Process control of filtered red blood cells: which counting method?

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SUMMARY. Various counting methods have been described and reported for process control of leucodepleted blood components. The recent production of high-efficiency leucocyte removal filters intensifies the need for sensitivity in determining the ever lower residual concentration of white cells (WBCs) in filtered units.

In order to assess which method was the most efficient and feasible in the laboratory for the control of WBC-reduced packed red blood cells, we compared the sensitivity of four counting methods: Nageotte chamber analysis, flow cytometry, the fluorochrome method by Borzini and Nageotte chamber analysis as modified by Prati.

We observed a difference in the post-filtration WBC content depending on which method of counting was used and we feel it reasonable to ask what method should be employed in blood component process control.

The answer must naturally consider that the method is for use by a large number of laboratories, while the sensitivity of the method needs to be appropriate to the goal desired.

Key words: filtration, flow cytometry, fluorochrome method, leucocytes, Nageotte chamber.

One of the most important aims of the BEST group is to improve the quality of leucodepleted blood components for greater safety of blood transfusion by validating protocols suitable for routine quality assurance.

Various counting methods have been described and reported for process control of leucodepleted blood components. The recent production of high-efficiency leucocyte removal filters intensifies the need for sensitivity in determining the ever lower residual concentration of white cells (WBCs) in filtered units.

Clearly the lower limit of accurate detection and the precision of the method used for counting residual WBCs are key factors in quality control of these blood components (Dumont et al., 1996).

In order to assess which method was the most efficient and feasible in the laboratory for the control of WBC-reduced packed red blood cells, we compared the sensitivity of four counting methods: Nageotte chamber analysis (Masse et al., 1991), flow cytometry (Wenz et al., 1991), the fluorochrome method by Borzini (Borzini et al., 1995a,b) and Nageotte chamber analysis as modified by Prati (Prati et al., 1996).

Blood units of 450 ± 50 mL were collected in quadruple systems with CPD. Each unit was centrifuged for 7 min at 1000g and blood components were separated by an automated separator (Terumo) into packed red blood cells without buffy coat and plasma; saline-adenine-glucose-mannitol was added to the packed red blood cells as the preservative solution.

To maintain the same volume and number of cells in all experiments, 3 units of packed red blood cells of compatible groups were pooled in a single bag. After thorough mixing in the single unit for 30 min at room temperature, the packed red blood cells were divided equally into three bags of about 280 mL each. Filtrations were performed on units stored at 4 °C for 24 h.

We used three filters designed by the manufacturers for high-efficiency leucocyte removal: Biofil R 01 Max (filter A), Terumo Imugard 400 (filter B) and Pall RCZL (filter C). For each filter, we evaluated the residual number of WBCs by each technique in 20 filtered units.

The filtration procedure was performed following the manufacturers' instructions.

We started the filtration procedure within 10 min of removing the packed red blood cells from the refrigerator. All filtration was carried out by gravity. No prefiltration rinsing of filters with saline solution was performed nor was any pressure applied to the filtration.
procedure. The distance between prefiltration and postfiltration packed red blood cells bags was about 120 cm.

We evaluated the prefiltration and postfiltration volumes of packed RBCs by dividing the net volume by the specific gravity (1.05). Both pre- and post-filtration samples were analysed by automated counting procedures in duplicate (Genius, SEAC, Florence, Italy); furthermore, the post-filtration specimens with a very low concentration of WBCs were counted by each of the following methods.

Nagelott chamber analysis. One hundred microlitres of the sample was mixed with 900 μL of Turk’s solution. Evaluation by light microscopy was performed by two investigators counting one full grid of the Nagelott chamber (50 μL), and the final results were expressed as a mean. We calculated the final WBC concentrations as follows: (number of counted cells x 10)/volume counted, where 10 is the dilution of the sample.

Flow cytometry. The flow cytometry count was performed as described by Wenz: 100 μL of packed RBCs was mixed with the following reagents: 600 μL of formic acid at 1.2 mL of distilled water, 265 μL of carbonate buffer (pH 7.4), 100 μL of paraformaldehyde (10 g L⁻¹ of distilled water) (Immunoprep EPICS, Coulter Diagnostics Hialeah, FL, USA) and 7 μL of polyethylene ester; 100 μL of a solution containing propidium iodide in phosphate buffer saline (0.1 g dL⁻¹, pL 7.4). Before the analysis we added 500 μL of phosphate buffer saline to each sample to reduce viscosity. Flow cytometry was performed using a flow cytometry EPICS (Coulter) and thresholds (electronic gate) were set using red fluorescence (FL2) vs. forward scatter (FSC) and FL2 vs. side scatter (SSC); we also used FSC vs. SSC cytograms to detect the WBCs by their specific light-scattering properties. The volume analysed was taken as the difference in milligrams in the weight of the tube before and after flow cytometric analysis. The final WBC concentration per millilitre was determined as follows: (number of fluorescence events per 16.72)/(volume counted), where 16.72 is the dilution.

Flow chromatographic technique. We followed the method described by Borzini. Briefly, 20 aliquots of the postfiltration sample (1 μL) were dispensed in two rows (20 wells) of a Terasaky tray. After addition of 5 μL of fluorochrome per well and 15 min incubation in the dark, the tray was centrifuged at 650g for 3 min to obtain an optimal cell sedimentation. Both living and dead WBCs were counted by a fluorescence microscope: the mean value of 20 replicates was taken to calculate the WBC concentration per microlitre and the WBC content in the postfiltration unit.

Nagelott chamber modified by Prati. Ten millilitres of the postfiltration sample was added to 40 mL of 3% paraformaldehyde. The tubes were centrifuged at 800g for 10 min. The supernatant was discarded and the moist pellet was resuspended with Turk’s solution to a final volume of 500 μL. The suspension was loaded in a single-grid Nagelott chamber. After 15 min incubation in a humid environment two investigators counted one full grid of the Nagelott chamber (50 μL), and the final results were expressed as a mean. The WBC concentration per microlitre was calculated by the following formula: (number of WBCs in 50 μL of sample x 10)/10.000.

In the absence of any reference counting method, the accuracy of the methods employed was confirmed by dilution studies. A sample of WBCs was serially diluted by adding WBC-depleted packed RBCs to reach a final dilution of 10⁻⁰.001 WBCs μL⁻¹. We evaluated the WBC concentration of the samples by the methods described and plotted the data obtained as a function of the expected concentration. The measured values of the experiments showed that the correlation coefficients ranged from 0.997 to 0.999 according to the sensitivity of the technique performed.

The data of the average residual WBCs content in the postfiltration units measured by the four counting methods are summarized in Table 1.

The count performed with the Nagelott method showed the number of leucocytes in the postfiltration unit to be 0.36 ± 0.15; 0.43 ± 0.2 and 0.29 ± 0.12 x 10⁶ with filters A, B and C, respectively. By flow cytometry and fluorochrome analysis we found about 1 log fewer leucocytes than with the Nagelott chamber. The absolute number of WBCs observed by flow cytometry was 0.06 ± 0.03, 0.04 ± 0.03 and 0.05 ± 0.03 x 10⁶ whereas by the fluorochrome method the absolute number was 0.04 ± 0.02, 0.04 ± 0.02 and 0.05 ± 0.03 x 10⁶, respectively, with filters A, B and C. With the Nagelott chamber analysis as modified by Prati we observed

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I log fewer leucocytes than by flow cytometry and fluorochrome analysis: 0.006 ± 0.004, 0.007 ± 0.004 and 0.005 ± 0.003 × 10^6 of total WBCs in the postfiltration units with filters A, B and C, respectively.

Flow cytometry and the Nageotte chamber method are the two techniques currently used in routine quality controls and validated by multicentre studies to a sensitivity of 0.1 WBCs μL⁻¹.

Our experience, as already described, yielded 1 log higher results with the Nageotte chamber than with flow cytometry. Flow cytometry showed results similar to those obtained by fluorochrome analysis. The fluorochrome method has been claimed to detect 0.5–1 log fewer results than the Nageotte chamber and this is in line with our results (P. Borzini, personal communication). The Nageotte chamber modified by Prati, based on a WBC concentration of 0.05 the original sample volume, raised the sensitivity of the method to 0.001 WBCs μL⁻¹ and the data obtained in our experiments showed that the average residual WBC content in the post-filtration units is 1 log less than by the flow cytometry and fluorochrome methods.

We observed a difference in the post-filtration WBC content depending on which method of counting was used. This could be related to the characteristics of the method employed because the various technical manipulations often lead to loss of cells. Thus the modifications made in the counting method aimed primarily at improving sensitivity might improve the technical manipulations to the detriment of the real count and consequently to an underestimation of the results.

Nonetheless, we feel it reasonable to ask what method should be employed in blood component process control to ensure that the haemocomponent will be really WBC-depleted to the expected value.

The answer must naturally consider that the method is for use by a large number of laboratories, while the sensitivity of the method needs to be appropriate to the goal desired. However, it must be remembered that the more the method is able to count extremely low concentrations of WBCs the more likelihood there is of underestimating the WBC content in the post-filtration unit and a consequent difficulty in reproducing countings.

Multicentre evaluation of such methods might profitably be a subject for national and regional workshops open to any group that needs to count at extremely low WBC levels. In fact, the development and perfection of counting methods to quantify very low numbers of leucocytes would make for good laboratory practice to safeguard for all patients needing highly purified cell suspensions.

REFERENCES


