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A Part of Sage

Comparison of two different autotransfusion devices: An ex-vivo study

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Abstract

Introduction: During surgery, blood is recovered from the surgical field and the autotransfusion device separates and washes the red blood cells (RBC's) after which these can be retransfused to the patient. Autotransfusion devices differ strongly in separation method and washing settings, which may lead to different RBC recovery rates and removal rates of contaminants. The objective of this study was to compare the autoLog IQ™ (Medtronic) and the Xtra® (LivaNova) in terms of RBC recovery, quality of the processed blood and processing time.

Methods: Human blood was heparinised and processed with both autotransfusion devices according to their standard protocols. Blood samples were taken from the collection reservoir and from the transfusion bag and analyzed for cell count, heparin removal and cell injury.

Results: The RBC recovery rates for both devices was 93%. Heparin was almost completely removed in both devices with >99.9%. The autoLog IQ™ showed significantly better removal of platelets (autoLog IQ vs Xtra; 86.1 ± 2.7 and 78.6 ± 4.4%, $p = 0.01$) and of leukocytes (autoLog IQ vs Xtra; 39.6 ± 2.7 and 13.4 ± 5.7%, $p < 0.001$). No other significant differences were observed in removal rates. The volume of RBC concentrated per minute was faster for the Xtra® (autoLog IQ vs Xtra, 21 ± 3 and 27 ± 2 mL RBC/min, $p = 0.007$).

Conclusions: In this study both the autoLog IQ™ and the Xtra® showed similar RBC recovery rates of 93% and almost all heparin was eliminated. The washing quality of the autoLog IQ™ device appears to be better, with better removal of platelets and leukocytes. Although both the autoLog IQ™ and the Xtra® devices use very different separation techniques and washing protocols, the difference in processing speed and various markers for cell damage in the end product seems trivial.

Keywords

autotransfusion, cardiac surgery, cell saver, CPB, recovery, wash quality

Introduction

The use of an autotransfusion device is a well-known method for blood conservation in cardiothoracic surgery and is strongly recommended to reduce the need for allogeneic red blood cell (RBC) transfusions.[1,2]

During surgery, blood from the surgical field is anticoagulated and aspirated into a reservoir in the autotransfusion device. This recovered blood is then introduced into the spinning bowl of the device. The centrifugal force of the spinning bowl separates the blood components by component density. The more dense RBC's are collected, and the effluent is discarded into the waste bag. These RBCs are then washed with 0.9% saline and collected in a transfusion bag before being reinfused into the patient. After the washing cycle, the autotransfusion device can remove more than 90%

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of the contaminants such as heparin, clotted material, fat, cell fragments as well as platelets, and leukocytes.[3,4]

Most current autotransfusion devices on the market use centrifugal separation followed by a washing process. However, large differences in the design of the bowls and washing settings (i.e.,: filling/washing speed, washing volume) exist. Studies using different autotransfusion devices with different bowl sizes and washing settings have shown differences in measured hemoglobin levels, leukocytes and platelet removal, and other substances in the washed red cells.[4–6]

In the present study, we compared two autotransfusion devices with very different bowl types and washing techniques.

The objective of this ex vivo study was to compare the AutoLog IQ™ and the Xtra® in terms of RBC recovery, quality of the processed blood and processing time.

Materials and methods

Study design and patient blood collection

The evaluation was performed as an ex vivo study. Blood was collected from ten haemochromatosis patients after informed consent (Ethical committee agreement: Z20.143). The phlebotomy bag (Macopharma, Moufaux, France), containing a citrate-dextrose solution, was pre-heparinized with 30 IU/mL (Leo Pharma BV, Amsterdam, The Netherlands). The phlebotomy blood bag was filled with 500 mL blood and was inverted several times to ensure adequate mixing. This blood was immediately used for the following steps.

The reservoir was prepared with NaCl (500 mL) and 50 IU/mL of heparin resulting in a hematocrit ~25% and a heparin concentration of circa 35–45 IU/mL. This to mimic the shed blood that is routinely collected intra- or post-operatively during cardiac surgery.

The blood was processed using the autoLog IQ™ (Medtronic, Minneapolis, MN, USA) or the Xtra® (LivaNova, Mirandola, Italy). Both autotransfusion devices processed blood, obtained from the 10 heamochromatosis patients, five times with five disposable sets. All tests were performed on the same autoLog IQ™ and Xtra® device. Blood samples were taken from the collection reservoir and the transfusion bag (the final/end product). Each sample was repeated three times and then analyzed for red cell recovery and markers of processed blood quality.

The time was recorded for every processing cycle.

Autotransfusion setups

Two autotransfusion devices were tested according to their standard protocols, the autoLog IQ™ and the Xtra®. The autoLog IQ™ uses a very specific and proprietary bowl design, the IntelliPath, and a relatively low wash volume. The Xtra® uses the Latham bowl design and uses relatively more volume for washing the blood cells.

The disposables were set up according to manufacturer's instructions for use (ALT2001 and X/225 bowl kits, respectively), but the same collection reservoir (EL2120, Medtronic), was used in both systems because this reservoir ensured adequate mixing. The EL2120 reservoir creates a funnel at the bottom, which allowed for more consistent hematocrit for this study.

The autoLog IQ™ is equipped with a propriety bowl, called Intellipath with a bowl size 135 mL. It operates at various speeds up to 10,000 r/min using and, according to the manufacturer uses, '*dynamic cell salvage technology with algorithm-driven intelligent blood sensing*'.

The bowl of the autoLog IQ™ is filled in two-stages with adaptive speeds in both stages (600 mL/min and 250 mL/min). During the standard wash cycle, 250 mL saline is pulsed through the bowl.

The Xtra® uses a Latham bowl, which operates at 5600 r/min. We used the standard adult bowl size of 225 mL and the standard default program (Pstd). The Pstd settings are 400 mL/min during the fill/wash stage and a wash volume of 800 mL.

Sample collection

One mL was taken from the collection reservoir, after rigorous mixing and was used for the blood gas analyzer (RapidPoint 500, Siemens Healtheneers, Den Haag, The Netherlands) for Heamoglobin (Hb) level to ensure the correct blood dilution before starting the procedure.

A sample of 10 mL was taken from the collection reservoir as a baseline sample (in). Another 10 mL sample was taken from the transfusion bag at the end of the autotransfusion procedure (out).

Three mL were used for whole blood laboratory tests, 3 mL were collected in an EDTA (ethylenediaminetetraacetic acid) blood collection tube and 3 mL were collected in a citrate (3.2%) tube. The EDTA and citrate blood samples were separated by centrifugation at 1500–2000*g* for 10–15 min. One mL of plasma was collected and stored at −80°C until analysis.

The whole blood samples from the collection reservoir and from the transfusion bag were analyzed by the hospital lab for cell count (Sysmex XN-9000, Sysmex Europe Norderstedt, Germany), including red blood cell count, total Hb, hematocrit (Hct), white blood cell count (WBC) and platelet count (PLT).

Plasma from the EDTA tubes was analyzed for free hemoglobin. Plasma from the citrate tubes were analyzed

for: Inhibition of X_a, potassium, lactate, albumin, glucose, triglycerides, and fatty acids.

Blood analysis

The following variables were analytically measured as follows.

The total and free Hb was determined by the method of Harboe (OD* = 2 × OD415-OD380-OD450) after dilution of the samples 100 times in 0.01% sodium carbonate.[7]

The potassium concentration was determined by the precipitated formation in the presence of cobalt nitrite, sodium nitrate, acetic acid, and ethanol. Precipitation is read at 600 nm in a spectrophotometer.[7]

Lactate was determined by the conversion of lactic acid to pyruvate and H_2O_2, which catalyzes substrate conversion by peroxidase (Diagnostic kit: Trinity Biotech Plc,Wicklow, Ireland) . The purple color was read at 540 nm.

The detection of free fatty acids was measured by Acrylodan labeled Intestinal Fatty Acid Binding Protein (ADIFAB) and is based on a change in the position of acrylodan fluorophore relative to the nonpolar binding pocket of the protein when it becomes occupied by a fatty acid (FFA sciences, San Diego,CA, USA). This shift goes from 432 to 505 nm upon excitation of 386–390 nm.

Triglycerides in the sample were hydrolyzed by lipase to glycerol and fatty acids. The glycerol was phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate(G3P) and adenosine-5-diphosphate in a reaction catalyzed by glycerol kinase (GK). Glycerol-3-phosphate was then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide by glycerophosphate oxidase (GPO). The hydrogen peroxide then reacted with 4-aminoantipyrine(4-AAP) and 3-hydroxy-2,4,6-tribomobensoic acid (TBHB) in a reaction catalyzed by peroxidase to yield a red-colored quinoneimine dye. The intensity of the color produced is directly proportional to the concentration of triglycerides in the sample when measured at 540 nm. The reagents used for this test were obtained from Biolabo SAS (Maizy, France).

Albumin was determined with the bromocresol green method.[8]

Glucose was determined by the conversion of glucose to D-glucono-1,5-lactone and H_2O_2, which catalyzes substrate conversion by peroxidase.[9] The color of OPD substrate is measured at 490 nm.

The inhibition of factor Xa was done to measure the effectiveness of the residual heparin to inhibit conversion of substrate S2222.[10]

Calculations

Calculations for the RBC recovery rate (RR) were done using this formula:

$$RR\% = 100\left[1 - \frac{V_{out} \times (Hct_{out})}{V_{in} \times (Hct_{in})}\right]$$

Formula 1: Recovery rate (RR); V_{out} = volume RBC bag; Hct_{out} = Hematocrit RBC bag; V_{in} = volume used to fill bowl; Hct $_{in}$ = hematocrit reservoir.

The removal rate was calculated according to Formula 2:

$$RR\% = 1 - \left[\frac{V_{out} \times \{c\}out}{V_{in} \times \{c\}in}\right] * 100\%$$

Formula 2: Removal Rate (ER); V_{out} = volume RBC bag; $\{c\}_{out}$ = concentration component in RBC bag; V_{in} = volume used to fill bowl; $\{c\}_{in}$ = concentration component in reservoir.

In both the recovery and removal rate calculations, the volume out (RBC bag) was taken to be the volume of the bowl sizes for the autoLog 135 mL and for the Xtra 225 mL. The volume used to fill the bowl (in) was used according to the volumes calculated by the devices.

RBC volume (mL) out was calculated with Formula 3:

$$\text{RBC volume} = (Hct_{out} * V_{bowl})/100$$

Formula 3: Hct out = Hematocrit RBC bag; V bowl = bowl size.

Processing time

The processing time was measured from the start of the cell saver filling process until the entire autotransfusion process was completed and the final product was in the transfusion bag.

Statistical analysis

Quantitative variables are presented as mean ± standard deviation (SD) or as median with interquartile ranges (IQR) where appropriate. Prior to analysis, data were tested for distribution using the Kolmogorov–Smirnov goodness of fit test. Student's t-test or Mann-Whitney test was used when appropriate for comparisons between two groups. Statistical analysis was performed with SPSS 22.0 (SPSS, Chicago, IL, USA).

Results

In our analysis, we excluded one set from the autoLog IQ^{TM}, due to the unreliability of data obtained. This set

had a RBC recovery rate of only 58%, while the other sets had an average of 93%. We concluded that there was probably a blood collection error and all the results from this set were discarded.

Table 1, shows the settings and procedural data of the two autotransfusion devices are displayed. The final RBC volume of the end-product differed significantly between both devices; autoLog IQ™ versus Xtra®, respectively 81 ± 8 and 130 ± 5 mL (p = 0.011). The processing time of one bowl was faster for the autoLog IQ™ 60 s compared to the Xtra® (224 ± 6 vs 284 ± 17 s, respectively; $p < 0.001$). However, the volume of RBC concentrated per minute was for the Xtra faster (AutoLog IQ vs Xtra, 21 ± 3 and 27 ± 2 mL RBC/min, p = 0.007).

In Table 2, the recovery rate of RBC's for both devices is shown. No significant difference was detected and both devices showed a recovery rate of 93%.

The removal rates of various contaminants are shown in Table 3. Anti-X_a, an indirect measure for heparin concentration, was almost completely removed in both devices with >99.9% and a significant difference was observed (p = 0.03). The concentration of residual heparin in the transfusion bag was 0.05 ± 0.2 and 0.12 ± 0.1 IU/mL (respectively autoLog IQ and Xtra, p = 0.18).

The autoLog IQ™ showed significantly better removal of platelets (autoLog IQ vs Xtra; 86.1 ± 2.7 and 78.6 ± 4.4%, p = 0.01) and of leukocytes (autoLog IQ vs Xtra; 39.6 ± 2.7 and 13.4 ± 5.7%, $p < 0.001$). No other significant differences were observed. The removal rates of both albumin and of triglycerides were >90% for both devices.

Table 1. Settings of the autotransfusion devices and procedure data.

	autoLog IQ™ (n = 4)	Xtra® (n = 5)
Protocol settings		
Wash volume (mL)	250	800
Fill speed (mL/min)	600/250	400
Bowl volume (mL)	135	225
Procedure data	(n = 4)	(n = 5)
Vin (mL)	454 ± 3*	622 ± 44*
RBC volume (mL)	81 ± 8*	130 ± 5*
Time* (s)	224 ± 6*	284 ± 17*
mL RBC/min	21 ± 3*	27 ± 2*

Data expressed as mean ± SD. * Significant difference $p < 0.05$ (V_{in} = volume used to fill bowl; RBC = red blood cells).

Discussion

The aim of this ex-vivo study was to compare two autotransfusion devices, the autoLog IQ™ and the Xtra® by processing human blood using their standard washing protocols. Both devices produced very comparable end products with a Hematocrit of 55–60% with RBC recovery rates of 93%. The removal rate of heparin was >99.9% for both devices, meaning that there was almost no residual heparin present in the transfusion bag, the end-product, for both autotransfusion devices. Platelets and leukocytes were removed significantly better with the autoLog IQ™ device.

A fundamental part of autotransfusion is the concentration of red blood cells. Both devices recovered 93% of the collected erythrocytes. Overdevest et al.[5] showed exactly the same recovery rate for the Xtra® in cardiac procedures as we found in our ex-vivo study. Another study showed a recovery rate for of only 78.6% in the autoLog device.[4] However, this publication is from 2002. In 2016, a different proprietary washing algorithm, called autoLog IQ™, was introduced and this updated blood process may be the reason for the better recovery rates.

The recovery of 93% of red cells in this ex-vivo setting can be considered as a good recovery rate. As far as the authors' knowledge, higher recovery rates for autotransfusion devices have not been reported.

Residual heparin (as indirectly measured by anti X_a) was almost completely removed with a removal rate of >99.9% of heparin in both devices. Both devices adhere to a threshold of 0.5 IU/mL, which is considered as clinically insignificant,[11] with a maximum heparin concentration of 0.12 IU/mL meaning that retransfusion of heparin via the RBC bag does not occur.

The almost complete removal of heparin is in consistent with previous studies as described in a review by Frank et al.[12] A more recent pilot study[13,14] showed in a clinical setting that heparin was eliminated for at least 96%. They showed that, the Xtra® and autoLog IQ™, among others, eliminated heparin from 3.6 to 3.8 IU/mL in the circulating blood to 0.10–0.15 IU/mL in the RBC bag. This result is supported by Vieira et al. who showed in a clinical setting that the Xtra® eliminated the level of heparin to ≤0.1 IU/mL.[14]

In addition to RBC recovery and heparin removal, the quality of the end product, the RBC bag, is also determined by the removal of contaminants. Both platelets and leukocytes are considered as markers of wash quality. During cardiopulmonary bypass (CPB), platelets and leukocytes are activated by the inflammatory response.[15] Platelet function is further impaired by the shear stress caused by the centrifugal forces used during blood processing.[16] In our study we showed that the autoLog IQ™ removed significantly more platelets and leukocytes than the Xtra® device. However, the absolute difference in platelet removal of 7% was small (86.1 ±

Table 2. Recovery rate of RBC in both autotransfusion devices.

	Hct in (%)	Hct out (%)	RBC recovery rate (%)
autoLog IQ™ (n = 4)	19 ± 1*	60 ± 7	93 ± 8
Xtra® (n = 5)	22 ± 2*	56 ± 2	93 ± 4

Data expressed as mean ± SD. * Significant difference p = 0.02 (Hct = hematocrit; RBC = red blood cells).

Table 3. Total amount In and Out and removal rates of contaminants in both autotransfusion devices.

	autoLog IQ™ (n = 4)	Xtra® (n = 5)
Anti X_a		
In (IU)	172676 ± 8321	27106 ± 8983
Out (IU)	6 ± 3	27 ± 21
Removal rate (%)	100.0 ± 0.0*	99.9 ± 0.0*
Platelets		
In ($\times 10^9$)	34 ± 11	63 ± 2
Out ($\times 10^9$)	5 ± 2*	13 ± 5*
Removal rate (%)	86.1 ± 2.7*	78.6 ± 4.4*
Leukocytes		
In ($\times 10^9$)	1.0 ± 0.4	1.5 ± 0.5
Out ($\times 10^9$)	0.6 ± 0.3*	1.3 ± 0.4*
Removal rate (%)	39.6 ± 2.7*	13.4 ± 5.7*
Lactate		
In (mg)	52.2 ± 30.1	70.7 ± 21.9
Out (mg)	14.5 ± 3.6*	29.9 ± 9.8*
Removal rate (%)	47.8 ± 48.0	57.0 ± 10.8
Free Hb		
In (mg)	29.5 ± 3.6*	39.2 ± 6.1*
Out (mg)	32.4 ± 28.9	31.3 ± 9.3
Removal rate (%)	−23.0 ± 89.4	20.9 ± 18.4
Glucose		
In (mg)	281 ± 102	286 ± 116
Out (mg)	3.2 ± 10.7	13.4 ± 14.6
Removal rate (%)	82.8 ± 35.7	96.9 ± 7.1
Potassium		
In (g)	9.4 ± 2.7	9.7 ± 2.4
Out (g)	4.9 ± 1.0	7.37 ± 1.94
Removal rate (%)	30.3 ± 46.4	22.7 ± 18.9
Albumin		
In (mg)	5519 ± 942*	7559 ± 763*
Out (mg)	214 ± 70*	658 ± 165*
Removal rate (%)	95.3 ± 3.2	91.3 ± 2.4
Triglycerides		
In (mg)	337 ± 194	339 ± 221
Out (mg)	12 ± 7	16 ± 8
Removal rate (%)	92.8 ± 8.6	94.8 ± 1.3
Free Fatty Acids		
In (nMol)	0.77 ± 0.10	0.76 ± 0.23
Out (nMol)	0.87 ± 0.31	1.21 ± 0.53
Removal rate (%)	−7.7 ± 32.9	−116.2 ± 196.0

Data expressed as mean ± SD * Significant difference $p < 0.05$.

2.7 and 78.6 ± 4.4% for the autoLog IQ™ and the Xtra® respectively) and the clinical implications seem questionable. The absolute difference of 27% in leukocyte removal rates of leukocytes between the two devices (autoLog IQ vs Xtra; 39.6 ± 2.7 and 13.4 ± 5.7%) appears significant. Both activated leucocytes and platelets contribute to an adverse effect of CPB. Activated leukocytes produce cytokines and other pro-inflammatory molecules that propagate the inflammatory response.[17] Activated platelets will induce a biological response that not only induces inflammation but causes additional activation of platelets and hemostatic disturbance.[18]

There are conflicting results in the literature regarding platelet and leukocyte removal rates. A study by Overdevest et al.,[19] which only tested the Xtra®, showed a platelet removal of 90–95% and leukocyte removal was between 30 and 40%. In our study we found a leukocyte removal rate of only 13% in the Xtra® device. A 2003 study showed that the autoLog device removed significantly more platelets and leukocytes (99 and 78%, respectively) than our results (86.1 and 39.6% respectively). A study by Amand et al. in a clinical setting with five different autotransfusion devices showed a leukocyte removal rate of 30–55%,[20] which is similar to our results with the autoLog IQ™ (39.6%) but not with the Xtra® (13.4%). During centrifugation, a small layer of platelets and leukocytes, called the buffy coat, is formed between the plasma and the red cells. This small layer may explain the contradictory results. Just a small change in the timing of the separation can result in a more or less of this buffy coat remaining in the final product. Perhaps the aforementioned change in the washing algorithm in the autolog IQ™ in 2016 has caused a small shift in the buffy coat separation.

In our study, there were no significant differences between the two devices in removal rates of various markers of cell damage, free Hb, potassium, lactate, free fatty acids and triglycerides. The negative removal rates for free Hb and free Fatty Acid can very well be explained by release after cell damage. The concentration of free Hb before processing was almost zero, which easily results in a significant increase during centrifugation and dilution in saline. Potassium can simultaneously be released by erythrocyte damage and lactate

will be formed by fermentation. This process occurs quite fast in the absence of organs. Important to note is that no significant differences between both devices were observed.

Glucose, albumin and triglycerides were properly removed between 83 and 97%, which is consistent with the results of other studies.[4,5] We found a free Hb removal rate of −23% in the autoLog IQ™ and 20% in the Xtra® (not significantly different) which seems low compared with other studies. Serrick et al.[5] showed a free Hb removal rate of 63% and 92% in five autotransfusion devices and de Somer et al found in the Xtra® a free Hb removal rate of circa 62%.[6] The discrepancy in the free Hb results may be due to the low concentrations of free Hb we found in the reservoir (0.06 mg/mL) and in the end product (0.1-0.3 mg/mL), while in a recent study a median level of 1 mg/mL during CPB was measured.[21] These low concentrations may be the cause of the unexpected results in % removal calculations, which may also be reflected in the large standard deviations. The low concentration of free Hb may be caused by the controlled blood sampling as used in our study which is in strong contrast with the clinical setting of cardiac surgery and the associated blood damage caused by suction and blood activation.[22]

In the event of major blood loss, the speed of the autotransfusion device in producing the final product can be lifesaving. The Xtra® was faster in producing of RBCs per minute, approximately 6 mL RBC/min, out of a total of 21-27 mL RBC/min, which seems a small difference in the clinical setting. The total number of RBC's per minute for both devices was very comparable to results found in other studies[5,6,19]

Our study has limitations. Our ex-vivo study is not performed in a clinical setting, which means that the blood we used was not exposed to surgical trauma and to the foreign surface area of the extracorporeal circuit. For example, platelet-leukocyte aggregates may form in a clinical setting[23] and these larger products may be better removed by centrifugation during the blood processing. The study is performed with one machine of both type of devices. We chose for this design to reduce the error of the procedure being performed by different machines. Conversely, the device used in our study may have a flaw that would cause the same error to be made over and over again in the autotransfusion procedure.

Conclusions

In this ex-vivo study both the autoLog IQ™ and the Xtra® showed similar RBC recovery rates of 93% and both devices eliminated almost all heparin. The washing quality of the autoLog IQ™ devices appears to be better, with better removal of platelets and leukocytes. Although both the autoLog IQ™ and the Xtra® devices use very different separation techniques and washing protocols, the difference in processing speed and various markers of hemolysis in the end product seems trivial.

Declaration of conflicting interests

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