New transfusion technology allows selective collection of different components via apheresis. This process involves collecting combinations of blood components, including red blood cells (RBC), from a single donation, which can reduce donor exposure and transmission of infectious diseases. Most RBC units used for transfusion are prepared from anticoagulated whole blood. Automated collection of RBC via apheresis can potentially provide a standardized, high-quality product with consistent RBC content. Hemolysis is an important parameter for measuring the quality of RBC. Hemolysis is caused by disruption of the RBC membrane and the subsequent release of hemoglobin (Hb). Hemolysis of RBC can occur during preparation and during storage. The extent of hemolysis is often defined as the percentage of free hemoglobin in relation to the hematocrit (HCT). According to the United States Food and Drug Administration (FDA) guidelines, hemolysis in stored RBC units should not exceed 1%. Recently, the FDA has added that 95% of units should meet quality standards and that the data for these units must show statistical certainty.

To evaluate the impact of the collection procedure on the in vitro quality of red blood cells (RBC), we studied 30 units of apheresis-prepared RBC (ARBC) and 30 units of manually collected RBC (MRBC). We performed assays on day 1 and day 21 of the study, evaluating red cell mass volume (RCM); rate of hemolysis; pH, and levels of sodium, potassium, adenosine triphosphate (ATP), 2,3-diphosphoglycerate (2,3-DPG) and glucose. Eight patients with aplastic anemia received RBC transfusions of both components and their post-transfusion hematocrit (HCT) levels were compared. On day 21, we observed a significant drop of sodium and glucose levels in the ARBC group, compared with the MRBC group (P < .05). ARBC group demonstrated higher RCM that provided significantly higher HCT values to our group of anemic patients (P < .05). Hemolysis was significantly lower in the ARBC group, compared with the MRBC group (P < .05). At day 21, both groups had no detectable 2,3-DPG. Specimens from both groups retained ATP in sufficiently healthy amounts. The ARBC group demonstrated higher RCM and lower hemolysis levels compared with the MRBC group.

Keywords: apheresis-prepared red cells, manually prepared red cells, hemolysis, in vivo assay, in vitro assay, storage days.

Abbreviations

RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; FDA, United States Food and Drug Administration; ARBC, apheresis-prepared RBC; MRBC, manually collected RBC; CPDA-1, citrate-phosphate-dextrose adenine; RCM, red cell mass volume; Na, sodium; K, potassium; ATP, adenosine triphosphate; 2,3-DPG, 2,3-diphosphoglycerate; ACD-A, acid citrate dextrose; SAGM, saline-adenine-glucose-mannitol; PAGGGM, phosphate-adenine-guanosine-glucose-gluconate-mannitol

1 Cairo University Blood Bank, Department of Clinical Pathology, Cairo University, Egypt; 2 Hematology laboratory, Department of Clinical Pathology, Cairo University, Egypt.

*To whom correspondence should be addressed.
E-mail: eimanhussein@ymail.com
Materials and Methods
We designed and performed the study according to the tenets of the Declaration of Helsinki. A total of 30 ARBC units versus 30 MRBC units were analyzed. RBC units were stored for 21 days, under standard blood-banking conditions at a mean (SD) temperature of 4 (2) °C. None of the units were leukoreduced.

We performed in vitro studies of RBC on day 1 and day 21, evaluating absolute red cell mass volume (RCM); pH; rate of hemolysis; and levels of HCT, sodium (Na), potassium (K), adenosine triphosphate (ATP), 2,3-diphosphoglycerate (2,3-DPG), glucose, and plasma Hb.

Collection and Preparation of RBC Units
All RBC units were collected from donors who all met the American Association of Blood Banks (AABB) eligibility criteria for donation. We recruited donors from the apheresis center and the Blood Bank at Cairo University Hospital, Egypt. Men who weigh at least 155 pounds qualify for automated donation at our center.

Manual RBC Preparation
We collected a mean (SD) of 450 (20 mL) of whole blood in triple blood bags, containing 63 mL of (CPD-A). We separated plasma from the RBC via centrifugation at 4200 g for 10 minutes.

Trima Accel Apheresis Protocol for RBC Collection
We performed apheresis via a single-needle procedure using a Gambro Trima Accel machine. The target end points were set to 250 mL of RBC and collected the RBC along with 6- × 10¹¹ to 9 × 10¹¹ platelets, depending on donors’ data.

The Trima Accel system is designed to optimize the efficiency of component collection while maintaining donor safety; it is an automated continuous-flow centrifuge that separates whole blood into components for collection based on donor blood volume and complete blood count. The system has a single-stage channel with all blood components flowing in one direction. The Trima Accel collects platelets, RBC, and plasma. Users interface with a touch-screen graphical monitor to manually configure and adjust collections before operating the machine. Users load the tubing set on the top of the device; then, they snap a cassette into the device to make the machine ready for use.

Whole blood is pumped into the machine and mixed with acid citrate dextrose (ACD-A) anticoagulant at a controlled ratio near the access needle. The blood components separate into layers over the entire circumference of the centrifuge, according to their density. Each component leaves the centrifuge via its own channel. RBC collection is initiated after platelet collection is completed. The components that are not collected are returned to the donor via return pump. In this study, we performed sessions using an initial anticoagulant ratio of 8:1 of whole blood to ACD-A.

In Vitro RBC Analysis
Samples of approximately 5 mL were collected anaerobically for testing from units immediately after collection, on day 1 and then subsequently on day 21. We performed in vitro assays of HCT, RCM, Na, K, ATP, 2,3-DPG, pH, glucose, plasma Hb, and rate of hemolysis using standard procedures. Specimens were immediately injected into a blood-gas analyzer to determine pH at 37 °C. Na⁺ and K⁺ concentrations were determined via flame photometry. For K⁺ levels of greater than 20 mmol/L, we used a urine standard for calibration. ATP levels were measured enzymatically, as described by Adams.² 2,3-DPG levels were measured as described by Rose and Liebowitz.³ Plasma Hb was measured using a modification of the Drabkin cyanmethemoglobin method⁴ which is read at 540 and 680 nm via spectrophotometer (Beckman Coulter, Inc, Brea, CA).

We calculated the absolute RBC mass volume using the following equation:

\[
\text{Absolute RBC mass volume} = \frac{\text{total RBC volume} \times \text{product HCT level}}{100}
\]

Percent hemolysis was determined using the following equation:

\[
\text{Percentage hemolysis} = \frac{(1 - \text{Hct}) \times \text{plasma Hb (g/L)}}{\text{Total Hb (g/L)}}
\]

In Vivo RBC Analysis
We evaluated a total of 60 RBC transfusions in 8 patients, of whom 3 were women and 5 were men; their mean (SD) age was 29 (6) years. All patients were being
treated for aplastic anemia and had thrombocytopenia. Their mean (SD) pretransfusion HCT level was 18 (3)%. None of the patients had bleeding, hemolysis, or sepsis. Each patient received transfusions of both products; all 8 patients received 4 transfusions each of MRBC. Six patients received 4 ARBC transfusions; the remaining 2 patients received transfusions of 2 units each of ARBC. All transfused units were stored for less than 7 days. We drew peripheral blood samples from patients with anemia 24 hours after transfusion of 1 unit and assessed their HCT levels using a calibrated cell counter (Cobas Micros, F. Hoffman La Roche, Ltd., Basel, Switzerland).

### Statistical Analysis
We performed standard statistical analysis of the test results, including calculation of descriptive statistics, mean, and SD. We used the Student’s t-test for comparative studies. A P value of less than .05 was considered to be statistically significant.

### Results

#### In Vitro Evaluation of RBC Count
MRBC had a volume of 240 to 300 mL per unit (mean [SD], 275 [35.5] mL/unit), RCM values of 158.5 to 240.0 mL/unit (192.5 [3.4] mL/unit), and HCT values of 66% to 80% (70% [9.5%]). ARBC had a volume of 240 to 250 mL/unit (mean [SD], 240 [8.5] mL/unit), RCM values of 204 to 237.5 mL/unit (207.84 [7.7] mL/unit), and HCT values ranging from 85% to 95% (86.6% [9%]).

ARBC showed less variability in HCT level and volume, compared with MRBC (P <.05). We observed a significant increase in hemolysis with storage in both products (P <.05). The platelet count was below the detection limit in both components. In vitro mean (SD) values for ARBCs and MRBCs on day 1 and day 21 are compiled in Table 1 and Table 2, respectively.

#### In Vivo Evaluation of RBC Count
The mean (SD) increase in HCT level after transfusion of ARBC was 4.5 (1.2%). The mean (SD) increase in HCT level after transfusion of MRBC was 2.4 (1.3%). The difference was statistically significant (P <.05).

### Discussion
We investigated the impact of collection procedure and storage on the in vitro characteristics of RBC, evaluating RCM; pH; rate of hemolysis; and levels of HCT, Na, K, ATP, 2,3-DPG, pH, glucose, and plasma Hb. We also prospectively studied the post-transfusion effectiveness of manually collected versus apheresis-collected RBC in patients with aplastic anemia.

Our evaluation of the HCT level of ARBC units revealed that the HCT of these units was not dependent on donor HCT as it was in whole-blood donation; this was due to the fact that apheresis machines collect RBC according to absolute RBC volume. ARBC showed significantly higher RBC mass that provided higher HCT values to our group of patients who received transfusions. The RBC mass difference between the 2 collections can also be explained by the presence of women in the whole-blood collection group or possibly a greater number of lower-weight individuals in that group.

We also observed low variability in the volume of ARBC in our study. Similar findings have been reported in previous studies.5,6

We observed a significant drop of Na and glucose on day 21 in the ARBC group compared with the MRBC group (P <.05). These data contrast with those reported by Picker et al,7 which revealed that the amounts of glucose consumed during 49 days of storage were higher for the ARBC group compared with the MRBC group, using saline-adenine-glucose-mannitol (SAGM) as an RBC-preservative solution.7 However, the lower levels of glucose in our ARBC group at day 21 may reflect the amount of glucose in the original anticoagulant rather than differences in the RBC metabolism between both products. Storage of both components showed no relevant drop in pH, and all units had a pH of 6.5 or greater.

It has been reported8 that damage in ARBC is minimal compared with that in MRBC. During manual RBC collection, the whole blood is typically poured onto the anticoagulant, and some of the RBC are instantly damaged by the acid, due to change in osmolarity. This phenomenon has been described as collection injury.8 By contrast, in automated collection, the anticoagulant is continuously added at a constant small ratio,2 which minimizes collection injury.
Holme et al reported a slightly lower rate of hemolysis (mean [SD], 0.44 [0.26] vs 0.61% [0.50%]), and lower supernatant K⁺ levels (50 [3] vs 53 [3] mEq/L) for ARBC prepared via the Haemonetics apheresis machine (Haemonetics Corporation, Braintree, MA) compared with manual units. These findings are in accordance to those of Moog and colleagues, who compared filtered SAGM ARBC units collected by Haemonetics machine with those collected manually. Our results demonstrated significantly higher levels of K⁺ and plasma Hb for the ARBC group on day 1 and day 21, when compared to the MRBC group. The plasma Hb values that we report for the ARBC group on day 1 (mean [SD], 37 [17] mg/dL) contrast with values reported in previous studies that used Trima and Amicus apheresis machines (Fenwal, Inc., Lake Zurich, IL).

Because of the different HCT values demonstrated in our study, it was essential to correct hemolysis for the HCT, to avoid overestimating the hemolysis percentage. We observed significantly lower rates of hemolysis in ARBC compared with MRBC.

Picker et al reported different results, in which MRBC had an advantage for hemolysis at the end of specimen storage and were superior in energy maintenance. This was indicated by less ATP degradation and K⁺ leakage, which could be a possible consequence of less citrate. The authors also demonstrated higher methemoglobin formation with ARBC, compared with MRBC, due to differences in glucose metabolism.

<table>
<thead>
<tr>
<th>Table 1. In Vitro Characteristics of ARBC and MRBC on Study Day 1</th>
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<tbody>
<tr>
<td>In Vitro Variable (Units of Measure)</td>
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<tr>
<td>Hematocrit (%)</td>
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<td>Absolute RBC mass volume (ml/U)</td>
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<td>Plasma Hg (mg/dL)</td>
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<td>K (mmol/L)</td>
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<td>Hemolysis corrected for hematocrit %</td>
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<td>Na (mmol/L)</td>
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<td>Glucose (mg/dL)</td>
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<td>ATP (mmol/L)</td>
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<tr>
<td>2,3-DPG (mmol/L)</td>
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<td>pH</td>
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ARBC, apheresis-collected red blood cells; MRBC, manually collected red blood cells; RBC, red blood cell; Hg, hemoglobin; K, potassium; Na, sodium; ATP, adenosine triphosphate; 2,3-DPG, 2,3-diphosphoglycerate

<table>
<thead>
<tr>
<th>Table 2. In Vitro Characteristics of ARBC and MRBC on Study Day 21</th>
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<tr>
<td>In Vitro Variable (Units of Measure)</td>
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ARBC, apheresis-collected red blood cells; MRBC, manually collected red blood cells; RBC, red blood cell; Hg, hemoglobin; K, potassium; Na, sodium; ATP, adenosine triphosphate; 2,3-DPG, 2,3-diphosphoglycerate
Mean percentages of hemolysis for stored ARBC, as reported in the literature, ranged from .05% to .8%\textsuperscript{1,7,10-12}. This variation may likely be related to the different apheresis machines and to the different additive solutions that various study groups used.

In the current study, we observed significant increases in hemolysis with storage in specimens from the ARBC and MRBC groups: from .09 (.03%) and .19 (.05%) to 0.41 (.22%) and .7 (.22%), respectively. All units met the United States standards of less than 1% hemolysis.

None of our RBC units were leukoreduced. It has been demonstrated\textsuperscript{13-15} that leukocytes can significantly contribute to RBC hemolysis during storage, due to the release of enzymes, especially proteases. Other studies\textsuperscript{16,17} showed an increase in hemolysis even with leukoreduction.

Many factors can also affect RBC quality, including the duration and force of centrifugation. The temperature of blood components during processing and storage can also contribute to hemolysis. The significant increase in hemolysis with MRBC may indicate that processing and/or storage of MRBC were not optimized.

Hemolysis in our MRBC group can also be related to the low Hb content of the specimens from those RBC units. A previous study\textsuperscript{18} demonstrated higher rates of in vitro hemolysis in RBC with low Hb content.

Several biochemical changes occur during storage that can affect recovery and survival of RBC in the recipient. The most dramatic changes are rapid decrease of ATP and 2,3-DPG. 2,3-DPG is important in the regulation of oxygen delivery by RBC.\textsuperscript{19} After transfusion, low levels of 2,3-DPG will increase in vivo. It takes at least 24 hours before normal levels are reached in healthy volunteers, although slower recoveries have been described in some patients.\textsuperscript{20} ATP is a critical energy source for the overall functioning of RBC. Heaton\textsuperscript{21} demonstrated that ATP levels should be higher than 2.7 µmol per g of Hb, to yield more than 90% chance for a 24-hour recovery of 75% or higher.\textsuperscript{21} Our ATP values of both components were well preserved during storage. Our data on ATP levels are consistent with those obtained in previous studies.\textsuperscript{5,22,23}

The initial mean (SD) concentrations of 2,3-DPG for specimens from our MRBC and ARBC groups were 2.25 (0.08) and 2.3 (0.09) mmol/L, respectively. On day 21, both components had no detectable 2,3-DPG. We suggest that the high citric acid content and associated acidity in ACD-A explains the poor maintenance of 2,3-DPG levels in both groups. Knutson and colleagues\textsuperscript{23} suggested that 2,3-DPG levels can be maintained by changing the anticoagulant used or by rapid cooling of the RBC unit immediately after preparation. Recently, it has been demonstrated\textsuperscript{19} that resuspension of RBC in phosphate-adenine-guanosine-glucose-gluconate-mannitol (PAGGGM) can provide a high-quality unit throughout 35 days of storage, with 2,3-DPG levels higher than 10 µmol per g of Hb, ATP levels higher than 5 µmol per g of Hb, and hemolysis levels of less than 0.2%.

Although the storage lesion is well documented, whether the age of RBC can adversely affect the outcome of transfusion remains in dispute.\textsuperscript{24-27} A 2006 study\textsuperscript{28} reported that transfusion of stored or recently donated blood to hypoxic patients has the same ability to restore evoked brain-stem potentials. In a previous study,\textsuperscript{29} however, it had been reported that transfusion of older stored RBCs produces extravascular hemolysis and circulating non–transferrin-bound iron, which may enhance complications such as infection. Four large multicenter trials are currently studying the impact of storage days on clinical outcomes. Strategies regarding storage age of RBC should await the results of such ongoing trials.\textsuperscript{30}

One limitation of the current study is that although measurement of HCT levels 24 hours after transfusion can reflect short-term viability, tagging studies may be required to draw definitive conclusions regarding RBC viability.

**Conclusion**

Compared with the MRBC group, the ARBC group demonstrated less variability in volume, higher RCM, and lower rates of hemolysis. Transfusion of ARBCs that had been stored for less than 1 week provided significantly higher HCT levels than transfusion of MRBCs for our cohort of patients with anemia. \textsuperscript{LM}

**Acknowledgments**

We thank the staff members of the Department of Internal Medicine, Hematology division, Cairo University, Egypt, for their cooperation. We also thank the staff members of the Cairo University Apheresis Center and the Blood Bank for their technical support.
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