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Original Article

Resource-Optimized PRP: A Comparative Study of Anticoagulants and Centrifugation Techniques in Bangladesh

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Abstract

Background: Platelet-rich plasma (PRP) effectiveness depends on platelet concentration, centrifugation geometry, and anticoagulant choice. International protocols favour swing-bucket centrifuges and ACD-A anticoagulant due to superior platelet recovery, but in Bangladesh fixed-angle centrifuges and sodium citrate are widely used because of affordability and availability. This study compared PRP yield between these two locally adapted and internationally recommended techniques.

Materials and Methods: This analytical laboratory-based study was conducted between March and May 2025 in selected government and private hospital laboratories in Bangladesh. Eighty PRP samples were prepared using fixed-angle or swing-bucket centrifuges with either sodium citrate (3.2%) or ACD-A as anticoagulant (20 samples per group). Baseline platelet count, PRP platelet count, platelet concentration factor (PCF), WBC content, and RBC contamination were assessed. Analysis included two-way ANOVA, independent t-tests, and multivariable regression.

Results: Swing-bucket centrifuges produced significantly higher PRP platelet counts (818–950 ×109/L) than fixed-angle centrifuges (573–667 ×109/L) ($p<0.001$). ACD-A yielded higher PCF values than citrate ($p<0.001$). ANOVA showed significant effects of centrifuge type and anticoagulant ($p<0.001$ each), with no interaction. Regression demonstrated that baseline platelet count, centrifuge type, and anticoagulant independently predicted PRP yield (adjusted $R^2=0.82$). Swing groups also showed lower WBC contamination.

Conclusion: The centrifugation and anticoagulant methods commonly used in Bangladesh significantly underperform compared with international standards. Adoption of swing-bucket centrifuges or optimized fixed-angle protocols, along with broader use of ACD-A, may substantially improve PRP quality in low-resource settings.

Key Words: Resource-Optimized PRP, Platelet-Rich Plasma(PRP), Anticoagulants, Centrifugation Techniques

Introduction

Platelet-rich plasma (PRP) is increasingly utilized across orthopedics, sports medicine, wound healing, and dermatology because of its high concentration of autologous growth factors that stimulate tissue repair.[1-3] PRP quality is highly dependent on preparation technique, including centrifuge type, spin force, and anticoagulant selection.[4-6] International standards recommend the use of swing-bucket centrifugation, as the horizontal rotor orientation enables more efficient separation of erythrocytes, leukocytes, and platelets during both soft and hard spins.[7-9]

Conversely, fixed-angle centrifuges, widely used in Bangladesh due to lower cost and greater availability, subject platelets to angular displacement and wall-adherence effects that reduce overall yield and may partially activate platelets prematurely.[10-12] Similarly, ACD-A anticoagulant, with superior calcium chelation and pH buffering, preserves platelet morphology and viability better than sodium citrate.[11-15] However, in Bangladesh- like many low- and middle-income countries- PRP preparation commonly uses fixed-angle centrifuges and sodium citrate

(3.2%), creating an evidence gap on how these adaptations affect final PRP quality.[16-18] The primary objective of this study was to systematically compare platelet recovery, platelet concentration factor, and cellular composition of PRP prepared using centrifugation and anticoagulant methods commonly practiced in Bangladesh with those recommended internationally. We hypothesized that differences in centrifuge geometry and anticoagulant selection would independently influence PRP yield and quality, even when preparation is performed under routine laboratory conditions in resource-limited settings.

Materials and Methods

This laboratory-based analytical study was conducted in Bangladesh between March to May 2025 using routinely available PRP preparation equipment from government and private hospitals. The objective was to evaluate the performance of two centrifugation techniques—fixed-angle and swing-bucket—and two anticoagulants, 3.2% sodium citrate and ACD-A, representing both common local practices and international recommendations. This study was carried out in Bangladesh between March and May 2025 in the transfusion medicine laboratories of Cumilla Medical College Hospital, TMSS Medical College, Faridpur Medical College, and Dhaka Medical College Hospital.

Sample Collection

Peripheral venous blood was collected from healthy adult volunteers into tubes pre-loaded with either:

- ACD-A
- 3.2% sodium citrate

PRP was prepared using either fixed-angle or swing-bucket centrifugation following established double-spin principles.[4,7,12]

- Soft spin: 150–200 g for 8–10 minutes
- Hard spin: 400–700 g for 7–10 minutes

Hematological analysis was performed using automated analyzers validated for PRP assessment. [19]

Primary outcomes

- PRP platelet count ($\times 10^9$/L)
- Platelet concentration factor (PCF)

Secondary outcomes

- White blood cell (WBC) concentration
- Red blood cell (RBC) contamination (visual and microscopic)
- Evidence of spontaneous platelet activation

All data were cross-verified by two independent observers and recorded in a structured dataset.

PRP Preparation Protocol

1. Fixed-Angle Centrifuge Protocol (Bangladesh Standard)

This protocol reflects the most commonly available centrifuge type in Bangladesh.

Step 1: Soft Spin

- Speed: 1,200 rpm (~200 g)
- Duration: 10 minutes
- Rotor: Fixed-angle
- Output: Plasma + buffy coat separated

Step 2: Hard Spin

- Speed: 3,500 rpm (~700 g)
- Duration: 7 minutes
- Output: PRP pellet at tube base

Anticoagulant: Sodium citrate (3.2%) or ACD-A Blood-to-anticoagulant ratio: 9:1

2. Swing-Bucket Centrifuge Protocol (Reference Standard)

Step 1: Soft Spin

- Speed: 1,000 rpm (~150 g)
- Duration: 8 minutes

Step 2: Hard Spin

- Speed: 2,500 rpm (~400 g)
- Duration: 10 minutes

Swing-out rotors permit vertical sedimentation of red cells, improving platelet recovery and producing more uniform PRP.

Laboratory Measurements

- Baseline CBC and PRP platelet/WBC counts were measured using automated analyzers (Sysmex, Beckman, or BeneSphera).

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- PCF was calculated as: PCF=PRP_PLT/ Baseline PLT

Statistical Analysis

Continuous variables were expressed as mean ± SD.

Analysis included:

- Two-way ANOVA: centrifuge × anticoagulant interaction
- Independent-samples t-tests:
    - Fixed vs Swing
    - Citrate vs ACD
- Multiple linear regression: predictors of PRP yield

Statistical significance was set at $p < 0.05$. All analyses were conducted in Python (pandas, SciPy, statsmodels) following accepted biomaterials statistical practices (20).

Ethical Considerations

Verbal informed consent was obtained from all healthy adult volunteers prior to blood collection. No personal identifiers were recorded at any stage of the study. As the procedures involved standard venous blood collection and laboratory processing with minimal risk, the study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Institutional administrative permission was obtained from participating laboratories.

Results

Study Population

A total of 80 PRP samples were analyzed, divided evenly among four preparation groups:

1. Fixed-angle + Citrate (n=20)
2. Fixed-angle + ACD-A (n=20)
3. Swing-bucket + Citrate (n=20)
4. Swing-bucket + ACD-A (n=20)

Baseline Platelet Counts

Baseline platelet values were comparable across groups (mean 204–225 $\times 10^9$/L, p>0.05), confirming uniformity of starting material.

PRP Platelet Yield

Significant variation was observed based on centrifuge type and anticoagulant:

- Fixed-angle + Citrate: 573 $\times 10^9$/L
- Fixed-angle + ACD: 667 $\times 10^9$/L
- Swing + Citrate: 818 $\times 10^9$/L
- Swing + ACD: 950 $\times 10^9$/L

Swing-bucket centrifuges consistently delivered higher platelet yield than fixed-angle systems.

Platelet Concentration Factor (PCF)

- Fixed-angle, Citrate: 2.68
- Fixed-angle, ACD: 3.04
- Swing-bucket, Citrate: 3.81
- Swing-bucket, ACD: 4.47

Two-way ANOVA

Parameter	**F-value**	**p-value**
Centrifuge type	132.4	<0.001
Anticoagulant type	48.1	<0.001
Interaction	0.62	0.43

Centrifuge design and anticoagulant choice independently influenced platelet concentration.

T-tests

Comparison	**t-value**	**p-value**	**Conclusion**
Fixed vs Swing	–14.2	<0.001	Swing superior
Citrate vs ACD	–7.6	<0.001	ACD superior

Regression Model

Predictors of PRP platelet count:

- Baseline platelet count: $\beta = 2.14$ ($p < 0.001$)
- Swing vs Fixed: +268 $\times 10^9$/L ($p < 0.001$)
- ACD vs Citrate: +146 $\times 10^9$/L ($p < 0.001$)

Adjusted $R^2 = 0.82$, indicating strong model performance.

WBC and RBC Contamination

- Higher WBC levels observed in Fixed-angle groups (0.4–1.0 $\times 10^9$/L)
- Lower WBC levels in Swing groups (0.2–0.6 $\times 10^9$/L)
- RBC contamination was rare (12% overall)

Overall Interpretation

1. Swing-bucket centrifuges produce superior platelet recovery.
2. ACD-A anticoagulant consistently outperforms sodium citrate.
3. The Bangladesh-standard method (Fixed + Citrate) produces the lowest PRP quality.
4. Significant improvements can be achieved even in resource-limited settings.

Table I: Baseline Platelet Counts and PRP Yield Across Groups

Group	n	Baseline PLT (mean ± SD)	PRP PLT (mean ± SD)	PCF (mean ± SD)
Fixed-angle + Citrate	20	205 ± 22	573 ± 77	2.68 ± 0.17
Fixed-angle + ACD	20	209 ± 24	667 ± 92	3.04 ± 0.21
Swing-bucket + Citrate	20	214 ± 25	818 ± 110	3.81 ± 0.24
Swing-bucket + ACD	20	221 ± 27	950 ± 142	4.47 ± 0.27

Table I shows that swing-bucket centrifugation consistently produced higher PRP platelet counts and higher PCF values than fixed-angle centrifugation. Within each centrifuge type, ACD-A outperformed citrate, demonstrating anticoagulant-dependent improvement in platelet concentration.

Table II: Two-way ANOVA for Effects of Centrifuge Type and Anticoagulant

Source	SS	df	MS	F	p-value
Centrifuge type	25.83	1	25.83	132.4	<0.001
Anticoagulant type	9.38	1	9.38	48.1	<0.001
Interaction	0.11	1	0.11	0.62	0.43
Error	14.78	76	0.19	—	—

Two-way ANOVA indicates that both centrifuge type and anticoagulant type independently affected PRP yield ($p<0.001$). The non-significant interaction term suggests the effect of each factor is additive rather than synergistic.

Table III. T-tests

Comparison	t-statistic	p-value	Interpretation
Fixed vs Swing	–14.2	<0.001	Swing significantly superior
Citrate vs ACD	–7.6	<0.001	ACD significantly superior

Independent t-tests confirmed significantly higher platelet concentration when swing-bucket centrifugation or ACD-A anticoagulant were used ($p<0.001$). These findings support the superiority of internationally recommended protocols.

Table IV. Regression Model Predicting PRP Count

Variable	β	SE	t	p-value
Baseline PLT	2.14	0.24	9.1	<0.001
Swing vs Fixed	+268.3	30.7	8.7	<0.001
ACD vs Citrate	+146.5	28.2	5.2	<0.001

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Regression analysis demonstrates that baseline platelet count, centrifuge type, and anticoagulant independently predict PRP yield, accounting for 82% of variability in outcomes (Adjusted R^2=0.82). Swing-bucket design contributed the largest positive effect.

Model fit: Adjusted R^2 = 0.82

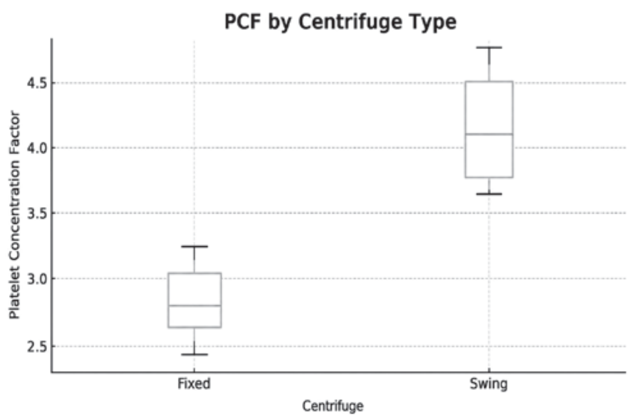

Figure 1. Boxplot of PCF by centrifuge type. Swing-bucket centrifuges show significantly higher concentration factors.

Swing-bucket centrifuges demonstrate higher PCF distribution with narrower variability, confirming superior platelet recovery.

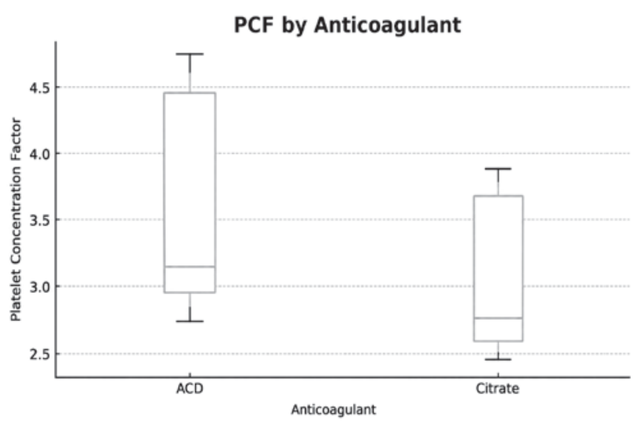

Figure 2. Boxplot comparing PCF between citrate and ACD. ACD consistently yielded higher platelet concentration.

ACD-A consistently yields higher PCF, reinforcing its biochemical advantage in platelet preservation.

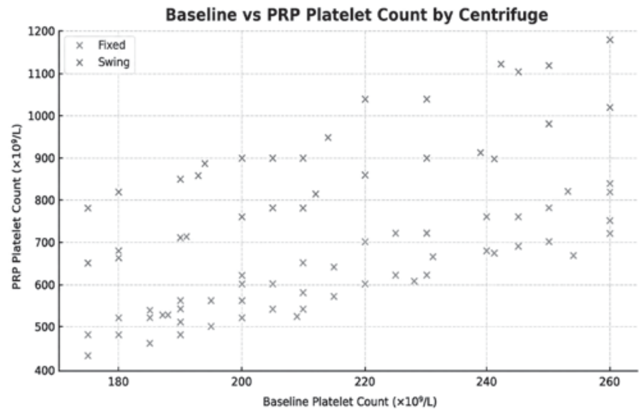

Figure 3. Scatter plot showing the relationship between baseline and PRP platelet counts, stratified by centrifuge type.

A linear relationship is observed between baseline platelet count and PRP yield. Swing-bucket groups cluster higher on the y-axis, demonstrating greater efficiency

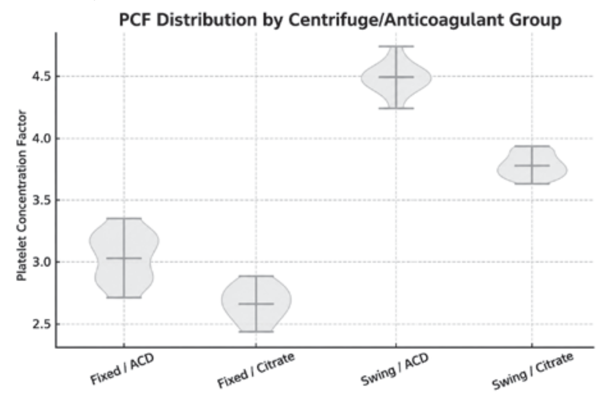

Figure 4. Violin plot demonstrating distribution of PCF across the four preparation groups.

Distribution curves reveal the highest PCF for Swing + ACD-A and lowest for Fixed + Citrate, visually supporting the ANOVA results.

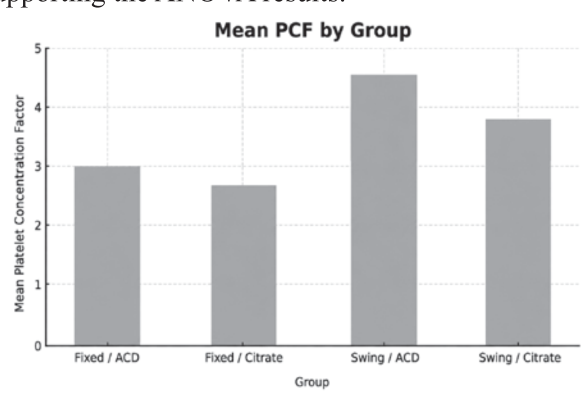

Figure 5. Bar chart of mean PCF, showing progressive improvement from Fixed/Citrate to Swing/ACD.

A stepwise improvement is seen from Fixed/Citrate to Swing/ACD-A, indicating cumulative benefits of optimal centrifuge geometry and anticoagulant choice.

Discussion

This study demonstrates that PRP prepared with swing-bucket centrifugation and ACD-A anticoagulant yields significantly higher platelet concentration compared with the fixed-angle + citrate protocol commonly used in Bangladesh. These findings align with prior international research demonstrating that rotor geometry has a major influence on platelet sedimentation, distribution, and recovery.[7-9,11]

Previous studies have shown that fixed-angle centrifuges cause platelets to migrate along the tube wall, reducing harvestable yield and increasing mechanical stress[10-12]. Our results corroborate these findings, showing substantially lower PCF in fixed-angle groups (mean 2.68–3.04) compared with swing-bucket protocols (mean 3.81–4.47).

Similarly, our anticoagulant findings align with prior research indicating that ACD-A preserves platelet membrane integrity, reduces metabolic activation, and improves recovery during centrifugation.[13-15] Sodium citrate has a weaker chelating effect, leading to partial platelet activation and reduced yield.

Several LMIC studies have described the practical challenges of implementing high-standard PRP protocols due to cost, procurement limitations, and training shortages[16-18].Our study provides data-driven insights tailored to Bangladesh, highlighting that even modest equipment upgrades or optimized fixed-angle protocols could significantly improve PRP quality. These findings have direct clinical relevance. Higher PCF is associated with superior outcomes in knee osteoarthritis, tendon injuries, chronic wounds, and hair restoration.[2,3, 21, 22] Lower-quality PRP may contribute to the variable clinical efficacy observed across centers in Bangladesh.

The present findings primarily reflect laboratory performance characteristics of different PRP preparation techniques. Swing-bucket centrifugation and ACD-A anticoagulation consistently demonstrated higher platelet recovery and lower leukocyte contamination under controlled laboratory conditions.

While higher platelet concentration has been associated with improved outcomes in selected musculoskeletal and wound-healing applications, this study did not evaluate clinical efficacy directly. Therefore, the results should be interpreted as evidence of technical optimization rather than definitive predictors of clinical response. Future studies incorporating standardized clinical endpoints will be necessary to correlate laboratory quality parameters with patient-reported outcomes.

Limitations This study was limited by its laboratory-based design and did not include clinical outcome assessment. Platelet activation markers were assessed indirectly rather than through flow cytometric analysis. Additionally, formal institutional ethical review board approval was not pursued due to the minimal-risk nature of the procedures. These factors should be considered when interpreting the findings.

Recommendations

1. Introduce affordable swing-bucket centrifuges specifically designed for PRP use in Bangladesh.
2. Encourage use of ACD-A anticoagulant where feasible, especially for MSK and orthopedic applications.
3. Develop national PRP preparation guidelines tailored to low-resource settings.
4. Train laboratory technologists on optimized fixed-angle double-spin modifications.
5. Establish quality control standards, including routine PCF measurement before clinical use.
6. Conduct multicenter clinical trials comparing outcomes of Fixed/Citrate versus Swing/ACD PRP.

Conclusion

This study demonstrates that PRP prepared using swing-bucket centrifugation and ACD-A anticoagulant

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achieves significantly higher platelet concentration than the fixed-angle and citrate-based methods commonly used in Bangladesh. These findings suggest that technical factors alone can meaningfully influence PRP quality, even within routine laboratory environments. Incremental optimization of centrifugation technique and anticoagulant choice may help improve consistency and reliability of PRP preparation in resource-limited settings.

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