Filtration of Shed Blood in Autotransfusion: Does gross filtration of shed blood impact end-product quality?

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Abstract

Introduction: Until recently, fine filtration of shed blood has been a standard practice in autotransfusion through modern cell salvage devices. The HEMAsavR blood collection canister has been produced as the first cell salvage reservoir which features a gross 1mm (1000µm) filter rather than conventional 20µm to 120µm filter media. Featuring less holdup volume than conventional cardiotomy reservoirs, the HEMAsavR may yield more red cells recovered with lower potassium (K+), lower plasma-free hemoglobin (PFHb), higher hematocrit (HCT) and higher hemoglobin (Hgb). The following article describes the impact of filtration of shed blood by comparing potassium removal, HCT, Hgb and plasma-free hemoglobin removal among three different filter media (20µm, 200µm and 1000µm). The aim of this study is to assess the end-product quality between each new protocol (HEMAsavR) and the control protocol (Haemonetics).

Methods: An equivalency test was performed between three reservoirs featuring different filter pore sizes, analyzing potassium (K+), hematocrit (HCT), hemoglobin (Hgb) and plasma-free hemoglobin (PFHb). Among each group, 20 trials were performed. K+, HCT, Hgb and PFHb samples were collected and analyzed at baseline, pre-wash, post-wash and post-filter after passing through a 40µm transfusion filter. Additionally post-filter samples from each group were analyzed for schistocytes and spur cells by

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a hematologist. The primary outcome used was percent K+ removal from baseline to post-filter equivalency between the three groups. We predict that the three groups will yield equal percent K+ removal.

Results: The mean post-filter K+ percentage removal was 72.7% for the 1000µm group, 70% for the 200µm group and 72.1% for the 20µm group. The mean post-filter PFHB percentage removal was -20% for the 1000µm group, 43.6% for the 200µm group and -19.7% for the 20µm group. The mean post-filter Hgb percentage gain was 108.7% for the 1000µm group, 161.3% for the 200µm group, and 138.9% for the 20µm group. The mean post-filter HCT percentage gain was 61.9% for the 1000µm group, 62.2% for the 200µm group, and 65.2% for the 20µm group. The 90% confidence interval calculated for our primary outcome (% K+ removal) fell within the generated equivalence limits. Additionally in all three samples acquired for RBC morphology scans, no schistocytes were noted and each of the three contained equal amounts (fewer than 5-10%) of spur cells.

Conclusion: We concluded that both the HEMAsavR and HEMAsavR + In Line Filter interventions were equivalent to the Haemonetics (control group) intervention with respect to percentage K+ removal. Thus, the novel design of a gross 1000µm filter was shown to produce an equivalent end-product quality to that of the more conventional 20-120µm filters used in traditional cell salvage reservoirs.

Keywords: Autotransfusion, Filtration, Blood Management, Quality Makers

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Introduction

Fine filtration of shed blood has been a standard practice in autotransfusion through cell salvage devices. During the process of autotransfusion, shed blood is aspirated from the operative field, passed through a cardiotomy filter and held in a collection reservoir for subsequent processing. Once a sufficient amount needed for processing has been sequestered, the health care practitioner responsible for autotransfusion then dispatches the collected blood through the cell salvage device, washing the initial waste into a final product of autologous packed red blood cells (pRBCs) (1). Over time, a multitude of autotransfusion devices making up a variety of configurations have been developed in order to optimize final products and yield higher quality washes. Multiple elements factor into the determination of pRBC product quality. Szpisjak, Edgell and Bissonnette (2000) performed an in-vitro analysis of cell-salvaged blood to assess whether potassium (K+) concentration correlated with other markers of cellular debris (free hemoglobin, leukocyte and platelet count). They found that potassium had a strong correlation with cellular debris and therefore a quality autotransfusion product should result in a decreased potassium concentration following processing (2). Similar studies have demonstrated results indicative of potassium free hemoglobin (PFHb), hematocrit (HCT), and hemoglobin (Hgb) as satisfactory markers of cell salvage quality (3,4). Autotransfusion circuits consist of an array of components which contribute to producing a high quality end product represented by these indicators. One of these components is the cardiotomy filter.

Cardiotomy filters prevent large particles from entering the cell saver bowl and interfering with the cell salvage process. The HEMAsavR blood collection canister (Ecomed Solutions, Mundelein, IL, USA) has been produced as the first cell salvage reservoir which features a gross 1mm (1000μm) filter rather than conventional 20μm and 120μm filter media. Its bidirectional dip tube and 1mm filter is a novel design intended to decrease shed blood holdup volume in comparison to levels seen with filter media used in conventional cardiotomy reservoirs (5). Because of these unique features, the HEMAsavR may yield higher volumes of

red cells recovered with similar qualities to traditional reservoirs. Referencing the quality indicators used in the previous studies described above, this can be illustrated by comparable potassium (K+), plasma-free hemoglobin (PFHb), hematocrit (HCT) and hemoglobin (Hgb) contents between the washed RBCs recovered via the HEMAsavR and traditional collection canisters. This investigation describes the impact of various levels of filtration on final end product quality of washed shed blood by comparing changes in K+, HCT, Hgb and PFHb among three intervention groups. The three groups evaluated were as follows:

1. 20µm (control group – Haemonetics Cell Saver Elite+ 3L (standard) collection reservoir) (Haemonetics Corporation Boston, MA, USA)
2. 200µm (HEMAsavR 1.8L collection reservoir + 200µm in-line filter)
3. 1000µm (HEMAsavR 1.8L collection reservoir).

The aim of this study was to assess the equivalency of end-product quality between each new protocol (HEMAsavRs) and the control protocol (Haemonetics).

Materials and Methods

Seven liters of fresh porcine blood was acquired on each morning of testing from the WholeStone Farms Packing Plant in Fremont, NE. A standard dose of 63 mL Anticoagulant Citrate Dextrose per 500 mL of blood (882 ml total) was used as anticoagulant upon collection. A schematic depicting study design and sample location is outlined in Image 6. Baseline samples were collected directly from the volume of shed blood prior to each iteration of testing to assess starting PFHb, K+, Hgb, and HCT prior to the introduction of filtering by each cell salvage reservoir. Approximately 125-150 ml of anticoagulated blood was then aspirated into the appropriate cell salvage reservoir, where it was then subjected to the unique filtration design/size of each intervention group. A “pre-wash” sample was drawn following cardiotomy filtration prior to centrifugation and washing. The blood was then spun via centrifugation in the Haemonetics Cell Saver Elite+ Autotransfusion System (Haemonetics Corporation Boston, MA,

USA) with a 70 ml cell saver bowl, and put through a wash cycle with isotonic normal saline (0.9% NaCl). After washing, the blood was placed in a holding bag and a “post-wash” sample was drawn. The product then passed through a 40 micron SQ40 transfusion filter (Haemonetics Corporation Boston, MA, USA) and a final product “post-filter” sample was collected to assess final end product values of PFHb, K+, Hgb, and HCT.

10 mL samples were drawn into syringes at each stage. Samples were analyzed for K+ and Hgb via the Epoc Blood Analysis System (Siemens Healthineers,AG Erlangen, Germany). A micro-capillary hematocrit reader was utilized for analysis of HCT. Lastly, PFHb was assessed with the HemoCue Plasma/Low Hb System after being mixed in 10 mL EDTA tubes (Beckton, Dickinson and Company, Franklin Lakes, NJ) and centrifuged via the Hettich EBA 280 centrifuge (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). These were spun at a recommended rate of 3500 RPM for 10 minutes per UNMC Medical Laboratory Science / Hematology recommendation (6). Plasma was pipetted from the meniscus forming the surface layer of each EDTA tube for analysis via the HemoCue (HemoCue America, a Danaher company, Brea, CA, USA).

Lastly, an additional “post-filter” sample was obtained for each of the three interventions in order to perform RBC morphology scans. Smears were made in duplicate for all three samples and smeared with Wright’s Stain for evaluation by a clinical hematologist.

An equivalency test was performed across each of the three test groups, analyzing K+, HCT, Hgb and PFHb. The study was reviewed and a sample size generated by Fang Qiu, a statistician at the University of Nebraska Medical Center College of Public Health. Percent K+ removal was selected as the primary outcome after sample size calculation using the PASS version 2005 statistical software (Number Crunching Statistical Systems, Kaysville, Utah, USA). This determined sample sizes of 18 in each group (Haemonetics, HEMAsavR + in line filter, and HEMAsavR) (total 54) were required to achieve 82% power at a 0.025 significance level (Bonferroni corrected 2 comparisons, alpha=0.05/2=0.025) to detect an equivalence using two

one-sided t-tests. This was done assuming equivalence limits of -4.6 and 4.6 and a standard deviation of 4. A sample size of n=20 per group was selected in order to account for any erroneous results or experimental error. The comparison (HEMAsavR) and control (Haemonetics) groups are to be considered equivalent if the 90% two-sided confidence interval for the difference in the % K+ removal falls wholly within the equivalence limit (-4.6, 4.6).

Results

All percent difference data as well as baseline and post-filter averages can be found in Table 1 and 3. The statistical results from collected data are referenced in Table 2. The mean post-filter K+ percentage removal was 72.7% for the 1000μm group, 70% for the 200μm group and 72.1% for the 20μm group (Image 1, Image 2). The mean post-filter PFHb percentage removal was -20% for the 1000μm group, 43.6% for the 200μm group and -19.7% for the 20μm group (Image 3. The mean post-filter Hgb percentage gain was 108.7% for the 1000μm group, 161.3% for the 200μm group, and 138.9% for the 20μm group (Image 4). The mean post-filter HCT percentage gain was 61.9% for the 1000μm group, 62.2% for the 200μm group, and 65.2% for the 20μm group (Image 5). The 90% confidence interval calculated for K+ percentage removal fell within the generated equivalence limits (-4.6, 4.6). This 90% CI of the protocol difference in the mean percent K+ removal was found to be (0.12,1.03) and (-2.59, -1.66) for the 1000μm group and 200μm group, respectively. Thus, the null hypothesis was rejected and equivalency was displayed between the new groups and control (20μm group).

In all three samples acquired for RBC morphology scans, no schistocytes (fragmented RBCs) were noted. All three of the samples contained fewer than 5-10% of spur cells, an RBC morphological abnormality caused by membrane deformity or prolonged storage. The usual preferred window for RBC morphology evaluation is within 8 hours. Due to staffing and availability during the investigation period, these scans were aged 6 days from initial testing. Thus, all morphology results are to be considered approximate and for observational use.

Discussion

The study demonstrated that the HEMAsavR and its gross-filter(1000μm) was found to be equivalent to a traditional cell saver reservoir's finer filtration (20μm) when using percent K+ removal as the primary outcome. Baseline and final potassium concentrations were similar between all trial groups, therefore yielding equivalent potassium % removal between the HEMAsavR and Haemonetics reservoirs. Although the 90% CI did not fall within the equivalence limit, the Hgb and HCT end-product quality indicators showed similar results across each group. We were unable to generate the statistical power required to claim equivalency for Hgb and HCT due to our selected sample size based on our manpower and equipment limitations. However, the overall averages in final Hgb seen across the three interventions were 11.5, 10.4, and 11.23 in the Haemonetics(20μm), HEMAsavR(1000μm), and HEMAsavR(1000μm + 200μm in-line filter) groups respectively (Table 3). The averages of final product HCT were 46.5, 44.3, and 46.3 across the same three groups (Table 3). These results are within the AABB's defined range (HCT = 40-60%) for processed salvage blood (7). Similarly, the HCT averages of 46.5, 44.3, 46.3 are all within the range/standard deviation of the results found in the study by Serrick et al. detailing wash properties of a Haemonetics-brand cell saver. Here, the average final Hematocrit produced by full bowls washed in the Haemonetics Cell Saver 5 were found to be 46.5 +/- 4.6% (8).

The lone irregularity in the results was PFHb. Results across each test group demonstrated increases in PFHb in both the HEMAsavR(1000μm) and Haemonetics groups, while the HEMAsavR(1000μm + 200μm in-line filter) group demonstrated a decrease in PFHb from baseline to final-product. The AABB states that in autotransfusion, "Residual free hemoglobin concentrations may be quite high (500-1000 mg/dL) despite greater than 90% removal" and therefore recommend that facilities determine a maximum allowable value for PFHb (7). This may aid in the explanation of the inconsistency within the PFHb data, for which the final PFHb

measured well below 500 mg/dL between all groups. All samples were analyzed uniformly, centrifuged at equal rates, following hematology standards, and the HemoCue analyzers underwent successful liquid quality control testing prior to the investigation. Because the trial groups were tested independent of each other on subsequent days, baseline data varied from day-to-day with respect to the differing lots of porcine blood. Baseline PFHb data averaged 68, 364, and 50.5 (mg/dL) for the HEMAsavR(1000μm), HEMAsavR(1000μm + 200μm in-line filter), and Haemonetics(20μm) groups respectively. Final PFHb values averaged 81, 205, and 60 (mg/dL) for the HEMAsavR(1000μm), HEMAsavR(1000μm + 200μm in-line filter), and Haemonetics(20μm) groups respectively (Table 3). As such, our PFHb % removal data varied considerably as described above. The HEMAsavR(1000μm) and Haemonetics(20μm) groups produced unanticipated increases (% gains) in PFHb while also averaging notably lower baseline starting values of PFHb in comparison to the HEMAsavR(1000μm + 200μm in-line filter) group. It was postulated by Szpisjak et al. that hemoglobin may be less effectively removed than potassium due to its larger molecular size (2). This may lend to the discrepancies seen with PFHb removal, and further validate our decision to use % K+ removal as our primary outcome. It is also possible that if subjected to further initial trauma, exposure, etc. (i.e. hemolysis), and in turn higher baseline PFHb values similar to the 364 mg/dL average seen in the HEMAsavR(1000μm + 200μm in-line filter) group, a % removal and decrease in PFHb from baseline to final product might have been observed in each of these two groups. This suggested reasoning would be more consistent with the expected trend in PFHb during the cell salvage process, with more comparable results to those seen in the HEMAsavR(1000μm + 200μm in-line filter) group. Additionally, the ability to test utilizing a single lot/sample of porcine blood for each test group may help account for this variability as well. In summary, the HEMAsavR(1000μm + 200μm in-line filter) group produced expected results while the other two interventions including our control group (Haemonetics) generated seemingly erroneous PFHb trends. Thus, further testing is necessary in order to expand upon these preliminary PFHb

findings and gain a more comprehensive understanding as to why inconsistent results were observed.

This investigation verifies the HEMAsavR's worth as an equal alternative to traditional standard cell salvage blood collection canisters. The similar final products observed across all three intervention groups in HCT and Hgb values, along with the equivalency of our primary outcome, percent K+ removal, across all three interventions show analogous performance between the groups. Additionally, despite this study's discrepancies in resultant PFHb data and the need for further evaluation, the HEMAsavR intervention groups fared similarly and better to that of the Haemonetics from baseline to post-filter averages. Overall, these findings illustrate no reduction in the effectiveness of the novel design of the HEMAsavR's 1000μm gross-filtration in comparison to standard cardiotomy reservoirs currently available on the market.

Acknowledgments

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Table 1:

outcome	group	N	Mean	Std Dev
K+ percentage removal at post-filter step	HEMA	20	72.7	0.94
	HEMA+InLineFilter	20	70	0.99
	Haemo	20	72.13	0.76
PFHB percentage removal at post-filter step	HEMA	20	-20.01	27.86
	HEMA+InLineFilter	20	43.59	6.08
	Haemo	20	-19.71	33.48
Hb percentage gain at post-filter step	HEMA	20	108.71	18.37
	HEMA+InLineFilter	20	161.26	16.69
	Haemo	20	138.94	9.9
HCT percentage gain at post-filter step	HEMA	20	61.95	9.86
	HEMA+InLineFilter	20	62.17	5.48
	Haemo	20	65.22	5.17

Table 2:

outcome	Control	other protocol	Mean Difference (other-Haemo)		equivalence limit	Assessment
			mean difference	90% CI		
K+ percentage removal at post-filter step	Haemo	HEMA	0.57	(0.12, 1.03)	(-4.6, 4.6)	reject not Equivalent
	Haemo	HEMA+InLineFilter	-2.13	(-2.59, -1.66)	(-4.6, 4.6)	reject not Equivalent
PFHB percentage removal at post-filter step	Haemo	HEMA	-0.30	(-16.72, 16.12)	(-4.3, 4.3)	Cannot reject Not equivalent
	Haemo	HEMA+InLineFilter	63.30	(50.19, 76.42)	(-4.3, 4.3)	Cannot reject Not equivalent
Hb percentage gain at post-filter step	Haemo	HEMA	-30.23	(-38.16, -22.3)	(-2.3, 2.3)	Cannot reject Not equivalent
	Haemo	HEMA+InLineFilter	22.33	(14.97, 29.68)	(-2.3, 2.3)	Cannot reject Not equivalent
HCT percentage gain at post-filter step	Haemo	HEMA	-3.26	(-7.5, 0.97)	(-2.3, 2.3)	Cannot reject Not equivalent
	Haemo	HEMA+InLineFilter	-3.05	(-5.89, -0.21)	(-2.3, 2.3)	Cannot reject Not equivalent

Table 3:

Haemonetics 20µm Group	Baseline Avg.	Post-Filter Avg.
Potassium	5.2	1.5
Plasma Free Hemoglobin	50.5	59.5
Hemoglobin	4.8	11.5
Hematocrit	28.2	46.5
HEMAsavR 200µm Group	Baseline Avg.	Post-Filter Avg.
Potassium	4.9	1.5
Plasma Free Hemoglobin	363.7	205.2
Hemoglobin	4.0	10.4
Hematocrit	27.3	44.3
HEMAsavR 1000µm Group	Baseline Avg.	Post-Filter Avg.
Potassium	5.315	1.5
Plasma Free Hemoglobin	67.9	81.4
Hemoglobin	5.4	11.2
Hematocrit	28.8	46.6

Image 1:

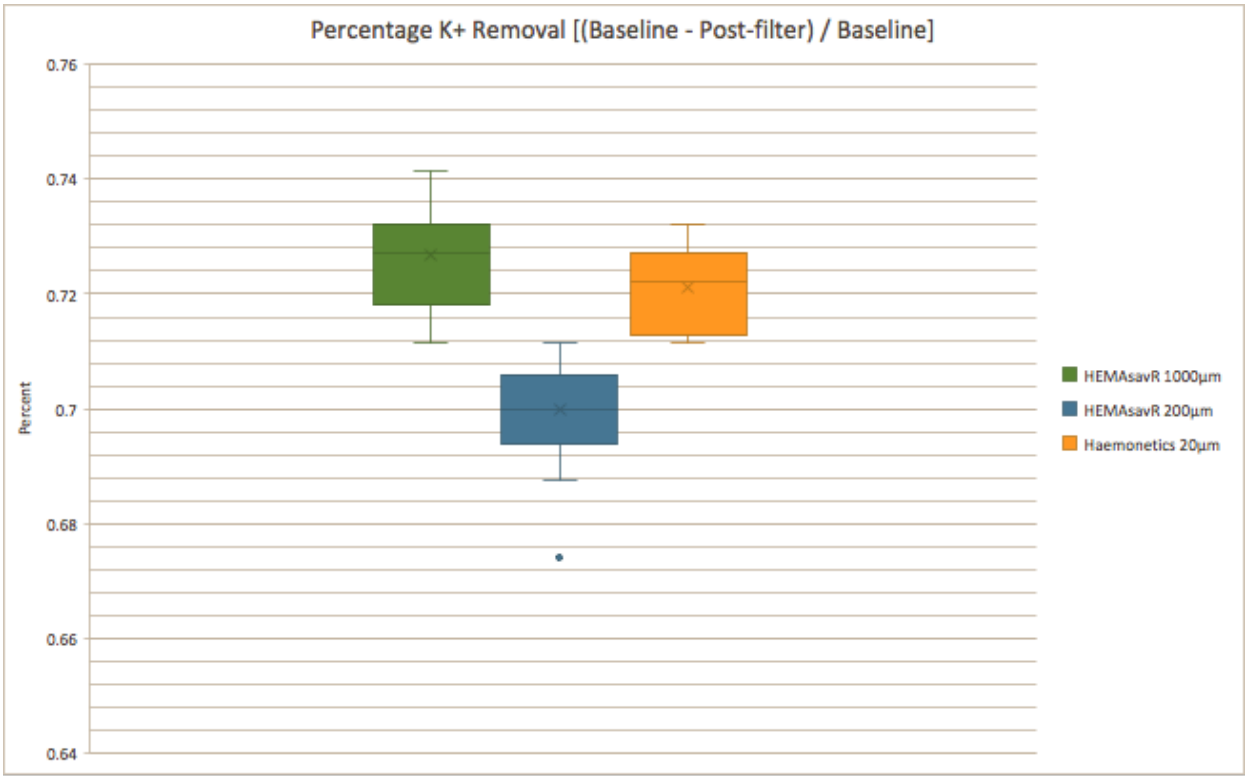

Percentage K+ Removal [(Baseline - Post-filter) / Baseline]
Percent
0.76
0.74
0.72
0.7
0.68
0.66
0.64
HEMAsavR 1000µm
HEMAsavR 200µm
Haemonetics 20µm

Image 2:

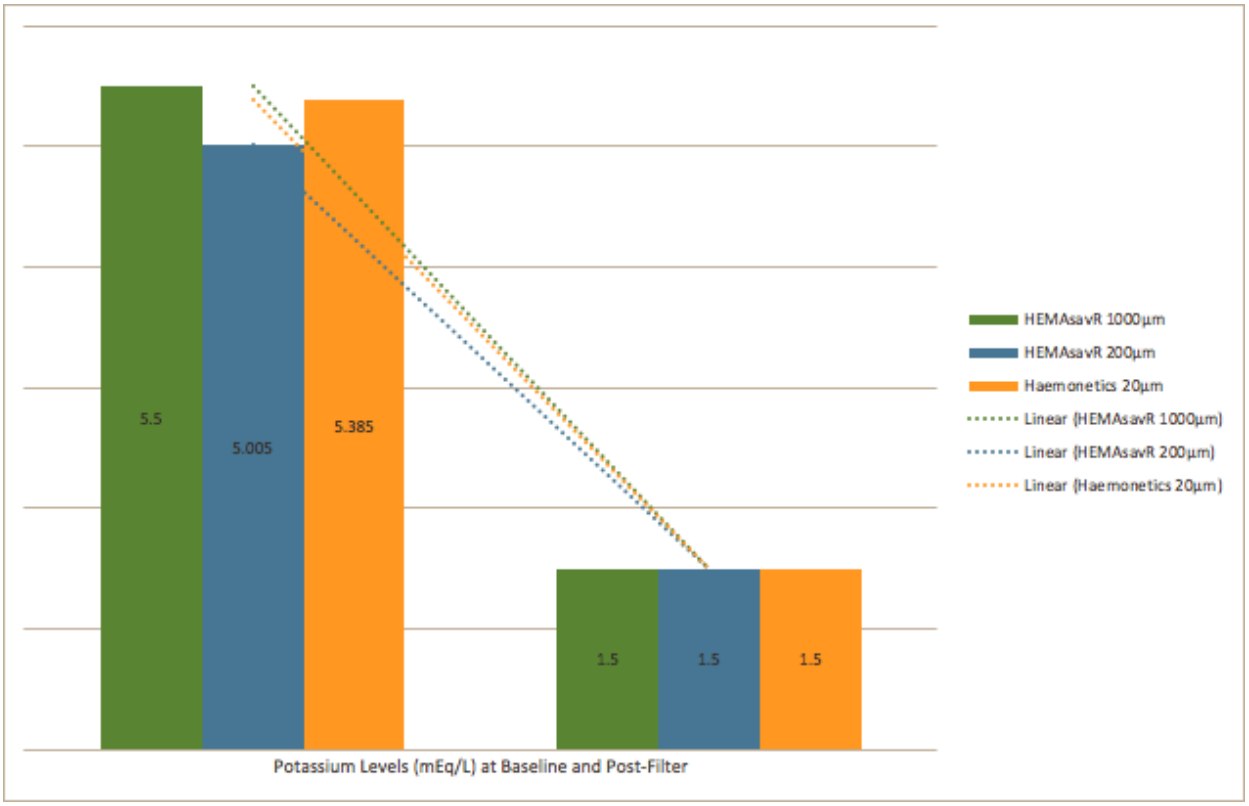

5.5
5.005
5.385
1.5
1.5
1.5
HEMAsavR 1000µm
HEMAsavR 200µm
Haemonetics 20µm
Linear (HEMAsavR 1000µm)
Linear (HEMAsavR 200µm)
Linear (Haemonetics 20µm)
Potassium Levels (mEq/L) at Baseline and Post-Filter

Image 3:

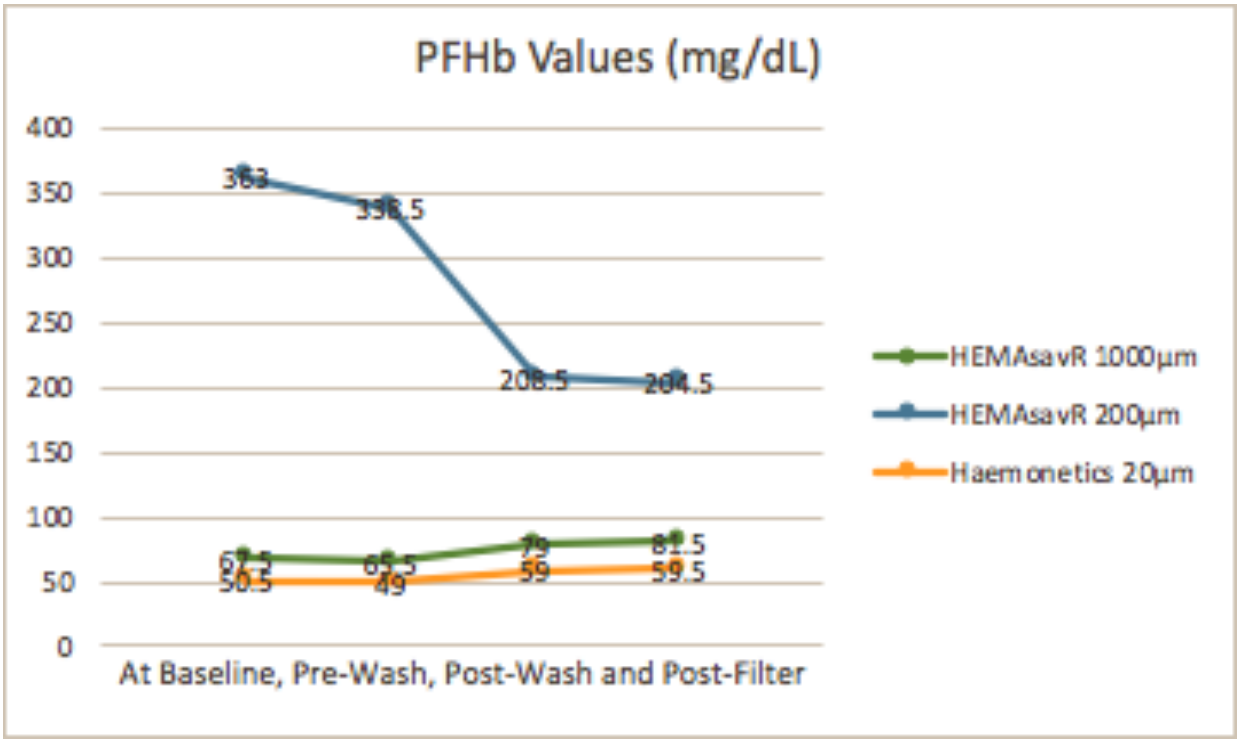

PFHb Values (mg/dL)
400
350
300
250
200
150
100
50
0
363
338.5
208.5
204.5
67.5
65.5
79
81.5
50.5
49
59
59.5
At Baseline, Pre-Wash, Post-Wash and Post-Filter
HEMAsavR 1000µm
HEMAsavR 200µm
Haemonetics 20µm

Image 4:

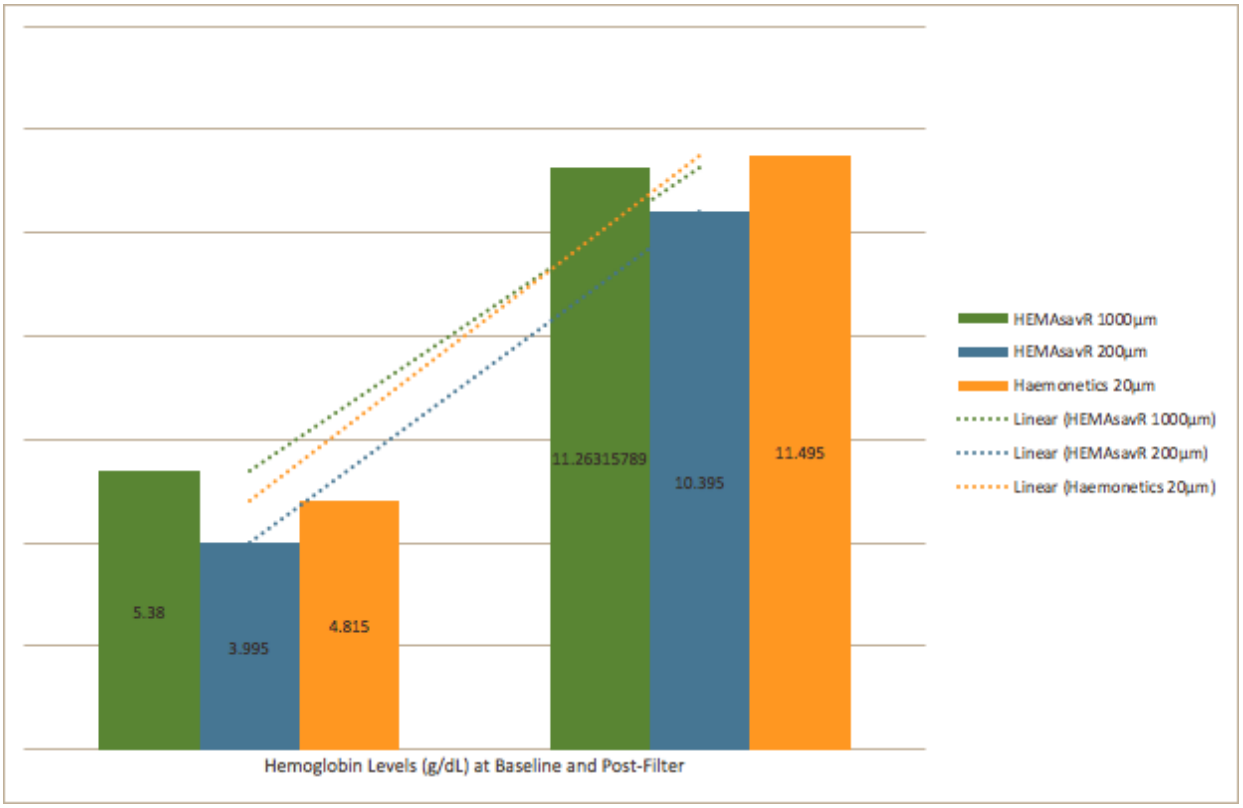

5.38
3.995
4.815
11.26315789
10.395
11.495
HEMAsavR 1000µm
HEMAsavR 200µm
Haemonetics 20µm
Linear (HEMAsavR 1000µm)
Linear (HEMAsavR 200µm)
Linear (Haemonetics 20µm)
Hemoglobin Levels (g/dL) at Baseline and Post-Filter

Image 5:

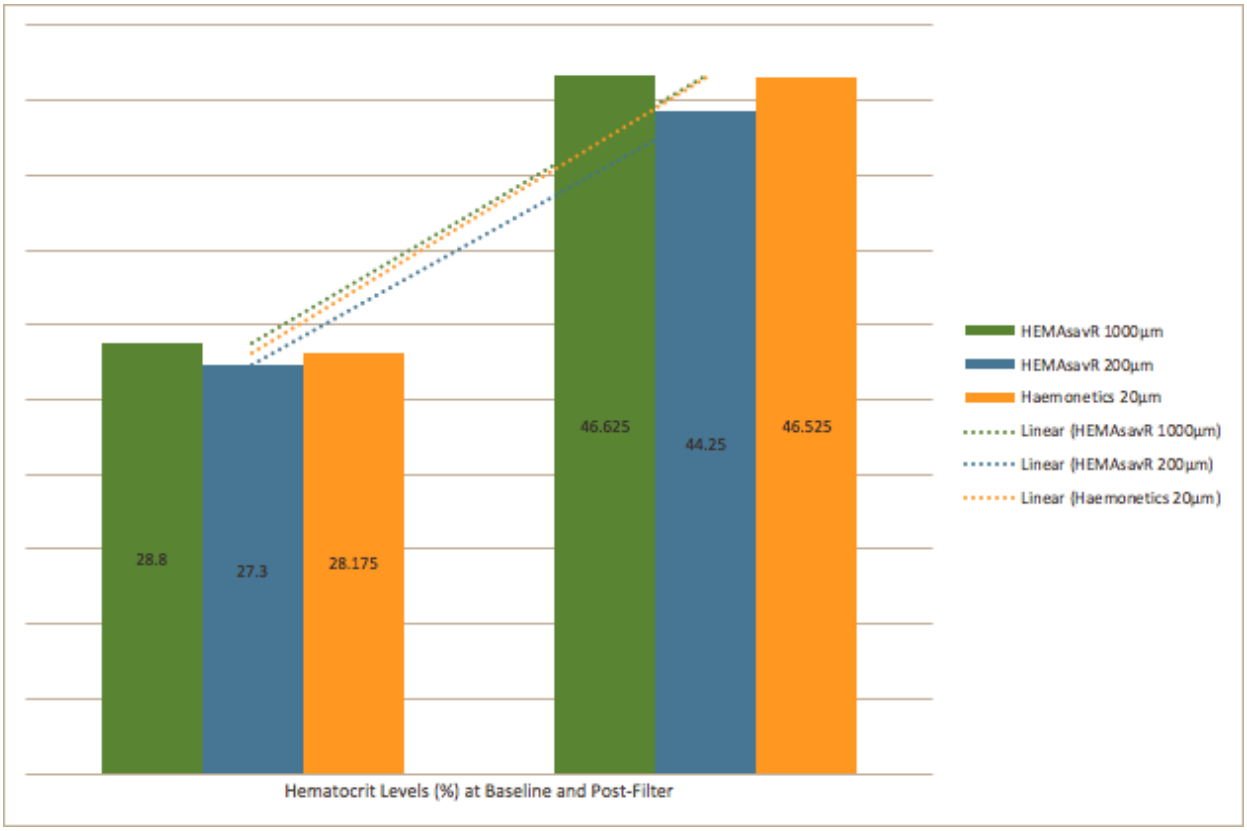
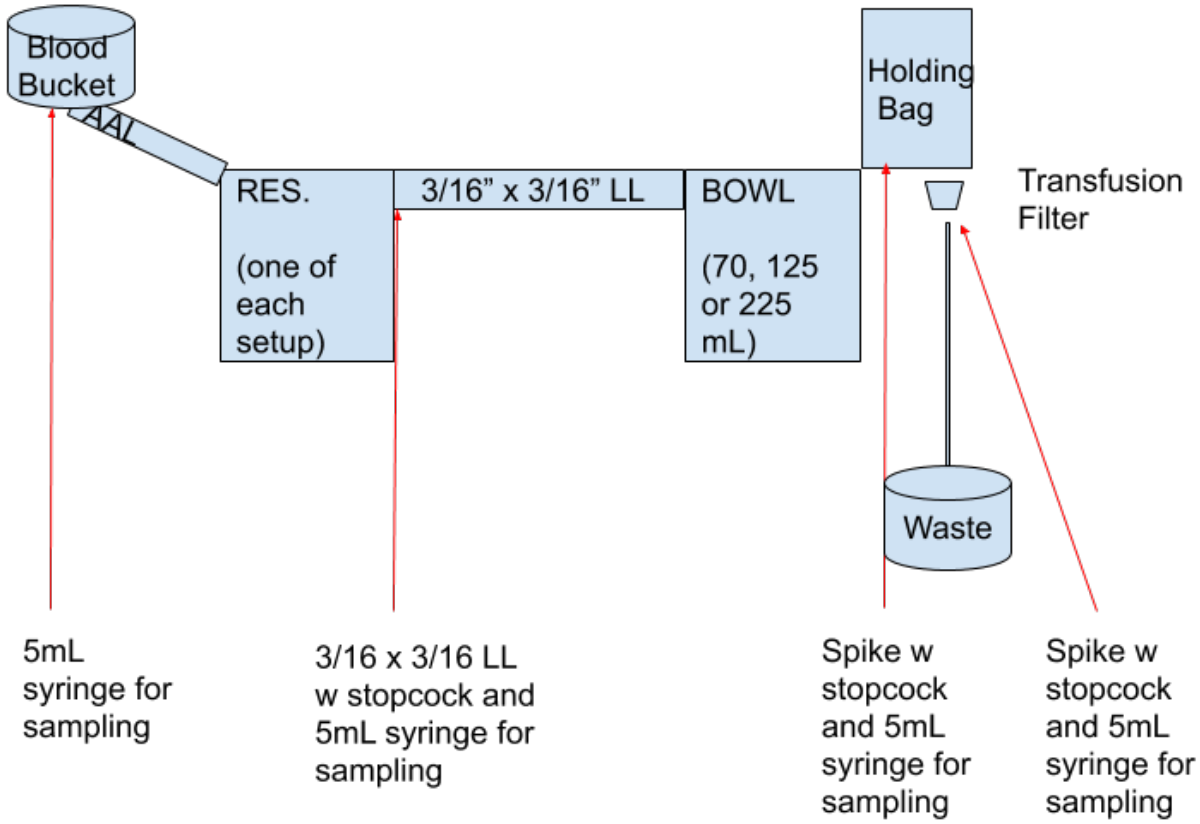

28.8
27.3
28.175
46.625
44.25
46.525
HEMAsavR 1000µm
HEMAsavR 200µm
Haemonetics 20µm
Linear (HEMAsavR 1000µm)
Linear (HEMAsavR 200µm)
Linear (Haemonetics 20µm)
Hematocrit Levels (%) at Baseline and Post-Filter

Image 6:


Blood Bucket
AAL
RES.
(one of each setup)
3/16" x 3/16" LL
BOWL
(70, 125 or 225 mL)
Holding Bag
Transfusion Filter
Waste
5mL syringe for sampling
3/16 x 3/16 LL w stopcock and 5mL syringe for sampling
Spike w stopcock and 5mL syringe for sampling
Spike w stopcock and 5mL syringe for sampling

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