Transfusion and Apheresis Science 54 (2016) 266–270

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Short Report

Enumeration of residual white blood cells in leukoreduced blood products: Comparing flow cytometry with a portable microscopic cell counter

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ARTICLE INFO

Article history:
Received 24 August 2015
Received in revised form 29 September 2015
Accepted 4 October 2015

Keywords:
Flow cytometry
Quality control
Leukoreduction
Apheresis
Automated blood cell counters

ABSTRACT

Transfusion of blood components is potentially associated to the risk of cell-mediated adverse events and current guidelines require a reduction of residual white blood cells (rWBC) below 1×10^6 WBC/unit. The reference method to enumerate rare events is the flow cytometry (FCM). The ADAM-rWBC microscopic cell counter has been proposed as an alternative: it measures leukocytes after their staining with propidium iodide. We have tested the Adam-rWBC for the ability to enumerate rWBC in red blood cells and concentrates.

We have validated the flow cytometry (FCM) for linearity, precision accuracy and robustness and then the ADAM-rWBC results have been compared with the FCM.

Our data confirm the linearity, accuracy, precision and robustness of the FCM. The ADAM-rWBC has revealed an adequate precision and accuracy.

Even if the Bland–Altman analysis of the paired data has indicated that the two systems are comparable, it should be noted that the rWBC values obtained by the ADAM-rWBC were significantly higher compared to FCM. In conclusion, the Adam-rWBC cell counter could represent an alternative where FCM technology expertise is not available, even if the risk that borderline products could be misclassified exists.

1. Introduction

The transfusion of blood components is potentially associated to the risk of cell-mediated adverse events associated to cell immunomodulation, alloimmunization to HLA antigens or graft versus host disease (GvHD) [1]. To limit the risk, current European guidelines require a reduction of residual white blood cells (rWBC) in blood components to a level below 1×10^6 WBC/unit (about 3.3 WBC/μL) [2]. Accordingly, the accurate enumeration of rWBC in leukoreduced products is mandatory for Transfusion Medicine services to ensure their quality.

Automated cell counters are routinely used for cell enumeration in whole blood samples but they cannot be utilized to enumerate rWBC because the cell concentration is far below the range of linearity of the instruments and because the high concentration of platelets (PLT) or red blood cells (RBC) in the sample to be tested could interfere with the count.

To date, the reference method to enumerate rare events is considered the flow cytometry (FCM), but this technique has drawbacks such as an elevated initial cost for

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http://dx.doi.org/10.1016/j.transci.2015.10.001
1473-0502/

equipment, the need for highly trained personnel and a lengthy procedure [3,4].

The ADAM-rWBC (NanoEnTek Inc., CA, USA) microscopic cell counter has been introduced on the market as an alternative counting method and is intended for the enumeration of rWBCs in leukoreduced blood products. The counter measures leukocytes on a plastic slide after their fluorescent staining with propidium iodide (PI) which only stains cells containing DNA. The system consists of a green LED (532 nm) laser and a CCD detector encased in a portable unit and a kit containing slides, buffers and a standard beads solution. The software performs the calculation of residual WBCs per μL that is automatically displayed.

To our knowledge, at present only two studies [5,6] have tested the ADAM-rWBC against a reference method; both studies compared the ability of ADAM-rWBC to quantify rWBC against a manual Nageotte counting chamber and an automatic FCM method in RBC concentrates [5] and in PLT concentrates [6]. With the present paper we have tested the Adam-rWBC for the ability to enumerate rWBC in both RBC and PLT concentrates.

2. Materials and methods

The two methods have been pairwise compared using real life samples: firstly we have validated the FCM according to the ICH guidelines [7] for linearity, precision, accuracy and robustness by using Leuko-Trol control kit (Beckman Coulter, CA, USA) and then the ADAM-rWBC results have been compared with the reference method. The Leuko-Trol reagents are used to monitor leukoreduced RBC and PLT products, including the dilution and staining process, method set-up and WBC enumeration.

For the FCM validation, four dilutions of Leuko-Trol Platelet Control and Leuko-Trol RBC Control Kit were loaded on a Cytomics FC500 flow cytometer (Beckman Coulter) and analyzed in three replicates on three separate days by two different operators for a total of 36 determinations each.

PLT concentrates were collected by cell apheresis from voluntary blood donors by using the Trima Acell system (Terumo BCT, USA) and leukodepleted RBC concentrates were obtained by using the CompoFlow Select in-line filtration system (Fresenius Kabi, Germany). Residual leukocytes were measured on both instruments in 36 PLT and 23 RBC units respectively.

Residual WBC enumeration in RBC and PLT concentrates was performed by using the LeukoSure (LeukoSure Enumeration Kit, Beckman Coulter) following the manufacturer's instructions: specimens have been lysed and permeabilized by using the LeukoSure Lyse Reagent to eliminate RBCs and prepare the cells for subsequent addition of the stain reagent which contains propidium iodide that binds only to double stranded DNA so that nucleated cells in the sample emit fluorescence in proportion to their DNA content: the stained cells represent the leukocyte component of the blood.

The enumeration method depends upon mixing 100 μL of LeukoSure Fluorospheres with an identical volume of sample to be tested. After analysis the absolute count for the specimen is calculated thus representing the absolute number of leukocytes in the specimen. A fixed number of 10,000 events were acquired for all analyses in a logical gate as specified in the LeukoSure manual. Only intact WBCs have been included in the detection gate as established with fresh samples.

Before starting each session, the Adam-rWBC was calibrated using a Standard Bead Solution. An aliquot of 100 μL of PLT or RBC products was stained with 400 μL of staining solution (r-Solution) and then 100 μL of the sample was pipetted onto a plastic slide and inserted in the instrument. The cell concentration was calculated from absolute cell count divided by sample volume. The declared range of linearity for ADAM-rWBC is between 0 and 100 WBCs/μL (http://www.nanoentek.com/upload/product/2/ADAM-rWBC_Brochure_Eng%20(V.1.1).pdf). Low and high Leuko-Trol platelet (2.0–20.0 WBC/μL) and RBC controls (2.2–23.0 WBC/μL) were run before starting each session on Adam-rWBC instrument (n = 10).

2.1. Statistical analysis

For the validation of the FCM method, the strength of the linear relationships between the expected and the obtained rWBC values was evaluated by using the linear regression coefficient (R^2).

The precision was estimated by calculating the coefficient of variation (CV) that was considered adequate when ≤20% for concentrations above [8] and ≤30% for concentration below 2×10^6 rWBC/μL respectively. The accuracy was estimated by calculating the percentage (%) error that was considered sufficient when ≤20%. The agreement between the two different instruments was estimated by using the Bland and Altman plots versus gold standard [9]. This approach was used since FCM is considered an established and high precise method already in clinical use. The difference in values obtained with the two methods represents the bias of the less established method relative to the more established one. The paired Student t-test was considered significant for $P \leq 0.05$.

When not differently indicated, results are expressed as mean ± standard deviation (mean ± SD). Data were analyzed by using the GraphPad PRISM 5.0 (GraphPad Software, Inc., San Diego, CA) and Excel 2010 (Microsoft Corp., US).

3. Results

The range of linearity of the rWBC determination in PLT and RBC concentrates by FCM is reported in Fig. 1. In Table 1 are reported the daily mean rWBC determinations for PLT and RBC leuko-Trol control cells at the four cell dilutions together with the coefficient of variation and the percent error.

On the ADAM-rWBC, the measured Leuko-Trol PLT low and high controls were 2.1 ± 0.5 (CV: 25.9; % error: 5.8) and 20.4 ± 2.6 (CV: 12.7; % error: 2.0) respectively and the measured Leuko-Trol RBC low and high controls were 2.0 ± 0.5 (CV: 24.0; % error: 9.8) and 24.8 ± 0.3 (CV: 1.2; % error: 7.7) respectively.

Fig. 2A and 2B report the Bland–Altman differences versus gold standard: the analysis revealed general concordance between methods even if an overestimation of the values obtained by ADAM-rWBC in both PLT and RBC concentrates was observed and the differences among the values (FCM – ADAM-rWBC) were outside the 95% confidence