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Short communication

Comprehensive Evaluation of Pooled Red Blood Cell Saline Suspensions Used in Routine Blood Typing: A Resource Optimisation Laboratory-Based Study

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1. Background

Preparation of 2–5% pooled A, B, and O red cell suspensions (RCS) in 0.9% normal saline (NS) is a routine and essential practice in most blood centres to ensure accurate reverse/plasma testing while performing ABO and Rh(D) blood typing [1]. These standardized RCS form the backbone of immunohematology (IH) testing because of their consistent antigen expression, reproducibility, and minimal background reactivity [2,3]. Despite their widespread use, the requirement to prepare RCS fresh each day is both labour-intensive and resource-consuming, particularly in high-volume transfusion laboratories. Current literature offers limited data on the biological and functional stability of 5% pooled RCS stored under controlled refrigeration [4]. Evidence-based guidance on safe reuse duration is lacking, even though such a protocol could substantially reduce workload, reagent consumption, and operational costs without compromising test accuracy. This study, therefore, primarily aimed to determine the stability and reusability window of 5% pooled RCS prepared in 0.9% NS when stored at 4 ± 2°C. Secondary objectives included assessing agglutination strength, hemolysis, pH changes, red cell morphology, and sterility over the storage period to establish a scientifically validated reuse protocol for routine IH laboratories.

2. Materials and methods

2.1. Study setting and design

This prospective, laboratory-based study was conducted in the Department of Immunohematology and Blood Transfusion, Himalayan Institute of Medical Sciences (HIMS), Swami Rama Himalayan University (SRHU), Dehradun. The work was undertaken as part of an intramural research initiative following approval from the Institutional Research Committee (Ref No.: HIMS/RC/2025/306; dated 18 August 2025) and the Institutional Ethics Committee (Ref No.: SRHU/HIMS/E-1/2025/283; dated 28 July 2025).

2.2. Preparation of the Pooled Red Cell Suspension (RCS)

A 5% pooled A Rh(D)-positive RCS was prepared using aliquots from three EDTA-anticoagulated donor samples, following the departmental standard operating procedure (SOP). Donor red cells were washed thoroughly with isotonic 0.9% normal saline (NS) and subsequently resuspended to achieve a final 5% concentration. The prepared suspension was stored at 4 ± 2°C, and stability assessments were performed daily for seven consecutive days at room temperature (22 ± 2°C) in the transfusion laboratory. Thus, in routine use, pooled RCS were retrieved intermittently from 4 ± 2°C storage, briefly exposed to room temperature during testing, and then returned to refrigeration. Therefore, the storage protocol at 4 ± 2°C, simulated routine laboratory handling and transient room-temperature exposure, while tests were performed, was not expected to significantly alter the stability or outcomes within the studied time frame.

2.3. Daily evaluation parameters

Daily testing consisted of noting down agglutination strength (Grade 4+ to 0) with anti-A and anti-Rh(D) antisera [Tulip Diagnostics Pvt Ltd., Goa, India], the time taken for agglutination to appear (in seconds), calculation of percent hemolysis both visually and spectrophotometrically at 540 /541 nm [Digital Photo Electric Colorimeter, TANCO, New Delhi, India]. Sterility was tested on nutrient and blood agar on day zero and day seven. pH was recorded using a calibrated pH meter [Deluxe Digital pH METER-101, Electronics India] at set intervals. Microscopy performed at 40× magnification examined red cell morphology. Testing was performed sequentially until either the expected serological agglutination reactions were no longer observed and/or visible hemolysis occurred.

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2.4. Percent hemolysis calculation

The percent hemolysis was calculated by comparing the absorbance of a sample to both a negative control (0% hemolysis, e.g., saline) and a positive control (100% hemolysis, typically achieved with distilled water to lyse all cells). The percentage of hemolysis was calculated using the formula [4]:

$$\%\ \text{Hemolysis} = \frac{\text{Test (Av)} - \text{NC (Av)} \times 100\%}{\text{PC (Av)} - \text{NC (Av)}}$$

where Av refers to the (absorbance value) reading at the selected wavelength(s). PC means positive control and NC means negative control. We continued the testing until a decline in expected agglutination reactions or visible hemolysis was observed. The detailed day-wise observations from day Zero to 7, including OD values, hemolysis %, pH, agglutination grade, and reaction time were noted and extracted from the laboratory work-up.

3. Results

A progressive increase in hemolysis was observed across the seven-day storage period. Percent hemolysis rose from 0.09% on Day 0 to 0.28% (Day 1) and 0.62% (Day 2), exceeding the accepted 1% limit by Day 3 (1.22%) and reaching 6.86% by Day 7, indicating a substantial decline in red cell membrane integrity beyond 48 h of storage. The pH showed only a mild and gradual decline, remaining within acceptable biological limits throughout the study period (6.90 on day 0, 6.86 on day 3, and 6.76 on day 7). The agglutination strength and reaction time exhibited a marked deterioration, with day 0 showing strong 4+ grade of strength of agglutination within 1 s, decreasing to 3+ grade of strength at 4 s on day 2, 2+ grade of strength at 8 s by day 3, and coming down to 1+ grade of strength at 20 s on day 7, indicating loss of expected reactivity after second day. Microscopy consistently revealed normal red cell morphology without echinocytes, spherocytes, or debris, and sterility was maintained throughout, with no microbial growth observed even on seventh day. A consolidated summary of stability trends is presented (Table 1).

4. Discussion

This study highlights a clear distinction between the biological stability (morphology, sterility, and pH) and functional stability (agglutination and hemolysis) of 5% RCS stored in 0.9% normal saline. Although biological stability remained largely preserved throughout the seven-day observation period, the progressive rise in hemolysis and decline in agglutination strength emerges as the principal limiting factors for their continued use in IH laboratory. Functionally, particularly antigenic reactivity measured through agglutination strength—is essential for the accuracy of routine blood grouping and reagent quality assurance [5]. The decline from a strong 4+ reaction on Day 0 to 2 + by Day 3 suggests diminishing antigen availability on the red cell surface. This reduction may be attributed to membrane instability, micro-hemolysis, and loss of surface integrity, all of which impair antigen–antibody interactions. Previous studies have similarly noted that stored RCS show a reduction in potency beyond 48–72 h, driven by biochemical shifts such as falling pH and increasing extracellular haemoglobin, which interfere with lattice formation during agglutination [6]. Additionally, the hemolysis trends observed in our study also have regulatory relevance. International standards—including those by the US FDA and the Council of Europe—permit up to 1.0% and 0.8% hemolysis, respectively, in stored red cell units prior to transfusion [7]. In our findings, the 1% threshold was crossed by Day 3, substantiating that functional degradation is well underway at this time point. Thus, even though the suspension remained biologically acceptable, its diagnostic reliability had already declined. These observations directly support our study objective of establishing a safe and resource-efficient reuse limit for pooled RCS. The evidence clearly indicates that functional parameters essential for accurate blood typing are preserved only within the first 48 h. From an immunohematological standpoint, ABO antigens i.e., A and B are structurally comparable carbohydrate antigens expressed on the red cell membrane. Therefore, deterioration in red cell membrane integrity, hemolysis, and storage-related biochemical changes would be expected to affect B antigen expression in a similar manner as that of A antigen expression. Nevertheless, the authors would like to acknowledge an important limitation in this research that a direct experimental evaluation of pooled B pooled RCS, similar to A pooled RCS, would strengthen generalizability, and this was left as an area for future investigation. Also, the exclusive use of 0.9% normal saline was intentional, reflecting the most economical and widely available reagent in resource-limited laboratories. Nevertheless, red cell storage solutions may enhance stability by reducing hemolysis and represent a potential avenue for extending shelf life in future studies. In fact, the reagent preparation practices vary globally and that stabilizing solutions may be considered where resources permit.

To sum up, although 5% pooled RCS in 0.9% NS maintained sterility, morphology, and acceptable pH levels for up to seven days, significant functional deterioration—marked by rising hemolysis and reduced agglutination—occurred after day 2. Therefore, we recommend that 5% RCS stored at 4 ± 2°C may be safely reused for ABO and Rh(D) typing for up to 48 h, but not beyond, to ensure accuracy in routine immunohematology workflows.

Ethical approval

Ethical clearance was sought and obtained from the Institutional Research Committee [Ref No.: HIMS/RC/2025/306; dated 18 August 2025) and the Institutional Ethics Committee (Ref No.: SRHU/HIMS/E-1/2025/283; dated 28 July 2025).

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Table 1
Summarizing the stability trends of 5% a pooled RCS in 0.9% normal saline.

Day	% Hemolysis	pH (units)	Agglutination strength (Grade) using anti-A antisera	Agglutination time (s)	Morphological changes in the red cells	Sterility
0	0.09	6.9	4+	1	Microscopy revealed intact, uniformly shaped red cells without debris or any other morphological changes	Sterile throughout
1	0.28	6.89	4+	2		
2	0.62	6.87	3+	4		
3	1.22	6.86	2+	8		
4	1.86	6.84	2+	13		
5	2.33	6.82	2+	15		
6	4.39	6.78	2+	18		
7	6.86	6.76	1+	20		

Disclosure of potential conflicts of interest

Authors declare no conflicts of interest.

CRediT authorship contribution statement

Manish Raturi: Conceptualization, Methodology, Investigation, Resources, Formal analysis, Writing – review & editing, Project administration, Validation. **Dushyant Gaur S.:** Investigation, Resources, Methodology, Formal analysis, Writing – original draft, Visualization, Validation. **Purshotam Paudel:** Writing – review & editing, Supervision, Validation. **Yashaswi Dhiman:** Writing – review & editing, Supervision, Validation. **Brajesh Upreti:** Investigation, Resources, Methodology, Formal analysis, Writing – original draft, Visualization, Validation.

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