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THE EFFECT OF THAWING FRESH FROZEN PLASMA AT VARIOUS TEMPERATURES ON IN VITRO COAGULATION FACTOR ACTIVITY

Brian Leslie Levy

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfillment of the requirements for the degree of
Master of Medicine

Johannesburg
2007

DECLARATION

I, Brian Leslie Levy declare that this thesis is my own work. It is submitted for the admission to the degree of Master of Medicine by the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

_____

_____day of _____, 2007

PRESENTATIONS ARISING FROM THIS STUDY

- Poster presentation at the Critical Care Congress, July 2006

- Poster presentation at the University of the Witwatersrand Health Sciences research day, August 2006

ABSTRACT

Thawing of fresh frozen plasma (FFP) in South Africa is not standardized and thawing at high temperatures may cause clotting factor activation and disseminated intravascular coagulation (DIC). This research project studies the in-vitro effects of thawing FFP at various temperatures on coagulation. Twenty units of FFP were each divided into 4 satellite bags which were respectively thawed at 22ºC, 37ºC, 45ºC and 60ºC and tested for Fibrinogen, D-Dimers, PT, PTT, r value, Alpha Angle and Maximum Amplitude (MA). FFP thawed at 60ºC showed significant differences suggesting clotting factor inactivation. FFP thawed at 45ºC showed significantly elevated D-Dimers. Clotting factors thawed at 22ºC may be partially inactivated. High thawing temperatures may activate and then denature the factors therein. Twenty two degrees may partially inactivate FFP until it is warmed to body temperature. The clinical implications and recommendations of this study are to thaw FFP at 37ºC.

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CHAPTER ONE

OVERVIEW OF THE STUDY

1.1 Introduction

In this chapter an overview of the study is provided. This includes the background to the study, the problem statement, the purpose, the objectives, the importance of the study, relevant definitions, overview of methodology, validity and reliability, ethical considerations and concludes with a brief outline of the study to determine whether Fresh Frozen Plasma (FFP) thawed at high temperatures causes activation of the clotting factors in FFP.

1.2 Background to the Study

The current practices as regards thawing of FFP in South Africa are not standardised. FFP is delivered from the South African Blood Transfusion Services (SABTS) frozen. Thawing of FFP is then at the discretion of medical staff prior to administration.

The current recommendation by the SABTS is to thaw FFP at 37ºC in a water bath (1). However, the common practice in South African hospitals is to thaw FFP in a non sterile bucket of water, the temperature of which is often influenced by the urgency with which the FFP is required. The average time to thawing of FFP at 37ºC is 35 to 45 minutes (2), which can be reduced by increasing the temperature of the waterbath.

There are two potential problems with this practice.

The first is one of bacterial contamination. Rhame and McCullough (3) report on 3 cases of pseudomonal septicaemia related to thawing of cryoprecipitate in a water bath and in view of this, recommend thawing blood products in protective plastic coverings. This, however, has been shown to prolong the thawing process (4).

The second potential problem with the practice of uncontrolled thawing of FFP is one of protein denaturation and / or possible activation when FFP is thawed at high temperatures to speed up the thawing process. Administering denatured protein, in particular coagulation factors, is potentially hazardous in its own right as it might initiate a Disseminated Intravascular Coagulation and / or cause renal failure.

Westphal et al (2), studied FFP thawed at 37ºC versus 56 ºC and showed no significant effect on the activity of clotting factors. The coagulation tests used were Prothrombin Time (PT), Partial thromboplastin times (PTT), Fibrinogen and factors II, IX, X, V and VIII assays. They do stress that the bags were removed as soon as they were deemed to be thawed in order to prevent protein denaturation. A confounding variable in the study was that unit to unit variability was not taken into account.

With concern for the interpretation of Westphal's results, not to mention a flawed study design, Plotz and Ciotola (4) undertook a study where satellite bags of FFP, thawed at 37ºC and 45ºC, were compared. Bags in the 45ºC were removed when 'slushy', i.e. before thawing was complete. No significant differences were found in coagulation parameters and factor levels measured, between the 37ºC and 45ºC, while thawing time was significantly reduced, bearing in mind that the satellite bags were smaller in volume than typical bags of FFP.

Of interest are other techniques used to reduce the thawing time of FFP. Initial reports on the use of microwave technology to thaw FFP found a decrease in coagulation factors which were deemed to be not clinically significant (5). In contrast to the study by Thompson (5), Luff, Kessler and Bell (6) found significant reductions in factors IX, X, XI and fibrinogen with prolonged PT and PTT. Total protein and albumin were similarly affected. With a change in microwave oven, with temperature regulation or water environment microwave thawing, no significant decreases in factors or albumin was detected, although, flocculent material was noted in all techniques. Whether this flocculent material was denatured proteins or debris from time of freezing is to be debated. In all studies the time to thawing of FFP was significantly reduced (6,7,8).

The potential deleterious effects of inducing clotting factor activation through the thawing process may be to induce a Disseminated Intravascular Coagulation (DIC) which in turn may precipitate a Systemic Inflammatory Response Syndrome (SIRS) (9,10). Widespread activation of the clotting cascade leads to clotting factor consumption through microvascular thrombus formation ultimately leading to factor depletion and bleeding, commonly seen in patients with major trauma and patients receiving multiple blood transfusions (9,10). Lack of coagulation inhibitors contributes to coagulation with microvascular thrombus formation ultimately leading to reduced capillary blood flow and tissue ischaemia and inflammation. Endothelial damage also contributes to the inflammatory process through neutrophil activation and the release of inflammatory cytokines e.g. Tumour Necrosis Factor – alpha (TNF α), Interleukin 1 and 6 (IL-1; IL-6) (11).

These pro-inflammatory cytokines may further induce coagulation. Monocytes release Tissue Factor which forms part of the initial steps in coagulation, activating platelets and binding and

activating Factor VII. Factor Xa was found to produce a proinflammatory response in endothelial cells (12).

In SIRS, an imbalance between pro coagulants and coagulation inhibitors exists. Numerous studies have shown relative 'exhausted' coagulation inhibitors and fibrinolysis, hence the recent findings where Activated Protein C (APC) improved outcome in patients with severe sepsis (13).

APC is anti-inflammatory (13). APC inhibits expression of Tissue Factor (TF), reduces neutrophil rolling, and inhibits proinflammatory cytokine production.

Trials such as the Kybersept (14) trial studying Anti Thrombin (AT) in sepsis, the Optimist trial (15) looking at Tissue Factor Pathway Inhibitor (TFPI) and trials studying Platelet Activating Factor acetyl hydrolase (Pafase) (16) explore different ways in which to correct the imbalance in coagulation created by sepsis and SIRS. AT also has anti-inflammatory properties in that it reduces Nuclear Factor (NF-κB) production. NF-κB is a transcription factor involved in immediate early gene activation during inflammation (17).

A further complication of a DIC is platelet consumption and thrombocytopenia. Platelet activation results in platelets binding not only to the endothelium and fibrin but also to neutrophils. This widespread platelet activation also contributes to microvascular thrombus formation and reduced blood flow. The thrombocytopenia may further contribute to the bleeding.

The potential deleterious effects of administering denatured clotting factors are difficult to ascertain as there are no studies where this has been directly addressed. The assumption can be made that intravenously administered denatured clotting factors would be sequestrated in the lung and kidney, potentially reducing capillary blood flow with endothelial dysfunction. This in turn could produce a localized inflammatory process leading to acute lung injury and / or acute renal failure.

1.3 Research Problem

Clinical experience and anecdotal reports suggest that the SABTS standardized method of thawing FFP at 37ºC is not adhered to and that FFP is thawed in a basin or bucket with water temperatures varying depending on the urgency to administer the FFP.

It has been shown that FFP thawed at high temperatures may denature clotting factors but does not induce clotting factor activation. These studies however were flawed in study design and hence the conclusions drawn may not be accurate.

The potential deleterious effects of administering activated clotting factors or denatured proteins, produced through the thawing process, are DIC and or SIRS in patients who by the very reason for requiring FFP, are already at risk for or have a DIC and SIRS.

No study to date has adequately addressed the issue of clotting factor activation and denaturation.

1.4 The purpose of the study

The purpose of this research project is to study the in-vitro effects of thawing FFP at various temperatures on coagulation parameters.

1.4.1 Hypothesis

There is no positive correlation between clotting factor activation and defrosting at high temperatures.

1.4.2 Aim

The aim of the study is to determine FFP activation with defrosting at different temperatures.

1.4.3 Objectives

To determine clotting factor activation at different temperatures (22°C, 37°C, 45°C, 60°C) by using the following tests:

- Fibrinogen
- D-Dimers
- PT
- PTT
- TEG analyses of r value, Alpha angle and Maximum Amplitude

1.5 Research Design

An experimental, quantitative, randomized, controlled study design was used to determine the in vitro effect of thawing FFP on clotting factor function.

1.6 Research Method and Sampling

1.6.1 Preparation of FFP

The FFP used in this study was prepared by the SABTS according to their standard operating procedures (SOP).

Adult units of FFP were each divided into 4 satellite bags prior to freezing and storage according to the SABTS SOP. Each satellite bag was assigned to one of 4 temperature groups viz. 22°C, 37°C, 45°C and 60°C. A total of 20 adult units of FFP were divided and stored for the purposes of this research project. Thawing temperatures were chosen based on similar temperatures used in other research on the effect of thawing temperatures on FFP.

1.6.2 Sampling

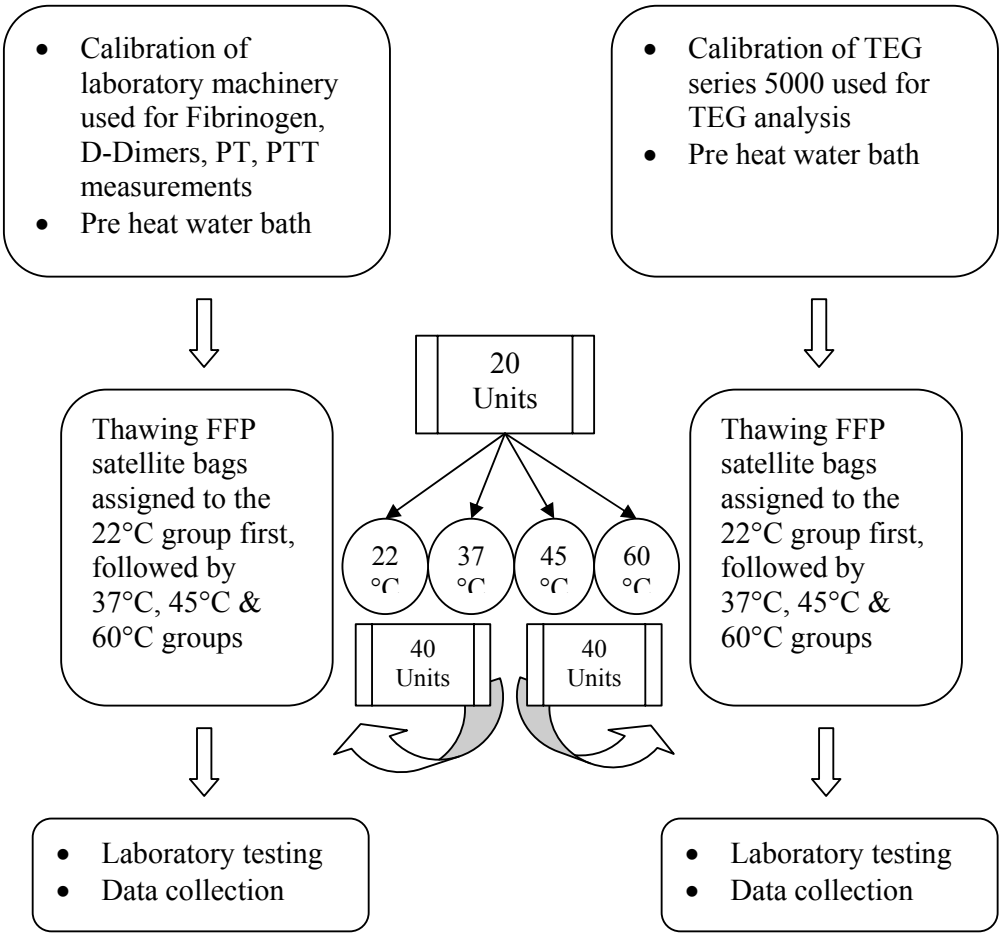


Figure 1.1: Flow diagram of sampling process

1.6.3 Laboratory work

The routine coagulation tests were performed by an experienced haematology registrar, with the assistance of qualified laboratory technicians of the Haematology Laboratory of the Johannesburg Hospital, using the laboratories SOP. The TEG tests were performed by the researcher using the TEG Series 5000 SOP. All laboratory equipment was calibrated prior to this research project.

FFP was thawed in a standard waterbath.

1.7 Data Collection

Data was collected by the investigator in hard copy and compiled on a SPSS data table.

1.8 Data analysis

Data was analysed using the appropriate parametric as well as non parametric tests, to describe differences between the variables thawed at different temperatures.

1.9 Reliability

Laboratory tests were performed using the standard operating procedures at the Haematology laboratory at the Johannesburg Hospital.

TEG tests were performed on calibrated TEG 5000 machines. Two samples from each satellite bag were simultaneously tested and the results thereof statistically compared with each other. No statistical difference was found between these 2 samples and is further discussed in Chapter 4.

1.10 Validity

The PT, PTT, Fibrinogen and D-Dimers provide a comprehensive analysis, and hence the effect of thawing temperature, on standard in-vitro coagulation pathways. The TEG provides a cost effective general overview of coagulation activity.

1.11 Ethics

Approval to conduct the study was received from the Committee for Research on Human Subjects (medical), ref: R14/49 Levy.

1.12 Overview of the study

This study will be presented as follows:

Chapter 1: Overview of the study

This chapter provides an overview of the study and includes the background to the study, the problem statements and purpose of the study and a brief explanation of the research methodology.

Chapter 2: Literature review

In this chapter, a review of the literature relevant to the various aspects of the study is covered.

Chapter 3: Research methodology

This chapter describes the research methodology used in this study including the research design, the study setting, the sampling process and data collection procedures.

Chapter 4: Data analysis and discussion of results

In this chapter the results of both the classic and TEG data will be analysed.

Chapter 5: Summary, conclusions, limitations and recommendations.

In this chapter a summary and conclusions from the main findings are presented, followed by a discussion of the limitations of the study and recommendations for clinical practice and for further research in this area.

1.13 Summary

Thawing of FFP in South Africa is done at the discretion of the attending medical staff. Current guidelines advise thawing FFP at 37ºC, however, this is often not the case when time is of the essence. There are potential serious deleterious effects of thawing FFP at high temperatures e.g. DIC and SIRS. These deleterious effects could be potentially fatal in the already compromised recipient. If knowledge of the effects of thawing FFP at high temperatures is known, this could aid in changing current practice and thereby potentially improving the outcome of patients receiving FFP.

CHAPTER TWO

REVIEW OF THE LITERATURE

2.1 Introduction

In this chapter a review of the literature is discussed. This includes an overview of prior research on thawing of FFP followed by a discussion on the potential effects of administering activated coagulation factors. To best understand the choice of clotting factor tests in this study we will review the coagulation pathway followed by a brief review of the conventional tests and Thromboelastography used to assess clotting factor function. A comparison of TEG versus conventional tests for coagulation is discussed followed lastly by the preparation and content of FFP.

2.2 Research on thawing of FFP

The South African Blood Transfusion Services currently recommends that FFP be thawed at 30 - 37°C (1). The average time to thawing of FFP at 37ºC in a water bath is 35 to 45 minutes (2), however FFP is often thawed at higher temperatures in order to expedite the thawing process.

Thawing of blood products in a non-sterile water-bath can potentially cause bacterial contamination. Rhame and McCullough (3) reported on 3 cases of pseudomonal septicaemia related to thawing of cryoprecipitate in a contaminated water bath. In view of this they recommended thawing blood products in protective plastic coverings despite a prolongation in the thawing process (4).

Various methods of thawing FFP have been studied in an attempt to expedite the thawing process. Westphal et al (2), studied FFP thawed at 37ºC versus 56 ºC and showed no significant effect on the activity of clotting factors. The coagulation tests used were Prothrombin time (PT), Partial thromboplastin times (PTT), Fibrinogen and factors II, IX, X, V and VIII assays. They do stress that the bags were removed as soon as they were deemed to be thawed in order to prevent protein denaturation. A confounding variable in the study was that unit to unit variability was not taken into account.

Due to concern for the interpretation of Westphal's results, not to mention a flawed study design, Plotz and Ciotola (4) undertook a study where satellite bags of FFP, thawed at 37ºC and 45ºC, were compared. Bags in the 45ºC were removed when 'slushy', i.e. before thawing was complete. No significant differences were found in coagulation parameters and factor levels measured, between the 37ºC and 45ºC, while thawing time was significantly reduced, bearing in mind that the satellite bags were smaller in volume than typical bags of FFP.

Microwave technology is another method which has been used to thaw FFP rapidly. Initial reports found a decrease in coagulation factors deemed to be not clinically significant (5). In contrast to the study by Thompson (5), Luff, Kessler and Bell (6) found significant reductions in factors IX, X, XI and fibrinogen with prolonged PT and PTT. Total protein and albumin were similarly affected. A further concern relating to microwave thawing was the uneven distribution of heat within the FFP being thawed, creating "hot spots" with the risk of clotting factor protein denaturation (5). There is anecdotal evidence, via personal communication, of 6 patients who developed DIC following a transfusion of FFP thawed in a conventional microwave oven.

A change in microwave oven technology, specifically designed for the purposes of thawing blood products e.g. the Transfusio-therm 2000R (Figure 2.1), has overcome these problems with the added benefit of reduced thawing times of FFP, no effect on clotting factors and also avoiding the risk of bacterial contamination (7,8,9,10). There is still concern, however, for detrimental effects on Packed Red Cells warmed in these devices (11).



Figure 2.1: The Transfusio-therm 2000R

2.3 Potential deleterious effects of activated clotting factors

2.3.1 Coagulation system

- **Platelets**

Thrombocytopenia is a common finding among patients with sepsis and is also used as an indicator of severity. Sepsis induced endothelial activation causes platelet activation by the exposure of TF as well as from circulating cytokines. This leads to platelet aggregation and adhesion not only to the endothelium and fibrin but also to neutrophils. Widespread platelet activation contributes to microvascular thrombus formation and reduced blood flow. The imbalance of the pro-coagulation versus anti-coagulation pathways together with platelet activation leads to thrombocytopenia (12,13).

- **Disseminated intravascular coagulation**

Widespread activation of the clotting cascade leads to clotting factor consumption ultimately leading to factor depletion and bleeding commonly seen in major trauma, massive blood transfusion patients (14,15). The hypothesis of administering activated clotting factors is that this could induce widespread activation of clotting factors leading to a consumptive coagulopathy or DIC. While there is no evidenced-based data to support that activated clotting factors alone may precipitate a DIC, it may still be a precipitating cause of DIC (16).

Should the administration of "activated" FFP precipitate a DIC, thrombocytopenia would once again be an associated complication.

2.3.2 Immune system

Endothelial damage or activation associated with sepsis, activates neutrophils with the resultant release of inflammatory cytokines e.g. Tumour Necrosis Factor – alpha (TNF α), Interleukin 1 and 6 (IL-1; IL - 6). These inflammatory mediators initiate coagulation (17). Monocytes also release TF which, as illustrated above, is part of the initial steps in coagulation (18).

The ancestral relationship of coagulation and inflammation is well illustrated in the Limulus Test. It was discovered that blood in the presence of the Limulus amebocyte coagulated when exposed to certain factors (19). This was also noted in the Horseshoe crab (Limulus polyphemus) when exposed to Gram-negative bacteria and similarities were also found in mammals (20). The Limulus Test is based on the ability of test material to initiate coagulation of Limulus lysate. It has been used as a sensitive test for bacterial endotoxin in

pharmaceutical preparations, food, water supplies as well as in blood, urine and cerebrospinal fluid (20). A study of different strains of Neisseria using the Limulus test found the Lipid A component of endotoxin to possess the majority of the procoagulant activity (21). Lipid A is also the major initiator of the inflammatory response (22).

While proinflammatory cytokines may induce coagulation, there is also data suggesting that coagulation itself can induce inflammation through the release of inflammatory cytokines (23,24). Factor Xa was found to produce a proinflammatory response in endothelial cells (18) and Activated Protein C (APC) was also found to be anti-inflammatory (25). APC inhibits expression of TF, reduces neutrophil rolling, and inhibits proinflammatory cytokine production. Anti-Thrombin (AT) reduces Nuclear Factor (NF-κB) production (10), NF-κB being a transcription factor involved in immediate early gene activation during inflammation (26).

The relationship between coagulation and inflammation is further highlighted by the imbalance that exists between pro coagulants and coagulation inhibitors in patients with sepsis. Numerous studies have shown a relative 'exhausted' coagulation inhibitors and fibrinolysis, hence the recent findings where APC improved outcome in patients with severe sepsis (25). Lack of coagulation inhibitors allows for unimpeded coagulation with microvascular thrombus formation and reduced capillary blood flow, and an associated increase in organ failure, morbidity and mortality (16, 23). Trials such as the Kybersept trial (27) looking at AT in sepsis, the Optimist trial (28) looking at TF Pathway Inhibitor (TFPI) and trials studying Platelet Activating Factor acetyl hydrolase (Pafase) (29) are exploring ways to correct the imbalance in coagulation created by sepsis.

The effect of administering activated clotting factors may however not be deleterious (16). Numerous studies have demonstrated a beneficial effect of administering activated factor VIIa in patients with ongoing blood loss (30,31) and activated Protein C has been shown to be of benefit in patients with severe sepsis as illustrated above (15, 17). Hence the effects of administering activated FFP may not be deleterious at all.

2.4 The coagulation system

The coagulation system is a complex system of interactions with the ultimate aim of haemostasis in the body. As illustrated above, it is linked to the immune system and has a marked effect on the inflammatory response in patients.

To follow is an overview of the coagulation system and the appropriate tests of the coagulation system.

2.4.1 Platelets (22,32)

Platelets play a key role in the first steps of the coagulation pathway.

- Vascular injury exposes subendothelial matrix, collagen, to soluble protein, von Willebrand factor (vWf). vWf attaches to the subendothelial matrix, exposing multiple Glycoprotein 1b (G1b) binding sites for the G1b, glycoproteins found on platelet membranes.
- Platelet adhesion to the subendothelial-vWf complex occurs and results in platelet activation
- Platelet activation exposes Glycoprotein IIb/IIIa (GIIb/IIIa) binding sites for further attachment of vWf and fibrinogen. At the same time dense granules are released from

platelets. These contain ADP which enhances platelet adhesion and activation, thromboxane A2 and serotonin which cause vasoconstriction, and calcium which is necessary for activation of proteins in the coagulation pathway. Alpha granules in platelets release fibrinogen, fibronectin, factor V, factor VIII and thrombospondin. Lastly, phosphatidylserine is exposed which serves as a platform for the initiation of the coagulation cascade.

2.4.2 Coagulation Pathway (19)

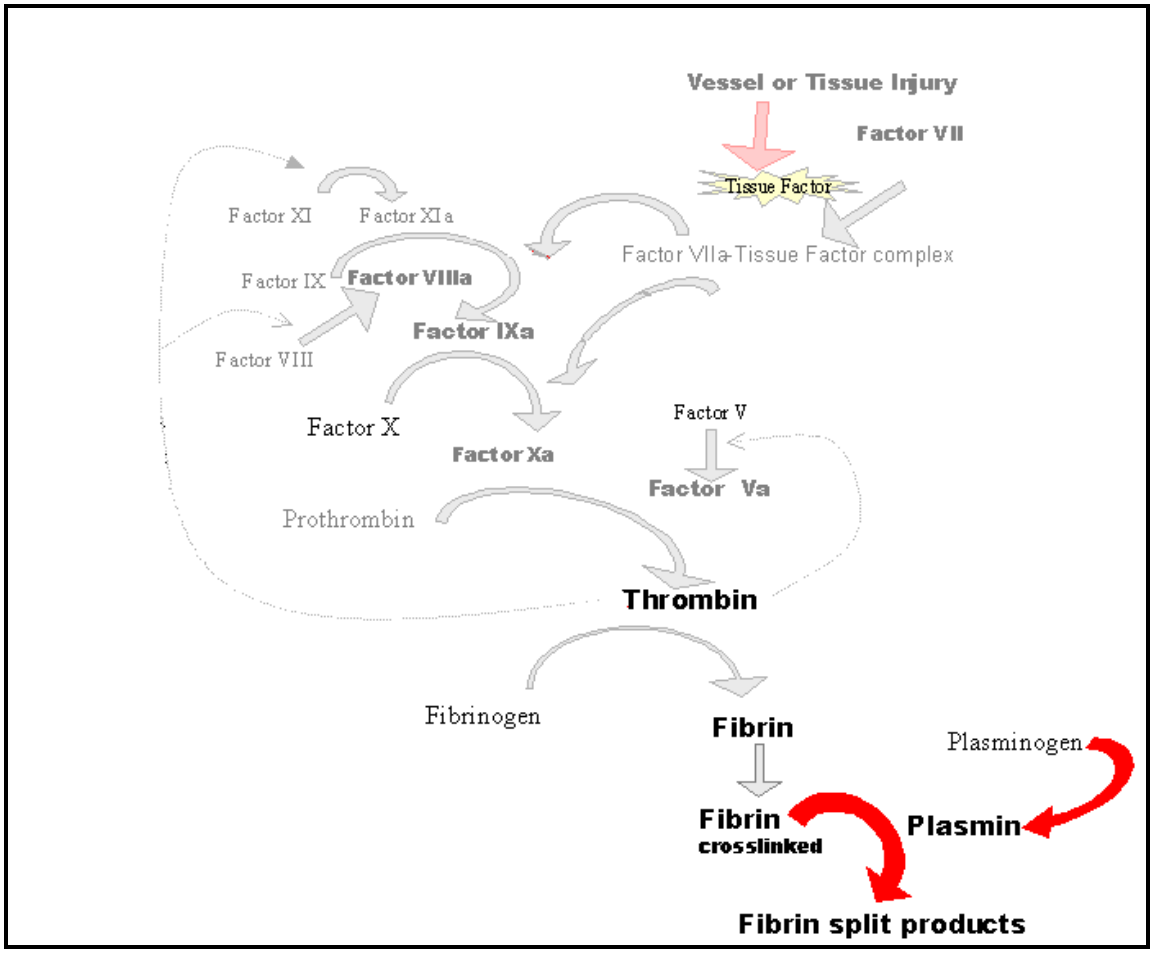


Figure 2.2: The coagulation pathway (adapted from Medinfo (33))

- Exposure of TF to factor VII (fVII) in the presence of calcium initiates the coagulation pathway.

- TF – fVII complex activates factor IX (fIXa) and factor X (fXa)
- fIXa in the presence of fVIII, further activates fX to fXa in large amounts facilitating a "thrombin burst"
- fXa converts prothrombin to thrombin (fIIa), facilitated by fVa
- Thrombin then
    - induces fVIII and fV
    - induces fXI to fXIa which in turn activates fIX to fIXa
    - enhances platelet aggregation and activation
    - converts fibrinogen to fibrin, which polymerises to form fibrin clot.
- fXIIIa (activated by fIIa and calcium) stabilises the clot through covalent bonds between the fibrin molecules.

2.4.3 Inhibition of the Coagulation Pathway (19,32)

- Prostaglandin I_2 released from intact endothelium causes vasodilation and inhibits platelet activation.
- Antithrombin, a serine protease inhibitor (serpin), and heparin sulphate from the endothelium, inhibit thrombin, fIXa, fXa, and fXIa.
- Protein C, activated by the thrombin-thrombomodulin complex, inactivates fVa and fVIIIa in the presence of co-factor, Protein S.
- TF Pathway Inhibitor (TFPI) binds to fXa and TF-fVIIa complex inhibiting this "extrinsic" component of the pathway.
- Tissue Plasminogen activator (tPA) is released from the damaged endothelium and stimulates the fibrinolytic pathway.

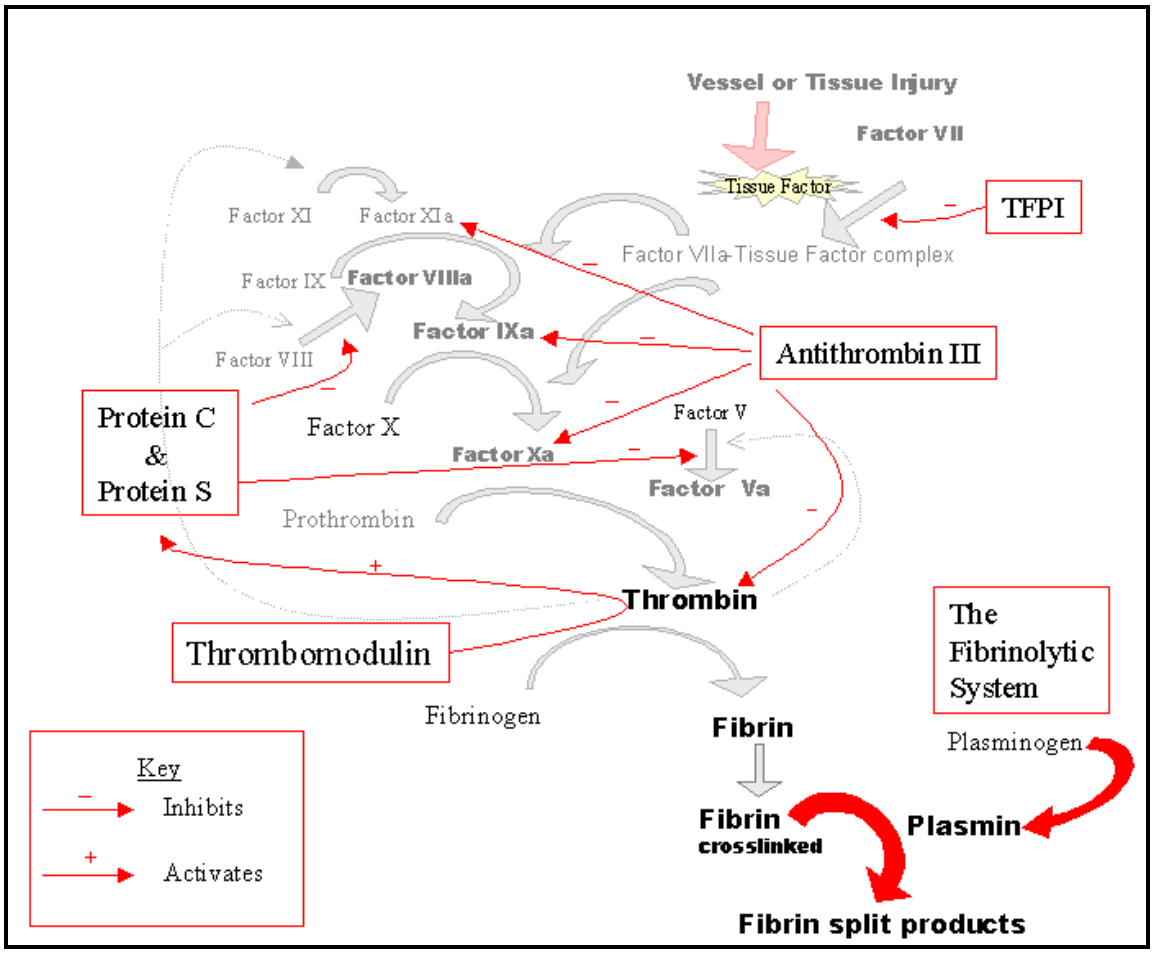


Figure 2.3: Inhibition of the coagulation pathway (adapted from Medinfo (33))

2.4.4 Fibrinolytic pathway (22,32)

- tPA and Urokinase are released from damaged endothelium and in response to thrombin. Plasmin is cleaved from plasminogen bound to fibrin within the clot.
- Plasmin degrades fibrin to D-Dimers and fibrin degradation products (FDPs)
- Plasmin also degrades fVa, fVIIIa and GPIb
- fXII is activated by the negatively charged subendothelium, which cleaves kallikrein from prekallikrein in the presence of kininogen.
- Kallikrein further cleaves plasmin from plasminogen and releases bradykinin from kininogen, the link to the pro-inflammatory process.

2.4.5 Regulation of Fibrinolysis (19,22,32)

- Plasminogen Activator Inhibitor I (PAI 1) is released from activated platelets, and inhibits tPA and Urokinase as well as inhibiting the activation of prekallikrein to kallikrein. Protein C inhibits PAI 1
- Alpha 2 antiplasmin and alpha 2 macroglobulin prevent the formation of plasmin from plasminogen and also inactivate free plasmin, creating a plasmin-alpha 2 antiplasmin (PAP) complex.

2.5 Conventional tests of the coagulation system (32)

2.5.1 Fibrinogen

As mentioned above, fibrinogen is cleaved to fibrin through the action of Thrombin.

Hypothesis of the test: A low fibrinogen level would suggest an activated coagulation cascade with the formation of increased amounts of thrombin and hence large amounts of fibrinogen being cleaved to fibrin.

2.5.2 D-Dimers

D-Dimers are breakdown products of fibrin. Elevated D-Dimers are found in situations where fibrinolysis is taking place.

Hypothesis of the test: D-Dimers should be elevated if FFP is activated leading to thrombus formation and subsequent fibrinolysis.

2.5.3 Prothrombin Time

To test the PT a 3.8% trisodium citrate anticoagulant is added to the specimen of plasma or in this case FFP, in a 9:1 ratio. This is then centrifuged to produce platelet poor plasma (PPP). Calcium + thromboplastin are then added and time to fibrin strand formation is measured, either by optical or electromechanical device. The PT tests the "Extrinsic Pathway".

Hypothesis of the test: Activated clotting factors would produce a shorter time to fibrin strand formation and hence a shorter PT. A prolonged PT would suggest factor deficiency or the equivalent e.g. denaturation.

2.5.4 Partial Thromboplastin Time

A specimen, in this case FFP, is "activated" by negatively charged Kaolin or Celite. Partial thromboplastin, also called cephalin, is a phospholipid component which is then added to this "activated" specimen. Time to fibrin formation is measured.

Hypothesis of the test: As with the PT, a short PTT would suggest factor activity. A prolonged PTT would suggest factor deficiency or the equivalent e.g. denaturation.

Large amounts of activated FFP could potentially also increase the PT and PTT due to factor depletion following activation.

2.6 Thromboelastography

A sample of celite or kaolin activated whole blood (0.4ml) is placed into a pre warmed cuvette to 36 C. A pin suspended from a torsion wire is then lowered into the cuvette. The cuvette is rotated backward and forwards in a small arc. As the fibrin strands interact with the activated platelets on the surface of the pin, the rotational movement of the cuvette is transmitted to the pin. The stronger the clot the more the pin moves. The Haemoscope is connected to a computer and the coagulation profile is then displayed on the screen as an outline of a thromboelastogram (31, 32, 34). Various parameters are evaluated. There are no normal values for FFP measured with TEG.

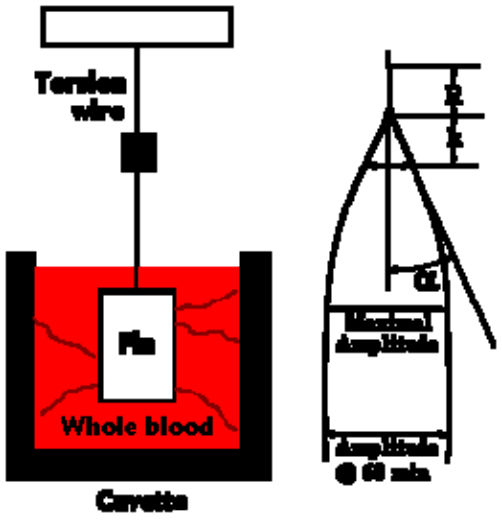


Figure 2.4: The thromboelastogram (32)

2.6.1 r value

This is the time taken from initiation of the test to the initial fibrin formation and pin movement. This value is indicative of factor activation.

Hypothesis of the test: A short R value would suggest factor activation as would be seen in 'Hypercoagulation' in Figure 2.5 and a prolonged R value would suggest factor deficiency.

2.6.2 Alpha Angle and K Value

The Alpha Angle is the angle between the line in the middle of the TEG tracing and the line tangential to the developing "body" of the TEG. The K value is the measure of time from the beginning of clot formation until the amplitude of the TEG reaches 20mm. Both the alpha angle and K value are indicative of factor activation and amplification. Only the Alpha Angle will be analysed in this study.

Hypothesis of the test: A widened Alpha Angle would imply clotting factor activation as would be seen in 'Hypercoagulation' in Figure 2.5 and a narrow angle would suggest factor deficiency.

2.6.3 Maximal Amplitude

The Maximum Amplitude (MA) is the greatest amplitude of the TEG tracing. It is a measure of platelet aggregation.

Hypothesis of the test: The MA is suspected to be larger if the clotting factors are activated even though FFP is platelet poor as would be seen in 'Hypercoagulation' in Figure 2.5. A narrow MA would imply clotting factor deficiency.

2.6.4 MA 60

The MA 60 is the amplitude of the TEG tracing at 60 minutes and is a measure of clot lysis. This value is mentioned for completeness and was not included in the research project because it does not contribute to the objective of determining clotting factor activation.

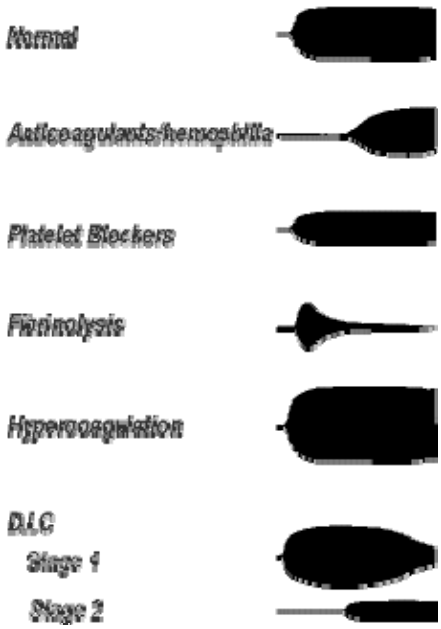


Figure 2.5: Overview of TEG interpretation (35)

2.7 Comparison of TEG versus conventional tests of coagulation

The traditional explanation of the coagulation pathway into intrinsic and extrinsic pathways has been superseded by a theory of enzyme complexes interacting with cells and coagulation factors until fibrin is formed.

Coagulation, or fibrinolysis, within flowing blood is obviously different to what one would find in the lab, using either conventional tests of coagulation or TEG. As discussed above, coagulation involves platelet interaction with clotting factors and a balance between fibrinolysis and fibrin formation. In the measurement of *haemostasis* it is argued that TEG provides a more global picture because it measures the net product of both the coagulation and fibrinolytic systems in that it is a whole blood investigation as apposed to conventional tests of a) coagulation namely PT and PTT, b) fibrinolysis namely D-Dimers and FDPs, and c) platelets, number or function (i.e. bleeding time) (34). As an example, a hypercoagulable state, as in the case of DIC, will show elevated D-Dimers and FDPs but may only show an elevated PT or PTT once the clotting factors become depleted, while the TEG may show an initial hypercoagulable state with increased clot lysis followed by delayed minimal coagulation (see Overview of TEG interpretation)

The TEG therefore provides for a continuous assessment of the developing clot, clot strength and stability as well as clot lysis.

Furthermore, in studies comparing TEG and conventional tests, Essell et al (36) found TEG (71%) to have similar sensitivities to bleeding time (71%) and platelet count (100%) but better specificity, 89% versus 78% and 53% respectively. Similar findings were reported comparing TEG to PT and APTT.

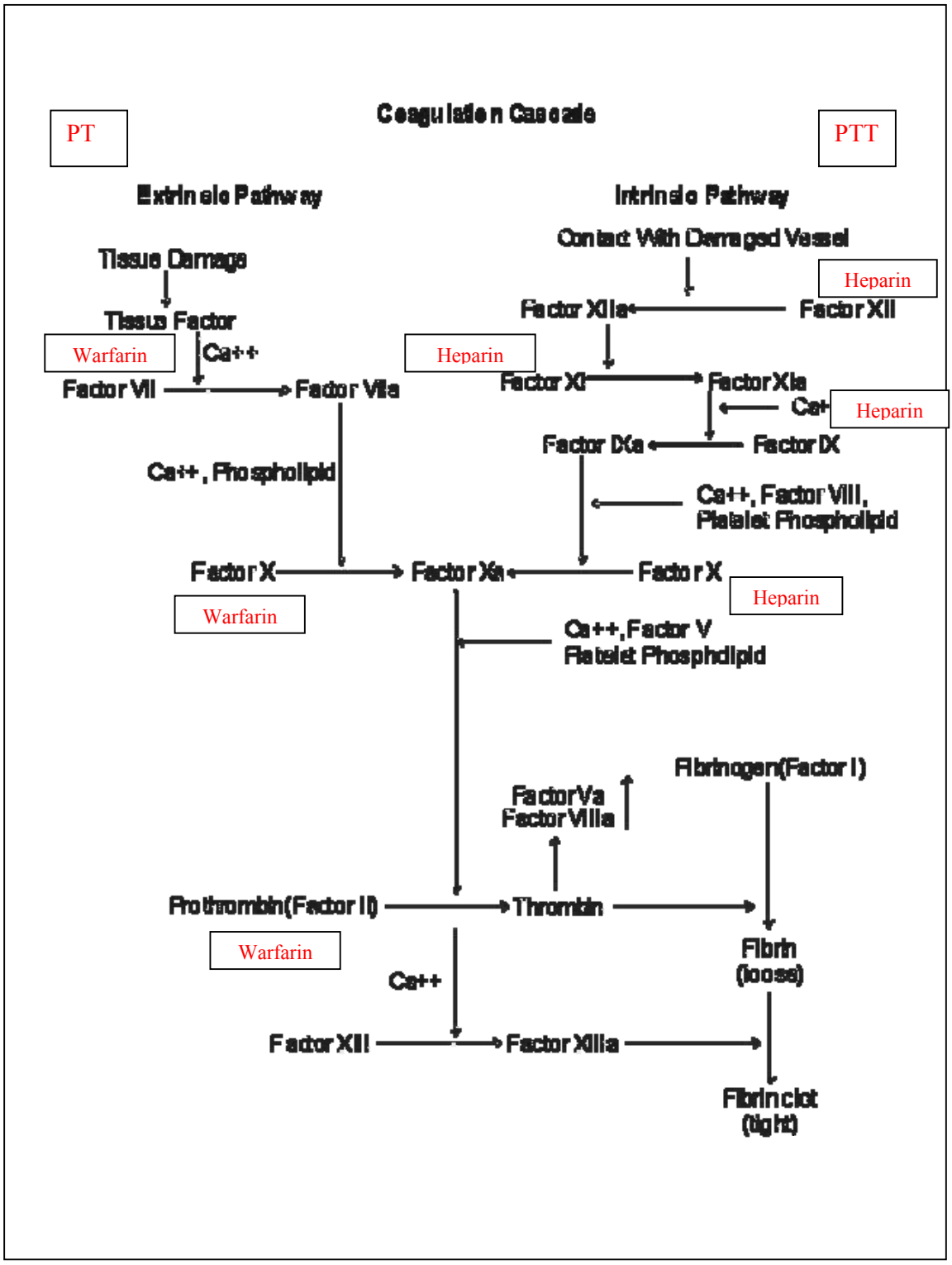


Figure 2.6: Extrinsic and Intrinsic Coagulation Pathways (19)

TEG has also been found to be a useful monitor in guiding transfusion algorithms as well as predicting which patients may bleed, particularly in cardiac and liver transplant surgery (37).

The traditional approach to the coagulation system of Extrinsic and Intrinsic pathways is still useful, however, for in vitro analysis and the understanding of the coagulation pathways. The PT and PTT are particularly useful for monitoring the effects of anticoagulants warfarin and heparin respectively or the ill effects of liver dysfunction and or vitamin K deficiency resulting in a prolonged PT.

2.8 Fresh Frozen Plasma

2.8.1 Preparation of FFP

Within 18 hours of donation, fresh frozen plasma (FFP) is separated from whole blood and frozen to below -18ºC. It contains all the clotting factors at normal physiological levels and is +/- 280 ml in volume.

The current recommendation by the South African Blood Transfusion Services is to thaw FFP at 30ºC - 37ºC and it should be transfused as rapidly as possible, 15 – 20 min per unit (1). The reason for this is the lability of the coagulation factors which deteriorate within a few hours.

2.8.2 Content of FFP

The content of FFP is given in Table 2.1 and Table 2.2 below.

Table 2.1: Average levels of coagulation factors in a unit of FFP (1)

Factor	Average levels per unit of FFP
Fibrinogen	200mg
Factor II	1.03 μ/ml
Factor V	0.64 μ/ml
Factor VII	1.21 μ/ml
Factor VIII	0.85 μ/ml
Factor IX	0.91 μ/ml
Factor X	1.25 μ/ml
Factor XI	0.79 μ/ml
Antithrombin	104%
Plasma pseudocholinesterase	3000 – 10000 iu/l

Table 2.2: Average levels of solutes in FFP (1)

Solute	Average levels per unit of FFP
Glucose	24.8 mmol/l
Potassium	3.0 mmol/l
Sodium	165 mmol/l
Chloride	79 mmol/l
Osmolarity	322 mmol/l
pH	7.9

2.9 Summary

The coagulation system is a complex interaction of enzymes, cells, clotting factors balancing coagulation and fibrinolysis. There are both conventional tests and TEG which can be used to assess the coagulation system with some evidence to suggest that TEG possibly gives a more global picture of the coagulation system.

FFP is collected, prepared and stored under stringent protocol by the SABTS. Consensus is that FFP should be thawed at 30°C and used shortly thereafter. Little evidence exists to corroborate detrimental effects of thawing FFP at higher temperatures.

The hypothesized effects of administering activated FFP includes thrombocytopenia, DIC and possibly induction of an inflammatory response. This effect was not looked at in this study as this study was confined to in vitro testing only.

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Introduction

A detailed explanation of the research methodology is discussed under the headings of FFP preparation, thawing of FFP, laboratory measurements and TEG studies. Standard operating procedures for the SABTS, Haematology laboratory at the Johannesburg Hospital and TEG 5000 series are provided as appendices.

3.2 Hypothesis

There is no positive correlation between clotting factor activation and defrosting at high temperatures.

3.3 Aim

The aim of the study is to determine FFP activation with defrosting at different temperatures.

3.4 Objectives

To determine clotting factor activation at different temperatures (22°C, 37°C, 45°C, 60°C) by using the following tests:

- Fibrinogen
- D-Dimers

- PT
- PTT
- TEG analysis of r value, Alpha Angle and Maximum Amplitude

3.5 Research design

An experimental, quantitative, randomized, controlled study design was used to determine the in vitro effect of thawing FFP on clotting factor function.

3.6 Research method

3.6.1 Preparation of FFP

The Fresh frozen plasma used in this study was prepared by the South African Blood Transfusion Services (SABTS) according to their standard operating procedures.

Donated blood units were centrifuged using either a Hettich or Sorval blood bag centrifuge and plasma was separated from the whole blood using the Optipress system. A quad pack of PL1240 bags were connected to the plasma unit using a Terumo Sterile Connecting Device. The contents of the plasma unit (220-290ml) were equally separated into the attached four bags. The aliquoted units were labeled and detached from each other using a heat sealer and the units were frozen using the blast freezing method. The units were stored below –25ºC. An initial ten adult units of FFP were divided into four satellite units as described above on which

Fibrinogen, D-Dimers, PT and PTT tests were performed. A further ten units were also divided into four satellite units on which TEG tests were performed.

3.6.2 Sampling

3.6.2.1 Calibration of laboratory machinery

The laboratory machinery that was to be used to measure Fibrinogen, D-Dimers, PT, INR and PTT, was calibrated by an experienced haematology registrar using the standard operating procedure of the Haematology Laboratory in the Johannesburg Hospital.

TEG 5000 series machines were used for TEG analyses of thawed FFP. These were calibrated by a representative of the manufacturer using the standard operating procedure for TEG calibration.

3.6.2.2 Thawing of FFP

Each of the satellite units of FFP was thawed in a standard water bath at 22ºC, 37ºC, 45ºC and 60ºC respectively. Thawing times were recorded but are not reported as they are expected to vary depending on the volume of each bag. There was no manual agitation of the bags except the minimum required to determine whether the contents were fully thawed. Bags were removed as soon as they were judged fully thawed. The laboratory test were initiated on bags that were thawed in 22°C followed by thawed FFP at 37°C, 45°C and then 60°C.

All satellite bags within each temperature group were defrosted at the same time in the "conventional" coagulation test arm, and tested simultaneously. Only those satellite bags within each temperature group which were to be tested immediately with TEG were thawed as each TEG could take between 30 and 60 minutes and only 4 TEG runs could be performed at one time. This was done in order to avoid a break in the cold chain or delayed testing once defrosted. Three satellite bags in the 22°C group which were however stored in a conventional freezer overnight, were found partially thawed. The results from these bags were not included in the statistical analysis.

3.6.2.3 Measurement of PT, PTT and D-Dimers and Fibrinogen

PT, PTT, D-Dimers and fibrinogen levels were measured according to the standard operating procedures of the Johannesburg Hospital Haematology Laboratory. PT, PTT and D-Dimers were tested according to standard laboratory operating procedures using the ACL Futura (Ilex Medical Systems). Fibrinogen assays were run on the ACL 7000 (Ilex Medical Systems). If the fibrinogen level of a particular sample was not detectable, that sample was diluted 1 in 2 with standard plasma of known fibrinogen level. If the fibrinogen level of the diluted sample was less than or equal to half of the fibrinogen level in the undiluted standard plasma, the fibrinogen level of the original sample was taken to be zero. This was recorded as 0.1g/l for statistical purposes.

3.6.2.4 Thrombo-Elastogram Studies

Four TEG series 5000 machines were used. One machine belonged to the Anaesthetic Department of the Johannesburg Hospital and the other machines were on loan from Manta-

Draeger. Each machine was calibrated and balanced according to manufactures specifications prior to running samples.



Figure 3.1: TEG series 5000 machine

'Cups and pins' were supplied by Manta-Draeger as were the Kaolin sample bottles. 'Cups and pins' and Kaolin sample bottles were all within their expiry date. TEG 'pins & cups' were placed in the TEG machine, according to the manufacturer's guidelines, prior to testing. 20uml of $CaCl_2$ was added to each cup in every test. Two samples from each unit of thawed FFP were tested. The samples were first placed in kaolin specimen bottles and shaken. A volume of 340uml of thawed FFP were sampled from kaolin specimen bottles within two minutes of FFP being added to the kaolin bottles, and added to the TEG cup and the TEG test started. R values, Alpha angles, Mean Amplitude (MA) were recorded. The $CaCl_2$ and 340uml of FFP were measured using a Pipette, set at these volumes.

3.6.3 Data collection and management

All standard coagulation tests was performed on one day with the help of an experienced haematology registrar (Dr M Isaacs) and experienced laboratory personnel of the Haematology Laboratory of the Johannesburg Hospital.

TEG testing was performed by the investigator with help from Dr Isaacs over a 2 day period.

Data was collected by the investigator in hard copy and compiled on a SPSS data table.

3.6.4 Data evaluation / analysis

Data was analysed using SPSS version 13 and Statistix 8. The sets of data for each test grouped for temperature were compared and analysed. The data was tested for normality using the Kolmogorov-Smirnoff test. Data that was found to be parametric was tested using the Two-Way ANOVA for differences in coagulation tests between each temperature group. Data that was found to be non-parametric was analysed by transforming the data using Van der Waerden's transformation and testing it using the Two-way ANOVA test. Post hoc analysis was performed using the Least Squares Differences. Due to the fact that there are no normal values for FFP using conventional or TEG studies, comparisons were tested between each temperature group and not against a control. Statistical analysis was done in consultation with statistician Professor P.J Becker, Medical Research Council, +27 12 339 8519/23.

3.6.5 Reliability

Laboratory tests were performed using the standard operating procedures at the haematology laboratory at the Johannesburg Hospital. TEG tests were performed on calibrated TEG 5000 machines. In order to prove the reliability of the TEG testing, 2 samples from each satellite bag were tested simultaneously. The results were tested using the paired Students t-Test and Wilcoxon Rank test. No statistically significant differences were found for either the r value ($p = 0.125$), Alpha Angle ($p = 0.932$) or Maximum Amplitude ($p = 0.144$). Further detail is provided in section 4.6.

3.6.6 Validity

The tests chosen to prove the hypothesis were done so in consultation with a haematologist. The results of these tests represent a comprehensive view of the effects of thawing temperature on the different components of the in vitro coagulation system.

3.6.7 Ethical considerations

Approval to conduct the study was received from the Committee for Research on Human Subjects (medical), ref: R14/49 Levy.

3.7 Summary

The study design and laboratory tests selected allow for a reliable assessment of the effects of thawing temperature on FFP clotting factors in order to prove or disprove the hypothesis of

clotting factor activation with increasing thawing temperature. The study design allowed for each unit of FFP to serve as its own control. The laboratory work was performed in keeping with SOPs and results analysed in consultation with a statistician.

CHAPTER FOUR

RESULTS AND STATISTICAL ANALYSIS

4.1 Introduction

This chapter contains the results of the laboratory work followed by the statistical analyses. The statistical analyses pertain to the assessment of clotting factor function at different thawing temperatures.

4.2 Laboratory results

The results of laboratory work are tabulated below.

Table 4.1: Fibrinogen data

FIBRINOGEN (g/l)								
Bags	22ºC	Bag no.	37ºC	Bag no.	45ºC	Bag no.	60ºC	Bag no.
1	3.07	**1**	3.21	**11**	3.21	**21**	0	**31**
2	3.72	**2**	3.99	**12**	3.85	**22**	1.91	**32**
3	4.47	**3**	4.65	**13**	4.53	**23**	2.99	**33**
4	5.52	**4**	6.04	**14**	5.71	**24**	2.9	**34**
5	3.72	**5**	3.94	**15**	3.81	**25**	1.33	**35**
6	2.92	**6**	3.24	**16**	3.07	**26**	0	**36**
7	5.09	**7**	5.43	**17**	5.25	**27**	3.39	**37**
8	5.09	**8**	5.52	**18**	5.34	**28**	0	**38**
9	4.86	**9**	5.01	**19**	5.01	**29**	2.34	**39**
10	2.94	**10**	3.1	**20**	2.97	**30**	2.36	**40**

E = Lab machine error report

Table 4.2: D-Dimer data

D-DIMERS (0.1 = 100ng/ml)								
Bags	22ºC	Bag no.	37ºC	Bag no.	45ºC	Bag no.	60ºC	Bag no.
1	0.124	**1**	0.12	**11**	0.142	**21**	.092	**31**
2	0.197	**2**	0.138	**12**	0.154	**22**	.136	**32**
3	0.113	**3**	0.09	**13**	0.138	**23**	.156	**33**
4	0.161	**4**	0.162	**14**	0.159	**24**	.137	**34**
5	0.14	**5**	0.097	**15**	0.34	**25**	.093	**35**
6	0.068	**6**	0.024	**16**	1	**26**	.109	**36**
7	0.21	**7**	0.203	**17**	1	**27**	.204	**37**
8	0.189	**8**	0.205	**18**	1	**28**	.313	**38**
9	0.241	**9**	0.242	**19**	1	**29**	.296	**39**
10	0.126	**10**	0.551	**20**	1	**30**	.594	**40**

E = Lab machine error report

Table 4.3: PT data

PT (sec)								
Bags	22ºC	Bag no.	37ºC	Bag no.	45ºC	Bag no.	60ºC	Bag no.
1	13.68	**1**	E	**11**	13.7	**21**	120	**31**
2	14.84	**2**	14.55	**12**	12.9	**22**	18.86	**32**
3	15.71	**3**	15.74	**13**	13.8	**23**	16.93	**33**
4	14.27	**4**	13.18	**14**	13.01	**24**	19.08	**34**
5	15.87	**5**	14.78	**15**	14.01	**25**	19.21	**35**
6	16.5	**6**	15.98	**16**	14.54	**26**	27.19	**36**
7	15.6	**7**	13.08	**17**	13.48	**27**	16.03	**37**
8	18.13	**8**	15.04	**18**	15.6	**28**	38.41	**38**
9	15.33	**9**	14.97	**19**	14.9	**29**	16.66	**39**
10	15.58	**10**	15.14	**20**	13.8	**30**	14.97	**40**

E = Lab machine error report

Table 4.4: PTT data

PTT (sec)								
Bags	22ºC	Bag no.	37ºC	Bag no.	45ºC	Bag no.	60ºC	Bag no.
1	28.9	**1**	29.4	**11**	30.4	**21**	120	**31**
2	27.7	**2**	29.1	**12**	28.7	**22**	32	**32**
3	39.6	**3**	41.8	**13**	41.5	**23**	48.4	**33**
4	22.7	**4**	23.3	**14**	23.5	**24**	27.3	**34**
5	33.4	**5**	34.4	**15**	34.9	**25**	43.1	**35**
6	25.1	**6**	25.6	**16**	27.1	**26**	44.2	**36**
7	33.5	**7**	34.2	**17**	34.8	**27**	38.3	**37**
8	31.9	**8**	32.7	**18**	34	**28**	66	**38**
9	36.3	**9**	38	**19**	38	**29**	41.9	**39**
10	37.4	**10**	39.4	**20**	39.4	**30**	42.6	**40**

E = Lab machine error report

Table 4.5: r-value data

r – VALUE (min)								
Bags / Bag no.	22ºC (Ch 1 & 2)		37ºC (Ch 1 & 2)		45ºC (Ch 1 & 2)		60ºC (Ch 1 & 2)	
1 / (**1,11,21,31**)	7	17.3	14.8	28.3	E	7.3	7.8	8
2 / (**2,12,22,32**)	E	E	7.6	7.4	6.8	7.2	28.2	28.2
3 / (**3,13,23,33**)	E	E	3.9	3.9	4.2	4.2	9.1	30
4 / (**4,14,24,34**)	E	E	11.2	13.1	7.9	11.6	8.9	8.1
5 / (**5,15,25,35**)	16.8	26.4	9.8	5.2	7	8.8	6	6.6
6 / (**6,16,26,36**)	4.8	4.1	4.6	4.8	4.3	9.3	5.3	4.1
7 / (**7,17,27,37**)	60.8	6.6	7.5	4.8	5	8.9	6.2	8.9
8 / (**8,18,28,38**)	9.3	9.9	9.2	9.1	8.9	16.4	13.9	3.5
9 / (**9,19,29,39**)	E	10.1	5.1	5.2	7.8	7	30	28.6
10 /(**10,20,30,40**)	10.1	E	6.9	7.9	7.2	E	0.2	7.7

Ch = TEG channel
E = TEG Error

Table 4.6: Alpha Angle data

ALPHA – ANGLE (deg)								
Bags	22ºC (Ch 1 & 2)		37ºC (Ch 1 & 2)		45ºC (Ch 1 & 2)		60ºC (Ch 1 & 2)	
1 / (**1,11,21,31**)	16.7	32.3	24.5	12.3	E	32.6	10.3	8.2
2 / (**2,12,22,32**)	E	E	36.4	62.9	54.3	38	0	0
3 / (**3,13,23,33**)	E	E	72.3	58	61.5	65.8	4.8	0
4 / (**4,14,24,34**)	E	E	60.3	36.3	49.5	58.1	27.7	31
5 / (**5,15,25,35**)	33.8	25.1	63.7	75.4	59.6	59.7	24.8	20.1
6 / (**6,16,26,36**)	38.4	45.5	75.3	74.7	67.6	42.7	38.1	50.9
7 / (**7,17,27,37**)	0	32.4	60.8	53.4	54.4	60	46.5	58.3
8 / (**8,18,28,38**)	53.2	52.4	41.7	51.3	52.5	38.7	32.4	40.7
9 / (**9,19,29,39**)	E	59.8	54	48.5	47.8	47.7	0	1.1
10 /(**10,20,30,40**)	44.4	E	42.4	57.5	36.9	E	77.5	50.5

Ch = TEG channel
E = TEG Error

Table 4.7: MA data

MA (mm)								
Bags	22ºC (Ch 1 & 2)		37ºC (Ch 1 & 2)		45ºC (Ch 1 & 2)		60ºC (Ch 1 & 2)	
1 / (**1,11,21,31**)	7.7	17.1	15.9	15.9	E	18.3	6.6	4.3
2 / (**2,12,22,32**)	E	E	21.6	21.5	22.2	21.5	0	0
3 / (**3,13,23,33**)	E	E	19.4	19.3	19.1	18	2.9	0
4 / (**4,14,24,34**)	E	E	25.3	24.9	23.7	25.3	16.1	13
5 / (**5,15,25,35**)	21	23.4	27.2	26.2	26.6	26.6	15.9	12.5
6 / (**6,16,26,36**)	25.2	28.3	24	24.9	24.2	23.5	21.1	21.2
7 / (**7,17,27,37**)	0	26.2	26.4	52.4	17.9	25.3	23	22.8
8 / (**8,18,28,38**)	22.9	22.2	20.5	22.7	22.3	22.3	21.2	21.1
9 / (**9,19,29,39**)	E	17.7	16.7	17.5	24.1	18.7	0	2.9
10 /(**10,20,30,40**)	27.9	E	24.1	23.3	20.7	E	15	18.9

Ch = TEG channel
E = TEG Error

4.3 Statistical analysis Fibrinogen, D-Dimers, PTT and PT

4.3.1 Descriptive statistics of the variables grouped for temperature

4.3.1.1 Fibrinogen

The means, confidence intervals and standard deviations of the fibrinogen results at the 4 different temperatures are listed in Table 4.8, followed by the Box and Whisker plot (Figure 4.1) of these values. There were no missing values.

The mean fibrinogen level is approximately 4 except in the 60ºC group where it is 1.7220.

4.3.1.2 D-Dimers

The means, confidence intervals and standard deviations of the D-Dimer results at the 4 different temperatures are listed in Table 4.9, followed by the Box and Whisker plot (Figure 4.2) of these values. There were no missing values.

The 45ºC group has a much wider inter-quartile range and a higher mean.

4.3.1.3 PTT

The means, confidence intervals and standard deviations of the PTT results at the 4 different temperatures are listed in Table 4.10, followed by the Box and Whisker plot (Figure 4.3) of these values. There were no missing values.

PTT levels in the 60ºC group were higher than in the other groups.

4.3.1.4 PT

The means, confidence intervals and standard deviations of the PT results at the 4 different temperatures are listed in Table 4.11, followed by the Box and Whisker plot (Figure 4.4) of these values. There was 1 missing value in the 37ºC group.

The PT in the 60ºC group is elevated compared to the other 3 groups.

Table 4.8: Descriptive statistics of Fibrinogen

Descriptives

	Temperature (deg C)			Statistic	Std. Error
Fibrinogen	22	Mean		4.1400	.31203
		95% Confidence Interval for Mean	Lower Bound	3.4341	
			Upper Bound	4.8459	
		Median		4.0950	
		Variance		.974	
		Std. Deviation		.98673	
		Minimum		2.92	
		Maximum		5.52	
	37	Mean		4.4130	.33779
		95% Confidence Interval for Mean	Lower Bound	3.6489	
			Upper Bound	5.1771	
		Median		4.3956	
		Variance		4.3200	
		Std. Deviation		1.141	
		Minimum		1.06819	
		Maximum		3.10	
				6.04	
	45	Mean		4.2750	.32379
		95% Confidence Interval for Mean	Lower Bound	3.5425	
			Upper Bound	5.0075	
		Median		4.2678	
		Variance		4.1900	
		Std. Deviation		1.048	
		Minimum		1.02391	
		Maximum		2.97	
				5.71	
	60	Mean		1.7220	.41702
		95% Confidence Interval for Mean	Lower Bound	.7786	
			Upper Bound	2.6654	
		Median		1.7250	
		Variance		2.1250	
		Std. Deviation		1.739	
		Minimum		1.31874	
		Maximum		.00	
				3.39	

Table 4.9: Descriptive statistics of D-Dimers

Descriptives

	Temperature (deg C)			Statistic	Std. Error
D-Dimers	22	Mean		.1569	.01657
		95% Confidence Interval for Mean	Lower Bound	.1194	
			Upper Bound	.1944	
		5% Trimmed Mean		.1572	
		Median		.1505	
		Variance		.003	
		Std. Deviation		.05239	
		Minimum		.07	
		Maximum		.24	
	37	Mean		.1832	.04565
		95% Confidence Interval for Mean	Lower Bound	.0799	
			Upper Bound	.2865	
		5% Trimmed Mean		.1716	
		Median		.1500	
		Variance		.021	
		Std. Deviation		.14436	
		Minimum		.02	
		Maximum		.55	
	45	Mean		.5933	.13678
		95% Confidence Interval for Mean	Lower Bound	.2839	
			Upper Bound	.9027	
		5% Trimmed Mean		.5960	
		Median		.6700	
		Variance		.187	
		Std. Deviation		.43253	
		Minimum		.14	
		Maximum		1.00	
	60	Mean		.2130	.04910
		95% Confidence Interval for Mean	Lower Bound	.1019	
			Upper Bound	.3241	
		5% Trimmed Mean		.1986	
		Median		.1465	
		Variance		.024	
		Std. Deviation		.15526	
		Minimum		.09	
		Maximum		.59	

Table 4.10: Descriptive statistics of PTT

Descriptives

	Temperature (deg C)			Statistic	Std. Error
PTT	22	Mean		31.6500	1.73399
		95% Confidence Interval for Mean	Lower Bound	27.7274	
			Upper Bound	35.5726	
		5% Trimmed Mean		31.7056	
		Median		32.6500	
		Variance		30.067	
		Std. Deviation		5.48336	
		Minimum		22.70	
		Maximum		39.60	
	37	Mean		32.7900	1.89581
		95% Confidence Interval for Mean	Lower Bound	28.5014	
			Upper Bound	37.0786	
		5% Trimmed Mean		32.8167	
		Median		33.4500	
		Variance		35.941	
		Std. Deviation		5.99508	
		Minimum		23.30	
		Maximum		41.80	
	45	Mean		33.2300	1.81304
		95% Confidence Interval for Mean	Lower Bound	29.1286	
			Upper Bound	37.3314	
		5% Trimmed Mean		33.3111	
		Median		34.4000	
		Variance		32.871	
		Std. Deviation		5.73334	
		Minimum		23.50	
		Maximum		41.50	
	60	Mean		50.3800	8.38975
		95% Confidence Interval for Mean	Lower Bound	31.4011	
			Upper Bound	69.3589	
		5% Trimmed Mean		47.7944	
		Median		42.8500	
		Variance		703.880	
		Std. Deviation		26.53073	
		Minimum		27.30	
		Maximum		120.00	

Table 4.11: Descriptive statistics of PT

Descriptives

	Temperature (deg C)			Statistic	Std. Error
PT	22	Mean		15.5510	.38555
		95% Confidence Interval for Mean	Lower Bound	14.6788	
			Upper Bound	16.4232	
		5% Trimmed Mean		15.5117	
		Median		15.5900	
		Variance		1.486	
		Std. Deviation		1.21920	
		Minimum		13.68	
		Maximum		18.13	
	37	Mean		14.7178	.33464
		95% Confidence Interval for Mean	Lower Bound	13.9461	
			Upper Bound	15.4895	
		5% Trimmed Mean		14.7386	
		Median		14.9700	
		Variance		1.008	
		Std. Deviation		1.00393	
		Minimum		13.08	
		Maximum		15.98	
	45	Mean		13.9740	.26427
		95% Confidence Interval for Mean	Lower Bound	13.3762	
			Upper Bound	14.5718	
		5% Trimmed Mean		13.9433	
		Median		13.8000	
		Variance		.698	
		Std. Deviation		.83569	
		Minimum		12.90	
		Maximum		15.60	
	60	Mean		30.7340	10.16686
		95% Confidence Interval for Mean	Lower Bound	7.7350	
			Upper Bound	53.7330	
		5% Trimmed Mean		26.6506	
		Median		18.9700	
		Variance		1033.650	
		Std. Deviation		32.15043	
		Minimum		14.97	
		Maximum		120.00	

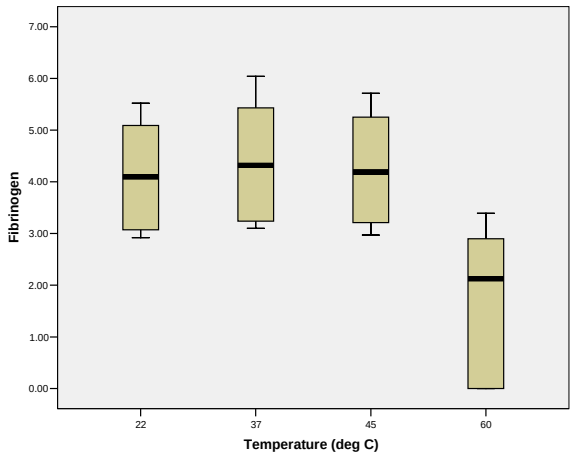


Figure 4.1: Box and Whisker plot of Fibrinogen

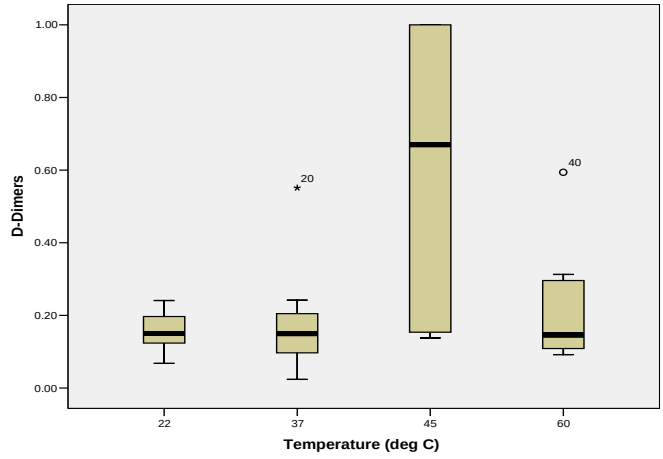


Figure 4.2: Box and Whisker plot of D-Dimers

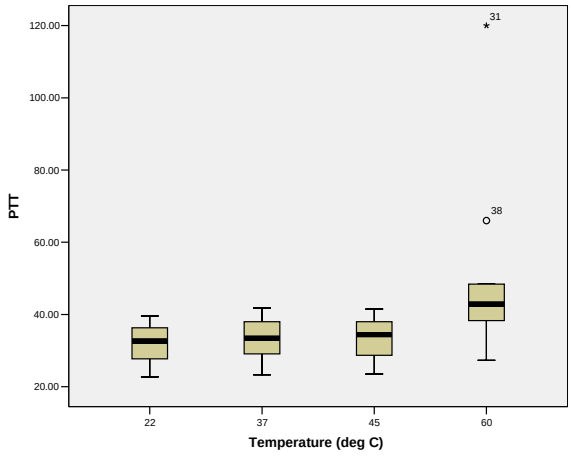


Figure 4.3: Box and Whisker plot of PTT

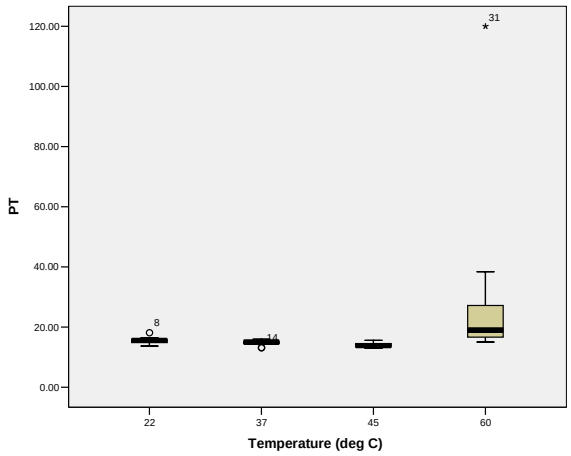


Figure 4.4: Box and Whisker plot of PT

It must be noted that 'Bag number 31' (which is the Satellite bag from Bag 1 assigned to the 60°C temperature group) comes up as an outlier in the PTT and PT analyses and also has the lowest Fibrinogen and D-Dimer levels when compared to the other bags. The reasons for this may be a discrepancy in the cold chain, collection process, or may simply be a cause of thawing at 60°C. It is unlikely that there was an intrinsic abnormality of the FFP itself as the sister satellite bags did not produce outliers in the other 3 temperature groups.

In order to ascertain whether any significant results would be a true difference or as a result of the outlier, statistics for Fibrinogen, D-Dimer, PTT and PT excluding Bag 31, were also conducted.

Table 4.12: Comparison of means and standard deviations in 60°C, bag 31 included and excluded

	Bag 31 Included		Bag 31 Excluded	
	Mean	Std Deviation	Mean	Std Deviation
Fibrinogen(g/l)	1.7220	1.3187	1.9133	1.2429
D-Dimers (100ng/ml)	.2130	.1553	.2264	.1584
PT (sec)	30.7340	32.1504	20.8156	7.4921
PTT (sec)	50.3800	26.5307	42.6444	10.8940

4.3.1.5 Fibrinogen excluding bag 31

The mean of the fibrinogen results with Bag 31 excluded (1.7220) is lower than the mean (1.9133) where bag 31 is not excluded.

4.3.1.6 D-Dimers excluding bag 31

The mean D-Dimer result with Bag 31 excluded is 0.2130.

4.3.1.7 PTT excluding bag 31

The mean PTT with Bag 31 excluded is 52.38s compared with 42.64s with Bag 31 not excluded.

4.3.1.8 PT excluding bag 31

The mean PT with Bag 31 excluded is 30.73s versus 20.81s, Bag 31 not excluded.

The fibrinogen and D-Dimers means were higher in the Bag 31 excluded calculations. Both the mean PT and PTT were lower in the Bag 31 excluded compared with Bag 31 included calculations. These differences were not tested for statistical significance, but rather an analysis of the differences between temperature groups (22°C, 37°C, 45°C, and 60°C) for both Bag 31 included and Bag 31 excluded was performed and follows.

4.3.2 Test for normality of data in each variable, including and excluding Bag 31

Each variable was tested for normality using the Kolmogorov-Smirnov Test (K-S). The following was found. P-P plots and K-S tables are shown in Appendix A.

The K-S statistic is significant for D-Dimers (P = 0.001), PTT (P = 0.014) and PT (P<0.000) in the Bag 31 included tests, implying that these variables do not follow a normal distribution. This is seen in the P-P Plots which show a non linear distribution of results. Fibrinogen however, follows a normal distribution. The K-S test where Bag 31 is excluded also shows D-Dimers (P = 0.001) and PT (P = 0.006) to be significant and hence does not follow a normal distribution, however PTT is now shown to follow a normal distribution (P=.917).

4.3.3 Statistical testing for differences in each variable grouped for temperature (Bag 31 included)

Based on the K-S test results, only the Fibrinogen variable could be tested using parametric tests. Fibrinogen was tested using a Two-way ANOVA, grouped for temperature and bag number, followed by a paired post hoc analysis using the Least Squares Difference (LSD).

The D-Dimers, PTT and PT variables were tested using the Van der Waerden's transformation of the data and tested with a Two-way ANOVA, grouped for temperature and bag number, followed by a paired post hoc analysis using the Least Squares Difference (LSD).

Tables of the statistics which follow can be viewed in Appendix A.

4.3.3.1 Fibrinogen

The $F = 42.32$ of Fibrinogen has a significance of $P < 0.000$ which implies a statistically significant difference in Fibrinogen results between the 4 temperature groups. Post Hoc analyses (equal variances assumed and equal variances not assumed) show statistically significant differences between the 60°C group and the other 3 groups ($P < 0.05$). The 60°C fibrinogen levels were significantly lower than the other in the other 3 groups. There was no significant difference between the 22°C, 37°C and 45°C groups.

4.3.3.2 D-Dimers

The Van der Waerden's transformation of D-Dimers tested with a Two-way ANOVA and post hoc analysis shows a significant difference ($P < 0.003$) between temperature groups with the significance coming from the difference between the 45°C group and the 22°C, 37°C and 60°C groups ($P < 0.05$), the 45°C D-Dimers being significantly higher. There were no significant differences between the 22°C, 37°C and 60°C groups themselves.

4.3.3.3 PTT

The Van der Waerden's transformation of PTT tested with a Two-way ANOVA and post hoc analysis shows a significant difference ($P < 0.000$) between temperature groups with the significance coming from longer PTT in the 60°C group compared with the 22°C, 37°C and 45°C groups ($P < 0.05$).

There were no significant differences between the 22°C, 37°C and 45°C groups.

4.3.3.4 PT

The Van der Waerden's transformation of PT tested with a Two-way ANOVA and post hoc analysis shows a significant difference ($P < 0.000$) between temperature groups. The PT in the 45°C group was significantly lower than in the 22°C, 37°C and 60°C groups ($P < 0.05$) while the PT in the 60°C was significantly longer than in the other 3 groups ($P < 0.05$).

4.3.4 Statistical testing for differences in each variable grouped for temperature (Bag 31 excluded)

To follow is the analysis of Fibrinogen, D-Dimers, PTT and PT where the outlier, Bag 31, is excluded from the analyses. This will tell us whether statistics generated above are in part due to the outlier as apposed to the thawing process.

Tables of statistics can be found in Appendix A.

4.3.4.1 Fibrinogen and PTT

Because Fibrinogen and PTT were found to follow a normal distribution, a Two-way ANOVA, grouped for temperature and bag number, followed by a paired post hoc analysis using the Least Squares Difference (LSD) was performed.

As in the case where 'Bag 31' was included, a statistically significant difference between groups is seen in both the Fibrinogen ($P = 0.000$) and PTT ($P = 0.002$) variables. This difference stems from a statistically significant difference between the 60°C and the 22°C, 37°C and 45°C groups in both the Fibrinogen and PTT variables ($P < 0.05$). There was no significant difference between the 22°C, 37°C and 45°C groups in the Fibrinogen and PTT variables as was the case above (i.e. Bag 31 included).

4.3.4.2 D-Dimers and PT

The D-Dimers and PT variables were tested using the Van der Waerden's transformation of the data and tested with a Two-way ANOVA, grouped for temperature and bag number, followed by a paired post hoc analysis using the Least Squares Difference (LSD).

Tables of the statistics to follow can be viewed in Appendix A.

As in the case where Bag 31 was included, significant differences occurred in both the D-Dimers ($P < 0.006$) and PT ($P < 0.000$) variables. The D-Dimers showed a significant difference between the higher values of the 45°C and those in the 22°C, 37°C and 60°C groups.

In the case of the PT variables significant differences were observed between all 4 temperature groups ($P < 0.05$), where the values in the 60°C group were higher than those in the 22°C which were higher than those in the 37°C which were higher than those in the 45°C group.

We can conclude from the results of 4.3.3 and 4.3.4 that the statistically significant differences found between groups was not due to the outlier (Bag 31) but rather due to the temperature in which FFP was thawed.

4.4 Analysis of r-values, Alpha Angles and Maximum Amplitude

Statistics were done on each set of TEG results i.e. r-value 1 & r-value 2, Alpha angle 1 & Alpha angle 2, and MA 1 & MA 2 as well as on the calculated average of each value from each satellite bag i.e. R-value 1 & R-value 2, Alpha angle 1 & Alpha angle 2, and MA 1 & MA 2.

Tables of all the statistical results pertaining to the TEG data analysis can be found in Appendix A.

4.4.1 Descriptive statistics of the variables grouped for temperature

4.4.1.1 R-value

The means in both R1 and R2 were higher in the 22ºC (18.13/12.4) and 60ºC (11.5/13.7) groups. The distribution, which seems to be similar in both the R1 and R2 groups, can be gauged from the Box and Whisker Plots in Figure 4.5 & 4.6

4.4.1.2 Alpha Angle

The means in both the Alpha Angle 1 and 2 were lower in the 60ºC group (26.2 / 26.08). The mean in the 22ºC Alpha Angle 1 group (31.08) was also lower than the 37ºC (53.14) and 45ºC (53.78) group, but this wasn't seen in the 22ºC group of Alpha Angle 2. See Figure 4.7 & 4.8

4.4.1.3 Maximum Amplitude

It is noticed once again that the means (12 / 11.6) in the 60ºC MA1 and MA 2 groups are lower than the means of the 22ºC (17.4 / 22.48), 37ºC (22.11 / 24.8) and 45ºC (22.3 / 22.1) groups. See Figure 4.9 & 4.10

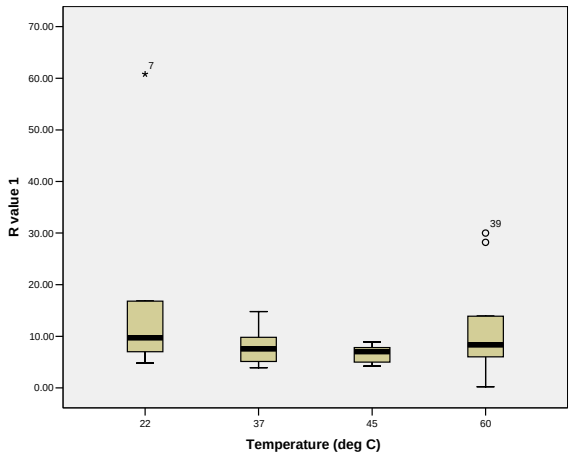


Figure 4.5: Box and Whisker Plot of r-value 1

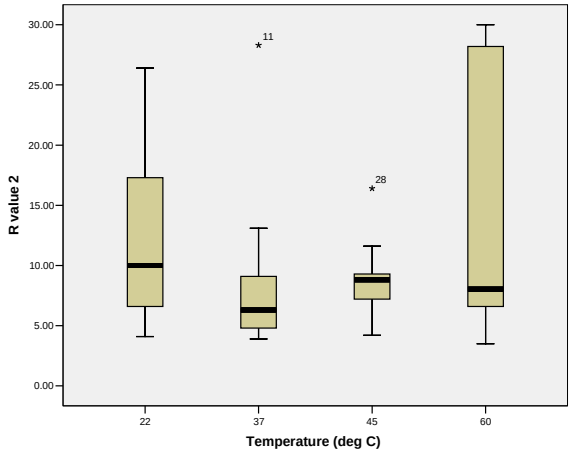


Figure 4.6: Box and Whisker Plot of r-value 2

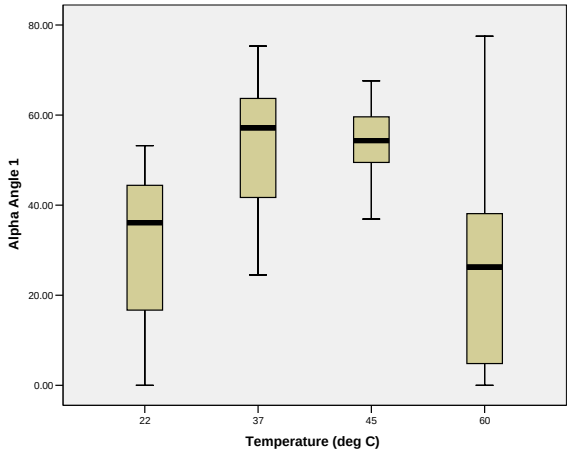


Figure 4.7: Box and Whisker Plot of Alpha Angle 1

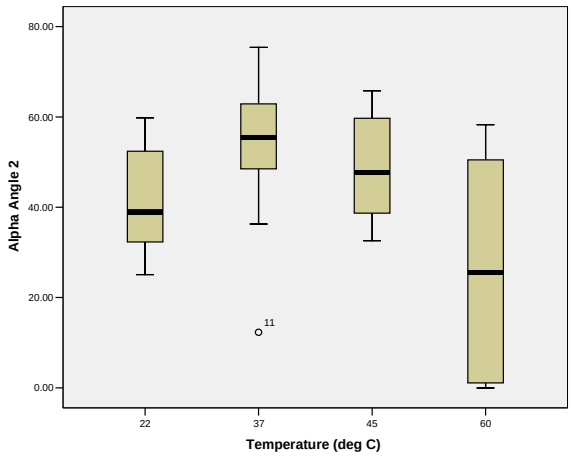


Figure 4.8: Box and Whisker Plot of Alpha Angle 2

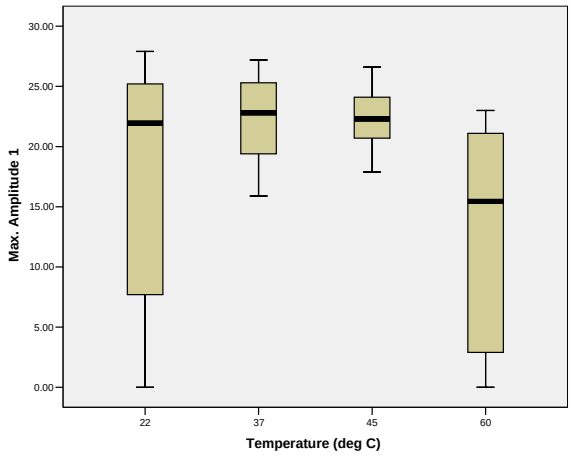


Figure 4.9: Box and Whisker Plot of MA 1

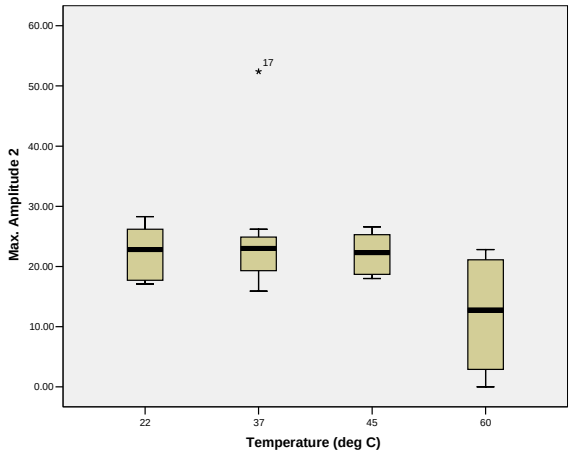


Figure 4.10: Box and Whisker Plot of MA 2

4.4.2 Test for normality of data in each variable

The Kolmogorov-Smirnov test for normality was used. The data for Alpha Angle 1 (P = 0.946) and 2 (P = 0.716)), MA (1 (P = 0.133) and 2 (P = 0.207)) follow a normal distribution whereas the data for R-values (1 and 2) (P = 0.002 / P = 0.009) do not follow a normal distribution.

In view of this a Two-way ANOVA, grouped for temperature and bag number, followed by a paired post hoc analysis using the Least Squares Difference (LSD), was used for Alpha Angle and MA, while a Two-way analysis on the Van der Waerden's transformation of r values was used to analyse the r values.

Tables of the statistics to follow can be viewed in Appendix A.

4.5 Statistical testing for differences in each variable grouped for temperature

4.5.1 R-value 1 and R-value 2 analysis

The p values for R1 (P = 0.1904) and R2 (P = 0.6423) suggest no statistically significant differences in the means of R-values within the different temperature groups.

4.5.2 Alpha Angle 1 and Alpha Angle 2 analysis

A statistically significant difference was found within the Alpha Angle 1 (P = 0.004) and Alpha Angle 2 (P = 0.009) groups.

4.5.3 MA 1 and MA 2 analysis

A statistically significant difference was found between the temperatures in both the MA 1 (P = 0.0056) and MA 2 (P = 0.0002).

4.6 Statistical analysis comparing R1 and R2, AA1 and AA2 and MA1 and MA2

Before continuing to determine which temperature groups were resulting in the statistical difference, I feel it is important to exclude a significant difference between R1 and R2, AA1 and AA2 and MA1 and MA2. A significant difference would suggest operator error or unreliable TEG equipment. If no significant difference is found then an average of each of these groups, labeled R-value 1 & 2, Alpha angle 1 & 2 and MA 1& 2, will be analysed.

Formula used for R-value 1 & 2, Alpha angle 1 & 2 and MA 1& 2

$$\text{R-value}_{ij}\ 1\ \&\ 2 = (\text{R-value}_{ij}1 + \text{R-value}_{ij}2)/2$$

$$\text{Alpha angle}_{ij}\ 1\ \&\ 2 = (\text{Alpha angle}_{ij}1 + \text{Alpha angle}_{ij}2)/2$$

$$\text{MA}_{ij}\ 1\&\ 2 = (\text{MA}_{ij}1 + \text{MA}_{ij}2)/2$$

The Wilcoxon Rank test for non-parametric data comparing pairs was used to compare the r values and a paired t-Test was used to asses the Alpha Angle and MA variables.

No significant difference was found between R1 and R2 (P = 0.125), AA1 and AA2 (P = 0.932) and MA1 and MA2 (P = 0.114).

4.6.1 Descriptive statistics for R-value 1 & 2, Alpha Angle 1 & 2 and MA 1 & 2, grouped for temperature

In view there being no significant difference between R-value 1 and R-value 2, Alpha Angle 1 and Alpha Angle 2, and MA 1 and MA 2, we now analyse the averages of these variables as explained above.

4.6.1.1 R-value 1 & 2

The mean values were higher in the 22ºC and 60ºC groups, with the 22ºC mean (16.3) being higher than the 60ºC mean (12.46). See Figure 4.11

4.6.1.2 Alpha Angle 1 & 2

The mean angle in 60ºC and 22ºC group were lower than in the 37ºC and 45ºC groups. Again the 22ºC group (32.98) being lower than the 60ºC group (26.14). See Figure 4.12

4.6.1.3 Maximum Amplitude 1 & 2

The mean MA 1 & 2 in the 60ºC group (11.9) was lower than in the other 3 groups. See Figure 4.13

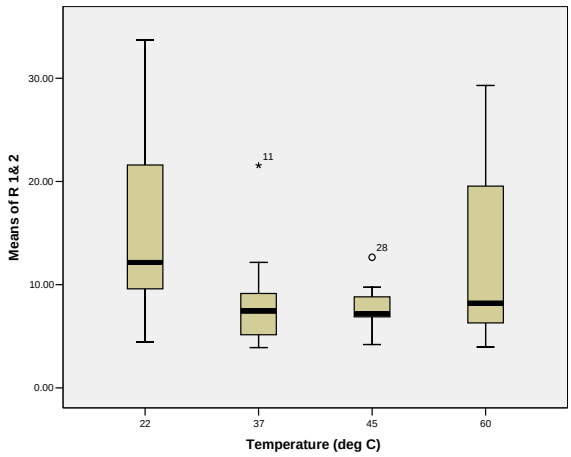


Figure 4.11: Box and Whisker Plot of r-value 1 & 2

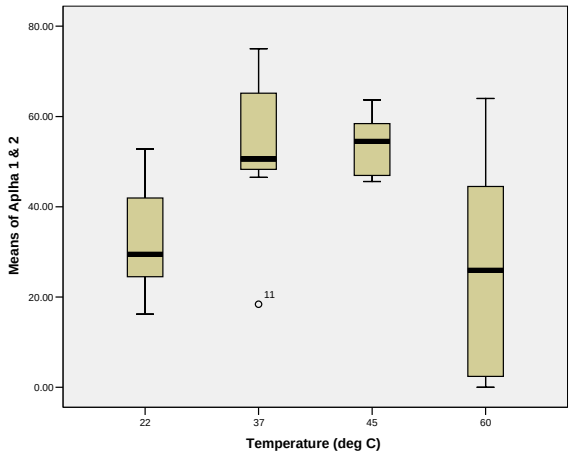


Figure 4.12: Box and Whisker Plot of Alpha Angle 1 & 2

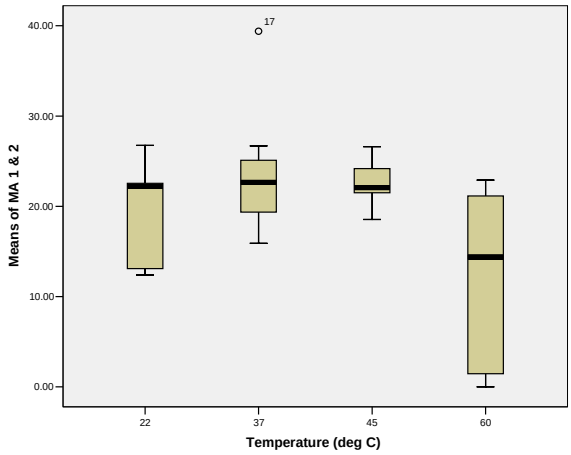


Figure 4.13: Box and Whisker Plot of MA 1 & 2

4.6.2 Test for normality of data in each variable

The Kolmogorov-Smirnov test for normality was used. The data for Alpha Angle 1 & 2, MA 1 & 2 follow a normal distribution with P = 0.209 and P = 0.131 respectively, whereas the data for R-values 1 & 2 do not follow a normal distribution (P = 0.013). In view of this a Two-way ANOVA, grouped for temperature and bag number, followed by a paired post hoc analysis using the Least Squares Difference (LSD), was used for Alpha Angle 1 & 2 and MA 1 & 2, while a Two-way analysis on the Van der Waerden's transformation of r values was used to analyse the r value 1 & 2.

Tables of the statistics to follow can be viewed in Appendix A.

4.6.3 Statistical testing for differences in each variable grouped for temperature

4.6.3.1 R-value1 & 2 analysis

The p values for R1 & 2 (P = 0.303) suggest no statistically significant differences in the means of the averaged R-values at different temperatures.

4.6.3.2 Alpha Angle 1 & 2 analysis

Using the Two-way ANOVA with Post Hoc analysis, a statistically significant difference was found in Alpha angle 1 & 2 (P = 0.002). The differences are seen in the 37°C and 45°C groups when compared with the lower values in the 22°C and 60°C groups (P < 0.05). The

values in the 37°C and 45°C groups are not significantly different from each other. The values in the 22°C and 60°C groups are likewise not significantly different from each other.

4.6.3.3 MA 1 & 2 analysis

Once again, using the Two-way ANOVA with Post Hoc analysis, a significant difference between the temperature groups in MA1 & 2 ($P = 0.000$) was found. Post Hoc analysis revealed a statistically significant difference between the 37°C group when compared with the lower values in the 22°C and 60°C groups ($P < 0.05$). There was also a significant difference between the higher values in the 45°C group when compared with the 60°C group ($P < 0.05$). There was no significant difference between the 45°C and 22°C as well as between the 22°C and 60°C groups.

4.7 Conclusion

Chapter 4 gives a comprehensive analysis of the data obtained in this research project through a stepwise approach to the statistical tests used and the reasons for the use thereof.

In summary, significant differences were seen between the 60°C group and the other temperature groups for fibrinogen (lower values in the 60°C group), PT and PTT (longer values in the 60°C group). Significant differences were also noted in the 60°C group when compared with the 37°C and 45°C groups in the Alpha Angle and MA data. This was also true for 22°C when compared with the 37°C and 45°C Alpha Angle and MA data, lower values in the 22°C and 60°C group. Significant differences were also between the 45°C group and the 22°C, 37°C and 60°C groups in the D Dimer and PT analyses, with the D Dimers in

the 45°C group being significantly higher and the PT being significantly shorter. Bag 31 was not found to be a cause of the findings in this study.

Chapter 5 follows with a detailed discussion of the findings in this chapter.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Introduction

This Chapter contains a detailed discussion around the statistical results of the laboratory work and the conclusion regarding the hypothesis of thawing FFP at high temperature causing clotting factor activation. Following on this is a discussion on TEG versus conventional laboratory testing as well as the implications the study has on daily practice and future research.

5.2 Discussion of statistical results

Statistically significant differences were found in variables fibrinogen, D-Dimers, PTT, PT, Alpha Angle and Maximum Amplitude in the 60ºC temperature group compared with these variables in the 22ºC, 37ºC and 45ºC temperature groups.

The mean fibrinogen level (1.72g/l) was significantly lower in the 60ºC group ($P < 0.05$). PT (30.73sec) and PTT (50.38sec) was significantly prolonged in this group ($P < 0.05$), while the Alpha Angle and MA were significantly shallower and narrower respectively when compared with the 37ºC and 45ºC groups ($P < 0.05$), but not the 22ºC group. The r value in the 60ºC group was prolonged when compared with the 37 ºC and 45ºC groups but not significantly so ($P > 0.3$).

The findings of low fibrinogen levels suggest clotting factor depletion. This is further corroborated by prolongation of the PT and PTT. A prolonged r value (albeit not significant), shallow Alpha Angle and narrow MA further suggest factor depletion with little factor activity. The most striking finding in this study is the significant difference seen in clotting factor activity, or rather inactivity, and factor depletion in FFP thawed at 60ºC. The most likely explanation for this would be protein denaturation when FFP is thawed at 60ºC.

There is, however, evidence to suggest factor activation at 45ºC. Significant differences were found in D-Dimers in the 45ºC group when compared with the other 3 temperature groups ($P < 0.05$). The mean D-Dimers in the 45ºC group was 0.59*10^2ng/l which is extraordinarily high. As an example, a D-Dimers of greater than 0.5*10^2ng/l is indicative of a deep vein thrombosis in clinical practice.

D-Dimers are produced through fibrinolysis. Fibrin is formed through the activation of thrombin which in turn is usually activated as the last step in the coagulation pathway. Hence elevated D-Dimers imply, at least, thrombin activation and fibrin formation.

Of further interest are the PT results. A significant difference between the PT of the 45ºC and those of the 22ºC and 37ºC groups was found ($P < 0.05$). The mean PT of the 45ºC (13.974 sec) group was shorter than the in the 22ºC (15.55 sec) and 37ºC (14.718 sec) groups. The faster time to coagulation further suggest clotting factor activation at this temperature.

D-Dimers in the 60ºC group (mean = 0.213*10^2ng/l) were non-significantly elevated when compared with 22ºC (0.157*10^2ng/l) and 37ºC (0.183*10^2ng/l). This may suggest a higher level of thrombin formation and hence fibrin production during the thawing process, again

suggesting the possibility of factor activation in FFP thawed at high temperatures. Statistically, however, this may also be due to random variation.

This study has confirmed the findings of previous research of clotting factor depletion in FFP thawed at 60ºC temperatures (4,5,6). This study is the only study to show clotting factor activation at a modest thawing temperature of 45ºC and possibly even at 60ºC. The study done by Plotz and Ciotola did not find any differences in clotting factor assay in FFP thawed at 37ºC and 45ºC (4). They concluded that thawing FFP at 45ºC was quicker and safe. The parameters they measured were PT, PTT, fibrinogen and Factor VIII assay, and as in our study, no significant differences were detected between the 37ºC and 45ºC groups at the 5% level of significance, in contrast to our study where there was statistically significant difference in PT in FFP thawed at 45ºC. Small sample size and the fact the laboratory work in the study by Plotz was performed on samples that were kept on ice after thawing, as opposed to this study where samples were tested immediately after thawing, may account for these differences in findings.

The TEG results showed significant differences in the 37ºC and 45ºC groups when compared with the 60ºC group in Alpha Angle and MA ($P < 0.05$), and in the 22ºC group when compared with the 37ºC and 45ºC groups for Alpha Angle and 37ºC group for MA ($P < 0.05$). No significant differences were found in r values. The 22ºC variables closely match the 60ºC variables with longer r values, narrower Alpha Angles and MA. This suggests that factor inactivity occurs in FFP thawed at 22ºC, bearing in mind however, that the only significant differences found were between this group and 37ºC and 45ºC groups for Alpha Angle and 37ºC for MA. Supporting this are the prolonged PT and PTT results found in this group which were not statistically significantly different from the 37ºC and 45ºC groups except when Bag

31 was excluded in the analyses. When Bag 31 was excluded, a significant difference was seen in the PT between the 4 temperature groups, with the PT in the 45ºC group < 37ºC group < 22ºC group < 60ºC group ($P < 0.05$).

Clotting factors and enzymes needed for cleavage, activation and clot formation are proteins which normally function at 37ºC. Therefore, at 22ºC the hypothermia may partially or completely inhibit the clotting cascade. This would explain the findings of delayed clotting. An argument against this theory would be the fact that TEG samples are kept at 37ºC during the test itself negating the effect of 22ºC hypothermia on the FFP.

There were no comparative studies testing FFP thawed at this temperature.

In summary, the findings of this study prove the alternate hypothesis that high thawing temperature is associated with clotting factor activation. This was evident at a temperature of 45ºC. FFP thawed at 60ºC was associated with delayed clot formation possibly on the basis of denatured proteins and may also have had factor activation as evidenced by a non significant elevation in D-Dimers at 60ºC. FFP thawed at 22ºC is dysfunctional and may need to be warmed to 'body temperature' to become more functional. The implications of these findings follow.

5.3 TEG versus conventional laboratory tests

TEG is a dynamic global measure of clotting factor interactions and hence coagulation, rather than a test of specific parts of the coagulation pathway, as are the conventional tests of coagulation.

The fibrinogen, PT and PTT provided clear evidence of clotting factor inactivation in FFP thawed at 60ºC, explaining and complimenting the significant findings of prolonged Alpha Angle and MA in this group. This was also suggested by a non-significant, longer r value in the 60ºC group. The D-Dimers and PT provided evidence of clotting factor activation in FFP thawed at 45ºC and possibly at 60ºC too, which was not detected, with TEG. The TEG, however, alludes to factor inactivation at 22ºC which would not have been evident using the conventional tests alone.

The TEG therefore, provides a more global dynamic overview of the coagulation process while the conventional tests provided a more specific analysis of parts of the coagulation pathway. Both TEG and conventional tests of coagulation were found to be relevant and complementary in this study.

5.4 Implications for daily practice

As elaborated on in Chapter 1, section 1.2, the current practice in South Africa, as regards thawing FFP is not standardised. Although the SABTS current recommendation is to thaw FFP in a water bath at 37ºC, this is often not followed and many techniques of thawing FFP are used in this country including: the use of non sterile water environment with unmeasured water temperatures ranging from cold to hot and microwave ovens either conventional or specifically designed.

FFP thawed at higher temperatures leads to clotting factor inactivation most likely on the basis of protein denaturation. This study also found evidence suggesting clotting factor activation. These findings are again cause for concern not only because of the potential of a

SIRS response and DIC from activated clotting factors but also because denatured proteins themselves could induce an inflammatory response, cause arteriolar obstruction with tissue ischaemia, of particular concern in kidney and lung parenchyma with the concomitant organ dysfunction and failure. This is not withstanding the obvious loss of clotting factor function in FFP thawed at these temperatures, defeating the purpose for which it was needed in the first place.

FFP thawed at 22ºC was shown to be significantly non-functional or inactivated. FFP thawed at this temperature may become functional at body temperature but this was not evident in TEG samples, which as already mentioned, are preheated to 37ºC. Thawing at 22ºC poses two problems of a) inactive FFP which may or may not become functional once warmed up and b) administering cold fluid to a patient who may be at risk for hypothermia already, as is the case in major surgery or trauma. Hypothermia per se is associated with increased bleeding from impaired coagulation further placing these patients at risk of coagulopathy and DIC.

Because this study was performed with meticulous attention to SOP for the various laboratory tests used and the validity of these tests to identify activation and function of the coagulation pathways, this study gives reliable credence to the its findings and hence we enable valuable and informed suggestions to be made with regards to the daily practicalities of FFP use.

Based on these findings it is recommended that FFP be thawed at temperatures of no greater than 37ºC to avoid activation and possible denaturation at higher temperatures, and the associated potential for complications. Furthermore FFP should not be thawed at temperatures that are too cold because of the risk of inactivated clotting factors as well as induced hypothermia in the patient. The optimal temperature for thawing is 37ºC.

Where FFP is needed urgently, facilities should be available to thaw the FFP in an appropriate and safe microwave oven. In South Africa, cost constraints is an important factor in service delivery yet clinicians are plagued and inundated by an inordinate amount of trauma often urgently requiring blood products. In view of this each blood bank which services a hospital which treats patients requiring emergency surgery, and possibly FFP urgently, should be in possession of a microwave oven specifically designed to thaw FFP, and issue the requested FFP in a thawed state.

FFP that is not required urgently could still be thawed in a waterbath at 37ºC but protected by a sterile packet or thawed in a sterile water bath to avoid the potential risk of bacterial contamination. Based on the findings in this study, the SABTS now supply, on request, FFP which has been thawed in a sterile waterbath at 37ºC.

FFP should be stored in appropriate refrigerators capable of reaching -25ºC. Conventional refrigerators run at temperatures +/- -8ºC and this may not be adequate for storage of FFP which has been issued but not used, for example, emergency stock which is kept in the operating theater complex for emergency situations.

5.5 Implications for further research

To date no study, that we are aware of, has used TEG analysis on FFP. This study therefore proposes the 37ºC TEG results to be used as normal values for future research. The reference ranges proposed are derived from the 95% confidence interval for each variable, r value,

Alpha Angle and Maximum Amplitude, of the FFP thawed at 37°C, and is tabulated in Table 5.1

Table 5.1: Proposed normal TEG values for FFP

	Mean	**Normal reference range**
R value	8.5 minutes	4.82 – 12.21 minutes
Alpha Angle	53.09 °	48.08 – 59.15 °
Maximum Amplitude	23.49 mm	20.57 – 24.60 mm

5.6 Conclusion and summary

This chapter reviewed and discussed the statistical analysis of the data collected. Statistical analysis of the FFP thawed at different temperatures suggests that FFP thawed at high temperatures activates and or denatures clotting factors. FFP thawed at 22ºC is relatively non-functional and may have other adverse side effects when administered. Therefore it is recommended that FFP be thawed at 37ºC.

The study therefore proves the hypothesis that FPP thawed at high thawing temperatures activates the factors therein.

Because the data collected was rigorous, reliable and valid, the findings and hence implications, for the thawing of FFP in South Africa are very important and relevant.

TEG was founded to be a useful technique in the qualitative analysis of FFP. Further to the hypothesis of the study I propose that the TEG values of FFP thawed at 37ºC in this study, be used as normal reference ranges for FFP undergoing TEG analysis in the future.

APPENDIX A

TABLES AND GRAPHS OF STATISTICAL TESTS

1. Tables of statistical results of data obtained from 'conventional' coagulation testing

Table A.1: Kolmogorov-Smirnov test for Fibrinogen, D-Dimers, PTT and PT (including bag 31)

		Fibrinogen	D-Dimers	PTT	PT
N		40	40	40	39
Normal Parameters(a,b)	Mean	3.6375	.2866	37.0125	18.8474
	Std. Deviation	1.54765	.29452	15.70617	17.20093
Most Extreme Differences	Absolute	.142	.310	.249	.415
	Positive	.066	.310	.249	.415
	Negative	-.142	-.204	-.181	-.365
Kolmogorov-Smirnov Z		.897	1.962	1.576	2.590
Asymp. Sig. (2-tailed)		.397	.001	.014	.000

a Test distribution is Normal.
b Calculated from data.

Table A.2: Kolmogorov-Smirnov test for Fibrinogen, D-Dimers, PTT and PT (excluding bag 31)

One-Sample Kolmogorov-Smirnov Test

		Fibrinogen	D-Dimers	PTT	PT
N		39	39	39	38
Normal Parameters[a,b]	Mean	3.7308	.2916	34.8846	16.1855
	Std. Deviation	1.44953	.29665	8.20326	4.47828
Most Extreme Differences	Absolute	.129	.310	.089	.277
	Positive	.068	.310	.089	.277
	Negative	-.129	-.200	-.069	-.232
Kolmogorov-Smirnov Z		.808	1.936	.556	1.708
Asymp. Sig. (2-tailed)		.531	.001	.917	.006

a. Test distribution is Normal.

b. Calculated from data.

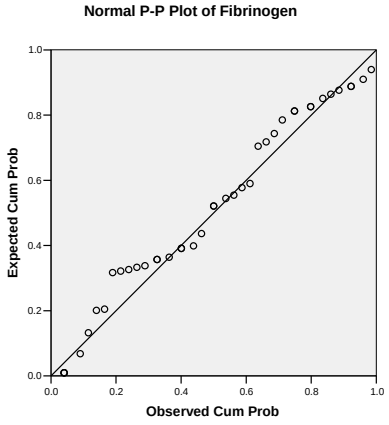


Figure A1: P-P Plot of Fibrinogen (including bag 31)

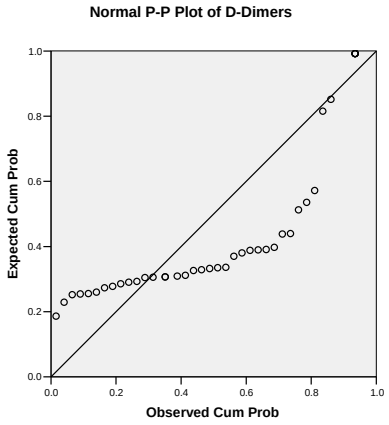


Figure A2: P-P Plot of D-Dimers (including bag 31)

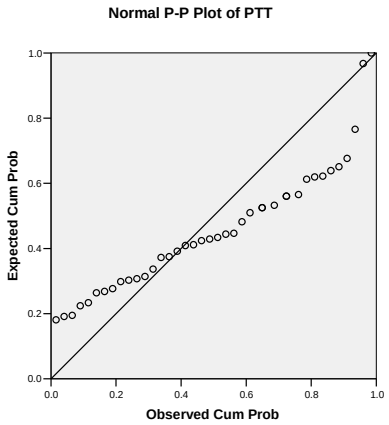


Figure A3: P-P Plot of PTT (including bag 31)

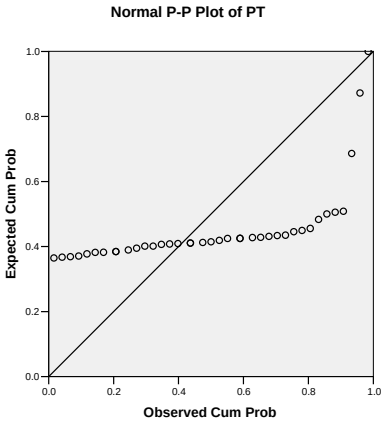


Figure A4: P-P Plot of PT (including bag 31)

Table A.3: ANOVA test of Fibrinogen

Source	DF	SS	MS	F	P
BAG	9	33.6352	3.7372	9.62	0.0000
TEMPERAT	3	49.2945	16.4315	42.32	0.0000
Error	27	10.4838	0.3883		
Total	39	93.4135			

Grand Mean 3.6375 CV 17.13

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	0.0149	0.01486	0.04	0.8492
Remainder	26	10.4690	0.40265		

Table A.4: Post-Hoc analysis of Fibrinogen

LSD All-Pairwise Comparisons Test of FIBRINOGEN for TEMPERATURE

TEMPERATURE	Mean	Homogeneous Groups
37	4.4130	A
45	4.2750	A
22	4.1400	A
60	1.7220	B

Alpha 0.05 Standard Error for Comparison 0.2787
Critical T Value 2.052 Critical Value for Comparison 0.5718

Table A.5: Analysis of variance of the Van der Waerden transformation of D-Dimers

Source	DF	SS	MS	F	P
BAG	9	2764.63	307.181	5.43	0.0003
TEMPERAT	3	996.35	332.117	5.87	0.0032
Error	27	1528.03	56.594		
Total	39	5289.00			

Grand Mean 20.500 CV 36.70

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	20.62	20.6226	0.36	0.5561
Remainder	26	1507.40	57.9770		

Table A.6: Post-Hoc analysis of D-Dimers

LSD All-Pairwise Comparisons Test of D-Dimers for TEMPERATURE

TEMPERAT	Mean	Homogeneous Groups
45	29.050	A
60	18.850	B
22	17.100	B
37	17.000	B

Alpha	0.05	Standard Error for Comparison	3.3643
Critical T Value	2.052	Critical Value for Comparison	6.9030

Table A.7: Analysis of variance of the Van der Waerden transformation of PTT

Source	DF	SS	MS	F	P
BAG	9	3110.50	345.611	12.90	0.0000
TEMPERAT	3	1495.00	498.333	18.60	0.0000
Error	27	723.50	26.796		
Total	39	5329.00			

Grand Mean 20.500 CV 25.25

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	12.324	12.3242	0.45	0.5080
Remainder	26	711.176	27.3529		

Table A.8: Post-Hoc analysis of PTT

LSD All-Pairwise Comparisons Test of PTT for TEMPERATURE

TEMPERATURE	Mean	Homogeneous Groups
60	30.900	A
45	18.300	B
37	17.600	B
22	15.200	B

Alpha	0.05	Standard Error for Comparison	2.3150
Critical T Value	2.052	Critical Value for Comparison	4.7500

Table A.9: Analysis of variance of the Van der Waerden transformation of PT

Source	DF	SS	MS	F	P
BAG	9	962.63	106.959	2.69	0.0232
TEMPERAT	3	2970.00	990.000	24.94	0.0000
Error	26	1032.17	39.699		
Total	38				

Note: SS are marginal (type III) sums of squares

Grand Mean 19.794 CV 31.83

Table A.10: Post-Hoc analysis of PT

LSD All-Pairwise Comparisons Test of PT for TEMPERATURE

TEMPERATURE	Mean	Homogeneous Groups
60	32.850	A
22	21.350	B
37	15.628	B
45	9.350	C

Alpha	0.05	Standard Error for Comparison	VARIES
Critical T Value	2.056	Critical Value for Comparison	VARIES

Table A.11: ANOVA test of Fibrinogen, Bag 31 excluded

Source	DF	SS	MS	F	P
BAG	9	30.6498	3.4055	8.70	0.0000
TEMPERAT	3	42.0989	14.0330	35.86	0.0000
Error	26	10.1744	0.3913		
Total	38				

Note: SS are marginal (type III) sums of squares

Grand Mean 3.6544 CV 17.12

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	0.17943	0.17943	0.45	0.5090
Remainder	25	9.99499	0.39980		

Table A.12: Post-Hoc analysis of Fibrinogen, Bag 31 excluded

LSD All-Pairwise Comparisons Test of FIBRINOGEN for TEMPERATURE

TEMPERATURE	Mean	Homogeneous Groups
37	4.4130	A
45	4.2750	A
22	4.1400	A
60	1.7897	B

Alpha	0.05	Standard Error for Comparison	VARIES
Critical T Value	2.056	Critical Value for Comparison	VARIES

Table A.13: Analysis of variance of the Van der Waerden transformation of D-Dimers, Bag 31 excluded

Source	DF	SS	MS	F	P
BAG	9	2540.16	282.240	4.87	0.0007
TEMPERAT	3	978.39	326.129	5.62	0.0041
Error	26	1507.47	57.980		
Total	38				

Note: SS are marginal (type III) sums of squares

Grand Mean 20.638 CV 36.90

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	19.52	19.5198	0.33	0.5720
Remainder	25	1487.95	59.5180		

Table A.14: Post-Hoc analysis of D-Dimers, Bag 31 excluded

LSD All-Pairwise Comparisons Test of D-Dimers for TEMPERATURE

TEMPERATURE	Mean	Homogeneous Groups
45	29.050	A
60	19.402	B
22	17.100	B
37	17.000	B

Alpha	0.05	Standard Error for Comparison	VARIES
Critical T Value	2.056	Critical Value for Comparison	VARIES

Table A.15: ANOVA test of PTT, Bag 31 excluded

Source	DF	SS	MS	F	P
BAG	9	1243.61	138.179	6.03	0.0001
TEMPERAT	3	655.70	218.567	9.54	0.0002
Error	26	595.73	22.913		
Total	38				

Note: SS are marginal (type III) sums of squares

Grand Mean 34.996 CV 13.68

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	45.294	45.2936	2.06	0.1639
Remainder	25	550.436	22.0174		

Table A.16: Post-Hoc analysis of PTT, Bag 31 excluded

LSD All-Pairwise Comparisons Test of PTT for TEMPERATURE

TEMPERATURE	Mean	Homogeneous Groups
60	42.312	A
45	33.230	B
37	32.790	B
22	31.650	B

Alpha 0.05 Standard Error for Comparison VARIES
Critical T Value 2.056 Critical Value for Comparison VARIES

Table A.17: Analysis of variance of the Van der Waerden transformation of PT, Bag 31 excluded

Source	DF	SS	MS	F	P
BAG	9	1087.27	120.808	3.49	0.0064
TEMPERAT	3	2432.50	810.833	23.42	0.0000
Error	25	865.50	34.620		
Total	37				

Note: SS are marginal (type III) sums of squares

Grand Mean 19.239 CV 30.58

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	14.087	14.0871	0.40	0.5345
Remainder	24	851.413	35.4755		

Table A.18: Post-Hoc analysis of PT, Bag 31 excluded

LSD All-Pairwise Comparisons Test of PT for TEMPERATURE

TEMPERATURE	Mean	Homogeneous Groups
60	31.183	A
22	21.350	B
37	15.072	C
45	9.350	D

Alpha	0.05	Standard Error for Comparison	VARIES
Critical T Value	2.060	Critical Value for Comparison	VARIES

2. Tables of statistical results for data obtained from TEG testing

Table A.19: Table of descriptive statistics of r value 1

Descriptives

	Temperature (deg C)			Statistic	Std. Error
R value 1	22	Mean		18.1333	8.69198
		95% Confidence Interval for Mean	Lower Bound	-4.2101	
			Upper Bound	40.4768	
		5% Trimmed Mean		16.5037	
		Median		9.7000	
		Variance		453.303	
		Std. Deviation		21.29091	
		Minimum		4.80	
		Maximum		60.80	
	37	Mean		8.0600	1.05158
		95% Confidence Interval for Mean	Lower Bound	5.6812	
			Upper Bound	10.4388	
		5% Trimmed Mean		7.9167	
		Median		7.5500	
		Variance		11.058	
		Std. Deviation		3.32539	
		Minimum		3.90	
		Maximum		14.80	
	45	Mean		6.5667	.56001
		95% Confidence Interval for Mean	Lower Bound	5.2753	
			Upper Bound	7.8581	
		5% Trimmed Mean		6.5685	
		Median		7.0000	
		Variance		2.823	
		Std. Deviation		1.68003	
		Minimum		4.20	
		Maximum		8.90	
	60	Mean		11.5600	3.12258
		95% Confidence Interval for Mean	Lower Bound	4.4962	
			Upper Bound	18.6238	
		5% Trimmed Mean		11.1667	
		Median		8.3500	
		Variance		97.505	
		Std. Deviation		9.87446	
		Minimum		.20	
		Maximum		30.00	

Table A.20: Table of descriptive statistics of r value 2

Descriptives

	Temperature (deg C)			Statistic	Std. Error
R value 2	22	Mean		12.4000	3.33706
		95% Confidence Interval for Mean	Lower Bound	3.8218	
			Upper Bound	20.9782	
		5% Trimmed Mean		12.0833	
		Median		10.0000	
		Variance		66.816	
		Std. Deviation		8.17411	
		Minimum		4.10	
		Maximum		26.40	
	37	Mean		8.9700	2.31718
		95% Confidence Interval for Mean	Lower Bound	3.7282	
			Upper Bound	14.2118	
		5% Trimmed Mean		8.1778	
		Median		6.3000	
		Variance		53.693	
		Std. Deviation		7.32758	
		Minimum		3.90	
		Maximum		28.30	
	45	Mean		8.9667	1.14758
		95% Confidence Interval for Mean	Lower Bound	6.3203	
			Upper Bound	11.6130	
		5% Trimmed Mean		8.8185	
		Median		8.8000	
		Variance		11.853	
		Std. Deviation		3.44275	
		Minimum		4.20	
		Maximum		16.40	
	60	Mean		13.3700	3.44216
		95% Confidence Interval for Mean	Lower Bound	5.5833	
			Upper Bound	21.1567	
		5% Trimmed Mean		12.9944	
		Median		8.0500	
		Variance		118.485	
		Std. Deviation		10.88506	
		Minimum		3.50	
		Maximum		30.00	

Table A.21: Table of descriptive statistics of Alpha Angle 1

Descriptives

	Temperature (deg C)			Statistic	Std. Error
Alpha Angle 1	22	Mean		31.0833	7.95916
		95% Confidence Interval for Mean	Lower Bound	10.6237	
			Upper Bound	51.5430	
		5% Trimmed Mean		31.5815	
		Median		36.1000	
		Variance		380.090	
		Std. Deviation		19.49589	
		Minimum		.00	
		Maximum		53.20	
	37	Mean		53.1400	5.19301
		95% Confidence Interval for Mean	Lower Bound	41.3926	
			Upper Bound	64.8874	
		5% Trimmed Mean		53.5000	
		Median		57.1500	
		Variance		269.674	
		Std. Deviation		16.42175	
		Minimum		24.50	
		Maximum		75.30	
	45	Mean		53.7889	2.94809
		95% Confidence Interval for Mean	Lower Bound	46.9906	
			Upper Bound	60.5872	
		5% Trimmed Mean		53.9599	
		Median		54.3000	
		Variance		78.221	
		Std. Deviation		8.84427	
		Minimum		36.90	
		Maximum		67.60	
	60	Mean		26.2100	7.68237
		95% Confidence Interval for Mean	Lower Bound	8.8313	
			Upper Bound	43.5887	
		5% Trimmed Mean		24.8167	
		Median		26.2500	
		Variance		590.188	
		Std. Deviation		24.29378	
		Minimum		.00	
		Maximum		77.50	

Table A.22: Table of descriptive statistics of Alpha Angle 2

Descriptives

	Temperature (deg C)			Statistic	Std. Error
Alpha Angle 2	22	Mean		41.2500	5.49465
		95% Confidence Interval for Mean	Lower Bound	27.1256	
			Upper Bound	55.3744	
		5% Trimmed Mean		41.1167	
		Median		38.9500	
		Variance		181.147	
		Std. Deviation		13.45909	
		Minimum		25.10	
		Maximum		59.80	
	37	Mean		53.0300	5.84483
		95% Confidence Interval for Mean	Lower Bound	39.8081	
			Upper Bound	66.2519	
		5% Trimmed Mean		54.0500	
		Median		55.4500	
		Variance		341.620	
		Std. Deviation		18.48297	
		Minimum		12.30	
		Maximum		75.40	
	45	Mean		49.2556	3.97454
		95% Confidence Interval for Mean	Lower Bound	40.0902	
			Upper Bound	58.4209	
		5% Trimmed Mean		49.2617	
		Median		47.7000	
		Variance		142.173	
		Std. Deviation		11.92362	
		Minimum		32.60	
		Maximum		65.80	
	60	Mean		26.0800	7.32442
		95% Confidence Interval for Mean	Lower Bound	9.5110	
			Upper Bound	42.6490	
		5% Trimmed Mean		25.7389	
		Median		25.5500	
		Variance		536.471	
		Std. Deviation		23.16184	
		Minimum		.00	
		Maximum		58.30	

Table A.23: Table of descriptive statistics of MA 1

Descriptives

	Temperature (deg C)			Statistic	Std. Error
Max. Amplitude 1	22	Mean		17.4500	4.51344
		95% Confidence Interval for Mean	Lower Bound	5.8478	
			Upper Bound	29.0522	
		5% Trimmed Mean		17.8389	
		Median		21.9500	
		Variance		122.227	
		Std. Deviation		11.05563	
		Minimum		.00	
		Maximum		27.90	
	37	Mean		22.1100	1.24744
		95% Confidence Interval for Mean	Lower Bound	19.2881	
			Upper Bound	24.9319	
		5% Trimmed Mean		22.1722	
		Median		22.8000	
		Variance		15.561	
		Std. Deviation		3.94474	
		Minimum		15.90	
		Maximum		27.20	
	45	Mean		22.3111	.90882
		95% Confidence Interval for Mean	Lower Bound	20.2154	
			Upper Bound	24.4069	
		5% Trimmed Mean		22.3179	
		Median		22.3000	
		Variance		7.434	
		Std. Deviation		2.72646	
		Minimum		17.90	
		Maximum		26.60	
	60	Mean		12.1800	2.84628
		95% Confidence Interval for Mean	Lower Bound	5.7413	
			Upper Bound	18.6187	
		5% Trimmed Mean		12.2556	
		Median		15.4500	
		Variance		81.013	
		Std. Deviation		9.00072	
		Minimum		.00	
		Maximum		23.00	

Table A.24: Table of descriptive statistics of MA 2

Descriptives

	Temperature (deg C)			Statistic	Std. Error
Max. Amplitude 2	22	Mean		22.4833	1.82965
		95% Confidence Interval for Mean	Lower Bound	17.7801	
			Upper Bound	27.1866	
		5% Trimmed Mean		22.4593	
		Median		22.8000	
		Variance		20.086	
		Std. Deviation		4.48170	
		Minimum		17.10	
		Maximum		28.30	
	37	Mean		24.8600	3.23866
		95% Confidence Interval for Mean	Lower Bound	17.5336	
			Upper Bound	32.1864	
		5% Trimmed Mean		23.8278	
		Median		23.0000	
		Variance		104.889	
		Std. Deviation		10.24155	
		Minimum		15.90	
		Maximum		52.40	
	45	Mean		22.1667	1.09202
		95% Confidence Interval for Mean	Lower Bound	19.6485	
			Upper Bound	24.6849	
		5% Trimmed Mean		22.1519	
		Median		22.3000	
		Variance		10.733	
		Std. Deviation		3.27605	
		Minimum		18.00	
		Maximum		26.60	
	60	Mean		11.6700	2.90968
		95% Confidence Interval for Mean	Lower Bound	5.0878	
			Upper Bound	18.2522	
		5% Trimmed Mean		11.7000	
		Median		12.7500	
		Variance		84.662	
		Std. Deviation		9.20121	
		Minimum		.00	
		Maximum		22.80	

Table A.25: Kolmogorov-Smirnov test for r value 1, r value 2, Alpha Angle 1, Alpha Angle 2, MA 1 and MA 2

One-Sample Kolmogorov-Smirnov Test

		R value 1	R value 2	Alpha Angle 1	Alpha Angle 2	Max. Amplitude 1	Max. Amplitude 2
N		35	35	35	35	35	35
Normal Parameters[a,b]	Mean	10.4029	10.8143	41.8314	42.3400	18.5257	19.9914
	Std. Deviation	10.60723	7.90317	21.66866	20.41491	8.05647	9.23801
Most Extreme Differences	Absolute	.311	.279	.089	.118	.197	.180
	Positive	.311	.279	.071	.079	.122	.180
	Negative	-.241	-.177	-.089	-.118	-.197	-.177
Kolmogorov-Smirnov Z		1.842	1.650	.525	.697	1.164	1.065
Asymp. Sig. (2-tailed)		.002	.009	.946	.716	.133	.207

a. Test distribution is Normal.

b. Calculated from data.

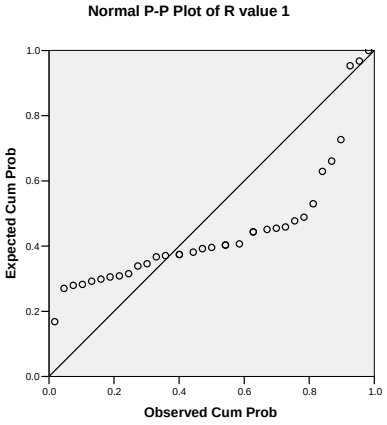


Figure A5: P-P Plot of r value 1

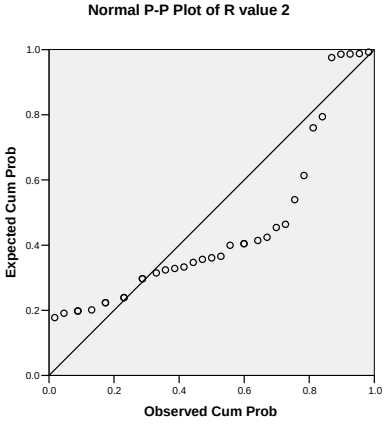


Figure A6: P-P Plot of r value 2

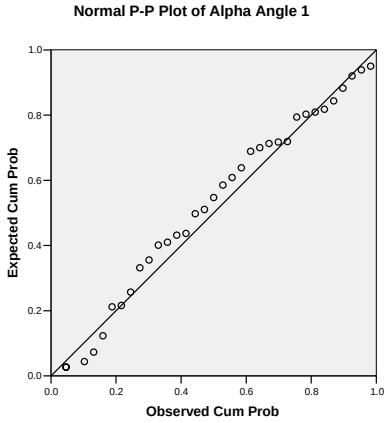


Figure A7: P-P Plot of Alpha Angle 1

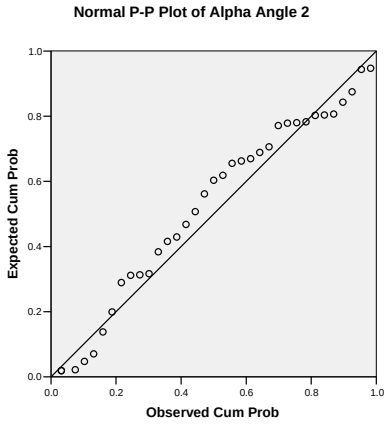


Figure A8: P-P Plot of Alpha Angle 2

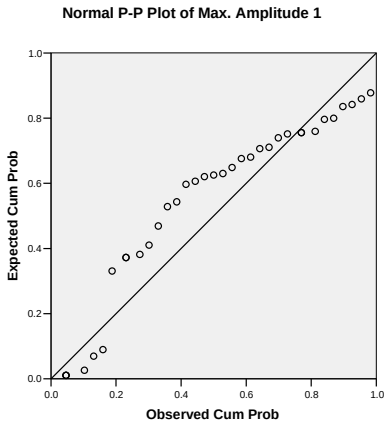


Figure A9: P-P Plot of MA 1

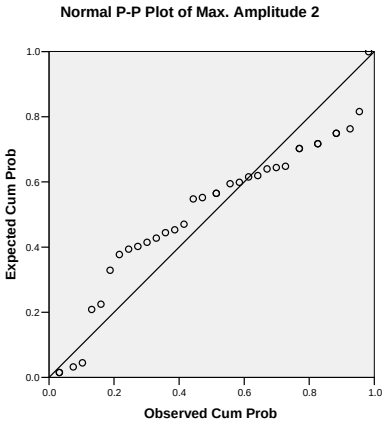


Figure A10: P-P Plot of MA 2

Table A.26: Analysis of variance of the Van der Waerden transformation of r value 1

Source	DF	SS	MS	F	P
BAG	9	1359.01	151.001	1.76	0.1366
TEMPERAT	3	446.19	148.731	1.74	0.1904
Error	21	1799.64	85.697		
Total	33				

Note: SS are marginal (type III) sums of squares

Grand Mean 18.856 CV 49.09

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	0.10	0.0983	0.00	0.9740
Remainder	20	1799.54	89.9771		

Table A.27: Analysis of variance of the Van der Waerden transformation of r value 2

Source	DF	SS	MS	F	P
BAG	9	778.24	86.472	0.69	0.7075
TEMPERAT	3	212.46	70.821	0.57	0.6423
Error	21	2619.24	124.726		
Total	33				

Note: SS are marginal (type III) sums of squares

Grand Mean 18.715 CV 59.68

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	10.51	10.509	0.08	0.7794
Remainder	20	2608.74	130.437		

Table A.28: ANOVA test of Alpha Angle 1

Source	DF	SS	MS	F	P
BAG	9	3398.81	377.65	1.21	0.3380
TEMPERAT	3	5512.14	1837.38	5.89	0.0041
Error	22	6866.16	312.10		
Total	34				

Note: SS are marginal (type III) sums of squares

Grand Mean 39.765 CV 44.43

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	343.44	343.441	1.11	0.3050
Remainder	21	6522.72	310.606		

Table A.29: ANOVA test of Alpha Angle 2

Source	DF	SS	MS	F	P
BAG	9	3366.20	374.02	1.25	0.3165
TEMPERAT	3	4414.27	1471.42	4.92	0.0092
Error	22	6579.73	299.08		
Total	34				

Note: SS are marginal (type III) sums of squares

Grand Mean 42.854 CV 40.36

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	325.12	325.120	1.09	0.3080
Remainder	21	6254.61	297.839		

Table A.30: ANOVA test of MA 1

Source	DF	SS	MS	F	P
BAG	9	681.118	75.680	1.94	0.0989
TEMPERAT	3	645.653	215.218	5.51	0.0056
Error	22	858.651	39.030		
Total	34				

Note: SS are marginal (type III) sums of squares

Grand Mean 17.880 CV 34.94

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	194.093	194.093	6.13	0.0219
Remainder	21	664.559	31.646		

Table A.31: ANOVA test of MA 2

Source	DF	SS	MS	F	P
BAG	9	1195.13	132.792	4.19	0.0029
TEMPERAT	3	985.66	328.555	10.37	0.0002
Error	22	697.13	31.688		
Total	34				

Note: SS are marginal (type III) sums of squares

Grand Mean 20.026 CV 28.11

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	27.957	27.9569	0.88	0.3596
Remainder	21	669.169	31.8652		

Table A.32: Wilcoxon Rank Test comparing r value 1 and r value 2, AA 1 and AA 2, MA 1 and MA 2

Ranks

		N	Mean Rank	Sum of Ranks
R value 2 - R value 1	Negative Ranks	11[a]	14.36	158.00
	Positive Ranks	19[b]	16.16	307.00
	Ties	3[c]		
	Total	33		
Alpha Angle 2 - Alpha Angle 1	Negative Ranks	16[d]	16.16	258.50
	Positive Ranks	16[e]	16.84	269.50
	Ties	1[f]		
	Total	33		
Max. Amplitude 2 - Max. Amplitude 1	Negative Ranks	16[g]	11.63	186.00
	Positive Ranks	13[h]	19.15	249.00
	Ties	4[i]		
	Total	33		

a. R value 2 < R value 1

b. R value 2 > R value 1

c. R value 2 = R value 1

d. Alpha Angle 2 < Alpha Angle 1

e. Alpha Angle 2 > Alpha Angle 1

f. Alpha Angle 2 = Alpha Angle 1

g. Max. Amplitude 2 < Max. Amplitude 1

h. Max. Amplitude 2 > Max. Amplitude 1

i. Max. Amplitude 2 = Max. Amplitude 1

Test Statistics[b]

	R value 2 - R value 1	Alpha Angle 2 - Alpha Angle 1	Max. Amplitude 2 - Max. Amplitude 1
Z	-1.533[a]	-.103[a]	-.681[a]
Asymp. Sig. (2-tailed)	.125	.918	.496

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

Table A.33: Table of descriptive statistics of r value 1 & 2

Descriptives

	Temperature (deg C)			Statistic	Std. Error
Means of R 1& 2	22	Mean		16.3000	5.16423
		95% Confidence Interval for Mean	Lower Bound	1.9618	
			Upper Bound	30.6382	
		5% Trimmed Mean		15.9917	
		Median		12.1500	
		Variance		133.346	
		Std. Deviation		11.54756	
		Minimum		4.45	
		Maximum		33.70	
	37	Mean		8.5150	1.63163
		95% Confidence Interval for Mean	Lower Bound	4.8240	
			Upper Bound	12.2060	
		5% Trimmed Mean		8.0472	
		Median		7.4500	
		Variance		26.622	
		Std. Deviation		5.15968	
		Minimum		3.90	
		Maximum		21.55	
	45	Mean		7.8313	.87433
		95% Confidence Interval for Mean	Lower Bound	5.7638	
			Upper Bound	9.8987	
		5% Trimmed Mean		7.7653	
		Median		7.2000	
		Variance		6.116	
		Std. Deviation		2.47299	
		Minimum		4.20	
		Maximum		12.65	
	60	Mean		12.4650	3.03114
		95% Confidence Interval for Mean	Lower Bound	5.6081	
			Upper Bound	19.3219	
		5% Trimmed Mean		12.0028	
		Median		8.2000	
		Variance		91.878	
		Std. Deviation		9.58532	
		Minimum		3.95	
		Maximum		29.30	

Table A.34: Table of descriptive statistics of Alpha Angle 1 & 2

Descriptives

	Temperature (deg C)			Statistic	Std. Error
Means of Aplha 1 & 2	22	Mean		32.9800	6.47778
		95% Confidence Interval for Mean	Lower Bound	14.9948	
			Upper Bound	50.9652	
		5% Trimmed Mean		32.8111	
		Median		29.4500	
		Variance		209.808	
		Std. Deviation		14.48476	
		Minimum		16.20	
		Maximum		52.80	
	37	Mean		53.0850	4.95238
		95% Confidence Interval for Mean	Lower Bound	41.8819	
			Upper Bound	64.2881	
		5% Trimmed Mean		53.7944	
		Median		50.6000	
		Variance		245.261	
		Std. Deviation		15.66082	
		Minimum		18.40	
		Maximum		75.00	
	45	Mean		53.6188	2.34037
		95% Confidence Interval for Mean	Lower Bound	48.0847	
			Upper Bound	59.1528	
		5% Trimmed Mean		53.5069	
		Median		54.4750	
		Variance		43.819	
		Std. Deviation		6.61956	
		Minimum		45.60	
		Maximum		63.65	
	60	Mean		26.1450	7.28724
		95% Confidence Interval for Mean	Lower Bound	9.6601	
			Upper Bound	42.6299	
		5% Trimmed Mean		25.4944	
		Median		25.9000	
		Variance		531.039	
		Std. Deviation		23.04429	
		Minimum		.00	
		Maximum		64.00	

Table A.35: Table of descriptive statistics of MA 1 & 2

Descriptives

	Temperature (deg C)			Statistic	Std. Error
Means of MA 1 & 2	22	Mean		19.4000	2.83262
		95% Confidence Interval for Mean	Lower Bound	11.5354	
			Upper Bound	27.2646	
		5% Trimmed Mean		19.3806	
		Median		22.2000	
		Variance		40.119	
		Std. Deviation		6.33394	
		Minimum		12.40	
		Maximum		26.75	
	37	Mean		23.4850	2.08141
		95% Confidence Interval for Mean	Lower Bound	18.7765	
			Upper Bound	28.1935	
		5% Trimmed Mean		23.0222	
		Median		22.6500	
		Variance		43.323	
		Std. Deviation		6.58201	
		Minimum		15.90	
		Maximum		39.40	
	45	Mean		22.5813	.85215
		95% Confidence Interval for Mean	Lower Bound	20.5662	
			Upper Bound	24.5963	
		5% Trimmed Mean		22.5819	
		Median		22.0750	
		Variance		5.809	
		Std. Deviation		2.41024	
		Minimum		18.55	
		Maximum		26.60	
	60	Mean		11.9250	2.85103
		95% Confidence Interval for Mean	Lower Bound	5.4755	
			Upper Bound	18.3745	
		5% Trimmed Mean		11.9778	
		Median		14.3750	
		Variance		81.283	
		Std. Deviation		9.01573	
		Minimum		.00	
		Maximum		22.90	

Table A.36: Kolmogorov-Smirnov test for R value 1 & 2, Alpha Angle 1 & 2, MA 1 & 2

One-Sample Kolmogorov-Smirnov Test

		Means of R 1& 2	Means of Aplha 1 & 2	Means of MA 1 & 2
N		33	33	33
Normal Parameters [a,b]	Mean	10.7258	42.0045	19.1439
	Std. Deviation	7.79465	20.43436	8.15288
Most Extreme Differences	Absolute	.277	.185	.203
	Positive	.277	.068	.145
	Negative	-.191	-.185	-.203
Kolmogorov-Smirnov Z		1.592	1.063	1.168
Asymp. Sig. (2-tailed)		.013	.209	.131

a. Test distribution is Normal.

b. Calculated from data.

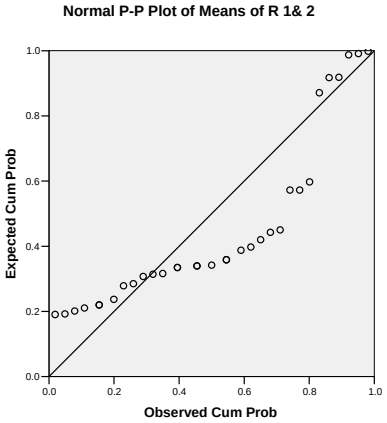


Figure A11: P-P Plot of r value 1 & 2

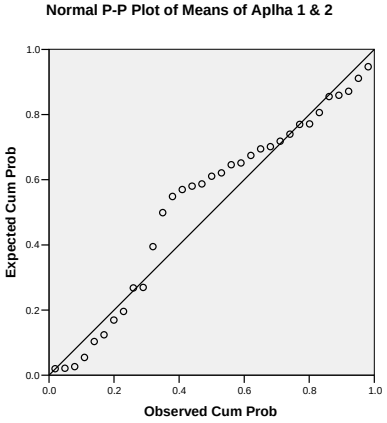


Figure A12: P-P Plot of Alpha Angle 1 & 2

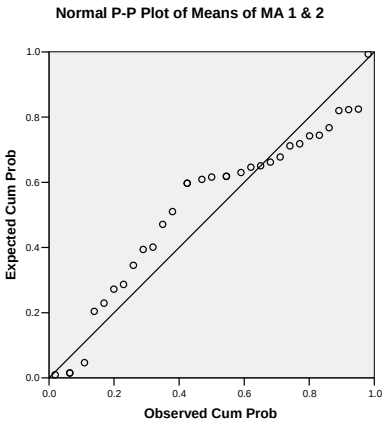


Figure A13: P-P Plot of MA 1 & 2

Table A.37: Analysis of variance of the Van der Waerden transformation of r value 1 & 2

Source	DF	SS	MS	F	P
BAG	9	1239.66	137.740	1.83	0.1286
TEMPERAT	3	294.66	98.219	1.30	0.3025
Error	19	1432.43	75.391		
Total	31				

Note: SS are marginal (type III) sums of squares

Grand Mean 17.750 CV 48.92

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	0.06	0.0636	0.00	0.9778
Remainder	18	1432.36	79.5758		

Table A.38: Post-Hoc analysis of r value 1 & 2

LSD All-Pairwise Comparisons Test of r value 1 & 2 for TEMPERATURE

TEMPERATURE	Mean	Homogeneous Groups
22	22.528	A
60	19.373	A
45	14.850	A
37	14.250	A

Alpha 0.05 Standard Error for Comparison VARIES
Critical T Value 2.093 Critical Value for Comparison VARIES

Table A.39: ANOVA test of Alpha Angle 1 & 2

Source	DF	SS	MS	F	P
BAG	9	3476.78	386.31	1.66	0.1653
TEMPERAT	3	5149.43	1716.48	7.37	0.0016
Error	20	4655.89	232.79		
Total	32				

Note: SS are marginal (type III) sums of squares

Grand Mean 41.051 CV 37.17

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	410.54	410.544	1.84	0.1912
Remainder	19	4245.34	223.439		

Table A.40: Post-Hoc analysis of Alpha Angle 1 & 2

LSD All-Pairwise Comparisons Test of ALPHA ANGLE 1 & 2 for TEMPERATURE

TEMPERATURE	Mean	Homogeneous Groups
45	53.191	A
37	53.085	A
22	31.841	B
60	25.687	B

Alpha 0.05 Standard Error for Comparison VARIES
Critical T Value 2.093 Critical Value for Comparison VARIES

Table A.41: ANOVA test of MA 1 & 2

Source	DF	SS	MS	F	P
BAG	9	737.508	81.945	2.80	0.0264
TEMPERAT	3	786.662	262.221	8.96	0.0006
Error	20	585.088	29.254		
Total	32				

Note: SS are marginal (type III) sums of squares

Grand Mean 18.644 CV 29.01

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	128.097	128.097	5.33	0.0324
Remainder	19	456.992	24.052		

Table A.42: Post-Hoc analysis of MA 1 & 2

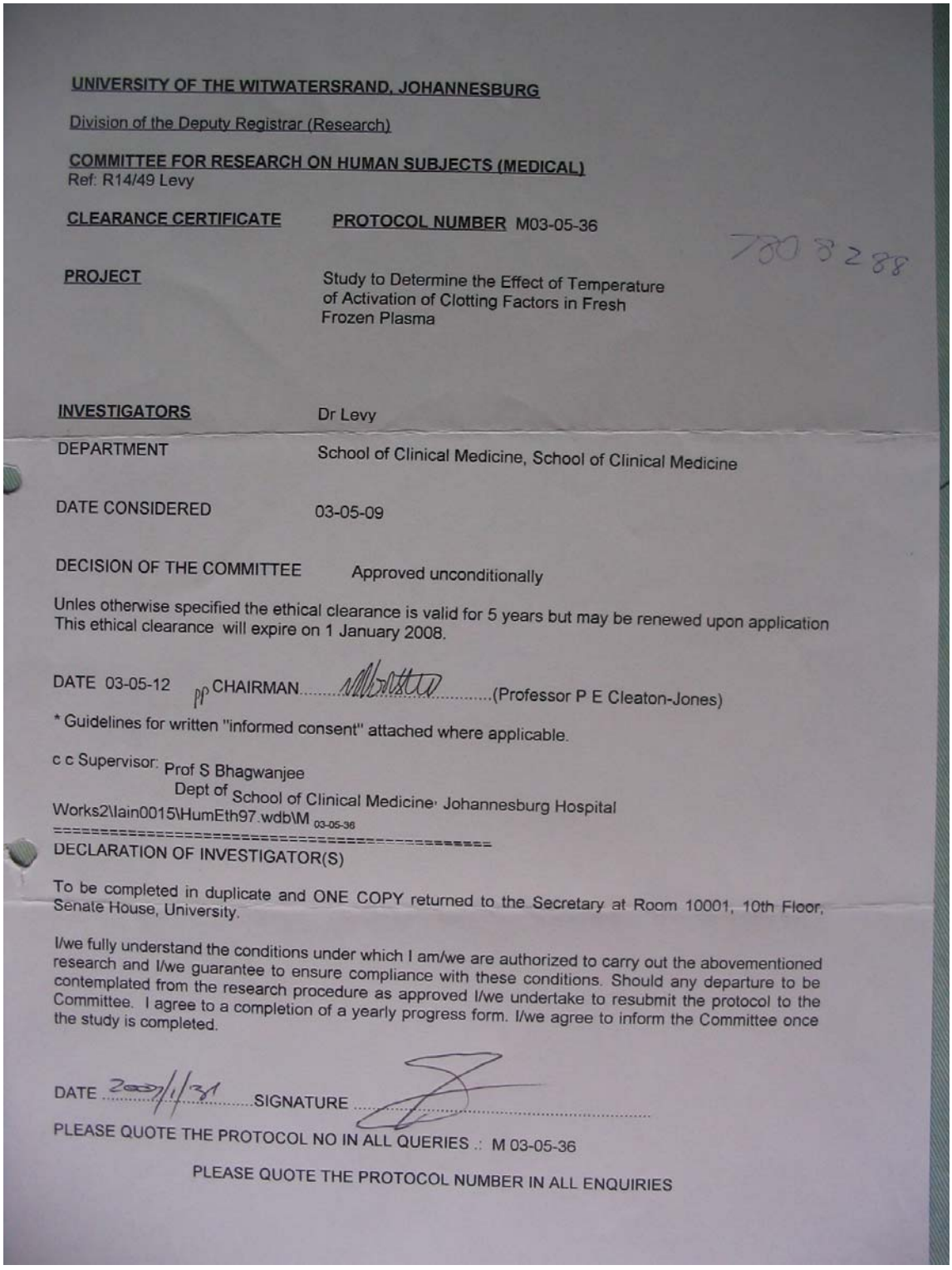
LSD All-Pairwise Comparisons Test of MA 1 & 2 for TEMPERATURE

TEMPERATURE	Mean	Homogeneous Groups
37	23.485	A
45	22.143	AB
22	17.018	BC
60	11.967	C

Alpha	0.05	Standard Error for Comparison	VARIES
Critical T Value	2.093	Critical Value for Comparison	VARIES

APPENDIX B:

COPY OF ETHICS CERTIFICATE



UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Levy

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M03-05-36

7003288

PROJECT Study to Determine the Effect of Temperature of Activation of Clotting Factors in Fresh Frozen Plasma

INVESTIGATORS Dr Levy

DEPARTMENT School of Clinical Medicine, School of Clinical Medicine

DATE CONSIDERED 03-05-09

DECISION OF THE COMMITTEE Approved unconditionally

Unles otherwise specified the ethical clearance is valid for 5 years but may be renewed upon application
This ethical clearance will expire on 1 January 2008.

DATE 03-05-12 pp CHAIRMAN .....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof S Bhagwanjee
Dept of School of Clinical Medicine, Johannesburg Hospital
Works2\Iain0015\HumEth97.wdb\M 03-05-36

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. I agree to a completion of a yearly progress form. I/we agree to inform the Committee once the study is completed.

DATE 2003/1/31 SIGNATURE .....

PLEASE QUOTE THE PROTOCOL NO IN ALL QUERIES .: M 03-05-36

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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