Quality of Processed Blood for Autotransfusion

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ABSTRACT

Centrifugal red blood cell washers for intraoperative autotransfusion process shed blood during surgery. In this study, the quality of processed fresh, human bank blood was assessed in a standardized laboratory setting during standard, medium, and high flow processing. Red cell recovery rates and plasma washout efficiencies were compared using three different devices. The accurate parameters measuring effectiveness and product quality were red cell mass (RCM) flow rate and the plasma washout efficiency. Cobe BRAT 2, a system with discontinuous flow (DF) and a cylindrical centrifuge bowl, permitted processing in standard and medium flow of 26 and 35 mL RCM/min, respectively, with washout of residual plasma albumin of 93.2 and 91.2%. The Medtronic Sequestra 1000, a DF system with a conical centrifuge bowl processed blood at 15 and 23 mL RCM/min and eliminated plasma albumin with 98.4 and 96.8% washout during standard and medium flow, respectively, with significant red cell loss occurring during medium flow. The respective speeds of high-flow programs with BRAT 2 and Sequestra 1000 were 15 and 22 mL RCM/min, related to a hematocrit in the holding bag, less than that of the incoming blood from the reservoir. Washout was 58.2 and 58.3%, respectively. Fresenius CATS, a continuous flow (CF) device, produced flow rates of 19, 24, and 43 mL RCM/min and plasma albumin elimination of 97.8, 94.4, and 93.3% in standard, medium, and high-flow, respectively. Holding bag hematocrits with CF exceeded that of DF. Standard, medium, and high-flow programs of CATS may be used without restriction.

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INTRODUCTION

The red blood cell recovery rate and washing efficiency of centrifugal devices used for intraoperative autologous transfusion (IAT) have an impact on the safety, purity, and potency of the processed product. Standard programs of most IAT devices have shown satisfactory performance during vascular surgery (1), cardiothoracic surgery (2), and major orthopedic surgery (3–7). However, accurate data on processing at high speeds are limited. The operating principles of centrifugal equipment are based either on discontinuous (DF) or continuous flow (CF). DF devices employ conical or cylindrical bowl geometries, which may affect results of the washing procedures. Manufacturers provide defined preprogrammed washing procedures with various washing speeds. In addition, operator-defined options are available, allowing for alteration of processing parameters of centrifuge speed and flow rates. Although changes that may affect the characteristics of the blood product are indicated, no exact data are provided.

The goal of this study is to perform a well-defined laboratory comparison of the processing speed and quality of the washed red cell product of three autotransfusion devices, each with operating principles based on different technology. Three speeds were studied in standard, medium, and high flow programs using nondistributable donor red cells reconstituted with compatible plasma and at three dilutions with saline, simulating conditions in the operating room.

MATERIALS AND METHODS

Washing programs were based on an ordinary requirement (standard flow), rapid (medium flow), and a very rapid (high flow), each representative of routine, rapidly processed, and emergency methods, respectively. The Cobe BRAT 2\textsuperscript{a}, the Fresenius CATS\textsuperscript{b}, and the Medtronic Sequestra 1000\textsuperscript{c} were employed during this study. Manufacturer-specified programs for DF using Sequestra 1000 with a conical centrifuge bowl and CATS with CF were selected as corresponding standard, medium, and high flow programs. The programs for comparable function on BRAT 2 with DF and a cylindrical centrifuge bowl were the standard flow program as specified by the manufacturer, medium and high flow are available as operator-defined procedures (Table 1).

Compatible fresh frozen plasma and nondistributable donor packed red cells, less than 5 days from the time of collection, were pooled to achieve an hematocrit (HCT) of approximately 40%. To simulate blood collected from a surgical field, an adjustment was made with normal saline to dilute pools with target HCT of approximately 30, 20, and 10%, each confirmed before processing. After thorough mixing, the pool was divided into separate bags and used as blood reservoirs. The devices were used with the same disposable sets for each respective instrument in the three corresponding programs. Variability between individual lots are not expected to influence test results significantly because of the known high reproducibility of the manufacturing process involved. A fresh portion of the identical blood pool was processed on each device in three separate consecutive runs in the automatic modes. The processing times using the 250-mL bowl with BRAT 2 and the 225-mL bowl with Sequestra 1000 were recorded each for one completely filled bowl until the system stopped automatically; residual packed red blood cells in the emptying lines to the holding bags were transferred by activating the “Empty” routine twice more. The processing time with CATS was measured during accumulation of an equivalent volume of 225 mL packed red cells in the holding bag as measured by digital display on the instrument’s control panel. To be consistent, the residual red cell volume in the emptying line to the holding bag was retained in the line. Volumes of blood from the reservoir and packed red cells in the holding bag were calculated from weights before and after processing. The weight differences were divided by densities according to measured HCT levels (8), respectively, to calculate volumes of blood drawn from reservoirs and packed red cells collected into holding bags. Volumes of five mL blood each were sampled from the reservoirs before and from holding bags after processing for CBC with the MAXM\textsuperscript{c} blood cell counter and tested within 2 hours. Similarly, separate samples were collected, the centrifuged supernatant separated and frozen at −30°C, pending batch analysis of plasma albumin measured immunochemically with the Behring BN Laser Nephelometer\textsuperscript{d} as the indicator for plasma washout.

Processing speeds, red cell recovery, rates of albumin elimination, and residual albumin transfusion were calculated as follows:

\[
\text{blood flow rate from reservoir (mL/min)} = \frac{\text{blood volume processed}}{\text{processing time}}
\]

\[
\text{PRBC flow rate into holding bag (mL/min)} = \frac{\text{Volume of PRBC}}{\text{processing time}}
\]

\[
\text{RCM flow rate into holding bag (mL/min)} = \frac{\text{Volume PRBC} \times \text{HCT}_{\text{PRBC}}}{\text{processing time}}
\]

\[
\text{RBC recovery rate in the holding bag (efficiency %)} = \frac{\text{PRBC volume} \times \text{HCT}_{\text{PRBC}}}{\text{Blood volume} \times \text{HCT}_{\text{blood}}} \times 100
\]

\textsuperscript{a} Cobe Laboratories, Inc., Arvada, CO 80004
\textsuperscript{b} Medtronic Inc., Parker, CO 80134
\textsuperscript{c} Fresenius Hemotechnology, Inc., Concord, CA 94520
\textsuperscript{d} Coulter, Miami, FL
\textsuperscript{e} Dade-Behring, Deerfield, IL
Albumin elimination rate (~\%)

\[
\frac{\text{Total Albumin}_{\text{PRBC}} - \text{Total Albumin}_{\text{blood}}}{\text{Wash volume (mL)}} = F
\]

*Volume PRBC* \(\times (1 - \text{HCT}_{\text{PRBC}})*\text{Albumin}_{\text{PRBC}}

Residual Albumin transfusion rate (mg/dL RCM)

\[
\frac{\text{Algorithm}_{\text{PRBC}} \times (1 - \text{HCT}_{\text{PRBC}})}{\text{HCT}_{\text{PRBC}}}
\]

Mean values and standard deviations were calculated for three replicates of each parameter and program based on three runs each using blood with a HCT of 10, 20, and 30\% \((N = 9)\). This represented the range of applications encountered clinically.

**STATISTICS**

Student’s t-test for unpaired data was used and probability of \(p < .05\) was considered significant. Statistically significant differences are indicated by the symbols °* | in the tables and figures for corresponding programs.

**RESULTS**

The differences between DF and CF are shown in Figure 1. In CF, blood from the reservoir was pumped into a rotating horizontal wash chamber, C-shaped like a spiral, hollow band. First, the red blood cells were concentrated on the outer aspect of the channel, then became resuspended in saline, reconcentrated on the outer aspect, and then transferred to the holding bag in a continuous flow of blood through the system. Plasma and saline wash waste were diverted to the waste bag.
HCT of the processed packed red cells was found to be consistently greatest in all CF (CATS) programs as compared with values obtained with the cylindrical DF (BRAT2) or the conical DF (Sequestra 1000) bowl systems. This was independent of the processing speed and HCT of the incoming blood. In DF, the HCT of packed red cells in the holding bag decreased progressively with greater processing speeds and with a greater HCT of the incoming blood (Table 2). High flow with BRAT 2 and Sequestra 1000 resulted in HCT even less than that of the blood in the reservoir before processing. As the speed was increased during processing with both BRAT 2 and Sequestra 1000, the volume of saline used per mL RCM became increased because of an insufficient red cell content in the bowls. However, with CATS, the volume of saline used per mL RCM decreased significantly (Table 3).

Apart from the medium flow program of Sequestra 1000, the range of red blood cell recovery rates was between 94 and 98% using all devices in all programs (Figure 2). During medium flow on Sequestra 1000, visible red cell loss to waste occurred with a diminished recovery rate of 86%. This was because of formation of an inverted saucer-shaped layer of red cells at the level of the rotating seal (Figure 3), caused by pressure diversion from the inlet port directly to the waste outlet. Red cell flow bypassed separation in the centrifugal field because of pressure buildup from force developed by rapid pump speeds once the bowl capacity was filled to about one-third.

In DF, processing reservoir samples with different HCT levels resulted in variation of the red cell mass flow rate into the holding bags (Table 4). Apart from incoming blood from the reservoir with high flow and an HCT of 10%, the red cell mass flow rate with CATS was found to be constant. The red cell mass flow rate into the holding bag increased progressively for all three devices from standard to medium flow. However, CATS high flow continued to show further increase in red cell mass flow rate, but both DF instruments showed a marked decline \( p < .0001 \). The flow rates with high flow for both BRAT 2 and Sequestra 1000 were less than those of standard...
flow because of premature triggering of the optical sensors by unseparated red blood cells. Wash cycles were initiated before filling the bowl properly, and the red cells were diluted further with saline wash solution. The CATS red cell mass high flow was 23% greater than the fastest program with the BRAT 2 (medium flow) and 87% greater than the fastest program of Sequestra 1000 (medium flow). Packed red blood cell flow rates proved to be misleading and erroneously indicated the greatest processing speed with DF in the high-flow programs (Table 5).

There was no visible hemolysis on naked eye examination of the supernatants; however, samples from the BRAT 2 with its cylindrical centrifuge bowl had a pale yellow color consistent with residual plasma. The depth of color increased with greater processing speeds. The washout efficiency was evaluated by comparing initial plasma albumin contents of the reservoir blood supernatant volumes before processing with those of corresponding packed red blood cells in the holding bags after processing. The supernatant residual albumin concentrations of samples taken from the holding bags after processing (packed red cells) are summarized in Table 6. Residual albumin concentration of supernatants in the holding bags increased progressively with greater processing speeds using all devices. Residual albumin concentrations using BRAT 2 was greatest in all three flow programs. In standard flow, comparison of the average albumin elimination rate with CATS and Sequestra 1000 showed no difference (Table 7). Both were greater than those of BRAT 2 ($p < .05$). In medium flow, the average albumin elimination rate with BRAT 2 was less than Sequestra 1000 ($p < .05$). In high flow, CATS was greater than both Sequestra 1000 and BRAT 2 ($p < .0001$). In all systems and programs, the albumin elimination rate decreased with an increase in HCT of the incoming blood. The mean albumin transfusion rates (Figure 4) in standard, medium, and high flow, respectively, with BRAT 2 were $447 \pm 340$ mg/dL, $604 \pm 514$ mg/dL, and $2,738 \pm 167$ mg/dL; with CATS were $90 \pm 44$ mg/dL, $337 \pm 284$ mg/dL, and $2,277 \pm 253$ mg/dL; and with Sequestra 1000 were $69 \pm 36$ mg/dL, $217 \pm 125$ mg/dL, and $2,277 \pm 253$ mg/dL. Compared with CATS and Sequestra 1000, residual albumin transfusion rates for BRAT 2 ranged up to six times greater in standard flow programs ($p < .01$) and up to three times greater in medium flow ($p < .01$). In high flow, the albumin transfusion rate of BRAT 2 and Sequestra 1000 were up to eight times greater as compared to CATS ($p < .0001$).

**DISCUSSION**

In confirmation of the value of intraoperative autologous transfusion practice, post-transfusion survival of salvaged red

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**Table 3. Saline volumes per red cell mass processed (mean ± standard deviation; mL saline/mL RCM processed) for three autotransfusion devices using three programs with three replicate procedures in each**

<table>
<thead>
<tr>
<th>Reservoir</th>
<th>Cobe BRAT 2</th>
<th>Fresenius CATS</th>
<th>Medtronic Sequestra</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stand</td>
<td>Med</td>
<td>High</td>
</tr>
<tr>
<td>Average All</td>
<td>8.9 ± 0.4</td>
<td>9.8 ± 0.8</td>
<td>30.1 ± 12.5</td>
</tr>
<tr>
<td>N = 9</td>
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</table>

$^* = p < .05$ between corresponding programs.
blood cells shed during surgery was studied; the radio-labeled chromium half-lives of shed red cells were reported previously to be comparable with normal values (9). In this report, quality of processed blood shed during surgery was evaluated in a defined environment using a single pool of donor banked blood to minimize variation for comparing test results. Red cells, suspended in freshly thawed, compatible frozen donor plasma were used to simulate operating room conditions in terms of blood viscosity and hemoglobin level. Comparable programs for each of the three systems were evaluated by using the same pool of blood with triplicate testing, showing close reproducibility within each program. Of the parameters studied, processing speed was the greatest factor influencing HCT, red cell recovery, and washout quality. Progressively greater processing speeds with DF devices led to decreased HCT of the packed red cells (PRBC) collected into the holding bag, and removal rates of plasma were reduced with all the devices to a varying degree.

Processed RBC must be prepared with a suitable HCT to affect a proper post-transfusion increment. The standard flow program of BRAT 2 and the standard and medium flow programs of Sequestra 1000 achieved a satisfactory HCT. HCT of the red cell products was inversely related to filling rates and the HCT of shed blood. The medium flow of BRAT 2 and high flow of both BRAT 2 and Sequestra 1000 yielded products with unacceptably low HCT. Because the separation capacity, defined by the radius of the bowl and centrifuge rotations per minute, is constant in all DF washing programs, a slower filling

### Table 4. Red cell mass (RCM) flow rates to the holding bags (mean ± standard deviation; mL/min) for three autotransfusion devices using three programs at reservoir hematocrits of 10, 20, and 30% as well as the means for all hematocrits

<table>
<thead>
<tr>
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</tr>
</thead>
</table>
|                      | Stand       | Med           | High              | Stand       | Med          | High
| 10% 10% 10% N = 3    | 19 ± 0.8    | 29 ± 1.2      | 12 ± 0.6          | 18 ± 0.3    | 23 ± 0.1     | 28 ± 0.4
| 20% 20% 20% N = 3    | 27 ± 0.5    | 37 ± 0.8      | 22 ± 0.0          | 19 ± 0.3    | 25 ± 0.7     | 49 ± 3.5
| 30% 30% 30% N = 3    | 32 ± 0.6    | 40 ± 0.3      | 34 ± 0.1          | 19 ± 1.4    | 24 ± 0.4     | 53 ± 1.7
| Average All N = 9    | 26 ± 5.6    | 35 ± 4.5      | 22 ± 9.0          | 19 ± 1.0    | 24 ± 0.9     | 43 ± 11.3

Average All = three replicate procedures in each program; ⁣|⁣ | | = | | | | | | | p < .05 between corresponding programs.

### Table 5. Packed red blood cell (PRBC) processing rate (mean ± standard deviation; mL/min) for three autotransfusion devices using three replicate procedures in three programs

<table>
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<tr>
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<th>Medtronic Sequestra</th>
</tr>
</thead>
</table>
|                      | Stand       | Med           | High              | Stand       | Med          | High
| Average All N = 9    | 58 ± 15     | 85 ± 17       | 136 ± 6           | 33 ± 2      | 47 ± 5       | 74 ± 21

Average all = means of all reservoir hematocrits; ⁣|⁣ | | = | | | | | | | p < .05 between corresponding programs.

### Table 6. Washed packed red blood cell supernatant albumin concentrations (mean ± standard deviation; mg/dL) for three autotransfusion devices using three programs

<table>
<thead>
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</tr>
</thead>
</table>
|                      | Stand       | Med           | High              | Stand       | Med          | High
| Average All N = 9    | 358 ± 258   | 395 ± 310     | 558 ± 265         | 114 ± 50    | 306 ± 212    | 474 ± 299

Average All = means of all programs with three replicates in each; ⁣|⁣ | | = | | | | | | | p < .05 between corresponding programs.
rate increases the time that the red blood cells settle in the centrifuge bowl, facilitating more concentration. With increased flow and increased HCT, packing of red blood cells is reduced and, at even greater flow, poorly separated red blood cells may trigger optical sensors, falsely detecting the appropriate PRBC filling level in the bowl and initiating a premature wash cycle. Residual air in the bowl is replaced with saline wash, further diluting red cell mass. Improper red blood cell sedimentation in respective centrifuge bowls of DF devices permits excess saline retention, leading to errors with false packed red blood cell flow rates and albumin concentration measurements.

HCT was maintained at a high level during progressively increased processing speeds with CATS (54–59%). As shown in previous clinical studies at standard flow, HCT using CATS was consistently greater than a variety of DF systems (1, 5, 7, 10). HCT was between 55 and 78% using the high flow wash (2–4, 11), also confirmed in the author’s operating room experience. While clinical experience with most DF equipment documented HCT results between 44 and 56% using standard flow (1, 5, 7, 10), no published data were found on the use of medium and high flow.

All laboratory and clinical data reviewed (1, 2, 5, 6) revealed no significant differences in RBC recovery using DF and CATS with standard flow programs. In medium flow of Sequestra 1000, diminished red cell recovery from red cell loss to waste was noted in the effluent line from the bowl, and was also noted in the author’s operating room experience at flow rates higher than 500 mL/min; at 1000 mL/min there was almost complete red cell loss to waste. This observation was not seen with BRAT 2 and may be related to the design of the conical centrifuge bowl.

This study describes the importance of accuracy in reporting the data for red blood cell processing speeds. Packed red blood cell flow rates are invalid because of variability of HCT of samples from the holding bag. A system yielding packed red blood cells with a lower HCT seems to process blood more rapidly, but the RCM flow rate is low, and a salvaged red cell unit of inadequate potency results. The RCM flow rate is the accurate measurement. Although RCM flow increased significantly from standard to medium flow using all three systems evaluated, increased pump speeds with high flow actually failed to compensate for progressive decline in HCT of the packed red blood cells in DF systems. This was attributable to inadequate separation of red cells in the bowl of DF. Using CATS, there was progressive increase in RCM flow rates with greater processing speeds; in contrast the high flow programs of BRAT 2 and Sequestra 1000 showed markedly decreased RCM flow rates. The manufacturers of CATS claim that a constant packed red blood cell flow rate and HCT is related to preprogrammed specification of the various programs. This is substantiated in this study, even with high flow, as in the emergency wash procedure. In CATS, the RCM flow rate to the holding bag remains constant because of automatic varia-

Table 7. Albumin elimination rates (mean ± standard deviation %) for three autotransfusion devices using three programs and three replicate procedures in each at 10, 20, 30%

<table>
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<tbody>
<tr>
<td></td>
<td>Stand</td>
<td>Med</td>
<td>High</td>
</tr>
<tr>
<td>10% N = 3</td>
<td>98.7 ± 0.4</td>
<td>98.4 ± 0.3</td>
<td>59 ± 0.4</td>
</tr>
<tr>
<td>20% N = 3</td>
<td>96.4 ± 2.5</td>
<td>96.1 ± 2.8</td>
<td>74.3 ± 18.2</td>
</tr>
<tr>
<td>30% N = 3</td>
<td>84.5 ± 0.7</td>
<td>79.3 ± 0.5</td>
<td>54.9 ± 2.8</td>
</tr>
<tr>
<td>Average All N = 9</td>
<td>93.2 ± 6.4</td>
<td>91.2 ± 8.7</td>
<td>62.8 ± 3.5</td>
</tr>
</tbody>
</table>

Average all = means for all hematocrits; * p < .05 between corresponding programs.

Figure 4: Mean holding bag supernatant albumin transfusion rates ± standard deviations (mg/dL/RCM) for three replicate processing runs using three autotransfusion devices in three programs; * p < .05; N = 9.
tion of incoming blood flow resulting from instrument adaptation to HCT of the blood from the reservoir. The processing speed with CATS may be modified during operation by selecting a processing program deemed more applicable while good product quality is maintained. In contrast, parameters of DF devices, including blood and saline flow, wash volume, and centrifuge speed, independently may be modified by the operator to increase processing speed. As shown in this study, this may lead to poor PRBC quality, even if used within permissible ranges that are recommended by the manufacturers. Changing processing parameters of DF during operation on quality may necessitate more stringent manufacturer limitations for the ranges of adjustable parameters to prevent transfusion of PRBC with questionable potency and purity.

As an indicator of plasma washout, plasma albumin endogenous in the samples proved to be a suitable analyte, because sampling or hemolysis did not interfere. Immunochemical analysis is relatively inexpensive, readily available in most clinical laboratories, and may be deferred while samples of red cell supernatant are maintained in appropriately frozen storage. Although used by many (1–3, 6–8, 11–13), potassium concentration is unreliable as a marker for washout because of spurious concentration increases from hemolysis or delay in separating supernatant from red cells. In addition, because measurement with the potassium ion selective electrode is optimized for testing in a plasma or serum medium, reproducibility was poor when measuring saline supernatants. Flame photometry, the appropriate analytical method, is very seldom used in clinical laboratories. Free hemoglobin and heparin measurements were also used by many (1–6, 12) but, because of very high dilution requirements for measurement, these methods may be too insensitive for levels encountered in shed blood. Fibrinogen and activated factor XII also were used (6) but may be unreliable markers, because erratic changes in concentration may be associated with incomplete anticoagulation.

All systems showed decreased washing efficiency with greater processing speeds. In contrast with the supernatant albumin concentration, which increased only slightly with greater flow rates, the albumin elimination rates decreased, and residual albumin transfusion rates increased very significantly. This observation was characteristic of DF systems, especially when comparing standard and high flow programs. These findings indicate that contaminating plasma proteins, including activated clotting factors, are transfused in relatively high amounts and could be significant in patients enduring estimated blood losses of one liter and more. The albumin concentration of supernatant in the holding bags is misleading; the albumin elimination and transfusion rates also reflect the variation in HCT of the packed red blood cells and are the proper parameters of quality control. Using DF devices reduced plasma washout with progressively greater processing speed related to a decreased HCT of the packed red blood cells, which reflected improper filling of the bowls. In DF, the phase of major elimination of plasma during red cell processing is when the plasma is separated to waste in a bowl that is filled properly. A respective separation of the plasma effluent during the filling cycle and the wash solution during the wash cycle into separate receptacles confirmed that 75% of plasma-free hemoglobin from shed blood was recovered in the separated plasma, and only 13% was found in the wash waste (9). Thus, the effect of the wash procedure in DF at the higher flow rates is minor and cannot compensate for insufficient plasma separation when bowls are filled incompletely.

The standard and medium flow programs of Sequestra 1000 may result in a satisfactory plasma protein elimination rate from shed blood in all surgical procedures. Using BRAT 2 with standard flow and parameters defined by the manufacturer, elimination of plasma is significantly less, and, in situations with poor blood quality, orthopedic surgery for example, using BRAT 2 may be questionable. In high-flow programs of the DF devices tested, the HCT and elimination of plasma are unacceptably low, and use of DF equipment is discouraged. All programs of CATS consistently yielded red cell products with preferred purity and potency. CATS provided red cell units with the greatest HCT, at the fastest processing speed and with the most elimination of plasma constituents, even when using high flow and may be recommended in all emergency situations without restriction. IAT is recommended as a viable transfusion option in a changing environment where autologous blood transfusion is accepted to be immunologically superior to allogeneic blood products and where costs and problems with the availability of blood products are increasing constantly.

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