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Brief Clinical Communication

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Ex vivo evaluation of the efficacy of canine fresh-frozen plasma thawed using a microwave plasma defroster

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Abstract

Background – Commercial microwave plasma defrosters (MPDs) are used globally in human medicine to safely thaw fresh-frozen plasma (FFP), but this technology has never been tested in a veterinary setting. This study was undertaken to assess the efficacy of a commercial MPD for the rapid thawing of canine FFP.
Study Design – Twenty-three units (twelve 120 mL and eleven 240 mL) of canine FFP were thawed using an MPD. Time-to-thaw and pre- and postthawing temperatures of the units were measured. Clotting factor activities (factors II, V, VII, VIII, IX, X, and von Willebrand factor), fibrinogen concentrations, prothrombin times, and activated partial thromboplastin times were measured.
Key Findings – The evaluated MPD effectively thaws plasma quickly for both 120 mL units (2.7 ± 0.08 min) and 240 mL units (3.9 ± 0.15 min) while maintaining clinically relevant activities of clotting factors and fibrinogen concentration. While some measurements of factor VIII activity fell below the reference interval, none fell below 40%. One 240 mL unit had von Willebrand factor activity <70%. There was no evidence of excessively heated plasma to indicate a safety concern.
Significance – The MPD evaluated in this study provides a useful means to rapidly thaw canine FFP for correction of factor-deficient coagulopathy.

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Keywords: clotting factors, coagulation, coagulopathy, critical care, dog, transfusion

Abbreviations

aPTT	activated partial thromboplastin time
FFP	fresh-frozen plasma
MPD	microwave plasma defroster
PT	prothrombin time
vWF	von Willebrand factor
WWB	warm water bath

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Introduction

Coagulopathy due to relative factor deficiency is common in critically ill or injured dogs. Causes of coagulopathies within the canine population include consumption of clotting factors due to hemorrhage or disseminated intravascular coagulation, vitamin K epoxide reductase antagonist rodenticide intoxication, acute traumatic coagulopathy, massive transfusion, or iatrogenic dilution secondary to aggressive resuscitation with fluids.[1,2] Replacement of clotting factors with fresh-frozen plasma (FFP) is recommended as part of the initial treatment of active or imminently life-threatening bleeding.[1] Conventionally, a recirculating warm water bath (WWB) is set to a temperature of 37°C and used to thaw FFP. Use of higher temperatures to decrease thawing time is contraindicated because warmer temperatures denature clotting factors and can trigger the precipitation of fibrinogen and other proteins.[3] The time required to thaw FFP via WWBs can result in significant

time delay to transfusion. This delay increases risk to dogs with life-threatening hemorrhage and can delay life-saving surgical interventions in the coagulopathic patient.[4]

To address the need for rapid administration of FFP in critically ill patients, microwave technology has been adapted to safely defrost FFP. Microwave defrosting of plasma was described in human medical literature as early as 1974 and in veterinary literature as early as 1987.[5,6] Purpose-built microwave plasma defrosters (MPDs) provide a proven method for providing FFP to human patients significantly faster than WWBs.[7] These MPDs have been implemented in human medical settings throughout the United States, Europe, and Asia. To the authors' knowledge, there are no studies evaluating the use of purpose-built MPDs in veterinary medicine.

The purpose of this study was to assess a commercial, purpose-built MPD,[a] to assess its ability to (1) rapidly thaw canine FFP to an appropriate temperature for transfusion and (2) maintain clinically useful clotting factor activities and fibrinogen concentration.

Figure 1: Image of the front and inside of the Flash Thaw-Vet, a microwave plasma defroster used to thaw plasma products for transfusion (image courtesy of Color Instruments, Inc.).

Materials and Methods

Twelve 120 mL and eleven 240 mL units of canine FFP were supplied by a commercial veterinary blood bank[b] and shipped to the study location using standard shipping practices with a shipping cooler and dry ice. Each unit was kept frozen in a consumer-grade, top mount refrigerator[c] at –20°C until the study was performed.[3] After removal from the freezer, unit surface temperatures were recorded via rapid calorimetry with infrared[d] and surface thermocouple[e] thermometers. The FFP was placed in a protective shield and defrosted in the MPD per manufacturer directions (Figure 1). The microwave's magnetron (which generates microwaves) operates via manufacturer proprietary algorithm while an FFP unit is mechanically rotated throughout the thaw cycle. Thaw cycles automatically cease when a FFP unit surface temperature of 20°C (chosen by the manufacturer) is detected by an infrared sensor within the rotating arm. Time-to-thaw was recorded with a stopwatch.

After a single automated thawing cycle, each unit was inspected for the presence of any visible solids (frozen plasma), discoloration, or bag defects. The thawed FFP unit temperatures were recorded immediately postthaw via infrared and thermocouple surface thermometers and a thermocouple probe[f] inserted into the access port to obtain a rapid temperature from inside the unit. The FFP units were gently manually agitated and samples were collected from each storage bag using aseptic technique and transferred into 1 mL sterile, additive-free sample vials.

The vials were immediately refrozen at –20°C and shipped to the Cornell University Comparative Coagulation Laboratory per laboratory recommendations within 24 hours. The following tests were performed: prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration, factor activity for clotting factors II, V, VII, VIII, IX, X, and for von Willebrand factor (vWF) using previously described laboratory methods.[4]

Statistics

The authors performed a power calculation to determine that a sample size of ≥11 units of each size tested were required to obtain representative clotting factor activities for each group with a confidence interval of 99.9%. All data were manually collected and analyzed with commercially available software.[g,h] A P value of <0.05 was considered significant.

Tests of normality were performed using Shapiro–Wilk tests, which identified the following datasets to be nonparametrically distributed: 120 mL unit PT, 240 mL unit factor VIII, 240 mL unit factor IX, and 240 mL unit frozen surface temperatures (infrared and thermocouple). All other datasets were normally distributed. Clotting factor activities, frozen surface temperatures (infrared and thermocouple), thawed surface temperatures (infrared and thermocouple), thawed internal temperatures, fibrinogen concentration, aPTT, and PT for 120 mL

Table 1: Results of thawing data using a microwave plasma defroster

Measurement (units)	Single units 120 mL	Double units 240 mL
Time-to-thaw (min)	2.7 ± 0.08*	3.9 ± 0.15*
Frozen temperature		
Infrared (°C)	−17.1 ± 0.98†	−16.6 ± 0.52 (-16.4)‡
Surface thermocouple (°C)	−7.34 ± 1.44†	−8.06 ± 0.77 (-8.4)‡
Thawed temperature		
Infrared (°C)	19.7 ± 0.68*	18.2 ± 0.66*
Surface thermocouple (°C)	19.6 ± 0.44*	18.3 ± 0.69*
Probe thermocouple (°C)	19.4 ± 0.52*	18.1 ± 0.70*

All reported as the mean ± SD. Nonparametric datasets include median values within parentheses.
*$P < 0.001$ between 120 and 240 mL groups.
†$P < 0.001$ within 120 mL group.
‡$P < 0.001$ within 240 mL group.

unit and 240 mL unit groups with normally distributed data were compared with unpaired *t*-tests and nonparametric groups were compared using Mann–Whitney *U*-tests. A one-way ANOVA was used to compare postthaw surface temperatures (infrared and thermocouple) and internal temperatures within 120 and 240 mL unit groups.

Results

All samples were confirmed by visual inspection for complete thawing with no evidence of frozen plasma, no discoloration, and no visible damage to the bag. Plasma time-to-thaw and temperature measurements are presented in Table 1. Statistically significant differences were detected when comparing frozen surface infrared and thermocouple temperature measurements between and within 120 and 240 mL unit groups ($P < 0.001$). Postthaw surface temperatures (infrared and thermocouple) between 120 and 240 mL units were found to be significantly different for all groups ($P < 0.001$). However, there was no significant difference found between any thermometry modality (infrared and thermocouple) when comparing postthaw surface and internal temperatures within 120 and 240 mL unit groups.

The aPTT was within reference interval for all samples tested. The PT of a single 120 mL FFP unit was prolonged at 17.6 seconds and all others were within reference interval. Clotting factor activity for factors II, V, VII, IX, and X were within reference interval for all samples tested (reference interval: >50% factor activity). Fibrinogen concentrations were within reference interval for all samples tested. Factor VIII activity of 1/12 of the 120 mL units and 6/11 of the 240 mL units was between 40–49%. All 120 mL FFP and 10/11 of the 240 mL units had vWF activities within reference interval. See Figure 2 and Table 2 for details.

Discussion

The results of this study support the use of the tested MPD to rapidly thaw canine FFP because it maintains clinically relevant activities of clotting factors and fibrinogen concentration. Additionally, the times-to-thaw for both 120 and 240 mL units would permit rapid administration with conventional storage practices.

The prethaw temperatures read via surface thermocouple thermometer and infrared thermometers were

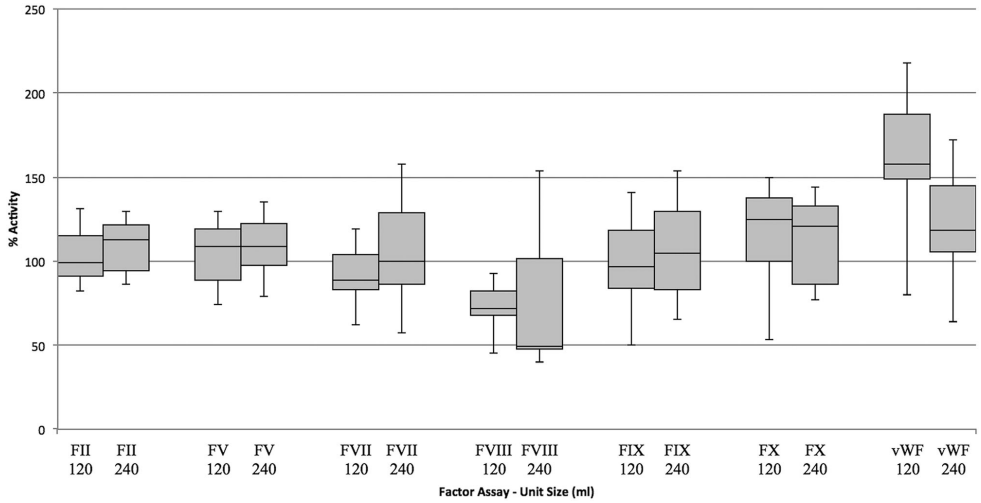

Figure 2: Box and whisker plot of coagulation factor activity of fresh frozen plasma units thawed in a microwave plasma defroster. Reference intervals for clotting factor activity for factors II, V, VII, VIII, IX, and X are >50%. Reference interval for vWF activity is 70–180%.

Table 2: Results of clotting factor activity and performance of functional clotting assays of FFP thawed in a plasma microwave defroster

Laboratory value (units)	Reference interval	120 mL units	240 mL units
FII (% activity)	>50	102 ± 15	110 ± 17
FV (% activity)	>50	103 ± 18	108 ± 17
FVII (% activity)	>50	93 ± 19	108 ± 31
FVIII (% activity)	>50	75 ± 20	84 ± 58 (49)
FIX (% activity)	>50	100 ± 36	117 ± 57 (105)
FX (% activity)	>50	119 ± 35	114 ± 28
vWF (% activity)	70–180	162 ± 37	134 ± 54
Fibrinogen (mg/dL)	150–490	298 ± 78	277 ± 73
PT (s)	11–15.5	12.9 ± 1.89 (12.3)	11.7 ± 0.93
aPTT (s)	8.5–15.5	12.9 ± 0.55	12.8 ± 0.64

FII, factor II; FV, factor V; FVII, factor VII; FVIII, factor VIII; FIX, factor IX; FX, factor X; vWF, von Willebrand factor.
All values reported as the mean ± SD. Nonparametric datasets include median values within parentheses. No statistically significant differences were found between 120 and 240 mL unit groups.

significantly different within 120 and 240 mL unit groups. The most likely cause of this discrepancy is the inability of the surface thermocouple to achieve the required large surface area of contact with the stiff, frozen bags. Albeit this initial discrepancy, the postthaw temperatures within groups agreed well between all 3 methods ($P > 0.68$), suggesting that the warm, flexible bags allowed better contact for the surface thermocouple thermometer. These findings highlight the advantage of verifying measurements with multiple modalities in experimental trials and may suggest the superiority of infrared thermometry in assessing frozen FFP units. Further studies are needed to establish a gold standard for rapid thermometry for similar research.

Factor VIII activity was lower than reference interval in 30% (7/23) of all study samples evaluated. Factor VIII is a labile factor and is thus sensitive to storage and heat. The decrease in factor VIII activity is of uncertain clinical relevance. Other studies have shown conventionally thawed plasma to have factor VIII activity similar to this study's results and considerable intradonor and interdonor variability in factor VIII activity regardless of thawing method.[4,8] Without paired samples thawed via WWB to compare factor VIII activity, it is unclear whether the factor VIII activity in this microwave-thawed plasma was less than that of traditionally (WWB) thawed plasma. Interestingly, there were more samples within the 240 mL unit group that fell below the reference interval (<50% factor activity) than in the 120 mL unit group despite no statistical significance. It would be expected that these larger volume units take longer to freeze and thaw due to their specific heat relative to volume, keeping the plasma above freezing and storage temperature longer than smaller units after initial collection. To the authors' knowledge, the 240 mL units in this study are the largest FFP units tested for hemostatic protein activity reported in the veterinary literature. Additional studies would be required to assess the clinical significance of this finding, especially when treating factor VIII deficiency (hemophilia A).

A weakness of this study is the lack of paired plasma units obtained from the same donor at the same time. This prevented direct comparison of microwave-thawed units to WWB-thawed units. However, the goal of the study was to determine whether clinically useful activities of clotting factors would be retained with microwave thawing in a clinical practice setting. The study was designed to reflect the common clinical practice of obtaining canine FFP from a third party blood bank with storage of this FFP under conditions commonly encountered in clinical practice. The clotting factor activities measured are comparable to the clotting factor activities of healthy dogs' pooled plasma at the laboratory, so it is reasonable to conclude that MPD-thawed plasma can provide necessary clotting factors, especially in urgent situations where WWB thawing is impractical.

Thawing FFP in anticipation of its need is sometimes practiced due to the time delay associated with thawing. Regardless of thawing method (WWB or MPD), storing at 4°C results in some unavoidable factor degradation, increases the risk of bacterial contamination, and can lead to waste of FFP.[4,9] MPDs eliminate the need to thaw FFP in anticipation of need, decrease exposure to WWB contaminants, and allow practices to conserve resources. This is especially important in practices that purchase plasma products.

A previous study evaluated the efficacy of a modified commercial microwave (a kitchen appliance) used to thaw FFP by attenuating the microwave energy with a plastic beaker filled with water on the center turntable.[8] This study showed significant decreases in activity of clotting factors II, IX, X, XI, vWF antigen, vWF collagen-binding activity, protein C, and concentrations of antithrombin and fibrinogen. These findings highlight the need for a purpose-built MPD designed for clinical use rather than modifications made to a kitchen microwave. Multiple human FFP studies demonstrate that purpose-built MPDs provide a safe, rapid method to thaw FFP while preserving clotting factors and albumin concentration, sometimes better than WWB control groups.[5,7,9]

Arguments have been made against the use of MPDs due to the perception of uneven heating that could result in localized protein denaturation within the FFP unit (often referred to as "hot spots"), but a study that used an array of surface thermometers and an internal thermometer showed no evidence of general or localized overheating.[10] Additionally, early

in their medical application, there was speculation that microwave energy could liberate dangerous amounts of phthalates (plasticizers) from the storage bags into the blood products. A comparison of MPD and WWB defrosted human FFP demonstrated no significant difference in phthalate levels between the 2 methods.[11]

Manufacturers of MPDs claim that there is significantly less FFP bag breakage due to the absence of warm water "shocking" and cracking the plastic. To the authors' knowledge, however, there are no controlled studies proving this phenomenon. Anecdotally, the authors have not encountered FFP bag breakage using the MPD tested in clinical practice but have encountered several bag breakages using a WWB. There is evidence that WWBs may provide a reservoir for bacterial growth and subsequent hospital acquired infection.[12]

MPDs provide a proven method to thaw FFP for human patients with less delay and fewer potential complications than WWBs while maintaining clotting factor activity. The current study confirmed the hypothesis that the studied purpose-built MPD is a useful option in clinical practice to rapidly thaw canine FFP while preserving clinically relevant clotting factor activities. Future studies are warranted to directly compare microwave and WWB methods for thawing canine plasma.

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Footnotes

[a] Flash Thaw-Vet, Color Instruments Inc., http://flashthaw.vet, Fort Lauderdale, FL.

[b] Blue Ridge Veterinary Blood Bank, Purcellville, VA.

[c] Top Mount Refrigerator (model #FRT18G4AWM), Frigidaire, Augusta, GA.

[d] Industrial infrared thermometer (model #IR-IND), ThermoWorks, American Fork, UT.

[e] Type-K thermocouple precision ribbon surface probe, ThermoWorks.

[f] Type-K thermocouple penetration probe, ThermoWorks.

[g] Microsoft Excel for Mac 2011, Redmond, WA.

[h] Real Statistics Resource Pack (Release 3.5.3), Charles Zaiontz, www.real-statistics.com.

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