# Stability of hemostatic potential of thawed plasma on storage at 2-6$^0$C for 5 days.

**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE M.D. DEGREE BRANCH XXI(TRANSFUSIONMEDICINEANDIMMUNOHEMATOLOGY) EXAMINATION OF THE TAMIL NADU DR.M.G.R. MEDICAL UNIVERSITY CHENNAI TO BE HELD IN MAY 2019.**

# Stability of hemostatic potential of thawed plasma on storage at 2-6$^0$C for 5 days.


**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE M.D. DEGREE BRANCH XXI(TRANSFUSION MEDICINE AND IMMUNOHEMATOLOGY) EXAMINATION OF THE TAMIL NADU DR.M.G.R. MEDICAL UNIVERSITY CHENNAI TO BE HELD IN MAY 2019.**

## DECLARATION

**This is to certify that this dissertation titled "Stability of hemostatic potential of thawed plasma on storage at 2-6$^0$C for 5 days" is a bonafide work done by Dr.A.Srivalli Register No.:201631053, in part fulfilment of rules and regulation from the M.D. BRANCH XXI (Transfusion Medicine and Immunohematology) Degree examination of the Tamil Nadu Dr. M.G.R Medical university, to be held in May 2019.**

I have independently reviewed the literature, standardised the data collection methodology and carried out the evaluation toward completion of the thesis.

Dr A. Srivalli,

**Register No.:201631053**

Post Graduate Registrar,

Department of Transfusion Medicine and Immunohematology,

Christian Medical College, Vellore.

## CERTIFICATE

This is to certify that this dissertation titled **"Stability of hemostatic potential of thawed plasma on storage at 2-6$^0$C for 5 days"** is a bonafide work done by **Dr.A.Srivalli Register No.:201631053**, in part fulfilment of rules and regulation from the M.D. BRANCH XXI (Transfusion Medicine and Immunohematology) Degree examination of the Tamil Nadu Dr. M.G.R Medical university, to be held in MAY 2019**.**

,

Dr. Joy John Mammen, MBBS, MD

Professor and Head,

Department of Transfusion Medicine and Immunohematology,

Christian Medical College, Vellore.

Dr. Anna Pulimod**,** MBBS, MD

Principal,

Christian Medical College, Vellore.

## CERTIFICATE

This is to certify that this dissertation titled **"Stability of hemostatic potential of thawed plasma on storage at 2-6$^0$C for 5 days"** is a bonafide work done by Dr.A.Srivalli, in part fulfilment of rules and regulation from the M.D. BRANCH XXI (Transfusion Medicine and Immunohematology) Degree examination of the Tamil Nadu Dr. M.G.R Medical university, to be held in May 2019.

The candidate has independently reviewed the literature, the data collection methodology and carried out the evaluation toward completion of the thesis.

Dr. Sukesh.C. Nair, MBBS, DCP, MD, FRCPA (HAEM)

Professor,

Department of Transfusion Medicine and Immunohematology,

Christian Medical College, Vellore

# Acknowledgements

I would like to express my deepest gratitude to my teacher, Dr Sukesh C Nair, Professor, Department of Transfusion Medicine and Immunohematology, for giving me the opportunity to complete my post-graduate dissertation under her able guidance. His expert advice, refined knowledge and dedication were of immense support to me.

I am thankful to professors, Dr. Joy Mammen and Dr. Dolly Daniel, Department of Transfusion Medicine and Immunohematology. Their depth of knowledge and attention to details were inspirational and helped me in building up the concept and executing the project. I would like to thank Dr. Ancy Abraham and Dr. Tulsi Geevar for their support during the project.

I would like to thank my colleagues Dr. Jess Elizabeth Raslam, Dr. Blessymol Varghese and Dr. Raja Vasanth who were of great support and helped me in executing this project whenever needed. I am thankful to each one of them. I express my heartfelt gratitude to all my teachers and colleagues in the Department of Transfusion Medicine & Immunohematology, for their constant encouragement and support. I am obliged to all the technologists and clerical staff of the haemostasis laboratory and Blood Bank for their share of input which was priceless. I am greatly indebted to Ms. Tuny Sebastian, Department of the Biostatistics for the meticulous analysis of the data and interpretation of the results. She has spent a considerable amount of time in shaping up the details and providing authentic outputs. I wish to thank my parents for being my pillars of strength. Above all, I would like to thank the Supreme being, whose grace and blessings enabled me to learn and improve myself while completing this endeavour.

***CONTENTS***

**PLAGIARISM CHECK: ORIGANILITY CERTIFICATE-i**

URKUND

## Urkund Analysis Result

| | |
|---|---|
| **Analysed Document:** | Study of stability of hemostatic potential of thawed plasma on storage at 2-60C for 5 days.docx (D42432394) |
| **Submitted:** | 10/11/2018 5:02:00 PM |
| **Submitted By:** | nmrsrivalli@gmail.com |
| **Significance:** | 2 % |

Sources included in the report:

Coagulation2_HP.pdf (D39810284)
https://www.duo.uio.no/handle/10852/36079
https://www.hindawi.com/journals/jbt/2016/6260792/
https://www.utmb.edu/bloodbank/blood-bank-transfusion-services/component-therapy/plasma
http://practical-haemostasis.com/Miscellaneous/Miscellaneous%20Tests/etp_assays.html
http://www.aabb.org/advocacy/regulatorygovernment/Pages/askfdatranscripts2010.aspx
https://www.utmb.edu/bloodbank/blood-bank-transfusion-services/component-therapy/handling-storage-returns
https://www.sciencedirect.com/topics/neuroscience/factor-vii

**Certificate-II**

This is to certify that this dissertation work titled Stability of hemostatic potential of thawed plasma on storage at 2-6$^0$C for 5 days" of the candidate A.Srivalli with Registration Number: **201631053** for the award of M.D Degree in the branch XXI (Transfusion Medicine and Immunohematology). I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows 2 percentage plagiarism in the dissertation.

Dr. Sukesh.C. Nair, MBBS, DCP, MD, FRCPA(HAEM)

Professor,

Department of Transfusion Medicine and Immunohematology,

Christian Medical College, Vellore

**ACRONYMS**

| | |
|---|---|
| **APTT** | **Activated partial thromboplastin time** |
| **FFP** | **Fresh Frozen Plasma** |
| **TP** | **Thawed plasma** |
| **TGT** | **Thrombin Generation Testing** |
| **CAT** | **Calibrated Automated Thrombography** |
| **TTI** | **Transfusion transmitted infection** |
| **WHO** | **World Health Organisation** |
| **TF** | **Tissue Factor** |
| **TFPI** | **Tissue Factor Pathway Inhibitor** |
| **PAI** | **Plasminogen Activator Inhibitor** |
| **NBTC** | **National Blood Transfusion Council** |
| **NACO** | **National AIDS Control Organisation** |
| **BCSH** | **British Committee for Standards in Haematology Guidelines** |
| **ACD** | **Acid Citrate Dextrose** |
| **FDP** | **Fibrin Degradation Products** |
| **DCA** | **Drugs and cosmetics Act** |
| **AABB** | **American Association of Blood Banking** |
| **FDA** | **Food and drugs Administration** |
| **ETP** | **Endogenous Thrombin Potential** |
| **Ttp** | **Time to Peak** |
| **DGHS** | **Directorate general of health services** |

# ABSTRACT

# ABSTRACT

**Back ground:**

Transfusion of fresh frozen plasma is still an important measure in emergency medicine to prevent disseminated intravascular coagulation after severe blood loss, but thawing procedures can delay its availability. On the other hand, the wastage of plasma, once thawed and not transfused within defined period, represents an inefficient handling of economic resources. To reduce wastage, we investigated the stability of hemostatic potential of thawed plasma when stored at 2-6 $^{0}$C.

**Aim:**

To assess the stability of hemostatic potential of thawed plasma when stored at 2-6 $^{0}$ C for 5 days using APTT, Factors V, VII and VIII and thrombin generation testing.

**Materials and methods:**

19 plasma units included in this study were separated from blood collected from donor and frozen overnight and thawed at 35.8$^{0}$C using plasma thawer. One set of plasma aliquots were stored at -70$^{0}$c and the other set of aliquots from each bag were stored as thawed plasma at

2$^{0}$-6 $^{0}$ C for 5 days. Factor V, VII, VIII levels, activated partial thromboplastin time and thrombin generation testing were done on first, third and fifth day of storage.

**Results:**

The mean levels of Factor V, VII and VIII of frozen plasma on day 5 of storage were 73.31%, 52.12% and 62.23% respectively. The mean levels of Factor V, VII and VIII of thawed plasma on day 5 of storage were 67.2%, 50.69% and 56.97% respectively. The mean change in values of TGT variables were calculated and was comparable between both the groups on day 5 of storage.

**Conclusion:**

This study highlights that thawed plasma can be stored at 2-6$^0$C for 5 days which could be used to restore haemostasis in bleeding patients as it has adequate thrombin generation capacity.

# INTRODUCTION

# INTRODUCTION

The World Health Assembly has highlighted the importance of guaranteeing blood safety and equity in blood availability by adopting many resolutions that has given larger priority to the current issue among varied world and national health agendas. There have been major inventions in the 20$^{th}$ century and that made component therapy possible, e.g. invention of anticoagulant and preservative solutions, refrigeration, plastic blood bags, component administration, infectious disease testing, high-risk donor screening, etc.

Transfusion medicine has undergone fascinating changes since its initiation in early twentieth century. One among these was the discovery of fact that the blood can be divided into individual components and they can stored and used separately. This is imperative in this field. Blood transfusion refers to the administration of blood and blood components. Strict adherence to proper indications and transfusion triggers for blood transfusion therapy is essential because of its potential adverse effects and costs of transfusion. Over the century, the significance of blood components in treating certain diseases or conditions has been recognized. Anticoagulated blood collected from the donor can be stored and transfused to a patient in an unmodified state. This component is called as “Whole blood”(1).

Blood is a complex fluid consists of different blood cells suspended in yellowish fluid called plasma. The blood cells is composite mixture of red blood cells, white blood cells and platelets. The plasma contains water, electrolytes, proteins

required for clotting, immunoglobulins and numerous metabolic substances. Since blood products and biologic technologies are inherently variable due to nature of source materials available at different places, processing blood and separating them into various components and biological products is a highly specialised process. Plasma is supernatant of centrifuged whole blood.(2) Adherence to proper indications for blood component therapy is essential because of its potential adverse effects and costs of transfusion.

WHO recommends that “National Blood System should be governed by the country’s own National Blood Policy and legislative framework to develop uniform implementation of standards and consistency in the quality and safety of blood and blood products”.

According to National blood transfusion council of India, Blood Transfusion Services must ensure that Blood/ Components (Whole Blood/ Packed Red Cells/ Plasma/ Platelets) are

- Available (Adequate Blood Collection to fulfil population’s need)
- Accessible (Enough reach where it is needed)
- Affordable (At reasonable costs)
- Safe (Not cause any harm, especially TTI)
- Of standard quality (Provide clinical gain)

The first objective of National Blood Policy is

"To reiterate firmly the Govt. commitment to provide safe and adequate quantity of blood, blood components and blood products" (3)

One of the major causes of death is uncontrolled bleeding associated with trauma-induced coagulopathy, often attributed to depletion in clotting factors, uncontrollable fibrinolysis, or both. Massive hemorrhage is one of the most challenging issue in critical care, affecting trauma patients, surgical patients, obstetric patients and gastrointestinal patients. Ensuring the availability of right component at right time is critical in saving the life of such patients.

Fresh frozen plasma is stored at $-18^0$c or lower for 1 year. During an emergency thawing FFPs takes 30-40 minutes which delays transfusion. This leads to increased mortality. This study aims at exploring the possibilities by which FFP can be made readily available to patients during their utmost need by storing at 2 $-6^0$c after thawing.

# AIM

# AIM:

Study of stability of hemostatic potential of thawed plasma on storage at $2^0$ to $6^0$ c for 5 days.

# OBJECTIVE

# OBJECTIVE:

1. To measure Activated partial thromboplastin time and levels of factor V, VII, VIII (labile factors) in thawed plasma on storage at 2-6$^0$ C on days 1, 3 ,5 and compare with that stored at -70$^0$C.

2. To measure Thrombin generation capacity of thawed plasma on storage at 2-6$^0$ C on days 1, 3 ,5 and compare with that stored at -70$^0$C.

# REVIEW OF LITERATURE

# REVIEW OF LITERATUE:

Death following trauma remains to be top 10 causes of death in India in the 2018. 20-40% of trauma related deaths is because of massive bleeding following injury and many of these deaths occur during first few hours of definitive care. At least 15% of trauma related deaths are potentially preventable by rapid hemorrhage control and aggressive transfusion strategies. A trauma patient's risk of death stems from combination of three factors

- Hypothermia
- Acidosis
- Coagulopathy

Around 25-35% of trauma patients are in coagulopathy at the time of admission. Coagulopathy, in simple terms is inability of blood to clot normally(4–6).

**Normal physiological mechanisms of coagulation:**

The cell based model of coagulation has replaced conventional cascade model coagulation. The cell based model of coagulation involves 3 phases that overlap with each other(7).

- Initiation phase.
- Amplification phase.
- Propagation phase.

Initiation phase:

Vascular endothelium and surface of blood cells express tissue factor on their surface following injury. Tissue factor interacts with plasma derived Factor VII which

in turn activates small amounts of Factor IX and Factor X. Factor Xa along with its co factor Va forms prothrombinase complex on the surface of cells expressing tissue factor. The prothrombinase complex converts small amounts of prothrombin to thrombin. The amount of thrombin generated in this phase is too small for fibrin clot formation but they play a very important role in amplification phase.

Amplification phase:

The small amounts of thrombin generated from initiation phase interacts with platelets, FVIII-Von Willibrand Factor complex. This culminates platelet plug formation, secondary hemostasis. Another function of the thrombin formed during the initiation phase is the activation of the FV and FVIII cofactors on the surface of activated platelets. The FVIII/vWF complex is dissociated and this allows vWF to mediate in platelet adhesion and aggregation at the site of injury. In addition to the above, small amounts of thrombin also activate FXI (FXIa) on the platelet surface during amplification phase. Thrombin activates FXI on platelet surface explaining why normal hemostasis remains unaffected even at low FXII levels. Simultaneously, due to chemotactic mechanisms, these factors are attracted to the surface of platelets where they rapidly began the propagating phase.

Propagation Phase:

Propagation phase involves migration of large number of platelets to the site of endothelial injury and production of tenase and prothrombinase complexes in large amounts. First the FIXa produced during the initiation phase binds to FVIIIa on the

platelet surface forming tenase complex. As FXa produced during the initiation phase cannot move out of the cell, large amounts of FXa must be generated on the surface of activated platelets by tenase complex. FXa on the surface of activated platelets rapidly associates with FVa forming prothrombinase complex which eventually leads to production of large amounts of thrombin.

Termination Phase:

Once the fibrin clot is formed, the entire process should gradually stop preventing complete occlusion of the vessel. Four natural anticoagulants are responsible for preventing further activation of clot,

- Antithrombin
- Tissue factor pathway inhibitor
- Protein C
- Protein S

Tissue factor pathway inhibitor forms a quaternary complex with TF/TFPI/FVIIa/FXa inactivates activated clotting factors and limits coagulation. Protein C, a vitamin K dependent glycoprotein present in plasma promotes proteolysis of FVa and FVIIIa cofactors. Protein S, another vitamin K dependent glycoprotein that acts as co factor for activated protein C. Antithrombin directly inhibits activity of thrombin.(8)

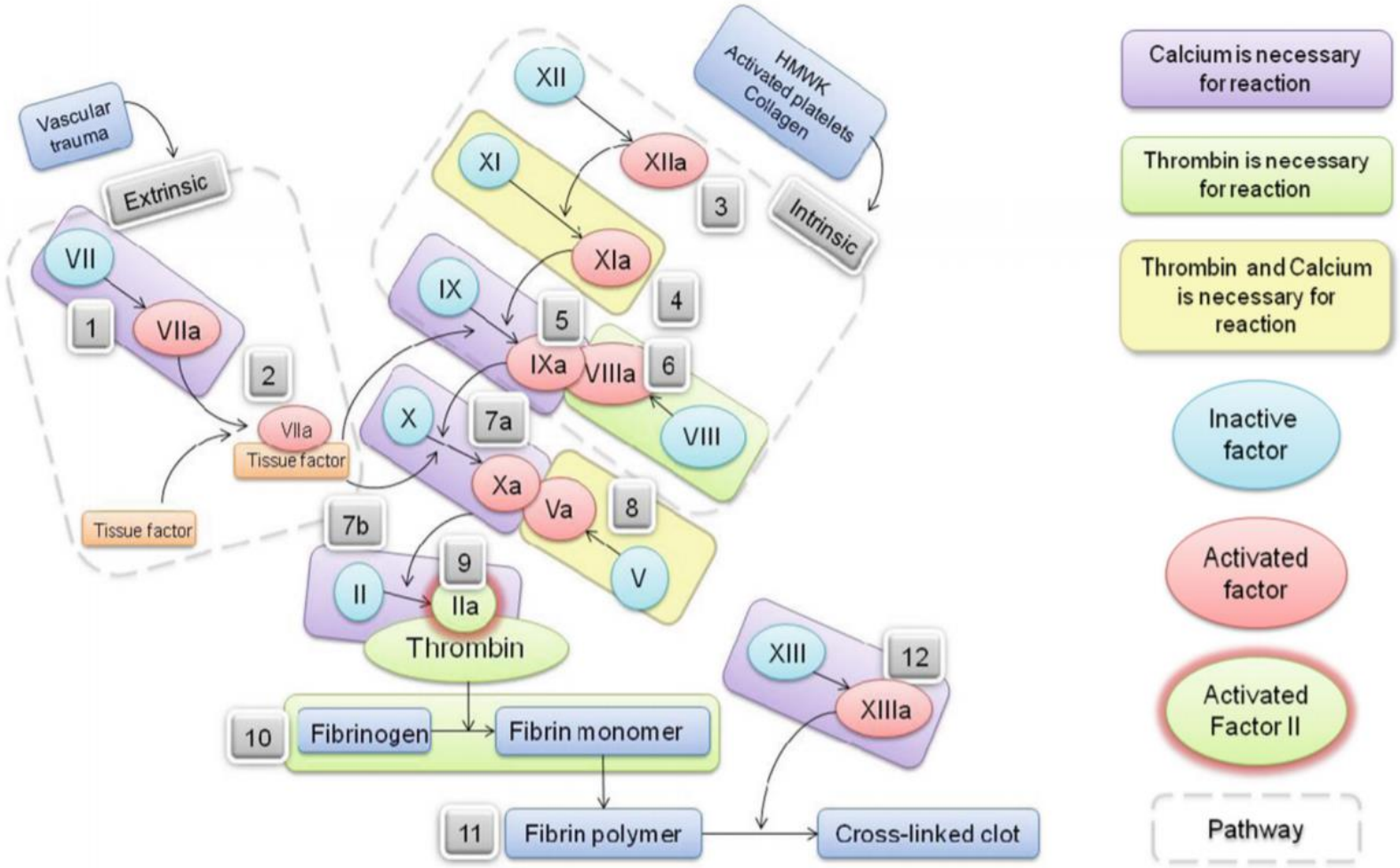



Figure 1: Conventional coagulation cascade

Adapted from Ultra structure of coagulation and inflammation Inflammation. 2015 Aug;38(4):1707-26.

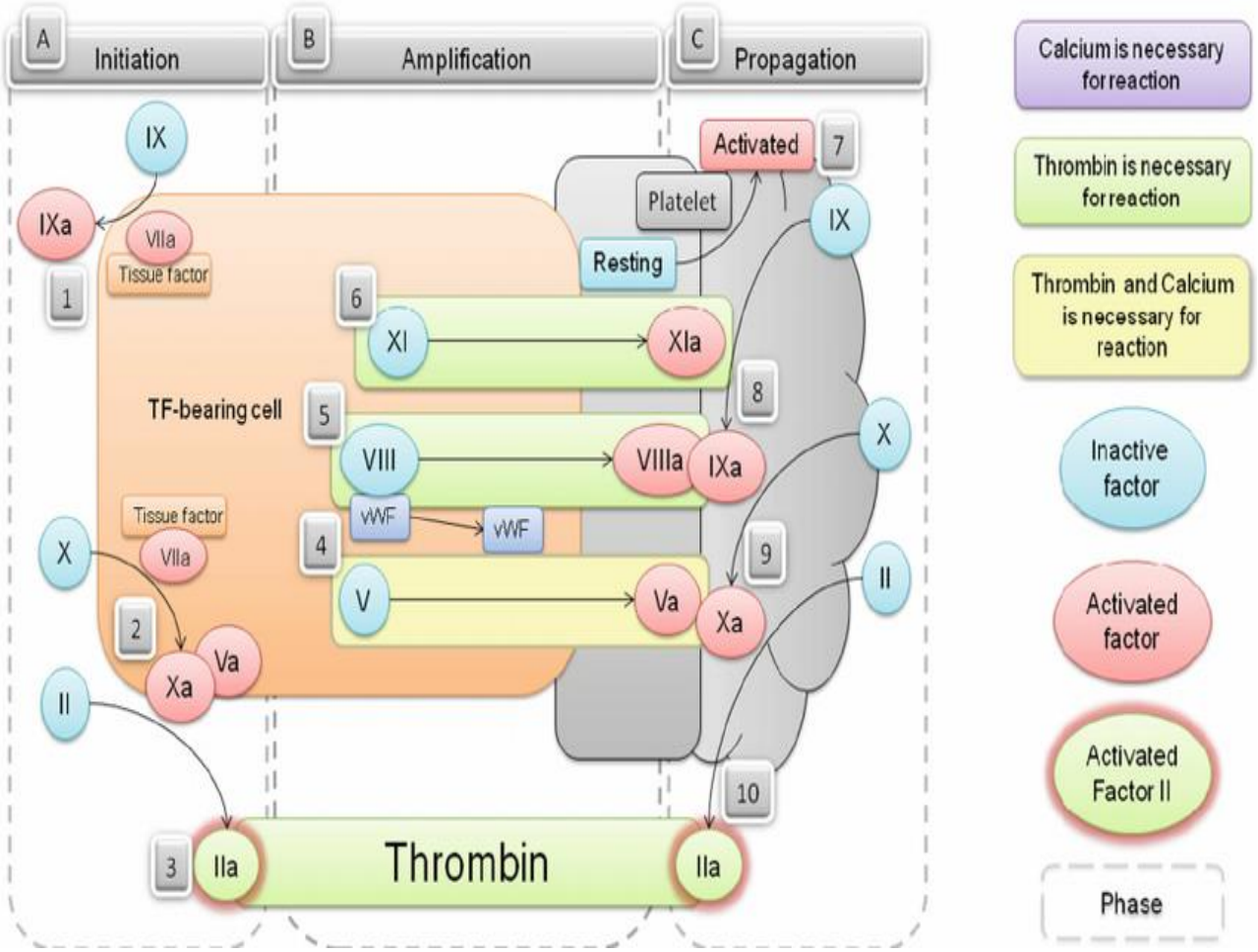



Figure 2: Adapted from Ultra structure of coagulation and inflammation Inflammation. 2015 Aug;38(4):1707-26.(9)

**Pathophysiology of trauma induced coagulopathy:**

1) Coagulation activation

- Procoagulants in systemic circulation
- Impairment of endogenous anticoagulant activity
- Thrombin generation in the systemic circulation

2) Hyperfibrinolysis

- Acute release of tissue plasminogen activator induced hyperfibrinolysis
- Coagulation activation induced fibrinolysis

3) Consumption Coagulopathy

Procoagulants in systemic circulation:

Several microparticles released into the systemic circulation in acute phase of trauma like platelet derived microparticle, endothelial derived microparticle, leucocyte derived microparticle and erythrocyte derived microparticle act as procoagulants during acute phase of trauma.(10,11)

Impairment of endogenous coagulant activity:

In severe trauma patients, the endogenous anticoagulant activities are immediately impaired by release of soluble thrombomodulin into the circulation and decreased protein C and resulting in dysregulation of coagulation activation.(11–15)

Thrombin generation in the systemic circulation:

The presence of procoagulants in systemic circulation and together with impairment of anticoagulation leads to thrombin generation. The thrombin has a very short half -life, which precludes measurement of plasma concentrations. Soluble fibrin and fibrinopeptide A are considered to reflect active thrombin because they reflect direct

action of thrombin on fibrinogen. (11–15)

Hyperfibrino(geno)lysis:

In severe trauma, hyperfibrinogenolysis, a combination of fibrinolysis and fibrinogenolysis, is frequently observed as a result of acute release of tissue plasminogen activator and coagulation activation. (11–15)

Shock induced hyperfibrinogenolysis:

Severe shock due to tissue hypoperfusion stimulates the endothelial cells and induces release of t-PA from Weibel-Palade bodies into the systemic circulation. Tissue plasminogen activator serves as a key enzyme in fibrinogenolysis be catalyzing cleavage of plasminogen to plasmin. (11–15)

Coagulation activation induced fibrinogenolysis:

Hyperfibrino(geno)lysis without shock is induced by coagulation activation and is recognized by elevation of D-dimer and fibrin/fibrinogen degradation product (FDP) levels which is usually seen in neuro trauma cases. In the acute phase of trauma, plasma Plasminogen activator inhibitor activity has not yet increased enough. Therefore, although trauma-induced coagulation activation reactively causes fibrino(geno)lysis, the fibrino(geno)lysis is not suppressed by PAI. (11–15)

Consumption coagulopathy:

Both coagulation activation and hyperfibrinogenolysis are simultaneously

observed in severe trauma cases leading to consumption of various coagulation factors and platelets. This leads to dilutional coagulopathy. Of all the coagulation factors, fibrinogen and factor V activity decreases more than the other factor activities. (11–15)

## Management of trauma induced coagulopathy:

Despite traumatic injury remaining a leading cause of death worldwide, few prospective randomized studies have been performed to elucidate the best method for haemorrhagic resuscitation. The PROMMTT study(16) revealed improved survival at 6 hours, for patients that received higher ratios of plasma to RBC or platelets to RBC. 1:1:1 (plasma: platelets: RBC) vs. 1:1:2 transfusion strategies in 680 severely injured patients (median injury severity score (ISS) 26.5). There was a 3.7% reduction in 30-day mortality in patients who received higher ratio of plasma (22.4% vs. 26.1%, respectively). In addition, patients in the 1:1:1 group achieved earlier haemostasis and had fewer deaths as a result of exsanguination at 24 h (9.2% vs. 14.6%, 95% CI -10.4% to -0.5%). In trauma, a recent series of retrospective clinical studies suggests that early and aggressive use of FFP at a 1:1 ratio with red blood cells (RBC) decreases mortality in cases of massive hemorrhage. this strategy – also known as hemostatic damage control or formula-driven resuscitation – has received substantial attention worldwide.

This emphasizes the need of early resuscitation with prewarmed plasma in acute trauma patients. This early formula-driven hemostatic resuscitation

proposes transfusion of FFP at a near 1:1 ratio with RBC, thus addressing coagulopathy from the beginning of the resuscitation and potentially reducing mortality.(17,18) Nevertheless, this strategy requires immediate access to large volumes of thawed universal donor FFP, which is challenging to implement.(19)

**Plasma:**

Plasma is prepared from whole blood and by apheresis. Plasma is generally frozen to maintain factor activity and to extend shelf life. Plasma is in some ways the most stable, and in others the least stable, of the components prepared from a unit of donated whole blood. Because plasma can be easily frozen and thawed without incurring significant losses of plasma protein activity, it can be stored for 1 to 2 years which is much longer than the allowable storage time for platelets (Platelets- 5 to 7 days) or red blood cells (RBCs; up to 42 days).(20)

Most indications for plasma transfusion reflect a need to restore hemostasis, by providing coagulation factors depleted in the patient by over anticoagulation, liver disease, or trauma; another significant area of utilization is for plasma exchange in thrombocytopenic thrombotic purpura. However, except in thrombocytopenic thrombotic purpura, high-level clinical evidence for the efficacy of plasma transfusion is not available. As per Indian drugs and cosmetic act & rules 1945 (DCA), one percent or 4 units per month of all components prepared and stored at appropriate temperatures and

conditions have to meet specific quality control parameter range as specified to prove blood components were prepared and stored appropriately(21).

Fresh Frozen Plasma (FFP) is one of the blood components prepared routinely from whole blood donations using the principle of density gradient centrifugation from the whole blood. Plasma is obtained as a supernatant, which is expressed out into the satellite bag and frozen (solidified) at a temperature less than -65ºC within 6 to 8 hours of collection, and then stored at a temperature less than $-30^0$C or below for 12 months from the date of collection(22),(23). If FFP is frozen between 8 to 24 hours after phlebotomy, it shall be labelled as "Plasma Frozen" within 24 Hours after Phlebotomy(24).

FFP or other plasma products are mainly used to treat coagulopathy due to multiple factor deficiencies or in therapeutic plasma exchange. DCA, National blood transfusion council (NBTC) under National AIDS control organisation (NACO) and other International organizations have prescribed quality control parameters for FFP and for other plasma components. As assessment of various factors is costly, most blood banks rely on PT and aPTT studies to assess quality of FFP.

**Fresh frozen plasma:**

FFP must be placed in the freezer within 8 hours of collection; within 6 hours if anticoagulated with ACD; or as directed by the manufacturer's instructions for use of the blood collection, processing, and storage system. FFP has a shelf life of 12 months when stored at –18 $^0$C or colder.(20)

**Thawed plasma:**

In contrast to its longevity in the frozen state, however, in liquid form

plasma has been reported to suffer significant losses in coagulation factor activity within 1 day, chiefly in the labile factors Factor VIII and FV. For clinical use plasma must be thawed at $37^0$C to transfuse it to patients. The procedure for thawing takes at least 30 mins and once thawed the plasma can be stored at 1-$6^0$C and must be used within 24 hours.

**NEED FOR THAWED PLASMA:**

During emergencies, time taken to thaw the fresh frozen plasma complicates the rapid provision of plasma therapy to patients in urgent need of this intervention because of the time required to thaw frozen plasma. The shelf life of thawed plasma is 24 hours leading to wastage of plasma products. Selecting an optimal permissible time for the storage of thawed plasma therefore requires a balance between maintaining high coagulation factor activities and minimizing plasma wastage. This challenge has intensified with changes in hospital practice such as the maintenance of stocks of thawed plasma for immediate transfusion to trauma patient(25).

The recently published BCSH guideline "A practical guide-line for the hematological management of major hemorrhage"(26), recommends that transfusion laboratories seeing major hemorrhage cases(27) should consider storing pre-thawed plasma on standby to allow FFP to be immediately available for resuscitation and management of hemorrhagic shock. Some centers are already doing this; how-ever this practice is leading to practical difficulties, including FFP wastage due to the current shelf-life of pre-thawed FFP being only 24 hours.

The Joint United Kingdom (UK) Blood Transfusion and Tissue Transplantation

Services' Professional Advisory Committee (JPAC) has reviewed the available data on FFP with the view to extending the shelf life of pre-thawed FFP to >24 hrs, and have agreed that:

**" The shelf life of thawed standard FFP can be extended from 24 h to 120 h, to enable rapid provision of FFP for the management of unexpected major hemorrhage with-out excessive wastage"** (19).

The hemostatic stability of thawed plasma can be measured using several coagulation tests like activated partial thromboplastin time, fibrinogen and factor assays. Apart from the conventional coagulation tests, global hemostatic tests such as thrombin generation test and other viscoelastic tests can also be used.

**Activated partial thromboplastin time:**

The test measures the clotting time of plasma after the activation of contact factors by the addition of phospholipid and $CaCl_2$ but without added tissue thromboplastin. Hence it indicates the overall efficiency of the intrinsic pathway.

The plasma is first preincubated with a contact activator such as kaolin, silica or elagic acid for a set period. During this phase of the test, FXIIa is produced, which cleaves FXI to FXIa, but coagulation does not proceed beyond this point in the absence of calcium. After recalcification, XIa activates FIX and coagulation follows. A standardised phospholipid is provided to allow the test to be performed on PPP. This test is dependent not only on the contact factors and on factors VIII and IX, but also on

the reactions with factors X, V, prothrombin and fibrinogen. It is also sensitive to the presence of circulating anticoagulants(inhibitors) and heparin(28)(29).

**Factor VII:**

Factor VII is a 50-kDa single-chain glycoprotein synthesized in the liver and secreted into the blood as a zymogen composed of 416 amino acids. Its biosynthesis requires vitamin K. It forms complexes with tissue factor and is converted to a serine protease called factor VIIa by limited proteolysis. Factor VIIa is responsible for conversion of factor X to factor Xa and factor IX to factor IXa in the presence of tissue factor and calcium ions.

**Factor V:**

Factor V is a 330-kDa glycoprotein containing 2196 amino acids. It is synthesized as a single chain molecule in liver and megakaryocytes and circulates in blood as an inactive cofactor. Factor V is activated to factor Va by thrombin and in turn is a cofactor in the conversion of prothrombin to thrombin catalysed by factor Xa in the presence of calcium and phospholipids.

## Factor assays:

**Assays based on the prothrombin time:**

The investigation of an isolated prolonged PT includes a one-stage factor VII assay.

**One-stage assay of factor VII, V**

**Principle.** The assays of factor VII and V are based on the PT. The assay compares the ability of dilutions of the patient's plasma and of a standard plasma to correct the PT of a substrate plasma. It is easily adapted to assay prothrombin or FX.

**Factor VIII:**

Factor VIII is a 330-kDa glycoprotein that participates in the middle phase of the intrinsic pathway of blood coagulation. It is synthesized primarily in the liver and endothelium and secreted into the blood, where it circulates as a complex with von Willebrand factor. Factor VIII accelerates the conversion of factor X to factor Xa in the presence of factor IXa, calcium, and phospholipids. For its action, factor VIII must undergo minor proteolysis by thrombin or other less defined proteases.

**Assays based on the activated partial thromboplastin time:**

An APTT-based assay (e.g. for FVIII) may be indicated after obtaining correction of a prolonged APTT by mixing with another plasma. An assay for FVIII is described, but this is easily adapted to FIX, FXI or contact factor assays by substituting the relevant factor-deficient plasma(28).

The typical coagulometric measurements, such as prothrombin time (PT) and activated partial thromboplastin time (aPTT), measure only the clotting time corresponding to the initiation phase of the coagulation process. Furthermore, the end-point of these tests occurs after the formation of only 5% of total thrombin. Therefore,

PT and aPTT reflect only the initial coagulation process while the formation of thrombin and fibrin is still occurring. A greater amount of thrombin is generated during the amplification and propagation phases, resulting in an exponential increase in thrombin, Evaluating individual coagulation factor content does not provide an overall picture of the hemostatic potential of the component. The global clotting assays, such as Thrombin generation test, assess the overall coagulation potential of plasma and specifically measure the dynamic processes of thrombin or clot formation and degradation. (30)

**Global hemostatic tests**

**Thrombin generation time**

The conventional coagulation tests like PT and APTT use the formation of a fibrin clot as the endpoint of the test and yet we know that this occurs when only approximately 5% of the total amount of thrombin that is generated. The generation of thrombin is a important part of the clotting cascade and as such an estimation of an how well a particular individual can generate thrombin may correlate with either a risk of bleeding or thrombosis and truly represents the hemostatic status of the patient. This technique evaluates the overall balance between procoagulant and anticoagulant forces including tertiary hemostasis and has provided new insights in our understanding of the coagulation cascade, as well as of the diagnosis of hypo coagulable and hypercoagulable conditions. Thrombin generated in the thrombin generation test can be quantitatively measured in both platelet-rich or platelet-poor plasma using the calibrated automated thrombogram method, which monitors the

cleavage of a fluorogenic substrate which is simultaneously compared to the known thrombin activity of a plasma sample which does not clot. The calibrated automated thrombogram method is an open system, which can make use of different antibodies, proteins, enzymes and peptides that are introduced to answer specific questions regarding hemostatic processes. The clotting times of the fibrinogen solution were used to estimate thrombin concentration by calibrating it against a thrombin standard. These so-called thromboplastin generation tests [TGTs] formed the basis of the 2-stage factor assay. (31)

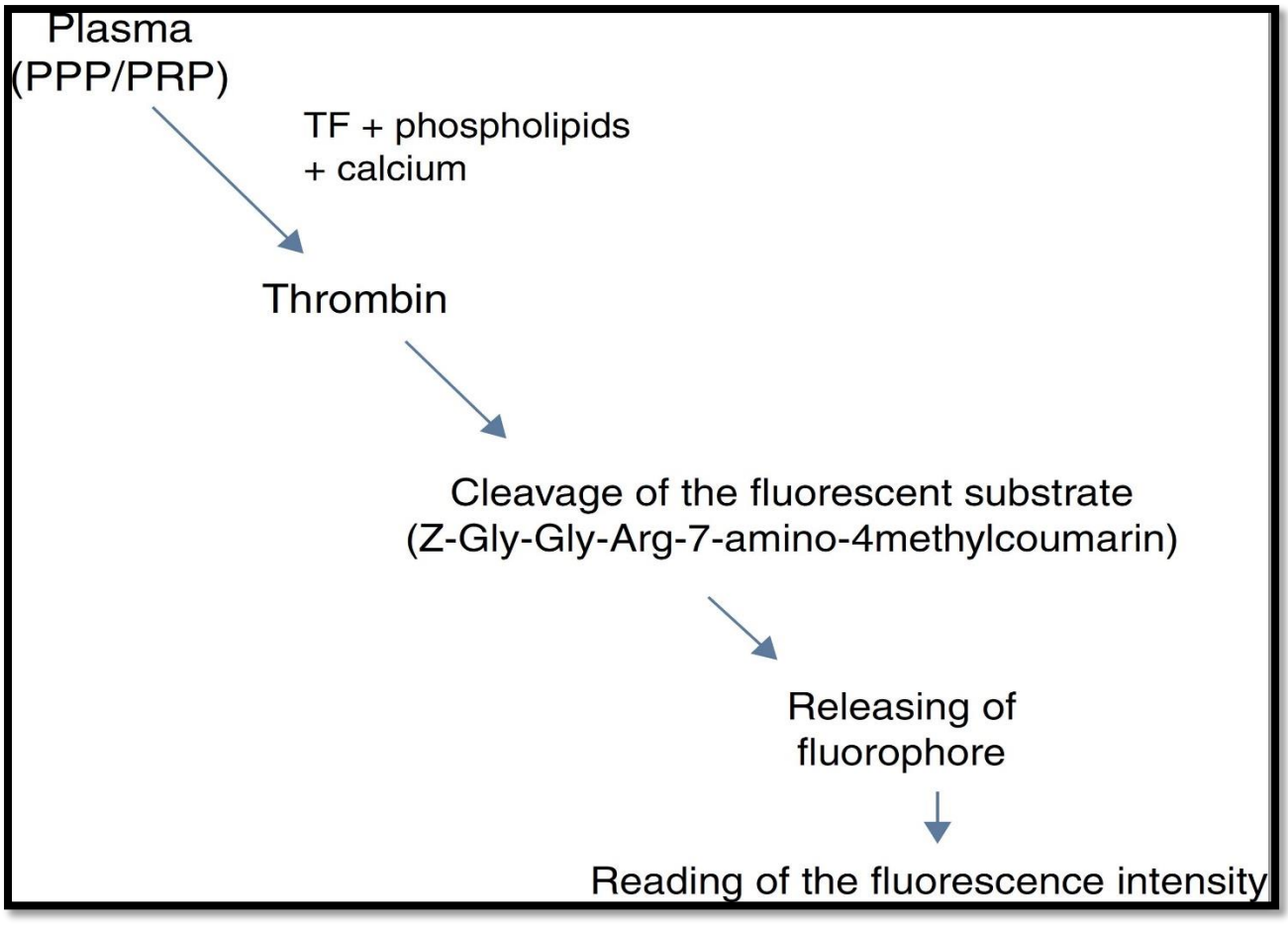



Figure 4: Calibrated automated thrombogram. Adapted from **Thrombin generation assays for global evaluation of the hemostatic system: perspectives and limitations.** (32)

The following variables of the TG curve will be measured:

- The lag time
- The time to the peak (ttP)
- The peak thrombin
- The area under the curve, known also as the endogenous thrombin potential
- The start tail(33)

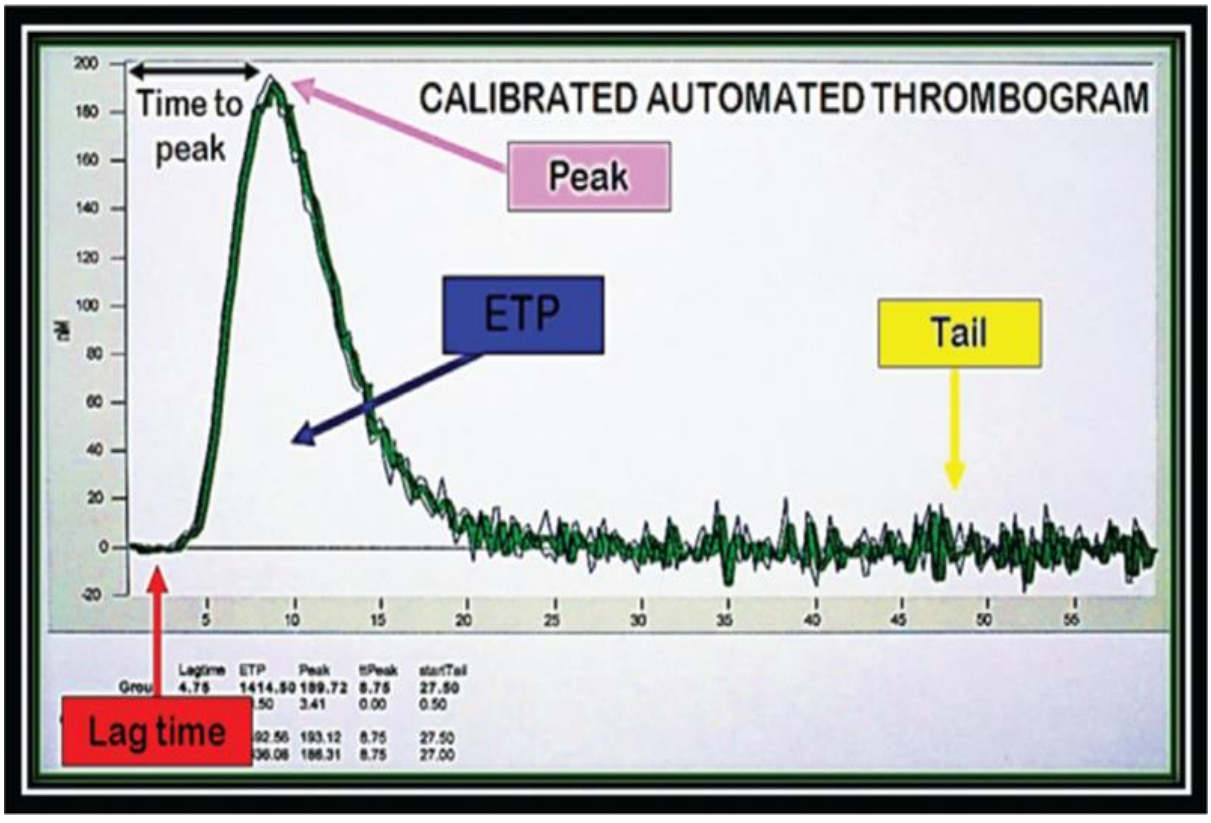



**Figure 5: Markers of thrombin generation. The image was obtained from the thrombinogram guide.**

**ETP = endogenous thrombin potential**(34)

**Key observations from the studies:**

Several studies have studied the stability of hemostatic potential of thawed plasma on storage at 1-6$^0$C. In 2001, Downes et al was the first one who studied the effects of storing thawed plasma at 1-6$^0$ C for 5 days. He observed that, FVIII, being heat labile, had the greatest decline in activity, with a drop of 28 percent from the original level of activity within the first 24 hours of storage. 4 Factor V is also heat labile (37°C), but it appears to be stable at 4°C. All other clotting factors are stable for over 5 days.5 Thawed plasma stored up to 5 days can safely be used to treat the coagulopathy of liver disease, thrombotic thrombocytopenic purpura, and disseminated intravascular coagulation and to reverse the effect of coumadin. Because all factors except fibrinogen decreased with extended storage, we elected at this time to use a more conservative expiration time of 3 days rather than 5 days. Extension to 3 days of the expiration time for thawed plasma reduced wastage from 5.1% to 1.1%(35).

In 2009 Heymann et al conducted a similar study with 20 fresh frozen plasma and found that all clotting factors and inhibitors remained within the reference range requested by quality assurance regulations. No FFP bag showed bacterial contamination(36).

In 2012 Sheffield et al experimented it with 27 units and reported that Factor FV and FVIII, but not FVII, declined significantly within 24 hours. Mean losses measured on day 5 of storage were 20, 14, and 41%, in FV, FVII, and FVIII, respectively; fibrinogen activity did not change. PT values were prolonged by 9% on Day 5(37).

With respect to the quality of thawed, stored plasma, previous studies showed that FFP, obtained from whole blood donations or apheresis, thawed and maintained

either at room temperature or at 1 to 6°C for 5 to 7 days, showed losses of 9% to 25% FV activity and 15% to 47% FVIII activity, with maintenance of fibrinogen and other factor levels(35) . However, with respect to FP24, two recent studies of 5-day refrigerated storage reported discordant results, at least with respect to FVIII losses, in that one reported negligible (3%) losses of activity on Day 5 after thawing , while the other cited 28% losses in line with the apheresis plasma studies(38) . Mean losses of FVIII of 45%, exceeding that reported in the latter study, were observed by Day 5 after thawing, if whole blood was processed to PLT-rich plasma (PRP) and the PRP was stored at room temperature for 24 hours before processing(39)

Though few studies show that there is no significant decrease in factor levels , in study done by Pati et al the thrombin generation time showed increased lag time , increased time to peak, decreased peak of thrombin generation , decreased area under the thrombin generation curve ( ETP) and increased time to start tail for Day 5 FFP(40).

Matijevic et al.(41) showed that although individual factor levels remained above 50% of normal levels in thawed FFP, after 5 days at 1–6°C, the Thrombin generation potential decreased significantly by 58%, as demonstrated at low tissue factor concentrations. In the assay with high tissue factor concentrations, Erickson et al. (42) demonstrated a decrease in the peak thrombin by mean of 26% in thawed PF24, during 5-day storage at 1–6°C. In contrast, Cookson et al. (43) found unaffected amount of TG by storage of PF24 (produced from whole blood, RT-FP24) for 6 days, but there was an increase in lag time and decreased rate of clot formation by ROTEM.

FVIII is the worst affected factor and is therefore an appropriate marker for validation studies; other factors are less affected .For FVIII, a significant proportion of units will be below the lower limit of normal at day 5 Most studies show that the largest decrease in FVIII activity occurs during the first 24 hours following thawing The reduction in FVIII may not be of such concern given that it is an acute phase reactant.

In bleeding trauma -patients the levels of FVIII do not reduce significantly in the first 24 hrs and therefore thawed FFP with lower levels of FVIII might be satisfactory, since replacement of FVIII in the immediate period may not be critical. It is not clear whether from a regulatory perspective there is an expectation for plasma at the end of its thawed shelf-life to meet the specification for FFP in terms of FVIII content. Several papers from John Holcomb s group(5) in the USA show that storage of plasma to day 5 results in much larger reductions in thrombin generation (lag time, peak thrombin (16-47%) and endogenous thrombin potential it may be due to the microparticle content of plasma due to differing methods of production or the length of time before plasma was frozen. They have shown a decrease in the platelet microparticle content of plasma during storage once thawed. Holocomb and few other groups have shown that the microparticle content of plasma contributes towards haemostasis in thrombin generation tests and ROTEM/ROTEG. John Holcomb s group hypothesise that the efficacy of FFP may in part be due to its effect in promoting vascular stability in addition to its haemostatic content.

Studies on pulmonary endothelial cells in culture show permeability induced by hypoxia can be reduced in the presence of FFP, but this effect is lessened in FFP

thawed and stored for 5 days (40).They have also shown in a rat model of hemaorrhagic shock that resuscitation with freshly thawed FFP appears to be superior to that with plasma 5 days after thawing (11).

The American Association of Blood Bank does not provide guidelines for quality control of FFP in regard to clotting factors. However, the Council of Europe requires testing of factor VIII level in 10 randomly selected units in their first month of storage (FVIII level should be >70% of the freshly collected plasma unit). There are no guidelines for other clotting factors. Regarding bleeding tendencies, approximately 40% of a single-factor activity is considered to be enough to support hemostasis in a single-factor deficiency. However, when multiple factors are depleted, which is often the case in major trauma with bleeding, it is difficult to estimate the necessary factor levels for adequate hemostasis. So use of global coagulation would help us in estimating the hemostatic potential of thawed plasma. If there is no significant change in hemostatic potential these Thawed FFPs will help us reducing the mortality among trauma patient(44).

# Materials and methods

## Materials and methods:

### Setting:

This study was conducted in Christian Medical College, Vellore, Tamil Nadu. It is a teaching hospital providing tertiary medical care service to the residents of Vellore and surrounding districts of Tamil Nadu and some parts of Andhra Pradesh and Kerala. It also serves as a referral centre for patients from rest of India and South East Asia.

This prospective observational study was carried out in the Departments of Transfusion Medicine and Immunohematology.

The study was approved by the Research and Ethics committee of the Institutional Review Board, Christian Medical College, Vellore.

This study was conducted in 2018 in the Hemostasis laboratory and blood bank of Department of Transfusion Medicine and Immunohematology.

### Sample size:

Based on the review of literature(45), sample size was calculated using the formula

$$n = \frac{4S^2}{d^2}$$

where, S-standard deviation of the difference in factor activity between day 0 and day 5

d- absolute precision

$$n = \frac{4\times(0.1)^2}{(0.05)^2}$$

n=15

Sample size required to prove statistical significance is 15.

A total of 19 bags (Irrespective of blood groups) were included in the study.

## STUDY PROTOCOL:

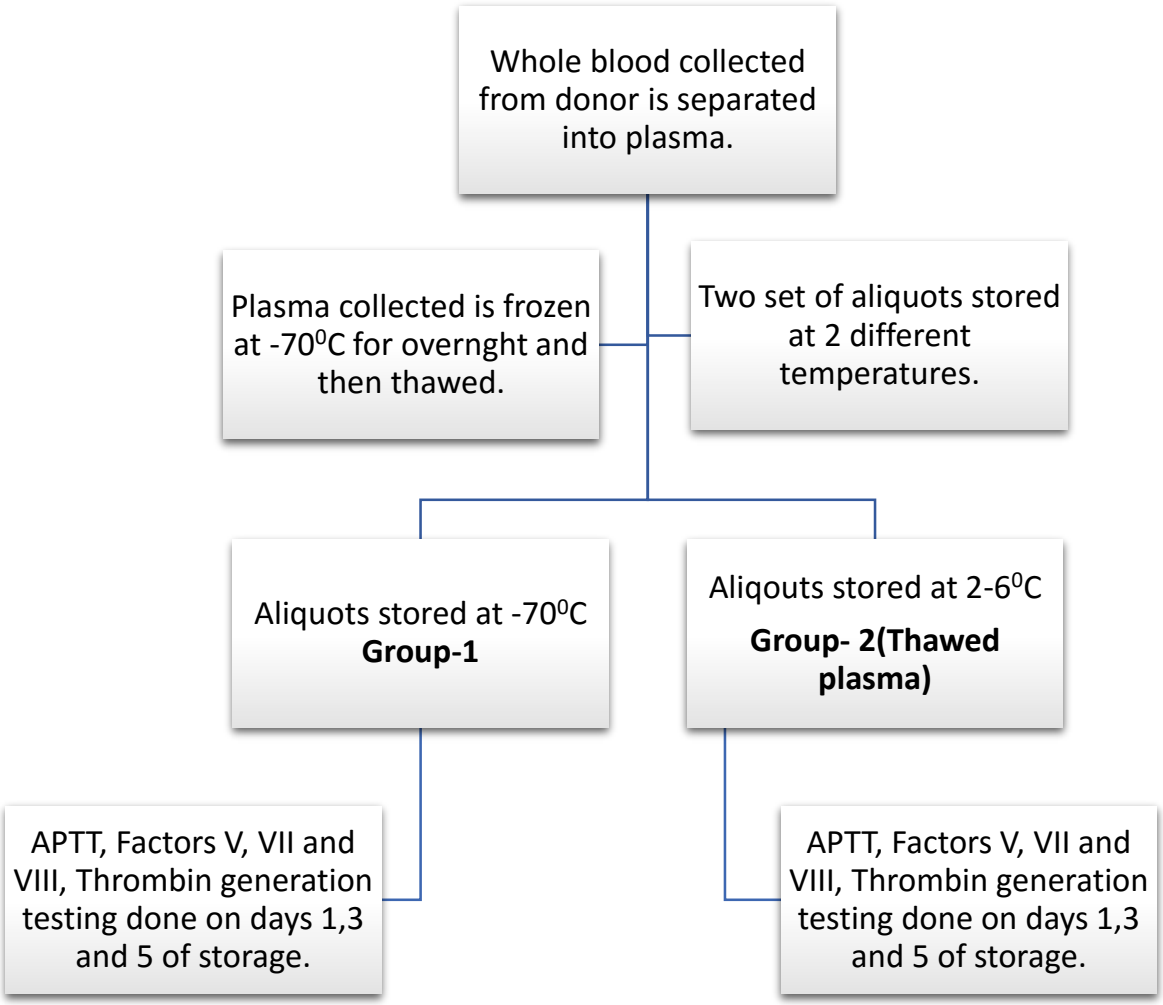
### Fresh frozen plasma:

For effective component preparation, 450 ml of blood (approximately 14 mL CPD per 100 mL Whole blood) from healthy donor is drawn into a triple / quadruple blood bag through a single venipuncture ensuring rapid and uninterrupted flow of blood. Plasma is separated from the whole blood collected from the donor using Hettich Rota Centrifuge. According to DGHS and AABB standards it is frozen at -70$^0$C within 6 hours of collection.(20,46) After overnight freezing it is thawed at 35.8$^0$C in a plasma thawer (MT204, Thermogenesis) for 20 minutes. It is separated into aliquots and stored at 2 different temperatures. One set of plasma aliquots at -70$^0$C and the other set of aliquots at 1-6$^0$C for 5 days. All the required tests for the study were in the plasma aliquots on Days 1,3 and 5 of storage. Activated partial thromboplastin time, Levels of Factors V, VII and VIII, thrombin generation testing were done on storage days 1, 3 and 5.

## Quantitative variables measured:

1) Activated partial thromboplastin time (APTT),
2) Factor V,
3) Factor VII,
4) Factor VIII and
5) Thrombin Generation testing.

**Diagrammatic algorithm of study protocol**



All the coagulation parameters were done using automated coagulometer SYSMEX CS 2000i.

## Automated coagulometer SYSMEX CS 2000i

Instrument Name: SYSMEX CS 2000i

Manufacturer: SYSMEX CORPORATION, JAPAN

The CS-2000i/CS-2100i is a fully automated blood coagulation analyzer for in vitro diagnostic use that can quickly analyze a large volume of samples with a high degree of accuracy. This instrument can analyze samples using coagulation, chromogenic, immunoassay and aggregation methods. It is a higher end coagulation analyzer which can perform multiple functions.

## Activated partial thromboplastin time(APTT):

### Principle:

APTT was measured using Clot based assay.

This test measures the clotting time of the plasma after the activation of contact factors but without adding tissue thromboplastin and so it indicates the overall efficiency of intrinsic pathway. For the activation of contact factors, the plasma is first pre- incubated for a set period with a contact activator such as Kaolin or elagic acid. During this phase of the test, factor XIIa is produced which cleaves factor XI to XIa but coagulation does not proceed beyond this in the absence of calcium. After recalcification, F XIa activates factor IX and coagulation cascade proceeds. Since

these tests are performed on platelet poor plasma a standardized phospholipid is included in the reagent (28)

## Factor VIII assay:

**Principle**

Single stage APTT based Factor assay

Clot based assay

The CS 2000i has the ability to automate the factor assays and perform multiple dilutions on patient samples (parallelism). The dilutions are used to determine if a low factor activity level is truly a deficiency or is due to an inhibitor in the sample that is interfering with the clotting time. By comparing the % activity results from the multiple dilutions, this determination can be made. For this study three point dilution was done(7,47,48).

## Factor V and VII assay:

**Principle**

**FACTOR ASSAY BASED ON PT-CS 2000i**

Single stage PT based Factor assay

Clot based assay

The assays of factor II, V, VII and X can be performed using a one-stage assay based on the prothrombin time. The assay compares the ability of dilutions of a standard or

reference plasma and test plasma to correct the prothrombin time of plasma known to be totally deficient in the clotting factor being measured(7,47,48).

## THROMBIN GENERATION TESTING:

### Instrument:

Thermo Fluoroskan Ascent FL & Thrombinoscope BV

### Principle:

Calibrated automated thrombography displays the concentration of thrombin in clotting plasma with or without platelets (PPP/PRP) by monitoring the splitting of a fluorogenic substrate, upon splitting by thrombin it releases the fluorescent AMC (7-amino -4-methyl coumarin), which is measured by a 390nm excitation and 460nm of emission filter set and comparing it to a constant known thrombin activity in parallel(49,50).

### Parameters measured(49,50):

### Lag time[min]:

Equivalent to clotting time, From addition of reagents until first burst of thrombin occurs.

### ETP[nM.min]:

Endogenous thrombin potential, amount of work that can potentially be done by thrombin, area under the curve.

**Peak [nM]:**

Maximum velocity of peak thrombin.

**TT peak[min]:**

Time to reach peak thrombin

**Start Tail[min]:**

Time when thrombin generation is stopped**.**

**Statistical methods:**

- Mean and standard deviation were used for the dissipation of variables.
- Box plot was used for data description.
- The normality was tested using histogram plots.
- Paired t test was used for testing the difference in Percentage (%) change between the two groups or the statistical significance of variables over time.
- P value <0.05 was considered as statistically significant.
- Software used R 2.12.0

# RESULTS

# Results:

A total of 19 units collected during the period from December 2017 to May 2018 were included in the study.

## APTT:

APTT of both the groups were measured on storage days 1, 3 and 5.

Mean APTT was calculated for the individual groups.

### Group 1:

| | Group 1-FFP stored at $-70^0$ C | |
|---|---|---|
| | Mean (secs) | Standard deviation |
| APTT on day 1 | 30.97 | $\pm$4.58 |
| APTT on day 3 | 32.16 | $\pm$4.57 |
| APTT on day 5 | 32.66 | $\pm$4.47 |

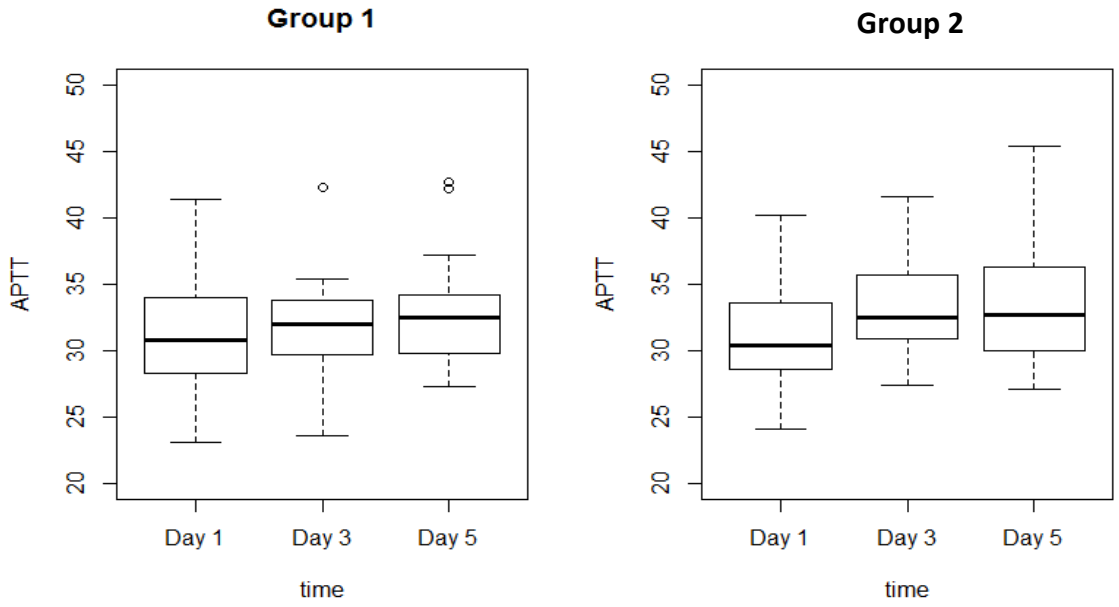
The mean APTT of Group 1 on Days 1, 3 and 5 of storage was 30.97, 32.16 and 32.66 secs respectively.

**Group 2:**

| | Group 2- Thawed plasma stored at 2-6$^0$ C | |
|---|---|---|
| | Mean (secs) | Standard deviation |
| APTT on day 1 | 30.92 | ±3.83 |
| APTT on day 3 | 33.17 | ±4.11 |
| APTT on day 5 | 33.47 | ±4.57 |

The mean APTT of group 2 on storage days 1,3 and 5 was 30.92, 33.17 and 33.47 respectively.

## Effects on APTT:




**Boxplot showing the APTT measurements on storage days 1,3 and 5 of both the groups**

## Factor V:

Factor V of all bags of both the groups were measured on storages days 1,3 and 5.

Mean Factor V for each group was calculated for all storage days.

### Group 1:

| | Group 1-FFP stored at $-70^0$ C | |
|---|---|---|
| | Mean (%) | Standard deviation |
| Factor V activity on day 1 | 76.35 | ±30.66 |
| Factor V activity on day 3 | 75.58 | ±20.93 |
| Factor V activity on day 5 | 73.31 | ±27.96 |

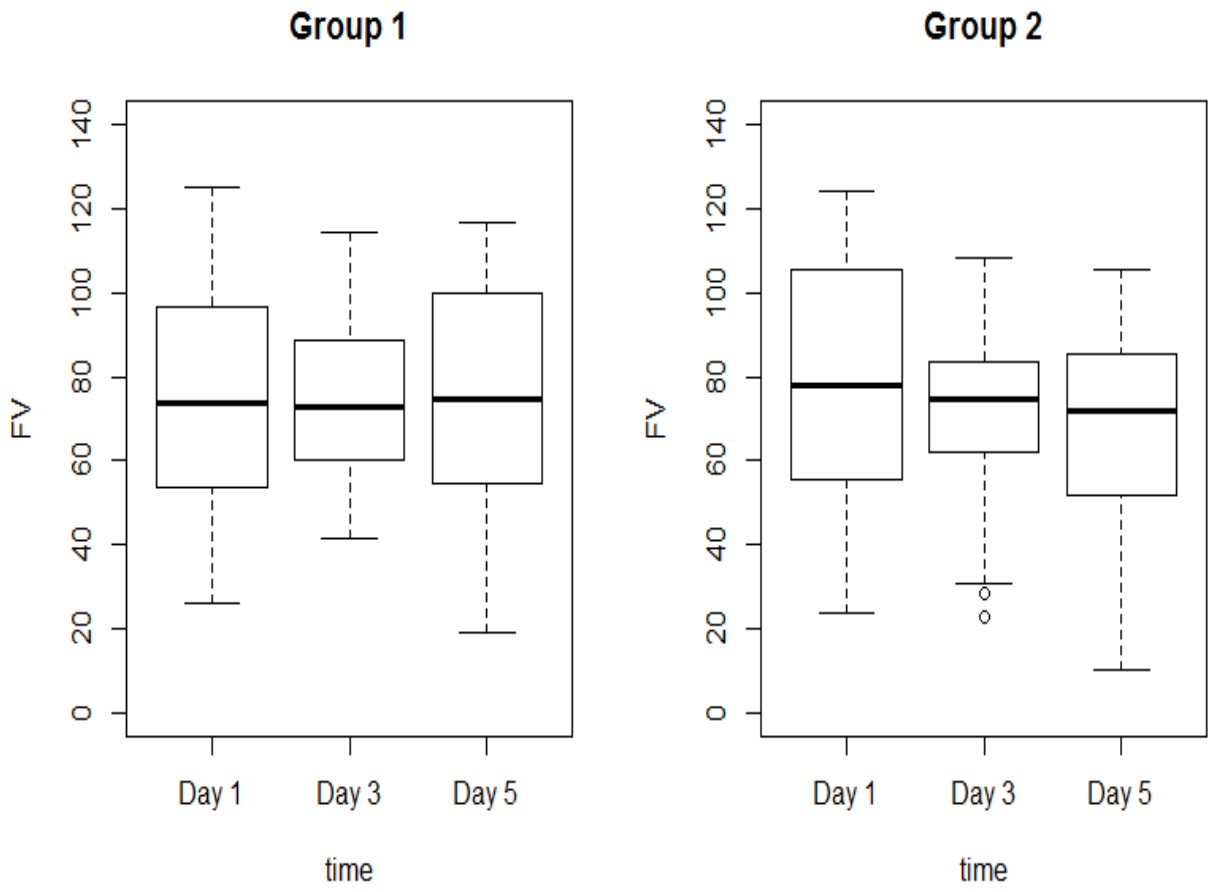
The mean Factor V activity of Group 1 on Days 1, 3, 5 of storage are 76.35%, 75.58%, 73.31% respectively.

### Group 2:

| | Group 2-Thawed plasma stored at $2\text{-}6^0$ C | |
|---|---|---|
| | Mean (%) | Standard deviation |
| Factor V activity on day 1 | 78.39 | ±31.22 |
| Factor V activity on day 3 | 70.44 | ±23.44 |
| Factor V activity on day 5 | 67.20 | ±26.56 |

The mean Factor V activity of Group 2 on Days 1, 3, 5 of storage are 78.39%, 70.44%, 67.20% respectively.

**Effects on Factor V:**




**Boxplot showing the Factor V measurements on storage days 1,3 and 5 of both the groups**

## Factor VII:

Factor VII of all bags of both the groups were measured on storages days 1,3 and 5.

Mean Factor VII for each group was calculated for all storage days.

## Group 1:

| | Group 1-FFP stored at $-70^0$ C | |
|---|---|---|
| | Mean (%) | Standard deviation |
| Factor VII activity on day 1 | 60.57 | ±23.32 |
| Factor VII activity on day 3 | 61.70 | ±21.64 |
| Factor VII activity on day 5 | 52.12 | ±27.68 |

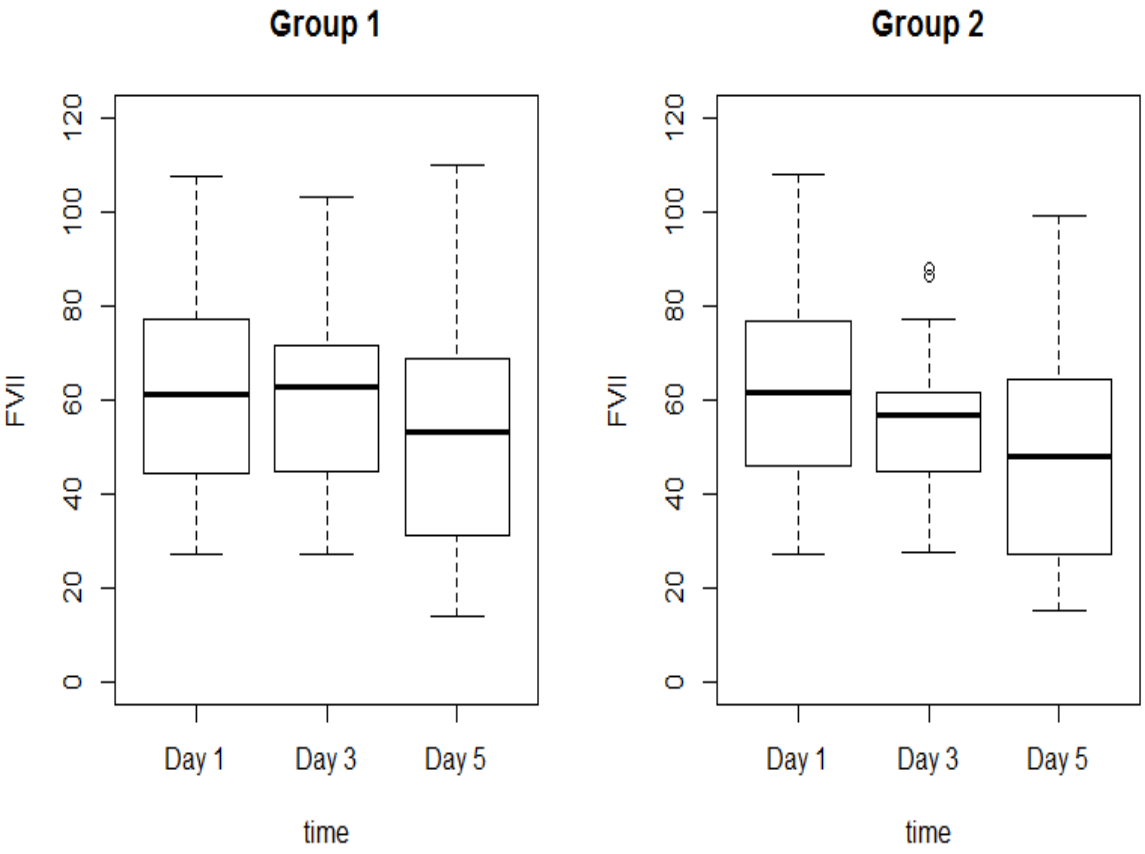
The mean Factor VII activity of Group 1 on Days 1, 3, 5 of storage are 60.57%, 61.70%, 52.12% respectively.

## Group 2:

| | Group 2-Thawed plasma stored at $2\text{-}6^0$ C | |
|---|---|---|
| | Mean (%) | Standard deviation |
| Factor VII activity on day 1 | 62.76 | ±24.51 |
| Factor VII activity on day 3 | 54.94 | ±17.47 |
| Factor VII activity on day 5 | 50.69 | ±25.39 |

The mean Factor V activity of Group 2 on Days 1, 3, 5 of storage are 62.76%, 54.94%, 50.69% respectively.

**Effects on Factor VII:**




**Boxplot showing the Factor VII measurements on storage days 1,3 and 5 of both the groups**

## Factor VIII:

Factor VIII of all bags of both the groups were measured on storages days 1,3 and 5.

Mean Factor VIII for each group was calculated for all storage days.

## Group 1:

| | Group 1-FFP stored at $-70^0$ C | |
|---|---|---|
| | Mean (%) | Standard deviation |
| Factor VIII activity on day 1 | 69.50 | $\pm$29.75 |
| Factor VIII activity on day 3 | 66.22 | $\pm$23.36 |
| Factor VIII activity on day 5 | 62.63 | $\pm$26.55 |

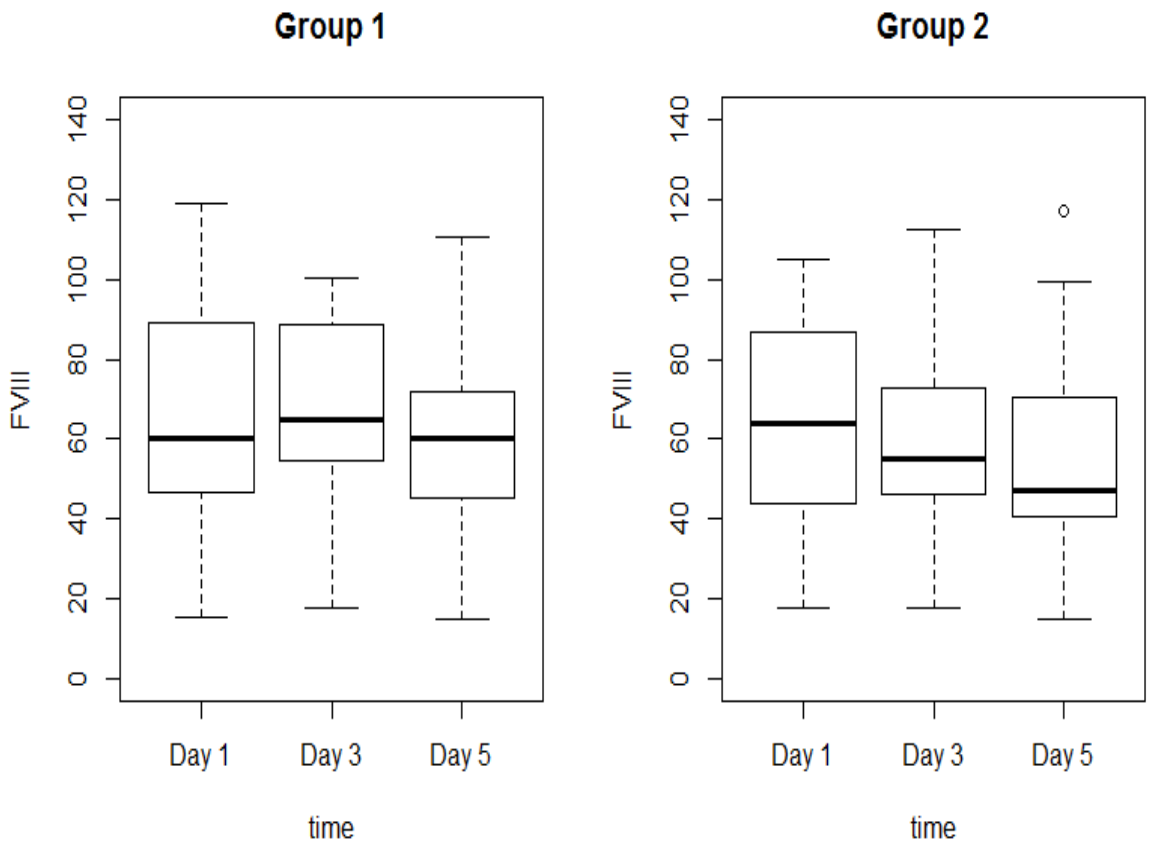
The mean Factor VIII activity of Group 1 on Days 1, 3, 5 of storage are 69.50%, 66.22%, 62.63% respectively.

## Group 2:

| | Group 2-Thawed plasma stored at $2\text{-}6^0$ C | |
|---|---|---|
| | Mean (%) | Standard deviation |
| Factor VIII activity on day 1 | 67.03 | $\pm$25.67 |
| Factor VIII activity on day 3 | 57.34 | $\pm$24.01 |
| Factor VIII activity on day 5 | 56.97 | $\pm$26.00 |

The mean Factor VIII activity of Group 2 on Days 1, 3, 5 of storage are 67.03%, 57.34%, 56.97% respectively.

## Effects on Factor VIII:




**Boxplot showing the Factor VIII measurements on storage days 1,3 and 5 of both the groups**

## Lag time:

Lag time of all bags of both the groups were measured using thrombin generation testing on storages days 1, 3 and 5. Mean Lag time for each group was calculated for all storage days.

## Group I:

| | Group 1-FFP stored at $-70^0$ C | |
|---|---|---|
| | Mean (min) | Standard deviation |
| Lag time on day 1 | 2.91 | $\pm$0.80 |
| Lag time on day 3 | 2.97 | $\pm$0.72 |
| Lag time on day 5 | 2.93 | $\pm$0.57 |

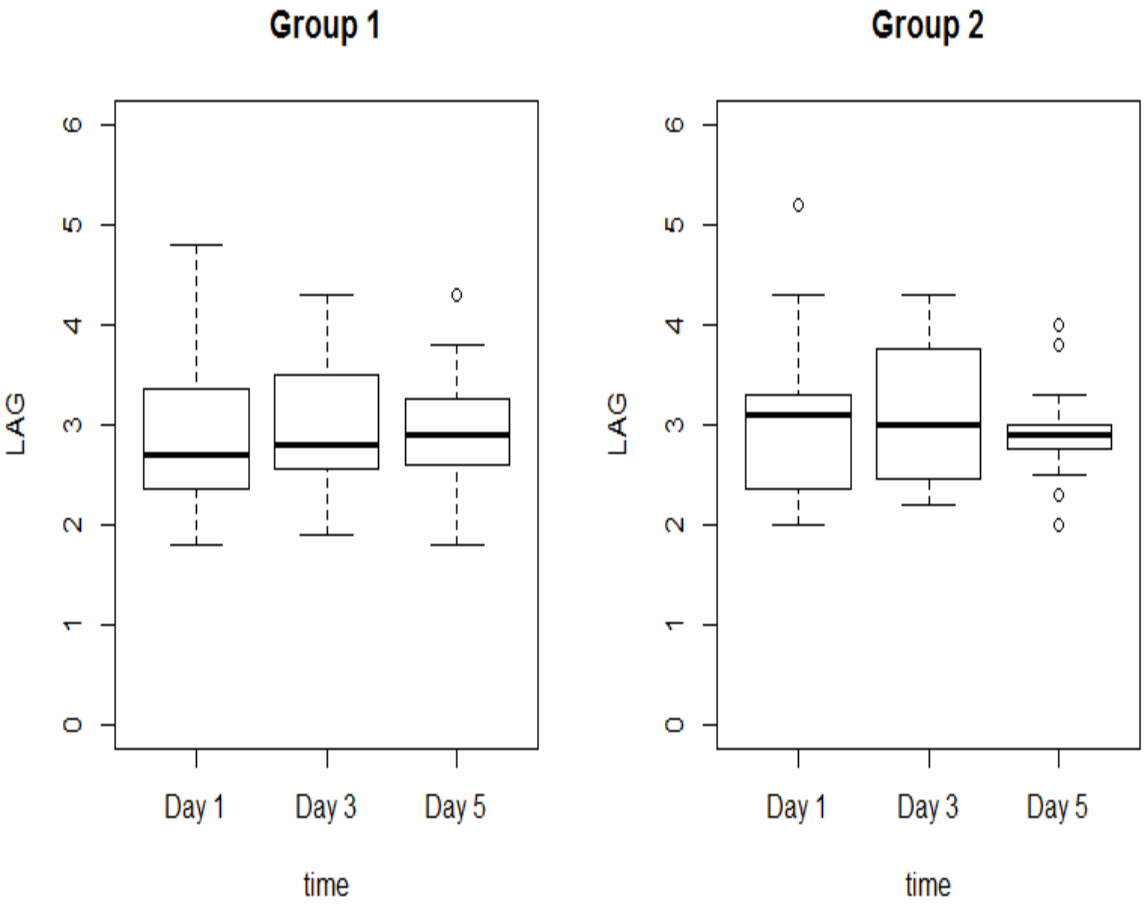
The mean Lag time of Group 1 on Days 1, 3, 5 of storage are 2.91, 2.97, 2.93 min respectively.

## Group 2:

| | Group 2-Thawed plasma stored at $2\text{-}6^0$ C | |
|---|---|---|
| | Mean (min) | Standard deviation |
| Lag time on day 1 | 3.02 | $\pm$0.78 |
| Lag time on day 3 | 3.11 | $\pm$0.76 |
| Lag time on day 5 | 2.93 | $\pm$0.47 |

The mean Lag time of Group 2 on Days 1, 3, 5 of storage are 3.02, 3.11, 2.93 min respectively.

**Effects on Lag time:**




**Boxplot showing the Lag time measurements on storage days 1,3 and 5 of both the groups**

## Endogenous thrombin potential:

The Endogenous thrombin potential is obtained from the thrombin generation testing on storage days 1, 3 and 5. Mean ETP for each group on each day were calculated.

## Group I:

| | Group 1-FFP stored at $-70^0$ C | |
|---|---|---|
| | Mean (nM.min) | Standard deviation |
| ETP on day 1 | 1553.04 | ±346.48 |
| ETP on day 3 | 1573.85 | ±247.47 |
| ETP on day 5 | 1601.75 | ±318.21 |

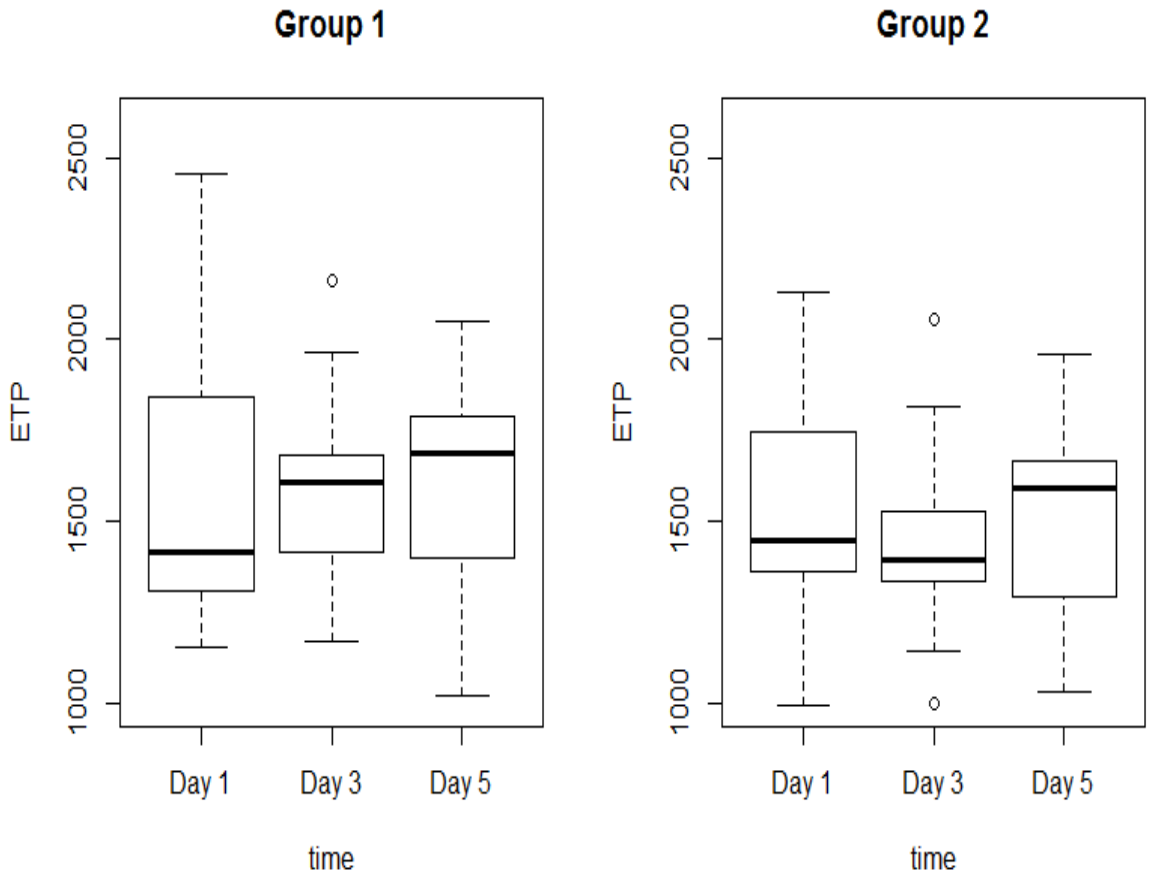
The mean ETP of Group 1 on Days 1, 3, 5 of storage are 1553.04, 1573.85, 1601.75 nM.min respectively.

## Group 2:

| | Group 2-Thawed plasma stored at $2\text{-}6^0$ C | |
|---|---|---|
| | Mean (nM.min) | Standard deviation |
| ETP on day 1 | 1514.37 | ±302.77 |
| ETP on day 3 | 1436.82 | ±261.77 |
| ETP on day 5 | 1499.03 | ±269.95 |

The mean ETP of Group 2 on Days 1, 3, 5 of storage are 1514.37, 1436.82, 1499.03 nM.min respectively.

**Effects on ETP:**




**Boxplot showing the Lag time measurements on storage days 1,3 and 5 of both the groups**

## PEAK:

The peak is obtained from the thrombin generation testing on storage days 1, 3 and 5. Mean ETP for each group on each day were calculated.

## Group I:

| | Group 1-FFP stored at -70$^0$ C | |
|---|---|---|
| | Mean (nM) | Standard deviation |
| Peak on day 1 | 366.67 | ±54.62 |
| Peak on day 3 | 418.11 | ±293.07 |
| Peak on day 5 | 359.98 | ±63.89 |

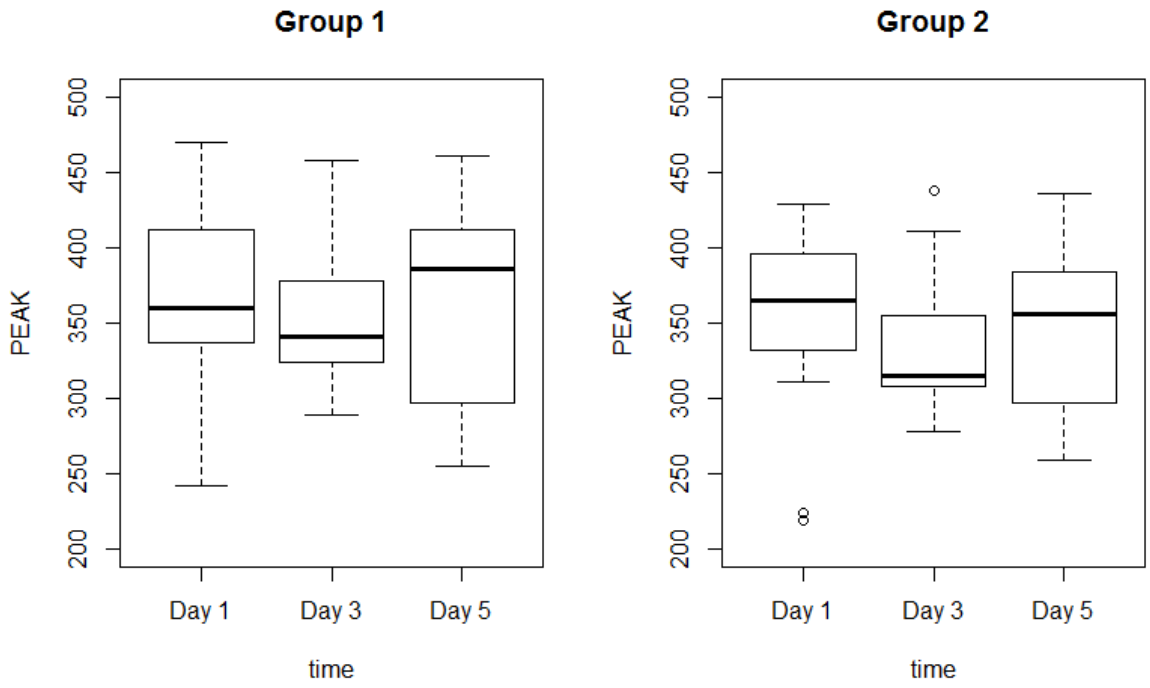
The mean Peak of Group 1 on Days 1, 3, 5 of storage are 366.67, 418.11, 359.98 respectively.

## Group 2:

| | Group 2-Thawed plasma stored at 2-6$^0$ C | |
|---|---|---|
| | Mean (nM) | Standard deviation |
| Peak on day 1 | 354.98 | ±59.17 |
| Peak on day 3 | 421.95 | ±398.02 |
| Peak on day 5 | 343.04 | ±53.23 |

The mean Peak of Group 2 on Days 1, 3, 5 of storage are 354.98, 421.95, 343,04 nM/min respectively.

## Effects on peak:




## Boxplot showing the peak measurements on storage days 1,3 and 5 of both the groups

## Time to Peak:

The Time to peak is obtained from the thrombin generation testing on storage days 1, 3 and 5. Mean ETP for each group on each day were calculated.

## Group I:

| | Group 1-FFP stored at $-70^0$ C | |
|---|---|---|
| | Mean (min) | Standard deviation |
| Time to Peak on day 1 | 4.75 | $\pm$1.11 |
| Time to Peak on day 3 | 4.83 | $\pm$1.04 |
| Time to Peak on day 5 | 4.78 | $\pm$0.87 |

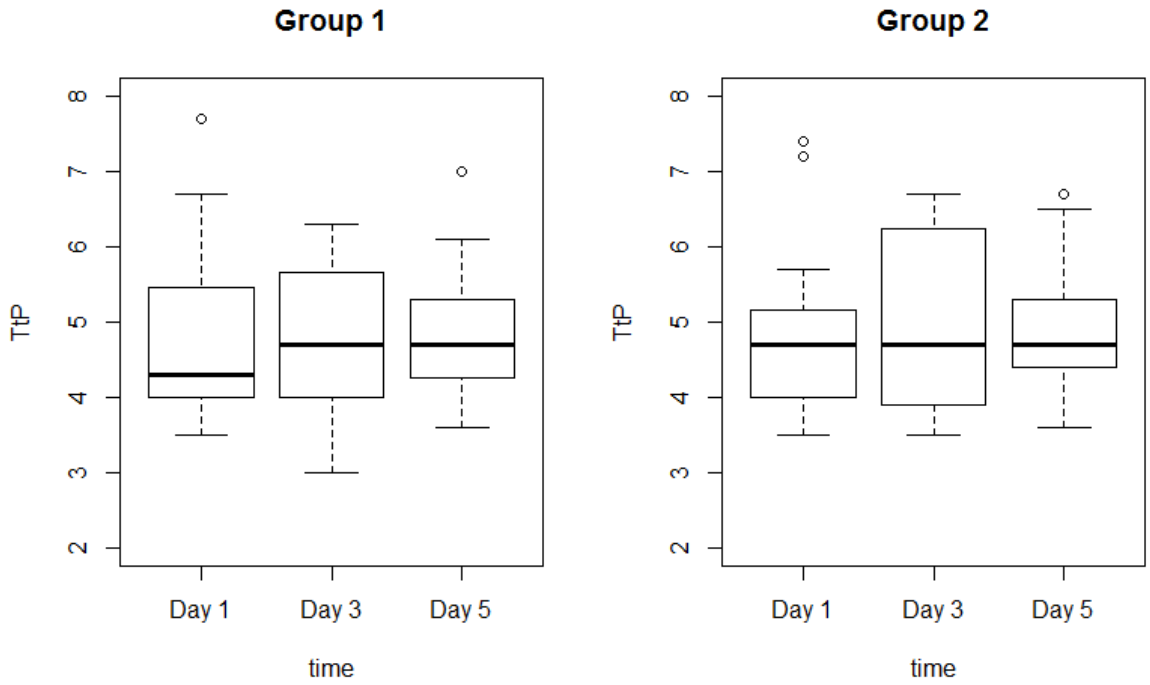
The mean Time to Peak of Group 1 on Days 1, 3, 5 of storage are 4.75, 4.83 and 4.78 respectively.

## Group 2:

| | Group 2-Thawed plasma stored at $2\text{-}6^0$ C | |
|---|---|---|
| | Mean (Min) | Standard deviation |
| Time to Peak on day 1 | 4.78 | $\pm$1.09 |
| Time to Peak on day 3 | 5.83 | $\pm$3.95 |
| Time to Peak on day 5 | 4.83 | $\pm$0.80 |

The mean Peak of Group 2 on Days 1, 3, 5 of storage are 4.78, 5.83 and 4.83 Min respectively.

## Effects  on Time to peak:




## Boxplot showing the Time to peak measurements on storage days 1,3 and 5 of both the groups

## Start tail:

The Start tail is obtained from the thrombin generation testing on storage days 1, 3 and 5.

Mean ETP for each group on each day were calculated.

## Group I:

| | Group 1-FFP stored at -70$^0$ C | |
|---|---|---|
| | Mean (min) | Standard deviation |
| Start tail on day 1 | 21.04 | $\pm$2.01 |
| Start tail on day 3 | 22.11 | $\pm$2.77 |
| Start tail on day 5 | 21.86 | $\pm$1.78 |

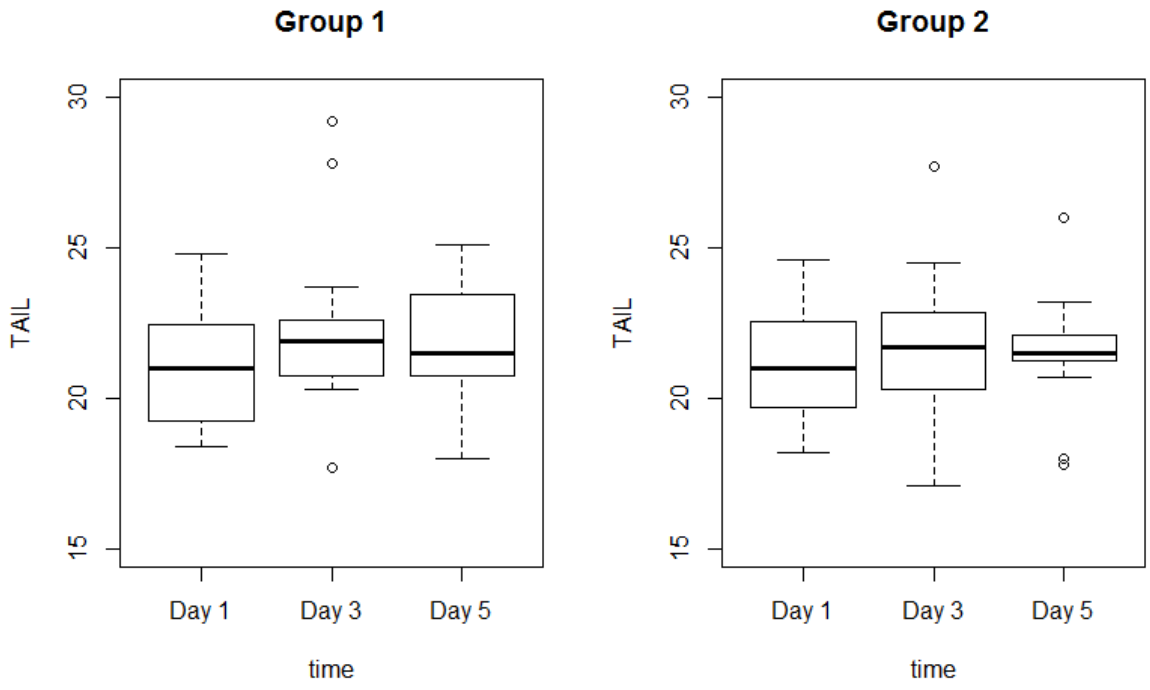
The mean Start tail of Group 1 on Days 1, 3, 5 of storage are 21.04, 22.11 and 21.86 respectively.

## Group 2:

| | Group 2-Thawed plasma stored at 2-6$^0$ C | |
|---|---|---|
| | Mean (Min) | Standard deviation |
| Start tail on day 1 | 21.13 | $\pm$1.89 |
| Start tail on day 3 | 21.64 | $\pm$2.49 |
| Start tail on day 5 | 21.55 | $\pm$1.72 |

The mean Peak of Group 2 on Days 1, 3, 5 of storage are 21.13, 21.64 and 21.55 Min respectively.

**Effects on Start tail:**




**Boxplot showing the Start tail measurements on storage days 1,3 and 5 of both the groups**

## Change in measurements from Day 1 to Day 5:

The difference in measurements of all variables between day 1 and day 5 were calculated.

**APTT:**

| . | Group1-FFP | | Group 2-TP | |
|---|---|---|---|---|
| | Mean(secs) | Standard deviation | Mean(secs) | Standard deviation |
| Change in APTT from Day 1 to Day 3. | -1.19 | ±4.12 | -2.25 | ±3.64 |
| Change in APTT from Day 3 to Day 5. | -0.41 | ±6.04 | -0.30 | ±4.29 |
| Change in APTT from Day 1 to Day 5. | -1.75 | ±4.35 | -2.55 | ±4.11 |

## Factor V:

| | Group1-FFP | | Group 2-TP | |
|---|---|---|---|---|
| | Mean (% activity) | Standard deviation | Mean (% activity) | Standard deviation |
| Change in Factor V activity from Day 1 to Day 3. | 0.77 | ±24.80 | 7.96 | ±23.37 |
| Change in Factor V activity from Day 3 to Day 5. | 2.27 | ±24.51 | 3.24 | ±18.57 |
| Change in Factor V activity from Day 1 to Day 5. | 3.04 | ±14.09 | 11.19 | ±19.96 |

## Factor VII:

| | Group1-FFP | | Group 2-TP | |
|---|---|---|---|---|
| | Mean (% activity) | Standard deviation | Mean (% activity) | Standard deviation |
| Change in Factor VII activity from Day 1 to Day 3. | -1.13 | ±9.43 | 7.82 | ±13.11 |
| Change in Factor VII activity from Day 3 to Day 5. | 9.58 | ±18.08 | 4.25 | ±15.98 |
| Change in Factor VII activity from Day 1 to Day 5. | 8.45 | ±14.84 | 12.07 | ±17.38 |

## Factor VIII:

| | Group1-FFP | | Group 2-TP | |
|---|---|---|---|---|
| | Mean (% activity) | Standard deviation | Mean (% activity) | Standard deviation |
| Change in Factor VIII activity from Day 1 to Day 3. | 3.28 | ±24.81 | 9.69 | ±26.71 |
| Change in Factor VIII activity from Day 3 to Day 5. | 3.58 | ±29.73 | 0.36 | ±31.17 |
| Change in Factor VIII activity from Day 1 to Day 5. | 6.87 | ±34.07 | 10.05 | ±22.76 |

## Lag time:

| | Group 1-FFP | | Group 2-TP | |
|---|---|---|---|---|
| | Mean (min) | Standard deviation | Mean (min) | Standard deviation |
| Change in Lag time from Day 1 to Day 3. | -0.02 | 0.87 | 0.09 | ±0.81 |
| Change in Lag time from Day 3 to Day 5. | -20.81 | 237.41 | 77.55 | ±249.59 |
| Change in Lag time from Day 1 to Day 5. | 0.05 | 0.83 | 0.18 | ±0.94n |

## Endogenous thrombin potential:

| | Group1-FFP | | Group 2-TP | |
|---|---|---|---|---|
| | Mean (nM.min) | Standard deviation | Mean (nM.min) | Standard deviation |
| Change in ETP from Day 1 to Day 3. | -20.81 | 237.41 | 77.55 | 249.59 |
| Change in ETP from Day 3 to Day 5. | -27.89 | 413.05 | -62.21 | 318.49 |
| Change in ETP from Day 1 to Day 5. | -48.71 | 388.55 | 15.34 | 296.22 |

## PEAK:

| | Group 1-FFP | | Group 2-TP | |
|---|---|---|---|---|
| | Mean (nM) | Standard deviation | Mean (nM) | Standard deviation |
| Change in Peak from Day 1 to Day 3. | -51.44 | 300.52 | -66.97 | 405.03 |
| Change in Peak from Day 3 to Day 5. | 58.13 | 287.56 | 86.59 | 405.23 |
| Change in Peak from Day 1 to Day 5. | 6.69 | 65.99 | 14.37 | 61.51 |

## Time to peak:

| | Group1-FFP | | Group 2 TP | |
|---|---|---|---|---|
| | Mean (min) | Standard deviation | Mean (min) | Standard deviation |
| Change in Time to Peak from Day 1 to Day 3. | -0.07 | 0.66 | -1.04 | 4.11 |
| Change in Time to Peak from Day 3 to Day 5. | 0.04 | 0.95 | 0.99 | 3.99 |
| Change in Time to Peak from Day 1 to Day 5. | -0.03 | 1.13 | -0.05 | 1.05 |

**Start tail:**

| | Group1-FFP | | Group 2-TP | |
|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation |
| Change in Start tail from Day 1 to Day 3. | -1.07 | 2.09 | -0.51 | 1.33 |
| Change in Start tail from Day 3 to Day 5. | 0.24 | 3.15 | 0.08 | 2.64 |
| Change in Start tail from Day 1 to Day 5. | -0.83 | 2.39 | -0.42 | 1.99 |

# DISCUSSION

# DISCUSSION:

During the last decade the indication of FFP transfusion is limited to Familial Factor V deficiency, Factor XI deficiency, severe liver disease, plasma exchange and Factor XIII deficiency and transfusion therapy during massive blood loss(51–54). The last indication is responsible for large number of casualty related mortality requires prompt administration of plasma. FFP is still the product of choice used to treat the loss of large volumes of blood, as it can prevent dilutional coagulopathy or the onset of disseminated intravascular coagulation (DIC) (52,53). However, the immediate availability of FFP is delayed by thawing procedures. Pre-thawing for possible use within the expiry time would result in large quantities of FFP being discarded.

The preparation and storage of FFP under optimal conditions (−20 °C) will result in products containing ≈ 1 U/ml of all coagulation factors and their inhibitors after thawing. Variations from 0·6 to 1·4 U/ml are caused by variation between individual donors or by suboptimal freezing procedures of donated plasma(55). The European Pharmacopoeia requires factor V and factor VIII activities of at least 0·5 U/ml of FFP after thawing(55). Previously several studies have been performed to evaluate clotting factors activity in both FFP immediately after thawing (35,56–62) and some studies have evaluated retention of clotting factors activity at 1 to 6°C for up to 28 days post thaw.

This study was conducted in our department to evaluate the stability of hemostatic potential of thawed plasma on storage at 2-6°C and then to compare with

that of storage at -70°C. We performed APPT, Factor V, Factor VII and Factor VIII were performed. The global hemostasis was tested using thrombin generation time.

**APTT:**

Activated partial thromboplastin time is the measure of intrinsic and common coagulation pathways. The activated partial thromboplastin time (aPTT) assay detects fibrin clot formation after addition of an activating agent, partial thromboplastin (phospholipids that lack Tissue factor), and recalcification of plasma. It assesses the activity of prothrombin, FV, FVIII, FIX, FX, FXI, and FXII, therefore providing information about the integrity of the contact activation (intrinsic) and common pathways.(7)

APTT was done on days 1,3 and 5 to see changes in the integrity of the coagulation cascade. The mean APTT on day 1 were almost similar in both the groups (Group 1, $APTT_1$: 30.97 secs; Group 2- $APTT_1$: 30.92 secs) whereas the mean APTT on day 3 and day 5 (Group 1, $APTT_3$: 32.16 secs; Group 2- $APTT_3$: 33.17 secs and Group 1, $APTT_5$: 32.66 secs; Group 2- $APTT_5$: 33.47 secs) were relatively prolonged in group 2 then group 1.

The mean change in APTT values in group 2 from day 1 to day 3 was -2.25secs (-7.97%) and that from day 1 to day 5 was -2.55 secs (-8.83%). The mean change in APTT values in group 1 from day 1 to day 3 was -1.19secs (-4.75%) and that from day 1 to day 5 was -1.75secs (-6.68%). Both the groups show that maximum changes occur with first 48 hours of storage. The changes in group 2 were relatively intensified in

comparison to that of group 1. Similar findings were observed in study conducted by *Alhumaidan et al*(60).

*Group 2- Thawed plasma*

| | Mean (%) | Standard deviation |
|---|---|---|
| *Change in APTT values from day 1 to day 3.* | -7.97 | ±12.63 |
| *Change in APTT values from day 1 to day 5.* | -8.83 | ±12.48 |

Table showing Mean change in APTT values across 5 days of storage in both the groups

Many studies have shown that such gross assessment using APTT does not reflect true quality of Plasma. There is decrease in level of coagulation factors FVIII and FIX in plasma under different conditions of blood collections, transport, storage, temperature and time which may show severe decrease in various coagulation factors especially heat labile factors. Assessment of coagulation factors such as factor V, factor VIII, fibrinogen and factor IX with respect to their quantity in FFP gives better quality than PT/aPTT.(63).

**FACTOR V:**

Since Factor V is a heat labile factor, we measured the levels of Factor V on storage days 1, 3 and 5 in both the groups. The mean Factor V activity of both the groups on day 1 was relatively similar (Group 1-76.35% and Group 2-78.39%). Group 2 showed relatively lower Factor V levels on Days 3 and 5. (Group 1, Factor $V_3$: 75.58%; Group 2- Factor $V_3$: 70.44% and Group 1, Factor $V_5$: 73.31%; Group 2- Factor $V_5$: 67.20%). The Factor V levels on day 5 in Group 2 was more than 50%.

The mean change in Factor V levels from Day 1 to Day 3 in Group 1 and Group 2 were 0.77% and 7.96% respectively. The mean change in Factor V levels from Day 1 to Day 5 in Group 1 and Group 2 were 3.04% and 11.09% respectively. The similar pattern of change in Factor V levels were observed in studies conducted by *Scott et al* (Loss of Factor V-31%), *Alhumaidan et al, Yazer et al* (Loss of Factor V-34%), *Sheffield et al* (Loss of Factor V-20%)and *Downes et al* (Loss of Factor V-16%)(37,57,59,60,62) . The percentage change in Factor V levels in both the groups are described below.

*Group 2-Thawed plasma*

| | Mean (% decrease) | Standard deviation |
|---|---|---|
| *Change in Factor V activity from day 1 to day 3.* | 0.2 | ±52.56 |
| *Change in Factor V activity from day 1 to day 5.* | 10.66 | ±32.28 |

Table showing Mean change in Factor V values across 5 days of storage in both the groups

**Factor VII:**

Factor VII levels were measured on storage days 1, 3 and 5. The mean Factor VII activity of both the groups on day 1 was relatively similar (Group 1-60.57% and Group 2-62.76%). Group 2 showed relatively lower Factor VII levels on Days 3 and 5. (Group 1, Factor $VII_3$: 61.70%; Group 2- Factor $VII_3$: 54.94% and Group 1, Factor $VII_5$: 52.12%; Group 2- Factor $VII_5$: 50.69%). The Factor VII levels on day 5 in Group 2 was more than 50%.

The mean change in Factor VII levels from Day 1 to Day 3 in Group 1 and Group 2 were -1.13% and 9.43% respectively. The mean change in Factor VII levels from Day 1 to Day 5 in Group 1 and Group 2 were 8.45% and 14.84% respectively. The similar pattern of change in Factor VII levels were observed in studies conducted by *Scott et al* (Loss of Factor VII-14%), *Alhumaidan et al, Yazer et al* (Loss of Factor VII-18%),

*Sheffield et al* (Loss of Factor VII-14%) and *Downes et al* (Loss of Factor VII-20%)(35,37,57,59,60) . It is evident that even after 5 days of storage at 2-6$^0$ C, Factor VII levels remains in the normal range.

*Group 2 Thawed plasma*

| | Mean (% decrease) | Standard deviation |
|---|---|---|
| *Change in Factor VII activity from day 1 to day 3.* | 8.1 | ±21.89 |
| *Change in Factor VII activity from day 1 to day 5.* | 18.55 | ±30.83 |

Table showing Mean change in Factor VII values across 5 days of storage in both the groups

**Factor VIII:**

Similar to Factor V, Factor VIII levels were measured on storage days 1, 3 and 5. The mean Factor VIII activity of both the groups on day 1 was relatively similar groups (Group 1, Factor $VIII_1$: 69.50.%; Group 2- Factor $VIII_1$: 67.03%). Group 2 showed relatively lower Factor VIII levels on Days 3 and 5. (Group 1, Factor $VIII_3$: 66.22%; Group 2- Factor $VIII_3$: 57.34% and Group 1, Factor $VIII_5$: 62.63%; Group 2- Factor $VIII_5$: 56.97%). The Factor VIII levels on day 5 in Group 2 was more than 50%.

The mean change in Factor VIII levels from Day 1 to Day 3 in Group 1 and Group 2 were 3.28% and 9.69 % respectively. The mean change in Factor VIII levels from Day 1 to Day 5 in Group 1 and Group 2 were 6.87% and 10.05% respectively. The similar pattern of change in Factor VIII levels were observed in studies conducted by *Scott et al* (Loss of Factor VIII-28%), *Alhumaidan et al, Sheffield et al* (Loss of Factor VIII-41%)and *Downes et al* (Loss of Factor VIII-40%)(35,37,57,59,60) . It is very clear that even after 5 days of storage at 2-6$^0$ C, Factor VIII levels remains in the normal range. In a study conducted by *Yazer et al*(59), unlike all other studies showed a very least percentage decrease in Factor VIII levels of 2.69% with nadir of 0.69U/ml.

*Group 2 Thawed plasma*

| | Mean (% decrease) | Standard deviation |
|---|---|---|
| *Change in Factor VIII activity from day 1 to day 3.* | 7.64 | ±42.8 |
| *Change in Factor VIII activity from day 1 to day 5.* | 11.26 | ±31.05 |

Table showing Mean change in Factor VIII values across 5 days of storage in both the groups

**Thrombin Generation Testing:**

Thrombin generated in the thrombin generation test can be quantified in platelet-poor plasma using the calibrated automated thrombogram method, which monitors the cleavage of a fluorogenic substrate which is simultaneously compared to the known thrombin activity. (30) Different to conventional coagulation tests, the thrombin generation test can be used for an overall evaluation of hemostasis whereas the results of conventional coagulation tests can only be used to evaluate specific characteristics of hemostasis, such as intrinsic and extrinsic pathways of coagulation. The following changes were observed in the individual variables of Thrombin generation testing done on storage Days 1, 3 and 5.

The change in mean lag time from Day 1 to Day 3 in both the groups were -0.06 and -0.09 mins respectively. The change in mean lag time from Day 1 to Day 3 in both the groups were -0.02 and 0.09 mins respectively.

The mean Endogenous thrombin potential on Day 5 in both groups were 1601.75nmol.min and 1499.03 nmol.min respectively. Similar change was noted in Peak of both groups on Day 5 which was 359.98nmol and 343.04 nmol respectively. The mean time to Peak on Day 5 showed mild prolongation in Group 2(4.83 min) in comparison to Group 1(4.78 min). The mean Start tail on Day 5 in group 1 and 2 were 21.86 min and 21.55min respectively.

In Group 2, there was decline in Peak thrombin from Day 1 to Day 5 by a mean of 1.96% against 0.68% in Group 1. Thrombin-generation assays provide a global measure of hemostasis, measuring both the propagation phase (with positive feedback

loops involving Factors V, VIII, and XI) and the termination phase (including down-regulation by anticoagulant pathways and plasma protease inhibitors) of coagulation. The resulting thrombin-generation curve reflects and integrates all procoagulant and anticoagulant reactions that regulate the formation and inhibition of thrombin. Similar to the findings of Scott and colleagues, we found that many factors were statistically significantly different on Day 5 relative to Day 0 post thaw; however, there is no indication that these differences amount to clinically significant disparities because, in every case, the individual factor levels were sufficient to support robust thrombin generation. Excessive activation of the coagulation cascade may be demonstrated by increased thrombin-antithrombin complexes, increased peak thrombin generation, or shortened non-activated partial thromboplastin time.

A decline of the haemostatic potential of thawed plasma during refrigerated storage was demonstrated in previous studies. *Matijevic et al.*(41,64) showed that although individual factor levels remained above 50% of normal levels in thawed FFP, after 5 days at 1–6°C, the Thrombin generation potential decreased significantly by 58%, as demonstrated at low tissue factor concentrations. In the assay with high tissue factor concentrations, *Erickson et al.* (42) demonstrated a decrease in the peak thrombin by mean of 26% in thawed PF24, during 5-day storage at 1–6°C. In contrast, Cookson et al. (43) found unaffected amount of Thrombin generation by storage of PF24 (produced from whole blood, RT-FP24) for 6 days, but there was an increase in lag time. Our study also demonstrated comparable stability of hemostatic potential in comparison to that stored at $-70^0$C. The changes in none of the parameters (FV, FVII, FVIII and TGT) were statistically significant (P value>0.05).

Our study demonstrated that during 5-day refrigerated storage of thawed plasma showed substantial reductions in Coagulation Factor levels. This was also accompanied by decrease in the Thrombin generation Capacities which was comparable in both the groups.

However, at the proposed outdate of 5 days, there were no differences in the overall haemostatic potentials of thawed plasma in comparison to the FFP. Thus, the usage of thawed plasma up to 5 days of refrigerated storage can be recommended. The recently published "PAMPer trial" (Prehospital Plasma during Air Medical Transport in Trauma

Patients at Risk for Hemaorrhagic Shock) by US Army and medical research showed that, In injured patients at risk for hemorrhage shock, the prehospital administration of thawed plasma was safe and resulted in lower 30-day mortality and a lower median prothrombin time ratio than standard-care resuscitation. (65)

Circular of Information for the use of human blood and blood components-2017 (66) prepared jointly by the AABB, the American Red Cross, America's Blood Centres, and the Armed Services Blood Program and it is recognized by the US Food and Drug Administration (FDA) suggests that thawed plasma can be used for

1) Emergency management of preoperative or bleeding patients who require replacement of multiple coagulation factors.
2) Initial Treatment of patients undergoing massive transfusions.
3) Warfarin reversal during emergencies.
4) Transfusion or plasma exchange with TTP.

# SUMMARY

# SUMMARY:

- Plasma is separated from blood collected from donor and then separated into 2 aliquots. Both the aliquots are frozen overnight.
- Plasma after thawing at 35.8$^0$C in a plasma thawer (MT204, Thermogenesis) for 20 minutes are stored as aliquots at $2^0$-6 $^0$C and -70$^0$C for 5 days.
- Factor V, VII, VIII levels; activated partial thromboplastin time, thrombin generation testing were done on first, third and fifth day. the values are compared.
- The mean increase in APTT of thawed plasma on day 5 on storage at $2^0$-$6^0$C was -2.55 secs (-8.83%).
- The mean Factor VII levels thawed plasma on day 5 on storage at $2^0$-$6^0$C was 50.69% and the percentage decrease from that of Day 1 was 18.55%.
- The mean Factor V levels thawed plasma on day 5 on storage at $2^0$-$6^0$C was 67.20% and the percentage decrease from that of Day 1 was 10.66%.
- The mean Factor VIII levels thawed plasma on day 5 on storage at $2^0$-$6^0$C was 56.97% and the percentage decrease from that of Day 1 was 11.26%.
- The peak thrombin levels of thawed plasma at day 5 of storage at $2^0$-$6^0$C was 343.0 nmol and was comparable with that of plasma stored at -70$^0$C.

- The time to Peak, Endogenous thrombin potential, lag time and start tail of both the groups were comparable.
- This would aid in immediate availability of thawed plasma during emergency situations.

# CONCLUSION

# Conclusion:

Most individual Hemostatic and anticoagulant factors had similar levels on Day 5 versus Day 1, with the exception of the known labile factors (Factors V and VIII) and Factor VII in which levels were decreased more than 10% but were comparable with changes shown in plasma stored at $-70^0$C.

Transfusion guidelines for the use of thawed plasma were set based on the test results of coagulation factors only. However, thawed plasma should provide a sufficient Thrombin generation capacity, to restore haemostasis and correct coagulopathy and bleeding in patients.

Thus, evidence-based quality criteria relevant for patient outcome appear to be needed for plasma for transfusion.

Such criteria could help us to ensure immediate availability of plasma to patients in hemaorrhagic shock during emergency admissions.

This would also aid in increasing the availability of Plasma by facilitating storage at centres with refrigerator facilities.

This would also help us in prehospital administration of plasma in addition to the initiation of standard resuscitation procedures in the prehospital environment, which may reduce the risk of downstream complications from hemorrhage and shock.(65,67)

# LIMITATION:

- In this study, the results of coagulation tests were only evaluated. The Possible benefits to patients as a result of the immediate availability of plasma should also be clinically evaluated.
- Well-designed clinical trial will be necessary to show the clinical and biological efficiency of the administration of thawed plasma, stored for several days at 2-6$^0$C.

# Bibliography

# Bibliography


1. Arya RC, Wander G, Gupta P. Blood component therapy: Which, when and how much. J Anaesthesiol Clin Pharmacol. 2011;27(2):278–84.

2. Gurevitz SA. Update and Utilization of Component Therapy in Blood Transfusions. Lab Med. 2011 Apr;42(4):235–40.

3. National Blood Transfusion Council (NBTC) | National AIDS Control Organization | MoHFW | GoI [Internet]. [cited 2018 Aug 22]. Available from: http://naco.gov.in/national-blood-transfusion-council-nbtc-0

4. Trauma-Induced Coagulopathy [Internet]. [cited 2018 Aug 23]. Available from: http://ccn.aacnjournals.org/content/34/4/54.full

5. Holcomb JB, Wade CE, Michalek JE, Chisholm GB, Zarzabal LA, Schreiber MA, et al. Increased plasma and platelet to red blood cell ratios improves outcome in 466 massively transfused civilian trauma patients. Ann Surg. 2008;248.

6. India [Internet]. [cited 2018 Aug 23]. Available from: http://www.healthdata.org/india

7. Key N, Makris M, O'Shaughnessy D, Lillicrap D. Practical Hemostasis and Thrombosis. :336.

8. Hoffman M. A cell-based model of coagulation and the role of factor VIIa. Blood Rev. 2003 Sep;17:S1–5.

9. Swanepoel AC, Nielsen VG, Pretorius E. Viscoelasticity and Ultrastructure in Coagulation and Inflammation: Two Diverse Techniques, One Conclusion. Inflammation. 2015 Aug;38(4):1707–26.

10. Hayakawa M. Pathophysiology of trauma-induced coagulopathy: disseminated intravascular coagulation with the fibrinolytic phenotype. J Intensive Care [Internet]. 2017 Jan 31 [cited 2018 Aug 21];5. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5282695/

11. Kushimoto S, Kudo D, Kawazoe Y. Acute traumatic coagulopathy and trauma-induced coagulopathy: an overview. J Intensive Care [Internet]. 2017 Dec [cited 2018 Aug 21];5(1). Available from: http://jintensivecare.biomedcentral.com/articles/10.1186/s40560-016-0196-6

12. Simmons JW, Powell MF. Acute traumatic coagulopathy: pathophysiology and resuscitation. Br J Anaesth. 2016 Dec;117:iii31–43.

13. White NJ. Mechanisms of trauma-induced coagulopathy. 2013;4.

14. Katrancha ED, Gonzalez LS. Trauma-Induced Coagulopathy. Crit Care Nurse. 2014 Aug 1;34(4):54–63.

15. Hayakawa M. Pathophysiology of trauma-induced coagulopathy: disseminated intravascular coagulation with the fibrinolytic phenotype. J Intensive Care [Internet]. 2017

Dec [cited 2018 Sep 6];5(1). Available from: http://jintensivecare.biomedcentral.com/articles/10.1186/s40560-016-0200-1

16. Holcomb JB, del Junco DJ, Fox EE, Wade CE, Cohen MJ, Schreiber MA, et al. The Prospective, Observational, Multicenter, Major Trauma Transfusion (PROMMTT) Study: Comparative Effectiveness of a Time-varying Treatment with Competing Risks. JAMA Surg. 2013 Feb;148(2):127–36.

17. Gonzalez E, Moore EE, Moore HB. Management of Trauma-Induced Coagulopathy with Thrombelastography. Crit Care Clin. 2017 Jan;33(1):119–34.

18. Holcomb JB, del Junco DJ, Fox EE, Wade CE, Cohen MJ, Schreiber MA, et al. The Prospective, Observational, Multicenter, Major Trauma Transfusion (PROMMTT) Study: Comparative Effectiveness of a Time-Varying Treatment With Competing Risks. JAMA Surg. 2013 Feb 1;148(2):127.

19. Green L, Cardigan R, Beattie C, Bolton-Maggs P, Stanworth SJ, Thachil J, et al. 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 Jun 1;n/a-n/a.

20. Technical manual. American Association of Blood Banks; 2014.

21. Fasano R, Luban NLC. Blood Component Therapy. Pediatr Clin North Am. 2008 Apr;55:421–45.

22. Chng WJ, Tan MK, Kuperan P, Wee Joo C. An Audit of Fresh Frozen Plasma Usage in An Acute General Hospital in Singapore. Singap Med J. 2003;44:574–8.

23. Saran R. Transfusion medicine Technical Manual. 2nd ed. New Delhi: Directorate General of Health Services; 2003.

24. Bethesda M, Grossman BJ, Hillyer CD, Westhoff CM, editors. Technical manual. 17th ed. Maryland: American Association of Blood Banks; 2014.

25. Sheffield WP, Bhakta V, Mastronardi C, Ramirez-Arcos S, Howe D, Jenkins C. Changes in coagulation factor activity and content of di(2-ethylhexyl)phthalate in frozen plasma units during refrigerated storage for up to five days after thawing. Transfusion (Paris). 2012 Mar;52(3):493–502.

26. Hunt BJ, Allard S, Keeling D, Norfolk D, Stanworth SJ, Pendry K, et al. A practical guideline for the haematological management of major haemorrhage. Br J Haematol. 2015 Sep 1;170(6):788–803.

27. Pati S, Matijevic N, Doursout M-F, Ko T, Cao Y, Deng X, et al. Protective Effects of Fresh Frozen Plasma on Vascular Endothelial Permeability, Coagulation, and Resuscitation After Hemorrhagic Shock Are Time Dependent and Diminish Between Days 0 and 5 After Thaw. J Trauma. 2010 Jul;69(Suppl 1):S55–63.

28. Bain BJ, Bates I, Laffan MA, Lewis SM. Dacie and Lewis practical haematology. 2017.

29. Chee Y. Coagulation. J R Coll Physicians Edinb. 2014;44(1):42–5.

30. Duarte RCF, Ferreira CN, Rios DRA, Reis HJ dos, Carvalho M das G. Thrombin generation assays for global evaluation of the hemostatic system: perspectives and limitations. Rev Bras Hematol E Hemoter. 2017;39(3):259–65.

31. Hemker HC. Recollections on thrombin generation. J Thromb Haemost. 2008 Feb 1;6(2):219–26.

32. Young G, Sørensen B, Dargaud Y, Negrier C, Brummel-Ziedins K, Key NS. Thrombin generation and whole blood viscoelastic assays in the management of hemophilia: current state of art and future perspectives. Blood. 2013 Mar 14;121(11):1944–50.

33. Thrombin Generation Assays [Internet]. [cited 2018 Sep 7]. Available from: http://www.practical-haemostasis.com/Miscellaneous/Miscellaneous%20Tests/etp_assays.html

34. View Image [Internet]. [cited 2018 Oct 6]. Available from: http://www.jahjournal.org/viewimage.asp?img=JApplHematol_2016_7_4_117_198509_f1.jpg

35. Downes KA, Wilson E, Yomtovian R, Sarode R. Serial measurement of clotting factors in thawed plasma stored for 5 days. Transfusion (Paris). 2001 Apr;41(4):570.

36. von Heymann C, Pruss A, Sander M, Finkeldey A, Ziemer S, Kalus U, et al. Thawing procedures and the time course of clotting factor activity in fresh-frozen plasma: a controlled laboratory investigation. Anesth Analg. 2006 Oct;103(4):969–74.

37. Sheffield WP, Bhakta V, Yi Q-L, Jenkins C. Stability of Thawed Apheresis Fresh-Frozen Plasma Stored for up to 120 Hours at 1°C to 6°C. J Blood Transfus [Internet]. 2016 [cited 2018 Oct 3];2016. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5143734/

38. Scott E, Puca K, Heraly J, Gottschall J, Friedman K. Evaluation and comparison of coagulation factor activity in fresh-frozen plasma and 24-hour plasma at thaw and after 120 hours of 1 to 6°C storage. Transfusion (Paris). 2009 Aug;49(8):1584–91.

39. Alhumaidan H, Cheves T, Holme S, Sweeney J. Stability of coagulation factors in plasma prepared after a 24-hour room temperature hold. Transfusion (Paris). 2010 Sep;50(9):1934–42.

40. S P, N M, Mf D, T K, Y C, X D, et al. Protective effects of fresh frozen plasma on vascular endothelial permeability, coagulation, and resuscitation after hemorrhagic shock are time dependent and diminish between days 0 and 5 after thaw., Protective Effects of Fresh Frozen Plasma on Vascular Endothelial Permeability, Coagulation, and Resuscitation After Hemorrhagic Shock Are Time Dependent and Diminish Between Days 0 and 5 After Thaw. J Trauma J Trauma. 2010 Jul;69 Suppl 1, 69(Suppl 1):S55, S55-63.

41. Matijevic N, Kostousov V, Wang Y-WW, Wade CE, Wang W, Letourneau P, et al. Multiple Levels of Degradation Diminish Hemostatic Potential of Thawed Plasma. J Trauma. 2011 Jan;70(1):71–80.

42. Erickson A, Waldhaus K, David T, Huang N, Rico S, Corash L, et al. Plasma treated with amotosalen and ultraviolet A light retains activity for hemostasis after 5 days post-thaw

storage at 1 to 6 º C: AMOTOSALEN/UVA-TREATED THAWED PLASMA. Transfusion (Paris). 2017 Apr;57(4):997–1006.

43. Cookson P, Lawrie A, Green L, Dent E, Proffitt S, Bashir S, et al. Thrombin generation and coagulation factor content of thawed plasma and platelet concentrates. Vox Sang. 2015 Feb;108(2):160–8.

44. Radwan ZA, Bai Y, Matijevic N, Junco DJ del, McCarthy JJ, Wade CE, et al. An Emergency Department Thawed Plasma Protocol for Severely Injured Patients. JAMA Surg. 2013 Feb 1;148(2):170–5.

46. DGHS manual.pdf.

47. CS-2100i_2000i_4p_EN.pdf [Internet]. [cited 2018 Sep 7]. Available from: http://www.sysmex.co.uk/fileadmin/media/f115/CS-2100i_2000i_4p_EN.pdf

48. Laboratory hemostasis: a practical guide for pathologists. New York: Springer; 2014.

49. Coen Hemker H, Hemker PW, Al Dieri R. The technique of measuring thrombin generation with fluorescent substrates: 4. The H-transform, a mathematical procedure to obtain thrombin concentrations without external calibration. Thromb Haemost. 2009 Jan;101(1):171–7.

50. Hemker HC, Giesen P, Al Dieri R, Regnault V, de Smedt E, Wagenvoord R, et al. Calibrated Automated Thrombin Generation Measurement in Clotting Plasma. Pathophysiol Haemost Thromb. 2003;33(1):4–15.

51. Mollison's Blood Transfusion in Clinical Medicine, 12th Edition [Internet]. Wiley.com. [cited 2018 Sep 30]. Available from: https://www.wiley.com/en-us/Mollison%27s+Blood+Transfusion+in+Clinical+Medicine%2C+12th+Edition-p-9781405199407

52. Hiippala S. Replacement of massive blood loss. Vox Sang. 1998;74 Suppl 2:399–407.

53. Patil V, Shetmahajan M. Massive transfusion and massive transfusion protocol. Indian J Anaesth. 2014;58(5):590–5.

54. Chidwick K, Matejtschuk P, Gascoigne E, Briggs N, More JE, Dash CH, et al. Massive Blood Replacement. Vox Sang. 1999;77(4):239–50.

55. European Pharmacopoeia (Ph. Eur.) 9th Edition | EDQM [Internet]. [cited 2018 Oct 1]. Available from: https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-9th-edition

56. Woodhams B, Girardot O, Blanco M-J, Colesse G, Gourmelin Y. Stability of coagulation proteins in frozen plasma. Blood Coagul Fibrinolysis [Internet]. 2001;12(4). Available from: http://journals.lww.com/bloodcoagulation/Fulltext/2001/06000/Stability_of_coagulation_proteins_in_frozen_plasma.2.aspx

57. Scott E, Puca K, Heraly J, Gottschall J, Friedman K. Evaluation and comparison of coagulation factor activity in fresh-frozen plasma and 24-hour plasma at thaw and after 120 hours of 1 to 6°C storage. Transfusion (Paris). 2009 Aug;49(8):1584–91.

58. Neisser- Svae A, Trawnicek L, Heger A, Mehta T, Triulzi D. Five-day stability of thawed plasma: solvent/detergent-treated plasma comparable with fresh-frozen plasma and plasma frozen within 24 hours. Transfusion (Paris). 2016 Feb 1;56(2):404–9.

59. Yazer MH, Cortese-Hassett A, Triulzi DJ. Coagulation factor levels in plasma frozen within 24 hours of phlebotomy over 5 days of storage at 1 to 6 degrees C. Transfusion (Paris). 2008 Dec;48(12):2525–30.

60. Alhumaidan H, Cheves T, Holme S, Sweeney J. Stability of coagulation factors in plasma prepared after a 24-hour room temperature hold. Transfusion (Paris). 2010 Sep;50(9):1934–42.

61. Keller MK, Pruss A, Sander M, Spies C, Schoenfeld H, Schuster M, et al. Thawed solvent/detergent-treated plasma: too precious to be wasted after 6 hours? Blood Transfus. 2012 Jul;10(3):360–7.

62. Buchta C, Felfernig M, Höcker P, Macher M, Körmöczi GF, Quehenberger P, et al. Stability of coagulation factors in thawed, solvent/detergent-treated plasma during storage at 4 degrees C for 6 days. Vox Sang. 2004 Oct;87(3):182–6.

63. Arya RC, Wander G, Gupta P. Blood component therapy: Which, when and how much. J Anaesthesiol Clin Pharmacol. 2011;27(2):278–84.

64. Pati S, Matijevic N, Doursout M-F, Ko T, Cao Y, Deng X, et al. Protective Effects of Fresh Frozen Plasma on Vascular Endothelial Permeability, Coagulation, and Resuscitation After Hemorrhagic Shock Are Time Dependent and Diminish Between Days 0 and 5 After Thaw: J Trauma Inj Infect Crit Care. 2010 Jul;69(Supplement):S55–63.

65. Sperry JL, Guyette FX, Brown JB, Yazer MH, Triulzi DJ, Early-Young BJ, et al. Prehospital Plasma during Air Medical Transport in Trauma Patients at Risk for Hemorrhagic Shock. N Engl J Med. 2018 26;379(4):315–26.

66. Circular of Information for the Use of Human Blood and Blood Components. :78.

67. Stubbs JR, Zielinski MD, Berns KS, Badjie KS, Tauscher CD, Hammel SA, et al. How we provide thawed plasma for trauma patients. Transfusion (Paris). 2015 Aug 1;55(8):1830–7.

# ANNEXURES

**OFFICE OF RESEARCH**
**INSTITUTIONAL REVIEW BOARD (IRB)**
**CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA**

**Dr. B.J. Prashantham,** M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

**Dr. Anna Benjamin Pulimood,** M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

**Dr. Biju George,** M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

May 02, 2017

Dr. A. Srivalli,
PG registrar,
Department of Transfusion Medicine,
Christian Medical College,
Vellore – 632 002.

Sub: **Fluid Research Grant NEW PROPOSAL:**
Stability of coagulation factors in thawed plasma and liquid plasma on storage at 20c-60c for 5 days.
Dr. A. Srivalli, Employment Number: 21287, PG registrar, Dr. Sukesh Chandran Nair, Professor, Ms. Ramya, Emp. No: 33021, Lab technician, Mr. Amal raj, Emp. No: 50327, Blood bank Instructor, Department of Transfusion Medicine and Immuno haematology.Ms. Tunny Sebastian, Employment Number: 32291, Lecturer, Department of biostatistics.

Ref: IRB Min. No. 10465 [OBSERV] dated 05.01.2017

Dear Dr. A. Srivalli,

The Institutional Review Board (**Blue**, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Stability of coagulation factors in thawed plasma and liquid plasma on storage at 20c-60c for 5 days" on January 05th 2017.

The Committee reviewed the following documents:

1. IRB Application format
2. Waiver of Consent
3. Data Form
4. Cvs of Drs. Sukesh, Ramya, Srivalli and Mr. P Amal Raj.
5. No. of documents 1 – 4.

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on January 05th 2017 in the BRTC Conference Room, Christian Medical College, Bagayam, Vellore 632002.



Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

**Dr. B.J. Prashantham,** M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

**Dr. Anna Benjamin Pulimood,** M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

**Dr. Biju George,** M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

| Name | Qualification | Designation | Affiliation |
|---|---|---|---|
| Dr. Biju George | MBBS, MD, DM | Professor, Haematology, Research), Additional Vice Principal , Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC, Vellore | Internal, Clinician |
| Dr. B. J. Prashantham | MA(Counseling Psychology), MA (Theology), Dr. Min (Clinical Counselling) | Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore | External, Social Scientist |
| Dr. Ratna Prabha | MBBS, MD (Pharma) | Associate Professor, Clinical Pharmacology, CMC, Vellore | Internal, Pharmacologist |
| Dr. Rekha Pai | BSc, MSc, PhD | Associate Professor, Pathology, CMC, Vellore | Internal, Basic Medical Scientist |
| Rev. Joseph Devaraj | BSc, BD | Chaplaincy Department, CMC, Vellore | Internal, Social Scientist |
| Mr. C. Sampath | BSc, BL | Advocate, Vellore | External, Legal Expert |
| Dr. Simon Pavamani | MBBS, MD | Professor, Radiotherapy, CMC, Vellore | Internal, Clinician |
| Dr. Jayaprakash Muliyil | BSc, MBBS, MD, MPH, Dr PH (Epid), DMHC | Retired Professor, Vellore | External, Scientist &Epidemiologist |
| Ms. Grace Rebekha | M.Sc., (Biostatistics) | Lecturer, Biostatistics, CMC, Vellore | Internal, Statistician |
| Mrs. Pattabiraman | BSc, DSSA | Social Worker, Vellore | External, Lay Person |
| Mrs. Sheela Durai | MSc Nursing | Professor, Medical Surgical Nursing, CMC, Vellore | Internal, Nurse |
| Dr. Balamugesh | MBBS, MD(Int Med), DM, FCCP (USA) | Professor, Pulmonary Medicine, CMC, Vellore | Internal, Clinician |



Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

**Dr. B.J. Prashantham,** M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

**Dr. Anna Benjamin Pulimood,** M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

**Dr. Biju George,** M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

| | | | |
|---|---|---|---|
| Dr. Santhanam Sridhar | MBBS, DCH, DNB | Professor, Neonatology, CMC, Vellore | Internal, Clinician |
| Mrs. Emily Daniel | MSc Nursing | Professor, Medical Surgical Nursing, CMC, Vellore | Internal, Nurse |
| Dr. Mathew Joseph | MBBS, MCH | Professor, Neurosurgery, CMC, Vellore | Internal, Clinician |
| Dr. Thomas V Paul | MBBS, MD, DNB, PhD | Professor,Endocrinology, CMC, Vellore | Internal, Clinician |
| Dr. Vivek Mathew | MD (Gen. Med.) DM (Neuro) Dip. NB (Neuro) | Professor, Neurology, CMC, Vellore | Internal, Clinician |
| Dr Sneha Varkki | MBBS, DCH, DNB | Professor, Paediatrics, CMC, Vellore | Internal, Clinician |
| Dr. Sathish Kumar | MBBS, MD, DCH | Professor, Child Health, CMC, Vellore | Internal, Clinician |

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "Stability of coagulation factors in thawed plasma and liquid plasma on storage at 20c-60c for 5 days."on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

*Fluid Grant Allocation:*

*A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 50,000/- INR (Rupees Fifty Thousand only) each will be released at the end of the first year as 2 nd Installment.*

Yours sincerely,

Dr. Biju George
Secretary (Ethics Committee),
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM,
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

IRB Min. No. 10465 [OBSERV] dated 05.01.2017

# DATA COLLECTION FORM

STUDY NUMBER:

Date:

| CONVENTIONAL COAGULATION TEST AND FACTOR ASSAY | | | | | | |
|---|---|---|---|---|---|---|
| | Day-1 | Day-1 | Day-3 | Day-3 | Day-5 | Day-5 |
| | Group- 1 FFP | Group-2 TP | Group- 1 FFP | Group-2 TP | Group- 1 FFP | Group-2 TP |
| **APTT (SECS)** | | | | | | |
| **F-V (% ACTIVITY)** | | | | | | |
| **F-VII (% ACTIVITY)** | | | | | | |
| **F-VIII (% ACTIVITY)** | | | | | | |

| THROMBIN GENERATION TESTING | | | | | | |
|---|---|---|---|---|---|---|
| | Day-1 | Day-1 | Day-3 | Day-3 | Day-5 | Day-5 |
| | Group- 1 FFP | Group-2 TP | Group- 1 FFP | Group-2 TP | Group- 1 FFP | Group-2 TP |
| **LAG TIME (MINUTES)** | | | | | | |
| **ETP (nM.MIN)** | | | | | | |
| **PEAK THROMBIN(nM)** | | | | | | |
| **TtP (MIN)** | | | | | | |
| **START TAIL** | | | | | | |

# DATA

| STUDY NUMBER | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 |
| APTT on Day 1 | 25.6 | 24.1 | 28.2 | 28.2 | 30.8 | 30.9 | 34 | 34 | 35.3 | 29.4 |
| APTT on Day 3 | 27.1 | 28.5 | 32.4 | 32.5 | 32.9 | 32.8 | 35.4 | 35.9 | 31.4 | 32.3 |
| APTT on Day 5 | 28.2 | 28.6 | 33.3 | 27.1 | 31.1 | 31.9 | 37.2 | 35.7 | 29.6 | 30.2 |
| FV on Day 1 | 93.9 | 86.5 | 73.6 | 78.1 | 41.6 | 44.8 | 62.3 | 61.8 | 77.9 | 122.6 |
| FV on Day 3 | 94.2 | 94.7 | 81.8 | 74.7 | 69 | 30.9 | 100 | 97.6 | 114.5 | 108.5 |
| FV on Day 5 | 73.9 | 62 | 50.6 | 81 | 58.7 | 49.5 | 74.4 | 78.2 | 79.7 | 71.9 |
| FVII on Day 1 | 87.9 | 102.3 | 46.3 | 47.2 | 35.1 | 35.2 | 40.7 | 41 | 61.2 | 73.6 |
| FVII on Day 3 | 103.2 | 86.4 | 70.8 | 49.4 | 42.2 | 56.9 | 49.3 | 42.7 | 64.9 | 57.9 |
| FVII on Day 5 | 58.6 | 90.4 | 31.4 | 82 | 24 | 14.9 | 26.5 | 25 | 70.9 | 68.5 |
| FVIII on Day 1 | 109.2 | 105.2 | 89.5 | 85 | 86.1 | 91.4 | 43.6 | 40.5 | 83.7 | 89 |
| FVIII on Day 3 | 59.1 | 56.4 | 64.7 | 49 | 54.8 | 34.7 | 21.8 | 21.2 | 91 | 78 |
| FVIII on Day 5 | 64.9 | 87 | 34.2 | 117.2 | 110.5 | 99.3 | 65 | 40.2 | 78 | 72.3 |
| LAG on Day 1 | 2.4 | 2.4 | 2.4 | 2.79 | 3.67 | 3.33 | 4.83 | 5.17 | 3.37 | 2.87 |
| LAG on Day 3 | 2.59 | 2.5 | 2 | 2.5 | 3.33 | 3.33 | 4 | 4.33 | 3.5 | 2.83 |
| LAG on Day 5 | 2.5 | 2.5 | 2.89 | 2.91 | 3 | 2.83 | 4.33 | 4 | 3.78 | 2.91 |
| ETP on Day 1 | 1980.03 | 1958.54 | 1898.68 | 1762.71 | 1357.77 | 1322.66 | 1845.58 | 1844.21 | 1646.36 | 1664.26 |
| ETP on Day 3 | 1781.03 | 1524.5 | 1740.01 | 1524.51 | 1487.03 | 1352.82 | 1673.66 | 1710.44 | 1646.5 | 1813.31 |
| ETP on Day 5 | 1981.1 | 1875.5 | 1608.1 | 1589.01 | 1156.93 | 1148.05 | 1504.19 | 1317.88 | 1689.5 | 1810.1 |
| PEAK on Day 1 | 438.29 | 427.64 | 434.6 | 414.6 | 344.72 | 320.14 | 413.33 | 399.19 | 313.95 | 357.71 |
| PEAK on Day 3 | 458.12 | 438.4 | 451.5 | 411.5 | 322.36 | 312.09 | 364.25 | 363.14 | 311.15 | 379.94 |
| PEAK on Day 5 | 461.5 | 436.24 | 401.05 | 410.12 | 260.12 | 258.79 | 297.7 | 262.06 | 323.1 | 353.51 |
| TtP on Day 1 | 3.91 | 4.07 | 4.07 | 4.07 | 5.5 | 5.33 | 6.67 | 7.17 | 5.54 | 4.4 |
| TtP on Day 3 | 2.98 | 3.5 | 4.01 | 3.98 | 5.5 | 5.33 | 6.33 | 6.5 | 6 | 4.67 |
| TtP on Day 5 | 3.59 | 3.59 | 4.01 | 4.01 | 5.17 | 5.33 | 7 | 6.67 | 6.1 | 5.51 |
| TAIL on Day 1 | 22.62 | 22.96 | 22.29 | 21.05 | 20 | 20.67 | 23.33 | 24 | 23.76 | 22.09 |
| TAIL on Day 3 | 23.62 | 22.9 | 21.95 | 23.12 | 20.33 | 20.17 | 22.5 | 23.33 | 23.67 | 22.83 |
| TAIL on Day 5 | 23.62 | 22.6 | 25.1 | 22.1 | 20.33 | 20.69 | 23.17 | 23.17 | 23.67 | 22.12 |

| STUDY NUMBER | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|
| | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 |
| APTT on Day 1 | 32.6 | 30.2 | 34 | 35.4 | 27.9 | 27.4 | 23.1 | 31.9 | 32.1 | 32.6 |
| APTT on Day 3 | 32.4 | 31.9 | 34 | 35.4 | 27.9 | 27.4 | 23.6 | 31.9 | 30.6 | 39.6 |
| APTT on Day 5 | 29.9 | 29.7 | 34.7 | 36.3 | 27.3 | 29.4 | 31 | 34 | 36.6 | 40.8 |
| FV on Day 1 | 125 | 124 | 96.8 | 97.6 | 115.3 | 113.7 | 122.6 | 115 | 30.2 | 29.6 |
| FV on Day 3 | 109.2 | 82.6 | 71 | 70.5 | 81.5 | 83.4 | 83.4 | 80.2 | 48 | 28.3 |
| FV on Day 5 | 85.8 | 77.1 | 99.7 | 88.2 | 105.2 | 105.6 | 116.6 | 82.6 | 23.5 | 13.8 |
| FVII on Day 1 | 107.8 | 108 | 27.2 | 27.1 | 62.6 | 61.6 | 79.4 | 67.1 | 43.6 | 44.6 |
| FVII on Day 3 | 99.3 | 88.2 | 26.9 | 27.4 | 62.6 | 61.4 | 76.8 | 67.5 | 40.4 | 35.8 |
| FVII on Day 5 | 99.9 | 99.3 | 31.2 | 26.9 | 68.9 | 48.1 | 83.9 | 56.9 | 13.8 | 16 |
| FVIII on Day 1 | 59 | 64 | 60.2 | 57 | 88.8 | 82.8 | 102.4 | 96.7 | 49.6 | 43.8 |
| FVIII on Day 3 | 63 | 54.9 | 54.5 | 47.1 | 93 | 72.7 | 86.7 | 65.9 | 80.2 | 74 |
| FVIII on Day 5 | 50 | 46 | 40.3 | 28.6 | 56.8 | 47.2 | 56.8 | 39.6 | 101.1 | 58.6 |
| LAG on Day 1 | 3.2 | 3.37 | 2.32 | 3.16 | 1.82 | 2.15 | 2.15 | 1.98 | 2.67 | 2.33 |
| LAG on Day 3 | 3.17 | 3.83 | 2.7 | 2.2 | 1.87 | 2.37 | 2.7 | 2.7 | 2.17 | 3 |
| LAG on Day 5 | 3.23 | 3.85 | 3.33 | 3.33 | 3 | 3 | 2.67 | 3 | 2.67 | 3 |
| ETP on Day 1 | 1480.38 | 1455.38 | 1309 | 1402.16 | 1217.85 | 1172.34 | 1415.24 | 1397.57 | 1374.54 | 1439.29 |
| ETP on Day 3 | 1606.51 | 1371.66 | 1365.68 | 1392.66 | 1166.35 | 996.68 | 1530.76 | 1290.68 | 1455.45 | 1366.71 |
| ETP on Day 5 | 1651.1 | 1491.58 | 2049.24 | 1690.21 | 1762.36 | 1641.47 | 1816.64 | 1634.83 | 1243.06 | 1265.87 |
| PEAK on Day 1 | 344.69 | 311.17 | 366.59 | 365.02 | 337.27 | 334.1 | 385.27 | 365.3 | 355.13 | 368.43 |
| PEAK on Day 3 | 381.41 | 283.7 | 301.67 | 313.23 | 341.25 | 307.58 | 334.07 | 343.2 | 333.02 | 306.68 |
| PEAK on Day 5 | 393.15 | | 418.12 | 386.08 | 419.59 | 383.72 | 406.1 | 372.55 | 292.82 | 273.15 |
| TtP on Day 1 | 5.38 | 5.71 | 3.99 | 4.83 | 3.49 | 3.82 | 3.82 | 3.49 | 4.17 | 3.67 |
| TtP on Day 3 | 5.33 | 6.5 | 4.54 | 3.87 | 3.53 | 3.87 | 4.54 | 4.2 | 3.67 | 21.5 |
| TtP on Day 5 | 5.58 | 6.51 | 5.33 | 5.33 | 4.67 | 4.67 | 4.33 | 4.67 | 4.33 | 4.67 |
| TAIL on Day 1 | 20.92 | 21.09 | 18.38 | 19.72 | 18.55 | 18.21 | 19.22 | 19.22 | 19.33 | 19.33 |
| TAIL on Day 3 | 20.3 | 21.5 | 21.9 | 20.3 | 17.72 | 17.06 | 21.9 | 18.72 | 20.67 | 21.5 |
| TAIL on Day 5 | 20.8 | 21.5 | 23.67 | 21.83 | 21.17 | 21.33 | 22.67 | 22.5 | 20.5 | 21.83 |

| STUDY NUMBER | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|
| | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 |
| APTT on Day 1 | 36.9 | 34.6 | 28.5 | 25.9 | 35.5 | 33.4 | 30.2 | 30.4 | 30.8 | 33.7 |
| APTT on Day 3 | 33.6 | 38.4 | 32 | 36.6 | 29.4 | 30.4 | 31.9 | 33.2 | 30 | 27.6 |
| APTT on Day 5 | 42.2 | 45.4 | 31.4 | 33.2 | 42.7 | 36.9 | 33.1 | 32.7 | 33.1 | 36.3 |
| FV on Day 1 | 25.9 | 51.1 | 47.1 | 23.7 | 52.2 | 50.7 | 55 | 59.7 | 83.7 | 81.8 |
| FV on Day 3 | 43.3 | 22.5 | 72.7 | 69 | 60.4 | 57 | 59.8 | 51.6 | 93.8 | 85.3 |
| FV on Day 5 | 19.2 | 10.2 | 52.6 | 40.7 | 43 | 53.3 | 56 | 50.3 | 100.8 | 90.4 |
| FVII on Day 1 | 45.2 | 79.7 | 76.8 | 57.4 | 52.2 | 53.5 | 77.9 | 80 | 102.6 | 106.5 |
| FVII on Day 3 | 47.1 | 46.8 | 72 | 53.1 | 42.2 | 34 | 65 | 59.3 | 90.3 | 77.1 |
| FVII on Day 5 | 16.8 | 23.1 | 43.7 | 60 | 31.8 | 36.8 | 59.4 | 57.9 | 109.9 | 81 |
| FVIII on Day 1 | 37.8 | 43.8 | 38 | 44.8 | 27.5 | 43.7 | 118.9 | 83.5 | 59.9 | 42.4 |
| FVIII on Day 3 | 61.5 | 45 | 65.2 | 29.8 | 42.2 | 93.8 | 65 | 112.5 | 90.3 | 50 |
| FVIII on Day 5 | 65.9 | 52.4 | 108.4 | 63.4 | 30.1 | 46.2 | 96.1 | 84.2 | 60.1 | 41.2 |
| LAG on Day 1 | 2.83 | 2.83 | 3.33 | 3.33 | 3.07 | 3.07 | 2.57 | 2.23 | 2.73 | 3.07 |
| LAG on Day 3 | 2.5 | 2.17 | 3.17 | 4 | 4.33 | 3.67 | 2.83 | 3 | 3.5 | 3.67 |
| LAG on Day 5 | 2.67 | 3 | 2.83 | 2.83 | 3.5 | 2.83 | 2.33 | 2.67 | 2.51 | 2.5 |
| ETP on Day 1 | 1214.03 | 990.69 | 1838.37 | 1726.81 | 1342.01 | 1446.22 | 2454.34 | 2132.93 | 1890.85 | 1798.31 |
| ETP on Day 3 | 1404.77 | 1369.09 | 1666.91 | 1531.56 | 1420.26 | 1139.58 | 2160.35 | 1705.29 | 1685.07 | 1448.24 |
| ETP on Day 5 | 1290.62 | 1248.19 | 1510.12 | 1324.31 | 1021.3 | 1062.33 | 1876.11 | 1957.71 | 1691.15 | 1469.38 |
| PEAK on Day 1 | 300.43 | 218.71 | 412.68 | 419.49 | 360.43 | 369.08 | 469.67 | 428.83 | 411.04 | 392.86 |
| PEAK on Day 3 | 325.06 | 326.63 | 389.26 | 315.17 | 359.31 | 287.41 | 351.22 | 346.01 | 375.31 | 327.89 |
| PEAK on Day 5 | 296.21 | 297.14 | 332.11 | 318.29 | 254.38 | 310.75 | 385.79 | 357.37 | 381.11 | 349.91 |
| TtP on Day 1 | 4.33 | 4.33 | 5.17 | 5 | 4.74 | 4.74 | 4.07 | 3.9 | 4.74 | 4.9 |
| TtP on Day 3 | 4 | 3.83 | 5 | 6.17 | 6.33 | 5.67 | 4.67 | 4.67 | 5.33 | 5.5 |
| TtP on Day 5 | 4.17 | 4.5 | 4.83 | 4.67 | 5.5 | 4.67 | 4 | 4.33 | 4.31 | 4.5 |
| TAIL on Day 1 | 21.5 | 22.17 | 21 | 20.83 | 19.93 | 19.93 | 24.77 | 24.6 | 22.27 | 22.93 |
| TAIL on Day 3 | 22.17 | 22.17 | 21.33 | 22.67 | 20.83 | 20.67 | 29.17 | 24.5 | 22.67 | 22.83 |
| TAIL on Day 5 | 21.5 | 21.33 | 21.67 | 21.17 | 19.5 | 18 | 23.33 | 26 | 21 | 21 |

| | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 |
|---|---|---|---|---|---|---|---|---|
| | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 | GROUP 1 | GROUP 2 |
| APTT on Day 1 | 41.4 | 40.2 | 28.9 | 29 | 28.4 | 29.2 | 24.2 | 26.9 |
| APTT on Day 3 | 42.3 | 41.6 | 42.3 | 31.4 | 34 | 35.4 | 27.9 | 27.4 |
| APTT on Day 5 | 32.5 | 31.7 | 28.5 | 30.3 | 34.7 | 36.3 | 27.3 | 29.4 |
| FV on Day 1 | 62.8 | 64.6 | 72.7 | 72.9 | 96.8 | 97.6 | 115.3 | 113.7 |
| FV on Day 3 | 41.6 | 66.9 | 59.3 | 80.7 | 71 | 70.5 | 81.5 | 83.4 |
| FV on Day 5 | 70.6 | 67.1 | 77.7 | 61.1 | 99.7 | 88.2 | 105.2 | 105.6 |
| FVII on Day 1 | 61 | 66.1 | 53.5 | 52.9 | 27.2 | 27.1 | 62.6 | 61.6 |
| FVII on Day 3 | 67.7 | 61.2 | 62.1 | 50 | 26.9 | 27.4 | 62.6 | 61.4 |
| FVII on Day 5 | 66.2 | 54.5 | 53.2 | 46.9 | 31.2 | 26.9 | 68.9 | 48.1 |
| FVIII on Day 1 | 15.2 | 17.5 | 102.1 | 102.6 | 60.2 | 57 | 88.8 | 82.8 |
| FVIII on Day 3 | 17.4 | 17.5 | 100.2 | 67.1 | 54.5 | 47.1 | 93 | 72.7 |
| FVIII on Day 5 | 14.6 | 14.6 | 60.1 | 68.7 | 40.3 | 28.6 | 56.8 | 47.2 |
| LAG on Day 1 | 4.33 | 4.33 | 3.5 | 3.5 | 2.32 | 3.16 | 1.82 | 2.15 |
| LAG on Day 3 | 3.67 | 4.17 | 3.83 | 4.17 | 2.7 | 2.2 | 1.87 | 2.37 |
| LAG on Day 5 | 2.33 | 2.33 | 1.83 | 2 | 3.33 | 3.33 | 3 | 3 |
| ETP on Day 1 | 1154.04 | 1124.58 | 1562.1 | 1559.81 | 1309 | 1402.16 | 1217.85 | 1172.34 |
| ETP on Day 3 | 1967.02 | 1316.01 | 1613.55 | 2056.4 | 1365.68 | 1392.66 | 1166.35 | 996.68 |
| ETP on Day 5 | 1057.21 | 1031.65 | 1712.89 | 1591.77 | 2049.24 | 1690.21 | 1762.36 | 1641.47 |
| PEAK on Day 1 | 242 | 223.5 | 332.68 | 329.75 | 366.59 | 365.02 | 337.27 | 334.1 |
| PEAK on Day 3 | 288.62 | 277.45 | 1613.55 | 2056.4 | 301.67 | 313.23 | 341.25 | 307.58 |
| PEAK on Day 5 | 281.58 | 277.83 | 397.5 | 357.44 | 418.12 | 386.08 | 419.59 | 383.72 |
| TtP on Day 1 | 7.71 | 7.38 | 5.5 | 5.5 | 3.99 | 4.83 | 3.49 | 3.82 |
| TtP on Day 3 | 6.33 | 6.67 | 5.83 | 6.33 | 4.54 | 3.87 | 3.53 | 3.87 |
| TtP on Day 5 | 4.33 | 4.33 | 3.67 | 3.83 | 5.33 | 5.33 | 4.67 | 4.67 |
| TAIL on Day 1 | 21.5 | 21.5 | 23.33 | 23.39 | 18.38 | 19.72 | 18.55 | 18.21 |
| TAIL on Day 3 | 27.83 | 21.67 | 21.83 | 27.67 | 21.9 | 20.3 | 17.72 | 17.06 |
| TAIL on Day 5 | 18 | 17.83 | 20.67 | 21.5 | 23.67 | 21.83 | 21.17 | 21.33 |