

# Rapid thawing of fresh-frozen plasma with radio wave-based thawing technology and effects on coagulation factors during prolonged storage at 4°C

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## Vox Sanguinis

**Background and Objectives** With traditional techniques the thawing time for fresh-frozen plasma (FFP) is about 20–30 min. A new technology using radio waves, radio wave thawing device (RTD), was applied for thawing FFP. With this technology the thawing time can substantially be reduced. The new technique was compared to an established method using Heated Air Technology (HAT). Variables subjected to assessment were temperature after thawing and analysis of factor V (FV), factor VIII (FVIII), protein S.

**Materials and Methods** Plasma was collected from 20 plasma donors. Each donation was aliquoted into two equal units of approximately 250 ml. The plasma units were frozen and stored below –75°C. The thawing time was pre-set to two time periods, one for each group, 23 min for HAT and 7.5 min for RTD. Thawed plasma was stored at 4 ± 2°C as liquid plasma. Samples were collected before freezing, after thawing, 1 week and 2 weeks after thawing.

**Results** The FV and FVIII levels were over 90% direct after thawing compared to before freezing values in both groups. At 2 weeks of storage the levels of FV and FVIII were approximately 70% and 50%, respectively, compared to before freezing values. Protein S levels decreased slightly during storage. No significant differences in the decline of quality were observed between the two groups.

**Conclusion** The new radio wave technology for thawing of FFP has a significant reduction of thawing time. The impact of thawing and storage on FV, FVIII, protein S does not significantly differ between HAT and RTD.

**Key words:** FFP, factor V, factor VIII, protein S, storage, thawing.

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## Introduction

The main indication for administration of fresh-frozen plasma (FFP) to patients is to replace deficient or dysfunctional coagulation factors, for example, some inherited or acquired coagulopathies, massive blood transfusions with demonstrated coagulopathy and plasma exchange for thrombotic thrombocytopenic purpura [1]. Plasma transfusions are also

used in daily practice to control massive bleeding in combinations with red blood cell and platelet concentrates [2].

When acute, massive transfusions are demanded, the thawing procedure may cause delay in delivery of fluid plasma ready for transfusion. One approach to circumvent this potential hazard is to store plasma in fluid phase at 4°C, a component that can promptly be used for urgent transfusion. Several trauma centres provide thawed AB plasma in their emergency departments [3] and we have recently reported that leucocyte-depleted apheresis and whole blood plasma stored for 14 days retain sufficient levels of coagulation factor V (FV) and factor VIII activity (FVIII) for maintenance of normal haemostasis [4].

An additional approach to circumvent the potentially deleterious effect of a delay in the thawing procedure is to use a technique that substantially shortens the thawing time. With traditional techniques, such as heated air and waterbath methods, the thawing time for FFP is about 20–30 min. Trials with the microwave technique for blood components were carried out in the 1970s [5], but the use declined due to reports of overheating and generation of hot spots [6], even though attempts have recently been made to overcome these obstacles [7]. An alternative to microwave heating is the radio frequency (RF) heating technique. RF heating is an attractive process due to rapid, uniform heating with small temperature gradients resulting in a low risk for hot spots. This technique has successfully been introduced in the food industry with promising applications including thawing, post-baking and sterilizing food products [8,9].

Given thawing time being a critical step in FFP administration in emergencies and that RF heating is a potential alternative to conventional heating, we considered the use of RF heating for FFP thawing. We therefore applied a new radio wave based thawing technology and evaluated thawing time and levels of coagulation factors during prolonged storage at 4°C. The study demonstrates that radio wave thawing technology offers a potential alternative to conventional heating for rapid FFP thawing without any disadvantageous effects on levels of critical coagulation factors.

## Materials and methods

### Preparation of FFP units

Plasma was collected by automated plasmapheresis (Haemonetics PCS2) from 20 voluntary blood donors in a double blood bag system. Nine out of 20 donors were blood group A and 11 out of 20 blood group AB. The volume ratio of CPD-50/ blood was 1 : 15 and the collected plasma volume 600 ml including anticoagulant. Each donation was aliquoted into two equal units of approximately 250 ml. The plasma units were frozen and stored below –75°C within 8 h from collection.

### Thawing procedures

Two modes of thawing procedures were applied in the present study, the Heated Air Technology (SAHARA-III Basic model, Sarstedt, Nümbrecht, Germany), designated HAT [10] and the RF technology (radio wave thawing device), designated RTD. The HAT technique was used according to manufacturer's instructions. The thawing time was pre-set to two time periods, one for each group, 23 min for HAT and 7.5 min for RTD. The RTD prototype device consists of a cavity equipped with a power supply, a frequency generator and an antenna. It generates radio waves with a frequency of 135.5 MHz, corresponding to a wave length of about 30 cm in plasma (compared to the wavelength of microwaves, approximately 2 cm), minimizing the risk for standing waves and hot spots in the device. Contrary to microwaves, the radio waves applied in the RTD device have efficient penetration properties. Hereby, high amounts of energy can be passed into the frozen material in a short time resulting in very rapid thawing. Two patents related to the radio wave technology and the thawing device are granted in the US (US 5977532 and US 6191404) and Europe (EP 752195 and EP 934681) and is described more in detail at [esp@cenet](mailto:esp@cenet), patent number #DE69828949T.

Temperature assessments on thawed plasma bags surfaces were done after thoughtful mixing with the use of the RayTek, Model ST 20 PRO, infrared temperature measurement instrument (Raytek Corporation, Santa Cruz, CA, USA) used in accordance with the manufacturer's instruction. After thawing the plasma was stored at 4°C for 2 weeks. All bags were subjected to daily visual inspection with special attention paid to leakage and aggregates.

### Plasma samples

Plasma samples were collected from the final plasma bags with sterile docking techniques before freezing, after thawing, 1 and 2 weeks after thawing.

To allow standardized serial analysis, samples were collected in plastic tubes from Sarstedt (Nümbrecht, Germany) from each plasma unit and frozen at –74°C within 30 min at 0.5 ml portions until further use. When required for analysis, samples were thawed for 10 min at 37°C. All plasma analyses were done within 1 h of thawing.

### Assays

#### *Clotting factors and inhibitor assays were done with Behring Coagulation System*

Instrument (Dade Behring Marburg GmbH, Marburg, Germany). Factor V was analysed with a one stage clotting assay according to the manufacturer with reagents from Dade Behring.

Factor VIII activity was analysed with a two-stage FVIII assay using a chromogenic substrate, kits from Chromogenix

**Table 1** Baseline plasma bag data of the two study groups

	HAT <sup>a</sup> (n = 20)	RTD <sup>a</sup> (n = 20)
Volume (ml)	250 (246–263)	252 (247–265)
Thawing time (min)	23 (23–24)	7.5 (7.5–8.2)
Temperature before thawing (°C)	–77 (–80––73)	–77 (–80––73)
Temperature after thawing (°C)	9 (1–22)	18 (5–28)

<sup>a</sup>Heated air technology (HAT) and radio wave thawing device (RTD).  
Data are expressed as medians (ranges).

(Milan, Italy). Free protein S (PS) was measured immuno-chemically, after adsorption to C4BP coated latex particles, by means of kits from Chromogenix (Milan, Italy).

### Statistical analysis

Quality variables were measured at four instances, a baseline measurement before freezing, directly after thawing, 1 week after thawing and 2 weeks after thawing. A single baseline measurement was used for both thawing techniques. The sample from each donor was then divided in two parts of approximately equal size before freezing. The donor acts as its own control.

For each quality variable a linear mixed-effects model unstructured covariance structure was estimated using differences between the baseline measurement and the three measurements taken after thawing. Results are presented as difference of means between the two thawing techniques (HAT-RTD) regarding changes from baseline, with confidence intervals and *P*-values from the statistical model.

## Results

### Basic characteristics

As described in Table 1, the volume and the temperature before thawing did not differ between the HAT and RTD methods. The thawing time was pre-set to approximately 23 and 8 min with the HAT and RTD methods, respectively. All thawed plasma batches were clear and free from visible particles and aggregates. The registered temperature was the surface temperature after thoughtful mixing of the plasma bag. The temperature after thawing was higher with the RTD method.

### Plasma levels of coagulation factors

The levels of FV and FVIII after thawing were > 90% compared to pre-freezing values (Table 2). As expected, we

found a gradual decrease in FV and FVIII levels during storage in both groups. At 2 weeks of storage the levels of FV and FVIII were approximately 70% and 40%, respectively, compared to before freezing values. Protein S levels decreased slightly, during storage. We found no significant differences in FV, FVIII and PS between the two groups at any observation point (Table 2).

## Discussion

In the present study, we demonstrate that the use of a new RTD substantially reduces the thawing time without any significant disadvantageous effects to a traditional thawing technique with regard to levels of coagulation factors during storage at 4°C for up to 14 days. We therefore propose that this technology offers a potential alternative to conventional heating for rapid FFP thawing.

The thawing time of FFP constitutes a critical step to secure the supply of plasma in emergencies. In the present study, we demonstrate that the use of RF heating allows a substantial reduction in thawing time. Based on preliminary experiments we decided to pre-set the thawing time to 23 and 7.5 min for the heating air and RF heating techniques, respectively. Despite this difference the post-thawing temperature was higher after RF heating. It is therefore justified to assume that the thawing time may be further shortened with the use of this technique.

The RTD technique did not have any disadvantageous effects to thawing with heated air technology with regard to levels of coagulation factors. In addition, storage for up to 14 days did not have any significant different effect on the content of coagulation factors between the RTD and HAT. These data on the impact of storage on coagulation factors are congruent with previous reports on recovered and apheresis plasma in which FV and FVIII are the most labile factors [4,11–13]. The levels of FV and FVIII at 2 weeks of storage are considered sufficient for normal blood coagulation, since blood usually coagulates appropriately when coagulation factor concentrations are at least 20–30% of normal. This assumption is based on the observation that FFP should be given in doses calculated to achieve a minimum of 30% of plasma factor concentration when FFP is given as replacement of an entire blood volume [14]. Moreover, endogenous FVIII is produced in a higher concentration in healthy individuals under stress such as bleeding, trauma and surgery and we therefore suggest that the levels of coagulation factors meet the criteria for adequate haemostasis support in the majority of emergencies. The rationale for including PS analysis is that reduced levels in different plasma preparations such as solvent-/detergent-treated plasma and Octaplas have been reported [15]. A larger sample size could reveal significant differences between the groups but the narrow confidence intervals indicate that such differences would

**Table 2** Effect of thawing procedure on factor V, factor VIII and protein S levels during storage

	Median (range)		Mean difference <sup>b</sup> (95% CI)	P-value
	HAT (n = 20)	RTD (n = 20)		
FV (kU/l)				
Before freezing <sup>a</sup>	1.27 (0.88–1.63)	1.27 (0.88–1.63)	0.	0.
After thawing	1.24 (0.91–1.63)	1.26 (0.88–1.61)	-0.0065 (-0.0484; 0.0354)	0.7488
Day 7	0.98 (0.74–1.40)	1.06 (0.76–1.38)	0.0115 (-0.0249; 0.0478)	0.5155
Day 14	0.88 (0.69–1.30)	0.90 (0.69–1.30)	0.0090 (-0.0181; 0.0361)	0.4950
Factor VIII (kIU/l)				
Before freezing <sup>a</sup>	1.20 (0.59–1.83)	1.20 (0.59–1.83)	0.	0.
After thawing	1.11 (0.58–1.73)	1.13 (0.60–1.64)	0.0185 (-0.0403; 0.0773)	0.5182
Day 7	0.50 (0.30–0.82)	0.53 (0.30–0.76)	0.0125 (-0.0192; 0.0442)	0.4194
Day 14	0.47 (0.28–0.72)	0.50 (0.28–0.74)	-0.0110 (-0.0438; 0.0218)	0.4914
Protein S (kIU/l)				
Before freezing <sup>a</sup>	1.08 (0.67–1.40)	1.08 (0.67–1.40)	0.	0.
After thawing	1.00 (0.59–1.34)	1.00 (0.68–1.42)	-0.025 (-0.0646; 0.0146)	0.2016
Day 7	1.00 (0.65–1.37)	0.98 (0.62–1.34)	-0.001 (-0.0229; 0.0209)	0.9248
Day 14	0.97 (0.60–1.33)	0.98 (0.63–1.33)	-0.018 (-0.0538; 0.0178)	0.3061

HAT, heated air technology; RTD, radio wave thawing device.

<sup>a</sup>Same primary plasma bags were used for split to HAT and RTD.

<sup>b</sup>Results are presented as the difference of means between the two thawing techniques, HAT and RTD, regarding changes from baseline. A positive difference indicates a smaller change for HAT than for RTD and a negative difference the opposite.

most likely be very small and therefore not of any clinical importance.

Electromagnetic techniques in the form of microwave warming devices have been tried with the aim to obtain a rapid thawing of FFP. Due to problems with uneven warming and thereby risk for denaturation of plasma proteins [16], this thawing technique is practically abandoned, even though attempts have recently been made to overcome these obstacles [7]. The main reason for the problems associated with the microwave warming is the short wave length that causes standing waves in the device and the fact that the resonance frequency of water is very close to the microwave frequency. Hence the melted water phase absorbs the supplied energy easier than the remaining ice thereby causing marked temperature gradients. An additional problem is the poor penetration of microwaves into the material to be thawed.

These draw-backs have essentially been taken care of with the radio wave technique. This technique is based on longer wavelengths, approximately 30 cm as compared to the wavelength of microwaves, approximately 2 cm. The radio wave technique was developed in the food industry to reduce the time for the centre of the heated material to reach desired temperature and to create a uniform temperature distribution [8,9]. This indicates that the difference between core and surface temperature is modest. However, additional temperature assessments to exclude the risk of 'hot spots' are to be done

before the technique is available for commercial use. In the present study, we also analysed labile coagulation factors that are frequently included in studies that evaluate plasma quality. These analyses are also to be completed with additional coagulation factors and other key factors in plasma, for example, ADAMTS13.

In emergencies there is a potential hazard in the supply of fluid plasma. Approaches to overcome this hazard are either to have fluid plasma in stock or to enable a rapid thawing of FFP. Two obvious advantages exist for keeping plasma stored at 4°C for 2 weeks. The procedure facilitates the plasma stock control and its availability for urgent transfusion. As a consequence the units that expire and go into discard will be reduced as well as the time-consuming thawing procedure.

We conclude that leucocyte-depleted apheresis plasma thawed by the RTD technique and stored for 14 days have the same level of critical coagulation factors as plasma thawed by heated air technology. We therefore propose that thawing based on this technology might become an alternative technique to reduce the thawing time for frozen plasma.

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