Ringer's lactate is compatible with saline-adenine-glucose-mannitol preserved packed red blood cells for rapid transfusion

Le lactate Ringer est compatible avec un culot globulaire conservé dans une solution saline d'adénine-glucose-mannitol pour les transfusions rapides

Brendan Levac · Joel L. Parlow, MD · Janet van Vlymen, MD ·
Paula James, MD · Angie Tuttle · Lois Shepherd, MD

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Abstract

Purpose Guidelines state that Ringer's lactate (RL) should not be co-administered with packed red blood cells (PRBC) due to a potential risk of clotting. The purpose of this study was to determine whether RL causes clotting in PRBC with the currently used preservative, saline-adenine-glucose-mannitol (SAGM).

Methods Phase 1: Samples from 12 units of SAGM-PRBC were diluted from 0–97.5% with RL and normal saline (NS), incubated for 30 min, and passed through 40 μ m filters. Additional samples were frozen and batch analyzed using an enzyme-linked immunosorbent assay (ELISA) to measure prothrombin activation fragment 1 + 2 (F1 + 2), indicative of thrombin generation. Packed red blood cells were also diluted, flushed with crystalloid using a rapid transfusion model, and filtered. Phase 2: Eight further units were serially diluted with RL and incubated for 30, 60, 120, 180, and 240 min. Fresh samples were analyzed by filtration and ELISA.

Results Phase 1: No clotting was seen during filtration or using the transfusion model with NS or RL. The F1 + 2 ranged from 2.28 to 154.37 pmol·L⁻¹ in NS dilutions and from 2.80 to 1675.93 pmol·L⁻¹ in RL dilutions, indicating coagulation in some samples. Phase 2: No clotting was observed within 60 min by filtration or ELISA. However, 4 of the 8 units showed clots in the filters of some dilutions between 120 and 240 min.

Conclusions No clotting was detected at any dilution of RL with SAGM-preserved PRBC within 60 min, but clotting was detected with extended incubation. The results indicate RL can be safely co-administered with PRBC during rapid transfusion (< 60 min).

Résumé

Objectif Selon les directives, le lactate Ringer (LR) ne devrait pas être administré conjointement à un culot globulaire en raison du risque potentiel de coagulation. L'objectif de cette étude était de déterminer si le LR provoquait une coagulation d'un culot globulaire conservé dans une solution saline d'adénine-glucose-mannitol (SAGM), l'agent de conservation actuellement utilisé.

Méthode Phase 1: Des échantillons tirés de 12 unités de culots globulaires avec SAGM ont été dilués de 0–97,5 % avec du LR et du sérum physiologique, incubés durant 30 min puis passés dans des filtres de 40 μ m. Des échantillons supplémentaires ont été congelés et analysés par lots à l'aide de la méthode ELISA (méthode immunoenzymatique) afin de mesurer F1 + 2, un indicateur de génération de thrombine. Les culots sanguins ont également été dilués et rincés avec des cristalloïdes à l'aide d'un modèle de transfusion rapide, puis filtrés. Phase 2: Huit unités supplémentaires ont été diluées en série avec du LR et incubées pour 30, 60, 120, 180 et

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B. Levac · J. L. Parlow, MD (✉) · J. van Vlymen, MD
Department of Anesthesiology and Perioperative Medicine,
Queen's University, Kingston General Hospital, 76 Stuart Street,
Kingston, ON K7L 2V7, Canada
e-mail: parlowj@queensu.ca

P. James, MD · A. Tuttle
Medicine (Division of Hematology), Queen's University and
Kingston General Hospital, Kingston, ON, Canada

L. Shepherd, MD
Pathology (Transfusion Medicine), Queen's University and
Kingston General Hospital, Kingston, ON, Canada

240 min. Des échantillons frais ont été analysés par filtration et par la méthode ELISA.

Résultats Phase 1: Aucune coagulation n'a été observée pendant la filtration ou lors de l'utilisation du modèle de transfusion avec le sérum physiologique ou le LR. Les valeurs $F1 + 2$ allaient de 2,28 à 154,37 $\text{pmol}\cdot\text{L}^{-1}$ dans les dilutions de sérum physiologique, et de 2,80 à 1675,93 $\text{pmol}\cdot\text{L}^{-1}$ dans les dilutions de LR, ce qui indique une coagulation dans certains des échantillons. Phase 2: Aucune coagulation n'a été observée au cours d'une période de 60 min par filtration ou ELISA. Toutefois, 4 des 8 unités ont généré des caillots dans les filtres de certaines dilutions entre 120 et 240 min.

Conclusion Aucune coagulation n'a été observée à quelque dilution de LR que ce soit avec des culots globulaires conservés par SAGM dans les premières 60 min, mais on a observé une coagulation lors d'une incubation prolongée. Ces résultats indiquent que le LR peut être administré conjointement à un culot globulaire de façon sécuritaire durant une transfusion rapide (< 60 min).

Normal saline (NS) and Ringer's lactate (RL) are commonly used first-line volume replacement solutions during rapid resuscitation for hypovolemia. Since NS contains super-physiologic concentrations of sodium and chloride, resuscitation with NS may cause hyperchloremic metabolic acidosis and disorders of electrolyte homeostasis.¹⁻³ Thus, RL is considered by some authorities to be a more appropriate volume replacement solution than NS for the resuscitation of a severely hypovolemic patient.¹⁻⁵ Under certain circumstances when blood is co-administered with crystalloid, it has been found that calcium-containing crystalloid solutions, such as RL, have the potential to overwhelm the calcium chelating ability of the citrate-based anticoagulant, citrate-phosphate-dextrose (CPD), resulting in clot formation.⁶ Conditions associated with an increased probability of clotting include a higher starting hematocrit (> 0.75-0.80), a greater than 1:1 ratio by volume of RL to packed red blood cells (PRBC), and an incubation time of > 30 min.^{7,8} These findings supported the caution expressed by the Canadian Blood Services (CBS) and American Association of Blood Banks, that only NS should be co-administered with PRBC.^{9,10} More recent research examining PRBC stored in different anticoagulants has put these guidelines into question. Ringer's lactate has been shown not to lead to clotting in CPD-preserved erythrocytes when mixed in typical dilutions.¹¹ When RL has been added to PRBC preserved with Additive Solution-3 (AS-3), no clotting occurred in a wide range of dilutions, when measured by macroscopic and

molecular means, and when using a simulated rapid transfusion model.¹²

As of July 2008, blood issued by the CBS is stored with a new preservative, saline-adenine-glucose-mannitol (SAGM). This preservative is added to PRBC following the centrifugation of whole blood collected in CPD, yielding a final hematocrit of 0.63 ± 0.07 . Unlike previous blood preservation solutions, SAGM does not contain additional citrate (Table 1). This conceivably could allow clotting to occur when lower volumes of RL are mixed with PRBC. To date, no studies have documented the effects of adding RL to SAGM-preserved PRBC. The purpose of the current study was to determine whether clotting can occur when RL vs NS is mixed with SAGM-preserved PRBC.

Methods

This study was approved by the Queen's University Faculty of Health Sciences Research Ethics Board. The study was carried out in two separate phases, as described below, in which we analyzed a convenience sample of 20 units of SAGM-preserved PRBC supplied by the Blood Transfusion Service at the Kingston General Hospital. All units had been collected by the CBS from volunteer donors, and they were prepared by adding CPD to the donated whole blood, centrifuging to acquire PRBC, and adding 100 mL of SAGM for preservation. A comparison of the constituents of SAGM with the previously used AS-3 is shown in Table 1. All of the PRBC units were used within the 42-day viability period, and all information identifying the donors was removed before experimentation. The PRBC were stored at 4°C, and both crystalloid solutions were stored at room temperature prior to the trials.

Table 1 Constituents of packed red blood cell preservatives¹⁰

	SAGM	AS-3
Sodium chloride	8.77	4.10
Adenine	0.169	0.30
Dextrose	9.0	11.0
Sodium phosphate	-	2.76
Sodium citrate	-	5.88
Citric acid	-	0.42
Mannitol	5.25	-
Volume (mL)	110	100
Shelf life (days)	42	42

Expressed in $\text{g}\cdot\text{L}^{-1}$ of packed red blood cells stored in Additive Solution 3 (AS-3) and the currently used preservative saline-adenine-glucose-mannitol (SAGM). For both preparations, citrate-phosphate-dextrose is added as anticoagulant to whole blood prior to centrifugation

Study phase 1

Samples from 12 units of PRBC were diluted, as outlined below, with both RL and NS (Baxter Corp., Toronto, ON, Canada) for three types of analysis: Part 1) filtration, Part 2) molecular analysis for evidence of thrombin activation, and Part 3) simulated rapid transfusion.

Part 1- filtration

For each unit of PRBC, two sets of seven progressively more dilute 10 mL samples of PRBC and crystalloid were prepared using both RL and NS (Table 2). The samples contained 0-97.5% crystalloid by volume. After mixing, these samples were incubated for 30 min and then strained through a 40-micron blood filter (Cell Strainer 40 μ m, Becton Dickinson, Swedesboro, NJ, USA). An observer blinded to dilution and type of crystalloid inspected the filters visually for evidence of clot.

Part 2- molecular analysis

Using the same 12 units of PRBC, two further sets of seven 20 mL samples (diluted with each of NS and RL) were prepared to the same dilutions as in Part 1. The samples were incubated for 30 min before centrifugation at 1,500G for 14 min at 4°C. The supernatant was pipetted into 2 mL micro vials, frozen at -80°C, and batch analyzed following completion of Phase 1 (range of storage time 10-77 days after collection). For analysis, the micro vials were thawed, and thrombin generation was measured using an enzyme-linked immunosorbent assay (ELISA) technique (Immulon* 4HBX Immunoassay Plates, Corning Inc, Kennebunkport, ME, USA), determining the concentration of prothrombin activation fragment 1 + 2 (F1 + 2), a specific indicator of thrombin generation. This technique is used to determine whether activation of the coagulation cascade has occurred

Table 2 Phase 1: Dilutions of PRBC with RL and NS for filtration after 30 min incubation ($n = 12$ units of PRBC). 20 mL samples of the same dilutions were incubated for 30 min prior to preparation for ELISA analysis

Dilution #	PRBC (mL)	Crystalloid (mL)	% Crystalloid
1	10	0	0
2	7.5	2.5	25
3	5	5	50
4	2.5	7.5	75
5	1.5	8.5	85
6	0.5	9.5	95
7	0.25	9.75	97.5

PRBC = packed red blood cells; RL = Ringer's lactate; NS = normal saline; ELISA = enzyme-linked immunosorbent assay

in any given sample. All measurements were completed in duplicate with standardized controls, and the mean value of each pair was reported (detection range of this assay 20-1,200 pmol·L⁻¹, control reference range 69-229 pmol·L⁻¹ for 5th-95th percentile).

Part 3

The remaining PRBC in the 12 bags of blood (approximately 150 mL) were used for the simulated rapid transfusion model, similar to that used by Albert *et al.*¹² Each unit of PRBC was attached to one limb of Y-type blood administration tubing with an integrated 170 micron filter (Baxter Corp., Toronto, ON, Canada). A 500 mL bag of crystalloid solution was attached to the other limb and used to prime the tubing. The fluid was suspended from a 1.5-m high intravenous pole, run through a blood warmer (Level 1 Hotline, Smiths Medical, Rockland, MA, USA) set at 37°C, exited through an 18-G catheter, and filtered using a 40-micron filter (details above). The crystalloid solution used for each trial was selected using a computer-generated randomization schedule such that 6 units of PRBC were mixed with each of RL or NS. The PRBC were diluted with 50 mL of the crystalloid, and the infusion took place over 15 min to model a relevant time frame for rapid transfusion in the setting of volume resuscitation. Following infusion of the blood-crystalloid mixture, the remaining crystalloid was used to flush the tubings until clear. Filters were then examined macroscopically for evidence of clotting.

Study phase 2

As seen in the Phase 1 results (below), despite an absence of clotting during the 30-min filtration experiment, elevated levels of F1 + 2 were seen in some of the stored samples containing RL, but not NS. Thus, it was left unclear whether the positive ELISA results might have been caused by coagulation occurring during the prolonged storage of the samples before analysis. Hence, Phase 2 of the study was set up to determine 1) whether ELISA analysis of *fresh* dilutions of PRBC and RL, rather than frozen batched samples, would demonstrate evidence of coagulation; and 2) whether longer incubation times before filtration would result in clotting. Since it was clear that NS did not lead to positive results in any of the tests, only RL was used in this phase of the study.

For each of 8 further units of PRBC, five progressively more dilute 60 mL mixtures of PRBC to RL solutions were prepared to a range of dilutions of 25-95% RL (Table 3). Samples for pure PRBC and the dilution of 97.5 RL were omitted in Phase 2 of the study. From each 60 mL mixture, 10 mL were distributed to each of five labelled test tubes,

Table 3 Phase 2: Dilutions of PRBC with RL for filtration ($n = 8$ units of PRBC). Each dilution was separated into 10-mL samples for analysis

Dilution #	PRBC (mL)	RL (mL)	% RL
1	45	15	25
2	30	30	50
3	15	45	75
4	9	51	85
5	3	57	95

PRBC = packed red blood cells; RL = Ringer's lactate

incubated for 30, 60, 120, 180, and 240 min, passed through 40-micron filters, and visually inspected for evidence of clotting.

Samples of 3 mL from the remainder of each of these mixtures were injected into vacuum tubes (Vacutainer Plus SST, BD Diagnostics, Franklin Lakes, NJ, USA), then centrifuged and analyzed for total and ionized calcium concentration (Biochemistry Laboratory, Kingston General Hospital, Kingston, ON, Canada) in order to determine whether any relationship existed between free calcium (i.e., not bound to citrate) and presence of clots.

For the ELISA analysis of fresh mixtures, 20 mL samples of the same dilutions as described above (five dilutions for each of 8 units of PRBC) were incubated for 30 min and centrifuged at 1,500G for 14 min at 4°C. The supernatant was pipetted into 2 mL micro vials and analyzed in duplicate using ELISA. Total duration available for the generation of F1 + 2 fragments during incubation and preparation of samples was at least 60 min, with results available in approximately 90 min.

Data analysis

For the filtration studies, any clotting seen, regardless of dilution, was considered a positive result for that unit of PRBC for any given incubation period. In Phase 1, F1 + 2

concentrations were compared between mixtures of NS and RL using Student's t tests on log transformed data. Since Phase 2 was performed only on samples diluted with RL, F1 + 2 was compared with the equivalent dilutions with NS from Phase 1 as controls. Serum calcium concentrations were compared between samples that showed clotting vs no clotting using unpaired Student's t tests. A P value of < 0.05 was considered significant.

Results

Phase 1

Following 30 min of incubation at room temperature, none of the dilutions of PRBC with either NS or RL showed any indication of clotting after visual inspection of the 40 micron filters (overall 95% confidence interval 0–3.5%, or 0–22% for any given dilution). No clot or debris was present to obstruct free flow through the filter.

The ELISA analysis of the frozen samples (stored 10–77 days) showed high concentrations of F1 + 2 in 5 of 12 units of PRBC, only in samples diluted with 75% RL ($P = 0.01$ vs NS) and 85% RL ($P = 0.006$ vs NS). The overall range of F1 + 2 was 2.8–1,675.9 pmol·L⁻¹ for RL and 2.3–154.4 pmol·L⁻¹ for NS. None of the dilutions $\leq 50\%$ RL contained elevated F1 + 2 (Figure).

Using the simulated blood transfusion model, no clots were present in the filters from any of the 12 units diluted with either NS or RL.

Phase 2

Filtration results of the serially diluted samples over the five time intervals are presented in Table 4. No clotting was observed in any of the dilutions at 30 or 60 min (95% confidence interval at each time period 0–7.2% overall, or

Figure Phase 1: F1 + 2 concentration (logarithmic scale) of various dilutions of packed red blood cells in Ringer's lactate (RL) or normal saline (NS). Samples were frozen and stored prior to analysis. Boxes represent the interquartile range, and dots indicate outliers beyond the 10th–90th percentile (brackets). * $P < 0.05$ from NS

Table 4 Phase 2: Number of units of PRBC diluted with RL showing clot formation ($n = 8$)

Percent RL	30 min	60 min	120 min	180 min	240 min
25	0	0	0	3	1
50	0	0	1	4	4
75	0	0	3	4	4
85	0	0	2	4	4
95	0	0	0	0	2

PRBC = packed red blood cells; RL = Ringer's lactate

Table 5 Total and ionized calcium concentration ($\text{mmol}\cdot\text{L}^{-1}$) of PRBC diluted with RL, in samples that showed clotting ($n = 4$) or no clotting ($n = 4$) in filtration study

Percent RL	Total Calcium		Ionized Calcium	
	Clotting	No clotting	Clotting	No clotting
0	0.50 ± 0.00	0.50 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
25	0.87 ± 0.03	0.74 ± 0.04	0.28 ± 0.03	0.30 ± 0.04
50	1.15 ± 0.03	1.06 ± 0.02	0.51 ± 0.02	0.58 ± 0.03
75	1.32 ± 0.02	1.28 ± 0.02	0.81 ± 0.05	0.85 ± 0.04
85	1.36 ± 0.02	1.34 ± 0.02	0.90 ± 0.01	0.92 ± 0.02
95	1.38 ± 0.02	1.39 ± 0.02	1.01 ± 0.01	1.02 ± 0.02

PRBC = packed red blood cells; RL = Ringer's lactate

0–31% for any given dilution). However, beginning at 120 min, evidence of clotting occurred in 4 of the 8 units.

The ELISA analysis of the samples (all diluted with RL) demonstrated F1 + 2 concentrations of $21.6 \pm 38.9 \text{ pmol}\cdot\text{L}^{-1}$ (mean $\pm$ SD) with a range of 2.0 – $228.7 \text{ pmol}\cdot\text{L}^{-1}$). These concentrations were all within the control reference range and below the lower end of the “physiologic” reference range. When compared with the values for the NS samples of Phase 1, there were no differences overall ($P = 0.60$) or at any level of dilution.

In the samples from the 4 units of PRBC that showed clotting after 120 min, total calcium was significantly higher and ionized calcium lower compared with samples that showed no evidence of clotting (Table 5). Differences were most notable in the dilutions of 50–75% RL ($P = 0.001$ for total and $P = 0.006$ for ionized calcium in 50–50 mixtures).

Discussion

Since July 2008, units of PRBC supplied by the CBS have been preserved with SAGM.¹⁰ This study was designed to determine whether RL causes clotting when used to dilute SAGM-preserved PRBC. Using a wide range of dilutions of PRBC with RL incubated for up to 60 min, we

demonstrated that there was no evidence of clotting, either at the macroscopic level or when using a molecular assay for indices of thrombin generation. Furthermore, no clotting occurred in a simulated model of rapid blood transfusion. However, clotting was observed to be present in some of the dilutions with RL, but not NS, when incubated for 120 min or more, while ELISA showed evidence of coagulation in some samples containing 75–85% RL that were stored for a prolonged period before analysis.

During the preparation of SAGM-preserved PRBC using the Buffy Coat method, whole blood is collected into bags containing CPD. These are centrifuged to produce platelet poor plasma, buffy coat, and red blood cells in three distinct layers. The PRBC are separated, and the additive/preservative solution SAGM is added. Compared with AS-3, the preservative formerly used by the CBS, SAGM-preserved PRBC lacks additional citrate beyond that contained in the CPD added prior to centrifugation.¹⁰

A number of studies have demonstrated advantages of RL over NS for initial resuscitation in hemorrhagic shock.^{1,2,14–16} The concern regarding the addition of RL to PRBC preserved with citrate as an anticoagulant relates to the calcium content of RL, which could potentially bind to the citrate to lead to clotting. Various approaches have been used in previous investigations to determine whether the dilution of PRBC with RL does cause clotting. Typically, blood mixed with NS or RL is filtered, and filter weights, flow rates, or visual inspection for clot formation are compared. King *et al.* utilized infusion pumps to push various mixtures of CPDA-1 preserved PRBC and crystalloid through filters, and found no significant difference in net filter weights between the samples diluted with RL and NS.⁷ This group also determined that an ionized calcium concentration of $\geq 0.23 \text{ mmol}\cdot\text{L}^{-1}$ is necessary to activate coagulation, corresponding to $> 100 \text{ mL}$ of RL added to a unit of PRBC. In a similar study, Cull *et al.* found clotting in mixtures of CPD-PRBC and RL only in dilutions with $> 50\%$ RL at up to 120 min of incubation. There was no significant difference in gravity transfusion flow rates between PRBC diluted with RL or NS.¹¹ Parlow *et al.* filtered solutions of CPDA-PRBC and RL or NS.¹⁷ Macroscopic clot debris was present in samples diluted with $> 70\%$ RL by volume and incubated for 30 to 60 min. No clot formation was visualized in dilutions that would be used clinically.

Using another approach, Rosenblatt *et al.* diluted supernatant plasma from red cell concentrates with varying amounts of RL, and used an ELISA technique to measure thrombin generation through quantification of the presence of F1 + 2 (the breakdown fragments following thrombin generation).¹⁸ The presence of F1 + 2 was detectable in very small quantities only in the samples in which the AS-3 PRBC supernatant was diluted with RL in a ratio of 1:20

and 1:10. In clinically relevant dilutions of 2:1 or higher, no F1 + 2 could be detected. Using these principles in a more clinically relevant model, Albert *et al.* tested for activation of the clotting cascade in AS-3-preserved PRBC using both filtration and ELISA analysis of F1 + 2.¹² No clotting was observed in any of a wide range of dilutions of NS or RL. Additionally, ELISA results showed that F1 + 2 values for both NS and RL were below the previously determined physiological level.¹⁹

The current study examined SAGM-preserved PRBC, which contains less added citrate than AS-3 (Table 1), suggesting potentially less buffering capacity of calcium contained in RL.¹⁰ Consistent with this, we did determine, contrary to the findings of Albert *et al.*,¹² that clotting can occur with the addition of RL, although the time frame required for this occurrence was much longer than that of the typical rapid blood transfusion scenario.

Phase 1 of this study showed no evidence of clotting in the filters following 30-min incubation throughout the entire range of dilutions. This time frame was chosen to reflect a maximum time required for a “rapid” blood transfusion (usually somewhat quicker in the clinical setting). Similarly, no clotting occurred during the simulated rapid transfusion model, including during flushing of the intravenous tubing with RL. However, at the molecular level, ELISA analysis of samples frozen and stored for a prolonged period of time revealed elevated levels of F1 + 2 in 5 of the 12 units tested, indicating activation of the coagulation pathway.

The second phase of the study was added to examine the effect of incubation time on the prevalence of clotting. In this phase of the study, no evidence of clotting occurred within 60 min of incubation with RL, but following incubation of 120 min or longer, 4 of 8 units showed evidence of clot formation. During this phase, the ELISA analysis was carried out on fresh, rather than frozen and stored samples. Samples were incubated for 30 min, but by the time full processing and analysis had taken place, approximately 90 min had elapsed following mixing of the samples. In this time frame, no levels of F1 + 2 beyond physiologic concentrations were determined.

As noted, the samples of PRBC with RL that led to a sharp increase in F1 + 2 concentration were the mid-range dilutions (50-75% RL to PRBC, Figure). As found in previous studies, lower dilutions of RL:PRBC were less likely to lead to clotting, since insufficient amounts of calcium were available to overwhelm the chelating ability of the citrate. Conversely, the extreme dilutions of RL:PRBC (95-97.5% RL) were less likely to lead to clotting, likely due to insufficient clotting factors to initiate the coagulation pathway at those high dilutions, even in the presence of excess ionized calcium. Interestingly, the mid-range of dilutions of samples that showed clotting also

contained significantly lower ionized calcium concentration and higher total calcium than the non-clotting samples (Table 5). This observation confirms the role of the calcium ion added in the form of RL in activating coagulation. The decrease in ionized calcium in these samples strongly supports the mechanism of binding to residual citrate ion in the PRBC. The samples with the same dilutions that did not clot may have lacked sufficient clotting factors to allow coagulation to occur, such that ionized calcium was not decreased.

In the current study, a convenience sample size of 20 units of PRBC was analyzed. Similar to most *in vitro* studies, it is not possible to determine an absolute number of samples that would be considered optimal. Thus, in order to maximize the reliability of the study, multiple methods were used to detect clotting, including macroscopic examination, molecular analysis, and simulated transfusion using warmed solution. The filtration method proved sensitive enough to detect clotting, but only in some of the samples that were incubated for 120 min or more. Thus, we are confident that clotting would have been detected had it been activated at an earlier stage. When the results of Phases 1 and 2 for dilutions with RL incubated for 30 min are combined, there was no clotting seen in any sample (95% CI for all dilutions 0-2.4%, or 0-14% for any given dilution).

In Phase 2 of the study, we intended to repeat the ELISA analysis on samples that were fresh and unfrozen following 30 min of incubation. However, due to the sample preparation time, a total of at least 60 min had elapsed during which the generation of F1 + 2 fragments could have occurred prior to binding of the sample to the antibody on the ELISA plates. Regardless, none of these samples diluted with RL showed evidence of coagulation, whereas the longer periods of incubation and storage in Phase 1 clearly resulted in high F1 + 2 values. This gives strength to the conclusion that PRBC should be co-administered with RL only during rapid transfusion. Finally, many practitioners do not dilute SAGM-PRBC with crystalloid due to the lower hematocrit than previous PRBC preparations. Interestingly, this lower hematocrit may actually be protective against clotting when mixed with RL.⁷ However, in situations where a small bore intravenous catheter is required, it may be necessary to dilute blood. In addition, when intravenous infusion lines are primed and later flushed with crystalloid, there is ample time for mixture of the blood with the crystalloid.

In summary, in the time frame inherent in the setting of rapid blood transfusion, such as for volume resuscitation in the operating room or emergency setting, there was no evidence that RL leads to clotting of PRBC. When considering the advantages of RL over saline for resuscitation of a severely hypovolemic patient, our group, consisting of

anesthesiologists, hematologists, and transfusion medicine specialists, supports the safety of co-administration of RL with PRBC in this setting only. However, this study confirms previous observations that blood should not be mixed with RL during slow transfusions.

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Conflicts of interest None declared.

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