

TRANSFUSION

Thawing times and haemostatic assessment of fresh frozen plasma thawed at 37°C and 45°C using water bath methods

Journal:	<i>Transfusion</i>
Manuscript ID	Trans-2019-0167.R1
Manuscript Type:	Original Research
Date Submitted by the Author:	n/a
Complete List of Authors:	Platton, Sean; Barts Health NHS Trust, Haemophilia Centre Elegbe, Oluwatola; Barts Health NHS Trust, Blood Transfusion Bower, Lucy; NHS Blood and Transplant, Component Development Cardigan, Rebecca; NHS Blood and Transplant, Components Development; University of Cambridge, Department of Haematology Lancut, Julia; Barts Health NHS Trust, Blood Transfusion McCullagh, Josephine; Barts Health NHS Trust, Blood Transfusion Green, Laura; Barts Health NHS Trust, Blood Transfusion; Blizzard Institute of Cell and Molecular Science; NHS Blood and Transplant
Key words:	FFP Transfusion, Hemostasis, Plasma Derivatives

SCHOLARONE™
Manuscripts

1
2
3 **Thawing times and haemostatic assessment of fresh frozen plasma thawed at 37°C and**
4
5 **45°C using water-bath methods**
6
7

8 Sean Platton,¹ Oluwatola Elegbe,¹ Lucy Bower,² Rebecca Cardigan,^{2,4} Julia Lancut,¹ Josephine
9 McCullagh,¹ Laura Green^{1,2,3}
10
11

12
13 ¹Barts Health NHS Trust

14
15 ²NHS Blood and transplant

16
17 ³Blizard Institute, Queen Mary University of London

18
19 ⁴ Department of Haematology, University of Cambridge

20
21
22 **Address for Correspondence:**

23 Dr Laura Green

24
25 The Royal London Hospital
26 4th Floor, Pathology and Pharmacy Building
27 80 Newark Street
28 London
29 E1 2ES

30 Phone: +44 208 957 2756

31 Fax: +44 208 957 2838
32
33

34
35
36 Email: laura.green@bartshealth.nhs.uk
37
38

39 **Sources of support:** Barkey Plasmatherm funded the reagent and plasma costs for
40 performing the haemostatic assays in the second stage of the study.

41
42 **Running head:** Haemostatic assessment of FFP at 45°C

43
44 **Abstract word count:** 249 words (including titles)

45
46 **Article word count:** 2615 words
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Abstract

BACKGROUND: The Barkey Plasmatherm (BP) can thaw plasma at 37°C and 45°C. No studies have assessed thawing times or haemostatic qualities of plasma thawed at 45°C with BP. This study assessed FFP thawing times using BP at 37°C and 45°C and Thermogenesis Thermoline (TT) at 37°C, and compared haemostatic quality of LG-Octaplas using BP at 37°C and 45°C with TT at 37°C.

STUDY DESIGN AND METHODS: Thawing time of FFP (pairs or fours) was assessed using BP at 37°C and 45°C (not pre-warmed, and pre-warmed) and TT at 37°C. Haemostasis was assessed in LG-Octaplas five minutes, 24 hours, 48 hours, and 120 hours post thaw using the three methods.

RESULTS: Thawing time for two units was 13.44' using TT, the same as using BP at 37°C (12.94' not pre-warmed; 12.20' pre-warmed) or 45°C (12.38' not pre-warmed), but longer than using BP pre-warmed to 45°C (11.31', $p < 0.001$). Thawing time for four units was 13.41' using TT, shorter than using BP at 37°C (17.19' not pre-warmed; 18.47' pre-warmed; both $p < 0.001$) or 45°C (15.03' not pre-warmed, $p = 0.012$; 15.22' pre-warmed, $p = 0.004$). There was no reduction in haemostatic markers in LG-Octaplas using BP at 37°C or 45°C compared to TT.

CONCLUSION: BP is quicker than TT by two minutes when thawing two units of FFP if it is pre-warmed to 45°C. BP is slower than TT by at least two minutes when thawing four units of FFP. There was no significant difference in the haemostatic qualities of plasma whether thawed at 37°C or 45°C.

Keywords: Plasma thawing; haemostasis; water-bath

Introduction

In the last decade there has been an increase interest in transfusing fresh frozen plasma (FFP) and cryoprecipitate early for the treatment of massive haemorrhage, with clinical trials suggesting that this practice improves outcome for patients who are bleeding due to trauma.^{1,2} In order to improve the availability of plasma components for management of bleeding, many countries have now extended the shelf life of thawed FFP from 24 hours to 5 days for thawed plasma and 14 days or more for liquid plasma.³⁻⁵ Although there have been no studies demonstrating a clinical difference between freshly thawed plasma, extended shelf life thawed plasma or liquid plasma, and the PROPPR study¹ used extended shelf life thawed plasma, *in vitro* data on thawed and liquid plasma has demonstrated that the haemostatic qualities of the components deteriorate over time.⁶ An optimal situation may be to have faster thawing methods that would allow for quick delivery of thawed plasma on demand, providing fresh components for patients. Fast thawing may also be desirable when stocks of thawed plasma are rapidly depleted, especially when multiple patients are being treated concurrently, or in centres where plasma is not often required and pre-thawing may lead to increased wastage.

There are several methods available for thawing FFP including water-bath, microwave oven, and dry-heat based methods: current national guidance in the United Kingdom for thawing plasma recommends that the optimal temperature at which the components should be thawed is 37°C (33°C – 37°C).^{4,7}

Barkey Plasmatherm (Barkey GmbH & Co. KG, Leopoldshöhe, Germany) is a water-based method that can thaw plasma at 37°C and 45°C temperatures. However, to our knowledge there have been no studies that have assessed the thawing time or the haemostatic qualities of plasma when thawed at 45°C temperatures in comparison with other thawing methods. The aims of this study are to: a) assess the thawing times between Barkey Plasmatherm at 37°C and 45°C and Thermogenesis Thermoline (Helmer Scientific, Indiana, USA) at 37°C, using variable volumes of single donor FFP; and b) compare the effect of thawing temperatures on the haemostatic qualities of LG-Octaplas (a pooled plasma component) using Barkey Plasmatherm (BP) at 37°C and 45°C and Thermogenesis

1
2
3 Thermoline (TT) at 37°C. A pooled component rather than multiple units of FFP were used
4 for the haemostatic assessment to reduce potential variation in clotting factors between
5 units.
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Review Only

Materials and Methods

Fresh frozen plasma (FFP) was supplied by NHS Blood and Transplant (NHSBT) and was derived from UK whole blood donation (475mL \pm 10%,) collected into 66.5mL CPD anticoagulant in top-and-top collection packs (FQE6283LB MacoPharma, Twickenham, UK) following NHSBT standard operation procedures.⁸ LG-Octaplas was supplied by Octapharma as part of routine delivery to hospital and was produced according to the manufacturer's specification

Assessment of thawing times

For the assessment of thawing times, single expired FFP units in pairs or in fours were assessed eight times using: a) Barkey Plasmatherm (BP) at 37°C, b) BP at 45°C, and c) Thermogenesis Thermoline (TT) at 37°C. The above experiment was repeated twice: 1) with the BP machine being switched on for <30 minutes, and 2) with the BP machine being switched on for at least four hours prior to the experiment. Assessment of thawing times was performed by two biomedical scientists using visual inspection for the absence of visible ice crystals, as this is the thawing end-point in most hospitals.

Assessment of haemostatic capacity

LG-Octaplas is a pooled plasma product that is likely to show less variability in haemostatic qualities than FFP, so 24 LG-Octaplas units (eight thawed in the TT at 37°C; eight thawed in the BP at 37°C; eight thawed in the BP at 45°C) were used for the assessment of haemostatic capacity stage of the experiment. A thawing time of 30 minutes at 37°C (30°C – 37°C) for LG-Octaplas is recommended by Octapharma Limited, and this timing was followed during the haemostatic assessment experiment for all three arms. After thawing, LG-Octaplas was stored at 2°C – 4°C and aliquots were taken for haemostatic testing at four different time-points: five minutes after removal from the thawing device (defined as time zero); 24 hours post thaw; 48 hours post thaw; and 120 hours post thaw – the latter timing

1
2
3 is the allowed extended shelf-life for thawed plasma in the UK.⁴ Samples were stored below
4
5 -70°C until testing was performed.⁷
6
7

8
9 Assays performed were prothrombin time (PT); activated partial thromboplastin time
10 (APTT); fibrinogen; factors II, V, VII, VIII and XI activity; free protein S antigen; protein S
11 activity; protein C activity; C1-inhibitor activity; and thrombin generation (TG). All assays
12 except for thrombin generation were performed on a Sysmex CS-2100 analyser. PT was
13 measured using Siemens Dade Innovin, and factor II, V and VII activity were measured by
14 one-stage assays using Innovin and Siemens factor deficient plasmas; APTT was measured
15 using Siemens Dade Actin FS, and factor XI was measured by one-stage assay using Actin FS
16 and Siemens factor deficient plasma; factor VIII assays were measured using Hyphen
17 Biomed Biophen VIII chromogenic reagents; protein C activity was measured using Siemens
18 Berichrom Protein C reagents; free protein S antigen was measured using Siemens
19 INNOVANCE Free PS reagents; protein S activity was measured using Hyphen Biomed
20 Hemoclot Protein S reagents; C1-inhibitor activity was measured using reagents from
21 Siemens; fibrinogen was measured by the Clauss method using Siemens Thrombin. All these
22 assays (except for PT and APTT) were calibrated using Siemens Standard Plasma and all
23 reagents were supplied by Sysmex UK (Milton Keynes, UK).
24
25
26
27
28
29
30
31
32
33
34
35
36
37

38 Thrombin generation was performed using the Calibrated Automated Thrombogram
39 Fluoroskan system (Thrombinoscope BV, Maastricht, The Netherlands) as described by
40 Hemker et al⁹ in conjunction with the manufacturer's PPP reagents, which gave reaction
41 concentrations of 5pM tissue factor and 4µM phospholipid. The following parameters of the
42 TG curve were measured: lag time; time to the peak (ttP); peak thrombin; and area under
43 the curve, known also as the endogenous thrombin potential (ETP).
44
45
46
47
48
49

50 51 *Statistical Analysis* 52 53

54 Data have been summarized as medians with 95% confidence intervals. Coagulation factors
55 were analysed using univariate statistical methods. Analysis of variance (ANOVA) was
56 performed, comparing concentration of each factor across arms and over time. For thawing
57 times, Fisher's multiple comparisons test was used; for haemostatic assays Tukey's multiple
58
59
60

1
2
3 comparisons test was used. For all tests a p value of below 0.05 indicated a statistically
4 significant difference.
5
6
7
8

9 **Results**

10
11
12
13 There was no statistically significant difference in the volumes of FFP thawed in each arm of
14 the experiment (Figure 1).
15
16
17

18 *Thawing times*

19
20
21
22 Data are summarised in Figure 1.
23
24
25

26 For every increase in volume of 1mL there was a mean increase in thawing times for two
27 units of 3.1 seconds at 37°C (TT or BP) and 1.9 seconds at 45°C; for four units the mean
28 increase was 2.5 seconds (TT at 37°C and BP at 45°C) and 2.8 seconds (BP at 37°C).
29
30
31

32
33 The thawing time for two units of FFP using the TT was 13.44 minutes (95% confidence
34 interval (CI) 11.78-15.10). The thawing time for two units of FFP using the BP at 37°C with
35 pre-warming was 12.20 minutes (95% CI 11.77-12.63) compared to 12.94 minutes (95% CI
36 11.09-14.79) without pre-warming. The thawing time for two units of FFP using the BP at
37 45°C without pre-warming was 12.38 minutes (95% CI 11.70-13.05). None of these times
38 were statistically significantly different from each other.
39
40
41
42
43
44
45

46 The thawing time for two units of FFP using the BP at 45°C with pre-warming was 11.31
47 minutes (95% CI 10.94-11.69); this was statistically significantly shorter than the thawing
48 time using the TT by 2.13 minutes (p=0.030).
49
50
51
52

53 The thawing time for four units of FFP using the TT was 13.41 minutes (95% CI 12.81-14.01)
54 which was not statistically significantly different from the thawing time for two units of FFP
55 using the TT. When using the BP to thaw four units of FFP, the thawing time was statistically
56 significantly longer than thawing in the TT whether using 37°C without pre-warming (17.19
57
58
59
60

1
2
3 minutes, 95% CI 16.21-18.16, $p < 0.001$) or with pre-warming (18.47 minutes, 95% CI 17.78-
4 19.16, $p < 0.001$) or when using 45°C without pre-warming (15.03 minutes, 95% CI 14.20-
5 15.86, $p = 0.012$) or with pre-warming (15.22 minutes, 95% CI 14.44-16.00, $p = 0.004$).
6
7
8
9

10 There was no statistically significant difference in thawing times using the BP whether the
11 device was pre-warmed or not.
12
13

14 *Haemostatic assays*

15

16
17
18
19 Data are summarised in Figures 2 and 3.
20
21

22
23 For PT there was no statistically significant difference at time 0 between TT and BP at 37°C
24 or BP at 45°C, and no statistically significant differences were seen within each thawing
25 method at 24 hours, 48 hours or 120 hours. For all thawing methods, APTT showed a
26 statistically significant increase over time but there was no statistically significant difference
27 at all time points (time 0, 24 hours, 48 hours or 120 hours) between TT and BP at 37°C or
28 45°C. Similarly, for fibrinogen there was no statistically significant difference at all time
29 points between the three thawing methods.
30
31
32
33
34
35
36

37
38 Factor VIII showed a statistically significant decrease over time regardless of thawing
39 method but there was no statistically significant difference between TT and BP at 45°C at
40 any time points, whereas factor VIII measured from the units thawed using the BP at 37°C
41 was statistically significantly higher at time zero (median 76.0iu/dL) than when using the TT
42 (median 57.7iu/dL, $p = 0.009$); there was no statistically significant difference in factor VIII
43 between units thawed at TT and BP at 37°C for any other time points. Factor V and protein S
44 activity showed similar statistically significant decrease over time as for factor VIII but no
45 statistically significant differences in results between TT and BP at 37°C or 45°C for any time
46 points.
47
48
49
50
51
52
53
54

55
56 Endogenous thrombin potential was statistically significantly reduced over time following
57 thawing, in line with the observed reduction of FVIII and FV. There was no statistically
58
59
60

1
2
3 significant difference at time 0 between TT and BP at 37°C or 45°C, and no statistically
4 significant differences at 24 hours or 120 hours. Results for TT at 48 hours were statistically
5 significantly lower than all other results: samples were run in the same batch as all samples
6 for TT at 120 hours and a reason for this apparent anomaly could not be identified. Peak
7 thrombin also statistically significantly reduced over time but there was no statistically
8 significant difference at all time points between TT and BP at 37°C or 45°C.
9
10
11
12
13
14
15

16 There were no statistically significant differences between temperatures or time points for
17 factor II, factor VII, factor XI, protein C activity, free protein S antigen or C1-inhibitor.
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Review Only

Discussion

The water bath method is the most commonly used method in the UK for thawing plasma, with the TT and BP being the most widely used devices. In this study we compared the two devices, and showed that with regards to thawing times TT was statistically significantly faster by at least two minutes when thawing four units of FFP, while BP at 45°C was statistically significantly faster by at least two minutes than TT when thawing two units of FFP. The reason for this is likely to be due to the difference in the contact surfaces area between the plasma units and the water between the two methods. In the TT the surface area is larger allowing for quicker thawing times, whereas in BP plasma units are placed close to each other thus, reducing the surface area with warm water and slowing down the thawing times.

In addition to thawing times, hospital transfusion laboratory who are considering the purchase of plasma thawing devices should also consider other factors, such as size, and maintenance of the device. For the two methods that we compared in this study, the TT is bigger in size than BP, allowing for up to ten units of plasmas being thawed at once – this can be a major advantage when dealing with mass casualties, however it will require more laboratory space to house. The BP can thaw a maximum of eight units (something that was not tested in this study), but four of these have to be placed below the cushions and need to be moved once first four units have been thawed and removed. Further, the cleaning and maintenance of TT is more demanding than BP: the TT requires the water to be changed regularly by laboratory staff, whereas the BP only requires the water to be replaced completely on an annual basis during the routine preventative maintenance; and in the TT spillages can enter the water, facilitating the need for complete replacement of the tank water, whereas the BP does not allow the water to come into contact with the product, as it uses a sealed tank method. Water is pumped into cushions and circulated. Should either the plasma bag or cushion rupture this is contained and easily cleaned.

In the UK, it is believed that temperatures close to 37°C may be optimal, and that thawing plasma at higher temperatures might affect the viability of plasma proteins.^{4,10} The current UK recommendations state that plasma should be thawed at 33°C – 37°C.⁴ However, to our

1
2
3 knowledge, there have been no studies that have compared BP at 45°C with 37°C thawing
4 using other methods. The clinical need to have plasma available quickly for the management
5 of major bleeding may have led to hospitals using a higher-than-recommended thawing
6 temperature for plasma. While the extension of the shelf-life of thawed plasma has
7 improved the availability of FFP in emergency situations, anecdotally this is still associated
8 with some degree of wastage, particularly in hospitals that do not use a lot of plasma on a
9 daily basis, and speeding up the thawing process for FFP could achieve a balance between
10 reduced wastage and improved availability. In this study we observed that heat labile
11 clotting factor levels and endogenous thrombin potential were statistically significantly
12 reduced over time after thawing with both BP and TT devices: these findings are consistent
13 with other studies^{4,6} but the clinical impact of these reductions is not known. In this study,
14 we did not see any statistically significant differences in any of the haemostatic markers of
15 LG-Octaplas thawed at 45°C using BP compared to thawing at 37°C with BP or TT. Our
16 results should give reassurance to clinicians and blood transfusion laboratories who are
17 considering introducing a 45°C thawing temperature with the BP device, in that we found
18 no evidence that this higher temperature of thawing for 30 minutes impairs the quality of
19 plasma, but it should be noted that a limitation of our study is that the temperature of
20 plasma after 30 minutes of thawing was not measured. There did not appear to be an
21 advantage to leaving the BP switched on, suggesting that it can be switched on at the same
22 time as thawing FFP is initiated. Further studies are required to validate our results.

23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42 In conclusion, compared to the TT, when thawing two units of FFP, the BP at 37°C is not
43 statistically significantly different, but at 45°C is statistically significantly quicker by more
44 than two minutes. When thawing four units of FFP the BP is statistically significantly slower
45 than the TT by two minutes. These results were not statistically different whether the BP
46 was pre-warmed or not. There was no statistically significant difference in the haemostatic
47 qualities of plasma between 37°C and 45°C thawing temperatures.
48
49
50
51
52
53
54
55
56
57
58
59
60

Conflict of interest

Barkey Plasmatherm funded the reagent and plasma costs for performing the haemostatic assays in the second stage of the study. The views expressed in this document are those of the authors and not those of the company. The company had no role in the study design, data collection/analysis or preparation of this article.

Roles

SP and LG wrote the first draft of the paper. OE and JM performed the thawing experiments. OE and SP performed the haemostasis assays. All authors were involved in study design and contributed to the writing of the manuscript.

Figure 1: Comparison of FFP volumes and thawing times for 2 and 4 units thawed together (n=8). TT: Thermogenesis Thermoline; BP: Barkey Plasmatherm; *: 0.01<p<0.05; **: 0.001<p≤0.01; ***: 0.0001<p≤0.001; ****: p<0.0001. Box plots show median, 5th and 95th percentile and minimum and maximum values.

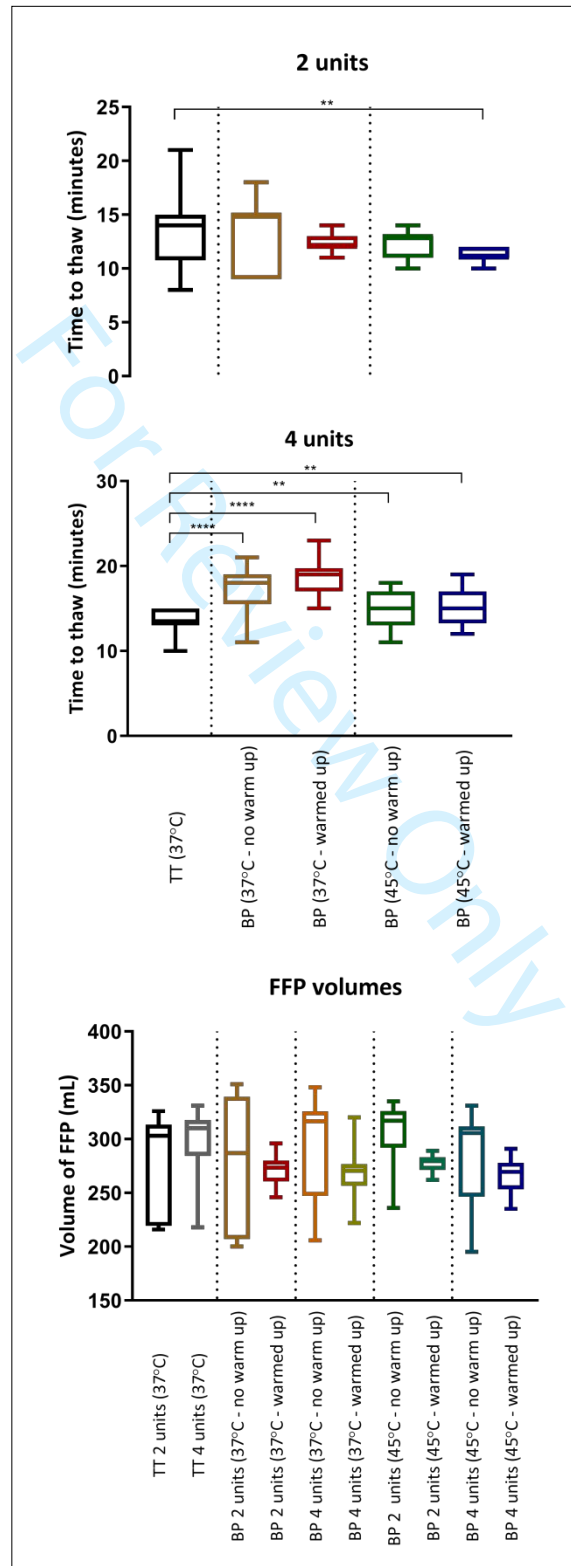


Figure 2: Comparison of clotting assays and thrombin generation in Octaplas following thawing by different methods. TT: Thermogenesis Thermoline; BP: Barkey Plasmtherm; PT: prothrombin time; APTT: activated partial thromboplastin time; ETP: endogenous thrombin potential. *: 0.01<p<0.05; **: 0.001<p≤0.01; ***: 0.0001<p≤0.001; ****: p<0.0001. Box plots show median, 5th and 95th percentile and minimum and maximum values.

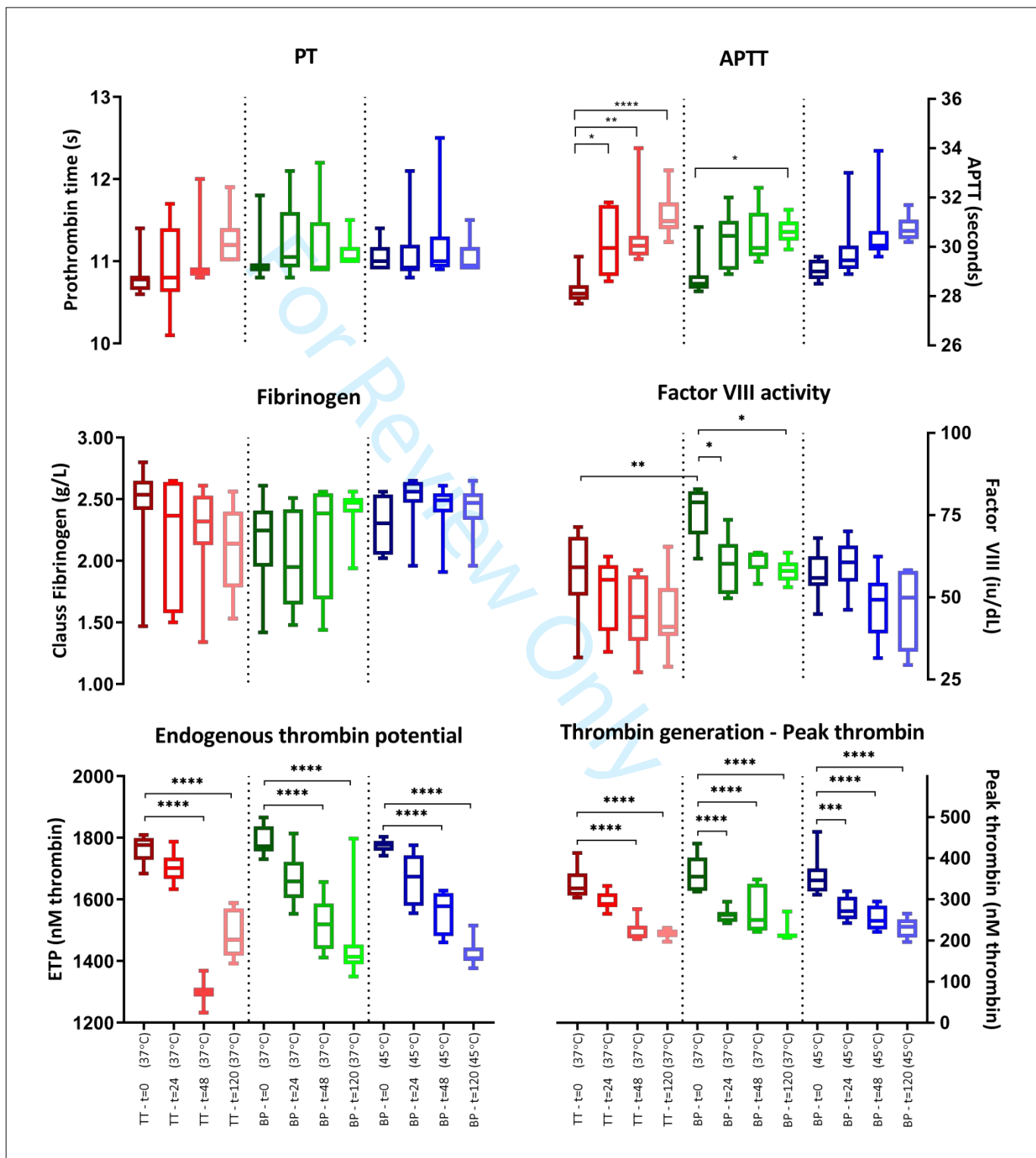
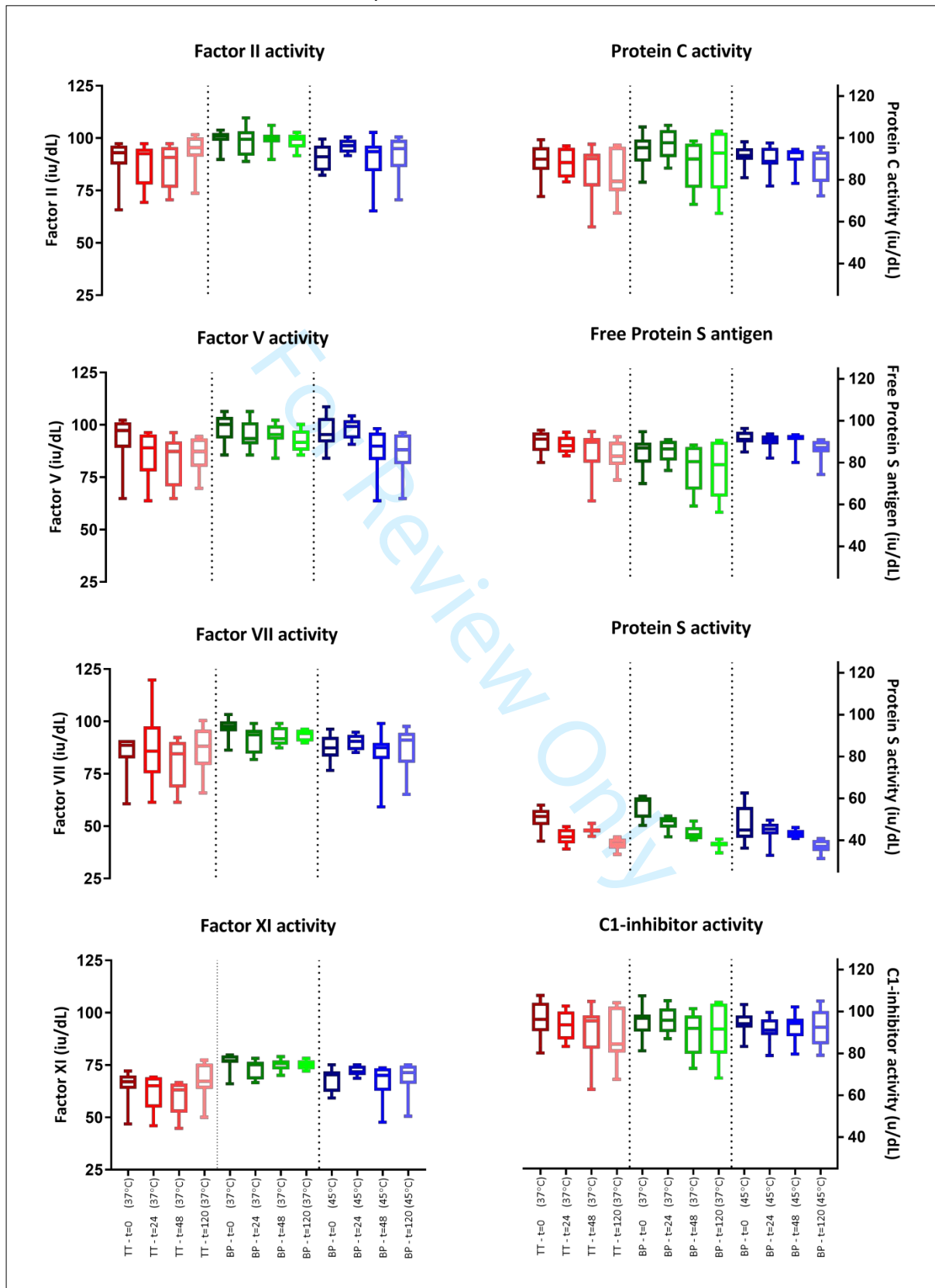


Figure 3: Comparison of haemostasis assays in LG-Octaplas following thawing by different methods. TT: Thermogenesis Thermoline; BP: Barkey Plasmatherm. Box plots show median, 5th and 95th percentile and minimum and maximum values.



References

1. Holcomb JB, Tilley BC, Baraniuk S, Fox EE, Wade CE, Podbielski JM, Del Junco DJ, Brasel KJ, Bulger EM, Callcut RA, Cohen MJ, Cotton BA, Fabian TC, Inaba K, Kerby JD, Muskat P, O'Keeffe T, Rizoli S, Robinson BR, Scalea TM, Schreiber MA, Stein DM, Weinberg JA, Callum JL, Hess JR, Matijevic N, Miller CN, Pittet JF, Hoyt DB, Pearson GD, Leroux B, van BG. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. *JAMA* 2015;**313**:471-82.
2. Sperry JL, Guyette FX, Brown JB, Yazer MH, Triulzi DJ, Early-Young BJ, Adams PW, Daley BJ, Miller RS, Harbrecht BG, Claridge JA, Phelan HA, Witham WR, Putnam AT, Duane TM, Alarcon LH, Callaway CW, Zuckerbraun BS, Neal MD, Rosengart MR, Forsythe RM, Billiar TR, Yealy DM, Peitzman AB, Zenati MS, Group PAS. Prehospital Plasma during Air Medical Transport in Trauma Patients at Risk for Hemorrhagic Shock. *N Engl J Med* 2018;**379**:315-26.
3. Green L, Cardigan R, Beattie C, Bolton-Maggs P, Stanworth SJ, Thachil J, Kallis Y, Zahra S. Addendum to the British Committee for Standards in Haematology (BCSH): Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryosupernatant, 2004 (Br. J Haematol 2004,126,11-28). *Br J Haematol* 2016;**178**(4):646-647.
4. Green L, Bolton-Maggs P, Beattie C, Cardigan R, Kallis Y, Stanworth SJ, Thachil J, Zahra S. British Society of Haematology Guidelines on the spectrum of fresh frozen plasma and cryoprecipitate products: their handling and use in various patient groups in the absence of major bleeding. *Br J Haematol* 2018;**181**:54-67.
5. Novak DJ, Bai Y, Cooke RK, Marques MB, Fontaine MJ, Gottschall JL, Carey PM, Scanlan RM, Fiebig EW, Shulman IA, Nelson JM, Flax S, Duncan V, Daniel-Johnson JA, Callum JL, Holcomb JB, Fox EE, Baraniuk S, Tilley BC, Schreiber MA, Inaba K, Rizoli S, Podbielski JM, Cotton BA, Hess JR. Making thawed universal donor plasma available rapidly for massively bleeding trauma patients: experience from the Pragmatic, Randomized Optimal Platelets and Plasma Ratios (PROPPR) trial. *Transfusion* 2015;**55**:1331-9.

- 1
 - 2
 - 3
 - 4
 - 5
 - 6
 - 7
 - 8
 - 9
 - 10
 - 11
 - 12
 - 13
 - 14
 - 15
 - 16
 - 17
 - 18
 - 19
 - 20
 - 21
 - 22
 - 23
 - 24
 - 25
 - 26
 - 27
 - 28
 - 29
 - 30
 - 31
 - 32
 - 33
 - 34
 - 35
 - 36
 - 37
 - 38
 - 39
 - 40
 - 41
 - 42
 - 43
 - 44
 - 45
 - 46
 - 47
 - 48
 - 49
 - 50
 - 51
 - 52
 - 53
 - 54
 - 55
 - 56
 - 57
 - 58
 - 59
 - 60
6. Backholer L, Green L, Huish S, Platton S, Wiltshire M, Doughty H, Curnow E, Cardigan R. A paired comparison of thawed and liquid plasma. *Transfusion* 2017;**57**:881-9.
7. Mackie I, Cooper P, Lawrie A, Kitchen S, Gray E, Laffan M. Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. *Int J Lab Hem* 2013;**35**:1-13.
8. Guidelines for the blood transfusion services in the United Kingdom. 8th ed. London: The Stationery Office; 2013
9. Hemker HC, Giesen P, al DR, Regnault V, de SE, Wagenvoord R, Lecompte T, Beguin S. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb* 2003;**33**:4-15.
10. Batty P, Hart D, Platton S. Optimization of pre-analytical heat treatment for inhibitor detection in haemophilia A. *Int J Lab Hem* 2018:1-8

Figure legends

Figure 1: Comparison of FFP volumes and thawing times for 2 and 4 units. TT:

Thermogenesis Thermoline; BP: Barkey Plasmatherm; *: $0.01 < p < 0.05$; **: $0.001 < p \leq 0.01$;

: $0.0001 < p \leq 0.001$; *: $p < 0.0001$. Box plots show median, 5th and 95th percentile and minimum and maximum values.

Figure 2: Comparison of clotting assays and thrombin generation in Octaplas following thawing by different methods. TT: Thermogenesis Thermoline; BP: Barkey Plasmtherm; PT: prothrombin time; APTT: activated partial thromboplastin time; ETP: endogenous thrombin potential. *: $0.01 < p < 0.05$; **: $0.001 < p \leq 0.01$; ***: $0.0001 < p \leq 0.001$; ****: $p < 0.0001$. Box plots show median, 5th and 95th percentile and minimum and maximum values.

Figure 3: Comparison of haemostasis assays in LG-Octaplas following thawing by different methods. TT: Thermogenesis Thermoline; BP: Barkey Plasmatherm. Box plots show median, 5th and 95th percentile and minimum and maximum values.