

Removal of an Effective Hemostatic Agent from Salvaged Blood by Combined Filtration and Cell Washing

Gary D. Reeder, CP1, Ann Gronda, PhD2 and James Drake, PhD2

Methods

Two in vitro studies were conducted utilizing identical laboratory methods, with study group 1 incorporating blood collection reservoirs(b) having a nominal filtration rating of 40 μ while study group 2 utilized collection reservoirs(c) rated at 150 μ . A total of ten experiments were conducted, in each study group, using 5.00 gm of Arista™ for each experiment.

Fresh human blood was obtained with citrate anticoagulant (ACD) from donors of similar blood type by a commercial blood bank(d) according to current Food and Drug Administration (FDA) and American Association of Blood Banks (AABB) standards. Each unit was tested for pathogens as required by blood banking regulations and standards prior to shipping via overnight Federal Express to the Hema Rx laboratory.

The hemostat was hydrated in 200 ml of normal saline prior to addition of 500 ml of human blood. The hemostat was allowed to hydrate with intermittent swirl mixing for approximately 20 minutes prior to blood addition. Prior hydration of the hemostat was necessary to reduce the potential of activating coagulation in the citrated blood on addition of dry microspheres. Blood and Arista™ were thoroughly mixed by gentle inversion of the blood bag on a mechanical rocker, and syringe collection of an unfiltered 35 ml sample for particle counting was obtained during the final minute of agitation.

Upon initial sample acquisition, the blood/hemostat mixture was introduced into a blood collection reservoir and allowed to pass through the filter/defoamer by gravity drainage. Following collection of an adequate processing volume of blood within the collection chamber, mixing of the blood/hemostat was achieved by gentle swirling of the reservoir.

Cell washing was initiated using an AP900/PRP centrifugal cell washing/pheresis system(e) as illustrated in Figure 1. Blood was aspirated from the filtration reservoir to the cell washing device, through sterile tubing, using an integral peristaltic roller pump. Transfer of blood from the filtration reservoir was not initiated until the centrifuge bowl had attained full rotational speed (5600 RPM). During the initial 300 ml of blood flow into the bowl, a blood sample for particle counting was syringe-aspirated from a luer port tubing connector previously inserted into the tubing between the collection reservoir and the cell washing bowl. The bowl FILL cycle continued until packed red cells were detected at the top shoulder of the centrifuge bowl by visual inspection. During this cycle, effluent plasma was “spilled” into a waste bag connected to the centrifuge bowl by a short length of PVC tubing.

On detection of red cells, the machine occluded the blood inlet line and opened the saline line, thereby initiating saline wash of the red cell pack. Washing was continued until 2000 ml of saline had been processed through the red cell pack, at which point the inlet lines were occluded and the centrifuge decelerated to a stop. After a five (5) second delay, the EMPTY cycle was initiated and the washed red cell pack was transferred to a sterile blood “holding



Figure 1: Centrifugal Cell Washing Machine

bag” using the integral peristaltic pump of the cell washing system. A sampling “spike”, with stopcock attached was inserted into one of the holding bag ports. After thorough mixing of the bag contents by gentle inversion, a sample of the contents was aspirated by syringe for subsequent particle counting. The various operating conditions for the cell washing process in each of the ten (10) replicate experiments were identical and are presented in Table 1.

OPERATIONAL PARAMETER	SETTING OR AGENT USED
Centrifuge Bowl Volume (ml)	225
Centrifuge Speed (RPM)	5600
Blood FILL Flowrate (ml/min)	300
Washing Agent Utilized	Millipore Filtered Normal Saline
Saline WASH Flowrate (ml/min)	300
Saline Wash Volume (ml)	2000
EMPTY Flowrate (ml/min)	300

Table 1: Operational Parameters

Following acquisition and storage of samples of the washed red cells, a Lipiguard™(f) transfusion filter was inserted into a port of the blood holding bag containing the washed red cells. The outflow port of this terminal filter was connected to a 150 ml blood transfer bag(g) with a dual spike PVC tubing. After thorough mixing by gentle inversion, the washed cell product was passed through this terminal filter by gravity drainage. A sample of this final red cell product was acquired by syringe aspiration for subsequent particle counting.

Electronic particle counts were conducted on a Model Z-2 particle counter(h) fitted with an aperture tube of 200µ diameter. Samples were placed on a tube rocker for approximately 15 – 20 minutes to mix while coming to room temperature. During that mixing time, a reagent background count of the Isoton diluent was obtained on the Z-2 particle counter. When adequately mixed, 3.20 ml of the unfiltered whole blood/hemostat was added to 76.80 ml of Isoton III(i) electrolyte (for a dilution of 1:50) in a Coulter counting beaker. Six (6) drops of ZAP-Oglobin reagent(j) were added to each diluted sample, and mixed by gentle swirling, to effect cellular lysis of erythrocytes. All other samples were diluted to either 1:50 or 1:10 prior to particle analysis. Each diluted sample was then electronically counted on the Z-2 instrument with continual mixing by an integral mixing paddle rotating within the beaker. The magnified image of the aperture allowed for observation of aperture obstruction by particles >200µ. If obstruction was observed, the count was aborted and repeated until unobstructed data collection was achieved. A minimum of six (6) replicate counts was obtained for all samples.

All data from the experiment series were entered into Excel™ spreadsheets for dilutional correction and calculations. Such calculations included calculation of mean results, standard deviation of sample group results and coefficient of variation (CV). Data for each experiment were graphically depicted and statistical significance between group means assessed by t test (Paired Two Sample for Means). Statistical significance was defined as $\alpha = 0.05$.

^a: Medafor, Inc., Minneapolis, MN

^b: Medtronic Perfusion Systems, Brooklyn Park, MN

^c: Haemonetics, Inc., Braintree, MA

^d: Interstate Blood Bank, Inc., Memphis, TN

^e: Electromedics, Inc., Parker, CO

^f: Terumo Medical, Somerset, NJ

^g: Charter Medical, Inc., Raleigh-Durham, NC

^h: Beckman-Coulter, Fullerton, CA

ⁱ: Beckman-Coulter, Fullerton, CA

^j: Beckman-Coulter, Fullerton, CA

Results

Mean particle counts for the initial whole blood/Arista mixture of study group 1, within each size channel reported by the Coulter Z-2, are graphically summarized as count distribution in Figure 2. The potential particle load presented to the cell washing system would be the total of all mean counts multiplied by the mean processed volume from the cardiotomy reservoir for each of the ten (10) experiments. Thus, the total Arista particles that would potentially be introduced into the cell washing system would approximate 80,800,958 if no removal by physical filtration occurred within the 40 μ blood collection reservoir.

Figures 3 and 4 show the particle size distribution after each stage of processing for study group 1, with 40 μ cardiotomy filtration and study group 2, with 150 μ cardiotomy filtration.

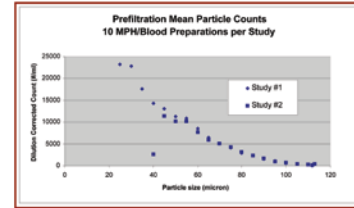


Figure 2: Initial Particle Size Distribution

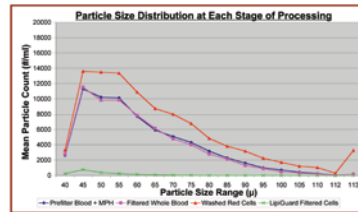


Figure 3: Initial Particle Size Distribution for Study Group 1

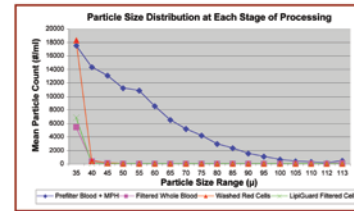


Figure 4: Initial Particle Size Distribution for Study Group 2

Discussion

This study was constructed to evaluate the ability of in vitro filtration and cell washing to adequately remove Arista™ hemostatic particles that might be aspirated with wound blood during autotransfusion procedures. There are two (2) primary safety concerns related to such potential contamination. The first is whether blood contaminated with Arista™ would have the ability to initiate intravascular coagulation upon transfusion to the patient. The second issue would be the potential for any Arista™ particles, returned to the patient's circulation, to act as an embolic obstruction for the microcirculation of the patient. Arista™ induces coagulation by absorption of crystalloid, with resultant concentration of clotting factors within the local vicinity of the microspheres. Such prolonged exposure to an aqueous environment, including wound residence, aspiration, reservoir dwell time, and salvaged blood washing with copious amounts of normal saline, will render any surviving particles fully hydrated. Thus, it seems obvious that any stimulation of intravascular coagulation, by such surviving Arista particles, would not occur.

The data for study group 1 demonstrated highly significant reduction in particles >30 μ after passing through the 40 μ blood collection reservoir (97.15% removal) with progressive depletion during cell washing (98.79% removal). Terminal filtration with the LipiGuard™ filter increased the removal rate to 99.47%. In study group 2, however, a particle reduction rate of 18.47% was seen after passing through the 150 μ blood collection reservoir. Cell washing of this collected blood/Arista mixture with 2000 ml of saline brought the total particle load reduction to only 32.91%. However, final filtration with the LipiGuard filter demonstrated a final removal efficiency of 98.75%.

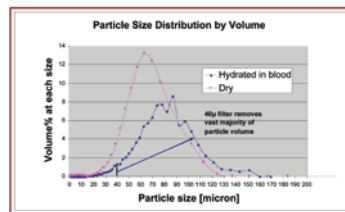


Figure 5: Initial Particle Size Distribution by Volume

These results concur with predicted results based on Arista's particle size distribution by volume, which correlates directly with gram load. Figure 5 shows that the vast majority of particle volume, and thus particle weight, should be removed by 40 micron filtration. For example, a worst-case scenario of 5g Arista mixed into one unit of blood should yield < 300mg of small particles in 250 ml of RBC's after cell salvage.

Thus, it is concluded that Arista™ hemostatic material can be effectively removed from salvaged blood by combined 40µ filtration and centrifugal cell washing.

Conclusions

1. Arista particles >30µ diameter are significantly reduced by 40µ filtered blood collection reservoir filtration, which should reduce potential embolic complications for the patient.
2. Arista particles >30µ diameter are not significantly removed by 150µ filtered blood collection reservoir filtration.
3. Centrifugal cell washing of Arista contaminated blood demonstrated that Arista particles are further depleted during the cell washing process.
4. Final filtration of the washed red cell pack with a combined screen/depth filter such as the LipiGuard transfusion filter brings the overall depletion of Arista to 99.47% when combined with 40µ collection reservoirs.
5. Final filtration of the washed red cell pack with a combined screen/depth filter such as the LipiGuard transfusion filter brings the overall depletion of MPH to 98.75% when used with 150µ collection reservoirs.
6. Thus, salvage processing through either a 40µ filtered blood collection reservoir or a 40µ screen/depth filter reduces a worst-case scenario 5.0 gram particle load to less than 300 mg per unit of salvaged blood.

