume on prothrombin time (PT) and activated partial thromboplastin time (APTT) values in healthy volunteers using 3.8% and 3.2% sodium citrate concentrations in the collection tubes using Dade/HLA system (Dade, Miami, Fla). For procedures, see the "Materials and Methods" section.

TABLE 3. THE EFFECT OF FILL VOLUME ON APTT AND PT VALUES IN PATIENTS RECEIVING ORAL ANTICOAGULANT THERAPY USING 3.2% AND 3.8% SODIUM CITRATE CONCENTRATIONS IN COLLECTION TUBES

Citrate/ Volume (mL)	No. of Specimens	PT (s) 100% Fill	PT (s) Underfilled	Percent Change	APTT (s) 100% Fill	APTT (s) Underfilled	Percent Change*
3.8%							
4.4-5.0	20	21.9 ± 8.8	22.9 ± 2.4	4	41.3 ± 7.0	40.6 ± 1.6	-2
4.0-4.4	11	31.7 ± 10.8	36.6 ± 4.4	14	49.4 ± 5.4	50.4 ± 1.7	2
3.5-3.9	32	24.3 ± 10.2	30.5 ± 2.8	21	42.7 ± 8.0	46.6 ± 1.9	8
3.0-3.4	27	29.2 ± 15.3	53.5 ± 7.6	70	46.3 ± 11.2	62.4 ± 4.9	32
2.5-2.9	31	23.1 ± 11.7	49.9 ± 6.4	103	44.8 ± 9.0	68.6 ± 4.3	52
2.0-2.4	12	24.6 ± 9.5	80.3 ± 11.2	214	45.5 ± 9.5	90.1 ± 9.3	95
1.5-1.9	5	20.4 ± 9.9	80.2 ± 16.2	293	40.6 ± 10.3	110.7 ± 22.7	167
3.2%							
4.0-5.0	8	15.4 ± 4.7	15.7 ± 5.0	2	39.0 ± 7.6	39.5 ± 8.0	2
3.5-3.9	15	25.5 ± 7.4	29.0 ± 9.7	13	46.5 ± 6.9	49.1 ± 7.7	6
3.0-3.4	22	22.7 ± 7.3	27.1 ± 9.7	18	47.0 ± 10.6	52.4 ± 12.7	11
2.5-2.9	12	21.1 ± 8.4	32.5 ± 16.3	52	45.0 ± 12.5	59.6 ± 24.3	32
2.0-2.4	36	24.9 ± 9.8	43.2 ± 25.7	66	47.1 ± 13.0	72.8 ± 26.7	55
1.5-1.9	17	19.1 ± 10.5	61.1 ± 51.8	201	39.4 ± 12.4	109.0 ± 37.4	189
1.0-1.4	20	18.1 ± 5.9	119.2 ± 52.1	596	39.2 ± 9.4	153.6 ± 36.0	308

APTT = activated partial thromboplastin time; PT = prothrombin time.

*For procedures, see the "Materials and Methods" section. One hundred percent fill indicates full tubes. The percent of change is calculated by the formula:

Percentage = (Underfilled-Full)/Full × 100

The APTT and PT results for patients receiving oral anticoagulation therapy drawn with one full and one underfilled tube are given in Table 3. Data were separated into groups defined by the volume of the underfilled tube and then compared with the corresponding full tube. A clinically significant difference was defined as a change in the coagulation test value of greater than 15% between the two tubes. In a 3.8% citrate tube, a clinically significant change occurred at an average volume of 80% for PT and at 70% for APTT

(see Table 3). Similarly, values obtained from a 3.2% citrate tube demonstrated clinically significant differences in the PT at an average volume of 70%, while clinically significant difference in the APTT was not demonstrated until a volume of 60% was reached.

DISCUSSION

To obtain accurate and reliable laboratory results, it is important to identify the effects of variables associated

with sample procurement, processing, and analysis. We evaluated two preanalytic variables: the effect of the citrate concentration and sample volume on APTT and PT determinations. Healthy volunteers and patients receiving oral anticoagulant therapy were studied.

To improve precision and accuracy in the clinical laboratory, guidelines for testing have been developed by the NCCLS, the standards-setting organization for clinical laboratories in the United States.¹ The NCCLS offers a variety of documents that provide valuable information for all areas of the clinical laboratory, including the performance of routine coagulation testing.¹ Most clinical laboratories adhere to guidelines established by NCCLS. Document H21-A2 addresses the collection, transport, and processing of blood specimens for coagulation testing.

The NCCLS recommends that coagulation samples be rejected if the evacuated tube is less than 90% of the expected filled volume or if the hematocrit is greater than 55% (0.55).¹ In both of these situations, the plasma volume is reduced, altering the blood/anticoagulant ratio. According to the NCCLS guidelines, the proportion of blood to anticoagulant should be a ratio of 9:1. Sodium citrate is the only acceptable anticoagulant for coagulation studies. Citrate inhibits clot formation by binding all of the available calcium in the plasma sample. The final concentration of assay-added calcium, however, depends on the blood/anticoagulant ratio, as well as the concentration of anticoagulant used.

The amount of calcium available in the assay mixture directly affects the APTT and PT results. When the concentration of anticoagulant is increased, as may occur when a higher citrate concentration is used or when the plasma volume is reduced, more calcium is bound by the anticoagulant and less is available for clot formation. This may prolong the PT or APTT. We previously demonstrated consistently longer APTT and PT values in samples from healthy volunteers and patients receiving anticoagulant therapy that were drawn into 3.8% *vs*

3.2% sodium citrate when evaluated with responsive reagents.² Given that more calcium is bound with a higher citrate concentration, it can be postulated that a higher citrate concentration would have a greater effect on clotting times when tubes are underfilled or if the hematocrit is significantly elevated. The current approved NCCLS guideline,¹ however, suggests that 3.2% or 3.8% sodium citrate can be used for coagulation testing. Furthermore, this guideline does not specify whether the minimum volume depends on the concentration of anticoagulant used.

We demonstrated that the results of APTT and PT assays depend on the filled volume. Clotting times prolonged in a consistent fashion as the filled volume was decreased. Healthy volunteers and patients receiving oral anticoagulant therapy demonstrated progressive prolongation of APTT and PT with decreasing total filled volume (see the Figure). Furthermore, the minimum sample requirements for accurate APTT and PT testing depend on citrate concentration (Table 4). Statistically significant differences in APTT and PT results between underfilled tubes and tubes filled to 100% are achieved at significantly lower total filled volumes when samples are drawn into 3.2% citrate *vs* 3.8%.

When using a higher citrate concentration, adequate sample volume is more critical than when a lower citrate concentration is used. For APTT assays for plasma in 3.8% citrate, a statistically significant difference in results was demonstrated in the samples in tubes that were less than 90% full compared with those in tubes that were more than 90% full. This finding agrees with the current NCCLS guideline.¹ For PT assays drawn into 3.8% citrate, a statistically significant difference was demonstrated in samples in tubes that were less than 80% full. In the samples drawn into 3.2% sodium citrate, however, only a 70% filled volume is necessary for accurate APTT results and a 60% filled volume for accurate PT results. Samples drawn into a lower citrate concentration therefore can be under filled to a greater extent than the samples drawn into a higher citrate concentration before a significant effect on results is noted. Using a lower concentration of sodium citrate, therefore, will permit more tolerance in filling of the tubes, thus ultimately reducing the number of specimens rejected by the laboratory.

Underfilling of evacuated tubes may be a significant problem in high-altitude regions such as Denver, Colo. As the altitude increases, atmospheric pressure decreases, reducing the pressure differential between the evacuated tube and the surrounding environment. Vacuum within manufactured tubes can also be lost when empty tubes are stored at higher temperatures,

TABLE 4. RECOMMENDED FILL VOLUMES FOR ACCURATE APTT AND PT TESTING BASED ON CITRATE CONCENTRATION

Citrate Concentration	Minimum Fill Volume (%)	
	APTT	PT
3.2%	70	60
3.8%	90	80

APTT = activated partial thromboplastin time; PT = prothrombin time.

presumably because the tubes expand and pressure equalization occurs around the rubber stopper. Rejecting fewer underfilled tubes for coagulation testing may ultimately reduce laboratory costs and help minimize unnecessary inconvenience and discomfort to the patient.

Data from this study suggest that samples from patients with polycythemia (hematocrit, > 55% [0.55]) may be acceptable when drawn into 3.2% sodium citrate, although this variable was not specifically studied. Ingram and Hills⁶ reported that citrate concentration may alter hematocrit results, because citrate concentrations above 3.0% are hyperosmolar with blood. They postulated that a higher citrate concentration would have a greater effect on clotting times when tubes are underfilled or a patient has a significantly elevated hematocrit.⁶

We evaluated only automated APTT and PT assays, so our results should not be extrapolated to other tests performed in the coagulation laboratory. Results we report may be specific for the reagents and instrument systems tested and should be regarded as guidelines only.

For a variety of reasons, we recommend the use of only one concentration of sodium citrate nationally. The concentration of citrate anticoagulant has a significant effect on APTT and PT results when responsive APTT and PT reagents are used.² Clotting times, as expected, are typically longer in 3.8% sodium citrate. We demonstrated that up to 25% of samples from patients receiving oral anticoagulant therapy can vary from 0.7 to 2.7 international normalized ratio units between 3.2% and 3.8% citrate concentrations. It is important, therefore, for laboratories to use a consistent concentration of anticoagulant. We advocate that NCCLS recommend the use of 3.2% sodium citrate only. This concentration is the current standard in Europe.

Underfilled samples are consistently associated with prolongation of the clotting times. Normal results from a filled tube of less than 70% suggest that

the true value is normal (regardless of sample volume). However, if the value from an underfilled tube is prolonged into the abnormal range (even by 0.1 second), another sample must be drawn.

We demonstrated that minimum volume requirements for APTT and PT testing depend on the citrate concentration. Based on our findings, we recommend that laboratories use 3.2% citrate. For samples drawn into 3.2% citrate, using our reagent/instrument system a 70% filled volume is required for accurate APTT testing and a 60% filled volume for PT assays. The lower concentration of anticoagulant allows significantly more leniency in the minimum sample volume requirements in an evacuated tube system and, therefore, will decrease the number of samples rejected because underfilled tubes. If 3.8% citrate is used, then a 90% filled volume is necessary for accurate APTT results and an 80% filled volume for PT results. This study supports our previous recommendation that a 3.2% sodium citrate concentration be the sole anticoagulant used in the coagulation laboratory.

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