Intraoperative cell salvaging: ex vivo evaluation of two swab-washing methods

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OBJECTIVE
To compare erythrocyte recovery by a cell salvage device between swab-washing by manual agitation or filtration.

SAMPLE
12 recently expired units of canine packed RBCs.

PROCEDURE
The packed RBC units underwent quality analysis before donation from a pet blood bank. Each unit was volume-expanded with anticoagulant and subsequently divided into 2 equal aliquots used to soak surgical swabs before washing. Two different swab-washing techniques were evaluated—standard swab-washing–manual agitation (SW-MA) and swab-washing–filtration (SW-F)—with a novel prototype device. The resulting bloody fluid was processed using the Cell Saver Elite Autotransfusion System (Haemonetics). The volume, manual PCV, CBC, and RBC mass, calculated as the product of the volume and PCV, were measured before and after salvaging. Last, the RBC mass recovery was recorded as a percentage.

RESULTS
The RBC mass recovered from SW-MA and SW-F averaged 85.73% and 83.99%, respectively. There was no significant difference in RBC recovery between the 2 methods ($P = .52$).

CLINICAL RELEVANCE
SW-MA and SW-F recovered a similar quantity of RBCs from blood-soaked swabs in an ex vivo setting.

Hemorrhage remains a common intraoperative complication. Despite advances in surgical equipment and professional training, blood loss can be significant, with an associated negative impact on the patient. Blood transfusions remain the gold standard in treating the systemic effects of hemorrhage. Intraoperative cell salvage (ICS) is a procedure that uses a specific device (cell saver) to collect and filter suctioned blood and bloody fluid. The cells are then washed, concentrated, and delivered to the patient as an autologous transfusion of packed RBCs (pRBCs). ICS is a common practice in human surgery, and it is starting to gain popularity in veterinary medicine.

In human medicine, it has been estimated that discarded blood-soaked swabs account for between 30% and 50% of total blood lost in surgery. With the availability of ICS, washing of blood-soaked surgical swabs has been recommended to improve RBC yield. Swab-washing is typically performed by a dedicated, trained member of staff according to an approved protocol. Surgical swabs are soaked and washed in a bowl filled with saline solution (0.9% sodium chloride), and subsequently aspirated and processed through the cell saver. Manual swab-washing is both time-consuming and labor intensive, requiring specifically trained staff. In addition, efficiency is operator and technique dependent, and it carries some health and safety hazards in the human field as a result of handling and agitating blood products. The technique of intraoperative swab-washing in veterinary medicine has not been reported to our knowledge.

The aim of this study was to compare RBC mass recovery following 2 different techniques of swab-washing using cell salvage. The first method is manual agitation. The second method involves using a novel apparatus assembled by one of the authors (AG) to aid filtration by gravity. We hypothesized that using both methods would result in similar RBC mass recovery.
Methods

Samples and equipment

For the study, units of recently expired canine pRBCs were received as a donation from a pet blood bank. The donated products were at least 4 weeks old. Hemolysis was greater than 0.8%, and the units were therefore considered unsuitable for transfusion.13 The pRBC units were collected, prepared, and stored according to the most recent literature guidelines.14–18 One of the authors (RF) performed quality analysis before shipment, measuring PCV, total hemoglobin, and hemolysis for each pRBC unit. Analysis and measurements were performed as reported previously in the literature.17,19 The pRBC units were transported within 24 hours of completion of analysis in suitable containers for biologic products. On arrival, blood products were stored at 2 to 6 °C in a dedicated refrigerator Compact Blood Bank (Labcold Medical Refrigeration). The temperature was maintained automatically within the optimal range and monitored continuously with a digital thermometer. In addition, manual PCV, hematocrit, blood type, and expiration date were recorded. The Cell Saver Elite Autotransfusion System (Haemonetics) was used for the salvaging procedure. The device recovers erythrocytes by centrifuging blood in batches in an appropriate-size bowl, which is determined depending on the expected blood loss (3 bowl sizes are available: 70, 125, and 225 mL). The RBC mass is then washed with 0.9% sodium chloride solution by complete or partial cycle, depending on the degree of filling of the bowl. To minimize cell damage, suctioning of the blood was performed using an auto-regulating vacuum system that operates at a maximum pressure of –150 mm Hg and adjusts vacuum levels automatically. Double-lumen suction tubing was used, allowing for a simultaneous flow of anticoagulant (Anticoagulant Citrate Dextrose Solution [ACD-A], Haemonetics). To aid swab-washing via filtration (SW-F), one of the authors (AG) assembled a filtration device (Figure 1) using 2 stainless steel elements: a meshed basket and a pan. Both components are readily available, inexpensive, and autoclavable. During swab-washing, the swabs are placed in the basket, which is kept suspended over the pan by two pins. As saline is poured over the swabs, the meshed design of the basket functions as a colander, allowing liquid to drain through the perforations via gravity while retaining the swabs. The pan collects the bloody fluid, which is then suctioned.

Data collection

The experiment was approved by the Royal College of Veterinary Surgeons ethics committee (reference 2020-11-Kalmukov). All blood units were processed in a clean operating room environment within 48 hours of being received. Two hundred milliliters of ACD-A were added to an equal volume of pRBCs, obtained from a single unit. These were mixed in a sterile bag and agitated gently for 5 minutes using a blood tube rocker (Test Tube Rocker, IDEXX). The blood–citrate solution was divided into 2 equal aliquots of 200 mL, and each was placed in separate plastic pans for swab-washing–manual agitation (SW-MA) and SW-F. All volumes were measured accurately by drawing into 50-mL syringes until the desired amount was reached. A sample (0.5 mL) from each pan was also obtained, and manual PCV was measured. The PCV was evaluated in triplicate, and the average result of the 3 measurements was recorded. The analysis was performed according to World Health Organization recommendations.20 The aliquots were processed further if the difference in values of PCV between bowls was not greater than 2%; otherwise, it was deemed that the pRBC–ACDA mixture was nonhomogenous. CBC measurement presalvage (SW-MA and SW-F) was performed on 1 aliquot. Measurements were randomized by flipping a coin, and were performed on an in-house automated analyzer (ProCyte Dx Haematology Analyser, IDEXX).

Each aliquot was soaked into ten 10- X 10-cm surgical swabs (gauze swabs, American folding, radiography detectable). Next, the 10 swabs from the SW-MA group were washed in a large bowl filled with 1,000 mL of sterile saline (Vetivex 1, Dechra Veterinary Products) using gentle continuous manual agitation for 15 seconds. The surgical sponges were subsequently gently squeezed manually and discarded. After all 10 swabs had been washed, the resulting blood–saline mixture was suctioned and salvaged. The 10 blood-soaked swabs from the SW-F group were fully individually and placed in the basket of the filtration device. One liter of normal sterile 0.9% saline (room temperature) was then poured directly over the swabs, which were allowed to drain, and were then squeezed gently before being discarded. The blood–saline mixture collected in the stainless steel pan was aspirated and underwent cell salvage. The Cell Saver Elite Autotransfusion System (Haemonetics) and a 70-mL bowl were used to process each aliquot for all procedures. In addition, a double wash function, which consists of a double cycle using 600 mL of saline, was used every time a partial bowl needed to be processed.1 At the end of each procedure, the salvaged volume of blood was measured automatically by the device and noted. Manual PCV and CBC values were acquired postsalvage for each aliquot. The RBC mass was calculated

![Figure 1—Novel prototype “swab washer.” Basket dimensions: width, 10 cm; height, 25 cm; depth, 5 cm. Mesh size: 0.5 X 0.5 cm. Pan dimensions: width, 15 cm; height, 30 cm; depth, 5 cm.](image-url)
by multiplying the volume of the salvaged blood by its PCV. This was recorded pre- and postsalvage for both swab-washing techniques. Their ratio was used to evaluate the recovery efficiency, recorded as a percentage, used for statistical analysis. Measurements/readings were only included in the study if no blood spillage occurred during the procedure. The same operator (IAK) performed the swab-washing for all samples and both methodologies.

Statistical analysis

The statistical analysis was conducted using R Studio (version 4.0.3, R Foundation for Statistical Computing). The practice of dividing each pRBC unit into 2 aliquots and using SW-MA on one and SW-F on the other was implemented to eliminate the effect of any difference between the pRBC units. Thus, the results were paired, and, for each pRBC unit, the results of prime focus were the percentages of RBC mass recovered for each method and the difference between them (ie, Percentage of RBC mass recovered for the SW-F aliquot – Percentage of RBC mass recovered for the SW-MA aliquot from the same pRBC unit). The number of observations was determined according to a power analysis. With a significance level of 5% and a power of 90%, an effect of size 1.03 would be detected with 12 pairs of samples/readings, which was considered sufficient, given the SDs reported in the literature. The variables PCV and hemolysis were recorded for each pRBC unit. In addition, for each aliquot, Hct and PCV were recorded, both before and after salvaging, along with the volume of salvaged blood.

These variables and, when appropriate, paired differences for each pRBC unit, were assessed for normality via Shapiro Wilks tests. If the normality assumption was determined to be reasonable, descriptive statistics were provided in the form of mean ± SD. When the normality assumption was rejected, descriptive statistics comprised median and interquartile ranges (IQRs). Tests were conducted on the differences in PCV, Hct, and volume salvaged between the SW-MA and SW-F groups. When the differences could be assumed normal, a paired t test was used; otherwise, a Wilcoxon signed-rank test was used. CIs were constructed for the percentage of RBC mass recovered for each method and for the percentage differences in RBC mass recovery. Tests were conducted to determine whether the order in which the aliquots were processed (ie, SW-MA first or SW-F first) or whether the blood was positive or negative had any effect on the difference in RBC mass recovery. When the normality assumption was reasonable, a 2-sample t test was used; otherwise, a Mann-Whitney test was used. A linear model was fitted to determine if the RBC mass value before processing influenced the difference in RBC mass recovery. For all analyses, P < .05 was considered significant.

Results

Twelve units of pRBCs were used for the study; 3 U were dog erythrocyte antigen 1.1 negative and 9 U were dog erythrocyte antigen 1.1 positive. Mean PCV was 60.42% ± 3.55%, and mean hemolysis was 1.69% ± 1.18%. After dilution of the blood, the mean Hct was 30.03% ± 2.64%. After creating the 2 aliquots and before salvaging, the mean PCV was 28.33% ± 2.02% for the SW-MA group and 28.25% ± 2.18% for the SW-F group. The PCV values for the 2 aliquots were identical for 11/12 pRBC units. For the remaining pRBC unit, the 2 aliquots had PCV values differing by 1%. Despite this discrepancy, there was no statistically significant difference between the PCV values for the aliquots used for SW-F and those used for SW-MA. The median volume of the salvaged blood was 141.00 mL (IQR, 138.25 to 143.25) for the SW-MA group and 141.00 mL (IQR, 138.75 to 143.00) for the SW-F group. The data were paired, with the difference between the volumes of salvaged blood from the two methods (SW-F – SW-MA) being adequately modeled by a normal distribution (P = .13). The mean difference between the volumes of salvaged blood from the 2 methods was –0.33 ± 1.72 mL. A paired t test identified no significant difference in the mean volume of salvaged blood between the two groups (P = .52). There was no difference (P = .71) in post-salvage PCV following SW-MA and SW-F (36.16% ± 8.26% and 36.5% ± 8.07%, respectively). The median Hct postsalvage was 36.75% (IQR, 33.03 to 40.60) in the SW-MA group and 37.90% (IQR, 34.25 to 40.73) in the SW-F group. A Wilcoxon signed-rank test indicates no difference between the Hct postsalvage values for the 2 methods (P = .13). The median and IQR for PCV rise after salvaging was also not different between the groups (P = .65), with median values of 7.00% (IQR, 4.75 to 9.00) for the SW-MA group and 6.50% (IQR, 4.50 to 8.75) for the SW-F group, and a mean difference between the 2 methods of 0.42% ± 3.09%. The median rise in Hct postsalvage was 7.20% (IQR, 5.88 to 8.65) for the SW-MA group and 7.35% (IQR, 5.43 to 10.05) for the SW-F group. There was no difference between the 2 methods when the rise in Hct postsalvage was compared (P = .13). One difference appeared to be an outlier. Omitting this value and rerunning the Wilcoxon signed-rank test on the remaining 11 differences confirmed the result of no significant difference (P = .23).

The median RBC mass recovery was 85.73% (IQR, 74.09 to 92.58) for the SW-MA group and 83.99% (IQR, 73.74 to 88.59) for the SW-F group. The data were paired, with the difference in percentage RBC mass recovery between the methods (SW-F – SW-MA) not being approximately normal (P = .03). Ultimately no difference between the 2 groups was found (P = .52). The difference in RBC mass recovered from 1 pRBC unit appeared to be an outlier. Omitting this value and rerunning the Wilcoxon signed-rank test on the remaining 11 differences confirmed the result of no significant difference (P = .21). Neither the order of processing (P = .47) nor the blood type (P = .06) affected the percentage of RBC mass recovery significantly. A linear model was fitted with the response variable being the difference in percentage RBC mass recovery and the explanatory variable being the RBC mass value before processing. The P value was .36, indicating no effect of
the RBC mass value before processing on the difference in percentage RBC mass recovery. Last, following the analysis of the CBC parameters before and after salvaging (Table 1), for the variables number of platelets (P = .03) and plateletcrit (P = .05), a difference was found between the groups. For the other variables, no statistically significant difference was identified.

Discussion

Our results demonstrate that RBCs can be recovered effectively via cell salvage with the swab-washing techniques described. A 90% efficiency when recovering human blood via direct suction with the same cell saver device used in this study has been reported.1 Our results with canine blood compare similarly with a mean RBC recovery of more than 80%. Our postsalvage median Hct values for SW-MA and SW-F were 36.75% and 37.90%, respectively. Haemonetics reports mean results that are higher, typically more 50%. Several factors could have led to this difference. First, Haemonetics reports performance using in-date human blood, which was concentrated using a larger processing bowl and harvested via direct suction (instead of swabs). Salvaging expired blood may also have led to increased destruction of cells, which we suspect have structural and functional changes. This phenomenon has not been evaluated extensively in veterinary medicine, but there is sufficient literature from human medicine regarding these changes.21,22 Methodology of swab-washing can also contribute to this difference if the washing is particularly harsh or abrasive, leading to mechanical cell destruction and lower yields. Although beyond the scope of our study, this theory could have been evaluated by measuring hemolysis postsalvage. More accurate conclusions about the differences in RBC recovery after direct suction and swab-washing could be assessed with a prospective study to compare both methods. Our preclinical data suggest that both swab-washing techniques allow for a high RBC yield during cell salvage. This practice could be precious in surgeries during which the blood is dispersed in swabs rather than suctioned from a body cavity (ie, maxillectomy/mandibulectomy), and valuable for cases prone to steady hemorrhagic "ooze" throughout a procedure. The median volume of salvaged blood after swab-washing was 141 mL for both groups. In 10/12 pRBC units, the volume of the salvaged blood ranged between 136 mL and 145 mL for each aliquot. In the remaining 2 U, the volumes postsalvage ranged between 64 mL and 67 mL. In those 2 U, the degree of hemolysis was more than 3.0% (3.48% and 3.2%), suggesting that the degree of damaged cells was greater in these units, which led to a lower recovery volume. Further studies are needed to evaluate whether the level of hemolysis in the blood to be salvaged affects the concentrating ability of the cell saver.

Unfortunately, publications regarding swab-washing are scarce in human literature and, to our knowledge, equivalent research in veterinary surgery—especially with canine blood—is undescribed. Ronai et al23 reported recovery of 87% ± 6% of human RBCs from blood-soaked surgical sponges in a similar swab washing model (manual agitation). Haynes et al24 reported RBC recovery after intraoperative swab-washing with manual agitation in patients undergoing elective aortic aneurysm repair, with a median of 67% (IQR, 33 to 98). Our results compare favorably with both these publications (SW-MA median, 85.73%; SW-F median, 83.99%), although it

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Table 1—Summary of the analysis of the CBC parameters before and after cell salvage.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Presalvage, median (IQR)</th>
<th>Difference between presalvage and SW-MA postsalvage, median (IQR)</th>
<th>Difference between presalvage and SW-F postsalvage, median (IQR)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs X 10¹²/L</td>
<td>4.59 (4.45 to 4.81)</td>
<td>-0.34 (-0.45 to 0.19)</td>
<td>-0.18 (-0.77 to 0.08)</td>
<td>.61</td>
</tr>
<tr>
<td>Hct, %</td>
<td>29.90 (28.88 to 32.63)</td>
<td>-7.20 (-8.65 to 4.40)</td>
<td>-7.35 (-10.05 to -3.60)</td>
<td>.13</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>10.85 (10.38 to 11.13)</td>
<td>-0.75 (-0.93 to -0.55)</td>
<td>-0.55 (-1.48 to -0.10)</td>
<td>.72</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>65.25 (62.35 to 67.78)</td>
<td>-10.05 (-10.95 to -8.60)</td>
<td>-12.95 (-13.63 to -9.55)</td>
<td>.13</td>
</tr>
<tr>
<td>MCH, pg</td>
<td>23.00 (22.90 to 23.58)</td>
<td>-0.05 (-0.13 to 0.10)</td>
<td>0.10 (-0.03 to 0.13)</td>
<td>.11</td>
</tr>
<tr>
<td>MCHC, g/dL</td>
<td>35.30 (33.98 to 36.48)</td>
<td>4.60 (4.40 to 4.95)</td>
<td>5.70 (4.75 to 6.40)</td>
<td>.09</td>
</tr>
<tr>
<td>RDW</td>
<td>20.65 (18.35 to 23.22)</td>
<td>-2.25 (-3.68 to -0.33)</td>
<td>-2.15 (-4.13 to 0.10)</td>
<td>.79</td>
</tr>
<tr>
<td>Reticulocytes, %</td>
<td>0.20 (0.18 to 0.40)</td>
<td>0.10 (0.00 to 0.20)</td>
<td>0.15 (0.00 to 0.20)</td>
<td>.17</td>
</tr>
<tr>
<td>Reticulocytes-hemoglobin, pg</td>
<td>22.50 (19.83 to 23.53)</td>
<td>1.45 (0.03 to 3.73)</td>
<td>1.55 (0.80 to 3.33)</td>
<td>.94</td>
</tr>
<tr>
<td>WBCs X 10⁹/L</td>
<td>0.01 (0.01 to 0.02)</td>
<td>-0.02 (-0.06 to -0.01)</td>
<td>-0.03 (-0.04 to -0.02)</td>
<td>.65</td>
</tr>
<tr>
<td>Platelets, K/μL</td>
<td>104.50 (39.50 to 204.25)</td>
<td>-138.00 (-308.25 to -65.75)</td>
<td>-335 (-595 to -179)</td>
<td>.03</td>
</tr>
<tr>
<td>Mean platelet volume, fl</td>
<td>7.40 (7.15 to 8.25)</td>
<td>-1.65 (-2.45 to -0.75)</td>
<td>-2.15 (-2.80 to -0.48)</td>
<td>.64</td>
</tr>
<tr>
<td>Plateletcrit, %</td>
<td>0.11 (0.05 to 0.15)</td>
<td>-0.14 (-0.34 to -0.07)</td>
<td>-0.34 (-0.76 to -0.14)</td>
<td>.05</td>
</tr>
</tbody>
</table>

IQR = Interquartile range; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; MCV = Mean corpuscular volume; RDW = Red cell distribution width; SW-F = Swab-washing–filtration; SW-MA = Swab-washing–manual agitation.

For each variable, a Wilcoxon signed-rank test was conducted to test the hypothesis that the change in a variable does not depend on the method of cell salvage used against the contrary alternative. P values are reported in the final column. For the variables platelets (P = .03) and plateletcrit (P = .05), a difference was found between the groups. For the other variables, no statistically significant difference was identified.
is worth mentioning that Haynes et al\textsuperscript{11} reported a clinical scenario with salvaging whole blood. This may explain slightly greater end results in our data because of greater initial Hct percentages. Our study found no statistically significant or clinically relevant difference between the percentage recovery of RBC mass for the 2 techniques, and no difference in the features of the erythrocytes evaluated via CBC analysis. This suggests that mechanical damage caused by the handling of the swabs might not be significant and would not affect overall efficiency, but further work in this area is warranted. Alternatively, it could imply that the cell salvage device is effective in filtering structurally damaged erythrocytes when harvested. These results lead to acceptance of our null hypothesis. The CBC analysis of the 2 groups did not reveal a significant difference between the groups, with the exception of platelets (\( P = .03 \)) and plateletcrit (\( P = .05 \)). Because the cell saver removes platelets from the suctioned blood, we suspect these values were artificially elevated (ie, influenced by fragmented erythrocytes). Structural changes were not evaluated by cytology—a limitation of our study.

In addition, evaluation of cell destruction and of the degree of cell changes were not the aims of our study, and these remain speculations with unknown relevance in a clinical setting. These aspects might be better evaluated with more targeted studies. Despite the unapparent advantage of either swab-washing method in terms of final blood product, there might be benefits in a clinical setting of passive filtration swab-washing, because it lends itself to be partly or fully automated. To our knowledge, there is only one automated swab-washing device available, and its efficacy was evaluated with human blood.\textsuperscript{11} Preliminary results were presented as a poster,\textsuperscript{11} describing an automated mechanical swab-washer as an alternative to swab-washing via manual agitation. The authors did not provide details regarding the device’s design or operation, subject to patent (WO2014029967A1). Similar conclusions regarding the device’s efficacy were found, with a range of erythrocyte recovery between 64% and 71%.\textsuperscript{11} By comparison, our filtration device has a corresponding range of RBC recovery between 74% and 89%, likely because of differences in study designs.

There are several limitations in our study. First, the use of donated, recently out-of-date pRBC units does not exactly mimic the real-life clinical scenario, because dogs that undergo ICS have younger erythrocytes than those used in our experiment. This difference in cell age could affect the efficacies of the 2 tested methods, both individually and in comparison with one another. However, using in-date banked blood or obtaining it via a direct donation from dogs cannot be justified ethically. Therefore, to obtain meaningful data, efforts were made to select expired units with a percentage of hemolysis conforming as closely as possible to blood used for transfusion. Moreover, because the cell saver device filters and discard hemolyzed erythrocytes, our results likely underestimate rather than overestimate the efficacy of the salvaging process.

A 70-mL processing bowl was used based on the planned volume of fluid for processing (200 mL/ aliquot). Haemonetics suggests increased RBC percentage recovery using larger bowl sizes, although this has not been evaluated for statistical significance.\textsuperscript{1} In addition, dilution of pRBC units may be a factor in the mechanical destruction of cells during processing, leading to relatively lower yields and affecting cell saver performance (full/partial cycle). The decision to use ACD-A to dilute the RBC mass was not a result of the lack of anticoagulant in the banked blood product bag, but a result of mimicking the amount of ACD-A used clinically. In our study, we decided to limit intra- and intraobserver variability when performing swab-washing as much as possible, but we acknowledge that this variability is inevitable in clinical scenarios, primarily when SW-MA is performed.

In conclusion, this study provides evidence of the efficacy of the Cell Saver Elite Autotransfusion System (Haemonetics) in salvaging canine blood after SW-MA or SW-F. The efficiency in harvesting erythrocytes between these 2 techniques did not differ statistically.

Acknowledgments

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Preliminary results were presented as an abstract at the British Small Animal Veterinary Association annual meeting (Manchester, UK, 2022).

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