How Low Can You Go? Minimizing Citrate Levels in Whole Blood Storage

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INTRODUCTION

- Whole blood (WB) use for trauma resuscitation is increasing
- Early 20th century studies (Rous and Turner et al) established optimal citrate concentrations for use as a common anticoagulant
- Military and far forward operations may require storage of empty anticoagulant-filled blood bags at temperature extremes
- The effects of cold exposure on the citrate concentration and minimum effective citrate required for whole blood preservation remains poorly defined

AIMS

- Evaluate the impact of reduced citrate concentrations on whole blood storage quality
- Define a minimum effective citrate concentration
- Assess the impact of cold exposure on empty anticoagulant-filled whole blood bag sodium citrate concentration

METHODS

- Whole blood citrate concentration gradient and storage lesion testing
  - Following IRB approval, whole blood samples were collected from 11 healthy volunteers and stored for 21 days
  - CP2D based sodium citrate concentrations tested: 4 mM (5%), 18 mM (20%), 31 mM (35%), 45 mM (50%), 63mM (60%), 72mM (80%), 90 mM (100%), 107mM (120%), and 125mM (140%)
  - Whole blood testing: coagulation potential by rotational thromboelastometry (ROTEM), deformability by ektacytometry, lactate accumulation by iStat, and flow cytometry for band-3 and microvesicle accumulation
- Blood bag cold exposure
  - Stability testing by Prompt Praxis Laboratories (Vernon Hills, IL) for mass spectrometry analysis
  - Testing groups: room temperature control and cold exposure (constant cold at -51° C and freeze thaw cycling between 21° C to -40° C)

RESULTS

	5% (4 mM)	20% (18 mM)	35% (31 mM)	50% (45 mM)	100% (90 mM)
Day 1	#5, #6	#5, #6	-	-	-
Day 14	All	#2	-	-	-
Day 21		#6	-	-	-

Table 1: Citrate concentration gradient (4 mM, 18 mM, 31 mM, 45 mM, and 90 mM) subjects in CP2D storage solution with visible whole blood clot formation over storage time points of day 1, 14, and 21

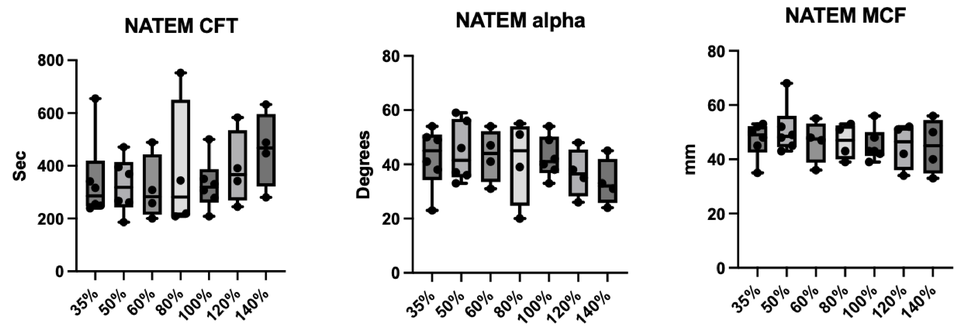

Figure 1: Rotational thromboelastometry analysis of citrate gradient whole blood samples after 21 days of storage by NATEM clotting time, clot formation time, alpha angle, maximum clot firmness. * indicates significance of $p<0.05$.

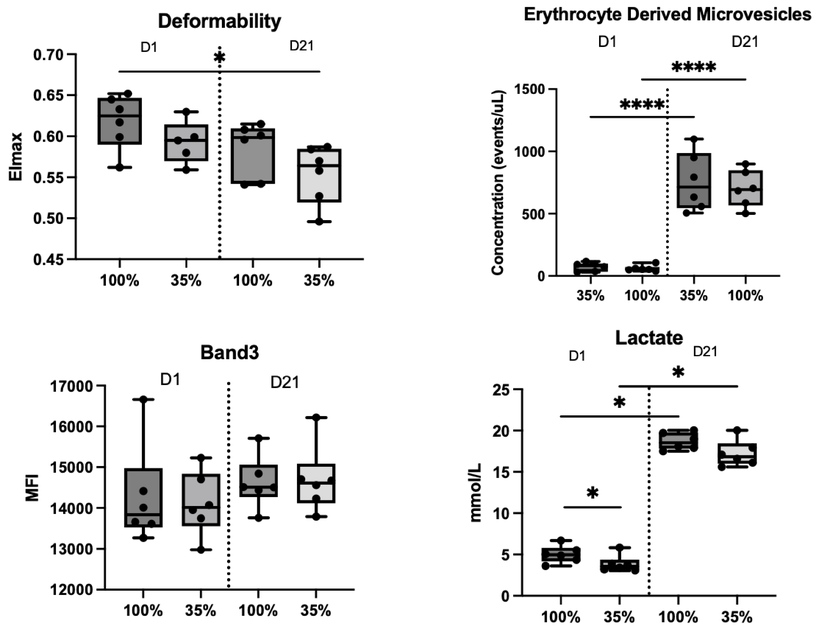

Figure 2: Storage lesion parameters of whole blood stored in standard citrate concentration compared to minimum effective concentration of 35% of standard. (A) Deformability (B) Band-3 expression (C) RBC derived microvesicles (D) Lactate. * indicates significance of $p<0.05$.

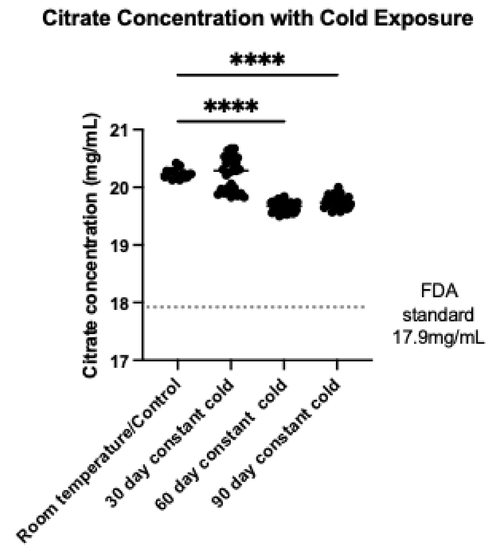

Figure 3: Concentration of sodium citrate from pre-filled whole blood storage bags after control and cold exposure of 30, 60, and 90 days of constant cold exposure at -51° C. * indicates significance of $p<0.05$.

CONCLUSIONS

- This study identifies the minimum effective citrate concentration where coagulation and storage parameters remain comparable across 21 days of storage
  - Minimum effective citrate concentration for 21 days of whole blood storage = 35% (31mM)
  - Cold exposure resulted in lower citrate concentrations; however, none were below the FDA approved ±10% range or below our established minimum effective concentration
- Cold storage of whole blood bags and reduced citrate concentrations do not appear to impact the storage or performance of whole blood

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