Transfusion and Apheresis Science 57 (2018) 544–548

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

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

Comparison between Nageotte and flow cytometric counting of residual leucocytes in freshly prepared leucocyte-reduced red blood cell components

Elias Kyriakou[a], Nikolaos Nearchakos[a], Stefanos Bonovas[b,c], Efstathia Makri[a], Katerina Pantavou[d], Georgios K. Nikolopoulos[d], Christine Kottaridi[e], Argyri Gialeraki[a], Panagiota Douramani[a], Maria Taichert[a], Violetta Kapsimali[f], Argirios E. Tsantes[a,*]

[a] Laboratory of Haematology & Blood Bank Unit, "Attikon" Hospital, School of Medicine, National and Kapodistrian University of Athens, 1 Rimini Str., 12462, Athens, Greece
[b] Department of Biomedical Sciences, Humanitas University, 20090 Pieve Emanuele, Milan, Italy
[c] Humanitas Clinical and Research Center, 20089 Rozzano, Milan, Italy
[d] Medical School, University of Cyprus, Palaios dromos Lefkosias Lemesou No.215/6, 2029, Strovolos, Nicosia, Cyprus
[e] 2nd Department of Pathology, "Attikon" Hospital, School of Medicine, National and Kapodistrian University of Athens, 1 Rimini Str., 12462, Athens, Greece
[f] Department of Microbiology, School of Medicine, National and Kapodistrian University of Athens, 17, Agiou Thoma Street, 11527, Athens, Greece

ARTICLE INFO

Keywords:
Leukoreduction
Nageotte
Flow cytometry
Leucocyte counting
Red blood cell unit

ABSTRACT

Background: Flow cytometry (FC) and Nageotte hemocytometry represent the most widely accepted methods for counting residual white blood cells (rWBCs) in leucocyte-reduced (LR) blood components. Our aim was to study the agreement between the two methods, under real working blood bank conditions.

Materials and methods: 94 freshly produced LR red blood cell (RBC) units were tested for rWBC concentrations by FC and Nageotte. To assess the precision of each method, we calculated the intra-assay coefficients of variation (CV), and followed the Bland-Altman methodology to study the agreement between the two methods.

Results: CV was 18.5% and 26.2% for the Nageotte and the FC, respectively. However, the agreement between the duplicate observations, using the binary cut-off threshold of 1×10^6 WBCs per unit to define the results as "pass/fail", was 71.9% for the Nageotte and 93.3% for the FC. Linear regression analysis did not show any correlation (R-squared = 0.01, p = 0.35) between the two methods, while the Bland-Altman analysis for the measuring agreement showed a bias toward a higher Nageotte count of 0.77×10^6 leucocytes per unit ($p < 0.001$) with the 95% limits of agreement (d ± 2 sd) ranging from -0.40×10^6 to 1.94×10^6 leucocytes per unit.

Conclusion: The absence of agreement between Nageotte and FC method, with the differences within d ± 2 sd being of high clinical importance, suggests that the two methods cannot be used for clinical purposes interchangeably. The Nageotte seems unsuitable for quality control even with a pass-fail criterion, under real working blood bank conditions.

1. Introduction

The presence of residual white blood cells (rWBCs) in blood products may result in a variety of adverse events due to transfusion of allogeneic leucocytes. Therefore, the universal removal of leucocytes from blood components has been established as a common practice in several countries, which has resulted in an increasing need to validate and monitor the leucoreduction processes. The concentration of WBCs in leucocyte-reduced (LR) blood components is below the level of accurate detection by standard hematology analyzers and presents a technical challenge. A residual number of 1×10^6 leucocytes per red blood cell (RBC) unit is currently the rejection limit for LR blood components set by the Council of Europe [1]. Several methods have been developed to count such low concentrations of WBCs [2]. Among the published methods, Nageotte hemocytometry has been the most widely accepted and used [3–5]. Masse et al. [4] compared several variations of this technique. The lower limit of accurate detection was approximately estimated at 2 WBC/μl, lower than 3.3 WBC/μl, i.e. the rejection level of LR blood components, and the method had been considered suitable for routine quality control testing [4]. However, this microscope-based counting technique is labor-intensive, time-consuming, and does depend upon individual observer training that may

* Corresponding author.
E-mail address: atsantes@med.uoa.gr (A.E. Tsantes).

https://doi.org/10.1016/j.transci.2018.06.002
Received 15 January 2018; Received in revised form 24 April 2018; Accepted 5 June 2018

interfere with the reproducibility of the results. This has led to the development of several other dedicated automated methods to count low levels of rWBCs, i.e. the automated volumetric capillary cytometer [6], flow cytometric methods [7,8], and polymerase chain reaction (PCR) techniques [9]. In the absence of a "gold standard" method, assay performance is hard to be assessed and, in most cases, counting assays for rWBCs in blood components were evaluated by dilution studies [3,4,6,10–14]. These studies demonstrated not only the feasibility of automated techniques for process control by blood collection facilities but also their improved precision and greater accuracy than what has been observed with the Nageotte hemocytometer method. Thus, flow cytometric methods were shown to be preferable to microscopic methods for counting rWBCs [10,12,13]. However, few studies have assessed in parallel the performance of flow cytometry (FC) and Nageotte hemocytometry on freshly produced LR blood components [15].

Our aim was to study the agreement between the two most widely accepted methods for counting rWBCs in leucodepleted blood units, i.e. the Nageotte hemocytometry and flow cytometry, under real working blood bank conditions.

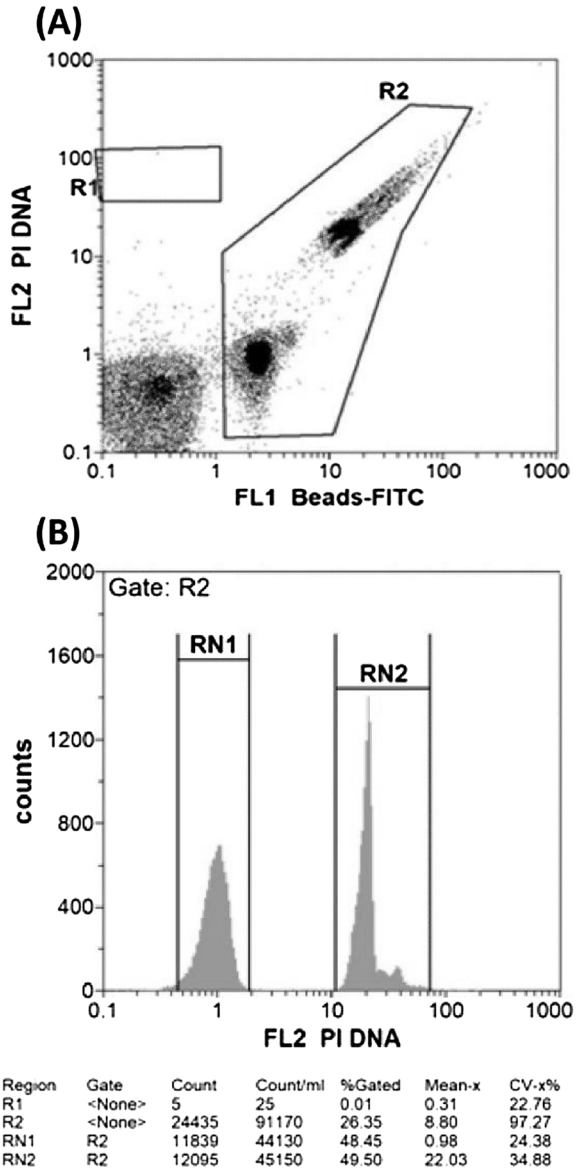
2. Materials and methods

This study was conducted in the Blood Bank Unit of "Attikon" University Hospital in Athens, Greece. Ninety-four freshly produced LR RBC units, collected into several different blood bag systems with integrated in line filters, were analyzed in the context of the routine program of leucoreduction process control over one year period (from July 2016 to July 2017). Thirty units were collected into Haemonetics bags (Haemonetics SA, Signy, Switzerland), 10 into MacoPharma blood bags (MacoPharma – Rue Lorthiois, Mouvaux, France), 20 into Fresenius blood bags (Fresenius Hemocare, Modena, Italia), and 45 were collected with the Atreus 3 Component Integrated Processing Set (Terumo BCT, Europe N.V. Zaventem, Belgium).

All RBC units were tested for rWBC concentrations by an automated FC method (LeukoFinderTM kit, CYTOGNOS SL, Salamanca, Spain) and Nageotte counting chamber within 24 h of collection. Flow cytometric cell count-tests were handled according to the manufacturer's instructions. Calibrated pipettes were used, with an accuracy of +/- 5% of the set volume and a CV < 5%, as the Blood Bank Unit of "Attikon" University Hospital meets the *requirements of ISO* 15189: 2012. The *LeukoFinderTM* kit is a single-platform method for the enumeration of rWBC by FC, which combines the detection of the fluorescence signal from a DNA marker incorporated into the nucleus of rWBCs allowing their discrimination, with the use of Perfect-Count MicrospheresTM for their absolute count. Labeling of rWBCs with the DNA marker enables them to be identified and discriminated from some non-nucleated populations, such us erythrocytes and platelets. The Perfect-CountTM Microspheres is a microbead-based single platform system, which assures the accuracy of absolute count results. Its unique internal quality control system contains two types of beads (defined as bead A and bead B) with densities around the upper and lower densities of peripheral blood cells. Variations of the ratio between beads type A and B warns about problems during sample preparation and/or acquisition, which could invalidate final results. This system may be used as a double reference standard, which, first, assures the accuracy of the assay and, second, ensures accurate calculation of the number of rWBCs per μL.

Briefly, 100 μL of each sample were transferred using the reverse pipetting technique to a flow cytometry tube. The cells were stained with 100 μL of the propidium iodide DNA labeling solution included in the kit, mixed gently, and incubated in the dark for 5 min. Then 100 μL of the Perfect-Count Microspheres included in the kit were added to the above mixture and followed by the addition of 550 μL PBS. After gentle mixing using the pipette, the sample data from the entire 850 μL volume was acquired, analyzed, and stored using the CyFlow Space *cytometer* (Sysmex-Partec GmbH, Münster, Germany).

For the flow cytometric analysis, the FSC (forward scatter) and SSC (side scatter) parameters were set on linear amplification, while FL1 and FL2 parameters were set on logarithmic amplification. The fluorescent signal emitted by cells stained by the DNA labeling solution was detected in the FL2 channel in the gated region R1, and the different sized beads A and B were detected in the FL1 channel in the gated region R2 (Fig. 1A). The absolute numbers of leukocytes and microspheres were determined by the unique TVAC (True Volumetric Absolute Count) feature of the CyFlow Space cytometer. The gated region R1 in the dot plot gives the absolute number of the stained rWBCs and the ranges RN1 and RN2 in the histogram plot provides the absolute number of each bead in the gated region R2 (Fig. 1B).



Region	Gate	Count	Count/ml	%Gated	Mean-x	CV-x%
R1	<None>	5	25	0.01	0.31	22.76
R2	<None>	24435	91170	26.35	8.80	97.27
RN1	R2	11839	44130	48.45	0.98	24.38
RN2	R2	12095	45150	49.50	22.03	34.88

Fig. 1. Flow cytometric analysis. (A) The R1 gate was used to select the residual leukocytes (rWBC) and the R2 gate was used to select the total microspheres. (B) The histogram range RN1 and RN2 shows the absolute count of the gated region R2.

The calculation of the absolute number of rWBCs in the LR bags was based on the following formula: Absolute Count (rWBC/μl) = [Nº of rWBC counted / Total Nº of microspheres counted (A + B)] × Nº of Perfect–Count Microspheres / μl (known concentration). Multiplying rWBC/μL by the volume of the pack (in μL) gives the total number of rWBCs in the entire pack.

For the Nageotte haemocytometer, the following procedure was

applied [16]: A hemocytometer chamber with 50-μL counting volume was used. 100 μL of the samples were diluted 1-in-5 with the lysing solution Leucoplate (Sobioda, Saint-Martin, France). A 50-μL aliquot was loaded onto a 50-μL gridded counting area of a Nageotte hemocytometer (Nageotte BriteLine Chamber, Hausser Scientific, Horsham, PA) and then incubated in a humidity chamber for 15 to 30 min. WBCs present in the entire 50 μL volume of the counting chamber were counted with the use of a 20x microscope objective. White cell concentration was calculated as follows: WBCs/ μL = (cells counted/50 μL) × 5, where 50 μL is the volume counted and 5 is the dilution factor resulting from the addition of lysing agent. For the total white cell content of the LR component holds that: WBCs / component = WBCs/ μL × 1000 μL / mL × volume in mL of the component.

2.1. Statistical methods

Summary statistics are presented with means ± standard deviations, medians and interquartile ranges (IQR), or percentages when appropriate.

To assess the precision of each method, we calculated the intra-assay coefficients of variation (CV). The CV of duplicate observations reflects the variance observed when replicate aliquots of the same specimen are processed and analyzed.

The agreement between the two methods was determined by the kappa statistic and the respective p-value. A residual number of 1×10^6 WBCs per unit – currently determined by the Council of Europe as the rejection limit for LR blood components – was used as cut-off to characterize the results as "pass" or "fail".

The guidance for combined graphical/statistical assessment of the agreement between two methods of clinical measurement includes a scatter diagram (concordance plot) combined with correlation and regression analysis, and a difference plot combined with calculation of the 2 sd limits of the differences between the methods (the so-called 95% limits of agreement) [17]. Accordingly, we plotted the data, and performed linear regression analysis to assess the correlation between the two methods. Then, we used the Bland-Altman plot to assess the agreement between the Nageotte method and the FC. The *x* axis shows the mean of the results of the two methods ([A + B]/2), whereas the *y* axis represents the absolute difference between the two methods ([B–A]). In addition, the plot includes the line for the mean difference (bias) and the observed 2 sd limits of the differences (precision) between the two methods. Bland and Altman suggest that provided the differences within d ± 2 sd (i.e. 95% limits of agreement) are not important, the two methods can be used interchangeably.

The paired Student's *t* test was used to determine whether the mean difference (bias) was significantly different from zero.

For all tests, a probability less than 0.05 indicates statistical significance. All p-values are two-tailed. Stata 14 was used for all statistical analyses (Stata Corp., College Station, TX, USA).

3. Results

3.1. Precision of each method

The estimated intra-assay CV was 18.5% for the Nageotte hemocytometry (32 duplicate observations) and 26.2% for the FC method (15 duplicate observations).

The agreement between the duplicate observations, using the binary cut-off threshold of 1×10^6 WBCs per unit to define the results as "pass/fail", was 71.9% for the Nageotte method (Table 1A) and 93.3% for the FC method (Table 1B).

3.2. Agreement between the two methods

Descriptive statistics of the study specimens processed and analyzed (n = 94) are presented in Table 2.

Table 1
2×2 contingency tables obtained using the binary cut-off threshold of 1×10^6 white blood cells (WBCs) per unit to characterize the results as "pass" or "fail".

A. Nageotte hemocytometry (32 duplicate observations): **Agreement = 71.9%**

		Count 1	
		"pass"	*"fail"*
Count 2	*"pass"*	**19**	**4**
	"fail"	**5**	**4**

B. Flow Cytometry (15 duplicate observations): **Agreement = 93.3%**

		Count 1	
		"pass"	*"fail"*
Count 2	*"pass"*	**14**	**0**
	"fail"	**1**	**0**

Table 2
Descriptive statistics of the study specimens processed and analyzed (n = 94).

Nageotte method (leucocytes per unit $\times 10^3$)	922 ± 523; 784 (542–1248)
Nageotte method (pass/fail)	63/94 (67.0%) vs. 31/94 (33.0%)
Flow cytometry (leucocytes per unit $\times 10^3$)	150 ± 217; 37 (4–246)
Flow cytometry (pass/fail)	93/94 (98.9%) vs. 1/94 (1.1%)

Data are presented as means ± standard deviations (SD); medians and interquartile ranges (IQR), or percentages when appropriate.

The agreement between the two cell count methods, as determined by the Kappa statistic, was poor (agreement = 66.0%, kappa = –0.02, p = 0.76).

The scatter diagram of the paired measurements of WBCs per unit ($\times 10^6$) as determined by the Nageotte method and the FC, for all study specimens (n = 94), is presented in Fig. 2. The visual inspection of the plot shows poor agreement between the two methods. Moreover, the linear regression analysis did not show any correlation (R-squared = 0.01, p = 0.35). Thus, not only the two methods showed poor agreement (as shown by the kappa statistic), but they are also not correlated (as shown in the linear regression analysis).

The Bland-Altman analysis for measuring agreement is presented in Fig. 3. It shows a bias toward a higher Nageotte count of 0.77×10^6 leucocytes per unit (p < 0.001) with the 95% limits of agreement (d ± 2 sd) ranging from -0.40×10^6 to 1.94×10^6 leucocytes per unit. Given that differences within d ± 2 sd (i.e. from -0.40×10^6 to 1.94×10^6 leucocytes per unit) are clinically important, it seems that the two methods cannot be used for clinical purposes interchangeably.

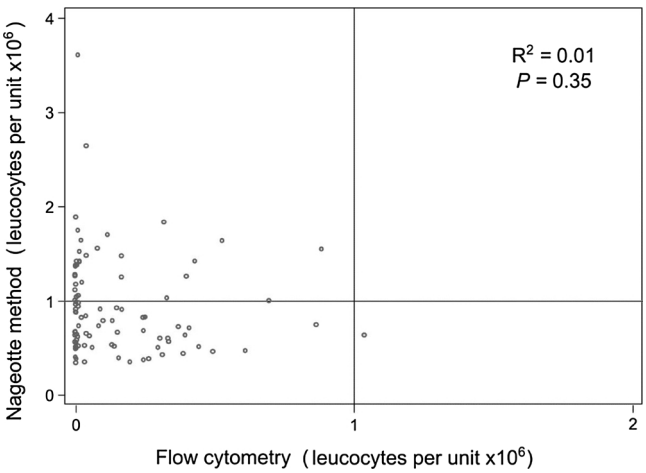


Fig. 2. Scatter diagram of paired measurements of white blood cells (WBCs) per unit ($\times 10^6$) as determined by Flow cytometry and Nageotte hemocytometry, for all study specimens (n = 94).

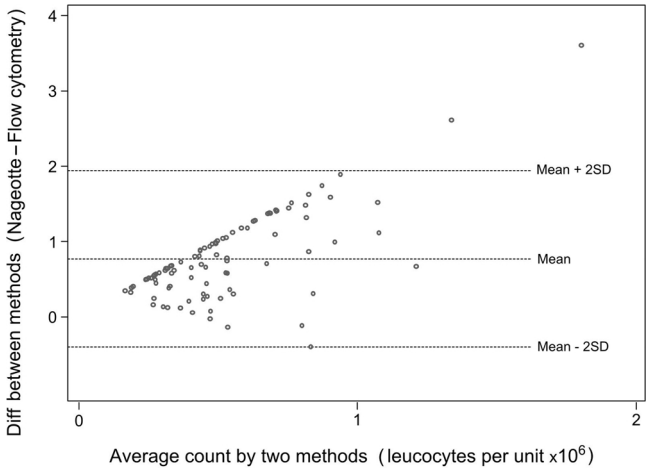


Fig. 3. Bland-Altman plot for measuring agreement between Nageotte hemocytometry and Flow cytometry (FC).

4. Discussion

In this study, we compared the performance of Nageotte hemocytometry and FC in terms of counting low WBC concentrations in leucodepleted RBC units, under real working blood bank conditions. In the absence of a "gold standard" method and dilution experiments, the precision of each assay, as well as the correlation and agreement between the two methods, were evaluated. No correlation was found between Nageotte hemocytometry and FC, while the two assays showed poor agreement to such an extent that their interchangeable use in clinical practice is probably not appropriate.

The Nageotte hemocytometry was the first practical method for the enumeration of rWBCs in LR blood components and had been considered suitable for routine quality control testing. However, it was labor intensive, showed low accuracy, and required both technical training and considerable experience [18]. In order to overcome these difficulties, alternative counting methods have been developed. Several single-site and multicenter studies compared WBC counts obtained by automated methods to results obtained by the Nageotte hemocytometry [6,19,20]. The experience with dilution studies demonstrated that the accuracy and precision of the Nageotte haemocytometer were poor at the rejection concentration of LR blood components and showed a bias to underestimation compared to results obtained with the automated methods [12–14]. The underestimation of leucocyte concentrations in the red cell concentrate samples might be partially due to incomplete lysis of red cells, preventing the leucocytes from settling, while leucocytes may be destroyed or lost during sample processing [12].

However, evidence regarding the performance of counting methods on freshly produced blood components under routine blood bank conditions are rare, and there are indications that in such samples the manual counting system may demonstrate higher absolute cell numbers than the automated staining systems [15]. In our study, the Nageotte counting yielded significantly higher rWBC results than the FC. The intra-assay CV for the Nageotte method was slightly better than that of FC. Nevertheless, due to overestimation of leucocyte concentrations with Nageotte haemocytometer compared to FC, about one third (9/32) of measurements of double replicate aliquots from the same specimen with Nageotte, were above and below the rejection limit, leading to inconsistent pass/fail results for labeling the RBC unit as leucoreduced. As a consequence, the agreement between the duplicate observations was only 71.9% indicating poor performance for Nageotte to a level around the cut-off dictated by the current standard. Regarding FC, despite its slightly worse precision in relation to Nageotte, all but one pair of measurements yielded concordant results in order to identify whether or not a unit meets existing standards and a very good agreement between the FC duplicate observations was found.

In a previous study on platelet components, although higher absolute rWBC numbers were also found by the Nageotte counting, all results were below the rejection level for rWBCs/platelet product [15]. The overestimation of leucocyte concentrations with Nageotte haemocytometer on freshly prepared blood components has been partially attributed to different methodical approaches between manual counting chambers and fully automated systems [15]. Besides variability in the counting assay, contradictory findings between spiked and freshly prepared samples may also be due to variability in the preparation of the diluted samples, while measurements at very low concentrations of cells, are prone to sampling errors [21]. Because the variation of the counted value is inversely proportional to the square root of the number of cells counted, a minimum number of cells must be counted to attain precision. Due to the fixed volume of the Nageotte chamber, the volume of sample analyzed per test could also negatively affect the method's performance [2].

In our analysis, when a pass/fail type of counting was employed, poor agreement was found between the two methods. Furthermore, they were not correlated, although they are assumed to measure the same quantity. This is in accordance with the absence of any correlation between the flow cytometric assay and the Nageotte counting chamber for the investigation of the rWBCs on buffy coat platelet concentrates [15].

The previously predominant methodology, Nageotte chamber counting, has recently been generally replaced by FC, which is currently reported as the main method of rWBC counting. However, the Nageotte method is still considered a reliable alternative for processes validation purposes [5], despite its several defects. Besides being time-consuming and laborious, it suffers from variability due to subjectivity. Moreover, in our analysis, the performance of Nageotte on freshly produced leucodepleted RBC components was found to be unreliable around the critical cut-off value and units could be incorrectly rejected as leucoreduced or incorrectly considered to be leucoreduced. Although no target samples were used in order to estimate the accuracy of the assays, if the Nageotte overestimation trend is real, an increased number of units could be incorrectly rejected as leucoreduced by using Nageotte in a process control program for leucoreduction. Under these circumstances, Nageotte is probably improper for the evaluation of new leucoreduction processes.

Several limitations of our study need to be acknowledged. Although in the absence of a "gold standard" method counting assays are usually evaluated by dilution studies, no spiked samples were applied. This was because the performance of both methods has been extensively investigated in previous dilution studies, while our aim was to evaluate them under real working blood bank conditions. Furthermore, because of the absence of samples with known leukocyte concentrations, we could not assess accuracy and sensitivity of the methods and it can be argued that there is uncertainty about which method's measurements are closer to the real values.

In conclusion, the absence of any agreement or correlation between Nageotte and FC, suggests that the two methods cannot be interchangeably used for clinical purposes. Moreover, the Nageotte chamber counting seems to be unsuitable for quality control with a pass/fail criterion or with determination of the actual leukocyte content of the RBC unit, under real working blood bank conditions. Testing of a larger number of samples will be required to document that these findings can be generalized. In such a case, Nageotte could not be recommended as an alternative for process control of LR RBC components.

Declarations of interest

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authorship contributions

AT, EK and VK contributed to the conception and design of the study. NN, EM, CK, AG, PD, and MT conducted the work and collected the data. SB, GN, and KP carried out the statistical analysis. All authors contributed to the interpretation of data for the work. AT, EK, and SB drafted the manuscript. All authors critically revised the paper for important intellectual content, and approved the final version to be published.

References

[1] Council of Europe. 18th ed. Guide to the preparation, use and quality assurance of blood components: recommendation no. R (95) 15. Strasbourg, France: European Directorate for the Quality of Medicines & HealthCare; 2015.
[2] Dzik S. Principles of counting low numbers of WBCs in WBC reduced blood components. Transfus Med Rev 1997;11:44–55.
[3] Lutz P, Dzik WH. Large volume hemocytometer chamber for accurate counting of white cells (WBCs) in WBC-reduced platelets: validation and application for quality control of WBC-reduced platelets prepared by apheresis and filtration. Transfusion 1993;33:409–12.
[4] Masse M, Naegelen C, Pellegrini N, Segier JM, Marpaux N, Beaujean F. Validation of a simple method to count very low white cell concentrations in filtered RBCs or platelets. Transfusion 1992;32:565–71.
[5] Dumont LJ, Papari M, Aronson CA, Dumont DF. Whole blood collection and component processing. In: Fung KM, Grossman BJ, Hillyer CD, Westhoff CM, editors. Technical manual. 18th ed.Bethesda: American Association of Blood Banks; 2014. p. 154–5.
[6] Adams MR, Johnson DK, Busch MP, Schembri CT, Hartz TP, Heaton WA. Automated volumetric capillary cytometry for counting white cells in white cell-reduced plateletpheresis components. Transfusion 1997;37:29–37.
[7] Wenz B, Burns ER, Lee V, Miller WK. A rare-event analysis model for quantifying white cells in white cell-depleted blood. Transfusion 1991;31:156–9.
[8] Barclay R, Walker B, Allan R, Reid C, Duffin E, Kane E, et al. Flow cytometric determination of residual leucocytes in filter-depleted blood products: an evaluation of Becton-Dickinson's LeucoCOUNT system. Transfus Sci 1998;19:399–403.
[9] Lee TH, Stromberg RR, Heitman J, Tran K, Busch MP. Quantification of residual white cells in filtered blood components by polymerase chain reaction amplification of HLA DQ-A DNA. Transfusion 1994;34:986–94.
[10] Rebulla P, Dzik WH. Multicenter evaluation of methods for counting residual white cells in leukocyte-depleted red blood cells. Vox Sang 1994;66:25–32.
[11] Prati D, Brandwein H, Capelli C, Dzik WH, Masse M, Myllylä G, et al. Multicenter evaluation of the 3% paraformaldehyde method for white cell counting in leukocyte-reduced red blood cells. Vox Sang 1996;70:241–5.
[12] van der Meer PF, Gratama JW, van Delden CJ, Laport RF, Levering WH, Schrijver JG, et al. Comparison of five platforms for enumeration of residual leucocytes in leucoreduced blood components. Br. J. Haematol. 2001;115:953–62.
[13] Dzik S, Moroff G, Dumont L. A multicenter study evaluating three methods for counting residual WBCs in WBC-reduced blood components: Nageotte hemocytometry, flow cytometry, and microfluorometry. Transfusion 2000;40:513–20.
[14] Szuflad P, Dzik WH. A general method for concentrating blood samples in preparation for counting very low numbers of white cells. Transfusion 1997;37:277–83.
[15] Schmidt M, Spengler HP, Lambrecht B, Hourfar MK, Seifried E, Tonn T. A new one-platform flow cytometric method for residual cell counting in platelet concentrates. Transfusion 2009;49:2604–11.
[16] Roback JD, Grossman BJ, Harris T, Hillyer CD. Technical manual. 17th ed. Bethesda: American Association of Blood Banks; 2011. p. 975–7.
[17] Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–10.
[18] Dzik WH. Leukocyte counting during process control of leukoreduced blood components. Vox Sang 2000;78:223–6.
[19] Dumont LJ, Dumont DF. Enhanced flow cytometric method for counting very low numbers of white cells in platelet products. Cytometry 1996;26:311–6.
[20] Dzik WH, Rebulla P. Multicenter evaluation of methods for counting residual white cells in leukocyte-depleted red blood cells. Vox Sang 1994;66:25–32.
[21] Dumont LJ. Sampling errors and the precision associated with counting very low numbers of white cells in blood components. Transfusion 1991;31:428–32.