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Overnight, room temperature hold of whole blood followed by 42-day storage of red blood cells in additive solution-7

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BACKGROUND: Overnight, room temperature hold (ONH) of whole blood before component processing offers several benefits. This study evaluated the storage and in vivo recovery characteristics of ONH red blood cells (RBCs) stored in additive solution-7 (AS-7).

STUDY DESIGN AND METHODS: We conducted a three-center, three-arm evaluation of a new blood collection system with AS-7 compared to leukoreduced RBCs processed within 8 hours and stored in AS-1 (control). Whole blood (500 ± 50 mL) from healthy research subjects (n = 240) was held at room temperature 0 to 2 hours, 6 to 8 hours, or ONH (18-24 hr) before component processing and storage at 1 to 6°C. RBCs were evaluated on Days 42 and 56 with a panel of in vitro assays. Subsets of the AS-7–stored RBCs were evaluated for ^{51}Cr 24-hour in vivo recovery and long-term survival.

RESULTS: Adenosine triphosphate (ATP) levels in ONH RBCs were not different than AS-7 RBCs prepared within 8 hours. ATP was higher in the ONH group on Day 42 than control, and ATP was maintained in all AS-7 groups through Day 56. ONH units had 0.36 ± 0.14% on Day 42 hemolysis (60/60 < 0.8%), and 0.54 ± 0.22% on Day 56 (10/60 > 0.8%, 2/60 > 1%). In vivo recoveries of stored RBCs were not different between the AS-7 arms at 42 days (p = 0.16; 27/27 ONH units > 75%), but the Day 56 ONH was significantly less than ONH on Day 42 (p = 0.008; 7/28 < 75%).

CONCLUSIONS: Overnight hold of whole blood at room temperature before component processing meets current regulatory requirements when RBCs are stored up to 42 days in AS-7.

The addition of storage solutions to red blood cells (RBCs) permits the extended storage at 1 to 6°C of RBCs for transfusion for up to 6 weeks in the United States and in several other countries. These additive solutions (ASs) provide osmotic stabilization, metabolic fuel sources such as glucose, buffers, and/or metabolic substrates such as adenine and phosphate. A new alkaline AS, AS-7 (SOLX, Hemerus Medical, LLC, St Paul, MN), is the culmination of decades of development work on an AS that will improve the metabolic energetics profile of refrigerator-stored RBCs through pH control early in the storage period.[1,2] In addition to the biochemical advantages that AS-7 may bring to RBC storage, the flexibility to hold CPD-anticoagulated whole

ABBREVIATIONS: ONH = overnight, room temperature hold; rWBC(s) = residual white blood cell(s).

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blood donations for up to 24 hours at room temperature before component processing offers significant logistic advantages to the blood collection and processing organization by reducing manufacturing night work and improving quality control. Overnight hold of whole blood before processing was initiated in Europe in the 1980s. They were motivated to be self-sufficient in the provision of blood products, with a special emphasis at that time on plasma. Allowance of overnight hold of the whole blood at approximately 20 to 24°C increased the utilization efficiency of all blood components (RBCs, platelets [PLTs], and plasma) while maintaining Factor VIII activity at acceptable levels before freezing plasma. They also observed that the yield of PLTs prepared from whole blood that was held before processing increased. This history has been recently reviewed by Pietersz.[3] Our objective in this study was to evaluate the quality and performance of RBCs prepared from whole blood held overnight at room temperature before processing and stored in AS-7 up to 8 weeks.

MATERIALS AND METHODS

We conducted a three-center, three-arm evaluation of the LEUKOSEP HWB-600-XL leukoreduction filtration system with CPD and SOLX AS for whole blood (test, Hemerus Medical, LLC) compared to the RZ2000 leukoreduction filter with CPD and AS-1 (control, 4R3335E, Fenwal, Inc., Lake Zurich, IL), a US Food and Drug Administration (FDA)-approved system for collection, whole blood leukofiltration, component processing, and storage. AS-7 is formulated with 26 mmol/L sodium bicarbonate, 12 mmol/L dibasic sodium phosphate, 2 mmol/L adenine, 80 mmol/L glucose, and 55 mmol/L mannitol with a pH of 8.5 at 237 mOsm/L. The control RBC group was selected solely as a comparator of current practice based on FDA guidance and was not intended to be used to assess overnight, room temperature hold (ONH) effects independent of or synergistically with all AS. Each participating center processed units in each arm in a manner where entry sequence in each arm was balanced over time, but was not subject to a formal randomization. Briefly, 500 ± 50 mL of whole blood was collected from healthy research subjects (n = 240) into CPD using either a test or a control collection system. Whole blood was held at room temperature, 20 to 24°C, individually on an open laboratory bench without cooling plates before filtration and component processing. Control units were whole blood filtered at room temperature after 6 hours, centrifuged with a validated hard centrifugation protocol (7268 × *g* 5 min, 6035 × *g* 10 min, or 5086 × *g* 10 min), and plasma was manually expressed. Control RBCs were placed in AS-1 (ADSOL, Fenwal, Inc.) for long-term storage at 1 to 6°C.

TABLE 1. Final leukoreduced RBC unit

Test arm	Volume (mL)*	Total Hb (g)*	Residual WBCs†
Control‡§	308 ± 17	60.6 ± 5.8‖	5.9×10^4 (2.0×10^7)
2 hr‡	311 ± 18	60.5 ± 6.4	5.1×10^4 (2.4×10^6)
8 hr‡	311 ± 17	59.4 ± 5.7	9.6×10^4 (1.4×10^6)
ONH‡	306 ± 16	58.1 ± 5.1	2.1×10^5 (2.2×10^6)

* Data are reported as mean ± SD.
† Data are reported as median (max).
‡ n = 60.
§ 8-hour AS-1, RZ2000 leukoreduction filter.
‖ One unit 44 g of Hb.

Test units were processed in the same manner within three treatment arms that varied by hold time before filtration and component processing: 2 hours, whole blood was processed and RBCs were placed at 1 to 6°C within 2 hours of collection; 8 hours, whole blood was processed after 6 hours and RBCs placed at 1 to 6°C within 8 hours of collection; and ONH, whole blood was held overnight at room temperature and processed after 18 hours and RBCs were placed at 1 to 6°C within 24 hours of collection. All test RBCs were placed in AS-7. Analytical methods, plasma results, and data from the control, 2-hour, and 8-hour groups are described in the companion articles.[4,5] Each center conducted all assays within their own institution. A formal, a priori comparison of analytical methods was not conducted; however, no significant center effects were observed for the study outcomes (data not shown).

This study was conducted after approvals by the FDA under an investigational new drug application, the local institutional review boards at each laboratory, and the Office of Research Protections Human Research Protection Office US Army Medical Research and Materiel Command. All subjects provided informed consent for participation. This study conforms to the Declaration of Helsinki.

Statistical analysis

Mixed-effects models were used to test hypotheses of treatment group and storage time effects ($p < 0.05$, PROC MIXED, SAS, Version 9.3, SAS Institute, Cary, NC). No corrections were made for multiple tests. Summary data were calculated with PROC MEANS (SAS).

RESULTS

Sixty units were evaluated in each study arm for in vitro characteristics. The final configuration of RBC units for storage was a volume of 309 ± 17 mL containing 59.6 ± 5.8 g of Hb per unit after component processing (Table 1). All units contained more than 45 g of Hb except one control at 44 g of Hb. One control unit had greater than 5 million residual WBCs (rWBCs) with the remaining

TABLE 2. Energetics and pH*

Analyte	Control†‡	2 hr†	8 hr†	ONH†
pH (37°C)				
Post§	7.02 ± 0.03	7.02 ± 0.04	6.96 ± 0.03	6.87 ± 0.03
Day 42	6.41 ± 0.05	6.46 ± 0.05	6.47 ± 0.05	6.48 ± 0.05
Day 56		6.37 ± 0.06	6.38 ± 0.06	6.39 ± 0.05
Biocarbonate (mmol/L)				
Post§	13.7 ± 1.4	19.3 ± 1.5	18.8 ± 1.4	18.0 ± 1.2
Day 42	7.8 ± 1.0	10.0 ± 1.5	10.2 ± 1.5	9.6 ± 1.6
Day 56		7.5 ± 1.4	7.5 ± 1.2	7.1 ± 1.1
Glucose (mmol/L)				
Post§	52.3 ± 6.5	41.5 ± 4.9	41.4 ± 4.2	41.3 ± 4.6
Day 42	39.4 ± 3.7	24.3 ± 3.6	25.8 ± 2.8	27.5 ± 3.5
Day 56		20.9 ± 3.1	22.6 ± 2.7	24.5 ± 3.2
Lactate (mmol/L)				
Post§	2.3 ± 0.4	1.0 ± 0.3	1.6 ± 0.4	3.4 ± 0.7
Day 42	26.5 ± 4.1	30.5 ± 4.8	27.7 ± 4.1	28.2 ± 3.6
Day 56		34.8 ± 6.4	31.5 ± 4.5	31.4 ± 5.0
ATP (μmol/g Hb)				
Post§	4.4 ± 0.7	4.1 ± 0.7	4.5 ± 0.7	4.4 ± 0.7
Day 42	3.6 ± 0.8	3.8 ± 0.7	4.0 ± 0.7	3.9 ± 0.8
Day 56		3.1 ± 0.6	3.2 ± 0.8	3.1 ± 0.7

* Data are reported as mean ± SD.
† n = 60.
‡ 8-hour AS-1, RZ2000 leukoreduction filter.
§ After processing, before storage.

59 less than 1 million per unit. AS-7 2- and 8-hour units had three of 120 greater than 1 million rWBC and none over 5 million. There were 6 ONH units with greater than 1 million rWBCs and 60 of 60 less than 5 million.

Spun hematocrit (Hct) levels for control units (60.2 ± 2.8%) were lower after processing following the addition of AS-1 compared to AS-7 units (71.6 ± 2%), likely reflecting osmolarity effects of the hypoosmolar AS-7 (237 mOsm/L) and the hyperosmolar AS-1 (462 mOsm/L). The control units' spun Hct levels remained unchanged over 42 days of storage, while AS-7 units declined to 65.2 ± 2.3% on Day 42 and 63.9 ± 2.3% on Day 56. This apparent change in RBC volume was not observed in the automated mean cell volume determination (AS-1 92.8 ± 5.8 fL and AS-7 92.9 ± 5.0 fL) or the automated Hct estimates since the automated systems perform large dilutions with isotonic solutions and the cell volume has time to respond before passing the detectors in the hematology analyzer.

Postprocessing extracellular pH for ONH was slightly less than the other arms reflecting lactic acid production during the additional time at room temperature. However, this difference in pH resolved by the end of storage (Table 2). Cellular energetics, as indicated by intracellular adenosine triphosphate (ATP) content, was not different in the ONH arm than observed with the AS-7 RBC 2- and 8-hour groups prepared within 8 hours of collection. Intracellular ATP was higher in the ONH group at 42 days of storage than observed in the control arm, and ATP was adequately maintained in all AS-7 groups through Day 56.

ONH units had a mean of 0.36 ± 0.14% hemolysis at 42 days of storage, 60 of 60 units with hemolysis less than 0.8%, the EU limit (Fig. 1A). By Day 56, hemolysis of ONH units increased to 0.54 ± 0.22% with 10 units greater than 0.8% and 2 units above the US limit of 1%. RBC microvesicles were not different between the AS-7 8-hour group and the ONH group at 56 days of storage (17 ± 12 and 17 ± 10 mg of protein/mL RBCs), but were less than the control AS-1 units on Day 42 (29 ± 18 mg of protein/mL of RBCs). As expected, potassium leakage is not different between treatment arms. ONH AS-7 RBC morphology scores at both 42 and 56 days of storage (76 ± 9 and 72 ± 8, respectively) were lower than AS-7 units processed within 8 hours, but were well maintained compared to Day 42 control AS-1 units (69 ± 8).[4]

In vivo 24-hour recoveries of stored RBCs were not different between the AS-7 arms at 42 days (p = 0.16), and 27 of 27 ONH units were greater than 75% recovery. Recoveries of Day 56 RBCs were not different between the 2- and 8-hour group, but the ONH group was significantly less (p = 0.008) with seven of 28 less than 75% (Fig. 2, Table 3). Likewise, there were no differences between conventionally processed groups and ONH units for 50% survival times (T50) estimates for 42-day-stored RBCs (p = 0.6). There were small declines in T50 for both the 8-hour and ONH groups at Day 56 (p = 0.04), but still within the reference ranges of 25 to 35 days. Also of note is the T50 estimates are extrapolated outside of the observed data range from Day 1 to Day 15 post infusion.

DISCUSSION

ASs work by providing nutrients and buffering for extended RBC storage. Much of the buffering comes from the additional 110 mL of volume for dilution of glycolytically produced lactic acid. AS-7 increases that buffer capacity further with the addition of phosphate and bicarbonate and increases the range of pH by starting as an alkaline solution. The resulting combination of increased pH range and greater buffer capacity means that stored RBCs can be more metabolically active longer before they reach the critical pH and begin to fail. The change in pH with AS-7, previously reported,[2] was not measured in this study. This effect is largely an intracellular phenomenon created by hypotonic RBC swelling, hydroxide for chloride anion shifts, and hemoglobin buffering. The use of hypoosmotic AS-7 results in an increase in RBC volume. This was only observed in the spun Hct levels in this study compared to AS-1, a hyperosmotic solution. This effect appears to be transitory. The increase

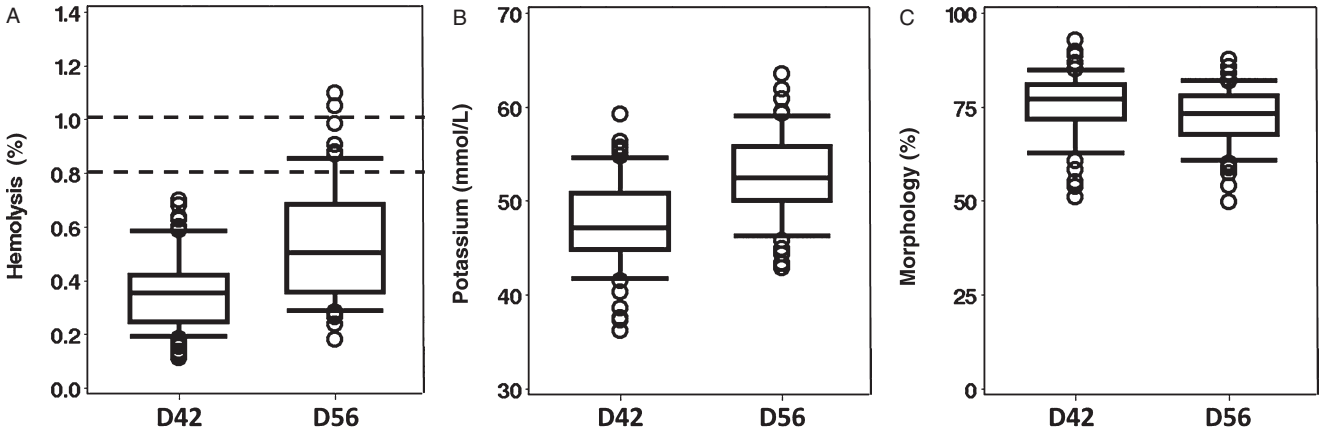

Fig. 1. ONH RBCs stored in AS-7 at the end of storage. (A) Percent hemolysis. The EU limit of 0.8% and the US limit of 1.0% are indicated. (B) Extracellular potassium concentration. (C) RBC morphology. D42 = Day 42; D56 = Day 56.

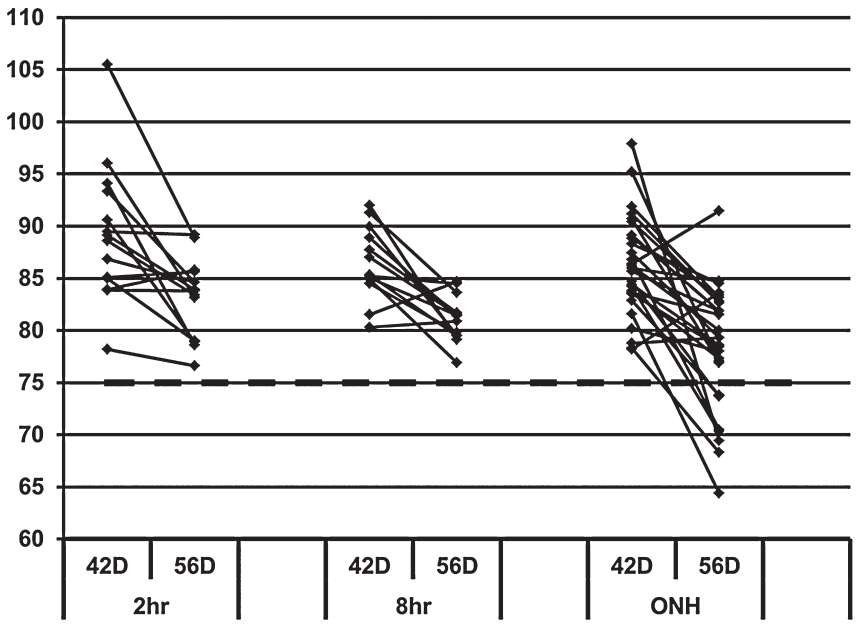

Fig. 2. In vivo 24-hour recovery of AS-7–stored RBCs. In vivo 24-hour recoveries (%) of ^{51}Cr labeled aliquots are shown by AS-7 group and the day of labeling. The US lower limit of 75% is indicated. Subject pairs are joined. n = 14, 2 hours; n = 13, 8 hours; n = 27, ONH 42 days (42D); n = 28, ONH 56 days (56D).

in pH observed with storage in AS-7 provides a favorable environment for maintenance of ATP even out to 56 days of storage. Overnight hold of whole blood at room temperature did not affect glucose consumption, pH maintenance, and intracellular ATP levels for AS-7–stored RBCs. Interestingly, with the leukoreduction filter medium and configuration used in this study, we observed a progressive increase in the median residual WBC levels from 2 to 8 hours to ONH. This may reflect a progressive relaxation of the activation state of both WBCs and PLTs during a rest period that influences the WBC removal efficiency of the filter. If true, we speculate that this is a filter- and/or a donor-dependent phenomenon independent of the RBC AS since all filtration in this study was performed before component processing and AS addition.

ONH AS-7 RBCs met key FDA performance criteria for 42-day storage with hemolysis less than 1.0% in 60 of 60 units and 24-hour in vivo recoveries greater than 75% in 27 of 27 subjects. ONH AS-7 RBCs fell short of these requirements when units were held to 56 days having hemolysis greater than 1% in 2 of 60 units and seven of 28 in vivo 24-hour recoveries less than 75%. In contrast, RBCs prepared and refrigerated with AS-7 within 8 hours met these requirements at 56 days. These differences were not marked by an increase in RBC microvesicle formation as the ONH Day 56 units were equivalent to the 8-hour Day 56 AS-7 units and less than observed at Day 42 in control AS-1 RBCs. Although Day 56 RBC morphologies in ONH AS-7 RBCs were lower than reported for AS-7–stored cells processed within 8 hours, the morphologies were better maintained than 42-day-old AS-1 RBCs. Therefore, overnight hold of whole blood at room temperature before component processing does meet current US and European regulatory requirements when RBCs are stored up to 42 days in AS-7.

A change in blood center practices to convert to the overnight hold of whole blood at room temperature will most likely be motivated by logistic and cost advantages as well as potential improvements in safety profiles. In this study, we did not explore these operational advantages. A review of the literature failed to uncover any quantitative studies exploring this process change in terms of industrial engineering and cost improvements. However, there

TABLE 3. In vivo recovery kinetics for AS-7–stored RBCs

	Recovery (%)			Survival (T50, days)		
Sample day	2 hr (n = 14)	8 hr (n = 13)	ONH (n = 28)	2 hr (n = 14)	8 hr (n = 13)	ONH (n = 28)
42	89.3 ± 6.6* (78.2)†	86.4 ± 3.5 (80.3)	86.2 ± 4.9‡ (78.2)	32.0 ± 8.7 (17.8)	34.8 ± 11.6 (22.1)	32.2 ± 7.1 (23.8)‡
56	83.3 ± 3.8 (76.6)	81.1 ± 2.2 (76.9)	78.5 ± 6.0 (64.41)	34.2 ± 9.7 (18.3)	27.6 ± 8.0 (17.5)	27.7 ± 6.2 (17.1)

* Data are reported as mean ± SD.
† Number in parentheses is minimum.
‡ n = 27.

are several qualitative observations from those who have made the conversion to overnight hold of whole blood. Pietersz notes that overnight hold avoids the need for both a day and a night shift in the components laboratory for immediate processing of units upon arrival, and the PLTs are not lost if processing does not occur within 8 hours of collection.[3] Shinar and colleagues[6] observed that multiple transportation trips from remote collection sites to the blood center during the day could be avoided, and efficient planning of the workload within the components laboratory was facilitated. Levin and colleagues[7] commented that all manufacturing may be performed the day after collection without a need to be concerned about staffing night shifts. Similarly, Thomas[8] mentions increased flexibility in whole blood collection and processing operations, with an improved likelihood of selecting more male plasma for distribution and reducing the risk of lung injury. These qualitative observations and conclusions from blood center operational experts are compelling even though they lack solid quantitative measures. Finally, there is a potential safety advantage of holding whole blood for an extended period because of the autosterilization effect of the WBCs against contaminating bacteria.[9] A recent report from the Canadian Blood Services observed a bacterial contamination rate (confirmed positive plus indeterminate) of 1.97 per 10,000 pools for PLTs prepared from buffy coat versus 6.25 per 10,000 pools for PLTs prepared from PLT-rich plasma, the former having an extended exposure to WBC at room temperature either in whole blood or in a buffy coat preparation.[10]

The results of this study are limited by the selection of the control condition (AS-1 processed within 8 hr of collection) and the absence of in vivo recovery assessments with the controls. To our knowledge, there are no published in vivo recoveries in healthy subjects for 6-week-stored AS-1 RBCs. To answer the broader question of the effects of ONH and AS storage support, control conditions including other AS (e.g., AS-3, AS-5, and PAGGSM) plus in vivo recovery kinetics would need to be examined. Such a large, all-encompassing experimental design will face several barriers such as costs, subject recruitment, exposure of more individuals to radioactivity, and the total time required to complete such a study. However, the data reported here from the current design that were driven by regulatory demands for product approval support overnight hold of whole blood at room temperature with subsequent storage of the RBCs in AS-7 for up to 42 days. Consideration of the bioequivalence of plasma prepared from room temperature, overnight held whole blood is presented in an accompanying article.[5] The combination of overnight hold with AS-7 storage of RBCs should offer important safety, logistic, and cost benefits to the blood supplier and patient.

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CONFLICT OF INTEREST

This study was sponsored by Hemerus Medical, LLC. Hemerus Medical, LLC, was acquired by Haemonetics, Inc., after the completion of this study. LJD, JAC, LAM, NR, PW, LH, AHS, and ZMS have disclosed conflicts of interest. JRH has patent and royalty rights to AS-7 and is a consultant of Hemerus, LLC, and Haemonetics Corporation. MZ was an employee and shareholder of Hemerus Medical, LLC, at the time of study and an employee of Haemonetics Corporation at the time of preparation of the manuscript.

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