Measurement of Pack Red Cells (PRC) Blood Components During Processing and Storage

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Abstract—The quality test of the PRC components is needed to ensure a consistent processing and storage processes for an optimal healing effect. Hemolysis during blood storage is the most severe manifestation of erythrocyte storage and is an important parameter for assessing the quality of PRC [1], [2]. This study aims to measure the level of PRC hemolysis as an indicator of quality during processing and storage. This is an observational descriptive study using a comparative analytical approach (student t-test). The previous researches on PRC on the bloods collected inside the building revealed that the average increase in hemolysis occurred on the 0th day along with the gradual increase in the next processing even though it was still under the standard of below 0.8% at the end of storage. It was also noted that PRC hemolysis on mobile unit (MU) collection significantly increased on 14th day. The most significant increase in hemolysis occurred at PRC drawing of MU on 7th day from 0.3 to 0.5. Furthermore, the PRC hemolysis of the two groups continued to increase variably until the end of the storage period, particularly on day 35 although it remained below the level of 0.8%. The t-test results show a significant level of (p) 0.001, that is lower than 0.05. Thus, it can be concluded that there is a significant score difference in the PRC hemolysis between the Blood Transfusion Unit blood sampling and mobile unit (MU) blood collection activities.

Keywords—Hemolysis, Blood Components, storage lesson, Packed Red Cell, PRC

I. INTRODUCTION

The Quality control of PRC should be carried out to ensure that the processing and storage process is carried out consistently to minimize the risk of transfusion reactions and provide an optimal healing effect. PRC quality standard indicators include volume, haemoglobin or hematocrit, hemolysis at the end of storage, and bacterial contamination. Hemolysis during blood storage is the most severe manifestation of erythrocyte storage and is an important parameter for assessing the quality of stored PRC and is associated with bacterial contamination [1][2].

This study aims to look at the hemolysis level of the PRC component as an indicator of quality during processing and storage. The results of the study are expected to be input for the government and BTS Center to make policies related to the obligation to conduct blood product quality testing to ensure quality and implementation of Good Manufacturing Practices (GMP).

II. METHOD

This is an observational descriptive study with a comparative analytical approach (student t-test). The study population was the total blood donated at UTD PMI Yogyakarta City within a period of one month. The research samples were randomly selected from blood donors who were willing to be involved as the research samples. The number of samples were selected according to standard specifications and quality control for blood components, namely a minimum of four bags per month [1].

The study population consists of the total blood donated at Blood Transfusion Services (BTS) of Indonesian Red Cross Yogyakarta City within a period of one month. The research sample was randomly selected from regular blood donors who were willing to be involved as the research sample. The number of samples were selected in accordance with the specifications and quality control standards for blood components, namely a minimum of four bags per month, plus one WB bag as a control. This study used 10 blood samples, consisting of five bags from the collection of BTS building and 5 bags from the mobile unit (MU) activity [1].

The tools used in this study were blood bank, thermometer, centrifuge, hematology analyzer, micropipette, and worksheet. The materials needed were hematology analyzer kit, double blood bag capacity of 350 ml with anticoagulant CPD-A1 for donor blood sampling. The study was conducted in 6 research steps starting from obtaining research permits and Research Ethics, determining the research sample, screening for infectious infections through blood transfusions, processing whole blood (WB) into PRC, measuring hemolysis, and analyzing data. The sample of the study was taken randomly from four bags of donor blood using the CPD-A1 anticoagulant double bag. As a control, one WB bag was taken with a single bag. The donor's blood sample was then screened for detecting HIV, HBsAg, HCV, and syphilis infection to obtain healthy blood. Whole Blood from double bags that have been screened and tested was then processed into PRC by centrifugation method. PRC’s were stored in a blood bank temperature of 2°C-6°C [3][4]. Blood bank temperature was monitored periodically every
day during storage, twice a day (every morning and evening).

Hemolysis examination involves three measurements, including measurement of hematocrit, measurement of total hemoglobin, and measurement of plasma hemoglobin. Hemolysis examination was carried out during processing and storage [5], [6]. Afterwards, the processing was done shortly after the completion of the previous processing. During the examination procedure, the PRC sample was removed from the blood bank then the bag tube was sealed using a hand sealer for at least 3 times, then it was homogenized slowly. Blood samples were taken aseptically from the bag tube, then it was transferred into a 0.5 ml volume tube. Samples were diluted using 0.9% NaCl solution in a ratio of 1:2, then identified and examined with a Sysmex automated hematology analyzer following the procedure from the manufacturer. The examination at this stage was considered as day 0, then the examination was repeated on days 7, 14, 21, 28, and 35 with the same procedure. The measurement results were recorded and documented every time it was examined [5], [7].

Total Hemoglobin measurement was carried out in conjunction with hematocrit examination, using a Sysmex automated hematology analyzer. Plasma Hemoglobin measurement was performed using the Hemocue Hb Low Plasma Analyzer. The examination at this stage was considered as day 0, then the examination was repeated on 7th, 14th, 21th, 28th, and 35th day with the same procedure. Measurement results were recorded and documented every time it was examined. At the final stage of measurement, the percentage of hemolysis was calculated by the following formula [5], [8]:

\[
\text{Hemolysis} = \frac{\text{Plasma Hb} \times (1-\text{Hct}) \times 100}{\text{B.Hb}}
\]

The data obtained were then tabulated and analyzed using statistical data analysis comparative test (student t-test). The results of the analysis were compared with the standard requirements [1].

III. RESULT AND DISCUSSION

A. Result of screening blood infection

The results of the blood screening test for 10 blood units against HIV, HBsAg, HCV, and syphilis showed non-reactive results, and thus the blood meet the quality standard for research samples.

B. Results of WB Processing into PRC

Whole Blood (WB) were processed into PRC by centrifugation method using a refrigerated centrifuge (Kubota Refrigerated Centrifuge) with a speed of 3,600 rmp at 4°C for 7 minutes which was then separated and a small amount of plasma was left to maintain the PRC hematocrit of 70%. Furthermore, the PRC is identified according to the manufacturing date, type of component, and expiry period, then stored in a blood bank temperature of 2°C-6°C up to 35 days according to the type of anticoagulant used in the bag, namely CPDA-1.

The average volume of the eight WB bags that were processed into PRC was 223.13 ml, which was still below the standard volume of PRC processed from WB with a capacity of 350 ml, namely between 218 ± 39 ml. Blood bank temperature monitoring resulted an average of 4.7°C, which was also still below the standard storage temperature [1].

C. Hematocrit (Hct) Measurement

The results of hematocrit measurement on PRC are indicating an increase in PRC hematocrit during the processing on day 0. Meanwhile, during storage, there was a decrease on 14th day for PRC from mobile unit activity and on 21st day for PRC taken inside the BTS building. Afterwards, the entire blood samples showed an increase until the end of its storage at the 35th day.

D. Measurement of Total Hemoglobin (Hb)

The results of Total Hemoglobin (Hb) measurement indicating the hemoglobin rate that varies greatly at each measurement stage. The hemoglobin rate of the PRC taken in the building increased until the 21st day, but decreased on the 35th day. The same result is also shown by the PRC taken from MU activities.

E. Plasma Hb Measurement

Hemoglobin is a protein that contains lots of iron and has an affinity for oxygen to form oxyhemoglobin in erythrocytes. The presence of free hemoglobin in the plasma is an indication of the rupture of erythrocytes which ends with the release of hemoglobin into the plasma. The results of plasma Hb measurement in this study shows that the percentage of plasma Hb in PRC that is processed from the WB taken in the BTS building and MU activities have increased during the processing and during storage. The results of PRC plasma Hb measurement at the time of processing (0th day) were 0.07 for PRC taken inside the building and 0.07 for PRC taken at MU activities. At the final stage of storage, the results of plasma Hb measurement were 0.24 for the PRC taken inside the building and 0.28 for the PRC taken from mobile unit.

F. Hemolysis Measurement

The results of the average hemolysis measurement are presented in table 1, indicating an increasing hemolysis scores on the PRC processed from the WB taken from inside the BTS building and on MU activities during processing and storage. However, at the end of the storage period, the average hemolysis experienced a drop. This may be attributed to a human error during the sampling or inspection process.

<table>
<thead>
<tr>
<th>TABLE I. RESULT OF HEMOLYSIS MEASUREMENT</th>
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<tbody>
<tr>
<td><strong>Origin</strong></td>
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<tr>
<td>In BTS Building</td>
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<tr>
<td>Mobile Unit</td>
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</table>
For the PRCs taken inside buildings, the average increase in hemolysis occurred on 0th day when further processing increased slowly even though at the end of storage it still met the standards. In contrast, the PRC taken from Mobile Unit indicated a significant increase on 14th day. The most significant increase of PRC hemolysis taken from Mobile Unit was on the 7th day from 0.3 to 0.5. Furthermore, the PRC hemolysis of the two groups continued to increase variably until the end of the storage period, namely on day 35, although it remained below the level of 0.8%.

This results are in line with the results revealed by Makroo, et al., 2011; Husein & Enein, 2014 in which the PRC hemolysis increased in all units. The increase in hemolysis varied from 0th day, namely at the time of processing to the end of the storage period, but all of which were still below the acceptable hemolysis standard of less than 0.8% [5, 9].

G. Data analysis

The research data were analyzed using the SPSS statistical test application. The tabulation results of hemolysis measurement data were analyzed using statistical analysis of comparative test (student t-test). Prior to data analysis, a data normality test was performed, with a value of p = 0.747 for the hematocrit, p = 0.383 for total hemoglobin, p = 0.978 for Hb plasma, and p = 0.487 for the hemolysis score. Because the value of p was > 0.05, the data distribution is considered normal and meets the statistical test requirements for t-test.

The t-test showed that the significance value of (p) was 0.001, less than 0.05. Thus, it can be concluded that there was a significant difference between the PRC hemolysis score on blood sampling at BTS and the hemolysis score on the PRC blood collection MU. Hemolysis is the rupture of erythrocytes followed by the release of free hemoglobin, ions, and several types of enzymes into the plasma. Increased PRC hemolysis can occur during processing, storage, blood transport to the patient's bedside, and during the transfusion process [5, 9]. The significant increase in hemolysis occurs during storage, because at the time of storage there are changes in the morphological shape of the red blood cell membranes and changes in blood biochemistry, which have an impact on changes in erythrocyte viability and function, known as storage lesion. Storage lesions are characterized by the rupture of the erythrocyte membrane followed by the release of free hemoglobin into the plasma referred to as hemolysis [10, 11].

In this study, the hemolysis scores at the end of the storage period for the two groups were still below the standard level of less than 0.8%, although there were significant differences in the PRC hemolysis score of BTS and MU. Such significant difference may be attributed to several factors, including the application of cold chain blood and compliance with procedures during blood collection, processing, storage, and transportation, so that erythrocytes can live optimally until the end of their storage period. Cold blood chain is the process of maintaining blood temperature from the time of collection, processing, storage, and distribution so that blood stays at an optimal temperature of between 2°C-6°C [5, 12-14]. Apart from hemolysis measurement, to see the quality of the PRC, it is necessary to test other parameters of the quality of the PRC, for example the test for bacterial contamination [1].

IV. Conclusion and Suggestion

Based on this study, this paper concludes that: Hematocrit measurement results of PRC blood samples showed an increase at the time of manufacture (day 0), but decreased during storage, and at the end of the storage period (day 35). The measurement result of the total Hemoglobin (Hb) varied widely at each measurement stage. In the PRC in buildings, the hemoglobin value increased until the 21st day, but decreased until the 35th day [5, 15-17]. The same results were also applied for the PRC of MU activities. The results of plasma Hb measurement indicates that the percentage of plasma Hb in the PRC processed from WB taken from the UTD building and MU activities increased during the processing and storage. The results of the PRC hemolysis measurement from the two groups continued to increase variably until the end of the storage period, namely on day 35 although it remained below the level of 0.8%. The t-test result shows that the significance value of (p) 0.001 is lower than 0.05. Thus, it can be concluded that there is a significant difference between the PRC hemolysis score on blood sampling at UTD and the hemolysis score on the MU blood sampling PRC.

Based on the previous conclusion, this paper suggest that: It is necessary to maintain the quality of PRC by properly maintaining and preserving the cold chain blood system. It is also vital to monitor room temperature, processing temperature, storage temperature, and transportation temperature properly as a way to maintain the quality of the blood until the end of its storage. 2) For further research on PRC hemolysis during processing and storage, it is essential to keep all research stages in accordance with the standard procedures to meet the expected research results. 3) PRC hemolysis examination during processing and storage can be carried out as part of the implementation of policies and quality systems in blood services.

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REFERENCES


