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Evaluation of two methods for counting residual leukocytes in leukoreduced packed red cells: Flow cytometry and fully automated cell counter

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Abstract:

BACKGROUND AND OBJECTIVES: Objective of the study is to explore the possibility of utilization of seven part fully automated hematology analyzer for enumeration of residual leukocytes (residual white blood cells [rWBCs]) in leukoreduced packed red cells (LR-PRCs) prepared from whole blood at a blood center as an alternate to the gold standard method, flow cytometry. In this study, we evaluate the performance characteristic of hematology analyzer against flow cytometry for the estimation of rWBCs in 39 LR-PRC units.

MATERIALS AND METHODS: PRCs prepared from whole blood donations by 39 donors were leukoreduced and their volumes were noted. The samples from these LR-PRCs were processed on HORIBA Yumizen H2500 hematology analyzer and Backman Coulter DxFlex (B5R3V5) Flow Cytometer and compared.

RESULTS: A total of 39 LR-PRCs were analyzed. The average volume of these LR-PRC units was 252 mL ranging from 227 to 285 mL/bag. The average rWBC count for all LR-PRC units as per flowcytometry method was 3.1×10^6/bag. There were 5 LR-PRC units with rWBC count more than 5×10^6/bag which did not fulfill the minimum quality control criteria of LR-PRCs as per the Indian standards (DGHS).

CONCLUSION: The new generation fully automated hematology analyzers could be simple, reliable, economical, and practically best method for assessing the efficacy of leukoreduction in LR-PRCs. They can be adopted by resource-constrained blood centers as an alternative to flow cytometry for this purpose. It can be practically applied to check the quality of leukoreduction in all blood component samples before release from blood center for transfusion to the patients.

Keywords:

Flow cytometer, hematology analyser, leukoreduced packed red cells, leukoreduction, residual white blood cell count

Background and Objectives

The use of leukocyte-reduced blood components significantly reduces or prevents many of the adverse transfusion reactions associated with donor white blood cells. Leukoreduction (LR) attenuates or eliminates the inflammatory response to red blood cell transfusion in humans. Leukoreduction is shown to lower the incidence of nonhemolytic febrile transfusion reactions, alloimmunization to leukocyte antigens and refractoriness

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to platelet transfusion, severe pulmonary dysfunction, graft-versus-host disease (GVHD), transfusion-transmitted cytomegalovirus (CMV), and immunomodulation all of which can contribute to morbidity.

As per the current Indian standards (DGHS), the total content of leukocytes in a leukoreduced packed red cell (LR-PRC) unit prepared by filtration should be $<5 \times 10^6$/unit and a minimum of 85% of red cells should be retained after leukoreduction.[1] A minimum of 75% LR-PRC units selected for quality control (QC) testing should comply with the above standards. It has been observed that antibodies against histocompatibility antigens tend to develop in the recipient, if the leukocyte content in a unit of transfused blood exceeds the figure mentioned above.[2] Therefore, accurately measuring residual white blood cells (rWBCs) in LR-PRCs is an essential requirement to decrease or prevent the adverse transfusion reactions associated with donor white blood cells.

As the transfusion of leukoreduced blood components became more common, there was a need for routine monitoring of the efficacy of leukoreduction of blood components. Several manual and automated methods for assessing rWBC count, which is a gold standard method in leukoreduced blood components, have been used such as manual microscopy (with Nageotte hemocytometer), microvolume fluorimetry, and flow cytometry.[3]

Conventional method like manual microscopy is time-consuming and subject to observer bias, whereas newer methods such as flow cytometry, may not be available at all the centers except few specialized tertiary care centers.[1]

Use of hematology analyzers for counting of rWBCs in leukoreduced blood components is practically useful and a standardized method. However, the detection level for rWBCs required to demonstrate leukoreduction was originally considered too low for hematology analyzers. The latest generation of hematology analyzers can detect this extremely low concentration of white cells found in leukoreduced blood components.[3]

The present study is done to evaluate the performance characteristic of hematology analyzer for rWBC enumeration in LR-PRCs and assess the potential impact of using analyzer in a routine blood center component preparation.[3] The use of analyzers shall remove the observer bias associated with manual microscopy and can be a simple, cost-effective alternative to verify the efficacy of leukoreduction. Furthermore, with this method, all LR-PRCs can be verified for rWBCs at the time of release which will ensure that all critical patients are transfused with leukoreduced PRCs of the highest quality.

Materials and Methods

Materials

LR-PRC samples, hematology analyzer HORIBA Yumizen H2500, Backman Coulter DxFlex (B5R3V5) Flow Cytometer.

Methods

Under sterile precaution, 450 mL (±10%) whole blood was collected from 39 eligible donors in standard quintuple blood bags with inline filters for prestorage leukoreduction of packed red cells as per the departmental standard operating procedures and national guidelines. In a closed system, PRCs were prepared from whole blood donation by 39 donors. These were leukoreduced using the inline filters and their volumes were noted. The samples of these LR-PRC units were collected in ethylenediaminetetraacetic acid tubes from properly stripped segments of these bags.

Samples were processed in HORIBA Yumizen H2500 hematology analyzer on the usual manual sampling mode after mixing 8–10 times by inversion for each sample and in Backman Coulter DxFlex (B5R3V5) Flow Cytometer to estimate the total leukocyte count. In this study, DuraClone IM Count which uses CD45+ as marker for total leukocyte count was used in the flow cytometer. A specific template for flow cytometer was created in Cytoflex software version 2.0 for acquisition and determination of absolute WBCs count. The same was used for all the 39 samples. QC was performed on each day of testing for both hematology analyzer and flow cytometry analyzer and found to be acceptable.

Principle of flow cytometry analysis[4]

The study was conducted on Backman Coulter DxFlex (B5R3V5) with 13 detectors and 3 lasers, using DuraClone IM Count which uses CD45+ as marker for total leukocyte count within 24 h. Furthermore, the mixing of a known number of beads with a known volume of whole blood can be used to determine the number of cells in the whole blood per unit volume.[10]

$$\text{Cell count}\left(\text{cells}/\mu\text{L}\right)=\frac{\text{Number of cell events}}{\text{Number of beads event}}\times\frac{\text{Total number of beads per test}}{\text{Volume of specimen}\left(\mu\text{L}\right)}$$

Principle of HORIBA YH2500[5]

µThe sample is forced into the center of the stream forming a single file by the patented Double Hydrodynamic Sequential System "DHSS" flow cytometry. The cells

also go through the aperture one by one to be counted and measured by electrical current (Impedance changes) which is the resistive measurement. Then, same cell crosses the light beam that arrives at a 0° angle determining the cells volume. Thus, two characteristics for each cell passing through this system are determined: Volume and absorbency.

rWBCs were counted for each LR-PRC unit to check the effectiveness of leukoreduction. Data from both the instruments were retrieved and analyzed to draw a comparison.

Results

A total of 39 LR-PRC samples were analyzed in this study. The average volume of LR-PRC units was 252 mL, ranging from 227 to 285 mL/bag. The average rWBC count for all units as per flowcytometry method was 3.1×10^6/bag. There were five bags with rWBC count more than 5×10^6/bag which did not fulfill QC criteria.

Results of total WBC counts by HORIBA YH2500 were analyzed against flow cytometry method. The correlation between the two methods was $r = 0.62$ and $R^2 = 0.39$ with 95% confidence interval. Although this looks like moderately significant correlation statistically, it can be justified by significantly low values. However, these values of rWBC are not clinically significant and are well within the acceptable criteria of 5×10^6 [Figure 1].

Bland–Altmann analysis was also performed for cell count by Flowcytometry and HORIBA YH2500 for WBC counts ($n = 39$). It shows good agreement with mean bias (standard deviation) between two methods as – 0.014 (0.024) $\times 10^3$/cumm [Figure 2].

As per the current Indian standards (DGHS), a minimum of 75% LR-PRC units selected for QC testing should comply with the above standards. Since, the rWBC count for these five bags was comparable by both methods used, this does not affect the outcome of the study. Therefore, according to the present study, seven part fully automated hematology analyzers give comparable results to flow cytometry in the estimation of rWBC in LR-PRCs.

Discussion

Leukoreduction is the crucial process for decreasing WBC concentration in blood components. The benefits of leukoreduction are plenty, namely, reducing the risk of various blood transfusion reactions such as febrile nonhemolytic reactions, refractoriness to platelet transfusions, human leukocyte antigen alloimmunization, transfusion-associated GVHD, reduction in storage lesion, reducing the risk of TRIM transfusion-related immunomodulation, and also reducing the risk of various transfusion-transmitted infectious such as Herpesviruses, CMV, retroviruses and various other virus, bacteria, protozoa, and prions transmission.[6]

The clinical indications for LR-PRC transfusion are in those patients who require regular multiple transfusions such as thalassemia, sickle cell disease, hemato-oncological patients, immunosuppressed patients, and high-risk recipients which includes low birth rate infants, oncology patients, bone marrow transplant patients as well as other transplant patients on immunosuppression.[7,8]

There are various methods for leukoreduction, comprising conventional methods such as red cells washing, centrifugation and buffy coat removal, freezing and deglycerolization of red cells which cause variable depletion of leukocytes. Newer methods such as filtration (especially 3rd and 4th generation leukofilters) and apheresis gives leukoreduction of $<5 \times 10^6$ WBCs/unit (99.99% efficacy).[9] Filtration can be performed before component storage at blood center (Pre storage leukoreduction) or during the transfusion (bedside/post storage filtration). The primary mechanism of leukocyte

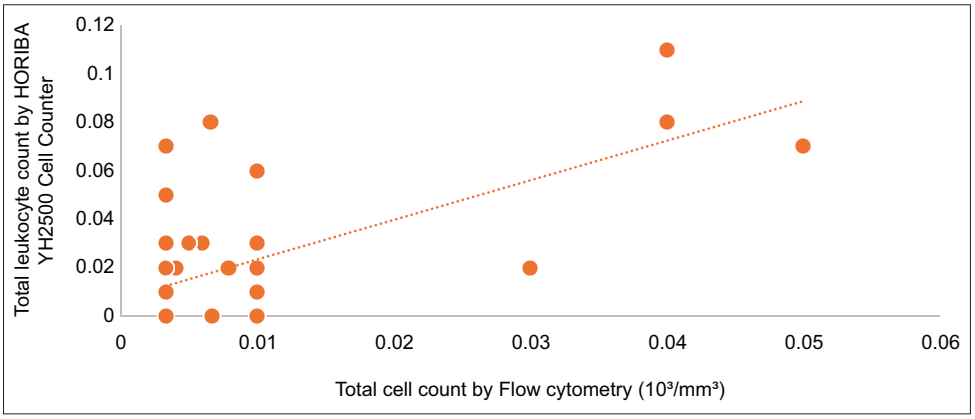

Figure 1: Correlation of total white blood cell count by flowcytometry and HORIBA YH2500 ($n = 39$) $r = 0.62$ and $R^2 = 0.39$ (regression coefficient)

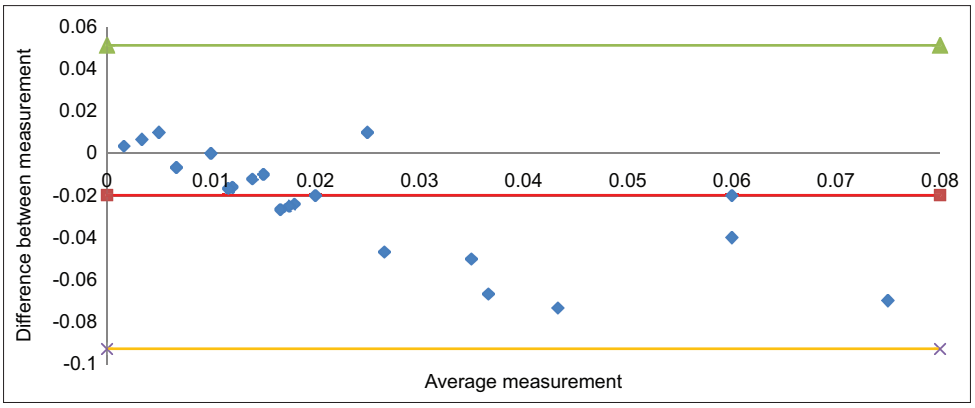

Figure 2: Bland–Altman plot demonstrating the degree of agreement between cell count by Flow cytometry and HORIBA YH2500 for white blood cell counts (n = 39). The solid red line in the center represents zero difference between the two methods. The dotted blue and yellow lines represent the upper and lower limits of agreement (95%) between the two methods, respectively

Table 1: Profile of the leukoreduced packed red cells

	PRCs bag volume	WBC count (10^3/mm^3) by flow cytometry	rWBC 10^6/ bag by flow cytometry
Average	252 mL/bag	0.0120	3.1
Range	227–285 mL/bag	0.003–0.050	0.7–13.4
Median	254 mL	0.010	2.4

PRCs=Packed red cells, WBC=White blood cell, rWBC=Residual WBC

Table 2: Comparison of measurement by Nageotte's and flow cytometry methods

Studies	WBC measurement		Correlation (r)
	Range (WBC/μL)	Mean (WBC/μL)	
van der Meer *et al*.[12]	1.97–7.40	113.05	0.91
Dzik[9]	0.3–18.4	0.017	0.9995
Javed *et al*.[13]	0.3–16.3	0.4	0.98

WBC=White blood cell

Table 3: Comparison of measurement by YH2500 analyzer and flow cytometry method

Blanco *et al*.[3]	1.02–4.14	5.22	0.9818 (R^2)
Present study	0.003–0.05	−0.014	0.6278

YH2500=Yumizen H2500

removal by filtration is the charge-based adhesion of negatively charged leukocytes to the filter material by Van der Waals and electrostatic forces.[10]

In our study, inline leukoreduction filter made up of polyester material is used for prestorage leukoreduction of PRCs [Table 1]. Ensuring QC of such product is an important quality and regulatory compliance requirement for blood centers. Volume of leukoreduced packed red cells and total rWBCs count in LR PRCs should be <5×10^6/unit as per the Indian standards.[11] In our study, we found five LR-PRC units had a total residual WBC count more than acceptable criteria. This signifies the importance of evaluating the residual WBC counts of each bag before transfusion to provide maximum benefit of leukoreduction to the patient.

As per van der Meer *et al*.,[12] flow cytometry gave accurate results with an acceptable coefficient of variation for low level counting as compared to Nageotte method. Majority of other studies also concluded similar findings. Nageotte method requires expertise and is subject to observer bias.[12] Many previous studies have compared hematology analyzers with Nageotte method. The present study is the only study apart from Blanco *et al*.[3] to compare the estimation of rWBC by hematology analyzer with flow cytometry.

Studies by Javed *et al*.[13] and Dzik[9] find the correlation between flowcytometry and Nageotte method as r = 0.91 and r = 0.99, respectively [Table 2]. The count range was 1.97–740 WBCs/μL and 0.3–18.4 WBCs/μL.[13] In the present study, the correlation between hematology analyzer and flow cytometry was found to be 0.6278. This can be attributed to the lower WBC counts in the study (Range: 0.003–0.05 × 10^3 WBCs/μL).

The results obtained from this study were comparable with that of the study done by Blanco *et al*.[3] on lower counts, where the comparison was done between hematology analyzer and flow cytometry method and the results between these two methods were in correlation.

Comparison by Bland–Altman method shows the mean difference between two methods was 15.3 units and 95% agreement limit was −10 to + 41[Table 3].[13] Whereas in the present study, the mean difference was −0.0147 and 95% agreement limit was -0.0618–0.0289 which is better. A bias of −0.014 was observed which was acceptable.

The use of Nageotte method can be subject to bias at such low counts. Flow cytometry is not available at all blood

centers as it is costly, requires preanalytical processing of the samples and requires expertise. Therefore, hematology analyzer can be a simple and cost-effective method of estimating rWBC in LR-PRCs and other leukoreduced blood components.

The limitations of this study include a sample size of 39 which is comparatively small in number and the use of only one hematology analyzer with capabilities to measure such low range samples. Therefore, it is needed to conduct similar studies with a larger sample size and on a variety of hematology analyzers so that the utilization of hematology analyzers for estimation rWBC in LR-PRCs in a blood center can be established.

Conclusion

The results of residual WBC estimation in leukoreduced blood samples by flow cytometric enumeration, taken as a reference method in this study and HORIBA Yumizen H2500 were comparable. Therefore, the present study shows that the new-generation fully automated seven-part hematology analyzers such as HORIBA YH2500 can be used for estimating rWBC in leukoreduced blood samples.

Furthermore, while processing samples in HORIBA Yumizen H2500 analyzers, there was no need of preanalytical sample preparation, any change in the software algorithm, or change of reagents for estimation of such extremely low concentration of white blood cells found in leukoreduced blood components.

The new generation fully automated hematology analyzers could be simple, reliable, economical, and practically best method for assessing the efficacy of leukoreduction in LR-PRCs and other leukoreduced blood components. In developing countries such as India, most blood centers cannot either afford the cost or do not have the required expertise for flow cytometry. Hematology analyzers can be adopted by these resource-constrained blood centers as an alternative to flow cytometry for improving blood transfusion safety, thereby decreasing financial burden without compromising quality. It can be practically applied to check the quality of leukoreduction in all blood component samples before release from blood center for transfusion to the patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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