

Comparison of Washing Efficiency and Recovery of Blood Cells Between Centrifugation, Coarse Filtration and Microfiltration Techniques to Prepare Autologous Blood for Transfusion

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Purpose: Cell salvage is the process by which blood lost in surgery is collected and washed or filtered to produce autologous blood for re-transfusion to the patient. Cell salvage aims to reduce the need for donor blood. Centrifugal cell salvage washing technique is a preferred medical treatment in order to retain lost red blood cells (RBCs) without contaminants. Although this technology very efficiently collects and washes shed blood, it is costly and often impractical or unavailable, especially in middle- or low-income countries. This study assessed two innovative filter devices as an alternative to centrifugal cell salvage technology: a coarse collection filter device (Hemafuse) and a microfiltration device (HemoClear). In contrast to centrifugal technology, both filter devices do not require electricity, nor costly equipment and extensive training. We compared the effectiveness of these filtration technologies to remove plasma constituents and recover and concentrate the cellular components with centrifugal technology (autoLog[®] device).

Methods: Whole blood was processed with each technology according to the device manufacturer's instructions. Before and after processing, the blood products were analyzed for supernatant solutes and cellular composition.

Results: The centrifugal technology confirmed its efficacy to remove potentially harmful solutes and capture red blood cells. The microfiltration technology (HemoClear) reached comparable levels of removal of solutes, with a potential advantage over centrifugal technology in the ability to also recover platelets. The coarse filtration technology (Hemafuse) had no washing capacity but, like the microfiltration technology, has the advantage of recovering platelets.

Conclusion: Innovative filtration devices represent an alternative to centrifugal technology in the preparation of autologous blood for reinfusion. The HemoClear technology for the first time enables the recovery of washed platelets and red blood cells. Clinical trials will have to be performed to investigate the clinical value of this new autologous blood product.

Keywords: cell salvage, blood salvage, cell salvage technology, cell saver, blood filter

Introduction

Globally there is a high demand for blood and blood products for transfusion.¹ The World Health Organization (WHO) reported 116 million units of red blood cell transfused worldwide. The WHO also reported a red blood cell shortage of nearly 17 million units.² Despite an overall increase in the number of blood donations collected in most WHO regions and World Bank Income groups, especially in lower- to middle-income countries, the number of blood collections is too low to meet the demand.¹ Most efforts to increase the availability of blood and blood products have focused on allogeneic blood donations.³ In Kenya, for example, non-remunerated blood collection drives were largely dependent on schools. This system has been hugely affected by the closure of schools during the COVID-19 pandemic, which has further increased blood shortages. Autologous

blood salvage systems have been proven to relieve the strain on donor blood systems.⁴ Cell salvage can effectively reduce both exposure to allogeneic blood and the volume of allogeneic blood transfused.⁵

Infusion of allogeneic red blood cells after significant blood loss is a valuable medical practice. Cell salvage, in which the patients' own blood is recovered during or after surgery and re-infused into the patient, is increasingly used.^{6,7} After collection and separation, the blood cells are concentrated and re-infused, while the residual plasma fluid, containing non-cellular components such as proteins, cations and pathological elements such as cytokines, chemokines and bacteria, is discarded.

A range of devices are available to assist in the salvage of the patient's own blood.⁸ The most commonly used device in high-resource settings is the centrifugal cell saver. The centrifugal cell saver washes and concentrates the RBCs efficiently. However, this device is often not available in lower-resource settings as consumables are expensive, and maintenance resources such as power supply, spare parts or skilled personnel are lacking. The Hemafuse (Sisu Global Health, Baltimore, United States) is a low-cost, handheld, mechanical coarse filter device for intraoperative autotransfusion of blood collected from internal bleeding, intended to replace or augment donor blood in emergency situations.¹⁰ The Hemafuse is available in Kenya and has a syringe-like design and includes a suction line and tip to collect blood from the surgical field. The suctioned blood is transferred to a reinfusion blood bag through a coarse dead-end filter (170 μm pores) by manual pushing down of the syringe plunger. Everything smaller than the pores, including the blood cells, passes through the filter, while large debris is filtered out.

The HemoClear device (HemoClear BV, Zwolle, The Netherlands) uses microfiltration technology to isolate blood cells from a liquid component.¹¹ This technology is used in Europe as a blood cell recovery filter. The device captures shed blood cells through highly specific (2–3 μm) crossflow filtration and removes solutes by its washing capability, yielding a high-quality cellular product.⁹ The HemoClear system is driven by gravity and can be used at patients' bedside. In short, a volume of collected blood is transferred to the HemoClear system and diluted with saline before entering the filter compartment. In the filter, the diluted blood flows along the filter membrane surface. Everything larger than the pores, including the blood cells, stays on the entry side of the membrane while everything smaller than the pores, including non-cellular components such as free hemoglobin, passes through the pores to the other side of the membrane. The compartments above and below the membrane are connected to two separate outlets. The outlet connected to the compartment above the membrane directs the blood cells to a reinfusion blood bag. The outlet connected to the compartment below the membrane directs the washing solution and non-cellular components to a waste bag.

This laboratory study was designed to compare the centrifugation (autoLog[®]), microfiltration (HemoClear) and coarse filtration (Hemafuse) techniques in their ability to remove non-cellular components and recover and concentrate the blood cells. Citrated whole blood was subjected to simulated salvage by the three technologies in accordance with each device manufacturer's instructions for use (Figure 1).

Materials and Methods

Blood Collection

All (non-remunerated) volunteer blood donors met standard donation criteria and gave their written, informed consent, in accordance with Sanquin Research's (Amsterdam, The Netherlands) guidelines and practices. This study was approved by the Institutional Medical Ethical Committee, in accordance with the standards laid down in the 1964 Declaration of Helsinki. A total of nine whole blood units, 500 mL \pm 10%, were collected in quadruple, bottom-and-top collection systems containing 70 mL of citrate-phosphate-dextrose (CPD, Fresenius Kabi, Emmer Compascuum, the Netherlands) at the Sanquin Blood Center (Sanquin, Amsterdam, the Netherlands). The whole blood units were placed on butane-1,4-diol cooling plates (Compocool, Fresenius Kabi) to allow their temperatures to adjust to 20 to 24°C.¹² The processing of the whole blood with the devices was initiated at around 16 hours after collection.

Blood Salvage

Processing with the autoLog (Centrifugation)

Three whole blood units were transferred to the autoLog[®] Autotransfusion System (Medtronic, Eindhoven, the Netherlands) centrifugal system's collection canister through an outer port, ie, the whole blood did not pass through the canister internal coarse



	autoLog	HemoClear	Hemafuse
	Centrifugation	Microfiltration (2-3 μm)	Coarse (170 μm) filtration
Technology	Automated, electrical	Passive, gravity-driven	Manual force
Washing feature	Yes	Yes	No
Standard washing volume	250ml per round, number of rounds automated	Equal to blood volume i.e. washing solution to be added in a 1:1 ratio	Not applicable
Washing solution	NaCl 0.9%	NaCl 0.9%	Not applicable
Washing volume used in study	750ml	500mL	Not applicable
Volume-reduction	Automatic	Optional, semi-adjustable	Not applicable
Equipment	Machine and consumable kit	Disposable kit	Limitedly reusable device and consumables kit

Figure 1 Overview of evaluated cell salvage technologies including washed centrifugation (autoLog), coarse filtration (Hemafuse) and washed microfiltration (HemoClear).

filter. The whole blood units were separated by centrifugal force and washed with 750 mL of isotonic saline in an automated program that consisted of three consecutive washing rounds, in accordance with the manufacturer's instructions.

Processing with HemoClear (Microfiltration)

In accordance with the manufacturer's instructions for use, the HemoClear system was primed manually with isotonic saline before use.⁹ Three units of whole blood were attached to the HemoClear system and diluted with 500 mL of isotonic saline before being filtered. After the first round of filtration, the cellular component was filtered again to concentrate the cellular product.

Processing with Hemafuse (Coarse Filtration)

In accordance with the manufacturer's instructions for use, the Hemafuse pump was assembled and de-aired before use. Three whole blood units were attached to the Hemafuse system and filtered through the coarse filter by manual force.

Analyses

Each whole blood unit and processed cellular unit was analyzed for cellular composition including numbers of red cells, platelets, leukocytes and hematocrit, and for supernatant-free hemoglobin, total protein, glucose and potassium concentrations. Total loads of each parameter were calculated from the measured concentration and the volume of the product. The cellular and non-cellular recoveries were calculated from the total load in the whole blood and the processed product.

Volume

The volume of blood components was calculated from the net weight and the specific gravity: 1.026 g/mL for plasma, 1.100 for RBCs. Based on the hematocrit values, the specific gravity of whole blood and diluted whole blood was calculated.^{13,14}

Hematological Parameters

Hematological parameters (cell count, hemoglobin concentration, hematocrit and mean corpuscular volume (MCV)) were obtained using a hematology analyser (Advia 2120, Siemens Healthcare Nederland BV, Den Haag, the Netherlands).

Hemolysis was determined as described previously by de Korte et al.¹⁵ Briefly, cell-free supernatants were obtained by centrifugation of the blood product at $12,000 \times g$ for 5 min followed by an additional centrifugation of the supernatant at $12,000 \times g$ for 5 min. Free hemoglobin was determined by absorbance measurement of supernatant at 415 nm (Eon plate reader, Bio Tek, Bad Friedrichshall, Germany), with correction for plasma absorption if necessary. Hemolysis was expressed as a percentage of total hemoglobin present in the blood product after correction for hematocrit.

Extracellular Potassium and Glucose

Samples of whole blood and processed blood were assayed for extracellular K⁺ and glucose using a blood gas analyzer (RapidLab 1265, Siemens Healthcare, Nederland B.V.).

Total Protein

Total protein was measured using the biuret method on Architect clinical chemistry analyzer (Abbott, Abbott Park, ILL).

Thromboelastography (TEG) Properties

TEG assays were performed using a TEG 5000 hemostasis system and plain cups and pins (Haemoscope Corp., Niles, IL). Blood samples were recalcified, and the intrinsic coagulation pathway was stimulated with kaolin.¹⁶ Four values that represent clot formation were determined by this test: the reaction time (R value), the coagulation time (K value), the angle and the maximum amplitude (MA).¹⁷ The R value represents the time until the first evidence of a clot is detected. The K value is the time from the end of R until the clot reading reaches 20 mm and this represents the speed of clot formation. The angle is the tangent of the curve made as the K is reached and offers similar information to K. The MA is a reflection of clot strength.

Statistics

The results are expressed as mean values \pm standard deviation. Paired two-sided Student's *t*-tests were performed to compare the whole blood measurements to the data acquired on the cellular and liquid components after processing. Significance was defined as $p < 0.05$. Based on past experience, a sample size of three whole blood units was expected sufficient to perform statistical analysis with power of at least 80%. The starting whole blood products were characterized by low variation (low standard deviation) as blood donors have to meet the standard donation criteria. Post hoc power calculations on the difference between whole blood and processed blood confirmed that the sample size of $n = 3$ for each parameter had a statistical power of at least 80%.

Results

Recovery of the Blood Cells

Volumes and mean cellular quantities for the pre-processed whole blood units, centrifuged (autoLog), microfiltered (HemoClear), and coarse filtered (Hemafuse) products are shown in Tables 1 and 2.

The whole blood units showed a normal cellular composition with a mean red blood cell concentration of $4.0 \pm 0.43 \times 10^{12}/L$, white blood cell concentration of $4.8 \pm 0.94 \times 10^9/L$ and platelet concentration of $175 \pm 31 \times 10^9/L$ (data not shown in table). The mean whole blood counts for red blood cells, white blood cells and platelets were $2.27 \pm 0.41 \times 10^{12}$, $2.56 \pm 0.64 \times 10^9$, and $105 \pm 23 \times 10^9$, respectively.

All three techniques resulted in efficient recovery of both WBC and RBC (Figure 2A). The amounts of RBC in the processed product did not significantly differ from the amounts in the whole blood. Mean platelet recovery for the

Table 1 Volumes and Cellular Quantities Before and After Whole Blood Processing with Each Technology

Parameter	Centrifugation (autoLog)		Microfiltration (HemoClear)		Coarse filtration (Hemafuse)	
	WB	Processed Product	WB	Processed Product	WB	Processed Product
Volume (mL)	549±0.3	281±30.4*	552±3.0	377±36.4*	552±1.6	510±38.6
Total WBC (x10 ⁹)	2.47±0.73	0.97±0.53* (39%)	2.56±0.64	2.06±0.45 (81%)	2.85±0.14	2.70±0.07 (95%)
Total RBC (x10 ¹²)	2.22±0.15	1.84±0.12 (83%)	2.27±0.41	2.13±0.35 (94%)	2.13±0.14	1.99±0.34 (93%)
Total Hb (g)	68±1.4	57±4.6 (85%)	66±4.6	61±3.6 (94%)	64±4.4	62±0.8 (97%)
Ht (%)	39.0±0.7	64.0±1.0*	37.4±2.4	52.2±5.1*	36.5±1.7	35.7±3.2
Total Plt (x10 ⁹)	89±10	4.0±1.1* (5%)	105±23	70±19*(67%)	96±20	88±17 (92%)

Notes: Values expressed as mean ± SD (% of recovery), n=3. *p<0.05 as compared to whole blood (Student's paired t-test).

Table 2 Quantities of Free Solutes Before and After Whole Blood Processing with Each Technology

Parameter	Centrifugation (autoLog)		Microfiltration (HemoClear)		Coarse filtration (Hemafuse)	
	WB	Processed Product	WB	Processed Product	WB	Processed Product
Total Glucose (mmol)	10.3±1.2	1.6±0.4* (16%)	11.9±1.3	2.2±0.2* (19%)	11.7±1.5	9.1±0.5 (80%)
Total protein (g)	32.4±1.7	0.7±0.1* (2%)	31.3±1.0	5.4±0.3* (17%)	30.7±2.1	28.2±4.0 (92%)
Total Free haemoglobin (mg)	10.1±8.9	74.6±8.8*	5.2±4.6	17.2±7.3	5.3±5.4	22.2±11.4
Haemolysis (%)	0.01±0.0	0.04±0.0	0.00±0.0	0.01±0.0	0.00±0.1	0.02±0.0
Total K ⁺ (mmol)	1.82±0.18	0.36±0.12* (19%)	1.82±0.32	0.74±0.08*(41%)	1.65±0.21	1.79±0.1 (110%)

Notes: Values expressed as mean ± SD (% of recovery), n=3. *p<0.05 as compared to whole blood (Student's paired t-test).

centrifugation technique was 5%. Both filtration-based technologies recovered a significantly greater amount of platelets, with the coarse filtration having the highest recovery of platelets, 92% versus 67% with microfiltration.

The mean hematocrit of the whole blood units was 37 ± 1.8%. Microfiltration and centrifugation concentrated the red blood cells to a hematocrit of 52 ± 5.1% and 64 ± 1.0%, respectively. The coarse filter did not result in concentration of the red blood cells, the obtained hematocrit, 36 ± 3.2%, was comparable to the whole blood hematocrit (Figure 2B).

Removal of Non-Cellular Components

Washing performance as measured by non-cellular loads in whole blood and the produced products is shown in Table 2. The concentrations of supernatant glucose, potassium and total protein were measured as indicators of the devices'

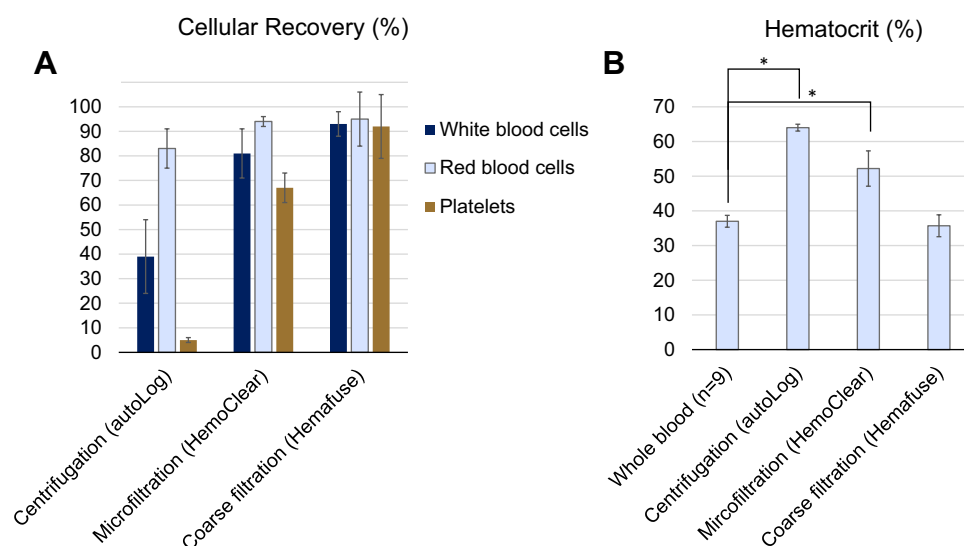


Figure 2 Recovery of the blood cells and hematocrit after processing with the different salvage technologies. (A) Cellular recovery. (B) Hematocrit (%). Values shown are mean ± SD (n=3). * p<0.05 as compared to whole blood (Student's paired t-test).

washing efficiency. Both the centrifugal and the microfiltration procedure effectively reduced the concentration of all solutes (Figure 3). Mean potassium concentrations in whole blood were low, around 3 mmol/L. After processing with both the centrifugal and microfiltration technologies, potassium concentrations were below 1 mmol/L (data not shown).

The coarse filtration (Hemafuse) device did not significantly reduce glucose, potassium, or total protein levels.

The mean-free hemoglobin concentration before processing was 11 ± 10 mg/L. The centrifugation procedure significantly increased mean-free hemoglobin concentration to 207 ± 22 mg/L. Processing with both microfiltration (37 ± 18 mg/L, $P < 0.001$) and coarse filtration (35 ± 19 mg/L, $P = 0.016$) resulted in significantly lower increase in free hemoglobin concentration (Figure 4). Also, total amounts of free hemoglobin after processing with microfiltration (17 ± 7.3 mg, $P = 0.001$) and coarse filtration (22 ± 11 mg, $P = 0.035$) were significantly lower as compared to the centrifugal device (75 ± 8.8 mg).

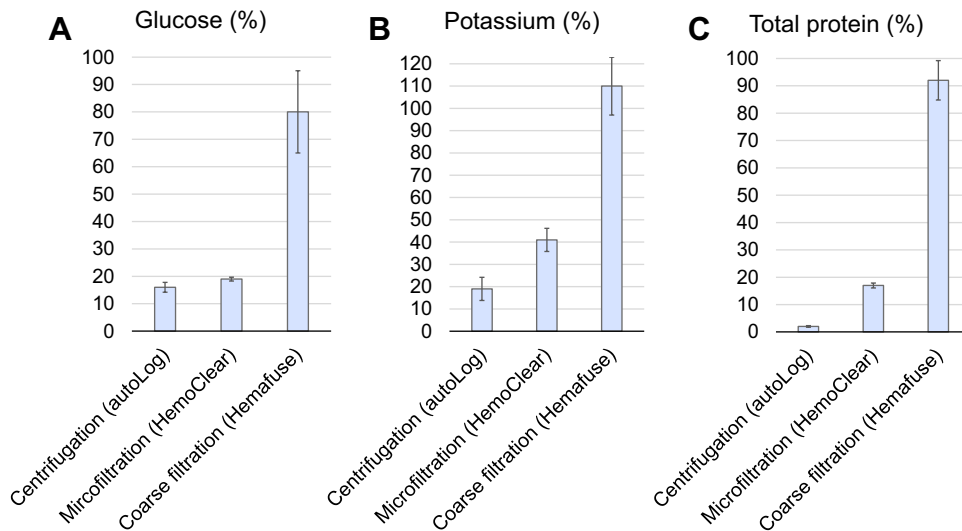


Figure 3 Recovery of solutes after processing with the different salvage techniques. Recovery of glucose (A), potassium (B) and total protein (C). Values shown are mean \pm SD (n=3).

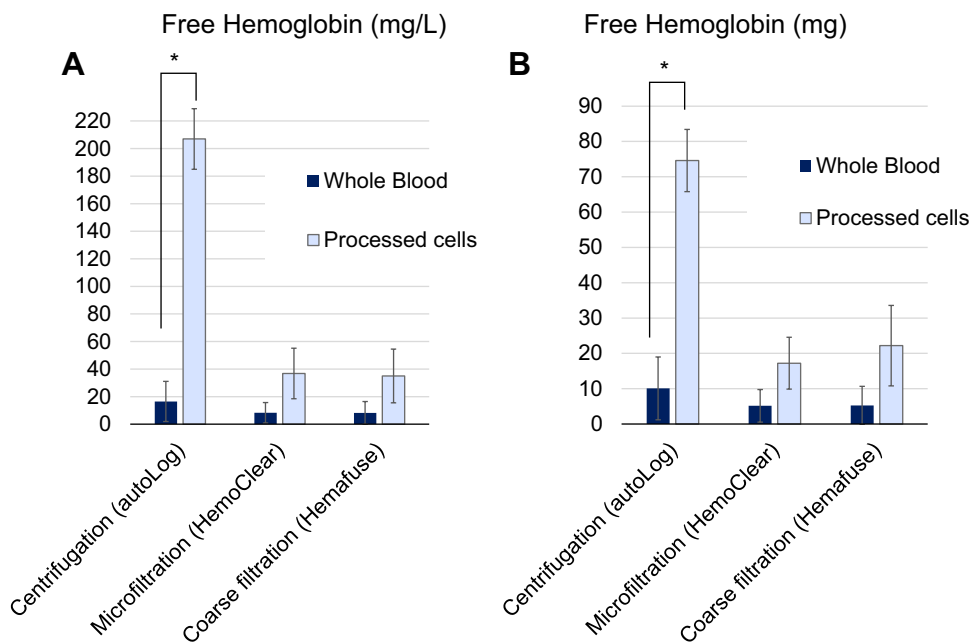


Figure 4 Release and removal of free hemoglobin. (A) Mean free hemoglobin concentration in milligram per liter. (B) Mean free hemoglobin total load in milligram. * $p < 0.05$ as compared to whole blood (Student's paired *t*-test).

Table 3 Thromboelastographic Properties Before and After Whole Blood Processing with Each Technology

	Centrifugation (autoLog)		Microfiltration (HemoClear)		Coarse filtration (Hemafuse)	
	WB	Processed Product	WB	Processed Product	WB	Processed Product
R (min)	8.9±0.3	52±9.6*	9.2±1.7	8.1±1.0	9.4±0.9	6.8±0.9*
K (min)	2.0±0.5	No coagulation	2.1±0.6	9.0±2.8*	2.3±0.4	1.8±0.4 *
Angle (°)	53±13.3		59±5.9	26±3.5*	52±7.6	63±6.0
MA (mm)	59±6.7		59±9.2	25±4.5*	64±1.7	66±2.0

Notes: Values expressed as mean ± SD (Recovery), n=3. *p<0.05 as compared to whole blood (Student's paired t-test).

Thromboelastography Properties

The mean thromboelastography properties for pre-processed whole blood units and microfiltered, coarse filtered and centrifuged products are shown in Table 3. For the units processed with the centrifugal device, no coagulation was measured. For the coarsely and microfiltered units, the R times, were comparable, 6.8 ± 0.90 min and 8.1 ± 1.0 min, respectively. Both were slightly, but significantly lower as the values obtained in whole blood. For the microfiltration technology, the mean K time, 9.0 ± 2.8 minutes, was significantly prolonged compared to the pre-processed mean time of 2.1 ± 0.6 minutes. In contrast, the angle and maximum amplitude were significantly decreased. After the coarse filtration, the K time slightly, but significantly, decreased to 1.8 ± 0.35 minutes. The angle and maximum amplitude remained comparable to the pre-processed values.

Discussion

In order to safely re-infuse shed blood cells, medical guidelines recommend the shed blood is washed with saline to remove harmful solutes.¹⁸ Centrifugal technology has been shown to effectively reduce the non-cellular load by about 90%.^{8,19,20} The centrifugal autoLog[®] procedure resulted in a removal of 98% of the total protein level. The reduction of smaller solutes was less effective, with about 20% of the small-molecule glucose and ion potassium left in the concentrated red cell product.

The HemoClear microfiltration technology achieved equal washing performance compared to the centrifugal autoLog[®] procedure and reduced the non-cellular components to a significantly greater extent than the coarse filtration technology (Hemafuse). Hemafuse showed no reduction in solute load. This finding is in line with expectations because the Hemafuse protocol does not include washing with saline. The Hemafuse procedure, using a 170 micron coarse filter, is effective in removing large debris but does not catch the smaller biologically active proteins, cations or small molecules.

A clinically relevant finding is the recovery of platelets by both filter technologies. Contrary to the centrifugal procedure, the filters recovered the majority of platelets together with the red cells. This is an important finding because administration of both red cells and platelets represents the most common co-transfusion.²¹ Storage of allogeneic platelet concentrates is limited to 5 to 7 days at room temperature.²² Therefore, maintaining hospital platelet concentrate inventory is logistically difficult and highly resource-intensive.^{23,24} Furthermore, platelet transfusion entails several risks to the patient, including allergic and febrile nonhemolytic reactions and sepsis. When platelet salvage is an option, these risks might be avoided.²⁵ The possibility to recover platelets with the red cells from shed blood could reduce pressure on blood banks to maintain adequate platelet stocks.^{1,26}

Based on the TEG results, the centrifugation washing procedure showed the complete depletion of coagulation. This is in agreement with the very low levels of platelets in the washed product. The results for the filtration technologies suggest that in the washed microfiltration product there still was coagulation. The decrease in cloth strength (MA) in the filtered product as compared to whole blood is most likely due to the removal of plasma coagulation factors such as fibrinogen. In the coarsely filtered product, coagulation was increased as reflected by a decrease in R and K times, suggesting some activation of the platelets. The TEG maximal amplitude was not influenced by the coarse filtration, and the lack of reduction of plasma components by this procedure might be involved in this. In a previous article on the microfiltration technology (HemoClear).⁹ It was shown that this processing method did not result in activation as measured by the expression of P-selectin (CD62P) on the surface of platelets. For the coarse filtration technology (Hemafuse) it should be investigated whether the recovered platelets are activated in processing.

Being processed shortly after donation, the whole blood free hemoglobin levels were very low at the start of processing. This finding is not representative of the practical cell salvage situation in which pre-processed shed blood contains substantial-free hemoglobin levels. In a previous article, the microfiltration HemoClear system was compared to a centrifugal device on reduction of high-free hemoglobin levels.²⁷ In this study postoperatively blood shed after elective cardiac surgery was collected over an extended period of 18–20 hours before processing with both technologies in a parallel set-up. The extended collection period yielded a mean-free hemoglobin concentration of 0.21 ± 0.14 mmol/L in the pre-processed samples. Processing with the centrifugal technology and microfiltration HemoClear device reduced the free hemoglobin load to statistically comparable values of 12.9% and 15.5%, respectively. The free hemoglobin levels reported in the current study might provide insight into the release of hemoglobin induced by the technologies, rather than their washing performance. The centrifugal procedure significantly increased free hemoglobin levels. This would be in line with previous findings that showed that centrifugal force exerted upon red cells results in sublethal injury and hemolysis.^{28,29}

In addition to this blood quality investigation, further research should be performed to evaluate the filtration technology's usability and cost-effectiveness.

Conclusions

Three technologies available for the preparation of autologous blood for transfusion were evaluated on the recovery of cellular and non-cellular components. The recovery of blood cells is the basis of effective autologous blood transfusion, whereas the removal of unwanted non-cellular contaminants is an imperative for safe cell salvage. Based on the results, we conclude that washing of blood cells with saline is necessary to remove non-cellular components and enable safe reinfusion. Both the centrifugation (autoLog) and microfiltration (HemoClear) technologies have a washing feature and effectively reduce the various non-cellular solutes. Whereas centrifugal technology only recovers the red blood cells, filtration technology unlocks the possibility to recover both platelets and red blood cells. Clinical trials should be performed to quantify the value of platelet salvage. The HemoClear microfiltration system would be a relatively simple procedure to produce washed blood cells of comparable quality as obtained with the centrifugal device, with as additional advantage the recovery of platelets.

The coarse filtration procedure with Hemafuse without a washing feature does not result in reduction of free solutes. The Hemafuse coarse filtration device presents a straightforward technique to filter out large debris from blood but is not suitable for salvage of blood cells contaminated with solutes such as free hemoglobin or bacteria.

Abbreviations

CPD, citrate-phosphate-dextrose; Hb, hemoglobin; Ht, hematocrit; K⁺, potassium; KEMSA, Kenya Medical Supplies Authority; MA, maximum amplitude; MCV, mean corpuscular volumes; Plt, platelet; RBC, red blood cell; TEG, thromboelastography; WBC, white blood cell.

Data Sharing Statement

Raw data files are available upon request from the corresponding author.

Ethical Approval

This study was approved by Sanquin's Research Medical Ethical committee, in accordance with the standards laid down in the 1964 Declaration of Helsinki.

Consent for Publication

HemoClear BV consents to the publication of these data.

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Disclosure

James Amenge purchased hemafuse devices and for presenting the study result in a virtual internal conference on bloodless surgery. He received manufacturer's training on hemafuse devices and used the hemafuse device in a patient. Dion Osemwengie is an employee of HemoClear BV. Arno Nierich holds stock in HemoClear BV. The other authors report no conflicts of interest in this work.

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