Impact of Sodium Citrate Levels in Coagulation Assays: A Controlled *ex-vivo* Study

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Introduction

Sodium citrate is a widely used anticoagulant (AC) in coagulation testing due to its ability to chelate calcium ions and temporarily prevent clot formation until additional calcium is supplemented.

The final plasma citrate concentration can be influenced by various factors, including the initial AC concentration (3.2, 3.8 or 4% w/v), blood-to-AC ratio (affected by under/over-filled vacutainers), patient conditions (such as hematocrit level, anemia, polycythemia, and body mass index), and the plasmapheresis method (centrifugation or membrane-based techniques).

Despite its extensive clinical use and numerous studies comparing the effects of 3.2% versus 3.8% citrated tubes on coagulation assays[1–3] in different populations, there is a lack of systematic dose-response investigations on the impact of varying citrate concentrations on routine coagulation assays and lupus anticoagulant (LA) testing.

This study aims to evaluate the sensitivity of selected coagulation and LA assays to the citrate concentration of a normal platelet poor plasma (PPP) sample without inter-patient variability and the impact of citrate tube brands.

Methods

Blood from a single healthy donor (F: 43 years old, hematocrit ~40%) was collected at a 9:1 v/v blood:AC ratio into 10 separate Falcon tubes containing 0.5 mL citrate at different concentrations (1.9% w/v or 64 mM, to 4.6% w/v or 156 mM, CAS: 6132-04-3 and 5949-29-1, pH 7.2). This wide range of starting citrate solution covers both commonly used 3.2% w/v (109 mM) and rarely used 3.8% w/v (129 mM) citrated blood collection tubes as well as potential pre-analytical variability.

A series of normal PPP samples were prepared via double-centrifugation following CLSI-H21-Ed6 and stored frozen at ≤-70 °C. All processed plasma samples were confirmed platelet-poor (<10,000 PLT/ µL, automated Cellometer). The citrate concentrations of these samples were quantified by an optimized Megazyme citric acid kit, the accuracy of which had been verified by spiking known citrate quantities into heparinized plasma samples.

Routine coagulation assays [IL's prothrombin time (PT, RecombiPlastin 2G), activated partial thromboplastin time (APTT-SP) and Stago STA-Fibrinogen], factor activity assays [CRYO*check*™ Normal Reference Plasma calibrator, Factor V and Factor VIII deficient, neat dilution] and LA assays [CRYO*check* LA Check™ and LA Sure™ (dRVVT assay), and CRYO*check* Hex LA™] were performed in triplicate on once-thawed PPP samples (37 °C water bath) using manufacturer assay configurations on a Stago STA-R Evolution analyzer (Stago OK diluent, 25 mM $CaCl_2$). Citrate dose-response curves were generated for each coagulation assay and analyzed for citrate sensitivity.

Observations

According to CLSI guideline recommendations for coagulation assays, blood should be collected in 3.2% buffered/unbuffered sodium citrate tubes,[4] which our experimental results indicate yields a final citrate concentration of ~16 mM in a healthy donor plasma.

APTT-SP and PT (RecombiPlasTin 2G) results shortened with increased citrate concentration in normal PPP samples, explaining rarely observed abnormally short PT and APTT results. A previous clinical study in a normal population between 3.2% vs 3.8% citrate tubes detected an opposite prolongation of results at increased citrate levels for Actin-FS and PTT-LA but no effect on PTT-A,[1] together these indicate that the citrate impact is reagent-specific.

Fibrinogen and factor V (PT) or factor VIII (APTT) assays showed clot time prolongation by increasing citrate concentration. Factor activity was artificially increased at low citrate levels, emphasizing the importance of pre-analytical citrate levels in plasma samples for accurate clinical factor assays.

dRVVT assay demonstrated a complex, non-linear response to citrate level, which consequently could influence the reference interval and dRVVT assay cutoff. No correlation was found between Hex LA results and citrate concentration, making it a more reliable LA assay where citrate variability is unavoidable.

Conclusions

When a change in plasma collection procedure or a shift in assay reference range is noticed, the assay-specific sensitivity to citrate concentration needs to be considered. This study highlights the sensitivity of various assays to citrate concentration in normal double-spun (PPP) plasma. Similar studies are warranted on abnormal plasma samples and other assay systems.

- APTT-SP showed the highest sensitivity to citrate concentration in a normal PPP plasma (-0.4 sec/mM, R^2 0.99).
- Lower citrate levels of a plasma sample could artificially increase fibrinogen quantification and factor V and factor VIII activity measurements.
- While a dRVVT lupus anticoagulant test exhibited complex, non-linear responses to citrate levels, the Hex LA assay result showed no systematic correlation to citrate concentrations, making it a more robust LA test in non-optimal citrated plasma samples.

References

1. Ratzinger, Franz et al. "The Effect of 3.2% and 3.8% Sodium Citrate on Specialized Coagulation Tests." Archives of Pathology & Laboratory Medicine (2018): 992–997.
2. Gosselin, Robert C et al. "Comparison of samples obtained from 3.2% sodium citrate glass and two 3.2% sodium citrate plastic blood collection tubes used in coagulation testing." American Journal of Clinical Pathology (2004): 843–8.
3. Adcock, D M et al. "Effect of 3.2% vs 3.8% sodium citrate concentration on routine coagulation testing." American Journal of Clinical Pathology (1997): 105–10.
4. CLSI. *Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays.* 6th ed. CLSI guideline H21. Clinical and Laboratory Standards Institute; 2024.

Results

When blood was collected from a healthy female donor following CLSI-H21 guidelines using a 3.2% w/v (109 mM) or 3.8% w/v (129 mM) starting citrate solution with a 9:1 blood-to-anticoagulant ratio, the final citrate concentrations were quantified to be 16.1 mM and 17.8 mM, respectively.

Routine coagulation assays showed varied responses to citrate concentration, suggesting reagent-specific citrate effects.

APTT and PT results were unexpectedly shortened with increasing citrate levels (R^2 = 0.99 and 0.76, respectively), while fibrinogen clot times prolonged (R^2 = 0.91).

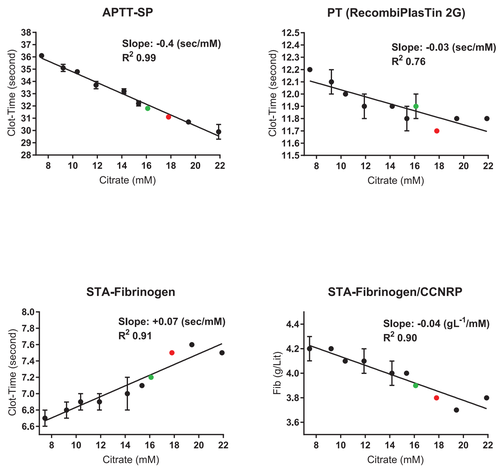

Figure 1: Citrate concentration variation in a normal PPP sample and the impact on APTT-SP and RecombiPlastin 2G (PT) assay, Mean±SD (n=3). Green and red data points represent the starting citrate concentrations of 3.2% w/v (APTT: 31.8, PT: 11.9 sec) and 3.8% w/v (APTT: 31.1, PT: 11.7 sec), respectively.

Figure 2: Citrate concentration variation in a normal PPP sample and the impact on STA-Fibrinogen assay clot time and Fibrinogen quantification (g/L), Mean±SD (n=3). Green and red data points represent the starting citrate concentrations of 3.2% w/v (7.2 sec, Fib 3.9 g/L) and 3.8% w/v (7.5 sec, Fib 3.8 g/L), respectively.

In factor V and VIII assays using the same calibrator, increased clot times were obtained with rising citrate concentration in PPP samples, with FV showing a strong correlation (R^2 = 0.92) and FVIII showing a weak correlation (R^2 = 0.34). Notably, a low level of citrate (~8 mM) artificially elevated factor V and VIII activity by 15–20%, highlighting the risk of misinterpretation in factor assays due to suboptimal plasma citrate level.

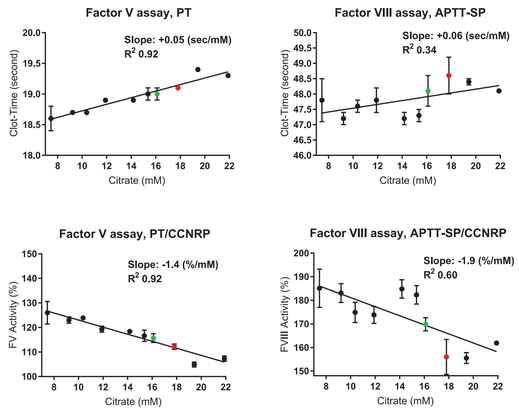

Figure 3: Citrate concentration variation in a normal PPP sample and the impact on factor V and VIII assay clot time and activity (%) measurement, Mean±SD (n=3). Green and red data points represent the starting citrate concentrations of 3.2% w/v (FV 115.7%, FVIII 169.8%) and 3.8% w/v (FV 112.2%, FVIII 156%), respectively.

Lupus anticoagulant assays displayed assay-specific sensitivity to citrate levels. dRVVT assays (LA Check, LA Sure) showed non-linear relationships with citrate concentration, influencing a variation of ± 0.1 on the dRVVT-ratio, without false-positive outcomes. Hex LA assay results showed no correlation with the citrate level (R^2 = 0.02), indicating no significant impact from citrate variation.

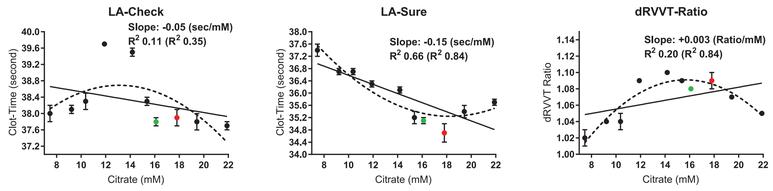

Figure 4: Citrate concentration variation in a normal PPP sample and the impact on CRYO*check* LA Check, LA Sure, and dRVVT-ratio, Mean±SD (n=3). Green and red data points represent the starting citrate concentrations of 3.2% w/v (CHK 37.8, SUR 35.1, R 1.08) and 3.8% w/v (CHK 37.9, SUR 34.1, R 1.09), respectively.

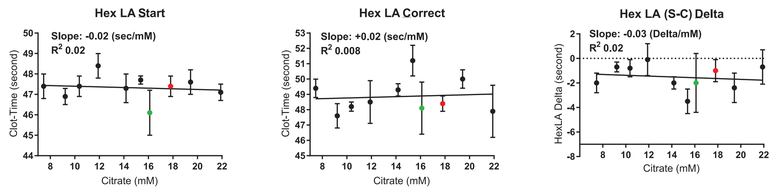

Figure 5: Citrate concentration variation in a normal PPP sample and the impact on the CRYO*check* Hex LA assay, Mean±SD (n=3). Green and red data points represent the starting citrate concentrations of 3.2% w/v (S 46.1, C 48.1, D 2 sec) and 3.8% w/v (S 47.4, C 48.4, D -1 sec), respectively.