Performance evaluation of Cell Sep PLX leukocyte reduction filter for platelet concentrates

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Introduction

White blood cells (WBCs) in blood products for transfusion can cause adverse effects in patients, such as febrile non-hemolytic anemia (FNHTR), HLA alloimmunization, platelet refractoriness, and transmission of infectious agents (cytomegalovirus, human T-lymphotropic virus type 1)[1-4]. And leukoreduction for blood components is associated with reduced recipient mortality and organ dysfunction in cardiovascular surgery patients [5,6]. It is essential to use leukoreduced blood components in order to prevent these transfusion related reactions caused by WBCs in blood products. Therefore, United States, Canada, Japan and some EU countries have made universal prestorage leukoreduction mandatory in all blood components since the early 2,000s [4,7]. In effect, after the implementation of universal prestorage leukoreduction, those countries reported that transfusion related reactions such as FNHTR, platelet refractoriness are decreased [8-11]. In

Korea, leukoreduced blood products have been utilized in indicated patients with insurance payments, Choi et al. reported that the leukoreduced Red blood cells (RBCs) and platelet concentrates (PCs) comprised about 10% and 50% of total blood component used, respectively [12].

American Association of Blood Banks (AABB) and The Council of Europe (CE) identify the standards of quality control for leukoreduced blood components [13,14]. Residual WBCs in leukoreduced RBCs should be $<5 \times 10^6$ per unit according to AABB's standards, and $<1 \times 10^6$ per unit according to CE's standards. In Korea, residual WBCs in RBCs should be $<5 \times 10^6$ per unit as those of AABB [15]. Residual WBCs in leukoreduced PCs obtained from whole blood should be less than 8.3×10^5 per unit (AABB's standards) and less than 2.0×10^5 per unit (CE's standards) in at least 95% of tested components. In Korea, residual WBCs in PCs should be $<8.3 \times 10^5$ per unit as those of AABB

Methods to count low level of leukocytes in blood components are in use, including Nageotte hemocytometry, flow cytometry and microfluorimetry. However, the most widely used Nageotte hemocytometry has some drawbacks. This method has lower precision than flow cytometry and microfluorimetry, and counts less residual WBCs than other methods count [16]. Latest studies showed that flow cytometry for residual WBCs count measurement was most appropriated [17-20]. This study evaluated the performance of the Cell Sep PLX (Sistar BH Korea, Seongnam, Korea) developed for post-storage leukocyte reduction of PCs

Materials and Methods

1. Types of leukoreduction filters

Cell Sep PLX leukoreduction filter is divided into one for the ward (A type) and one for the blood bank(N type) depending on where leukoreduction performs, and one for the less than 6 PC units (5) and one for the less than 12 PCs units (10) depending on how many leukoreduction performs. Therefore, Cell Sep PLX leukoreduction filter consisted of 4 types (PLX-5A, PLX-10A, PLX-5N, PLX-10N). This study investigated the performance of the PLX-5A/5N and PLX-10A/10N with 6 and 10 PCs, which units were the most commonly used, respectively.

2. Preparation of platelet concentrates

PCs for this study were supplied from Korean Red Cross Seoul Seobu blood center as only research use. PCs for research use was judged to be unappropriate for transfusion, because those showed >65 IU/L alanine aminotransferase (ALT). However, those blood products were normally processed from whole blood according to platelet rich plasma (PRP) method, so those had the same properties as other PCs except ALT.

3. Leukoreduction of platelet concentrates

Leukoreduction for PCs was performed according to Cell Sep PLX type and manufacturer's instruction. The procedures of leukoreduction for PCs are as follows. To make pooled product, 6

units of PCs (5A/5N types) or 10 units of PCs (10A/10N types) were randomly pooled into a pre-filtration transfer bag. After closing all clamps of leukoreduction filter, pre-filtration transfer bag and post-filtration bag connected to a leukoreduction filter. Filtration time was determined by gravity and roller clamp control (A type). It kept the distance between the pre-filtration bag and the post-filtration bag to achieve ahead difference of approximately 1 meter. 4 mL of sample was taken before and after filtration for leukocytes and PLTs measurement. Filtration time was determined by time needed to empty the pre-filtration transfer bag.

4. WBCs and PLTs enumeration

Pre-filtration WBC counts, pre- and post-filtration PLT counts were measured by using an automated hematology analyzer (ADVIA 2120i; Siemens Diagnostics, Tarrytown, NY). And post-filtration WBC counts were measured using Nageotte hemocytometry and flow cytometry. In Nageotte hemocytometry method, a blood component sample was diluted with Turk's solution at a 1:9 ratio. When the chamber was filled with diluted sample, leukocytes were counted after 10 minutes rest. Enumeration performed by flow cytometry was performed with LeucoCOUNT Kit(cat no. 340523; BD Biosciences, San Jose, CA). In TruCOUNT tube that contain counting beads, 100 uL of blood sample and 400 uL LeucoCOUNT reagent containing propidium iodide (PI) and RNase were pipetted. Well mixed samples were incubated for 5 minutes in the dark, and Measurements were made on the flow cytometer (FACS Canto II; BD Biosciences). According to manufacturer's instruction, the number of residual WBCs per uL was calculated with the equation.

$$\text{Absolute WBC count} = \frac{WBC\ events(R2)}{Bead\ events\ (R1)} \times \frac{Bead\ count\ per\ tube}{Stained\ sample\ vol}$$

5. Leukocyte removal rate and platelet recovery

Leukocyte removal rate was calculated with the equation in Nageotte hemocytometry and flow cytometry, respectively.

$$\text{Leukocyte removal rate}\ (\%) = (100 - \frac{Total\ Leukocyte\ count\ after\ filtration}{Total\ Leukocyte\ count\ before\ filtration}) \times 100$$

Platelet recovery was calculated with the equation;

$$\text{Platelet recovery}\ (\%) = \frac{Total\ Platelet\ count\ after\ filtration}{Total\ Platelet\ count\ before\ filtration} \times 100$$

6. Institutional review board approval

All experiments and analyses performed in this study were approved by the Severance Hospital's institutional review board (1-2013-0027).

Results

1. Residual leukocyte counts and Leukocyte removal rate

Determination of residual leukocyte counts, which was the most important evaluation indicator of leukoreduction filter, was performed by Nageotte hemocytometry and flow cytometry. Residual leukocyte counts measured by Nageotte hemocytometry were 0.04 x 10^6/6 units in 5N type, and 0.40 x 10^6/10 units in 10N type. According to Korean biological products standards and experimental laws, residual leukocyte counts in leukoreduced blood products should be <8.3 x 10^5/unit. Therefore, the criteria are in accord with <5.0 x 10^6/6 units or <8.3 x 10^6/10 units. Therefore, all types of filters met the criteria (Table 1). Residual leukocyte counts measured by flow cytometry were 0.08 x 10^6/10 units in 10A type and 0.27 x 10^6/10 units in 10N type. However, as we mentioned, all results were in accord with Korean biological products standards and experimental laws (Table 1). Leukocyte removal rate was 99.88% in Nageotte hemocytometry and 99.87% in flow cytometry, which showed excellent performance.

Table 1. Pre- and Post-filtration total leukocyte count and leukocyte removal rate

Filter type	No. of used filters	No. of filtered platelet concentrate per filter	Total leukocyte count (x 10^6/pooled unit)			Leukocyte removal (%)	
			Pre-filtration	Post-filtration (NC)	Post-filtration (FCM)	NC	FCM
5A	5	6	126.50 ± 31.98	0.13 ± 0.06	0.18 ± 0.18	99.90	99.88
5N	5	6	90.15 ± 24.57	0.04 ± 0.05	0.09 ± 0.04	99.95	99.90
10A	5	10	213.53 ± 50.07	0.15 ± 0.12	0.08 ± 0.07	99.93	99.96

10N	5	10	141.40 ±	33.24	0.40 ±	0.22	0.27 ± 0.16	99.72	99.77
Total	20	-	142.89 ±	57.62	0.18 ±	0.19	0.16 ± 0.15	99.88	99.87

Values are expressed as mean ± SD

Abbreviation: NC, Nageotte chamber hemocytometry; FCM, flow cytometry

2. Platelet recovery

10N type filters showed the lowest platelet recovery, 85.9% and 5N type filters showed the highest platelet recovery, 91.5%. All evaluated filters had a platelet recovery of more than 80% , meeting manufacturer's requirement(Table 2).

Table 2. Pre- and Post-filtration total platelet count and platelet recovery

Filter type	No. of used filters	No. of filtered platelet concentrate per filter	Total platelet count (x 10^{11}/unit)		Platelet recovery (%)
			Pre-filtration	Post-filtration	
5A	5	6	2.74 ± 0.36	2.48 ± 0.27	90.85
5N	5	6	2.78 ± 0.58	2.54 ± 0.49	91.50
10A	5	10	4.30 ± 0.28	3.73 ± 0.25	87.19
10N	5	10	4.60 ± 0.85	3.90 ± 0.61	85.91
Total	20	-	3.61 ± 1.02	3.16 ± 0.79	88.86

Values are expressed as mean ± SD

3. Filtration time

Filtered volume per minute of A type filters for ward were 7.6 mL, 8.2 mL in 5A type and 10A, respectively. This result showed appropriate flow rate for safe blood transfusion. And in case of N type for blood bank, filtered volume per minute were 40.2 mL, 39.2 mL in 5N type and 10N type, respectively. This results was also good enough to meet the safe blood transfusion requirement다

(Table 3).

Table 3. Filtration time and filtered volume per minute

Filter type	No. of used filters	No. of filtered platelet concentrate per filter	Filtration time (sec)	Filtered volume per minute
5A	5	6	1,721 ± 54	7.6 ± 0.5
5N	5	6	342 ± 22	40.2 ± 3.9
10A	5	10	3,244 ± 1,334	8.2 ± 2.2
10N	5	10	589 ± 52	39.2 ± 3.6
Total	20	-	1474 ± 1327	23.8 ± 16.2

Discussion

When a unit of whole blood is collected, approximately 2 x 10^9 WBCs are present. With blood component processing, about 90%, 8% and 2% of these cells remain with RBC, platelet concentrate and fresh frozen plasma, respectively. [21]. Leukocytes in blood products can cause febrile non hemolytic transfusion reaction, HLA alloimmunization, PLT refractoriness and cell-mediated virus infections. This study was conducted in order to evaluate the performance of newly developed Cell Sep PLX leukoreduction filter for PLT, according to the US and EU standards. Cell Sep PLX leukoreduction filter for PLT was performed clinically effective leukocyte reduction, PLT recovery and filtration time.

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[별지 제1호 서식]

연구용 혈액(검체) 공급통보서

제제 / 구 분	혈 액 제 제						검체	비고
	WB	RBC	PLT	FFP	FP	기타		
연구용혈액		units	300단위					
계		units	300단위					

위 혈액(검체)을 연구용으로 사용코자 공급을 요청합니다.

2013 년 6 월 10 일

주관연구책임자 김신영 (서명 또는 인)

13.6.10

대한적십자사 서부혈액원장귀하

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심 의 일 자	2013년 6 월 3 일
과제승인번호	1-2013-0027

세브란스병원 연구심의위원회의 심의 결과를 다음과 같이 알려 드립니다.

Protocol No.

연 구 제 목	혈소판제제용 Cell Sep PLX 백혈구 제거 필터(여과방식자동성분 분리장치)의 임상적 성능 평가
연 구 책 임 자	김신영 / 세브란스병원 진단검사의학과
의 뢰 자	세브란스병원
연구예정기간	2013-06-03 ~ 2013-11-19
지속심의 빈도	면제
과 제 승 인 일	2013-06-03
위 험 수 준	Level I 최소위험
심 의 종 류	신규과제
심 의 내 용	-임상 연구계획서(국문) -증례기록서 -제품정보
I R B 회 의	제5위원회
참 석 위 원	제5위원회 신속심의자
심 의 결 과	승인, 피험자 동의 면제
심 의 의 견	-