Journal of Insights in Basic and Applied Sciences
مجلة رؤى في العلوم الأساسية والتطبيقية
ISSN: 3106-1737
Volume 2, Issue 3, 2026, Pages: 742-747
Journal homepage: https://jibas.com.ly/index.php/jibas/en

Impact of EDTA as an Anti-coagulant on Prothrombin Time Outcomes

Ali Guma Azbida[1*], Faraj Sagar[2] , Abtihal Salih[3], Ayah Abdulkarim[4], Ayah Mohammed[5], Dua Ahmed[6], Malak Salah[7]
[1,2,3,4,5,6,7] Department of medical laboratory, Faculty of Medical Technology, Azzaytuna University, Libya

تأثير مادة *EDTA* كمضاد للتخثر على نتائج زمن البروثرومبين

علي جمعة ازبيده[1*]، فرج صقر[2]، ابتهال صالح[3]، آية عبد الكريم[4]، آية محمد[5]، دعاء أحمد[6]، ملاك صلاح[7]
[1،2،3،4،5،6،7] قسم المختبرات الطبية كلية التقنية الطبية جامعة الزيتونة ليبيا

[*]Corresponding author: a.azbida@azu.edu.ly

Received: June 27, 2026	Accepted: August 25, 2026	Published: September 17, 2026

Abstract:
Background: A number of blood tests, including prothrombin time (PT), are frequently used in clinical settings to evaluate patients' coagulation status. Sodium citrate as anticoagulant ensures accurate measurements of clotting tests, such as prothrombin time (PT), while ethylenediaminetetraacetic acid (EDTA) helps to preserve whole blood and cellular components and less suitable for PT testing. Objectives: This study aimed to assess the possibility of using EDTA as an anticoagulant when performing PT testing and determine its effect on the results by measuring PT (using both EDTA and sodium citrate as anticoagulants) for 50 healthy volunteers living in Tarhuna. Results: The results of this study showed that when citrate was used as an anticoagulant, PT was normal in 88% of the samples, and INR was normal in 86%. In contrast, when EDTA was used as an anticoagulant, PT was elevated in 98% of the samples, while INR was above normal in 92% of them. Conclusions: This study concluded that EDTA as an anticoagulant is not suitable to use in coagulation testing. Further studies are recommended to identify potential alternative anticoagulants to citrate that can be used when performing coagulation tests, and more studies to find an equation that can be used to correlate the results of citrate and EDTA in emergency situations where citrate is unavailable.

Keywords: PT, sodium citrate, EDTA and INR.

الملخص
الخلفية: يُستخدم عدد من فحوصات الدم بما في ذلك زمن البروثرومبين (PT) بشكل متكرر في الفحوصات السريرية لتقييم حالة تخثر الدم عند المرضى. يضمن سترات الصوديوم كمضاد للتخثر قياسات دقيقة لاختبارات التخثر مثل زمن البروثرومبين (PT)، بينما يساعد EDTA في الحفاظ على الدم الكامل ومكوناته الخلوية وهو أقل ملاءمة لاختبار PT. الهدف: هدفت هذه الدراسة إلى تحديد إمكانية استخدام EDTA كمضاد للتخثر عند إجراء اختبار PT وتأثيره على النتائج من خلال قياس زمن البروثرومبين (باستخدام كل من EDTA وسترات الصوديوم كمضاد للتخثر) لخمسين متطوعاً سليماً يعيشون في ترهونة. النتائج: أظهرت نتائج هذه الدراسة أنه عند استخدام السترات كمضاد للتخثر، كان زمن البروثرومبين PT طبيعياً في 88% من العينات، وكان INR طبيعية في 86% منها. في المقابل، عند استخدام EDTA كمضاد للتخثر، ارتفع PT في 98% من العينات، بينما كان INR أعلى من المعدل الطبيعي في 92% منها. الاستنتاجات: خلصت هذه الدراسة إلى أن استخدام EDTA كمضاد للتخثر غير مناسب في اختبارات التخثر. وتوصي الدراسة بإجراء المزيد من

الأبحاث لتحديد بدائل محتملة لمضادات التخثر المستخدمة في اختبارات التخثر، بالإضافة إلى المزيد من الأبحاث لإيجاد معادلة تربط نتائج استخدام السترات وEDTA في حالات الطوارئ التي لا يتوفر فيها السترات.

الكلمات المفتاحية: زمن البروثرومبين، سترات الصوديوم، كمضاد للتخثر

Introduction

Prothrombin time is one of several blood tests routinely employed in clinical settings to assess patients' coagulation status. More specifically, PT is used to evaluate the extrinsic and common pathways of coagulation, which helps detect deficiencies of factors II, V, VII, and X as well as low fibrinogen levels; also, PT is used to monitor oral anticoagulant drugs. **(Levy, et al, 2014; Shahangian, et al, 2006; Poller, 2004).** The international normalized ratio INR represents the ratio of the patient's prothrombin time divided by a control prothrombin time value obtained using an international reference thromboplastin reagent developed by the World Health Organization (WHO). The implementation of INR for reporting PT findings has reduced results discrepancies arising from varying thromboplastin. **(Le, et al, 1994; Levy, et al, 2014; Poller, 2004)**

Sodium citrate acts as an anticoagulant by binding to calcium ions and forming calcium citrate complexes; as a result, there is less free calcium available, which is necessary for the coagulation process. Because it helps keep clotting factors stable and guarantees precise measurements of clotting times, such as in PT and activated partial thromboplastin time (APTT) assays, sodium citrate is especially crucial in coagulation investigations. However, sodium citrate is less suitable than EDTA for maintaining cellular components or nucleic acids since it can interfere with calcium-dependent cellular functions. EDTA is a strong anticoagulant that acts by chelating divalent cations, especially calcium (Ca^{++}); by sequestering Ca^{++}, EDTA effectively prevents their participation in the coagulation cascade, thereby inhibiting the activation of calcium-dependent clotting factors and preventing blood clot formation. Because of this characteristic, EDTA is very useful in maintaining whole blood and cellular constituents. Furthermore, EDTA is frequently used in molecular biology applications because it preserves the integrity of nucleic acids. **(Rattan, & Jeyaseelan, 2024)**

Some studies have been conducted to clarify the effect of EDTA on PT testing, such as the study of Della Valle et al 1999, which assessed the impact of anticoagulant antibodies on PT assays in plasma from patients with lupus anticoagulant (LA) who were taking oral anticoagulation; their findings indicated that the INR results could vary significantly depending on the type of anticoagulant used. **(Della Valle, et al, 1999**)

Vitamin K-dependent coagulation factors (II, VII, and X) are measured by clotting techniques called PT assays. The Quick assay, Owren's assay, and PT dry chemistry test cards are the three primary PT assay types that are commonly used. Mattsson et al 2001 conducted a study to assess the reliability of commercially accessible PT assays in monitoring the anticoagulant activity of melagatran (a reversible direct thrombin inhibitor), and they concluded that PT assays and INR cannot be used to monitor melagatran. **(Mattsson, et al, 2001)**

In another comparative study carried out by Horsti 2001, samples were taken from 107 patients taking oral anticoagulant medication; samples were collected using tubes containing citrate and EDTA anticoagulants. The PT values and INR for both were measured. The results demonstrated that no clinically significant difference was observed between the PT and INR values obtained from citrate and EDTA samples. This makes it possible to use EDTA samples for PT measurement under certain conditions with citrate calibrators. **(Horsti, 2001)**

Additional research by Banfi et al 2007 emphasized that while EDTA is commonly used for haematological analyses due to its cell-preserving properties, its application in coagulation testing is limited. The study reaffirmed that calcium chelation by EDTA results in inhibition of coagulation pathways in vitro, which generally renders it inappropriate for PT assessments. **(Banfi, et al, 2007)**

Based on what was mentioned above, blood coagulation testing, particularly PT and INR, plays a critical role in diagnosing coagulation disorders and monitoring anticoagulant therapy. The accuracy of these tests heavily depends on the type of anticoagulant used during blood sample collection. While sodium citrate is widely accepted and standard for PT testing due to its ability to temporarily chelate calcium without permanently inhibiting coagulation, the use of EDTA as an anticoagulant, predominantly employed in hematology for hemocytometry, is generally considered inappropriate for coagulation studies because of its strong calcium-binding properties, which can potentially alter test results. However, some studies suggest that EDTA may produce PT and INR results comparable to citrate under certain conditions. Despite this, there remains a paucity of comprehensive research explicitly comparing the effects of EDTA versus citrate on PT outcomes, particularly in diverse patient populations and clinical settings. Given the widespread availability and use of EDTA in laboratories, understanding its impact on coagulation parameters is crucial to ensure the accuracy and reliability of PT measurements.

Therefore, this study is vital because it addresses a significant gap in current laboratory practice by systematically evaluating the possibility of using EDTA as a practical alternative to citrate in PT testing. Also, its findings could lead to potential adjustments in sample collection protocols, particularly in resource-limited settings where EDTA use might be more accessible, which ensures the accuracy of PT and INR test results, which directly impacts the treatment and monitoring of patients, especially those receiving anticoagulant therapy. This study also contributes to the broader scientific understanding of anticoagulants' effects on coagulation assays, which contributes to improving laboratory standards and practices.

In summary, this study seeks to generate evidence regarding the compatibility of EDTA use with PT testing, which may contribute to simplifying laboratory procedures and improving diagnostic accuracy. It also aims to highlight the impact of using EDTA as an anticoagulant in PT testing.

Research Objectives:

This study aims to investigate the impact of EDTA as an anticoagulant on PT results and INR, contrasting these findings with those obtained using sodium citrate as an anticoagulant for the same test.

Materials and methods:

Study area, population, and duration: This study was conducted in the Tarhuna district (northwest of Libya) on 50 healthy volunteers (randomly selected) during the period from February to May 2025.

Blood samples collection: Using sterile blood collection equipment's, 4 ml of venous blood was collected from each participant; immediately after collection, 2 ml of blood was transferred into tubes containing EDTA, while the remaining 2 ml was transferred into 3.2% sodium citrate tubes**.**

All samples were centrifuged at 4000 rpm for 15 minutes, plasma was separated, and they were transported promptly to the laboratory under appropriate conditions to maintain sample integrity for subsequent laboratory analyses.

Analysis method:

The test was carried out using the CoaDATA504 device and the Biomaghreb Calcium Thromboplastin reagent for determination of the prothrombin time..

Data Analysis:

The obtained results were presented as tables and figures to guarantee accurate and trustworthy findings. The results are expressed as numbers and percentages to illustrate the comparison between the laboratory analysis results for both anticoagulants used.

Results:

Fifty healthy volunteers residing in the Tarhuna district were randomly selected, and their PT and INR were measured.

The results of the current study showed that, when citrate was used as an anticoagulant, 44 (88%) samples had normal prothrombin time, while 6 (12%) had elevated PT. In contrast, 49 (98%) samples had elevated PT, and just one sample had a normal PT when EDTA was used as an anticoagulant. Notably, no results below the normal range of PT were recorded either when employing citrate or EDTA as an anticoagulant.

In the same context, there were differences in findings between using citrate and EDTA in the present study regarding INR results; the results showed that, when using citrate, 43 samples, representing 86%, had INR results within the normal range, and 6 samples gave an INR rate higher than the normal range, while the result was lower than normal in one sample. Looking at the analyses' findings when utilizing EDTA as an anticoagulant, it is evident that a large number of samples (46) displayed results higher than normal limits in opposition to 4 samples that fell within the normal range. These results are described in Figure1.

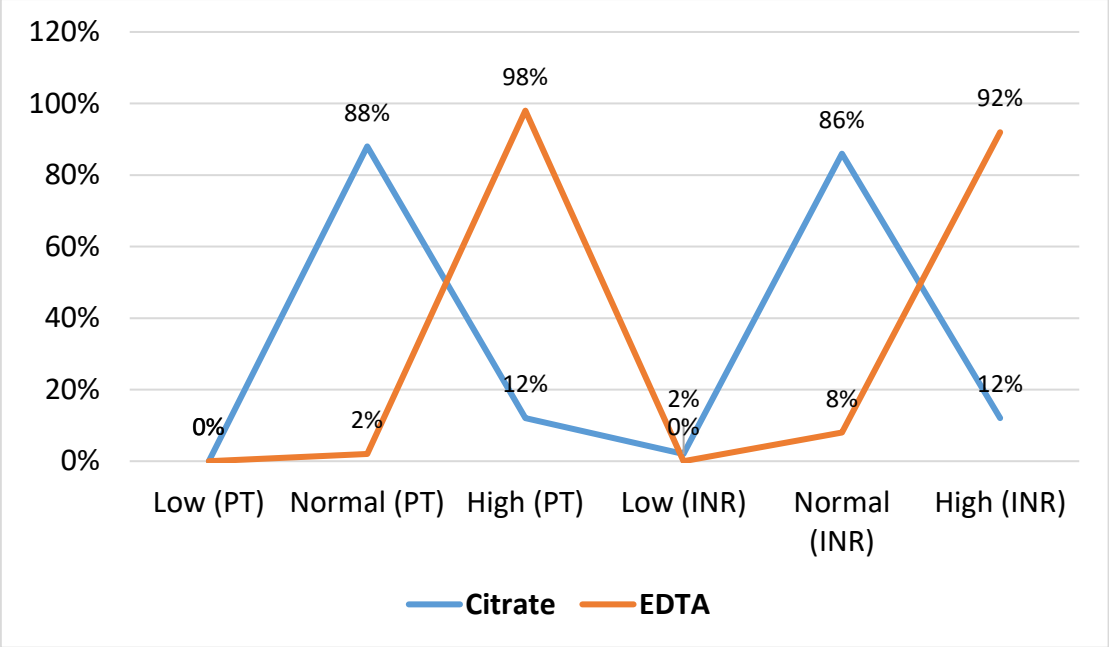

Figure 1. Shows the distribution of laboratory findings (PT & INR) for the study sample by use of both of "citrate and EDTA" as an anticoagulant.

As illustrated in Table 1, forty-nine (98%) out of the total samples show an elevated PT, while just one sample had a normal PT by EDTA, among those 49 samples, only 5 samples had elevated PT, and the remaining 44 had normal prothrombin time by using citrate. Similarly, 43 of the 49 samples "which had elevated PT using EDTA" had normal INR, whereas 5 samples had increased INR using citrate. At the same time, the only sample that had normal PT using EDTA showed elevated levels in PT and INR when using citrate.

Table 1 Shows the distribution of PT results using EDTA as an anticoagulant comparison to PT and INR findings using citrate.

EDTA			Citrate		
				PT	INR
PT	high	49	high	5	5
			normal	44	43
			low	//	1
	normal	1	high	1	1
			normal	//	//
			low	//	//

This study's findings also revealed that 46 samples had INR levels higher than normal using EDTA, among them 43 samples with normal PT and 3 samples with elevated PT using citrate, while 41 of them had normal INR, 4 had high INR, and only one sample was lower than the normal INR range using citrates. It was discovered that none of the four samples with normal INR using EDTA had higher than normal PT or INR, while one case had normal PT and two cases had normal INR. As shown in Table 2.

Table 2 Elucidate the distribution of INR results using EDTA comparison to PT and INR findings using citrate as an anticoagulant.

EDTA			Citrate		
				PT	INR
INR	high	46	high	3	4
			normal	43	41
			low	//	1
	normal	4	high	//	//
			normal	1	2
			low	3	2

Additionally, the data demonstrated that five of six samples with raised PT using Citrate also had elevated PT using EDTA, whereas only five of 49 samples with elevated PT using EDTA also had elevated PT using Citrate.

On the other hand, it was noted through the results of the current study that the number of samples that had an elevated INR using citrate was 6, four of which were also elevated using EDTA, while among 46 samples that had an elevated INR using EDTA, only 4 of them were found to have an elevated INR using citrate.

When citrate was used as an anticoagulant, it was discovered that 5 of 6 samples with an elevated PT also had an elevated INR.

Also, when using EDTA as an anticoagulant, it was found that among 49 samples that had an elevated PT result, 46 of them also had an elevated INR result.

Discussion:

Citrate samples are only used for coagulation tests, while EDTA samples have long been used in many haematological measurements because they provide the best preservation of blood cells morphology. As a result, the possibility of using EDTA for coagulation tubes appears to be practical, convenient, and cost-effective. **[M Albayaa, 2011]**

The purpose of this study is to assess if EDTA can be used as an anticoagulant to perform PT testing as an alternative to sodium citrate.

These results demonstrate a significant difference between PT results using citrate and EDTA as anticoagulants when collecting blood samples. As evident from the results of this study, the use of EDTA produced significantly elevated PT compared to the use of citrate, "where most citrate samples showed normal results". This is largely acceptable given that all participants are healthy individuals “not receiving any anticoagulant medications and not suffering from any cardiovascular disease". It is also worth noting that neither citrated nor EDTA samples were found to produce any PT readings below the normal range.

This is attributed to the fact that EDTA is a potent chelating agent that continue binding to Ca^{++} ions even when more calcium is introduced, making it less suitable for coagulation tests than citrate, while sodium citrate is used in coagulation tests due to it reversible chelation of calcium, and its effect can be readily countered by the addition of calcium ions. **(Banković Radovanović, et al, 2020)**

The results of the current study also showed significant differences with respect to INR similar to those observed in PT.

Although some studies have demonstrated the feasibility of using EDTA in the PT assays, the results of the current study indicate that it is preferable not to use EDTA as an anticoagulant when collecting blood samples for the PT test.

This is consistent with some previous studies conducted to determine the possibility of using EDTA as an anticoagulant instead of citrate, e.g. The study conducted in Iraq by M Albayaa 2011, which reported that, the use of EDTA samples for PT testing is unacceptable and may lead to serious discrepancies in INR estimation. **[M Albayaa, 2011]**

Also results of Della Valle et al 1999 indicated that, the INR results could vary significantly depending on the type of anticoagulant used. **(Della Valle, et al, 1999)**

The study by Banfi et al, 2007 highlighted that the use of EDTA in coagulation testing is limited because EDTA-induced calcium chelation inhibits coagulation pathways in vitro making it generally unsuitable for PT assessments**(.Banfi, et al, 2007)**

On the other hand, the findings of the current study are incompatible with other studies, such as the findings of Triantaphyllopoulos et al 1955 while studying the action of EDTA on PT, which noted that occasionally a shorter prothrombin time was obtained with the EDTA specimen than with the oxalated. **(Triantaphyllopoulos, et al, 1995)**

The Horsti 2001 findings showed that no difference between the PT and INR values obtained from citrate and EDTA samples were noted; this makes it possible to use EDTA samples for PT measurement under certain conditions with citrate calibrators. **(Horsti, 2001)**

Conclusions and recommendations:

Added anticoagulants could influence the specimen's composition and cause issues with some tests; as a result, some anticoagulants are more suitable for particular tests, while others might not be. Decades have been spent searching for the ideal anticoagulant; regretfully, there is still no common anticoagulant for laboratory testing. The current study, which was conducted on healthy volunteers, aimed to investigate the possibility of using EDTA as an anticoagulant in PT testing and showed that there were differences in the results of the PT and INR between EDTA and citrated blood samples since the results were normal in most samples when using sodium citrate and higher than normal when using EDTA as an anticoagulant, so this study concluded that it is not appropriate to use EDTA as an anticoagulant in coagulation tests.

At the end of this study the following are recommended: Further studies are recommended to identify possible alternative anticoagulants to citrate that can be used when performing coagulation testing. Search for an equation that can be used to correlate the results of citrate and EDTA in emergency situations where citrate cannot be used. In laboratories, avoid using EDTA when performing coagulation tests except when absolutely necessary "with calibration using citrate".

References:

[1] Banković Radovanović, P., Živković Mikulčić, T. and Simović Medica, J. (2020). Unexpected abnormal coagulation test results in a 2-year-old child: A case report. Biochemia Medica (Zagreb), 30(1), 158-163 p.011002. doi:10.11613/BM.2020.011002.

[2] Banfi, G., Salvagno, G.L. and Lippi, G., (2007). The role of ethylenediamine tetraacetic acid (EDTA) as in vitro anticoagulant for diagnostic purposes. Clinical Chemistry and Laboratory Medicine, 45(5), pp.565-576. doi:10.1515/CCLM.2007.110.

[3] Della Valle, P., Crippa, L., Garlando, A. M., Pattarini, E., Safa, O. M. I. D., D'angelo, S. V., & D'Angelo, A. R. M. A. N. D. O. (1999). Interference of lupus anticoagulants in prothrombin time assays: implications for selection of adequate methods to optimize the management of thrombosis in the antiphospholipid-antibody syndrome. Haematologica, 84(12), 1065-1074. Retrieved from https://pubmed.ncbi.nlm.nih.gov/10586206/

[4] Horsti, J. (2001). Use of EDTA samples for prothrombin time measurement in patients receiving oral anticoagulants. Haematologica, 86(8), 851-855. Retrieved from https://www.haematologica.org/haematologica/article/view/2101

[5] Le, D. T., Weibert, R. T., Sevilla, B. K., Donnelly, K. J., & Rapaport, S. I. (1994). The international normalized ratio (INR) for monitoring warfarin therapy: reliability and relation to other monitoring methods. Annals of internal medicine, 120(7), 552-558. DOI: 10.7326/0003-4819-120-7-199404010-00004. PMID: 8116992

[6] Levy, J. H., Szlam, F., Wolberg, A. S., & Winkler, A. (2014). Clinical use of the activated partial thromboplastin time and prothrombin time for screening: a review of the literature and current guidelines for testing. Clinics in laboratory medicine, 34(3), 453-477. DOI: 10.1016/j.cll.2014.06.005. PMID: 25168937

[7] Mattsson, C., Menschiek-Lundin, A., Wåhlander, K., & Lindahl, T. L. (2001). Effect of melagatran on prothrombin time assays depends on the sensitivity of the thromboplastin and the final dilution of the plasma sample. Thrombosis and Haemostasis, 86(02), 611–615. Retrieved from https://pubmed.ncbi.nlm.nih.gov/11522011/

[8] M Albayaa, I. (2011). Control of Oral Anticoagulant Therapy Using EDTA Plasma. Kerbala Journal of Medicine, 4(10), 1122-1127. Retrieved from https://medicine.uokerbala.edu.iq

[9] Poller, L. (2004). International Normalized Ratios (INR): the first 20 years. Journal of Thrombosis and Haemostasis, 2(6), 849-860. doi: 10.1111/j.1538-7836.2004.00775.x.

[10] Rattan, S., & Jeyaseelan, C. (2024). Emerging trends in synthesis and catalysis in chemistry. Springer. https://doi.org/10.1007/978-981-97-5169-3

[11] Shahangian, S., LaBeau, K. M., & Howerton, D. A. (2006). Prothrombin time testing practices: adherence to guidelines and standards. Clinical chemistry, 52(5), 793-794. doi: 10.1373/clinchem.2005.065433.

[12] Triantaphyllopoulos, D. C., Quick, A. J., & Greenwalt, T. J. (1955). Action of Disodium Ethylenediamine Tetracetate on Blood Coagulation: Evidence of the Development of Heparinoid Activity during Incubation or Aeration of Plasma. Blood, 10(5), 534-544. PMID: 14363334. Retrieved from https://pubmed.ncbi.nlm.nih.gov/14363334/