Evaluating Vortexing Times to Disaggregate Platelet Clumps in EDTA Specimens

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ABSTRACT

EDTA-dependent pseudothrombocytopenia (PTCP) causes spurious low platelet counts, necessitating costly repeat testing and patient recollections. This study evaluated the efficacy of varying vortexing durations to mechanically disaggregate platelet clumps and restore accurate counts. A total of 120 adult EDTA-anticoagulated specimens flagged for platelet clumps by a Beckman Coulter DxH 900 were included. Samples were assigned to 1-, 2-, or 3-minute vortexing groups. Peripheral blood smears were reviewed pre- and postvortex to confirm the presence of clumps and assess disaggregation. The analyzer's platelet clump flag demonstrated a low positive predictive value (34.2%), with only 41 samples containing true clumps. Among these true-positive samples, successful disaggregation occurred in 29% of samples in both the 1-minute and 3-minute groups, with no statistically significant difference in efficacy across durations ($P = .09$). All other complete blood cell count parameters remained stable postvortexing (Pearson $r > 0.99$). However, vigorous agitation induced new platelet clumps in 29% of samples that were initially clump negative. These findings suggest that although vortexing is a viable noninvasive corrective action that can resolve true PTCP cases, it is not without risk. Therefore, a "smear-first" protocol is recommended, in which microscopic confirmation of clumps precedes mechanical disaggregation to avoid inducing artifacts in false-positive specimens.

ABBREVIATIONS: CBC - complete blood cell count, HCT - hematocrit, HGB - hemoglobin, Ig - immunoglobulin, PPV - positive predictive value, PTCP - pseudothrombocytopenia, RBC - red blood cell, SOP - standard operating procedure, WBC - white blood cell.

INDEX TERMS: hematology, complete blood count, platelets, platelet count, EDTA.

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INTRODUCTION

In the clinical hematology laboratory, the complete blood cell count (CBC) is one of the most commonly performed laboratory tests. Although automation has streamlined workflow, samples flagged for platelet aggregation require manual intervention. Flagged platelet clumps prevent the release of automated results, necessitating further assessment. This phenomenon, often identified as EDTA-dependent pseudothrombocytopenia (PTCP), is an in vitro artifact caused by anticoagulant-induced platelet clumping. Although the prevalence in the general population is estimated at only 0.1%,[1,2] the clinical implications are significant. Failure to recognize PTCP can lead to spurious thrombocytopenia diagnoses, resulting in unnecessary bone marrow aspirations, cancellation of surgeries, or inappropriate treatments.[3-5]

The pathophysiology of EDTA-PTCP is immunologically mediated. In the presence of EDTA, the calcium concentration decreases, inducing a conformational change in the platelet membrane glycoprotein IIb/IIIa complex. This change exposes cryptantigens, allowing naturally occurring autoantibodies (usually immunoglobulin [Ig] G or IgM) to bind, bridging platelets and causing agglutination.[2,6] This clumping leads automated analyzers to undercount platelets and often misclassify the large clumps as white blood cells (WBCs).

A case study by Tangella et al documented a 17-year-old patient presenting with recurrent fever and low platelet counts but no history of bleeding.[7] The patient was subjected to serology testing and bone marrow aspiration before a peripheral smear confirmed platelet clumps. The standard resolution for such cases involves recollecting a patient's specimen using an alternative anticoagulant, such as sodium citrate.[2] However, recollection increases turnaround time, health care costs, and patient discomfort. Other methods, such as heating samples or adding pharmaceuticals,[6,8-10] have been proposed but may not be practical for high-volume routine workflows.

To avoid the burden of specimen recollections, mechanical disaggregation via vortexing has been studied. In a foundational study, Gulati et al focused on disaggregating clumps by vortexing samples at maximum speed for 1 to 2 minutes.[11] Reviewing 188 flagged samples, they found that 43.6% showed complete disaggregation and 49.5% showed partial disaggregation. More recently, Mundt validated this approach using Sysmex XN series analyzers.[12] Mundt observed that vortexing for 1 to 2 minutes

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resolved flags in the majority of samples, allowing for result release without recollection. Similarly, Zhu et al employed a vortex method on 221 PTCP specimens, reporting significant increases in platelet counts without statistically significant changes to mean platelet volume, red blood cell (RBC), or WBC parameters, further suggesting that mechanical disaggregation is a reliable alternative to secondary venipuncture.[13]

Although Gulati et al, Mundt, and Zhu et al used vortexing times ranging from 1 to 2 minutes,[11-13] there remains a lack of consensus regarding the optimal duration required to maximize disaggregation while maintaining cell integrity. The investigators' laboratory currently adheres to a policy of manual smear review and patient recollections when platelet clumps are observed. This study aims to evaluate the effectiveness of varying vortexing times to successfully disaggregate platelet clumps. It is hypothesized that optimized vortexing times will increase the recovery of accurate platelet counts, reducing the need for patient recollections.

METHODS

This study was conducted in the clinical hematology core laboratory at Wellstar MCG Health. Residual EDTA-anticoagulated whole-blood samples analyzed by a Beckman Coulter DxH 900 hematology analyzer were identified for potential inclusion during the period of July 1, 2025, through December 21, 2025. Inclusion criteria comprised samples flagged for platelet clumps by either of 2 DxH 900s used in the investigators' laboratory, collected from patients aged 18 years or older, and analyzed within 24 hours of sample collection. Samples were excluded if they exceeded the 24-hour stability window, were collected from pediatric patients (<18 years old), or possessed insufficient volume for repeat testing. A total of 120 samples meeting these criteria were selected for this study.

On initial flagging by the analyzer for platelet clumps, the standard operating procedure (SOP) for the investigators' laboratory was to review a baseline peripheral blood smear. Following procedure, this smear was prepared by the Beckman Coulter DxH SMS and reviewed microscopically to confirm the presence of platelet clumps (prevortex evaluation).

Baseline CBC parameters associated with this prevortex smear were recorded and included WBC, RBC, hemoglobin (HGB), hematocrit (HCT), and platelet counts. A separate prevortex smear review and all subsequent study procedures were performed solely for the purposes of this study. These procedures were conducted outside of the laboratory's normal SOP and were not part of routine patient testing.

Samples were assigned to experimental groups using a systematic alternating assignment method (eg, 1, 2, and 3 minutes; repeating) to ensure even distribution. Vortexing was performed using a Fisher Scientific Vortex Mixer (speed range 300–3200 rpm) set to speed setting 5 (midrange intensity ~1500 rpm). Following agitation, samples were placed on a sample rotator for a minimum of 2 minutes to re-equilibrate and minimize the microbubbles caused by vortexing prior to aspiration. For postvortex CBC parameters, samples were reanalyzed on 1 of the 2 DxH 900 analyzers, selected according to instrument availability within the routine operational workflow. Following reanalysis, a postvortex smear was prepared using the DxH SMS. All sample analyses occurred after the DxH 900s and DxH SMS met quality control requirements according to the laboratory's procedures. The DxH 900s additionally met correlation study requirements according to the laboratory's procedures.

A prevortex and postvortex peripheral blood smear was prepared for every sample. Microscopic evaluation for the presence or absence of platelet clumps was performed by a single trained laboratory scientist systematically scanning the smear. The process of scanning each smear included reviewing the feathered edge, the lateral edges, and the monolayer, ensuring comprehensive coverage of the smear; any aggregation of 3 or more platelets observed during the scanning process resulted in a classification of platelet clumps present.[14]

The independent variable was the vortexing duration (1, 2, or 3 minutes). Dependent variables included the presence or absence of platelet clumps on the peripheral smear and the quantitative CBC parameters (WBC, RBC, HGB, HCT, and platelet counts).

The positive predictive value (PPV) of the DxH 900 platelet clump flag was calculated using manual smear review as the reference standard. The number of samples with persistent platelet clumps across the 3 vortexing time groups were compared using Fisher exact test. To assess sample integrity and ensure vortexing did not cause hemolysis or cellular damage, pre- and postvortex values for WBC, RBC, HBG, HCT, and platelet counts were compared using a Pearson correlation coefficient. A P value of less than 0.05 was used for statistical significance. Microsoft Excel (version 16.103.4) and R (version 4.5.2) were used for data analysis. This study was reviewed and approved by the Augusta University institutional review board as a minimal-risk project (2189545).

RESULTS

A total of 120 samples were selected for this study, all of which were flagged for platelet clumps by the DxH 900. On microscopic review of the prevortex peripheral blood smears (the reference standard), true platelet clumps were confirmed in 41 samples (true-positive samples). The remaining 79 samples were determined to be false-positive results because no clumps were visible on the smear despite the analyzer flag. With 41 confirmed true-positive findings out of 120 flagged samples, the PPV of the DxH 900 platelet clump flag for this cohort was 34.2%.

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Table 1. Microscopic evaluation for platelet clumps

Group	Prevortex	Postvortex	Total, *N*
1 minute	Absent	Absent	23
		Present	3
	Present	Absent	4
		Present	10
2 minutes	Absent	Absent	18
		Present	9
	Present	Absent	0
		Present	13
3 minutes	Absent	Absent	20
		Present	6
	Present	Absent	4
		Present	10

For the 41 true-positive samples, successful disaggregation (defined as the absence of clumps on the postvortex smear) was observed in 4 of 14 samples (29%) in the 1-minute group and 4 of 14 samples (29%) in the 3-minute group (Table 1). In contrast, the 2-minute vortexing group resulted in 0 of 13 samples with disaggregation. A Fisher exact test analysis comparing the disaggregation rates across the 3 time intervals indicated no statistically significant difference in efficacy between the vortexing durations ($P = .09$).

To assess whether mechanical agitation compromised cellular integrity, Pearson correlation coefficients were calculated comparing prevortex and postvortex CBC parameters (Table 2). The analysis demonstrated excellent stability across all cell lines, with strong positive correlations for WBC, RBC, HGB, HCT, and platelet counts (Figure 1). An incidental finding was observed in the 79 samples that were initially absent of clumps on the peripheral smear (Table 1). Following vortexing, 18 of these 79 samples (29%) demonstrated new platelet clumping on the postvortex smear.

DISCUSSION

The primary objective of this study was to evaluate the efficacy of varying vortexing durations to mechanically disaggregate platelet clumps in EDTA-anticoagulated specimens, thereby offering a workflow alternative to patient recollections. Our findings suggest that mechanical disaggregation, although not a universal solution, possesses distinct clinical utility as a noninvasive corrective action step. Although the overall success rate for complete disaggregation was approximately 29% in the 1- and 3-minute groups, this represents an opportunity to spare patients a second venipuncture.

Our results contextualize the findings of Mundt and Zhu et al,[12,13] who reported that vortexing was a reliable method for resolving platelet clumps. Mundt, using Sysmex XN series analyzers, discovered that vortexing eliminated platelet clump flags and allowed for the release of results in the majority of flagged cases. Similarly, Zhu et al demonstrated platelet clump disaggregation and significant increases in platelet counts postvortexing without compromising other hematologic parameters.[13] In our cohort, using the Beckman Coulter DxH 900, the success rate for true-positive samples was modest. The complete lack of disaggregation in the 2-minute group (0%) is curious and likely attributable to the small sample size of confirmed positive results in that subgroup, yet it underscores the unreliability of mechanical disaggregation. This discrepancy between our findings and prior studies may stem from differences in analyzer technology (impedance vs optical counting methods), the severity of clumping in the patient populations, or the intensity of sample vortexing. Even with a modest success rate, the ability to resolve platelet clumping without a recollection supports the integration of vortexing into the routine hematology workflow.

A significant finding of this study is the low PPV of the automated platelet clump flag (34.2%). The majority of flagged samples (79/120) contained no clumps on microscopic review. This aligns with recent findings by Woo et al,[14] who reported a similarly low PPV (41.5%) for platelet clumping flags on a Sysmex XN-9000. These high rates of false-positive results do not negate the value of vortexing but rather dictate where it should be placed in the laboratory workflow. Because "blind" vortexing of all flagged samples carries a risk of inducing artifacts in false-positive specimens, we propose a smear-first protocol. In this model, the laboratory scientist first reviews the peripheral smear to confirm the presence of clumps. If clumps are

Table 2. Complete blood cell count parameter comparisons

Parameter	Prevortex, Mean (±SD)	Postvortex, Mean (±SD)	Pearson Correlation, *r*	Significance, *P* Value
WBC, 10^3/μL	9.3 (±6.1)	9.1 (±5.9)	0.99	<.01
RBC, 10^6/μL	3.66 (±0.96)	3.65 (±0.96)	1.00	<.01
HGB, g/dL	10.9 (±2.9)	10.8 (±2.9)	1.00	<.01
HCT, %	32.4 (±8.7)	32.2 (±8.7)	1.00	<.01
PLT, 10^3/μL	181 (±117)	199 (±125)	0.97	<.01

HCT, hematocrit; HGB, hemoglobin; PLT, platelet; RBC, red blood cell; WBC, white blood cell.

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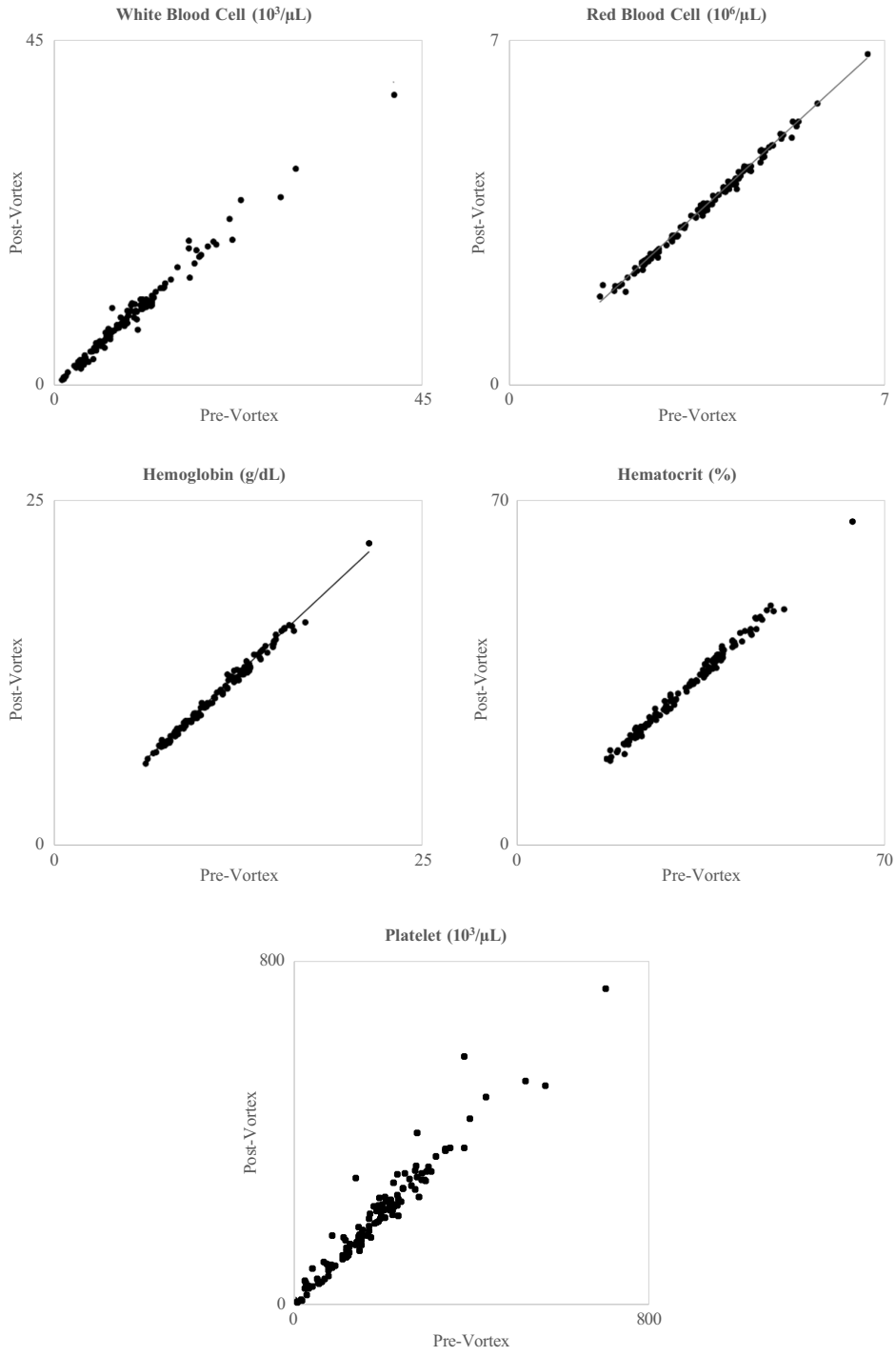

Figure 1. **Complete blood cell count parameter correlations.**

confirmed, vortexing is used as the immediate corrective action. If successful, the result is released; if unsuccessful, the laboratory proceeds to a sodium citrate recollection or pharmaceutical intervention.

A critical finding in our study was the introduction of new platelet clumps in 18 (29%) of the 79 samples that were initially negative for clumps. This phenomenon suggests that mechanical agitation is not a benign intervention for all specimens. Platelets are sensitive to shear stress, and vigorous vortexing may activate platelets in certain unstable samples, paradoxically creating the very artifact the method intends to resolve. Although we confirmed the findings of Zhu et al and Mundt regarding the stability of WBC, RBC, and HGB parameters (Pearson $r > 0.99$),[12,13] the potential to degrade platelet integrity in false-positive samples presents a clinical risk. The proposed approach of a smear-first protocol maximizes clinical utility. By verifying the presence of clumps first, the

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laboratory eliminates the risk of inducing aggregation in false-positive samples. Subsequently, by applying vortexing to the confirmed cases, the laboratory can potentially reduce the volume of recollection requests by approximately 30%.

Our study has several limitations. First, although the total sample size ($N = 120$) was sufficient to assess the PPV of the platelet clump flag, the number of confirmed true-positive samples ($N = 41$) limited the statistical power of subgroup analyses (eg, the 2-minute vortex group). Second, this single-center study used the DxH 900; results may differ on platforms employing alternative detection methodologies, such as fluorescence flow cytometry. Third, the inclusion criteria of less than 24 hours from collection led to lag times between prevortex and postvortex analysis. Additionally, because of prioritization of routine clinical workflow, there were delays between vortexing and reanalysis, which may have allowed platelets to reaggregate prior to the postvortex measurement. Fourth, 2 DxH 900 analyzers were used for prevortex and postvortex analysis based on instrument availability, and usage was not tracked during data collection; therefore, potential analyzer-specific variation could not be assessed. Finally, a fixed vortex speed was used; it remains unclear if altering the intensity would impact efficacy. Future research should explore if varying speeds, rather than duration, yield higher disaggregation rates and investigate the impact on time and resources for a smear-first vortexing protocol compared with sample recollection or pharmaceutical treatment.

In conclusion, mechanical disaggregation via vortexing is a potentially viable, cost-effective tool for managing EDTA-PTCP. Although it does not replace the gold standard of sodium citrate recollection for refractory cases,[2] it serves as an effective intermediate step. We recommend laboratory professionals explore a protocol that combines initial microscopic verification with a 1- to 3-minute vortexing step for confirmed clumps. This strategy balances patient safety by preventing artifact introduction while reducing the burden of unnecessary recollections.

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