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Evaluation of Leukoreduction in Packed Cell Units Filtered by Home-Made Bedside Filters: Pre and Post Revision of Product Technology and Materials According to Standard Values

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Abstract

Introduction: Leukocytes causing a wide variety of side effects after transfusion are present in all blood products prepared by standard methods. As a consequence, the use of filter technology for leukoreduction has been widely practiced .According to AABB accreditation in 1996, leukoreduced blood components must contain less than 5×106 leukocytes per unit, but sometimes this value is higher even in leukoreduced products. In this study we did absolute leukocyte count in filtered (home made bedside filter) packed cell units by two methods of true count as standard method and CD45 MoAb.

Materials & Methods: 93 packed cell units were stored at 4ºc and filtered by two types of home-made filters according to manufacturer's instructions. Furthermore, eight packed cell units were filtered by Europe certified control group filters (bio-fil). Sample preparation was done according to True count kit and CD45 MoAb procedures and analysis was performed by flowcytometry (EPICS-XL,coulter) and (Partec PAS III).The results were then analyzed by chi2 test via SPSS.

Results: The mean values of leukocyte count/unit by anti CD45 and True count method were 9×106 and 10×106 respectively in 55 pre-revised filter bags; these figures were 4.2×106 and 4.8×106 in 30 post-revised filters, whereas the mean leukocyte count/bag in eight control filters was 2.3×106,we selected randomly eight test units out of 53 and 30 pre-revised and post-revised filtered bags, respectively(equal with number of units in the control group) to compare the test and control groups The mean values of leukocyte count/bag in pre-revised test group was 7.9±5.4×106 and in postrevised test group was 4.2 ×106 but in the control group it was 2.3 ×106 (p value<0.05).

Discussion: According to the results, the mean leukocyte count/bag in pre-revised group was higher than AABB standard. 38.2 % of bags had lower and 61.8 % had higher leukocyte count than the standard value (48.9 to 74.6 % with CI=95 %), this indicates the necessity of revision in product technology of homemade filters. relevant manufacturer revised the product technology and material accordingly, so that new filters (post-revised group) reduced leukocytes within standard limits (leukocyte count in 6 bags were out of standard range).In post-revise filter group, 20 % had a leukocyte count of more than 5×106 while 80% showed less than this value (5.7%-34.3% with CI=95%).There is a significant difference between control and pre-revised test groups (p=0.03).In post-revised test group, despite significant differences, the mean values of leukocyte count/bag were within normal standard range. The results of this research caused home-made filter production with higher quality.

Keywords: leukoreduction, Filtration, flowcytometry, true count beads, anti CD45 antibody

Introduction

Leukocyte contamination of blood components causes a wide variety of side effects after transfusion (1).Examples of these adverse effects are transmission of cell-associated infectious agents and prions, febrile non-hemolytic transfusion reactions, intransigence to Platelet transfusion, graft versus host disease, generalized immunosuppression, and an increased graft rejection rate of marrow or kidney transplantations (2,3).As leukocytes in blood can cause so many undesirable effects, blood transfusion services have decided to leukodeplete all cellular blood components. Various techniques have been used for removing leukocytes from blood components.

Blood filtration by leukodepletion filters is a commonly used method .Leukocyte filters can reduce 3-4 log orders of magnitude. Leukoreduction aphresis is also practiced in some centers, but filtration is known as the most effective and perfect method that may be immediately carried out at blood centers after shortly storage or at the bedside (post storage) (4).

In 1996, FDA started to permit blood products to be labeled "leukocyte reduced" if they contained less than $5x10^6$ leukocytes (5), but sometimes filtered blood products contain more than this number causing some adverse effects(4).To evaluate residual leukocyte count in leukodepleted products, different methods have been developed. Flowcytometry has been applied widely because of it's high specificity and sensitivity. The products were commonly adopted to get Propidium Iodide (PI) for labeling nucleated cell DNA, or fluorochrome conjugated monoclonal anti leukocyte antibody (anti CD45).The labeled nucleated cells are passed through the laser beam in order to be counted (6).National blood transfusion services began to look at automated leukocyte counting methods for assessment of leukodepletion effectiveness in late 1997.Today, fluorescent standard beads are known as commercial quality assured products (7).

In this study, we evaluated leukodepletion in packed cells filtered by two groups of home-made bedside filters (technology and material underwent pre and post-revision).At first, we investigated the filters that were produced before 1386(according to Iranian Calendar); material and product technology were revised. After data acquisition and informing the manufacture then, the post-revised filters were taken into account in order to be qualified; Furthermore Europe certified bedside filters were evaluated as the control group.

Materials & Methods

93 packed cell units were collected from volunteer donors at Tehran Blood Transfusion Center. Units were sent to flowcytometry lab within 3 hours at 4ºc. Units had to be filtered within 24 hours. Two ml. of each sample was taken before and after filtration. Three group filters were used including 8 Europe certified filters as control group, 55 pre-revised home-made bed side filters, and 30 post-revised home-made bed side filters. Filtration was carried out according to manufacturers' instructions. In the anti CD45 method, 100µl of a well mixed sample was added to 10 µl MoAb CD45

***Table 1**: Leukocyte count in 52 packed cell bags filtered by the pre-revised home made bed side filters (in this group, 3 samples were omitted because of high SD)*

Leukocyte count	Post filter			Pre filter	
	True count/bag	CD45 count/bag	WBC/µl	CD45 count/bag	WBC/µl
Total Number of filtered samples	52	52	52	52	52
Number of omitted samples	3	3	3	3	3
Leukocytes mean	8.0×10^6	7.2×10^6	85	2351.8×10^6	9409
Standard deviation	5.3×10^6	4.4×10^6	132	893.1×10^6	2816
min	0.9×10^6	0.8×10^6	0	632.0×10^6	3800
max	19.8×10^6	18.6×10^6	700	4525.8×10^6	17100

***Table 2**: Leukocyte count in 30 packed cell bags filtered by the post-revised home_ made bed side filters*

Leukocyte count	Pre filter			Post filter	
	True count/bag	CD45 count/bag	WBC/µl	CD45 count/bag	WBC/ µl
Total Number of filtered samples	30	30	30	30	30
Leukocytes mean	4.5×10^6	4.2×10^6	0.10	2943.0×10^6	10303
Standard deviation	3.6×10^6	3.1×10^6	0.305	974×10^6	3102
min	0.6×10^6	0.6×10^6	0	1566.0×10^6	5800
max	18.7×10^6	17.1×10^6	0.10	4994.0×10^6	18100

***Table 3**: Leukocyte count in 8 packed cell bags filtered by control group bed side filters*

Leukocyte count	Post filter			Pre filter	
	True count/bag	CD45 count/bag	WBC/µl	CD45 count/bag	WBC/ µl
Total Number of filtered samples	8	8	8	8	8
Leukocytes mean	2.3×10^6	1.9×10^6	25	2303.3×10^6	10271
Standard deviation	1.4×10^6	0.8×10^6	46	492.1×10^6	1639
min	0.8×10^6	0.9×10^6	0	1641.8×10^6	8700
max	4.7×10^6	3.4×10^6	100	3012.7×10^6	13700

(DAKO, Cat No=F861) and still another 100µl to 10 µl of isotope control as negative control (DAKO, Cat No=X927). Samples were incubated at 4ºc for 30 minutes followed by addition of 1 ml. of phosphate buffer. It was analyzed using flowcytometry (Partec PAS III-Germany).For counting by true count method, 100µl of each well mixed samples was placed in true count beaded tubes to each one of which 400 µl of leucocount reagent was added, then they were mixed by vortex and incubated in a dark room and low temperature for a period of minimum 5 minutes to maximum 60 minutes up before counting begins. The Flowcytometry (EPICS XL-USA)was set on optimal predetermined conditions, according to leukocount instructions(9).Absolute leukocyte count per bag was calculated according to kit instruction(9) and bag volume. The results were analyzed via the chi^2 test and SPSS.

Results

The mean values of leukocyte count in 55 units filtered by the pre revised home-made bed side filters were higher than 10×10^6 in true count method and 9×10^6 in anti CD45. Three ones out of 55 samples had more than 20×10^6 leukocytes and because of high standard deviation, we analyzed the results with and without these 3 samples these. Results are shown in table 1.

The mean values of leukocyte count in 30 units filtered by the post-revised home made bedside filters were 4.5×10^6 in true count method and 4.2×10^6 in the anti CD45. Six out of 30 samples had more than 5×10^6 leukocytes per bag (Table 2).

The mean values of leukocyte count in 8 units filtered by the control group filters were 2.3×10^6 in true count method and 1.9×10^6 in anti CD45 (Table 3)

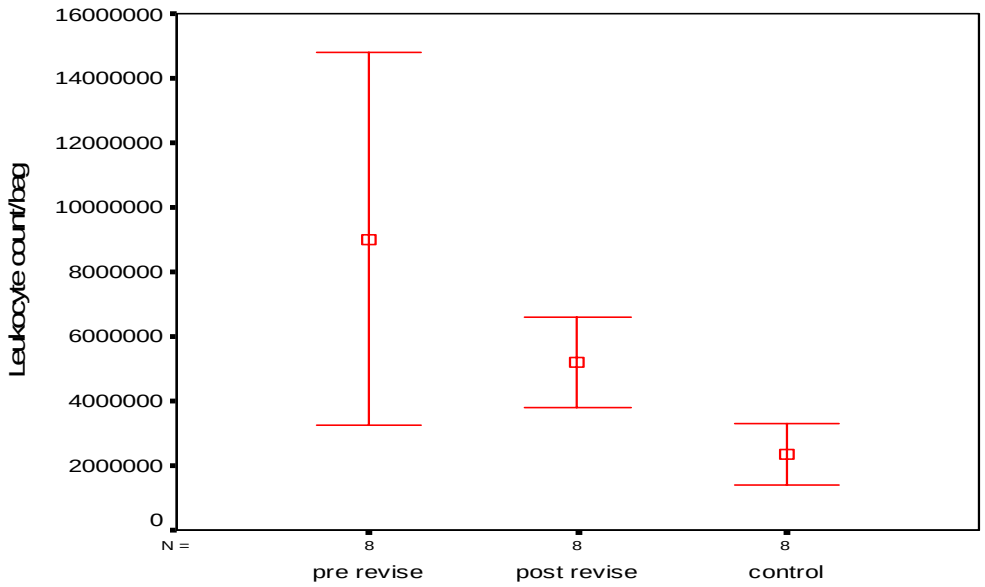
To compare these filters, we selected 8 samples of two home-made filters groups randomly and here after compared the results with 8 control filters (Table4).

Discussion

A variety of methods including leukocytes marked via the Mo Anti CD45 and use of standard

***Table 4**: Comparison of means and residual leukocyte counts according to standard value in different filter groups (p<0.05)*

Filter group	True count /bag		Mean of leukocyte ± SD	Total number
	Number >5x10^6	Number <=5x10^6		
Control group	0 0 %	8 100 %	2.3 ± 1.4	8 100 %
First group home made	4 50 %	4 50 %	7.9 ± 5.4	8 100 %
Second group home made	1 12.5 %	7 87.5 %	4.5 ± 3.6	8 100 %



***Graph 1**: Comparison of means and residual leukocyte counts in test and control groups (p<0.05)*

beads are currently available for the quality control of leukoreduced cellular blood components by the flowcytometry(10).Among these methods, the flowcytometry can detect even 25000 cells per bag. Today standard beads are golden standard method for the quality control of filters (11). In this study, we used both of these methods. According to the results, in 55 packed cells filtered by the pre revised-filters, residual leukocytes were about twice of the standard value. 38.2 % of filters reduced leukocytes to a level below 5×106 per bag and 61.8 % to a level above this number. The FDA permits blood products to be labeled "leukocyte reduced" if they contain less than 5×106 leukocytes. Therefore, more than half of blood products filtered by pre-revised group were not possible to be labeled as "leukocyte reduced".

It has been observed that in filter production technology, a few batch of filters in a series of products fall out of range making bias in final results (12, 13); this calls for a larger number of samples to be studied. In the present study, similarly the results showed that 3 samples had more than 25×106 leukocytes causing an increase in SD; they were thus omitted to avoid bias. As a result, the mean of leukocyte count in the rest of 53 samples was calculated to be 8×106.

All of the control filters reduced leukocytes within standard values and the mean of leukocyte count /bag in this group was 2.3×106(within FDA

standard). These results demonstrated the accuracy of counting method and controlled filtration condition. In addition, a significant difference between the control and test groups was verified. The home-made filter manufacturer was then notified of the results and they decided to revise product technology and materials. After revision, we gained 30 randomly selected post-revised filters in order to filter 30 packed cells under the same pre-revised-phase conditions. The mean of leukocyte count in the post-revised group was within the FDA standard though it showed a meaningful difference with the control group. Overall, the mean leukocyte count/bag in all groups even in the pre-revised filters showed 3 log reductions.

Comparison of an equal number (8 samples) of randomly selected samples from each group showed a meaningful difference between the control and test groups (p value=0.03).This analysis also demonstrated necessity of product technology revision.

As mentioned, all samples were counted by two methods. The mean leukocyte count in true count was about 0.5-1×106 more than the anti CD45 method. This difference in counting can be attributed to instrumental sampling inaccuracy of the exact volume in anti CD45 method. Furthermore, because of leukocyte adhesion to filter fibers in filtration procedure, surface receptors may be damaged; this would present Mo Anti CD45 from efficient binding to cells (14, 15) so that leukocyte count will be lower than its real value. We compared leukocyte count in different groups via the true count value method.

Finally, quality control on the pre-revised home-made filters caused an important decision to adapt that the quality of products must be improved parallel to universal standards. The authors recommend a periodic documented quality control on the home- made filters in order to prevent any deviation.

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