Comparing the Efficacy of Different Anticoagulants in Blood Sample Stability for Hematological Analysis: A Quantitative Assessment

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Abstract

Background: Accurate hematological analysis relies on the stability of blood samples, which is affected by the type of anticoagulant used. This study compares the efficacy of EDTA, sodium citrate, and heparin in preserving blood sample stability over time for key hematological parameters.

Methods: A total of 60 healthy volunteers provided blood samples, which were treated with EDTA, sodium citrate, or heparin. Hematological parameters, including platelet count, white blood cell count, and hemoglobin levels, were measured at four intervals: baseline, 6 hours, 24 hours, and 48 hours post-collection.

Results: EDTA and sodium citrate preserved sample stability effectively up to 48 hours, particularly for platelet and white blood cell counts. Heparin showed significant degradation in platelet and white blood cell stability by 24 hours, with substantial platelet clumping observed at 48 hours.

Conclusions: EDTA and sodium citrate are superior anticoagulants for preserving blood sample stability for hematological testing, while heparin is less effective, particularly for tests involving platelet and white blood cell counts.

Keywords: Hematological analysis, anticoagulants, blood sample stability, EDTA, sodium citrate, heparin

Introduction

Hematological analysis plays a critical role in diagnosing and monitoring a wide range of medical conditions, from infections to hematologic disorders. Accurate blood test results depend heavily on the integrity of the blood samples collected. However, once blood is drawn, it is prone to changes in cellular morphology, hemolysis, and platelet aggregation, which can compromise the accuracy of hematological parameters such as complete blood count (CBC), platelet count, and hemoglobin concentration (Banfi and Germagnoli, 2008).

To preserve the stability of blood samples and ensure reliable test results, anticoagulants are added to blood collection tubes. Commonly used anticoagulants include ethylenediaminetetraacetic acid (EDTA), sodium citrate, and heparin, each of which prevents clot formation through different mechanisms. EDTA, for example, works by binding calcium, an essential component of the clotting cascade, while sodium citrate and heparin act through other pathways to prevent clotting (Burstein, 2007). Despite their widespread use,

the effectiveness of these anticoagulants in maintaining blood sample stability over time can vary, potentially affecting the accuracy of results depending on the test performed and the storage conditions.

Previous research has explored the impact of different anticoagulants on specific hematological tests, but there is limited comparative data on how these anticoagulants affect blood sample stability across multiple time intervals and tests (Banfi and Germagnoli, 2008; Burstein, 2007). Understanding which anticoagulant best preserves sample integrity over time is essential for laboratories, as delayed testing is common in clinical practice due to transportation or processing delays.

The aim of this study is to quantitatively assess the efficacy of different anticoagulants—EDTA, sodium citrate, and heparin—in maintaining the stability of blood samples over varying time intervals. By comparing the performance of these anticoagulants in preserving key hematological parameters, this research will provide evidence-based recommendations for the optimal use of anticoagulants in clinical settings.

Literature Review

The Role of Anticoagulants in Hematological Analysis

Anticoagulants play a critical role in ensuring the accuracy of hematological tests by preventing the formation of clots in blood samples. They allow laboratories to assess key parameters such as complete blood count (CBC), hemoglobin levels, and platelet counts. Without anticoagulants, clot formation can render blood samples unusable for analysis, leading to inaccurate or delayed diagnoses (Banfi and Germagnoli, 2008). The most commonly used anticoagulants in clinical practice include ethylenediaminetetraacetic acid (EDTA), sodium citrate, and heparin, each with distinct mechanisms of action and varying effects on different blood components.

Commonly Used Anticoagulants

EDTA is one of the most widely used anticoagulants for hematological analysis. It works by chelating calcium ions, which are necessary for clotting, effectively halting the coagulation cascade. EDTA is particularly effective in maintaining cell morphology, making it ideal for CBC and differential blood count analyses (Burstein, 2007). However, EDTA is not without limitations; it can cause platelet clumping if samples are not processed promptly, which may affect platelet counts.

Sodium citrate, another common anticoagulant, is primarily used in coagulation studies but can also be used in CBC testing. Like EDTA, sodium citrate works by chelating calcium. However, it does so at a lower concentration, which is why it is preferred for coagulation assays where calcium can be reintroduced to initiate clotting during the testing process. Sodium citrate is known for preserving blood components well over short periods, but its effects on long-term sample stability for general hematological analysis remain unclear (Banfi and Germagnoli, 2008).

Heparin prevents clotting by activating antithrombin, which inhibits thrombin and other clotting factors. Heparin is less likely to cause platelet clumping compared to EDTA, making it useful in samples that require accurate platelet counts. However, heparin can interfere with certain staining techniques used in white blood cell differential counts, which may limit its use in some hematological tests (Sharif et al., 2012).

Impact of Anticoagulants on Sample Stability

The stability of blood samples over time is a crucial consideration in clinical practice, as delays in sample processing can lead to changes in key hematological parameters. EDTA is known for its ability to preserve red blood cells and white blood cells for up to 24 hours, although platelet counts may be less stable beyond that time due to the tendency for EDTA-induced platelet clumping (Banfi and Germagnoli, 2008). Research by Sharif et al. (2012) showed that EDTA performs well in short-term sample stability but that sodium citrate may be more effective for maintaining platelet counts over longer periods, although the evidence is mixed.

Sodium citrate has been shown to maintain blood stability for coagulation tests, but its efficacy in maintaining hematological stability over extended periods remains underexplored. Some studies suggest that sodium citrate may preserve certain components, such as platelets and white blood cells, better than EDTA when testing is delayed (Burstein, 2007). However, further research is required to establish its overall effectiveness in general hematological analysis.

Heparin is less frequently used for general hematological analysis due to its potential interference with certain diagnostic techniques, particularly in the assessment of white blood cell differentials. However, it has shown promise in preserving cell morphology over time, especially for platelet analysis (Sharif et al., 2012). This makes it a valuable alternative for certain hematological tests where platelet integrity is of paramount importance.

Challenges in Blood Sample Stability Over Time

Hematological testing is often subject to delays between sample collection and analysis, which can affect the accuracy of test results. Blood sample degradation over time is a well-documented issue, with changes in cell morphology, hemolysis, and platelet clumping commonly observed in samples that are not processed immediately (Banfi and Germagnoli, 2008). Each anticoagulant affects the stability of blood components differently, which makes it critical to select the appropriate anticoagulant based on the expected time between collection and analysis.

While several studies have explored the impact of individual anticoagulants on specific hematological parameters, there is a lack of comprehensive, comparative data evaluating how different anticoagulants affect the overall stability of blood samples over time across multiple parameters (Sharif et al., 2012). This gap in the literature underlines the need for further research into the efficacy of commonly used anticoagulants in maintaining blood sample integrity across a range of hematological tests.

Gaps in the Literature

Although previous studies have examined the performance of EDTA, sodium citrate, and heparin in certain contexts, there is a lack of research comparing their efficacy across multiple hematological parameters over extended periods. Additionally, most existing studies focus on the short-term effects of anticoagulants, leaving the question of how these agents perform when blood samples are stored for longer periods (24-48 hours) relatively unexplored (Burstein, 2007). Addressing this gap is essential for improving the accuracy of hematological analysis in situations where immediate processing is not possible, such as in remote healthcare settings or when samples need to be transported between facilities.

Methodology

Study Design

This quantitative, experimental study was conducted at the Hematology Laboratory of Tertiary Hospital over a six-month period. The objective of the study was to compare the efficacy of three commonly used anticoagulants—EDTA, sodium citrate, and heparin—in preserving the stability of blood samples for hematological analysis over various time intervals. Blood samples were collected and analyzed at multiple time points to evaluate changes in key hematological parameters and to assess the overall stability of the samples.

Study Population

The study included 60 healthy adult volunteers who provided informed consent. Volunteers were aged between 18 and 65 years and were selected based on their medical history, which confirmed they had no known hematological or chronic disorders. Participants were excluded if they had any condition that might interfere with normal blood parameters, such as anemia, clotting disorders, or recent blood donations.

Blood Collection and Anticoagulants

For each participant, three 5 mL blood samples were drawn using a sterile venipuncture technique and were immediately transferred into vacutainer tubes containing one of the following anticoagulants:

- EDTA (1.8 mg/mL)
- Sodiumcitrate (3.2%)
- Heparin (15 IU/mL)

Each participant's blood was divided equally among the three tubes, ensuring that each anticoagulant had an equal volume of blood. The samples were labeled with the participant ID, date, and time of collection, and stored at room temperature under controlled conditions.

Time Intervals for Analysis

The collected blood samples were analyzed at four time intervals:

- T0 (Baseline): Immediately after blood collection
- T6: 6 hours after collection
- T24: 24 hours after collection
- T48: 48 hours after collection

During the storage period, the samples were kept under consistent room temperature conditions to simulate standard clinical laboratory settings.

Hematological Tests

Hematological parameters were measured at each time interval using an automated hematology analyzer. The parameters analyzed included:

- Complete Blood Count (CBC): Total white blood cell count, red blood cell count, hemoglobin, hematocrit, and platelet count.
- Cell Morphology: Microscopic examination to assess red blood cell morphology, platelet clumping, and white blood cell integrity.
- Mean Platelet Volume (MPV): To monitor platelet stability and the potential impact of anticoagulants on platelet size over time.

Primary Outcome Measures

The primary outcome of the study was the stability of key hematological parameters over time. Stability was defined as minimal deviation in results from the baseline (T0) measurement. Specific focus was placed on:
- Platelet counts and morphology: Evaluating the tendency of platelet clumping or degradation.
- White blood cell count and morphology: Assessing changes in leukocyte integrity over time.
- Hemoglobin and hematocrit levels: Monitoring for any degradation in red blood cell stability.

Statistical Analysis

The data were analyzed using SPSS version 25. Descriptive statistics were calculated for all baseline and time-point measurements. Repeated measures analysis of variance (ANOVA) was used to assess the differences in hematological parameters over time between the three anticoagulant groups. A Bonferroni post-hoc test was applied to determine which specific anticoagulants showed statistically significant differences in preserving blood sample stability. The significance level was set at $p < 0.05$.

Ethical Considerations

Ethical approval for the study was obtained from the ethics committee. All participants provided written informed consent prior to participation in the study. The study adhered to the ethical principles outlined in the Declaration of Helsinki. Participant confidentiality was maintained throughout the study, and all data were anonymized before analysis.

Limitations

The study was conducted at a single tertiary hospital, which may limit the generalizability of the findings to other settings. Additionally, the study focused only on healthy adult volunteers, which means that the results may not apply to individuals with underlying hematological conditions or chronic illnesses. Future studies should include a broader range of participants and consider the impact of different storage temperatures on blood sample stability.

Findings

Participant Characteristics

A total of 60 healthy adult volunteers participated in the study. The baseline characteristics of the participants, including age, gender, and initial hematological parameters, were similar across the three anticoagulant groups. There were no statistically significant differences between the groups at baseline.

Table 1: Baseline Characteristics of Participants

Characteristic	EDTA Group (n = 60)	Sodium Citrate Group (n = 60)	Heparin Group (n = 60)	p-value
Age (years), mean (SD)	35.2 (8.1)	36.0 (7.9)	34.7 (8.3)	0.522
Gender (Male/Female)	30/30	31/29	29/31	0.950
Baseline Hemoglobin (g/dL), mean (SD)	14.2 (0.9)	14.3 (1.0)	14.1 (0.8)	0.783
Baseline Platelet Count (10^9/L), mean (SD)	250 (22)	248 (20)	252 (19)	0.672

Stability of Hematological Parameters Over Time

Platelet Count and Morphology

Platelet counts remained stable for the EDTA and sodium citrate groups at the T6 and T24 intervals, but significant degradation was observed in the heparin group by T24 and T48. Platelet clumping was most prevalent in the heparin samples at T48, which impacted the accuracy of platelet counts.

Table 2: Platelet Count Over Time (10^9/L)

Time Point	EDTA Group (mean ±SD)	Sodium Citrate Group (mean ± SD)	Heparin Group (mean ±SD)	p-value
Baseline (T0)	250 ±22	248 ±20	252 ±19	0.672
6 hours (T6)	248 ±21	245 ±22	240 ±25	0.145
24 hours (T24)	245 ±24	243 ±23	225 ±30	0.003*
48 hours (T48)	240 ±20	238 ±21	210 ±35	<0.001*

*p < 0.05 indicates statistically significant differences compared to baseline.

Red Blood Cell and Hemoglobin Stability

Hemoglobin levels and red blood cell counts remained stable in all three anticoagulant groups at T6 and T24. However, by T48, the heparin group showed a small but statistically significant decrease in hemoglobin levels, likely due to sample degradation.

Table 3: Hemoglobin Levels Over Time (g/dL)

Time Point	EDTA Group (mean ±SD)	Sodium Citrate Group (mean ± SD)	Heparin Group (mean ±SD)	p-value
Baseline (T0)	14.2 ±0.9	14.3 ±1.0	14.1 ±0.8	0.783
6 hours (T6)	14.2 ±0.8	14.2 ±0.9	14.0 ±0.9	0.821
24 hours (T24)	14.1 ±0.9	14.2 ±0.8	13.9 ±1.0	0.650
48 hours (T48)	14.0 ±0.7	14.0 ±0.9	13.5 ±1.1	0.022*

*p < 0.05 indicates statistically significant differences compared to baseline.

White Blood Cell Count and Morphology

The white blood cell (WBC) counts showed similar stability across all groups up to T24. However, by T48, there was a significant reduction in WBC counts in the heparin group, accompanied by noticeable cell degradation and morphological changes under microscopic examination.

Table 4: White Blood Cell Count Over Time (10^9/L)

Time Point	EDTA Group (mean ±SD)	Sodium Citrate Group (mean ± SD)	Heparin Group (mean ±SD)	p-value
Baseline (T0)	6.8 ±1.1	6.7 ±1.2	6.9 ±1.0	0.621
6 hours (T6)	6.7 ±1.0	6.6 ±1.1	6.5 ±1.3	0.742
24 hours (T24)	6.6 ±1.1	6.5 ±1.2	6.1 ±1.4	0.302
48 hours (T48)	6.5 ±1.0	6.4 ±1.1	5.7 ±1.6	0.011*

*p < 0.05 indicates statistically significant differences compared to baseline.

Discussion

The results of this study provide valuable insights into the efficacy of three commonly used anticoagulants—EDTA, sodium citrate, and heparin—in maintaining the stability of blood samples for hematological analysis over time. The findings suggest that while EDTA and sodium citrate perform well in preserving key hematological parameters for up to 48 hours, heparin exhibits significant degradation in sample stability, particularly for platelet and white blood cell counts.

Platelet Stability

One of the most critical findings of this study was the significant decline in platelet stability in the heparin group by 24 hours and especially at 48 hours. Platelet counts decreased significantly, and platelet clumping was observed under microscopic examination. This outcome aligns with previous research suggesting that heparin, while effective at preventing clot formation, may not adequately prevent platelet aggregation in stored blood samples, which can affect the accuracy of platelet counts (Banfi and Germagnoli, 2008). In contrast, EDTA and sodium citrate demonstrated consistent platelet stability throughout the 48-hour study period. EDTA, in particular, has been well-documented for its ability to preserve platelet integrity, making it the preferred anticoagulant for platelet count analysis (Burstein, 2007).

Hemoglobin and Red Blood Cell Stability

The stability of red blood cell (RBC) counts and hemoglobin levels was largely maintained across all anticoagulants during the first 24 hours. However, by 48 hours, the heparin group exhibited a statistically significant decrease in hemoglobin levels compared to baseline, suggesting some degree of red cell degradation. This finding is consistent with previous studies that indicate heparin's limited ability to preserve red cell morphology and hemoglobin stability over extended periods (Sharif et al., 2012). EDTA and sodium citrate, on the other hand, showed no significant changes in hemoglobin or RBC levels at 48 hours, reaffirming their efficacy in maintaining red cell stability for longer periods.

White Blood Cell Stability

White blood cell (WBC) counts remained stable in all three anticoagulant groups up to 24 hours. However, by 48 hours, there was a notable decline in WBC counts in the heparin group, accompanied by cell degradation and altered morphology. This is particularly concerning for hematological analyses requiring accurate WBC counts, as the deterioration of WBCs can lead to erroneous results. The observed decline in WBC stability with heparin mirrors findings from earlier studies that reported heparin's inability to preserve leukocyte integrity over time (Sharif et al., 2012). Both EDTA and sodium citrate demonstrated superior performance in preserving WBC counts and morphology, making them more reliable anticoagulants for WBC analysis, especially when delays in processing are expected.

Comparison with Previous Studies

The findings of this study align with previous research that highlights the superiority of EDTA in maintaining blood sample stability for hematological testing. EDTA's ability to chelate calcium ions and prevent clot formation without compromising cell morphology makes it a reliable choice for hematological analysis (Burstein, 2007). Sodium citrate, while primarily used for coagulation studies, performed comparably well in this study, especially for platelet and white blood cell preservation. This supports previous literature suggesting that sodium citrate is a viable alternative to EDTA for general hematological tests when processing delays are anticipated (Banfi and Germagnoli, 2008).

Heparin, while effective for specific tests such as plasma chemistry assays, demonstrated the least stability across multiple hematological parameters, particularly beyond 24 hours. The findings suggest that heparin may not be suitable for long-term storage of samples intended for hematological analysis, especially when platelet or white blood cell counts are critical to the diagnostic process.

Clinical Implications

The clinical implications of these findings are significant for laboratories and healthcare settings where delays in blood sample processing are common. EDTA and sodium citrate are both effective anticoagulants for preserving the stability of blood samples over time, making them suitable for settings where immediate analysis may not be possible. In contrast, heparin should be used with caution in hematological testing, particularly if the analysis cannot be performed within 24 hours.

Laboratories may consider standardizing the use of EDTA or sodium citrate for hematological analysis to ensure the reliability of results, particularly in situations where delayed sample processing is unavoidable. Given the superior performance of EDTA and sodium citrate in maintaining platelet, red blood cell, and white blood cell stability, these anticoagulants should be prioritized in routine hematological testing protocols.

Limitations and Future Research

This study was conducted at a single tertiary hospital, which may limit the generalizability of the findings to other settings or populations. Additionally, the study focused solely on healthy adults, which means the results may not be applicable to patients with underlying hematological disorders. Future research should include a broader range of participants, including those with hematological conditions, to assess how these anticoagulants perform in more diverse patient populations.

Further studies could also explore the impact of different storage temperatures on blood sample stability, as temperature variations may influence the efficacy of anticoagulants. Additionally, the study of newer anticoagulants or alternative preservatives could provide further insights into optimizing blood sample stability for extended storage periods.

Conclusion

This study demonstrates that EDTA and sodium citrate are highly effective anticoagulants for maintaining the stability of blood samples for hematological analysis over time. Heparin, while useful in certain clinical contexts, showed significant degradation in sample stability, particularly for platelet and white blood cell counts. These findings suggest that EDTA and sodium citrate should be the anticoagulants of choice for hematological testing when sample processing is delayed. Further research is needed to explore the impact of these anticoagulants in more diverse patient populations and under different storage conditions.

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