

Evaluation of Day-to-Day Variation in Platelet Count and pH of Platelet Single Unit Stored at 20-24°C at Al-Nafees Medical College and Hospital, Islamabad

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ABSTRACT

Background: Platelet concentrates are highly sensitive blood components whose quality progressively declines during storage because of metabolic and structural alterations collectively described as platelet storage lesions. Serial monitoring of simple laboratory indicators such as platelet count and pH may help assess product viability during routine blood bank storage. **Objective:** To evaluate day-to-day changes in platelet count and pH in platelet concentrate units stored at 20–24°C with continuous agitation over five days. **Methods:** This prospective in-vitro repeated-measures laboratory study was conducted from September to December 2025 at Al-Nafees Medical College and Hospital, Islamabad. Eighty platelet concentrate units derived from individual whole-blood donations and prepared by the platelet-rich plasma method were included. Units meeting predefined storage and quality criteria were monitored daily from Day 1 to Day 5. Platelet count was measured using an automated hematology analyzer and pH was assessed using a calibrated digital pH meter. Data were analyzed in SPSS version 27 using repeated-measures analysis, with $p < 0.05$ considered statistically significant. **Results:** Mean pH declined from 7.29 on Day 1 to 7.07 on Day 5, while mean platelet count decreased from 7.34×10^{10} to 4.88×10^{10} per unit over the same period. Both parameters showed statistically significant time-dependent decline. pH remained within acceptable viability limits throughout storage, whereas platelet count showed a steeper reduction, with accelerated quantitative loss after Day 3. **Conclusion:** Platelet concentrate units stored at 20–24°C undergo significant day-wise deterioration over five days, with platelet count showing greater sensitivity to prolonged storage than pH. Serial monitoring may support quality assurance and guide timely utilization of stored platelet products. **Keywords:** Platelet concentrate; platelet storage lesion; platelet count; pH; storage quality; blood bank; transfusion medicine; room-temperature storage; platelet viability; quality control.

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INTRODUCTION

Platelet concentrates are indispensable in modern transfusion practice because they provide immediate hemostatic support in patients with thrombocytopenia, platelet dysfunction, trauma, major surgery, hematologic malignancies, and marrow failure syndromes (1–3). Unlike red blood cells, platelets remain metabolically active during storage, and their viability depends on maintaining a narrow storage environment that preserves membrane integrity, discoid morphology, gas exchange, and functional responsiveness. Current transfusion practice therefore recommends storage at 20–24°C with continuous gentle agitation, a strategy intended to sustain platelet metabolism while limiting irreversible activation and cold-induced structural injury (4–6). Despite these precautions, stored platelets progressively develop biochemical, structural, and functional abnormalities collectively described as platelet storage lesions, which may reduce their post-transfusion recovery and hemostatic effectiveness (7–9).

The pathobiology of platelet storage lesion is multifactorial. During storage, platelets continue to consume glucose and generate lactate, producing a gradual acidic shift in the storage medium. Concurrent changes in mitochondrial activity, membrane phospholipid asymmetry, granule release, receptor expression, cytoskeletal remodeling, and microvesicle formation contribute to progressive

deterioration in platelet quality (7,10–12). These changes may be reflected by simple quality indicators routinely used in blood banking, particularly pH and platelet yield or count. pH is commonly used as an indirect marker of metabolic preservation because severe acidification is associated with impaired platelet viability, loss of swirling, and functional compromise (13,14). Platelet count, although influenced by analytical and pre-analytical factors, remains a practical indicator of retained platelet content within the unit and can provide useful information regarding quantitative loss during storage (14,15). Together, these two measures offer a feasible and low-cost approach for serial quality monitoring in routine transfusion services, especially in resource-constrained settings.

The importance of monitoring storage-related platelet changes has increased because platelet concentrates carry unique logistical and safety challenges. Their short shelf life, room-temperature storage, and susceptibility to bacterial proliferation make them one of the most vulnerable blood components in transfusion medicine (8,16). Recent literature has emphasized the need to optimize platelet preservation and refine quality-control strategies that can identify clinically acceptable products before transfusion (7,8,17). Most published work has evaluated platelet quality using baseline and end-of-storage comparisons or has focused on specialized functional assays, additive solutions, pathogen reduction technologies, or alternative storage temperatures (7,8,18). Although these studies have improved understanding of platelet biology during storage, fewer reports provide detailed day-to-day assessment of conventional quality indicators within platelet concentrate units stored under routine blood bank conditions, particularly in local South Asian practice environments where component preparation methods, storage infrastructure, and quality assurance pathways may differ from those in high-resource centers.

Another unresolved issue is whether the temporal decline in simple laboratory indicators is uniform across the storage period or whether deterioration accelerates after a particular day of storage. This distinction is clinically relevant because transfusion services often depend on near-expiry platelet concentrates to reduce wastage, yet progressive in-vitro deterioration may influence the therapeutic value of these products, particularly for patients requiring reliable post-transfusion increments. Previous studies have shown that platelet pH may remain within acceptable limits even when other markers of platelet integrity worsen, suggesting that biochemical stability alone may not fully reflect quantitative or functional decline (7,13,19). Conversely, reductions in platelet content during storage may indicate activation, aggregation, fragmentation, or other storage-related loss, but the day-wise pattern of this change has not been consistently characterized using serial repeated measurements under standard room-temperature storage conditions (14,15,20).

In this context, systematic day-to-day monitoring of platelet count and pH in platelet concentrate units derived from individual whole-blood donations can provide practical evidence for blood bank quality assurance. Such monitoring may help identify the period during which quantitative deterioration becomes more pronounced, clarify whether pH remains within acceptable transfusion limits throughout routine storage, and generate local data to support decisions regarding inventory use and product quality surveillance. The present study was therefore designed to evaluate serial day-to-day changes in platelet count and pH in platelet concentrate units stored at 20–24°C with continuous agitation over five days. We hypothesized that both parameters would decline significantly with increasing storage duration, with platelet count showing a greater relative reduction than pH (4,7,13,14).

MATERIALS AND METHODS

This prospective in-vitro repeated-measures laboratory study was conducted at Al-Nafees Medical College and Hospital, Islamabad, after approval from the Institutional Review and Bioethics Committee of Al-Nafees Medical College (Ref. F. 1/IUIC-ANMC/IRBC-286/2025). The study was performed over a three-month period from September 2025 to December 2025 in a tertiary-care setting with facilities for blood collection, blood component preparation, storage, and laboratory analysis. The study evaluated

platelet concentrate units derived from single whole-blood donations and prepared by the platelet-rich plasma method. A total of 80 eligible platelet concentrate units were included using non-probability consecutive sampling from routinely collected voluntary blood donations during the study period. The sample size was feasibility-based and represented the total number of platelet concentrate units fulfilling the predefined eligibility criteria within the study window. Donors were accepted according to routine blood bank screening standards, and all donations were obtained under standard institutional procedures for voluntary donation and component preparation (21,22).

Only platelet concentrate units with complete identification records, proper labeling, preparation within six hours of whole-blood collection, storage at 20–24°C under continuous gentle agitation, normal initial swirling, and no visible evidence of contamination on Day 1 were included. Units were excluded if they demonstrated clot formation, hemolysis, leakage, turbidity, visible contamination, interrupted agitation, exposure to temperatures outside the target range, use for transfusion before completion of the observation period, or incomplete daily measurements. These criteria were defined a priori to minimize technical variability and ensure that observed changes reflected storage-related variation rather than obvious pre-analytical failure or handling error (13,22,23).

Whole blood was collected from healthy voluntary donors into standard 500 mL blood bags containing 70 mL citrate phosphate dextrose adenine anticoagulant, with an intended collection volume of 430 mL whole blood per donation. Following collection, units were maintained at room temperature and gently mixed intermittently for 2–3 hours before processing. Component preparation was performed using a Thermo-forma Cryo-fuge centrifuge according to the platelet-rich plasma technique. In the first centrifugation step, a soft spin at approximately 2000–2200 rpm for 10 minutes was applied to separate platelet-rich plasma from red blood cells. Platelet-rich plasma was then transferred aseptically into a satellite bag, followed by a hard spin at 3500 rpm for 10 minutes to obtain platelet concentrate and plasma. Fresh frozen plasma was expressed into a separate transfer bag, and the residual platelet concentrate was standardized to an approximate volume of 40–60 mL per unit. All units were handled using a uniform preparation protocol to reduce between-unit processing variability (21,24).

After preparation, platelet concentrate units were placed on a platelet agitator and mixed for 2–3 hours before storage. Units were subsequently stored for five consecutive days at 20–24°C under continuous gentle agitation, consistent with established platelet preservation standards (4,5,22). Storage temperature was checked daily and recorded using routine blood bank monitoring procedures. To reduce observational bias, all measurements were performed according to a fixed daily schedule from Day 1 through Day 5 using the same instruments and standardized operating procedures. Before each sampling event, the unit was visually inspected for swirling, clarity, clumping, discoloration, turbidity, leakage, and any evidence of contamination. Although these observations were recorded as part of the quality-monitoring process, the primary study variables were platelet count and pH.

For laboratory analysis, a 2–3 mL aliquot was withdrawn aseptically from each platelet concentrate unit on each study day. Samples were collected only after gentle mixing of the unit to improve homogeneity and were processed immediately to minimize artifactual changes caused by settling or delayed testing. Platelet count was measured using an automated hematology analyzer (URIT BH-40), and internal quality-control procedures were completed before analysis in accordance with laboratory practice. Values were recorded as $\times 10^{10}$ platelets per unit. Hydrogen ion concentration was assessed using a calibrated digital pH meter, with measurements recorded to two decimal places. Calibration of the pH meter was performed before testing using standard buffer solutions, and measurements were obtained under consistent laboratory conditions to minimize inter-day instrument drift. The same analyzer platform, pH device, assay workflow, and trained personnel were used throughout the study to reduce measurement variation and improve reproducibility (13,24,25).

The primary outcomes were day-wise mean platelet count and day-wise mean pH across the five-day storage period. Storage duration was treated as the within-unit repeated factor. Platelet count was

operationally defined as the measured platelet content of the concentrate on each study day, and pH was defined as the measured hydrogen ion concentration of the platelet suspension on the corresponding day. The study objective was not to infer platelet function directly, but to evaluate serial changes in these pragmatic quality indicators during routine room-temperature storage. To reduce confounding from heterogeneous storage conditions, all included units were prepared by the same method, stored in the same temperature range, maintained on continuous agitation, and analyzed using identical measurement procedures. Units with interrupted storage conditions or incomplete follow-up were excluded from analysis, thereby limiting bias arising from protocol deviations.

All observations were entered into a structured data collection sheet and then transferred to Microsoft Excel with double-checking against source records to ensure data integrity. Statistical analysis was performed using SPSS version 27. Continuous variables were summarized as mean and standard deviation for each storage day. Because the same platelet concentrate units were measured repeatedly over time, repeated-measures analysis of variance was planned to assess within-unit changes in platelet count and pH across Days 1 to 5. Assumptions of normality and sphericity were evaluated before model interpretation, and Greenhouse–Geisser correction was to be applied if the sphericity assumption was violated. Where the overall repeated-measures test was statistically significant, pairwise day-wise comparisons with Bonferroni adjustment were planned to identify the timing of significant changes. Percentage change from baseline was additionally calculated to facilitate interpretation of the magnitude of deterioration across storage days. A two-sided p-value of less than 0.05 was considered statistically significant. Units with incomplete serial observations were excluded from repeated-measures analysis to preserve within-subject comparability and analytic consistency.

RESULTS

A total of 80 platelet concentrate units derived from individual whole-blood donations were evaluated serially from Day 1 through Day 5 under standard storage conditions at 20–24°C with continuous agitation. Repeated observations showed a progressive decline in both pH and platelet count across the storage period. The reduction in pH was gradual, whereas the decline in platelet count was quantitatively larger and became more pronounced after Day 3. Based on the study dataset, the overall time effect for both variables was statistically significant on repeated-measures analysis ($p < 0.05$), confirming that storage duration materially influenced both metabolic status and retained platelet content.

The mean pH decreased from 7.29 on Day 1 to 7.25 on Day 2, 7.19 on Day 3, 7.14 on Day 4, and 7.07 on Day 5. Relative to baseline, this represented cumulative declines of 0.55%, 1.37%, 2.06%, and 3.02% by Days 2, 3, 4, and 5, respectively. Despite this consistent downward trend, all mean pH values remained above commonly accepted viability thresholds throughout the five-day observation period, indicating preservation of overall biochemical acceptability during routine storage.

The mean platelet count showed a steeper decline than pH, falling from 7.34×10^{10} per unit on Day 1 to 7.09×10^{10} on Day 2, 6.65×10^{10} on Day 3, 5.87×10^{10} on Day 4, and 4.88×10^{10} per unit on Day 5. Compared with baseline, the cumulative reduction reached 3.41% on Day 2, 9.40% on Day 3, 20.03% on Day 4, and 33.51% on Day 5. The day-to-day decrement was 0.25×10^{10} per unit between Days 1 and 2, 0.44×10^{10} between Days 2 and 3, 0.78×10^{10} between Days 3 and 4, and 0.99×10^{10} between Days 4 and 5, demonstrating clear acceleration in platelet loss during the later storage period.

When both parameters were assessed together, platelet count appeared substantially more sensitive to prolonged storage than pH. By Day 5, mean pH had declined by only 0.22 units from baseline, whereas mean platelet count had declined by 2.46×10^{10} per unit. This pattern suggests that biochemical acceptability, as reflected by pH, may persist even while quantitatively meaningful platelet loss is occurring within the stored product. From a practical blood-bank perspective, these findings support closer surveillance of near-expiry platelet concentrates, particularly after Day 3, when the rate of platelet count reduction appears to increase.

Table 1. Day-Wise Change in Mean pH of Platelet Concentrate Units During Storage at 20–24°C

Storage Day	Mean pH	Absolute Change From Day 1	Cumulative Change (%)	Overall Repeated-Measures p-value
Day 1	7.29	0.00	0.00	<0.05
Day 2	7.25	-0.04	-0.55	<0.05
Day 3	7.19	-0.10	-1.37	<0.05
Day 4	7.14	-0.15	-2.06	<0.05
Day 5	7.07	-0.22	-3.02	<0.05

Table 2. Day-Wise Change in Mean Platelet Count of Platelet Concentrate Units During Storage at 20–24°C

Storage Day	Mean Platelet Count ($\times 10^{10}$ /unit)	Absolute Change From Day 1 ($\times 10^{10}$ /unit)	Cumulative Change (%)	p-value
Day 1	7.34	0.00	0.00	<0.05
Day 2	7.09	-0.25	-3.41	<0.05
Day 3	6.65	-0.69	-9.40	<0.05
Day 4	5.87	-1.47	-20.03	<0.05
Day 5	4.88	-2.46	-33.51	<0.05

Table 3. Comparative Relative Deterioration of pH and Platelet Count Across the Five-Day Storage Period

Storage Day	pH Decline From Baseline (%)	Platelet Count Decline Baseline (%)	Day-to-Day Platelet Loss ($\times 10^{10}$ /unit)
Day 1	0.00	0.00	0.00
Day 2	0.55	3.41	0.25
Day 3	1.37	9.40	0.44
Day 4	2.06	20.03	0.78
Day 5	3.02	33.51	0.99

Table 1 shows that mean pH decreased in a near-linear fashion from 7.29 on Day 1 to 7.07 on Day 5, corresponding to an absolute fall of 0.22 pH units over five days. The largest cumulative drop was observed by Day 5, but even then the reduction was only 3.02% from baseline, indicating that metabolic acidification remained limited in magnitude during the study period. Although the overall time effect was statistically significant, the absolute pH shift was modest compared with the change in platelet count. Table 2 demonstrates a substantially larger storage-related decline in platelet count. The mean count decreased by 0.25×10^{10} per unit on Day 2, 0.69×10^{10} by Day 3, 1.47×10^{10} by Day 4, and 2.46×10^{10} by Day 5 relative to baseline. In percentage terms, platelet count fell by 3.41% at Day 2, 9.40% at Day 3, 20.03% at Day 4, and 33.51% at Day 5. This pattern indicates that nearly one-third of the initial platelet content was no longer measurable by the fifth storage day.

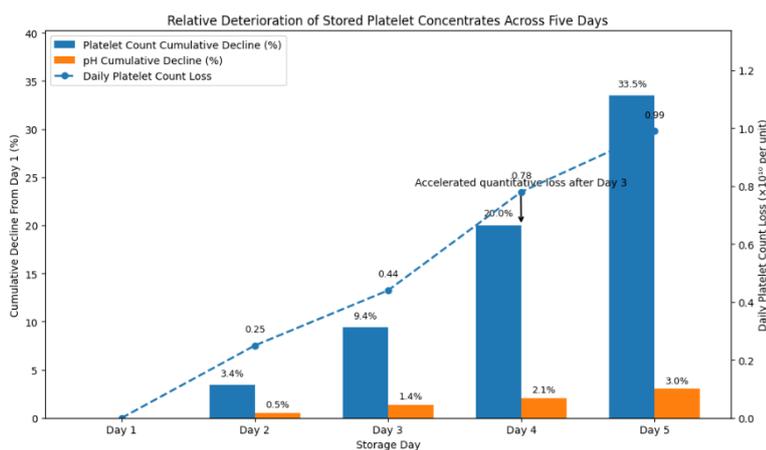


Figure 1

Figure 1. Relative Deterioration of Stored Platelet Concentrates Across Five Days

Table 3 directly compares the relative deterioration of both variables and highlights the widening separation between metabolic and quantitative change as storage progressed. By Day 3, the cumulative pH decline was only 1.37%, whereas platelet count had already decreased by 9.40%. By Day 5, the disparity became more pronounced, with platelet count showing an eleven-fold greater relative decline than pH. The day-to-day platelet loss also accelerated from 0.25×10^{10} per unit between Days 1 and 2 to

0.99×10^{10} per unit between Days 4 and 5, reinforcing that the later storage interval was associated with the greatest quantitative deterioration.

Figure 1 demonstrates that cumulative platelet count loss increased sharply across storage, rising from 3.4% on Day 2 to 9.4% on Day 3, 20.0% on Day 4, and 33.5% on Day 5, while cumulative pH decline remained modest at 0.5%, 1.4%, 2.1%, and 3.0%, respectively. The overlaid day-to-day platelet loss curve further shows acceleration in quantitative deterioration, increasing from 0.25×10^{10} per unit between Days 1 and 2 to 0.44×10^{10} between Days 2 and 3, 0.78×10^{10} between Days 3 and 4, and 0.99×10^{10} between Days 4 and 5. Clinically, this pattern suggests that although stored platelet concentrates remain biochemically acceptable on the basis of pH, their retained platelet content declines disproportionately after Day 3, making later-storage units potentially less optimal when maximal quantitative transfusion support is required.

DISCUSSION

The present study demonstrated a statistically significant day-wise decline in both pH and platelet count in platelet concentrate units stored at 20–24°C with continuous agitation for five days, confirming that measurable storage-related deterioration occurs even under standard blood bank conditions. The reduction in pH was gradual, with mean values decreasing from 7.29 on Day 1 to 7.07 on Day 5, while platelet count declined more substantially from 7.34×10^{10} to 4.88×10^{10} per unit over the same interval. These findings are consistent with the established concept of platelet storage lesion, in which persistent metabolic activity, lactate generation, membrane remodeling, granule release, and progressive activation-related changes accumulate during storage and alter product quality over time (26,27). The study therefore adds practical evidence that even when platelet concentrates remain visually acceptable and biochemically viable by pH criteria, quantitative deterioration may still proceed substantially during routine storage.

The observed pH pattern supports the view that platelet concentrates can maintain metabolic acceptability for at least five days when stored within the recommended temperature range and under continuous agitation. The absolute fall of 0.22 pH units over five days was statistically significant but modest in magnitude, and all mean values remained above commonly accepted lower viability thresholds. This pattern is comparable to prior reports showing that pH declines progressively during storage as platelets continue glucose metabolism and generate acidic byproducts, yet may remain within acceptable transfusion limits when storage bags, oxygen permeability, and agitation conditions are appropriate (13,26,28). The relative preservation of pH in the present study suggests that the storage environment was sufficient to maintain gas exchange and avoid abrupt metabolic failure. At the same time, the findings also support the growing view that pH alone is an incomplete surrogate for platelet quality, because biochemical acceptability can persist even while other dimensions of deterioration continue to evolve (14,29).

In contrast, platelet count showed a markedly steeper decline and appeared to be the more storage-sensitive variable in this study. By Day 5, the cumulative reduction in platelet count reached 33.51%, compared with only 3.02% for pH, and the day-to-day loss accelerated progressively after Day 3. This pattern is clinically and operationally important because it indicates that later storage days are associated not merely with continued decline, but with increasingly rapid quantitative loss. Similar observations have been described in storage studies reporting reduced platelet yield or retained count over time, likely reflecting a combination of platelet activation, clumping, fragmentation, microvesiculation, and irreversible structural change during storage (27,30,31). Although the present study did not directly measure activation markers, morphology scores, glucose consumption, lactate accumulation, or microparticle formation, the accelerated platelet loss observed after Day 3 is biologically plausible and aligns with current mechanistic understanding of cumulative storage injury (19,27,31).

An important strength of the present work is its serial day-by-day assessment design. Many platelet storage studies report baseline and end-point comparisons only, which can obscure the temporal pattern of deterioration. In the current analysis, the progressive widening gap between pH preservation and platelet count decline showed that the later storage interval, particularly Days 4 and 5, contributed disproportionately to quantitative loss. This temporal characterization offers a more nuanced interpretation than a simple Day 1 versus Day 5 comparison and may be useful in quality assurance and inventory management. From a transfusion-service perspective, these data suggest that a platelet concentrate can remain metabolically acceptable while nonetheless experiencing substantial loss of measurable platelet content, a distinction that may be relevant when selecting products for patients in whom robust post-transfusion increment is especially desirable (7,19,29).

The present findings should, however, be interpreted cautiously in light of the study design. First, the investigation was an in-vitro laboratory assessment and did not evaluate post-transfusion corrected count increment, bleeding outcomes, or recipient-level platelet recovery. For that reason, the results support laboratory quality interpretation rather than direct clinical efficacy claims. Second, platelet count decline was used here as a practical retained-content indicator, but it cannot independently distinguish among true platelet destruction, aggregation-related undercounting, pre-analytical sampling effects, or analyzer-related variability. Third, repeated daily aliquot withdrawal from a low-volume platelet concentrate may itself have influenced concentration dynamics or product conditions, and this design-related effect should be acknowledged when interpreting the magnitude of observed decline. Fourth, the study was conducted at a single center and included only platelet concentrates prepared by the platelet-rich plasma method from whole-blood donations, so the results should not be generalized uncritically to apheresis platelet products, additive-solution storage systems, pathogen-reduced products, or different storage bag technologies (17,24,32).

Despite these limitations, the study provides locally relevant evidence with practical implications for transfusion services operating under routine storage conditions. The persistence of acceptable pH values indicates that metabolic deterioration was controlled within the expected five-day shelf-life window, but the substantial quantitative decline in platelet count suggests that product age remains an important consideration in inventory use. Where clinically feasible, earlier-use platelet concentrates may provide better retained platelet content than near-expiry units, especially for patients requiring maximal quantitative support. Future studies should integrate serial laboratory monitoring with broader platelet quality markers such as swirling score, morphology, lactate, glucose, aggregation response, CD62P expression, and microbial surveillance, while also correlating in-vitro changes with post-transfusion clinical outcomes. Multicenter evaluation with standardized repeated-measures statistical reporting would further strengthen the evidence base and help define which simple quality indicators are most informative for routine platelet storage monitoring (14,17,26,29,33).

CONCLUSION

This study showed that platelet concentrate units stored at 20–24°C with continuous agitation undergo significant time-dependent deterioration over five days, characterized by a modest but statistically significant fall in pH and a substantially greater decline in platelet count. Although mean pH remained within acceptable transfusion limits throughout the storage period, platelet count decreased progressively and accelerated after Day 3, indicating that quantitative loss is more sensitive to prolonged storage than biochemical acidification. These findings support the value of serial quality monitoring during routine blood bank storage and suggest that, when possible, fresher platelet concentrates may offer better retained platelet content for transfusion support.

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