

Stability of Selected Hematological Parameters in Stored Blood Samples

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Abstract

1.1 Background: Apheresis platelets are a mainstay therapy in our tertiary cancer centre wherein 3000 donors are tested annually. Platelet drives to register willing donors are conducted. The complete blood counts to determine the eligibility for platelet donation is done on the first donor visit. However, it can be challenging to test these samples on that day due to reasons like manpower shortages, weekends, and a single cell counter in blood banks. National guidelines for blood banks in India do not elaborate on the ideal storage time for such blood samples. Hence we embarked on this study to determine the sample stability.

Aim: The aim was to study the stability of blood samples given at first visit for registration as platelet donors. Specific hematologic parameters were studied at time intervals of 4, 48 and 72 hours.

Materials and Methods: Eligible donor's venous blood sample was collected in K2-EDTA (Ethylenediaminetetraacetic acid) vacutainers for cell counts. This was tested on an automated cell counter (Hemocount, Human, Germany) within 4 hours at room temperature. Then, the samples were stored at 4°C and retested at 48 and 72 hours.

Results: 969 blood samples were tested for hemoglobin, WBC (White blood cell) and Platelet count. There was no statistical difference in mean values of hemoglobin and WBC counts at three time intervals. Even though difference in mean platelet count was statistically significant ($p < 0.001$), it did not impact on donor acceptance criteria.

Conclusions: Specific hematologic parameters (Hb, WBC, platelet) were found to be stable at 4°C for 72 hours. Hemoglobin (Hb) was the best preserved parameter followed by WBC and platelet count.

Keywords: Stored blood sample; Stability; Hematologic parameters

Introduction

Apheresis platelets are a mainstay supportive and therapeutic product in our tertiary cancer centre. This leads to a high demand of platelet concentrates (PC). Recently the focus is on getting PC from apheresis donors rather than as random donor platelets traditionally obtained from blood donations. This change is driven by supportive evidence showing efficacy of apheresis platelets over random donor platelets especially in multiply transfused oncology patient [1]. As a result of this, transfusion services face a challenging task of recruiting voluntary plateletpheresis donors. To combat this need, platelet drives are being conducted in our institute since November 2009 to register willing donors.

So far, according to the author's knowledge, there have been no reports on the ideal storage duration for blood samples in a blood bank setting. Also, national guidelines for blood banks in India, do not elaborate on the ideal storage time for blood samples of donors to be tested for complete blood counts. Hence, it may have to be done on the day of collection, preferably within 6 hours, as samples are assumed to undergo changes on storage. This hinders the prospect of feasibility to conduct platelet donor recruitment drives on weekends, holidays and at distant places due to manpower shortages and availability of a single cell counter in blood banks. This project was undertaken to study the stability of selected complete blood count parameters (Hb, WBC, Platelet) important for donor selection, over a period of 72 hours.

Sample stability is defined as the capability of a sample to retain the initial value of a measured quantity for a defined period within specific limits when stored under defined conditions [2]. Only few studies investigating the stability of hematologic parameters are available to date [3,4]. It is well known that handling of blood samples, as well as method of storage can significantly influence the results of hematological determinations. Consequently, results of hematological

determinations of improperly stored or handled blood samples can yield misleading results [5-9]. Butarrello and Goosens in their studies concluded that refrigeration of human blood samples is recommended to stabilize blood and minimize artifactual changes [9,10].

Materials and Methods

Participants

Donors who had willingly registered for screening of platelet donation were included in the study. Donor selection for this study was random. As per Drugs and Cosmetics Act, 1940 (D&C Act), the criteria for platelet donation includes a hemoglobin value > 12.5 g/dl and platelet count $> 150 \times 10^9/L$. Preliminary screening for platelet donation included hemoglobin, weight, medical history.

Sampling and instruments

3 ml venous blood sample was collected in EDTA (Ethylenediaminetetraacetic acid) vacutainer tubes (K2 EDTA, 5.4 mg) from 969 healthy donors aged between 18 to 50 years. The sample collection was done using standard aseptic precautions and in a minimal amount of time. The sample was thoroughly, but gently mixed with the anticoagulant immediately after filling the tube. Blood samples were tested within 4 hours on an automated 3 part differential

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cell counter (Humacount 30, Human, Germany) for hemoglobin, WBC and platelet counts. The samples were stored at 4°C in a monitored blood bank refrigerator and were retested at 48 and 72 hours from the time of collection. They were allowed to reach room temperature (22-24°C) and mixed well manually before being retested in the automated cell counter.

Analysis

The cell counter values were entered into excel sheet format for documentation and analysis. SPSS version 18 was used for statistical analysis. Paired t-test was used to compare each hematological parameter at time intervals of 4, 48 and 72 hours. Mean, standard deviation, confidence intervals were calculated for each hematological parameter. We considered p values of less than 0.05 as statistically significant. One way Analysis of variance (ANOVA) was applied to each hematological parameter to calculate the variation between baseline value and the other two time periods.

Ethics

The study was approved by the Institutional Review Board (IRB) and funded by Department of Atomic Energy/Government of India.

Results

EDTA samples from 969 platelet donors were tested on 3 part cell counter and the following parameters were studied: hemoglobin, WBC and platelet count at 4, 48 and 72 hours after collection. The null hypothesis formulated for the study was that there is no significant difference in the counts of the hematological parameters at different time intervals.

Stability of haemoglobin: The mean values for hemoglobin (g/dl) were 14.25, 14.30, and 14.32 at 4, 48 and 72 hours respectively. The difference in the mean values was not statistically significant ($p=0.45$). Of the 969 blood samples, we found 964(99.5%) to be within 10% variation from the baseline value at 48 hours. Similarly, 963 (99.4%) samples were found to be within 10% variation from the baseline value at 72 hours (Figures 1 and 2).

Stability of WBC count: The mean values for WBC count ($\times 10^9/L$) were 7.63, 7.60, 7.58 at 4, 48 and 72 hours respectively. The difference in the mean values was not statistically significant ($p=0.79$). Of the 969 blood samples, we found 892(92.1%) to be within 10% variation from

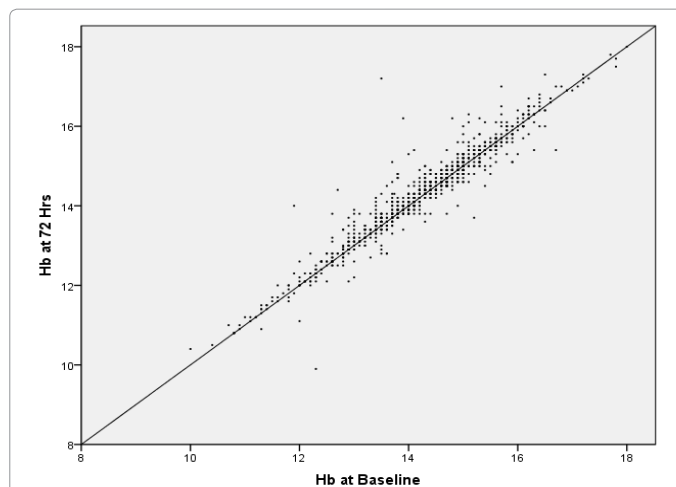


Figure 2: Hemoglobin values at 72 hours plotted against mean baseline haemoglobin.

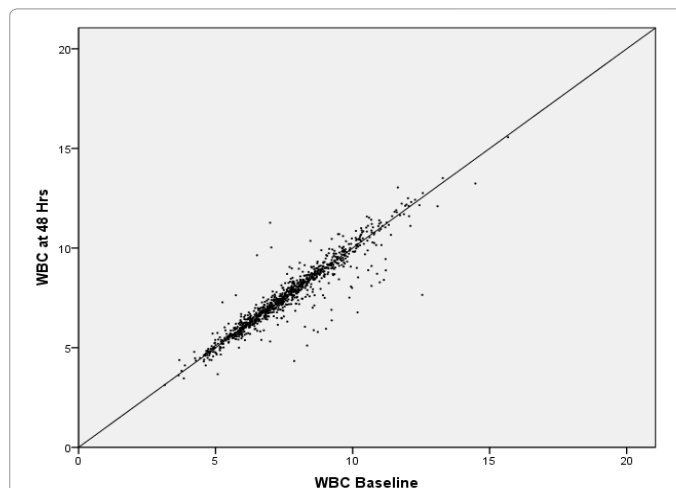


Figure 3: WBC count at 48 hours plotted against mean baseline WBC ct.

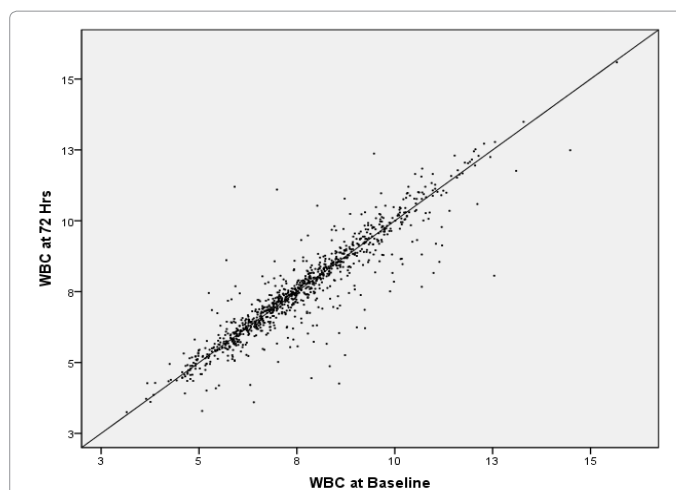


Figure 4: WBC count at 72 hours plotted against mean baseline WBC ct.

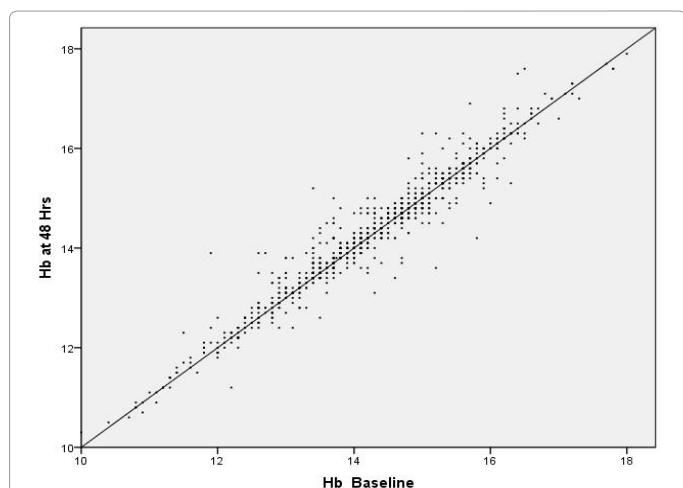


Figure 1: Hemoglobin values at 48 hours plotted against mean baseline haemoglobin.

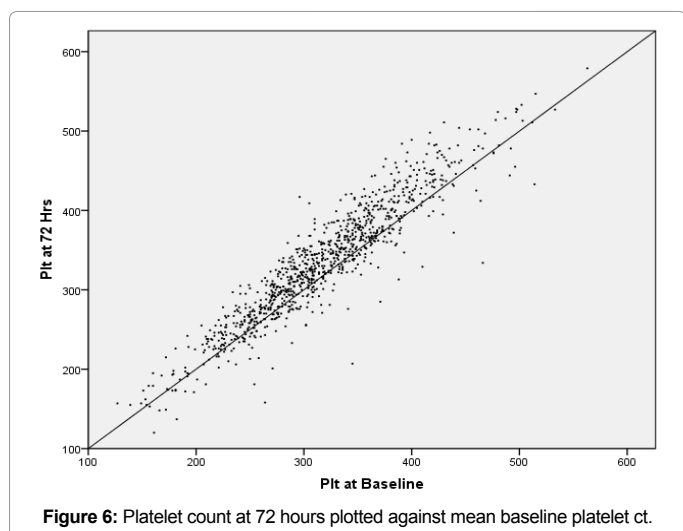
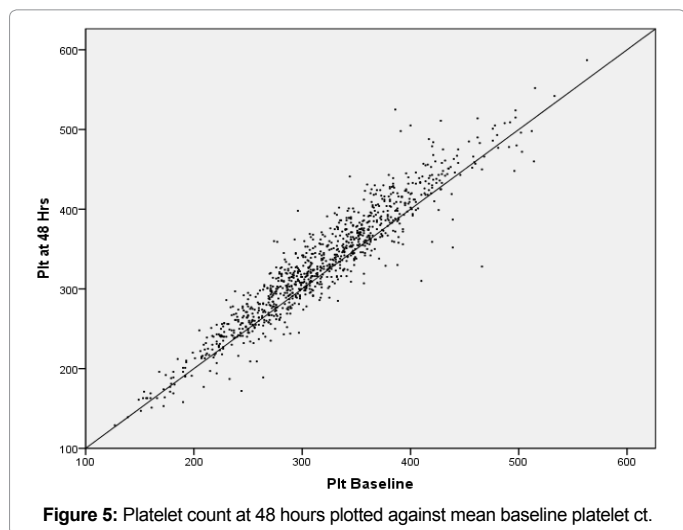
the baseline value at 48 hours. Similarly, 832 (85.9%) samples were found to be within 10% variation from the baseline value at 72 hours (Figures 3 and 4).

Stability of platelet count: The mean values for platelet count ($\times 10^9/L$) were 317.76, 330.71, 334.58 at 4, 48 and 72 hours respectively. The difference in the mean values was statistically significant ($p < 0.001$). However, this difference at three time intervals did not impact on donor acceptance criteria. Maximum difference in mean platelet counts at the three time intervals was $17 \times 10^9/L$ (Figures 5 and 6) Of the 969 blood samples, we found 756(78%) to be within 10% variation from the baseline value at 48 hours. Similarly, 680 (70.2%) samples were found to be within 10% variation from the baseline value at 72 hours (Tables 1-4).

Discussion

Platelet drives have been initiated in our tertiary cancer setup since November 2009. As platelet support forms an integral treatment modality in cancer patients, the demand is very high [11]. In order to have a robust platelet donation setup, voluntary donors are tested and enrolled in our platelet registry on a regular basis.

Delayed sample analysis is not a rare circumstance in clinical and laboratory practice [12]. Blood samples may have to be stored for longer periods at times. Platelet donor registration drives conducted on weekends leads to accumulation of a large number of blood samples for testing. In such a scenario, with only one dedicated cell analyser in



Time interval (hours)	Hemoglobin (g/dl)		WBC count ($\times 10^9/L$)		Platelet count ($\times 10^9/L$)	
	Mean	1SD(Standard deviation)	Mean	1SD	Mean	1SD
4	14.25	1.19	7.6	1.72	317.8	67.43
48	14.30	1.19	7.6	1.73	330.7	72.15
72	14.32	1.20	7.6	1.76	334.6	73.54
p value	0.45		0.79		< 0.001	

Table 1: Mean of specific hematological parameters at 3 time intervals, n=969.

Deviation from Baseline value	48 hours	72 hours
<10 %	99.5 (964 / 969)	99.4 (963 / 969)
10-20%	0.5 (5 / 969)	0.5 (5 / 969)
>20 %	Nil	0.1 (1 / 969)
Total %	100	100

Table 2: Deviation from Baseline value for Hemoglobin at 48 and 72 hours.

Deviation from Baseline value	48 hours	72 hours
<10 %	92.1 (892 / 969)	85.9 (832 / 969)
10-20%	5.5 (53 / 969)	9.4 (91 / 969)
>20 %	2.4(24 / 969)	4.7 (46 / 969)
Total %	100	100

Table 3: Deviation from Baseline value for WBC count at 48 and 72 hours.

Deviation from Baseline value	48 hours	72 hours
<10 %	78 (756 / 969)	70.2 (680 / 969)
10-20%	20 (194 / 969)	25.5 (247 / 969)
>20 %	2 (19 / 969)	4.3 (42 / 969)
Total %	100	100

Table 4: Deviation from Baseline value for Platelet count at 48 and 72 hours.

the blood bank, waiting time for complete blood counts (CBC) tests is increased. Ideally, CBC testing should be performed immediately after sample collection, but in cases where a delay is unavoidable, $4^{\circ}C$ is considered to be the temperature of choice.

Sample testing accuracy depends on how the sample is collected, continues with how it is handled until it is run, and finally comes down to the mechanics of how the tests are run [13]. For venous blood, K2 EDTA is the anticoagulant of choice for counting and measuring cell size in automated cell counters. It is preferred over K3 EDTA due to lack of dilution effects on the sample and due to lesser influence on the hematocrit, since K3 determines a decrease in MCV (Mean corpuscular volume) due to erythrocyte shrinkage [9]. We collected 969 blood samples from a clean venepuncture in K2 EDTA tubes in a minimal amount of time. Specimen collection, transport and storage are major determinants of the preanalytical variability and might influence the accuracy and precision of laboratory testing, as the greatest part of laboratory errors occurs within the preanalytical phase [14,15].

Cellular elements are known to have a limited stability in blood containing EDTA [16]. We stored the blood samples in a monitored blood bank refrigerator at $4^{\circ}C$ for a period of 72 hours. Conservation of specimens at low temperatures is known to improve stability of some CBC and DLC (Differential leucocyte count) parameters, but there are no systematic studies that take into account all of the available instruments and consider normal specimens as well as a variety of pathologies, where a delay in analysis might generate completely different results [9]. Storing samples at $4-8^{\circ}C$ increased stability in most analytes. RBC (Red blood cell) count and haemoglobin level is almost unaffected by storage at $4-8^{\circ}C$ or RT over 72 hours. This temperature range also extends stability of leukocytes [17]. Refrigeration is recommended to improve accuracy of CBC results for blood samples

[18]. Artfactual changes are known to be more pronounced in samples stored at higher temperatures [19]. The desirable approach to measure hematologic parameters is a timely processing of blood samples, when this option is unavailable, the most suitable alternative is sample storage under refrigeration, excluding those parameters more sensitive to the aging of the specimens [12].

Analyte stability of hematologic parameters varies not only according to the investigated parameter but also according to storage temperature and the employed measurement system. Of the 969 blood samples tested in our study, we found that haemoglobin in 964(99.5%) to be within 10% variation from the baseline value at 48 hours. Similarly, 963 (99.4%) samples were found to be within 10% variation from the baseline value at 72 hours. Of all the three parameters tested in this study, Hemoglobin was the best preserved. Imeri et al also found that RBC count and hemoglobin level were almost unaffected by storage at 4-8°C or RT over 72 hours [17]. Gulati et al. used a Coulter GEN-S counter(Beckman-Coulter, Hyaleah, FL) and kept specimens at room temperature. Their erythrocyte counts and hemoglobin concentrations were stable for 7 days [20]. The first study in literature where whole blood samples kept at room temperature for 72 hours were analysed for hematologic parameters was in 1973 wherein WBC, Hemoglobin, RBC and MCV were found to be stable [21]. These findings were confirmed at room temperature and 4°C by further studies in 1981 by Cohle et al. Thus they concluded that blood from normal healthy donors can serve as an adequate control for the coulter counter for three days if kept at 4°C [6]. Avian studies on turkeys have also shown consistent results with 4°C storage for 72 hours, which significantly decreased changes regards to PCV, MCV and haemoglobin [22]. Literature cites various studies on animal blood samples for stability after storage; however our study is one amongst few to look at healthy human samples.

In our study, of the 969 blood samples, we found WBC ct in 892(92.1%) to be within 10% variation from the baseline value at 48 hours. Similarly, 832 (85.9%) samples were found to be within 10% variation from the baseline value at 72 hours. Thus, in our study the results for hemoglobin and WBC ct were in accordance with our null hypothesis. Gulati et al found leukocyte and platelet counts remained constant for 4 days at RT [20]. Imeri et al also found that storing the samples at 4-8°C extended the stability of leukocytes and reticulocyte counts [17]. Butarello et al stored human samples at 4°C upto 72 hours and found that WBC counts which were systematically low when obtained by the optical channel of the analyser were stable with the impedance channel [9].

For platelet counts we found that the mean values for platelet count ($\times 10^9/L$) were 317.76, 330.71, and 334.58 at 4, 48 and 72 hours respectively. This difference in mean was statistically significant ($p < 0.001$), which is not in accordance with the null hypothesis. However, this difference did not impact on donor acceptance criteria as the maximum difference in mean platelet count was $17 \times 10^9/L$ which is clinically insignificant. Prior studies on platelet counts have proved that sample storage affects the platelet volume and causes clumping, which in turn is responsible for the low platelet values. This problem can be overcome by intermittent mixing of samples to provide consistent results [6,23,24]. Hence, we practiced mixing for all our blood samples before subjecting them to cell counts on the analyser.

An aspect of specimen conservation that must be addressed is the difference in behaviour of automated counters using optical or impedance methods. Previous studies reveal improvement of stability of CBC counts, as determined on the basis of the principle of focussed flow impedance, especially if sample is refrigerated [25]. Impedance

counting, first described by Wallace Coulter in 1956, depends on the fact that red cells are poor conductors of electricity, whereas certain diluents are good conductors; this difference forms the basis of the counting systems. Our instrument was based on the impedance principle, which was calibrated against appropriate reference standard material and controlled daily with the use of proprietary controls. The impedance method counts and sizes cells (WBC and Platelet) by detecting and measuring changes in electrical impedance, when a particle in a conductive liquid passes through a small aperture. For Hemoglobin, the lysed sample dilution is measured by a photometric method. The reagent lyses the red blood cells releasing hemoglobin which through a chemical process forms methemoglobin. This is measured by a photometer on the chamber.

As opposed to Hematology laboratories, it is essential to note that blood banks need to do testing of only Hemoglobin, Platelet count and WBC count, as the eligibility for platelet donation do not require other parameters. The blood samples are from healthy individuals and not patients with possibly distorted morphology of cells. NABH (*National Accreditation Board for Hospitals & Healthcare Providers*) and NACO (*National AIDS Control Organisation*) guidelines for blood banks have not specified ideal storage period and temperatures for donor samples tested on cell counter. Also, manual methods for reconfirmation of results are not practised in blood banks. Personnel performing routine blood bank tests have to perform cell counter tests in extra time and there is no dedicated manpower allotted for cell counter tests as in hematology laboratories. Blood banks usually have cell counters functioning on impedance principles as against the optical principle cell counters of hematology labs.

Conclusions

Blood samples for testing specific hematologic parameters of platelet donors were found to be stable at 4°C for up to 72 hours. Hemoglobin was the best preserved parameter followed by WBC and platelets. This can facilitate to increase our platelet donor registry by organizing long distance and weekend platelet camps. Hindrances due to manpower shortages, equipment breakdowns and weekend testing can be overcome with extended sample storage at 4°C. The study can be instrumental in formulating blood sample storage guidelines for blood count evaluations in transfusion services.

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