Quality of Red Blood Cells Using Autotransfusion Devices: A Comparative Analysis

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Abstract: Cell salvage devices are routinely used to process and wash red blood cells (RBCs) shed during surgical interventions. Although the principle theory of cell saving is the same, the actual process to achieve this is very different from one device to another. The purpose of this study was to compare the quality of washed, concentrated RBC produced by five very different cell-saving devices, specifically the Cobe BRAT 2, Medtronic Sequostra 1000, Haemonetics Cell Saver 5, Medtronic Autolog, and the Fresenius CATS. Reservoir and washed red blood cells were analyzed for hematocrit (Hct), platelets (PLT), leukocytes (WBC), potassium (K+), heparin, plasma-free hemoglobin (PFH), RBC mass recovery and recovery rate. The Haemonetics and BRAT 2 had the highest RBC recovery. All devices adequately removed heparin and potassium. The Medtronic Autolog had the highest removal of platelets and PFH; whereas, the BRAT had the lowest. Although the Autolog had the highest leukocyte removal, leukocytes were not adequately washed out by any of the autotransfusion devices. In conclusion, although all cell-saving devices use the same theory of centrifugation, the actual quality of the washed RBC product differs widely from one device to another. Keywords: cell savers, autotransfusion device, quality, washed, red blood cells.
A variety of contaminants are intentionally or unintentionally added or aspirated with the blood. Some of these include heparin or other anticoagulants, antibiotics, and irrigants such as saline, and also such debris as clot, fat, and bone fragments. Autotransfusion systems should be able to remove at least 90% of these contaminants (4).

Although the principle theory of cell salvaging and washing is the same, the actual process to achieve this varies from one device to another. This variation between the different types of cell-saving devices may also result in differences in the quality of the concentrated RBC product. The purpose of this study was to compare the quality of washed, concentrated RBC produced by five very different cell-saving devices specifically the Medtronic Autolog, Cobe BRAT 2, Medtronic Sequestra 1000, Haemonetics Cell Saver 5, and the Fresenius Continuous Autotransfusion System (CATS). All devices produce washed, packed red blood cells by centrifugal cell savage.

METHOD

Cardiac surgery patients for whom cell salvage was required were divided into five groups based upon the type of autotransfusion device. Although this is an adult study, only the low-volume bowls were used for all the autotransfusion devices to avoid unnecessary waste. All devices were evaluated using the manufacturers recommended protocols in the automatic mode.

1. Medtronic Autolog: The Medtronic Autolog (Medtronic Inc., Minneapolis, MN) uses a tall cylindrical bowl with two indentations in the sides to increase mixing of cells. It operates at a speed of 10,000 rpm with a volume of 135 mL. Initially, the bowl fills at 600 mL/min, slowing down to 250 mL/min during the secondary bowl fill. During the wash cycle, wash fluid is pulsed through the bowl, which, along with the indentations, increases turbulence and, thus, the washing efficiency. The wash volume used is 250 mL.

2. Cobe Baylor Rapid Autotransfusion Device (BRAT): The BRAT 2 (COBE Cardiovascular, Denver, CO) uses the Baylor 135 mL cylindrical centrifuge bowl at a speed of 4400 rpm. The bowl is filled at a recommended rate of 200 mL/min, washed at 400 mL/min, and emptied at 200 mL/min. The recommended wash volume using this bowl is 1000 mL.

3. Medtronic Sequestra 1000: The Sequestra 1000 (Medtronic Inc., Minneapolis, MN) uses the Latham type bell-shaped bowl. The bowl size used was 125 mL. It operates at a centrifugal speed of 5600 rpm. Both fill, wash and empty rates are standard at 300 mL/min. The recommended wash volume for cardiac cases is 1000 mL.

4. Fresenius CATS: The Fresenius CATS (Fresenius HemoCare, Inc., Germany) uses a closed spiral-washing chamber for a continuous washing process. The centrifuge speed varies from 1400–2400 rpm with a prime volume of 210 mL. The protocol used in this study was the Quality Wash in which there is a 5:1 wash solution to blood ratio.

5. Haemonetics Cell Saver 5: The Cell Saver 5 (Haemonetics Corp., Braintree, MA) also uses the Latham bowl technology; however, the bottom of the bowl has impellers to improve mixing. The bowl size used was the low volume 125 mL bowl. The centrifuge spins at 5650 rpm, fills, and washes at 300 mL/min, then empties at 500 mL/min. The wash volume was 750 mL.

Operators were limited to those who have been properly trained to use the autotransfusion devices. The same device was used for each case throughout the study. Each autotransfusion machine was set up according to manufacturers’ specifications using the recommended fill, wash, and empty rates in the automatic mode, with a maximum controlled suction of 150 mmHg. The cell saver was set up as per usual protocol with a 150-μL reservoir. Two luer connectors with stopcocks were placed; first, after the reservoir; and second, before the blood bag for sampling.

Before processing, the reservoir was agitated to ensure adequate mixing. The fill cycle was started, and the time was recorded. Blood was withdrawn from the reservoir line and placed in appropriate containers without using needles to avoid hemolysis. Once the cycle was completed (i.e., start of fill to end of empty), the time was recorded, and the blood was withdrawn from the holding bag through a stopcock. Reservoir and process volumes were recorded, and samples were sent to the lab.

If there were not enough RBCs to perform an effective wash (i.e., bowl is not completely filled), the contents were returned to the reservoir until enough blood had been salvaged from the operating field. This procedure was repeated each time a bowl was processed.

Analysis of Blood Samples

Complete Blood Count: 3 mL samples were placed in EDTA vacuum containers and labeled. The blood was then analyzed for platelets, white blood cells (WBC), total red blood cells and hematocrit (Hct) using a coulter counter.

Potassium: 3 mL of blood was collected in a dry heparinized blood gas syringe and analyzed for potassium levels using the Nova pHox (Nove Biomedical Corp., Waltham, MA) blood gas analyzer.

Heparin Analysis: 4.5 mL samples were collected in citrate containers and centrifuged twice for 20 minutes each. After centrifugation the citrated plasma was then frozen at −70°C. Once enough samples were collected they were analyzed for heparin using a chromogenic assay utilizing Anti-Factor 10a.
Plasma Hemoglobin: 5 mL of blood was collected in the heparinized tubes and centrifuged twice for 20 minutes. Plasma was then taken off and analyzed for plasma free hemoglobin (FH) utilizing a spectrometric assay.

Processing Time: The processing time for all devices was also recorded. The times were measured from the start of processing to the production of the final product (i.e., start of filling to the end of the empty cycle).

Red Blood Cell Recovery: The red blood cell mass recovery in terms of a percentage value, as well as the red blood cell mass-processing rate was calculated. The starting and ending hematocrits and liquid volumes in the collection reservoir and holding bag were recorded and the recovery calculated using the following equation:

\[
\text{RBC Recovery} = \left( \frac{V_{HB} \times \text{Hct}_{HB}}{V_R \times \text{Hct}_R} \right) \times 100\%
\]

Where:
- \( V_{HB} \) = Holding Bag volume
- \( \text{Hct}_{HB} \) = Holding Bag hematocrit
- \( V_R \) = Reservoir Volume
- \( \text{Hct}_R \) = Reservoir hematocrit

Statistical Analysis
Statistical analysis of all data was done using the single factor ANOVA. A two-tailed \( p \) value of less than 0.05 was considered statistically significant.

RESULTS
A total of 65 cardiac operations were entered in the study. None of the patients were on drugs that directly affect red blood cell integrity such as sulphonamide. Table 1 shows the number of patients and process procedures for each of the autotransfusion devices. All findings are summarized in Tables 2 and 3.

Red Blood Cell Recovery
There were no significant differences between the various autotransfusion devices with regards to the starting Hct. However, the CATS gave a significantly higher Hct than all the other devices (\( p < 0.05 \)) with a mean of 66% ± 7% while the Haemonetics Cell Saver 5 had a significantly lower Hct than all the rest (46 ± 4.6%, \( p < 0.05 \)). Also the Autolog had a significantly higher Hct than both the BRAT and the Sequestra (Figure 1).

Table 1. Number of patients included in the study and the actual number of processes done with each cell saver.

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Processes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequestra</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>BRAT 2</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>Haemonetics</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>Autolog</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>CATS</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

When looking at the percentage of RBCs recovered using the equation:

\[
\text{RBC Recovery} = \left( \frac{V_{HB} \times \text{Hct}_{HB}}{V_R \times \text{Hct}_R} \right) \times 100\%
\]

\[
\text{Hct}_{HB} = \text{Holding Bag hematocrit}
\]

\[
V_R = \text{Reservoir Volume}
\]

\[
\text{Hct}_R = \text{Reservoir hematocrit}
\]

a totally different picture arises (Figure 2). Haemonetics has a significantly higher RBC recovery than the CATS, Autolog or Sequestra (94 ± 16%, \( p < 0.05 \)) while the BRAT 2 had a significantly higher RBC recovery than the Autolog and Sequestra (94 ± 18%, \( p < 0.05 \)).

Processing Times and Volumes:
The processing times and volumes are summarized in Table 4. Since the CATS system is a continuous system, only the devices that use bowl technology were used in this comparison. The Sequestra and BRAT 2 had the longest process times at 6.1 ± 0.7 min and 6.1 ± 2.1 min respectively (\( p < 0.05 \)). There was no significant difference between the Haemonetics and the Autolog. The Haemonetics had the lowest processing volumes of all the devices (437 ± 114 ml, \( p < 0.02 \)).

RBC Mass Recovery Rate:
To compare all the autotransfusion machines the RBC mass recovery rate was used. This was calculated by dividing the volume of RBCs recovered by the time it took to process the RBCs as expressed in the following equation:

\[
\text{RBC Mass Recovery} = \frac{V_{HB} \times \text{Hct}_{HB}}{\text{Process Time}}
\]

Where:
- \( V_{HB} \) = Volume of RBCs in the holding bag.
- \( \text{Hct}_{HB} \) = Hct of the blood in the holding bag.

The calculated RBC mass processing rate, as depicted in Figure 3, shows that the Haemonetics had a significantly higher RBC recovery rate than that of all the other devices (22 ± 7 ml/min, \( p < 0.05 \)) while the Sequestra had the poorest recovery rate (10 ± 2 ml/min, \( p < 0.05 \)). The Autolog had a significantly higher recovery rate than the BRAT 2 and CATS (18 ± 5 ml/min vs. 14 ± 5 and 14 ± 5 ml/min respectively, \( p < 0.05 \)).

Quality of Wash
The Quality of the washed blood was assessed by calculating the percent removal of the contaminants of leukocytes, platelets, plasma free hemoglobin, heparin and potassium using the following equation:
% Removal = \left[1 - \frac{V_{HB} \times [\text{Sub}_{HB}]}{V_{R} \times [\text{Sub}_{R}]}\right] \times 100%

where: 
- \(V_{HB}\) = Volume in the Holding Bag
- \([\text{Sub}_{HB}]\) = Concentration of substance in the Holding Bag
- \(V_{R}\) = Processed volume in the reservoir
- \([\text{Sub}_{R}]\) = Concentration of substance in the reservoir

Results are summarized in Table 5.

**Heparin Removal:** All cell savers removed heparin adequately with over 98% removal (Figure 4). However, the Haemonetics had the greatest heparin removal with 99% ± 6% (\(p < 0.05\) vs. all).

**Potassium Removal:** There were no statistically significant differences between the groups for potassium removal. (Figure 5).

**Plasma Free Hemoglobin (PFH):** The Autolog had the highest PFH removal of all the autotransfusion devices (92% ± 4, \(p < 0.05\)) (Figure 6). The Sequestra had a significantly higher removal than the BRAT 2 (\(p < 0.05\)). The PFH removal of the CATS was very inconsistent as reflected by the very large standard deviations.

**Platelet Removal:** As shown in Figure 7 the Autolog had a significantly higher platelet removal than all the other devices (99% ± 1%, \(p < 0.05\)) while the platelet removal of the BRAT 2 was significantly lower than any of the other machines (68% ± 20%, \(p < 0.05\)).

**Leukocyte Removal:** All autotransfusion devices did a very poor job of removing leukocytes (Figure 8).

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**Table 2.** Summary of complete blood count for all cell savers.

<table>
<thead>
<tr>
<th></th>
<th>Pre Hct (%)</th>
<th>Post Hct (%)</th>
<th>Pre Plt ((\times 10^9/L))</th>
<th>Post Plt ((\times 10^9/L))</th>
<th>Pre WBC ((\times 10^9/L))</th>
<th>Post WBC ((\times 10^9/L))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequestra</td>
<td>15 ± 3.7</td>
<td>57 ± 6.1</td>
<td>65.1 ± 47.2</td>
<td>24.7 ± 44.2</td>
<td>4.27 ± 1.52</td>
<td>7.1 ± 2.9</td>
</tr>
<tr>
<td>BRAT 2</td>
<td>15 ± 3.8</td>
<td>54 ± 8.0</td>
<td>79.9 ± 28.4</td>
<td>89.2 ± 70.9</td>
<td>5.98 ± 3.37</td>
<td>15.1 ± 5.45</td>
</tr>
<tr>
<td>CATS</td>
<td>14 ± 4.6</td>
<td>66 ± 7.0</td>
<td>67.6 ± 38.3</td>
<td>28.0 ± 25.6</td>
<td>3.37 ± 1.23</td>
<td>9.7 ± 4.2</td>
</tr>
<tr>
<td>Haemonetics</td>
<td>15 ± 4.2</td>
<td>46 ± 4.6</td>
<td>63.9 ± 27.7</td>
<td>24.6 ± 21.0</td>
<td>3.71 ± 1.41</td>
<td>8.1 ± 3.4</td>
</tr>
<tr>
<td>Autolog</td>
<td>17 ± 2.9</td>
<td>62 ± 2.2</td>
<td>61.2 ± 22.8</td>
<td>3.7 ± 2.1</td>
<td>4.78 ± 1.5</td>
<td>4.9 ± 2.7</td>
</tr>
</tbody>
</table>

**Table 3.** Summary of heparin, potassium, and plasma-free hemoglobin in the reservoir (pre) and holding bag (post) of the various cell savers.

<table>
<thead>
<tr>
<th></th>
<th>Pre Heparin (units/mL)</th>
<th>Post Heparin (units/mL)</th>
<th>Pre K+ (mmol/L)</th>
<th>Post K+ (mmol/L)</th>
<th>Pre PFH (mg/L)</th>
<th>Post PFH (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequestra</td>
<td>11.8 ± 5.0</td>
<td>0.61 ± 0.36</td>
<td>4.7 ± 1.4</td>
<td>1.7 ± 0.31</td>
<td>3078 ± 1508</td>
<td>1476 ± 793</td>
</tr>
<tr>
<td>BRAT 2</td>
<td>11.4 ± 6.2</td>
<td>0.80 ± 0.65</td>
<td>5.2 ± 2.0</td>
<td>2.0 ± 0.71</td>
<td>1788 ± 1288</td>
<td>3093 ± 2803</td>
</tr>
<tr>
<td>CATS</td>
<td>11.5 ± 7.9</td>
<td>0.73 ± 0.86</td>
<td>4.9 ± 2.2</td>
<td>2.6 ± 0.47</td>
<td>2322 ± 1031</td>
<td>2942 ± 2716</td>
</tr>
<tr>
<td>Haemonetics</td>
<td>13.0 ± 11.5</td>
<td>0.19 ± 0.17</td>
<td>4.9 ± 1.5</td>
<td>1.4 ± 0.39</td>
<td>2386 ± 952</td>
<td>1224 ± 797</td>
</tr>
<tr>
<td>Autolog</td>
<td>7.75 ± 5.0</td>
<td>0.39 ± 0.33</td>
<td>5.6 ± 1.8</td>
<td>2.2 ± 0.15</td>
<td>3640 ± 1341</td>
<td>1324 ± 466</td>
</tr>
</tbody>
</table>

**Figure 1.** Cell saver comparison of hematocrit before and after final processing.

**Figure 2.** Comparison of the red blood cell recovery.

**Table 4.** Processing times, reservoir, and final RBC volumes.

<table>
<thead>
<tr>
<th></th>
<th>Reservoir (mL)</th>
<th>RBCs (mL)</th>
<th>Process Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Sequestra</td>
<td>539 ± 121</td>
<td>105 ± 27</td>
<td>6.1 ± 0.7</td>
</tr>
<tr>
<td>BRAT 2</td>
<td>649 ± 283</td>
<td>146 ± 4.6</td>
<td>6.1 ± 2.1</td>
</tr>
<tr>
<td>Haemonetics</td>
<td>437 ± 114</td>
<td>123 ± 3.0</td>
<td>4.4 ± 1.7</td>
</tr>
<tr>
<td>Autolog</td>
<td>548 ± 148</td>
<td>110 ± 17</td>
<td>3.8 ± 0.7</td>
</tr>
<tr>
<td>CATS</td>
<td>1213 ± 543</td>
<td>241 ± 151</td>
<td>10 ± 3.0</td>
</tr>
</tbody>
</table>
Autolog was significantly better than the rest with 78% ± 11% (p < 0.05) followed by the Sequestra with 66% ± 14% (p < 0.05 compared to CATS, BRAT 2 and Haemonetics). The BRAT 2 had the worst removal with 30 ± 26% (p < 0.05).

DISCUSSION

While all cell saving devices use the same theory of centrifugation and washing this study clearly shows that the actual RBC product produced by the different cell savers varies significantly from one device to another. The hematocrit value of blood salvaged from cases in this study varied from values as low as 10% with an average of approximately 15%. The first challenge for effective blood salvage is to raise the hematocrit value to an acceptable
level. By centrifugation we hope to reach hematocrit levels above 50–55%. All of the cell savers in this study easily reached this goal with the exception of the Haemonetics Cell Saver 5, which had an average hematocrit of 45%. Surprisingly, when you take into account the total RBC recovery the Haemonetics Cell Saver 5 had the highest recovery even though it had the lowest hematocrit. This is a very significant clinical finding that for the most part is ignored when comparing or evaluating cell savers. Thus inferring that the hematocrit is of little significance compared to the amount of RBCs that are recovered. Ideally all cell savers should be able to recover 100% of the RBCs with no wastage, however, the BRAT 2 and more consistently the Cell Saver 5 were the only cell savers that came close to this at approximately 94%. Therefore a lower hematocrit is clinically insignificant as long as you are retrieving most of the RBCs.

Active suctioning and air to blood interfaces can cause a high degree of hemolysis. Whole blood with hemolysed RBCs has very high levels of PFH, potassium, protein and lipid content. Electrolytes and proteins, especially potassium and albumin, are good markers or indicators of washing efficiency with at least 90% removal constituting an effective wash. Potassium concentration, a simple, rapid and reliable assay, has been advocated as a marker of washing adequacy. Values below 2 mEq/L are held to reflect sufficient cleansing (2,5) and all the autotransfusion devices in this study stayed within this range removing 90% of the potassium.

An index of hemolysis in the blood salvaged within the cardiotomy reservoir is PFH levels. PFH were higher than 400 mg/dL in some of the cardiac cases in this study and may be several times this level in orthopedic cases. Adequate washing should remove 90–98% of this free plasma hemoglobin (6). Only the Autolog consistently removed over 90% of the PFH in this study with the poorest removal being the BRAT 2 at 63%. This is surprising since the Autolog only has a wash volume of 250 mL of saline while the BRAT 2 has a wash volume of 1000 ml. The biggest difference between the two systems is the configuration of the cell washing system. The Autolog pulses the wash and has two indentations on the side of the bowl to increase the turbulence and improve mixing and washing of the blood.

Another important indicator of how adequately the salvaged RBCs are washed is the platelets and leukocytes. A safe cell salvage product should remove most of these contaminants. Again, in this study, the Autolog had the best platelet and leukocyte removal while the BRAT 2 had the worst. Although the Autolog had a significantly higher leukocyte removal that of the other cell savers it was still inadequate (approximately 78%). Bull et al. claim that in rare circumstances, activation of leukocytes and platelets trapped on the inner wall of the disposable centrifuge chamber can release leukoattractant and thromboplastic materials, which result in a systemic syndrome called “the salvaged blood syndrome” (7). This syndrome involves elements of disseminated intravascular coagulation, capillary leak, and adult respiratory distress syndrome. Activated leukocytes circulating throughout the body can cause great tissue damage when they enter organs. Upregulation of adhesion receptors can increase their binding to endothelial cells and migration into extra-vascular tissues leading to edema. Activated leukocytes also release their granules, which contain destructive elements such as oxygen free radicals, elastase, and cathepsin G. The later of which are capable of degrading fibrin, potentially contributing to the instability of newly formed clots (8). There is still little evidence that leukocyte depletion of the cell-salvaged product is of clinical benefit (9), however, at this institution leukocyte/lipid reducing filters have been adopted as a standard of care.

The degree of heparin washout should also be monitored during cell salvaging. More than 95% heparin washout is expected (6). Umlas et al., have shown that the washed, concentrated salvaged blood should have heparin levels of less than 0.2 to 0.4 international units per ml of supernatant fluid after washing (10). However this study has shown levels to vary from a mean of 0.19 to 0.80 U/ml. This still is an adequate washout considering that all cell savers removed approximately 99% of the heparin.

In conclusion, this study has shown that the variation in process between the different types of cell saving devices also results in drastic variations in the quality of concentrated RBC product. This is an important finding that the autotransfusionist must consider when providing adequate patient care.

REFERENCES

*Figure 8. Percentage of leukocytes removed during the wash cycle.*


