Characteristics of thawed pooled cryoprecipitate stored at refrigerated temperature for 24 hours

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Background. The need for thawed cryoprecipitate is growing. However, according to current guidelines, the shelf-life of pooled thawed cryoprecipitate at room temperature is limited because of possible bacterial contamination and loss of clotting factor activity. Here we assessed microbial growth and retention of clotting activity in cryoprecipitate stored at 4 °C after thawing to see whether its shelf life could be safely extended.

Materials and methods. Pooled thawed cryoprecipitate units (n=10) were maintained at room temperature for 6 hours and then placed at 1-6 °C for 18 hours after thawing. We examined the cryoprecipitate pools for fibrinogen, factor VIII, and von Willebrand factor activity at the following time points: 0 hours (immediately after thawing), after 6 hours at room temperature, and after 24 hours at 1-6 °C. A 5-mL aliquot from each pool was collected for aerobic and anaerobic bacterial cultures at the 24-hour time point.

Results. Mean fibrinogen concentration and von Willebrand factor activity were similar at each time point, but factor VIII activity decreased significantly over the storage period. Bacterial growth was not detected in any cultured pooled sample.

Discussion. Extended storing of thawed cryoprecipitate at 1-6 °C does not appear to increase the risk of bacterial contamination or affect coagulation factor activity.

Keywords: fibrinogen, factor VIII, von Willebrand factor.

Introduction

Cryoprecipitate is made from fresh-frozen plasma and contains fibrinogen, factor VIII, von Willebrand factor (VWF), factor XIII, and fibronectin. Although originally used to treat bleeding disorders such as haemophilia A and von Willebrand's disease, cryoprecipitate is currently used primarily to replenish fibrinogen levels and to manage major bleeding because of its effective haemostatic properties. Recent clinical studies in bleeding management indicate the importance of maintaining fibrinogen content at a higher level (150-200 mg/dL) than that previously established as a therapeutic set point (100 mg/dL)[1,2]. Thus, cryoprecipitate use has increased, especially in treatment areas in which large-volume bleeding is managed, such as operating rooms, obstetric practice, and emergency departments. Moreover, cryoprecipitate is being increasingly used as an early intervention in an algorithm-driven option to maintain effective haemostasis in massive transfusion protocols[3,4]. In the European Union, cryoprecipitate is not available for clinical situations in many countries in which solvent/detergent-treated fibrinogen concentrate is the only form of fibrinogen available for therapeutic use[5]. However, cryoprecipitate remains a therapeutic option for fibrinogen replacement in the United Kingdom, the United States of America, and other countries such as Canada, Brazil, and Australia[6]. Cryoprecipitate is preferred by many facilities because it is less expensive than fibrinogen concentrate and contains additional therapeutic constituents (e.g., factor XIII)[7,8].

Current Food and Drug Administration guidance and AABB (formerly, the American Association of Blood Banks) standards require that cryoprecipitate must be transfused within 6 hours after thawing when individual bags are stored at room temperature or within 4 hours after pooling. The short shelf-life is due primarily to concerns of loss of clotting activity of the labile factors. Presently, cryoprecipitate is used more for its fibrinogen content in attaining haemostasis in bleeding patients or patients at risk for bleeding. Cryoprecipitate transfusion requires careful planning and coordination because of the limited shelf-life of the product; a patient's status may quickly change, eliminating the need for an already thawed cryoprecipitate, thus leading to product waste because of this limited shelf-life. According to the AABB National Healthcare Safety Network, the wastage rate is 7% (range, 0-33%) among reporting participants[9].

Thus, developing strategies to reduce potential product waste is important.

One strategy is to increase the storage time after thawing for cryoprecipitate. In a study of the properties of thawed cryoprecipitate, Green *et al*.[10] found no statistically significant decrease in fibrinogen, factor VIII, factor XIII, or VWF activities in cryoprecipitate units that had been stored at 20-24 °C for up to 24 hours[10]. However, extended storage of thawed cryoprecipitate at room temperature may increase the risk of bacterial contamination. Contamination may also occur as a consequence of the processes used to thaw and store cryoprecipitate. Although there are case reports of *Pseudomonas* septicaemia after plasma transfusion[11,12], the risk of bacterial contamination should be negligible with proper unit handling techniques, such as sterile docking, plastic bag overwraps, and sterilisation of 37 °C water baths. Thus, in the present study, we examined microbial growth and the retention of clotting factor activities in cryoprecipitate stored at 4 °C for up to 24 hours after thawing.

Materials and methods

Cryoprecipitate units were prepared using centrifugation techniques. Donor plasma was frozen within 8 hours of collection. Fresh-frozen plasma units were stored overnight at 1 °C to 6 °C to yield the cryoprecipitate. The units were centrifuged at 4,500 *g* for 15 minutes, and the liquid plasma was expressed off to render wet cryoprecipitate. These units were frozen within 1 hour. Individual cryoprecipitate units were thawed in a water bath with overwrap at 37 °C. The thawed cryoprecipitate units were combined into ten pools of five units each by using sterile docking devices. We maintained the cryoprecipitate pools at room temperature for 6 hours and then stored them at 1 °C to 6 °C for 24 hours after thawing. We examined the cryoprecipitate pools for fibrinogen, factor VIII and VWF activity at the following time points: 0 hours (immediately after thawing), after 6 hours at room temperature, and after 24 hours at 1 °C to 6 °C. A 5-mL aliquot from each pool was collected for aerobic and anaerobic bacterial culture at the 24-hour storage time point. Fibrinogen activity was examined using the Clauss method (a functional assay based on the time for fibrin clot formation), and factor VIII activity was assessed using a one-stage partial thromboplastin time assay. VWF ristocetin cofactor activity (concentration and functional activity of the plasma VWF) was measured on a Siemens BCS-XP analyser (Tarrytown, NY, USA).

A repeated measure analysis of variance (ANOVA) was performed to assess the intra- and inter-variability among the cryoprecipitate bags for fibrinogen, factor VIII, and VWF activity. Wilk's λ was used to determine whether there was a statistically significant effect of time of storage on fibrinogen, factor VIII, and VWF activity; partial η^2 was used for the effect size. A p-value <0.05 was considered statistically significant.

Results

Cryoprecipitate units were clear of precipitate on initial thawing. After refrigeration for 24 hours, we observed precipitates but were able to easily resuspend the aggregates into solution by placing the refrigerated units in a 37 °C water bath. The average volume of the 10 bags of pooled cryoprecipitate was 122.7±16.2 mL. The mean values for fibrinogen and factor VIII levels and for ristocetin cofactor activity are presented in Table I. Fibrinogen content

Table I - Fibrinogen, factor VIII, and von Willebrand factor activity at 0 hours and after 6 hours at room temperature and 24 hours at refrigerated temperature.

	Mean ± SEM	95% confidence interval	
		Lower bound	*Upper bound*
Fibrinogen (mg)			
0 h	2,266.78±167.15	1,888.66	2,644.90
6 h at RT	2,240.71±144.69	1,913.41	2,568.01
24 h at 1-6 °C	2,193.18±148.99	1,856.15	2,530.22
Factor VIII (%)			
0 h	421.82±47.53	314.30	529.34
6 h at RT	376.32±33.78	299.90	452.74
24 h at 1-6 °C	350.14±30.98	280.06	420.22
VWF activity (%)			
0 h	859.83±121.47	585.05	1,134.61
6 h at RT	775.92±142.49	453.59	1,098.25
24 h at 1-6 °C	804.34±104.29	568.41	1,040.27

SEM: standard error of mean; h: hours; C: Celsius; RT: room temperature; VWF: von Willebrand factor.

was determined to be constant at 0, 6, and 24 hours (at different temperatures) in the ten cryoprecipitate pools (Wilks's λ=0.52, F[2, 8]=3.66, p=0.07, partial η^2=0.48). Statistical analysis revealed no change in ristocetin cofactor activity between 0 hours (at room temperature), 6 hours (at room temperature), and 24 hours at 1 °C to 6 °C in ten bags of pooled cryoprecipitate (Wilks's λ=0.72, F[2, 8]=1.55, p=0.27, partial η^2=3.09). However, repeated measures ANOVA analysis showed a significant decrease in factor VIII activity over the storage period (Wilks's λ=0.46, F[2,8]=4.73, p=0.04, partial η^2=0.54). Bacterial growth was not detected in any of the pooled aliquots submitted for culture.

Discussion

The results of our study indicate that storage of cryoprecipitate for extended shelf-life intervals has no effect on fibrinogen levels or VWF activity but may significantly decrease factor VIII activity levels. In addition, we found that the 24-hour storage period did not result in bacterial contamination of the cryoprecipitate. Thus, our study indicates the safety of a longer storage period for thawed cryoprecipitate.

The primary clinical purpose of cryoprecipitate is to support fibrinogen levels, but the current limits on its shelf-life seem more relevant to the historical role of cryoprecipitate as a factor VIII concentrate. Current Food and Drug Administration and AABB standards appear overly restrictive and may contribute to the high levels of product waste reported in the AABB Biovigilance programme[9]. As early as 1990, Saxena *et al.*[13] reported that the shelf-life of thawed cryoprecipitate may be safely extended for up to 24 hours. AABB standards currently allow use of thawed fresh-frozen plasma that has been stored for up to 5 days at 1 °C to 6 °C. The use of this practice for plasma provision has not been associated with an increase in cases of bacterial contamination. Sidhu *et al.*[14] reported no bacterial growth in thawed fresh-frozen plasma stored at 1 °C to 6 °C for 5 days after thawing. In the ANZSBT 2013 publication on Extended Life Plasma (ELP), sterility studies on 107 ELP bags stored under controlled conditions at 2 °C to 6 °C showed no bacterial growth in any sample after 7 days, and the same testing was performed on a total of more than 200 ELP bags after the initial results[15]. In addition, cryo-poor plasma has been shown to remain sterile after being stored for up to 120 hours at 1 °C to 6 °C[16]. Thus, there is little to no apparent risk of bacterial contamination in cold-stored, non-pooled plasma products.

Because cryoprecipitate units must be pooled as part of the preparation for transfusion, the risk of bacterial growth from product contamination associated with pooling must be considered. Recently, Canadian Blood Services reported significant bacterial growth in cryoprecipitate units that had been spiked with bacteria and maintained at room temperature[17]. Although bacteria grow in spiked samples stored at room temperature, the low inherent risk of bacterial contamination in cell-free products such as plasma and cryoprecipitate may be a mitigating factor. Pooling does add risk for contamination; however, pooled plasma maintained for up to 24 hours is commonly used in some therapeutic apheresis centres.

Green *et al.*[10] showed that the haemostatic characteristics of fibrinogen and factor VIII in thawed cryoprecipitate pools stored at ambient temperature for up to 72 hours were stable but reported statistically significant reductions in factor VIII levels after 24 hours, which is similar to our findings. However, that study did not assess the sterility of the product during the extended period of storage. In contrast, a strategy of storing cryoprecipitate at 1 °C to 6 °C affords additional protection against bacterial growth.

Bacterial organisms that contaminate blood components need time and appropriate conditions to grow to detectable levels in blood products[18]. Therefore, in our study, we simulated conditions in which cryoprecipitate units were maintained at room temperature for a minimal time followed by storage at 1 °C to 6 °C for a total of a 24 hour. Our finding of no detectable bacterial growth supports the notion that cryoprecipitate is a non-cellular blood component with negligible risk of contamination. Additional culture data will help to bolster bacterial safety claims; however, the current practice of storing a similar product such as thawed plasma for up to 5 days for plasma distribution suggests that undue concern is not warranted[19,20]. These data and those of Green *et al.*[10] suggest that revision of current Food and Drug Administration and AABB guidelines could result in greater efficiency of cryoprecipitate utilisation without undue risk. This could be of significant importance as the use of cryoprecipitate products increases.

Our study has limitations pertaining to the small sample size. The primary intent of this study was to serve as a proof of principle. These findings should be verified in a larger number of samples to ensure the safety profile before wide-scale clinical application. Given the rarity of plasma bacterial contaminants, testing the large number of components required for establishing a definitive safety profile and using methods to increase yield such as a larger sample volume may not be practicable.

Conclusions

Our findings suggest that extended storage thawed cryoprecipitate may be considered a safe product similarly to extended storage thawed plasma, but

these results need confirmation in larger studies. This approach may increase product availability to treat patients with bleeding in a timely manner.

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Authorship contributions

AB and MR designed the study. LK conducted the laboratory testing included in the study. ES performed the statistical analysis and wrote the manuscript with contributions from AB.

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