A new tool in white blood cell reduction for packed red cells: 5 Log depletion

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SUMMARY. The recent development of new filters used for leucocyte reduction aims at restricting the number of leucocytes to a threshold where their undesirable effects can be minimized or excluded. In this paper we describe the performance of a new filter named BIO RO1 MAX and claimed by the manufacturer to perform 5 Log10 depletion. The results show that the efficiency of the filter reached 5 Log10 depletion and the absolute number of white blood cells in the post-filtration units is always less than $2 \times 10^4$ with considerable safety in the prevention of transfusion reactions.

Key words: filtration, leucocytes, red blood cells.

Leucoreduction of cellular blood components may provide several advantages, including reduced risk of primary alloimmunization and its consequences, decreased frequency of febrile reactions and removal of cell-associated viruses (Wenz, 1990; Brand, 1990; Reverberi & Menini, 1990).

Several reports and the Council of Europe advise that the leucodepleted packed red blood cells (RBC) ought to have a number of leucocytes $< 10^6$ to avoid HLA alloimmunization (Berlin & LeCren, 1994). Manufacturers have thus engaged in a search for new filters with a greater efficiency of leucodepletion in order to restrict the number of leucocytes to a level at which the undesirable effects of white blood cells (WBC) can be minimized or excluded (Sirchia et al., 1990; Pietersz et al., 1992).

In a previous paper we described the efficiency of WBC reduction achieved by a new generation of filters with $4 \log_{10}$ depletion and an absolute number of residual leucocytes in the haemocomponents $< 0.5 \times 10^6$ (Bontadini et al., 1994).

A new filter is now available for the depletion of packed RBC, named Biofil BioR 01 MAX (Biofil, Medolla, Italy) and claimed by the manufacturer to perform $5 \log_{10}$ depletion.

In this paper, we describe the performance of the BioR 01 MAX designed for filtration of a single bag in the blood bank or transfusion service laboratory. In our experiments we used 30 packed RBCs in saline–adenine–glucose–mannitol solution, 15 of them with buffy coat (BC) and 15 without, in order to evaluate if the number of WBCs and platelets is associated with improved filter efficiency or not (Steneker et al., 1993). A modified counting method by Nageotte chamber, claimed to be suitable for low concentrations of WBCs, was used (Prati et al., 1994, 1996).

MATERIALS AND METHODS

We performed 30 experiments; in the first group we utilized 15 units of packed RBCs with BC and in the second group 15 units of RBCs without BC.

Blood samples of 400 ± 50 mL were taken from healthy donors and collected in quadruple systems with CPD (Terumo Corporation, Tokyo, Japan). We centrifuged the whole blood for 7 min at 1000g. Fifteen units were separated by an automated blood components separator AC 212 Terumo (Terumo Corporation) into packed RBCs with BC and plasma. The same procedure was repeated with a further 15 units, this time separated into RBCs without BC and plasma. Saline–adenine–glucose–mannitol solution was added to each unit. The packed RBCs were stored at a temperature of 4 ± 2°C for 24 h. We performed filtrations after 24 h from collection.

The filtration procedures were performed by the BioR 01 MAX filter (Biofil) following the manufacturer's instructions. The filter was designed for filtration of a single bag in the laboratory of blood bank. All filtrations...
The time of filtration ranged from 5 min to 8 min and 30 s

**Table 1. Summary of absolute number of WBCs in pre- and post-filtration RBC units, and Log_{10} depletion post filtration**

<table>
<thead>
<tr>
<th>WBC</th>
<th>RBC with BC</th>
<th>RBC without BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-filtration (x10^9)</td>
<td>2.5 ± 0.8</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>range (x10^9)</td>
<td>(1.3-4.4)</td>
<td>(0.5-1.6)</td>
</tr>
<tr>
<td>Post-filtration (x10^4)</td>
<td>1.0 ± 0.4</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>range (x10^4)</td>
<td>(0.2-1.5)</td>
<td>(0.2-1.5)</td>
</tr>
<tr>
<td>Log_{10} depletion range</td>
<td>(5.1-5.8)</td>
<td>(5.0-5.7)</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.7</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Values are the mean ± SD of 15 experiments plus the range.

**Volume and RBC recovery**

The mean volume in the pre-filtration units was 322 ± 45 mL and 296 ± 28 mL while it was 280 ± 44 mL and 265 ± 24 mL in the post-filtration units, respectively, in the group of RBCs with and without BC.

Haemacrit was evaluated in the samples before and after filtration by the automated counter (Table 2). The RBC recovery was similar in the two groups (89.7 ± 4.8% and 91.4 ± 4.3%) and no significant difference was observed ($P = 0.38$).

**Platelet reduction**

The mean number of platelets in the RBC prefiltration was different in the two groups. It was 0.65 ± 0.14 x 10^{11} in the group with BC and 0.18 ± 0.08 x 10^{11} in the group without BC.

The mean depletion post-filtration was 43 ± 9% in the first group and 57 ± 14.3% in the second with a mean of the absolute number of 0.1 ± 0.04 x 10^{11} and 0.07 ± 0.01 x 10^{11}, respectively. Data are summarized in Table 2.

**Time of filtration**

The time of filtration ranged from 5 min to 8 min and 30 s without any significant difference in the two groups ($P = 0.4$).

**Statistical analysis**

We performed statistical analysis by using an unpaired t-test to compare the Log depletion, the RBC recovery and the time of filtration of the group of RBCs with and without BC. The $P$ value was considered significant when it was less than 0.05. The mean, standard deviation

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and coefficient of variation were used to show the results in a concise fashion.

DISCUSSION

In this study we have evaluated the efficiency of a new commercial filter named Biofil BioR 01 MAX used for WBC reduction of packed RBCs and claimed by the manufacturers to achieve $5 \log_{10}$ depletion.

The results showed that this new filter was able to reduce WBCs by $5 \log_{10}$. A few points deserve consideration.

The efficiency of the filter reached $5 \log_{10}$ depletion independently of the initial number of leucocytes in the RBCs with an excellent reproducibility of data in all experiments performed. Moreover, the variation of the absolute number of WBCs in the prefiltration units does not greatly affect the residual number of WBCs in the post-filtration units, which is less than $2 \times 10^4$ in the experiments performed.

In view of the application of the new counting method and to support the performance of the new filter, we evaluated the residual WBCs in the post-filtration units with the filter Biofil R01 Plus claimed to achieve $4 \log_{10}$ depletion. The results showed a mean number of residual WBCs in the post-filtration units of $8.3 \pm 1.2 \times 10^4$ and a mean $\log_{10}$ depletion of $4.1 \pm 0.2$ (A. Bontadini et al., unpublished data).

The recovery of RBCs ranges from a mean of $89.7\%$ in the units with BC to a mean of $91.4\%$ in the unit without BC, similar to data previously reported using a filter with less efficient leucodepletion (Pietersz et al., 1992; Bontadini et al., 1994). This is in accordance with the AABB Standards that require a recovery of at least $80\%$ of the original red blood cells (Standard Committee American Association of Blood Banks, 1991) and with the new guidelines recently promulgated by the US FDA regarding leucoreduction of blood which report that leucodepletion devices should not sacrifice more than $15\%$ of the therapeutic blood elements.

Even if the number of platelets in the prefiltration unit was different, after filtration the residual platelets were always less than $0.15 \times 10^11$ per unit; this is important in alloimmunized patients with a high titre of antibodies that must be transfused with two or more units of RBCs. Although the greatest number of leucocytes and platelets were found in the group of packed RBCs with BC, they did not interfere with the RBC recovery too much.

In conclusion, given the limited number of experiments performed, our data suggest that the BioR 01 MAX is very likely more efficient by $1 \log_{10}$ leucoreduction than the latest generation of filters described until now (Pietersz et al., 1992; Bontadini et al., 1994).

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