Thawing of Pooled, Solvent/Detergent-Treated Plasma octaplasLG®: Validation Studies Using Different Thawing Devices

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Keywords

Solvent/detergent treated plasma · S/D plasma · octaplasLG · Plasma thawing · Biochemical profile

Summary

Background: The aim of this study was to perform validation of the thawing process for solvent/detergent-treated plasma octaplasLG® using different thawing devices. Optimized settings for temperature and thawing time should be defined based on the results of both temperature measurements and extensive biochemical characterization studies. **Methods:** octaplasLG units were thawed using water bath systems (i.e. MB-13A, Quick-Thaw® DH4), dry tempering systems (i.e. plasmatherm, SAHARA-III), and microwave oven (i.e. transfusio-therm® 2000). Optimized thawing conditions were defined. Subsequently, using the selected thawing conditions, octaplasLG units were thawed and tested on product release parameters. **Results:** The fastest thawing was observed for the microwave oven. All octaplasLG units thawed by different devices and optimized thawing conditions were clear and free of solid and gelatinous particles, indicating no protein denaturation or overheating. In addition, no significant differences were found in the coagulation and inhibition activity and hemostatic potency of octaplasLG when thawed by the different devices tested. All parameters after thawing were within the product release specification levels. **Conclusion:** Our study demonstrated that octaplasLG can be thawed using all above listed devices without any negative influence on the plasma quality, presupposed that optimized settings defined for this plasma product are used.

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Introduction

Proper and standardized storage and thawing procedures are presupposed to maintain the quality of human fresh frozen plasma (FFP), as specified by guidelines for the use of human blood and blood component products [1–6]. Frozen blood products must be thawed at 30–37 °C [1–3] or 37 °C [4–6]. Thawing at higher temperatures may lead to protein destruction and precipitation, and severe transfusion reactions in the patients [6–7]. On the other hand, thawing at 1–6 °C or lower temperatures may form insoluble cryoprecipitated components, thereby reducing the clotting properties of the product [1, 4, 8, 9]. There are several ways for thawing of FFP. The most common thawing process uses a recirculating water bath [1–5]. In some countries like Germany [6], validated dry heating systems should be used to avoid potential microbial contamination.

Selection of appropriate thawing devices in hospital use depends on both the maximum number of plasma units thawed in parallel (i.e. thawing of single bags vs. large volume plasma exchange) and the urgency of thawed plasma bag requirements (i.e. for planned operations vs. in emergency situations).

Pooled, solvent/detergent-treated plasma octaplas® was developed as an alternative to FFP with the aim to increase pathogen safety during plasma transfusion [10, 11]. octaplas was first licensed in Europe in 1992. The second-generation octaplasLG® product with implemented prion removal step is available in Europe, Canada, and the USA [12, 13]. octaplasLG is a pharmaceutical product, thus, guidelines issued for human blood and blood components are not obligatory for this product. The aim of this study was to perform validation of the octaplasLG thawing process using different thawing devices available on the market, including water bath systems, dry tempering ovens and microwave oven. For each thawing device optimized settings for temperature and thawing time were defined based on multiple temperature measure-

ments during the thawing procedure. The quality of octaplasLG after thawing, using the different thawing devices and optimized predefined thawing conditions, was investigated and confirmed. All relevant product release parameters were tested, supplemented by investigation of temperature-sensitive parameters and markers of coagulation activation.

Material and Methods

Plasma Units

octaplasLG used in the thawing studies was produced at Octapharma AB, Stockholm, Sweden [12–14]. Seven octaplasLG batches of different blood groups (i.e., 2 A units, 1 B unit, 2 AB units AB and 2 O units) were thawed per thawing device used.

According to the manufacturer instructions, octaplasLG should be stored at -18°C or lower temperatures. Preliminary studies showed that the storage temperature from -18°C up to -70°C has no significant impact on the thawing times (data not shown). In this study, all octaplasLG units were pre-stored at $-28 \pm 3^{\circ}\text{C}$ before thawing. octaplasLG units are delivered sealed in a secondary, heat-sealed outer wrapper, which protects the infusion parts and plasma product from possible contamination when thawing in a water bath system. During the studies, all octaplasLG bags were thawed in the outer wrapper independent of the thawing device used.

Thawing Devices

Conventional open water bath system (i.e. MB-13A) from Julabo GmbH, Seelbach, Germany, was used as control thawing device in the study. Alternative thawing devices included the open water bath circulator from Helmer Scientific US (QuickThaw[®] DH4; supplier Hettich AG, Bäch, Switzerland), the plasmatherm dry tempering system from Barkey GmbH & Co. KG (Leopoldshöhe, Germany), the SAHARA-III dry tempering system from Sarstedt AG & Co (basic model, supplier Transmed Medizintechnik GmbH & Co. KG, Bad Wünnenberg, Germany) and the transfusio-therm[®] 2000 microwave oven from EIC Umwelt- und Medizintechnik Ltd (Heilbad Heiligenstadt, Germany). The maximum thawing capacity of the devices was 3 units (for SAHARA-III and transfusio-therm 2000) or 4 units (for QuickThaw DH4 and plasmatherm). All alternative thawing devices included agitation of bags as well as temperature controls; end of thawing was indicated by optical and/or acoustical signals. In the conventional water bath no agitation of bags was possible.

Temperature Measurements

Product surface temperature was measured by a calibrated infrared-thermometer (testo-831, Testo GmbH, Vienna, Austria) and recorded every 3 min during the thawing process. In addition, the product was optically inspected every 3 min to be free of ice. Finally, the plasma temperature within the bag was determined at the end of the thawing process by introducing a calibrated thermometer (i.e., testo-110, Testo GmbH) into the plasma. For all devices, times to defrost plasma (i.e., 'ice-free') and to reach 30°C product temperature were defined.

Measurement of Plasma Quality after Thawing

octaplasLG units were thawed by different thawing devices and optimized settings. octaplasLG after thawing was divided into several aliquots, rapidly frozen and stored at $\leq -28 \pm 3^{\circ}\text{C}$ until parallel testing. No plasma aliquots were re-frozen again.

Thawed octaplasLG units were first visually inspected on clarity and the presence of solid and gelatinous particles. Subsequently, plasma aliquots were tested on product release parameters.

Visual control, pH, osmolality, activated coagulation factors (NaPTT), coagulation factors II–XI (FII–XI), ADAMTS13, protein C, protein S, and plasmin inhibitor (also known as α_2 -antiplasmin) were assessed according to European Pharmacopeia (Ph. Eur.) methods. Total protein levels were quantified by the Biuret method, activated partial thromboplastin time (aPTT) was tested by

coagulation test, while fibrinogen was tested by the Clauss method. As marker of coagulation activation, activated FVII (FVIIa) was tested using a prothrombin time-based clotting assay (Diagnostica Stago, Asnières, France). Finally thrombin generation assay (TGA) was performed using the Technothrombin TGA test kit and the RC High trigger from Technoclone GmbH (Vienna, Austria). For more details of the test methods see [12, 16].

The Student's paired t-test was used to assess statistically significant differences between the quality of octaplasLG units thawed by the conventional water bath circulator versus alternative thawing devices and those thawed for 30 min versus 60 min. A p value of < 0.05 was considered statistically significant.

Results

Validation of Thawing Conditions

Thawing of octaplasLG units was started as soon as the set thawing temperature in the device was reached, as indicated by the temperature display (for water bath and dry tempering systems) or protocol printer (for dry tempering systems). For the microwave oven, no pre-heating time was required.

Thawing results are summarized in table 1. For all thawing devices tested, thawing times were significantly shorter during thawing of single octaplasLG units versus thawing at maximum capacities (i.e. parallel thawing of 3–4 bags).

As expected, the fastest thawing was observed for transfusio-therm 2000. 30°C product temperature was reached after 5 min in case of single unit thawing or after 7–10 min in case of parallel thawing of 3 bags.

Using the QuickThaw DH4 device, due to the gentle agitation of bags during thawing, both the time to defrost octaplasLG and the time to reach 30°C product temperature could be reduced by 6–9 min compared to the conventional water bath system (i.e. from 21/21–24 to 12/18 min and from 24/24–30 to 16/24 min for 1/4 bags, respectively).

octaplasLG units were thawed in the dry tempering systems plasmatherm (at 37°C water temperature) and SAHARA-III (using the fast tempering function) at comparable times. The product was ice-free after 21–24 and 27–35 min, while 30°C product temperature was reached after 24–37 and 35–40 min, during thawing of single bags and thawing at maximum thawing capacity, respectively.

Optimized thawing conditions selected for the different thawing devices and used in the biochemical characterization studies are presented in table 1 (see last column). The following thawing conditions were defined: 20–25 min for the QuickThaw DH4, 30–45 min for plasmatherm, 25–40 min for SAHARA-III as well as 5–10 min for transfusio-therm 2000, depending on the number of plasma bags. Control octaplasLG units were thawed with the conventional water bath for 30 min.

Biochemical Profile of Thawed Plasma

After thawing using the validated thawing conditions, octaplasLG units were investigated on plasma quality (table 2).

All octaplasLG bags thawed by different devices and optimized thawing conditions were clear and free of solid and gelatinous particles, indicating no local overheating or protein denaturation. Total protein levels varied between 52 and 54 mg/ml, while pH

Table 1. Temperature measurements and recommendations for thawing of octaplasLG units using different thawing devices

Thawing device	Maximum thawing capacity	Thawing of single units		Thawing at maximum capacity		Thawing recommendations*
		time to ice-free, min	time to 30 °C, min	time to ice-free, min	time to 30 °C, min	
MB-13A	n.s.	21	24	21–24	24–30	minimum 30 min at 37 °C for up to 4 bags (thawing time should not exceed 60 min)
QuickThaw DH4	4 bags	12	16	18	24	20 min at 37 °C for single bag or 25 min at 37 °C for up to 4 bags
plasmatherm	4 bags	24	27	27–35	35–40	30 min at 37 °C for single bag or 45 min at 37 °C for up to 4 bags
SAHARA-III	3 bags	21	24	30–35	35–39	using the fast tempering program, 25 min for single bag or 40 min for up to 3 bags
transfusio-therm 2000	3 bags	n.d.	5	n.d.	7–10	for up to 3 bags, reach 30 °C set temperature (indicated by optical and acoustical signal)

*All octaplasLG units should be thawed in the secondary overwrap. n.s., not specified; n.d., not determined.

Table 2. Product release parameters and activation markers for octaplasLG after thawing (mean ± standard deviation, n = 7)

Parameters	Plasma thawing devices				
	MB-13A	QuickThaw DH4	plasmatherm	SAHARA-III	transfusio-therm 2000
Visual control	passed*	passed*	passed*	passed*	passed*
pH value	7.46 ± 0.08	7.45 ± 0.06	7.44 ± 0.06	7.42 ± 0.07	7.46 ± 0.06
Osmolality, mosmol/kg	349 ± 3	349 ± 3	348 ± 3	349 ± 2	348 ± 7
Total protein, mg/ml	52 ± 3	53 ± 3	54 ± 2	53 ± 2	53 ± 3
aPTT, s	31 ± 1	30 ± 1	31 ± 1	30 ± 1	29 ± 1
NaPTT, s	406 ± 24	417 ± 35	415 ± 37	401 ± 48	401 ± 27
Fibrinogen, mg/ml	3.8 ± 0.8	3.8 ± 0.8	3.8 ± 0.8	3.8 ± 0.7	3.7 ± 0.5
Factor II, IU/ml	1.13 ± 0.03	1.12 ± 0.02	1.12 ± 0.03	1.13 ± 0.05	1.14 ± 0.04
Factor V, IU/ml	0.89 ± 0.09	0.88 ± 0.09	0.86 ± 0.09	0.86 ± 0.09	0.88 ± 0.10
Factor VII, IU/ml	1.21 ± 0.05	1.21 ± 0.05	1.19 ± 0.04	1.15 ± 0.04	1.16 ± 0.07
Factor VIII, IU/ml	0.95 ± 0.17	0.98 ± 0.19	0.93 ± 0.14	0.96 ± 0.20	0.92 ± 0.16
Factor IX, IU/ml	1.09 ± 0.08	1.10 ± 0.07	1.06 ± 0.09	1.08 ± 0.07	1.11 ± 0.10
Factor X, IU/ml	1.04 ± 0.16	1.03 ± 0.15	1.02 ± 0.17	0.98 ± 0.13	0.98 ± 0.11
Factor XI, IU/ml	0.93 ± 0.05	0.93 ± 0.04	0.90 ± 0.04	0.92 ± 0.03	0.91 ± 0.05
ADAMTS13, IU/ml	1.09 ± 0.08	1.06 ± 0.09	1.08 ± 0.15	1.07 ± 0.10	1.06 ± 0.11
Protein C, IU/ml	1.05 ± 0.12	1.06 ± 0.13	1.04 ± 0.10	0.95 ± 0.10	0.96 ± 0.11
Protein S, IU/ml	0.54 ± 0.06	0.58 ± 0.04	0.59 ± 0.07	0.62 ± 0.07	0.60 ± 0.07
Plasmin inhibitor, IU/ml	0.51 ± 0.03	0.53 ± 0.03	0.52 ± 0.04	0.50 ± 0.06	0.51 ± 0.03
Factor VIIa, mIU/ml	68 ± 8	68 ± 7	66 ± 7	69 ± 8	70 ± 10

*The thawed plasma is clear to slightly opalescent and free of solid or gelatinous particles.

value and osmolality were both within the physiological range and product specifications. There were no statistically significant differences observed in the global coagulation parameters (i.e., aPTT and NaPTT levels), important coagulation and protease inhibitors, as well as FVIIa activation marker during thawing by different devices (i.e., all p values were > 0.05). Activities of all coagulation factors in all plasma samples after thawing were within the normal ranges for single-donor FFP (mean activities were ≥ 0.9 IU/ml). With regard to the protease inhibitors tested, protein C activity levels were 0.9 IU/ml or higher, while protein S and plasmin inhibitor

activities were 0.5 IU/ml or higher, i.e., well within both the Ph. Eur. limits on human plasma pooled and treated for virus inactivation [17] and the product release specifications for octaplasLG [12]. In addition, there was no coagulation activation observed in any of the plasma bags thawed, and FVIIa levels were in the normal range for plasma. Finally, thrombin generation parameters (fig. 1A) were comparable in octaplasLG units thawed with different devices and within assay variances.

Additional studies demonstrated that thawing times at 37 °C up to 60 min have no impact on the plasma quality (see fig 1B for

Fig. 1. Comparison of TGA parameters in octoplasLG units. **A** Thawing by conventional water bath (i.e. MB-13A, $\square$), QuickThaw DH4 ($\diamond$), plasmatherm (Δ), SAHARA-III ($\circ$) and transfusio-therm 2000 ($\ast$) devices; mean results for 7 units per device are shown. **B** Thawing at 37 °C for 30 min ($\square$) or 60 min ($- -$) by MB-13A; single results for 7 units are shown.

Fig. 2. Comparison of octoplasLG quality after thawing at 37 °C for 30 min (black column) and 60 min (white column) using conventional water bath system (i.e. MB-13A). Results for coagulation factors and protease inhibitors are shown as mean values ($n = 7$ units/device) $\pm$ standard deviation. p values are indicated.

TGA parameters and fig. 2 for coagulation factors and inhibitors). Coagulation factor and protease inhibitor activities as well as TGA parameters were comparable in octoplasLG® units thawed for 30 or 60 min, without any statistically significant differences between the two groups.

Discussion

According to the manufacturing instructions, octoplasLG units should be thawed at 37 °C by using water bath systems or specifically approved thawing devices. Pre-heating over 40 °C should be avoided. Gentle agitation during thawing may facilitate the thawing process. After thawing, octoplasLG units should be free of solid

or gelatinous particles. Thawed octoplasLG is stable for 24 h at 2–8 °C or for 8 h at room temperature. Thawed octoplasLG units should not be re-frozen again. Nonused products should be discarded.

The aim of our studies was to perform a validation of thawing devices for octoplasLG units available on the market, including both temperature monitoring and biochemical characterization studies. Alternative thawing devices used were separated into water bath systems, dry tempering systems, and microwave ovens. The water bath system from Helmer Scientific US uses both controlled temperature and agitation to achieve homogenous temperature distribution, which reduced thaw times compared to the conventional water bath circulator (i.e. MB-13A) by 6–9 min. Due to the direct contact with water, water bath systems may carry the risk of bacte-

rial contamination. However, octaplasLG is delivered sealed in a secondary overwrap, thus avoiding the risk of possible bacterial contamination when thawing in open water bath systems. The dry tempering systems are sealed systems developed with the aim to exclude the direct contact of plasma bags with water and thus lower the potential for contaminating plasma units with microbes. The plasmatherm device uses close water bags, while the SAHARA-III thawing device uses warming plates and hot fans for heating. Both dry heat ovens required longer thawing times than water bath systems with agitation. The thawing results for octaplasLG were in agreement with those of previous studies performed with SAHARA-III for blood [15] or octaplas thawing [16]. As expected, the fastest thawing times were achieved by using the microwave oven transfusio-therm 2000; i.e., octaplasLG units were defrosted already after 5–10 min, depending on the number of bags thawed in parallel. In the past, there were some concerns related to possible local overheating and the formation of precipitated denatured proteins (mainly albumin and fibrinogen) when using the old-generation microwave ovens [4, 18]. In the present study, all octaplasLG units were clear and free of solid or gelatinous particles after thawing, indicating no local overheating during the thawing process. In addition, coagulation factor and protease inhibitor activities were comparable to those observed in octaplasLG units thawed with water bath or dry tempering systems. Similar studies performed for FFP [19, 20] and octaplas [16, 21] described no protein denaturation during thawing using the transfusio-therm 2000 device.

Validation of thawing using different thawing devices in the present study was performed with focus on octaplasLG. For the

thawing of other plasma and/or blood products, separate validation studies have to be performed.

In conclusion, our study showed that octaplasLG can be thawed using all tested devices without any negative influence on the plasma quality, presupposed that optimized settings defined for this plasma product are used. Thawing devices tested enable standardized thawing and warming of plasma bags. However, for all devices it is of high relevance to consider the number of bags to be thawed in parallel. octaplasLG units should be thawed in the outer wrapper. Thawing times should not exceed 60 min. After thawing, all octaplasLG units were clear and free of solid and gelatinous particles. There were no significant differences observed in global coagulation parameters, coagulation factors, protease inhibitors, and markers of activated coagulation in octaplasLG units thawed by different thawing devices. All parameters in all units remained within both normal plasma range and product specifications.

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Disclosure Statement

All authors are employees of Octapharma.

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