The Level of Coagulation Factors in Fresh Frozen Plasma in Rwanda

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To all Rwandan blood donors.

To all patients that suffer from coagulopathies.

ACKNOWLEDGEMENT

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LIST OF ABBREVIATIONS AND ACRONYNOMS

AABB:	American Association for Blood Bank
APTT:	Activated Partial Thrombin Time
AT:	Antithrombin
CPDA-1:	Citrate Phosphatase Dextrose Adenine-1
FFP:	Fresh Frozen Plasma
Kg:	Kilogram
NBTC	National Blood Transfusion Center
PC:	Protein C
PS:	Protein S
PT:	Prothrombin Time
RPM:	Revolutions per minute
TF:	Tissue Factor
TRALI:	Transfusion Related Acute Lung Injury
USA:	United State of America
vWF:	von Willebrands factor

OPERATIONAL DEFINITIONS

1. **Aphresis** is a procedure in which blood is temporarily withdrawn, one or more components are selectively removed, and the rest of the blood is reinfused into the donor. The process is used in treating various disease conditions in the donor and for obtaining blood elements for the treatment of other patients or for research (Mosby's Medical Dictionary, 2009).
2. **Coagulation factors** are plasma proteins that are involved in blood coagulation in case of a cut that ends up in bleeding. These factors are thirteen in number and they follow in cascade intervention resulting into blood clot (Hoffbrand *et al.,* 2002).
3. **Fresh frozen plasma** is the liquid portion of human blood that is centrifuged, separated with blood cells, solid at minus 18 degrees C or colder within eight hours of blood donation. It contains all coagulation factors, inhibitors, albumin, and electrolytes in normal concentration, and was be used for blood transfusion (Spence, 2006)
4. **Haemostasis** is the maintenance of blood in a fluid state and prevention of blood loss from blood vessels while balancing between clotting and anti-clotting (Hoffbrand *et al.,* 2002).

ABSTRACT

Fresh frozen plasma (FFP) is indicated in the treatment and prevention of bleeding disorders associated with insufficient management of massive blood loss, liver disease, disseminated intravascular coagulation or reverse anticoagulant therapy. FFP is therefore used in transfusion medicine but its storage deteriorates with time depending on conditions and time which has never been evaluated in Rwanda. Therefore this study was aimed at determining the levels of coagulation factors in fresh frozen plasma stored up to the period of three months under -18^{o}C. A crossectional prospective study was conducted and a total of 18 FFP samples were collected from Kigali, Butare and Ruhengeri blood donor centres during the period between August and November 2013. A total of 72 samples were analysed at baseline, after one, two and three months of storage. Samples were analyzed for coagulation factors, prothrombin time and activated partial thrombin time using an automated ACL 7000 coagulation analyzer. One-way ANOVA test was used to compare means at 95% confidence interval and results were considered statistically significant if p-value ≤0.05. There was a significant decrease in fibrinogen 10% (p-value .002), FII 8% (p-value = .003) , FV 16% (p-value <.001), FVII 13.5% (p-value < .001), FX 14.9% (p-value < .001), FXIII 14.8% (p-value < .001), ATIII 6.7% (p < .001) and PC 7% (p-value < 0.003), from baseline to three months of FFP storage. However, there was change in the levels of other coagulation factors and inherited inhibitors were not significant.

Findings from this study confirmed that all coagulation factors and inhibitors in plasma are still retained under minus 18ºC up to three months. Nevertheless, there is a need to evaluate further possible retention of these factors up to 12 months of storage.

CHAPTER ONE

1.0 INTRODUCTION

Coagulation factors are plasma proteins that play a major role in the blood coagulation process. There are thirteen (13) clotting factors labeled in Roman numbers: I to XIII (Pallister *et al.,* 2002). These factors maintain haemostasis which is the balance between clotting and anti-clotting (Hoffbrand, 2002). In case any factor becomes deficient either through blood loss or dilution during transfusions with other blood products and or by crystalloids it can lead to heavy bleeding without clotting or taking too long to clot. This condition is improved by replacement with fresh frozen plasma (O'Shaughnessy *et al.,* 2004).

United States of America and European guidelines for production of blood and blood product define Fresh Frozen Plasma (FFP) as the liquid portion of human blood that is centrifuged and separated from blood cells, solid at minus 18 degrees Centigrade (-18^0C) or colder within eight hours of blood donation (Spense, 2006). It contains all coagulation factors in normal concentration (0.5 to 2IU/ml or 50-200%) (Hematol, 2010).

Previous studies have shown that within 24 hours of phlebotomy and storage at 1 to 6°C, most activities of coagulation factors could still be preserved even at the end of the fifth day of storage relative to their initial post thaw levels, including FV and FVIII activity, which could be decreased by 2.69% to 0.69IU/ml on day 5 (Mark *et al.,* 2008). It was shown that at the time of thawing, the activity of Protein C and S could be at the low end of the normal range and declining to slightly below its normal level by Day 5. In addition, von Willbrand Factor activity could remain within its normal range by day 5.In a study done in Germany, FVII after thawing was found to have decreased significantly while fibrinogen, FV, FVIII, F IX, FXI, FXII, von Willbrand Factor-Antigen(VWF-Ag) and protein S (PS) remained unaffected by freezing, storage, and thawing (Von

Heymann *et al.,* 2006).

In another study, it was found that FVII decreased significantly whereas F IX and FX remained stable without a significant decrease (Ben-Tal *et al.,* 2003). This was contrary to previous studies on rate of decrease observed in FX factor after thawing, and stability in F IX despite missing data on time between baseline and after thawing for FII, FVIII, FXI, FXII, FXIII, Protein C (PC), and VWF-Ag (Christian *et al.,* 2009).. With regard to freezing and thawing-associated stability of clotting factors and inhibitors, it confirmed a need to consider that most clotting factors with the exception of fibrinogen, FII, FV, FVII, FX, and FXIII could be unstable with time. This should also apply that vWF-Ag and the inhibitors PC, Antithrombin and PS could be a stable inhibitor protein (Christian *et al.,* 2009).

The prolongation of PT observed in this studies could be due to either deficiency or inhibition of the extrinsic pathway (FVII), possible mild factor X, V, and II deficiencies, inhibitors, disseminated intravascular coagulation, therapy with anticoagulant or liver diseases (Kamal *et al.,* 2007). For an isolated result showing APTT prolongation, that could suggest a deficiency or inhibitor of one or more of the intrinsic pathway clotting factors XII, XI, IX, and VIII, liver diseases, use of oral anticoagulant therapy or disseminated intravascular coagulation (Kamal *et al.,* 2007).

According to United State of America, guideline on FFP, FFP should be stored at less or equal to minus 18 degrees C for up to twelve months with approval from food and drug administration. While in Europe, FFP at -18°C to -25°C is stored only up to three months. In Rwanda, an American guideline was adopted despite the varied genetic, race, environmental and physiological conditions. However, no similar studies have been conducted to evaluate the protocol in Africa including Rwanda.

1.1. Problem Statement

Inherited bleeding disorders due to deficiency of coagulation factors VIII and IX known as hemophilia A and B respectively, are relatively common and frequent clinical problem. These are followed by von Willbrand factor deficiency; the other factor deficiencies are rare as stated by World Federation of Hemophilia report on the annual global survey 2011. They are associated with increased morbidity, faster progression to other complications such as anemia, decreased survival time and an increased mortality.

For these reasons, it is imperative that clinicians in coagulopathy care are prepared to manage bleeding disorders related to coagulation factor deficiencies using fresh frozen plasma and factor concentrate where they are available and affordable. Understanding the use and indications of fresh frozen plasma and knowing the level of each coagulation factor and the level required for plasma to be used for treatment, we need to measure at least labile factors before the use of FFP to ensure that hemophiliac individuals and other patient suffering from bleeding disorders due to coagulation factors disorders was receive appropriate treatment and to improve the quality of preparation and use of FFP.

In Rwanda there are many resources limited Referral, District hospitals and health centers laboratories, where factor assay are not done systematically on patients with bleeding disorders attending these centers and fresh frozen plasma is used without knowing the concentration of coagulation factors it contains. This could lead to the circulatory overload without any improvement; if many bags are used with low level of factors. Patients with bleeding disorders due to coagulation factor deficiency are treated like other routinely bleeding patients who are not appropriate to these patients. Also the education about coagulopathy management and prevention given by health workers in most health centers and hospitals in Rwanda is not specific to the

bleeding disorders related to coagulation factors deficiency and is not based on some frequent characteristics and etiologic causes of coagulopathy, this can lead to inappropriate management. While we may determine at least APTT and PT for patient with bleeding disorders to know exactly the cause, if prolonged we do substitution test to know whether prolongation is due to coagulation factors deficiency or by inhibitors, also these test must be done on stored FFP before the use. This study is aimed to determine the level of coagulation factors in fresh frozen plasma in Rwanda.

1.2. Justification of the study

Data from this study will be used at guiding the management of patients with factor deficiency and monitoring using FFP with known level of coagulation factors. This study shed light on level of fibrinogen, FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII, AT or FIII, vWF, PC, and PS in current used plasmas which was remains elusive. The need for levels of coagulation factors data in donated blood is important in order to provide information on quality of fresh frozen plasma prepared by National and Regional Centers for Blood Transfusion. It will also serve as basis to renew policies and procedures regarding storage conditions and quality control prior to clinical use of fresh frozen plasma.

Rwanda has adopted guidelines which have not been assessed using our population while coagulation factors level can vary depending on several conditions including race, age, blood groups and other environment condition. In this study, I chose to measure the level of coagulation factors in fresh frozen plasma prepared and used in Rwanda were measured because there is no existing data in Rwandan population.

In addition to ascertain stability or decrease it is first necessary to establish levels in fresh frozen material as this allow for comparison to be made. It is on that account that the method of sample assay for factor level before freezing was chosen with a three month follow-up to look for possible

changes in coagulation factor level. The aim of this study was to determine whether the levels of coagulation factors in fresh frozen plasma stored at -18^{0}C after one week, a month, two months and three months are adequate for their intended purpose.

1.3. Research Question

1. What is the level of coagulation factors in fresh frozen plasma prepared by NBTC in Rwanda?
2. What is the level of coagulation inherited inhibitors (Anti-Thrombin, Protein C, and Protein S) in fresh frozen plasma prepared by NBTC in Rwanda?
3. What is prothrombin time of fresh frozen plasma prepared by NBTC in Rwanda?
4. What is activated partial thrombin time of fresh frozen plasma prepared by the NBTC in Rwanda?
5. What is the impact of storage conditions (temperature -18oC and time period) on the levels of coagulation factors in fresh frozen plasma over time?

1.4. Objectives

1.4.1. Broad objective

To determine the level of coagulation factors in fresh frozen plasma from Rwandan blood donors

1.4.2. Specific objective

1 To determine the level of coagulation factor (Fibrinogen, FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII and vWF) assays in fresh frozen plasma

2 To determine the level of inherited inhibitors, Anti-Thrombin (AT III), Protein C (PC), and Protein S (PS).

3 To determine Prothrombin Time (PT) time in fresh frozen plasma.

4 To determine Activated Partial Thrombin Time (APTT) of fresh frozen plasma.

5 To determine the impact of storage conditions (temperature -18^{o}C and time period)on the levels of coagulation factors in fresh frozen plasma over time

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Haemostasis

This is the maintenance of blood in a fluid state and prevention of blood loss from blood vessels while balancing between clotting and anti-clotting (Hoffbrand *et al.,* 2002).

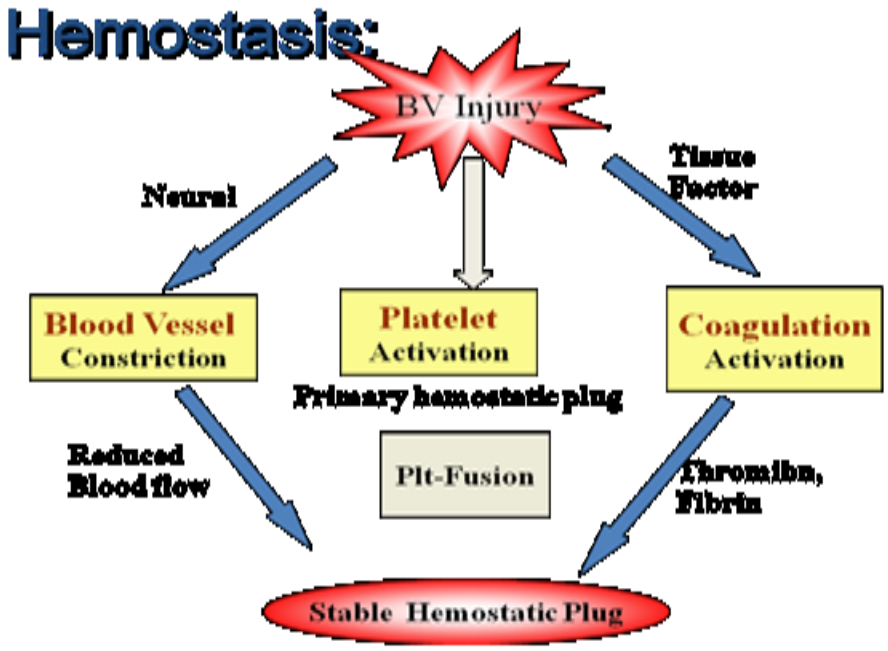

Figure 2. 1: Haemostasis process in case of Blood Vessel (BV) injury (Beth et al., 2010)

2.1.1. Coagulation factors and inhibitors in haemostasis

2.1.1.1. Coagulation factors

Coagulation factors are plasma proteins that are involved in blood coagulation in case of a cut that ends up in bleeding. These factors are thirteen in number and they follow in a cascade intervention resulting in the clotting of blood (Hoffbrand *et al.,* 2002). These coagulation factors labeled in standardized roman numbers includes; Factor I, Fibrinogen: a protein present in blood plasma;

converted to fibrin when blood clots; Factor II, Prothrombin: a protein in blood plasma that is the inactive precursor of thrombin, Factor III, Thrombokinase, Thromboplastin: this is an enzyme liberated from blood platelets that converts prothrombin into thrombin as blood starts to clot, Factor V and VI, Accelerator factors: they are prothrombin accelerators, Factor VII, Proconvertin and stable factor: this factor is formed in the liver under the influence of vitamin K, Factor VIII, Antihaemophliac factor: a coagulation factor whose absence or low level in plasma is associated with haemophilia A or Classical haemophilia, Factor IX, Christmas factor: this is a coagulation factor whose absence or low level in plasma is associated with haemophilia B or Christmas disease., Factor X, Prothrombinase or Tenase: coagulation factor that is converted to an enzyme that converts prothrombin to thrombin in a reaction that depends on calcium ions and other coagulation factors, Factor XI, Plasma thromboplastin antecedent: coagulation factor whose deficiency results in a hemorrhagic tendency Factor XII, Hageman factor: coagulation factors whose deficiency results in prolongation of clotting of venous blood and Factor XIII, Fibrinase: in the clotting of blood thrombin catalyzes factor XIII into its active form(Pallister and Watson, 2002).

2.1.1.2. Inhibitors

These are plasma protein or antibodies that bind to coagulation factor antigens hence physiological leading to the limitation of blood coagulation in haemostasis (Hoffbrand *et al.,* 2002)Physiological anticoagulants or inhibitors can be genetic such as; protein C, protein S, Antithrombin, tissue factor pathway inhibitor in contrast to acquired inhibitors such as anticoagulant drugs (Spense, 2006).

2.2. Fresh Frozen Plasma (FFP) preparation and composition

Fresh frozen plasma is a blood component that has been available since 1941 (O'Shaughnessy *et al.,* 2004). It was initially used as a volume expander, but is currently indicated for the management and prevention of bleeding in patients with coagulopathies (Erber *et al.,* 2006).

FFP is prepared from single units of whole blood approximately 250ml by centrifugation at 2800 rpm for 10 minutes or plasma collected by aphaeresis using automated machine that collect only plasma, usually 500ml (Spence, 2006). FFP is collected in citrate-containing anticoagulation solution, frozen within 8 hours and stored at -18ºCfor one year (Spence, 2006).

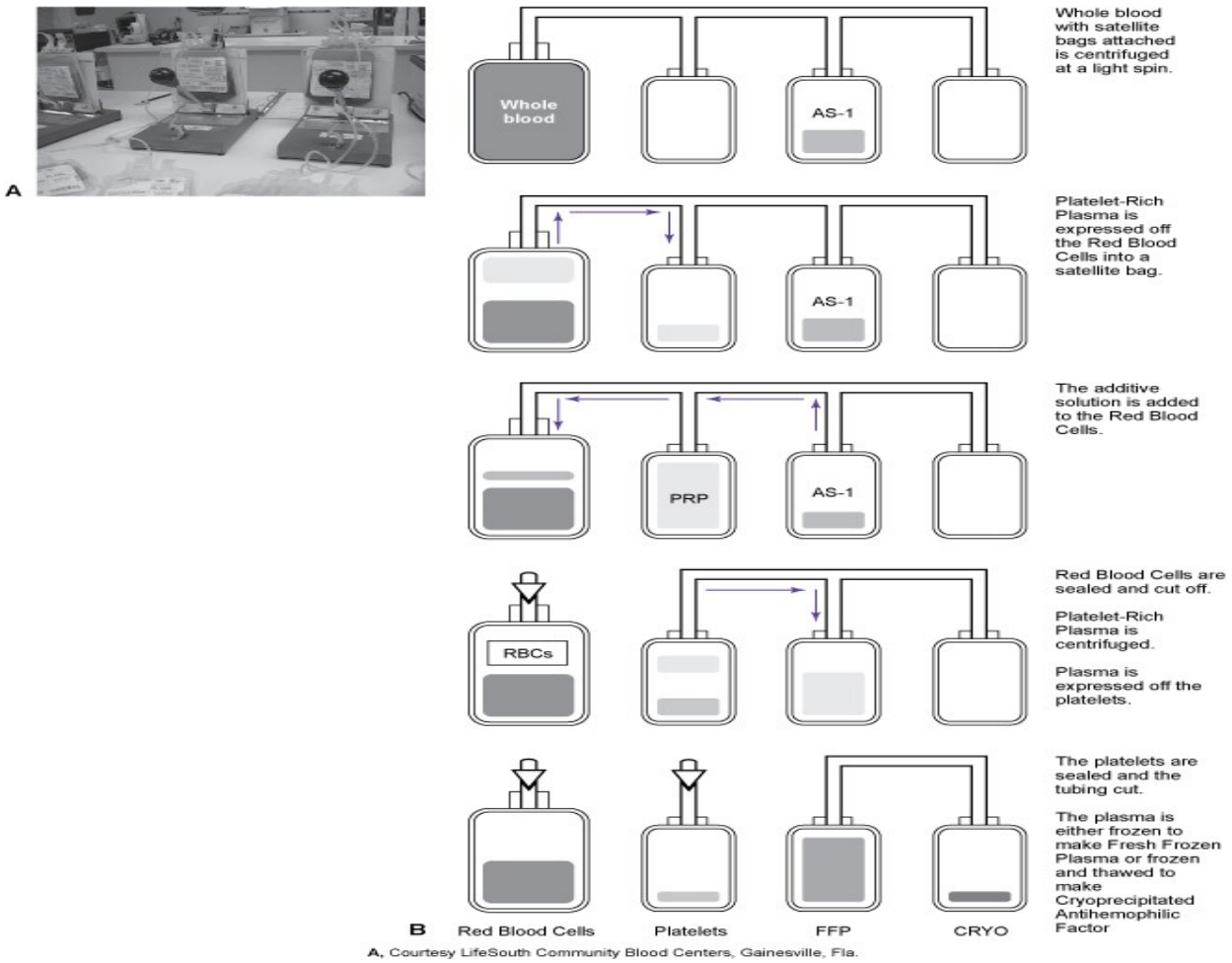

Figure 2. 2: Fresh frozen plasma preparation (Spence, 2006)

United States of America and United Kingdom guidelines for production of blood and blood product define FFP as the liquid portion of human blood that is centrifuged, separated from blood cells, solid at -18^{0}C colder within eight hours of blood donation.

FFP contains all of the clotting factors at normal range 0.5-2IU/dl, including fibrinogen (400 to 900 mg/unit), plasma proteins particularly albumin, electrolytes, physiological anticoagulants or inhibitors such as protein C, protein S, Antithrombin, tissue factor pathway inhibitor and added anticoagulants (Spense, 2006).

Fresh frozen plasma is commonly thawed in a water bath over 20 to 30 minutes before use, but United State, Food and Drug Administration(FDA) approved that, microwaves can thaw it in two to three minutes (O'Shaughnessy *et al.,* 2004). After thawing, the activity of labile clotting factors such as FV and FVIII decline gradually and most countries recommend FFP use within 24 hours (Triulzi, 2002).

In a study done in Turkey on the level of labile FVIII and stable FIX in FFP produced from whole blood stored at 4°C overnight showed that holding blood at 4ºC overnight resulted in a 25% loss of FVIII and in a 9% loss of FIX compared to plasma frozen within 8 hours of donation(Neval *et al.,* 2012)

2.3. Indication and complications of Fresh frozen plasma

Fresh-frozen plasma (FFP) is frequently used to treat minor to major coagulation factor deficits to prevent blood loss in patients requiring massive transfusion (O'Shaughnessy *et al.,* 2004).Other indications for treatment with FFP are disseminated intravascular coagulation, liver disease patients and reversal of therapy with vitamin K antagonists (Stanworth, 2007). Mainly emergency situations, transfusion of FFP delivers clotting factors without aggravating existing dilutional

coagulopathy induced by crystalloid or colloid volume replacement solutions (Gonzalez *et al.,* 2007).

Fresh frozen plasma like other blood products can cause immunological and or non-immunological complications to the recipients (O'Brien *et al.,* 2007). The important immunological complications caused by FFP is Transfusion-Related Acute Lung Injury (TRALI); which is due in most cases to passive transfer of leucoagglutinins mostly anti-Human Leucocytes Antigens (HLA) class I or class II or granulocyte antibodies, in blood donor plasma, leading to endothelial and epithelial injury, alveolar damage and inflammatory changes, mediated by cytokines and other inflammatory mediators (Popovsky, 2006 and Renaudier *et al.,* 2009). TRALI is the commonest cause of transfusion-related death (Popovsky, 2006 and Renaudier *et al.,* 2009). Non- immunological complications are transmission of diseases such as Hepatitis B Virus, Hepatitis C Virus, Cytomegalovirus, Epsilon Barr Virus, syphilis, malaria, Human Immunodeficiency Virus, (O'Brien *et al.,* 2007). The other important non-immunological complication is transfusion associated circulatory overload which is the rise in blood volume and venous pressure after the transfusion of blood and/or plasma. This is dangerous especially for pregnant women, patients with severe anaemia, and the elderly with compromised cardiovascular function, (Popovsky, 2006 and Renaudier *et al.,* 2009).

2.4. Coagulation factor and inhibitor levels in fresh frozen plasma

Fresh frozen plasma is used up to 5 days after thawing and stored between 1-6^{o}C, although in five days old plasma the activity of FVIII is expected to drop by above 50%, and the activity of FV and FVII drops to about 20% (Shehatta *et al.,* 2001)

The German guidelines for the production of blood and blood components and for the utilization of blood products require that preparation of FFP must be accomplished within 24 hours after

collection and after thawing be administered as fast as possible. In clinical practice, FFP storage of 6 to 24 hours for thawed plasma is reported without loss of clotting factor activity (Ben-Tal *et al.,* 2003).For thawed FFP stored beyond this time it is assumed that clotting factor activity falls below the minimum standard level recommended for use in the transfusion guidelines (Paul-Ehrlich-Institut,2007).Owing to uncertainty about preservation of clotting factors 2.2% of all FFP units were disposed after thawing, as shown in a report investigating the blood product usage of Charité-Universitätsmedizin Berlin in 2003. The substantial economic burden on health care givers by wastage of FFP may be reduced by prolonged storage of thawed FFP, which may also allow for rapid access to clotting factor replacement in emergencies. To be effective this strategy requires stability of clotting factors in thawed FFP during storage (Von Heymann *et al.,* 2005). The aim of their study was to investigate the quality of FFP with regard to clotting factor and inhibitor activity and evaluate if there is higher bacterial contamination rate when stored at 4 ± 2°C over a period of 6 days. This previous studies have confirmed a significant decrease of FVII and the relative stability of FVIII, F IX, FXI, FXII, VWF-Ag, and FPS. The decrease of fibrinogen (299 to 290 mg/dL, $p < 0.001$) and FV (130% and 116%, $p < 0.001$) which was not in accordance with previous results done in 2005, showed an insignificant increase of both factors (Von Heymann *et al., 2006).*

A study done on Coagulation factor levels in plasma frozen within 24 hours of phlebotomy over 5 days of storage at 1 to 6°C, in America by Mark *et al.,* 2008 , showed that, the activities of most factors were well preserved by the end of the fifth day of storage relative to their initial post-thaw (Day 0) levels. At the time of thawing, the activity of Protein S was at the lower end of the normal range and it declined to slightly below its normal level by Day 5. vWF activity remained within

its normal range by Day 5. The PT, INR, and APTT demonstrated modest prolongations during storage (Mark H, 2007).

Previous work in German has described a significant decrease of FVII after thawing while fibrinogen, FV, FVIII, and protein S remained unaffected by freezing, storage, and thawing (Von Heymann *et al., 2006).*

FVII decreased significantly in a former investigation by Ben-Tal and colleagues, whereas F IX and FX were stable without a significant decrease (Ben-Tal *et al., 2003).* This is contrary to the significant decrease of FX after thawing in the results of the study done by Christian *et al*, 2009, whereas F IX was stable, too. The time course of FII, FVIII, FXI, FXII, FXIII, PC, and VWF-Ag between baseline before thawing and immediately after thawing was not measured before in that study. With regard to freezing and thawing-associated stability of clotting factors and inhibitors, Christian *et al.,* conclude from previous and new data that most clotting factors with the exception of fibrinogen, FII, FV, FVII, FX, and FXIII must be considered unstable. This applies also to VWF-Ag and the inhibitors PC and Antithrombin, while PS seems to be a stable inhibitor protein (Christian *et al.,* 2009).

2.5. Prothrombin time, Activated partial thrombin time and coagulation factor

The prothrombin time is a measure of the integrity of the extrinsic and final common pathways of the coagulation cascade. This consists of tissue factor and factors I, II, V, VII and X. The test is performed by adding calcium and thromboplastin, an activator of the extrinsic pathway, to the serum sample then measuring the time (in seconds) required for fibrin clot formation. The reference range for prothrombin time is 9.5-13.5 seconds (Jackson, 2005).

The prolongation of PT can be due to deficiency or inhibition of the extrinsic pathway (FVII), but mild factor X, V, and II deficiencies are also possible causes, also disseminated intravascular

coagulation and liver disease,(Kamal *et al.,* 2007). The APTT is the time taken from the addition of calcium to the formation of a fibrin clot. Platelet poor plasma [PPP] is incubated at 37°C then phospholipids (cephalin) and a contact activator such as Kaolin, micronized silica or ellagic acid are added followed by calcium all pre-warmed to 37°C. Addition of calcium initiates clotting and timing begins. The APTT in contrast to the PT, measures the activity of the intrinsic and common pathways of coagulation. It is also known as Kaolin Cephalin Clotting Time (KCCT) (Jackson, 2005). An isolated result showing APTT prolongation suggests a deficiency or inhibitor of one or more of the intrinsic pathway clotting factors XII, XI, IX, and VIII, liver diseases and disseminated intravascular coagulation (Kamal *et al.,* 2007).

In the US, FFP is stored at less or equal to -18°C for up to twelve months with approval from food and drug administration and at - 65°C or below for 7 years. In Europe, the shelf life of FFP is low, if at below -25°C for up to three years and if at -18-25°C, up to three months. In Rwanda, currently, this American guideline is still adopted contrary to this diverse evidence of variations.

CHAPTER THREE

3.0 METHODS AND MATERIALS

3.1. Study Area

Three blood transfusion centers in Rwanda namely: Kigali, Ruhengeri in the Northern Province, and in Butare in the Southern Province.

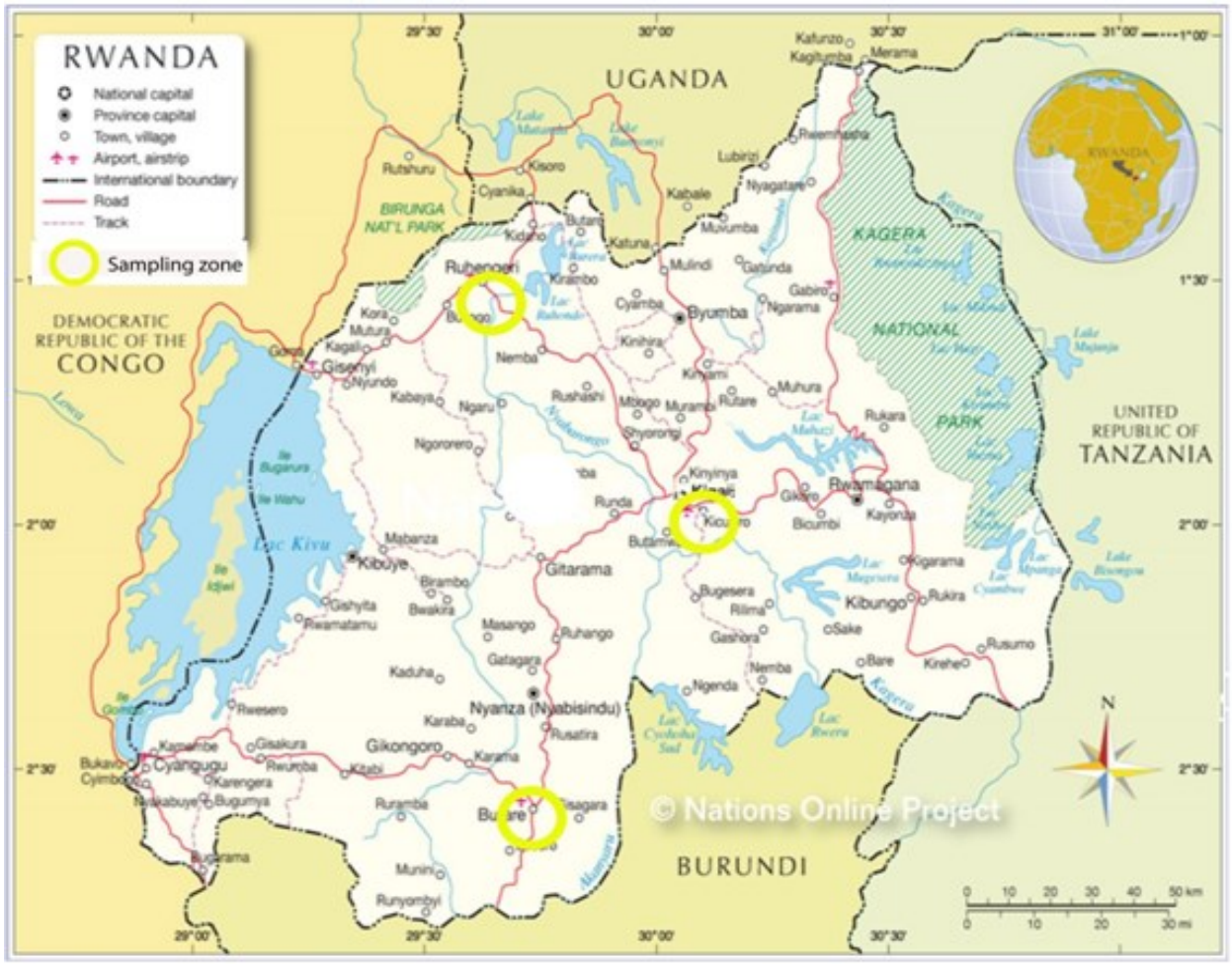

Figure 3. 1: Rwanda Google map accessed on 19 March, 2014

3.2. Study Design

This was a prospective descriptive study.

3.3. Study population

This study targeted daily prepared fresh frozen plasma from Kigali, Butare and Ruhengeri blood transfusion centers. All these centres were recruited into the study since they are only centres involved in preparation of FFP in Rwanda

3.4. Sample size

The minimum sample size was eighteen bags, six from each blood transfusion center.

This sample size was calculated using the formula below:

$n = \frac{\mathrm{N}}{1+\mathrm{N}\ (\mathrm{e})^2}$ (Ejaz *et al.* 2011)

n: required sample size

N: Study population, daily prepared fresh frozen plasma in the three centers $= 18$, six are prepared daily in each center for blood transfusion

e: Standard error (5% or 0.05)

$$n = \frac{18}{1 + 18\ (0.05)^2} = 18.045 \sim \mathbf{18}$$

Given the high cost of reagents to determine coagulation factors and being a descriptive study to explore the system of FFP preservation (baseline to three months), Kigali Health Institute Research Scientific Review Committee advised to collect specimens from FFP prepared within same period and to use the above formula to get the minimum sample size. This sample size was enough to determine the modification during the period of preservation. However the sample size used could not allow us to generalize the coagulation factors among Rwandan blood donors.

3.5. Sampling procedure

Eighteen blood donors, six each from 3 sites of the National Center for Blood Transfusion (NCBT) were sampled for this study. Each donor fulfilled the criteria for blood donation as set by the NCBT and gave informed consent for participation in this study (Appendix 1).Four plasma samples were then drawn from each FFP bag prepared according to NCBT standard operating procedures (Appendix 6).

3.5.1. Inclusion criteria

All blood bags from donors that fulfill the blood donation requirements as set by the NCBT (Appendix 1).

3.5.2. Exclusion criteria

Other blood products prepared at the NCBT that are not fresh frozen plasma.

3.6. Sample collection

Whole blood was collected from blood donors and used to prepare fresh frozen plasma. The whole blood units (450±50 ml) were collected in triple bags containing 63 mL Citrate Phosphatase Dextrose Adenine-1(CPDA-1) and centrifuged within 8 hours of collection. A semi- automated extractor was used to separate the plasma within 30 minutes of centrifugation and put in a fresh plasma bag (Appendix 5).

To determine the level of coagulation factors in FFP, 72 samples of plasma were collected from eighteen fresh plasma bags (4 plain tubes of 5ml from each FFP bag). A total of 18 tubes were immediately used to test for baseline levels of coagulation factors while 54 tubes with plasma were immediately frozen at-18^{o}C as plasma is normally performed at Rwanda blood transfusion centers.

3.7. Laboratory procedures

3.7.1. Specimens type

Whole blood was collected into blood bags with CPDA-1 which is preferred for haemostasis tests as it preserves clotting factors.

3.7.2. Specimens handling and storage

Eighteen tubes of plasma were kept at room temperature and tested within two to four hours of collection for the baseline values while 54 tubes were frozen at -18^{o}C. The frozen tubes were then transported to the laboratory in 3 batches of 18 after 1 month, 2 months and 3 months of freezing for testing. The transportation was done in a cool box at 4 to 8°C as recommended by the NCBT to prevent brisk thawing, and took two to three hours. Testing was then done within two to four hours upon reception at the laboratory.

3.7.3. PT, APTT and Factor assay

Laboratory analysis was done at the Kigali Health Institute, Biomedical Laboratory Sciences Department. The coagulation factors were analyzed using the ACL 7000 coagulation analyzer (Instrumentation Laboratory Company, USA). The same analyzer was used to measure thrombin time and activated partial thrombin time. All tests were done according to the laboratory SOPs, equipment and reagent manufacturer's instructions (Beckman Coulter, 2010)

3.8. Data Management and Analysis

Socio-demographic and medical information was obtained from the blood donors files. Results from the laboratory tests were entered into a hard cover register and a password protected Microsoft Excel database. Samples were identified by a unique study number for confidentiality. The information from the files included gender, age, demographic location, results of transmissible disease and blood group. Results were analyzed using SPSS version 20 (IBM corporation, 2012).

One Way ANOVA was used to compare the change in PT, APTT and coagulation factors at collection, after 1 month, 2 months and 3 months of freezing. Based on reference values PT, APTT, levels of coagulation factors and inhibitors were graded.

Table 3. 1: Reference range and cutoff value for parameters measured

Parameter	**Reference range**	**Cutoff**	**Abnormal**
PT (seconds)	11-15	15	> 15
APTT (seconds)	27-35	35	> 35
Fibrinogen (mg/dl)	150-400	150	< 150
Coagulation factors (%)	50-200	50	< 50
Inherited inhibitors (%)	50-200	50	< 50

3.9. Ethical considerations

This study was approved by the Rwanda National Ethics Committee and a research permit was also obtained from Ministry of Education before excursion (Appendices 7 and 8).

CHAPTER FOUR

4.0 RESULTS

4.1. Variation in coagulation factors

There was a statistically significant decrease in level of fibrinogen (11mg/dl) p-value .002, FII (11.5%) p-value .003, FV (16%) p-value .001, FVII (13.5%) p-value .001, FX (14.9%) p-value .001 and FXIII (14.8%) p-value .001 at 95% C.I with a cutoff p-value of .05. Change in the levels of other coagulation factors such as FVIII (4.5%), FIX (3.9%), FXI (6.1%), FXII (3.5%), and vWFAg. (7.61%) was not statistically significant (Table 4.1).

Table 4. 1: Mean of level of coagulation factors from baseline up to three months of storage

Variable	**Normal range**	**Mean at baseline**	**Mean after 1month**	**Mean after 2month**	**Mean after 3month**	**p-value**	**Interpretation**
FII (%)	50-200%	121.1	116.1	114.9	109.6	**0.003**	**Significant decrease**
FV (%)	50-200%	116.5	111	106.9	100.5	< 0.001	**Significant decrease**
FVII (%)	50-200%	112.6	110.6	106.4	99.1	$\mathbf{< 0.001}$	**Significant decrease**
FVIII (%)	50-200%	119.1	119.1	117.3	114.66	0.056	Not significant
FIX (%)	50-200%	115	115	113.5	111.1	0.38	Not significant
FX (%)	50-200%	110.7	105.9	101.1	95.8	$\mathbf{< 0.001}$	**Significant decrease**
FXI (%)	50-200%	122.1	122.1	120.8	116	0.07	Not significant
FXII (%)	50-200%	111.6	111.6	111	108.1	0.26	Not significant
FXIII (%)	50-200%	103.5	95.3	91.7	88.7	$\mathbf{< 0.001}$	**Significant decrease**
vWF (%)	50-200%	129.4	127	124.6	121.8	0.051	Not significant
Fibrinogen (mg/dl)	150-400	308.7	305.6	303.3	297.7	**0.002**	**Significant decrease**

4.1.1. Mean of factor V on time point versus sex, age, blood group and weight

The level of FV was found with the highest decrease of 5% at each observation regardless of sex, age, blood group and weight as shown in figure 4.1 below.

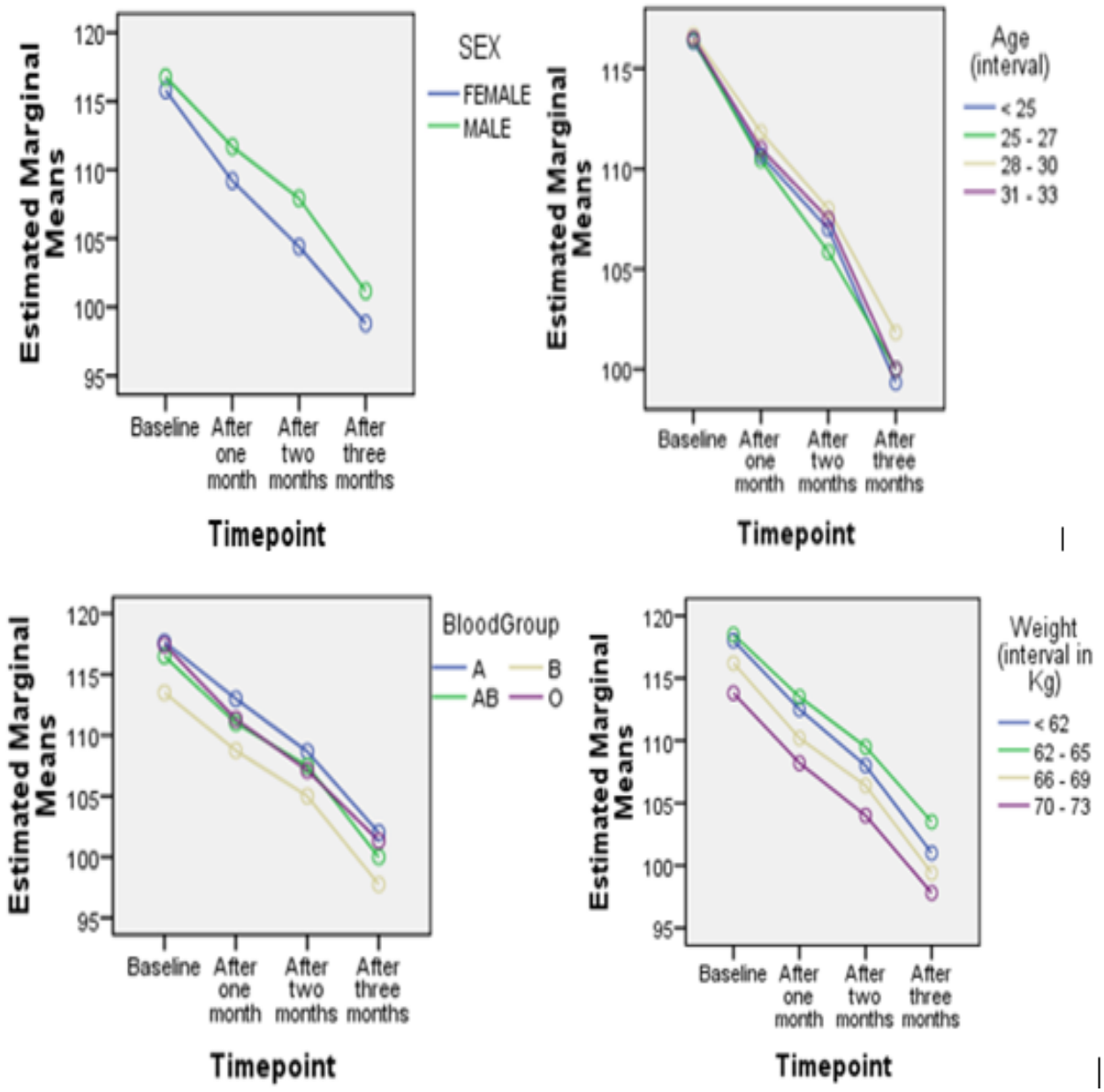

Figure 4. 1: Mean level of FV in % based on sex, age, blood group, weight and the time point (Where the X-axis shows the time point while Y-axis shows the estimated marginal mean)

4.1.2. Mean of factor VII on time point versus sex, age, blood group and weight

The decrease in factor VII was observed regardless of sex, age, blood group or weight as shown in figure 4.2 below.

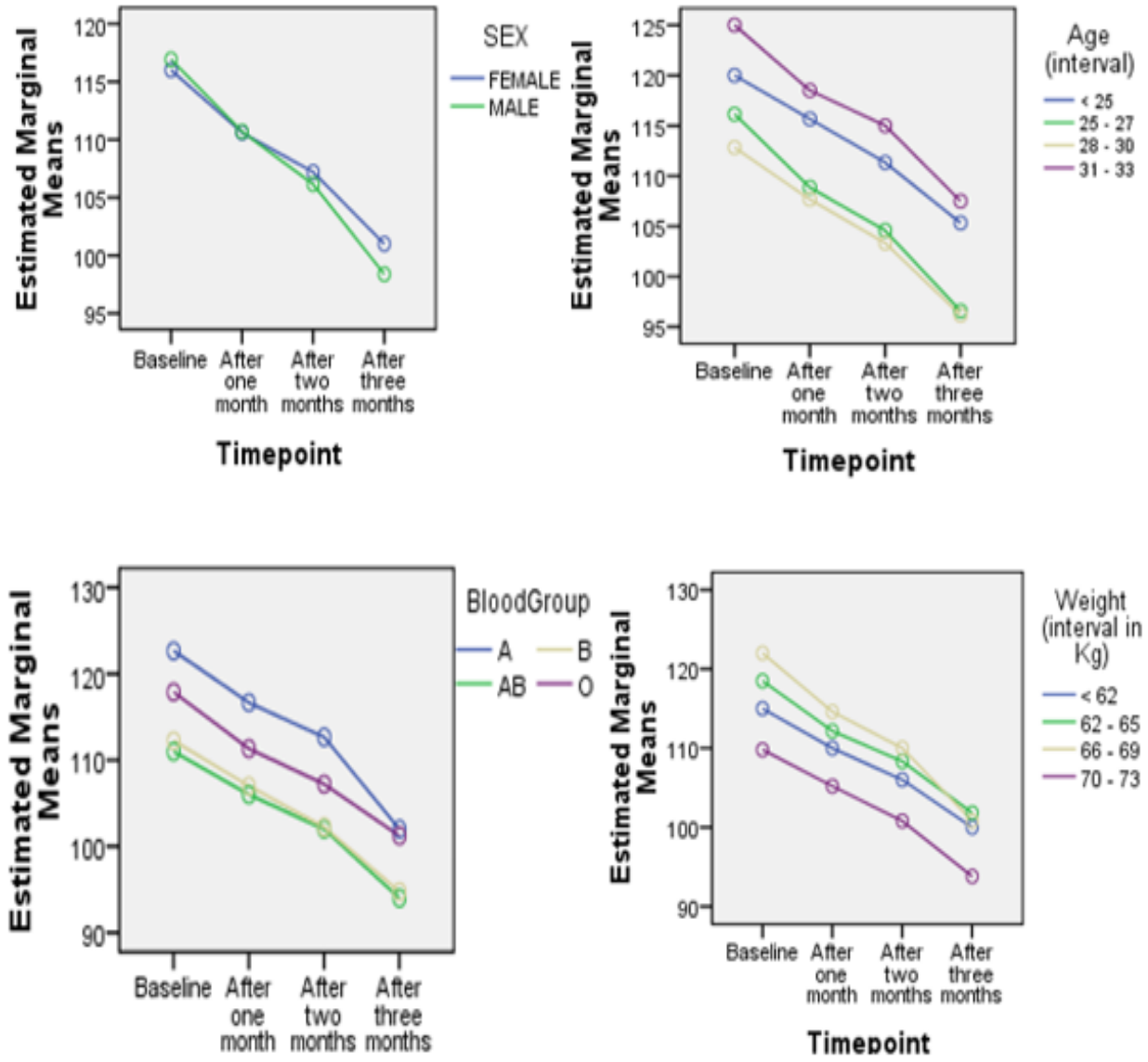

Figure 4. 2: Mean level of FVII in % based on sex, age, blood group, weight and the time point (Where the X-axis shows the time point while Y-axis shows the estimated marginal mean)

4.1.3. Mean of factor X on time point versus sex, age, blood group and weight

Factor X decreased regardless of sex, age, blood group or weight as shown in figure 4.3 below

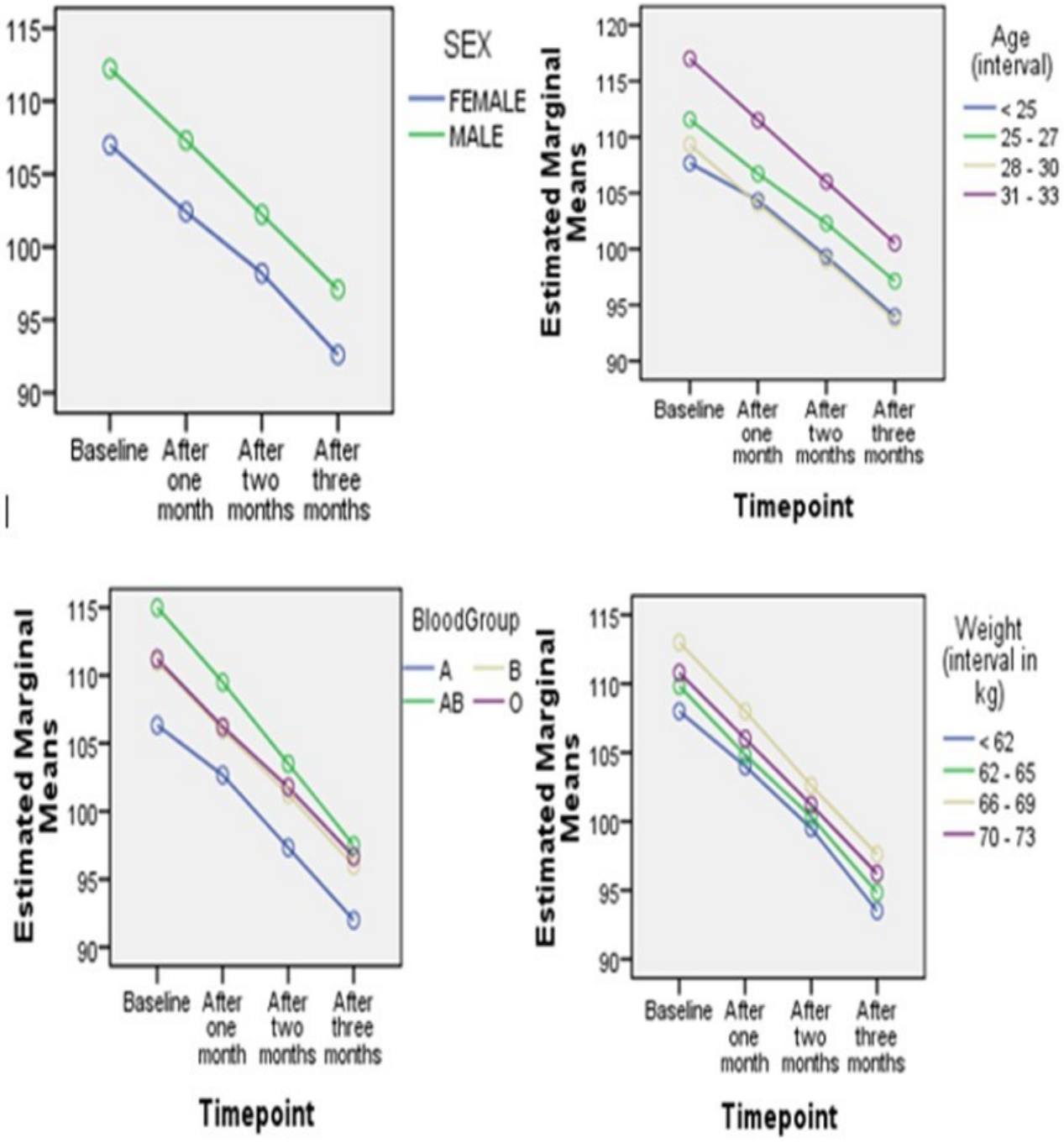

Figure 4. 3: Mean level of FX in % based on sex, age, blood group, weight and the time point (Where the X-axis shows the time point while Y-axis shows the estimated marginal mean)

4.1.4. Mean of factor XIII on time point versus sex, age, blood group and weight

The decrease in APTT was not found to be related to sex, age, blood group or weight of blood donors as shown in figure 4.4 below

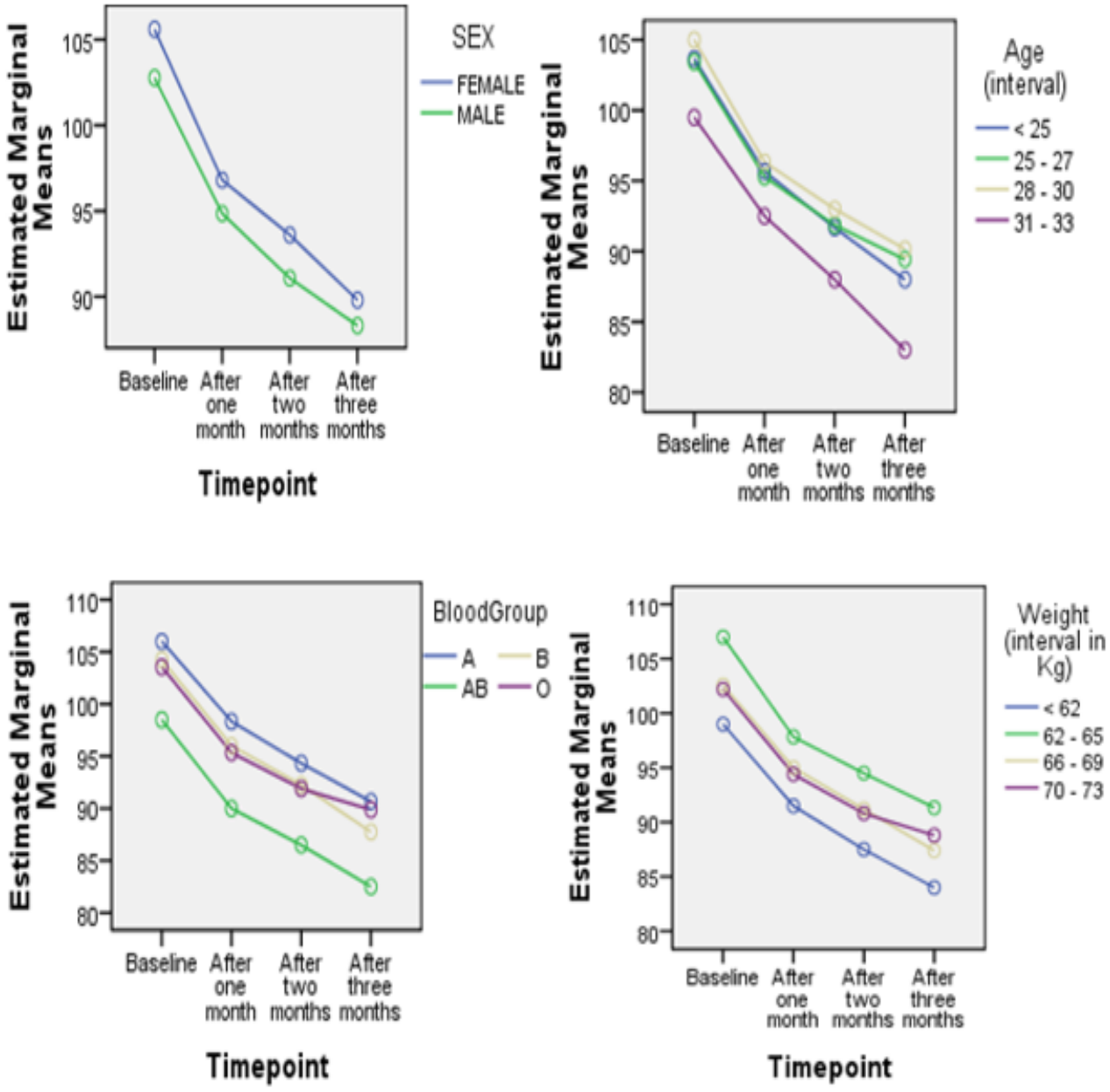

Figure 4. 4: Mean level of FXIII in % based on sex, age, blood group, weight and the time point (Where the X-axis shows the time point while Y-axis shows the estimated marginal mean)

4.2. Variation in inherited inhibitors

There was a statistically significant decrease in level of ATIII (6.7%) p-value .001 and PC (7%) p-value .003 at 95% C.I with a cutoff p-value of .05. No significant decrease was observed in FPS from baseline to three months of storage (Table 4.2).

Table 4. 2: Mean level of inherited inhibitors from the baseline to three month of storage

Variable	Mean at baseline	Mean at after 1month	Mean at after 2month	Mean at after 3month	p-value	Normal range	Interpretation
AT III	102.8	101.5	98.7	96.1	< 0.001	50-200%	**Significant decrease**
PC	104.3	102.5	100.1	97.3	0.003	50-200%	**Significant decrease**
PS	104	104	102.4	99.8	0.132	50-200%	Not significant

The decrease in ATIII was not related with sex, age, blood group and weight of blood donors as shown in the figure 4.5 below:

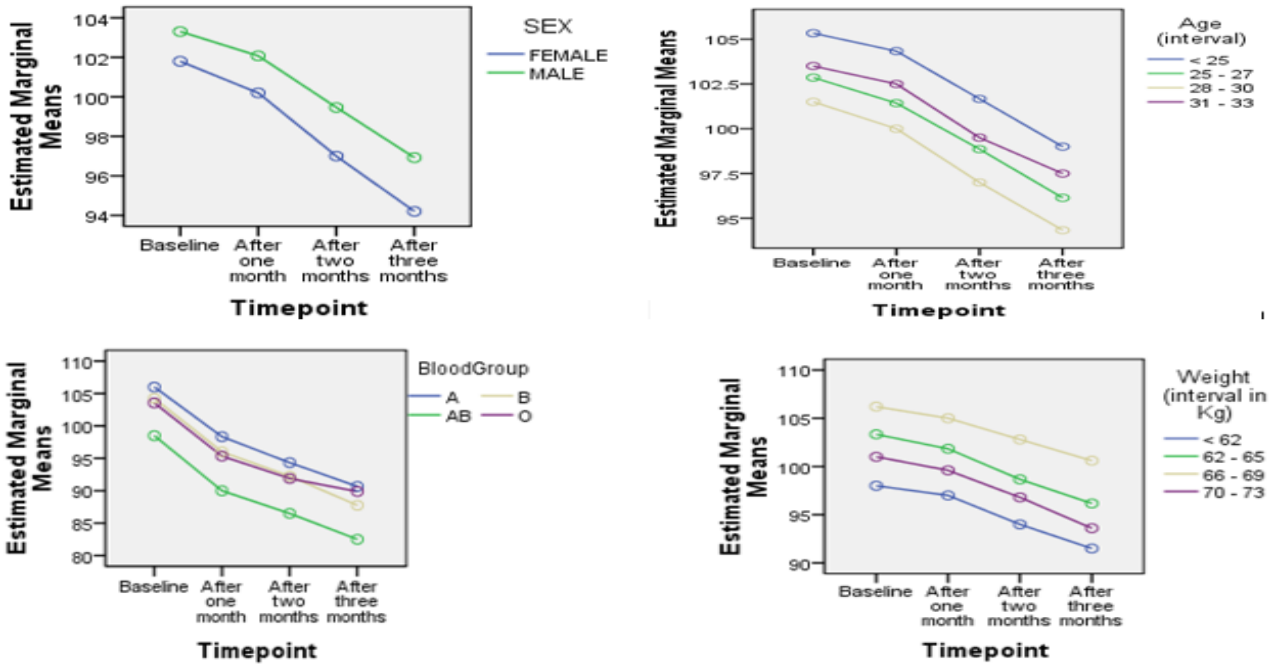

Figure 4. 5: Mean level of FXIII in % based on sex, age, blood group, weight and the time point (Where the X-axis shows the time point while Y-axis shows the estimated marginal mean)

4.3. Variation in Prothrombin Time

There was an increase of 1.56 seconds in PT between the baseline and month 1. No change was observed at month two and month three. The increase in PT was not statistically significant at 95% confidence interval with a cutoff p-value of 0.05 as shown in Table 4.3

Table 4. 3: Mean of PT from baseline to three months of storage

Variable	Normal range	Mean at baseline	Mean after 1month	Mean after 2month	Mean after 3months	p-value	Interpretation
PT (Seconds)	11-15	11.33	12.82	12.87	12.89	0.065	Not significant

PT increased regardless of sex, age, blood group or weight (Figure 4.6)

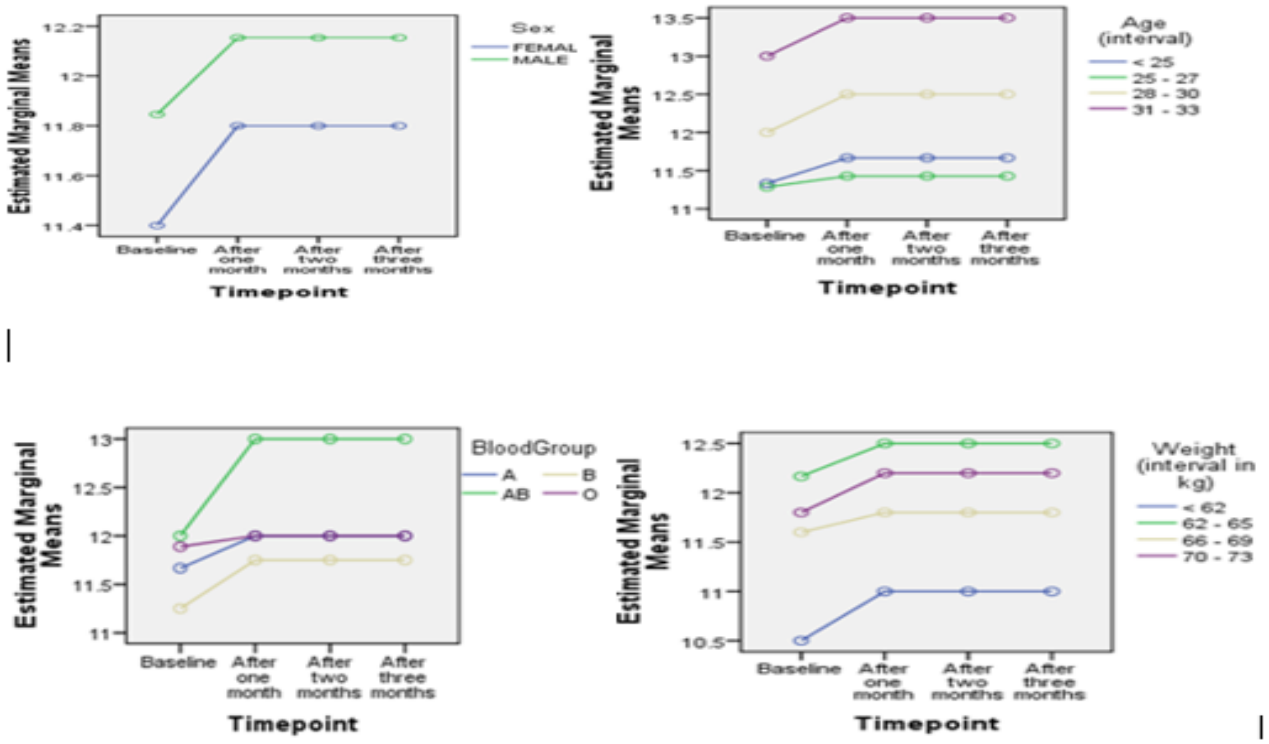

Figure 4. 6: Mean level of FXIII in % based on sex, age, blood group, weight and the time point (Where the X-axis shows the time point while Y-axis shows the estimated marginal mean)

4.4. Variation in APTT

The APTT mean increased from 26.32 seconds to 27.33 seconds, between baseline and one month. No change was noted at month two and three of storage. At 95% confidence interval with a cutoff p-value of 0.05, the increase of APTT was not statistically significant.

Table 4. 4: Mean of APTT from the baseline up to three month of storage

Variable	Normal range	Mean at baseline	Mean after 1month	Mean after 2month	Mean after 3month	p-value	Interpretation
APTT (seconds)	25-35	26.32	27.33	27.34	27.34	0.08	Not significant

The increase in APTT was not related to sex, age, blood group or weight of blood donors as shown in figure 4.7 below:

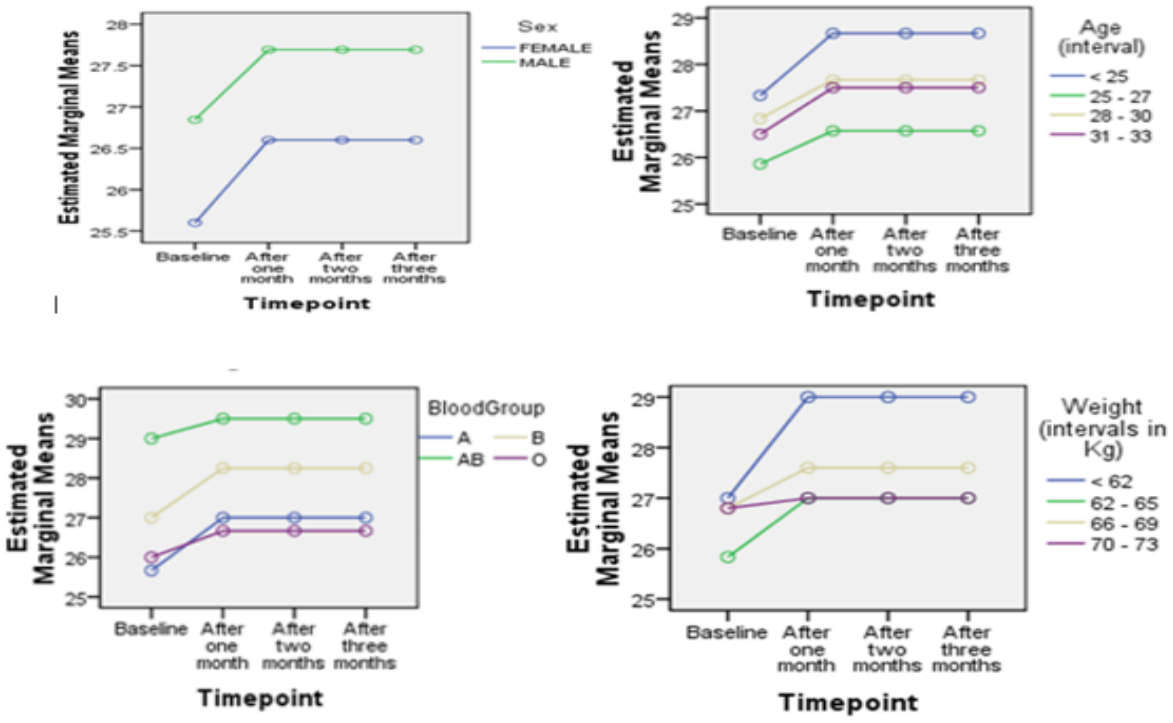

Figure 4. 7: Mean level of FXIII in % based on sex, age, blood group, weight and the time point (Where the X-axis shows the time point while Y-axis shows the estimated marginal mean)

CHAPTER FIVE

5.0 DISCUSSION

Fresh frozen plasma (FFP) is indicated for the treatment and prevention of bleeding disorders due to deficit in minor or major coagulation factors, management of massive bleedings, liver disease, disseminated intravascular coagulation or reverse anticoagulant therapy. FFP is used in transfusion medicine but it could be associated with many risks if used inappropriately. This study was aimed at determining the level of coagulation factors in fresh frozen plasma in Rwanda after being stored three months at minus 18^0 C.

The results of this study shows that all coagulation factors; inhibitors; FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII, vWF, FPS, PC and ATIII including PT and APTT, remained within the normal range (Table 4.1, Table 4.2, Table 4.3 and Table 4.4). However, these factors had variation in their levels at different periods. For example the PT and APTT showed an increase of 1.56 (p-value = 0.06) and 1.03 (P-value = 0.08) seconds respectively, from baseline up to one month of storage and from one month up to three months of storage these factors remained stable.

All clotting factors and inhibitors in this study remained within the reference range (Table 4.1 and Table 4.2) and these findings were similar to those obtained in Germany (Von Hermanny *et al.,* 2005. The levels of coagulation factors found in this study were varied from those found in Germany subjects. However these variations could be associated with a divergent in temperatures and time period of storage. The decrease of coagulation factor level in FFP is always directly proportional to the time period and temperature of storage (AABB, 2002).

Contrary, the reported decrease of coagulation factor level in this study were however lower than those reported among American subjects within 24hours over 5 days of storage at 1 to 6°C, for FV, FVII and FVIII activity in 40%, 20% and 50% respectively at day 5 (Mark *et al., 2007*).

However, vWF activity remained within its normal range by Day 5 (Mark *et al.*, 2007). The observed variation could have possibly been led by varied temperature and frozen time after phlebotomy.

Nevertheless, these findings in regard to stability of FV, (Nifong *et al.,* 2002) was slightly different from this study where a decrease was statistically significant (P value < 0.001). The significant decrease of FVII levels in our study was similar to those observed by Ben-Tal *et al*. but contrary to FX which was stable in their study (Ben-Tal *et al.,* 2003*).* However, similar findings were observed regarding levels in decreases for FX factor after thawing (Christian *et al*, 2009).

In a study conducted by Agus N. *et al.* (2012) at blood hold at 4°C overnight resulted in a 25% loss of FVIII ($p<0.05$) and in a 9% loss of FIX (not significant). However, in this study, the levels were lower.. The difference observed in this study may have been attributed to the different storage conditions (temperature and time or length of storage) and sample size differences and storage temperatures.

The increase in PT and APTT was recorded irrespective of sex, age, blood group and body weight of blood donors. The results of this study are comparable to the findings done on coagulation factor levels in plasma frozen within 24 hours of phlebotomy over 5 days of storage at minus 18°C (Mark *et al.,* 2007) in which modest prolongations of PT and APTT 1.56 and 1.03 seconds were recorded respectively. The fairly similar findings of these two studies indicate that plasma stored at minus 18ºC could be safely used after three months as recommended by European Guidelines for blood bank, 2007.

The findings of this study in PT and APTT were however prolonged than the time reported in previous studies in USA (Von Heymann *et al.,* 2006; Thompson *et al*, 2006). This difference could be associated with diverse storage time and temperature.

CHAPTER SIX

6.0 CONCLUSION AND RECOMANDATIONS

6.1. Conclusion

- This study found a significant decrease in fibrinogen, FII, FV, FVII, FX, FXIII, ATIII and PC, in the measurements at the baseline, after one month, two months and three months of FFP storage.
- Change in the levels of other coagulation factors tested for (FVIII, FIX, FXI, FXII and vWF) and inherited inhibitor (PS) were not statistically significant. These coagulation factors were stable from the baseline up to three months of storage.
- The findings of this study reveal that all coagulation factors and inhibitors could still be retained in normal range in fresh frozen plasma stored at -18 ºC for three months.

6.2. Recommendations

Although the findings of this study reveal that all coagulation factors and inhibitors in plasma could still be retained in normal range in fresh frozen plasma stored under minus 18ºC for three months, there is a need to determine the retention of these factors after 12 months of storage.

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APPENDICES

Appendix 1: English; blood donor medical questionnaire

BLOOD DONOR NUMBER	DONATION NUMBER

BLOOD DONORMEDICAL QUESTIONNAIRE

Tick where applicable ☒
Please answer the following questions correctly. This was help to protect you and the patient who receives your blood. Thank you!

Names:

……..……

Sex: Male ☐ Female ☐

Date of Birth: ……..….. Place of Birth (District-Sector):

……

Occupation: …….

Place of Residence

District: ……..……..…

Cell: ……..……..…

Sector:……..… Village (Umudugudu): ……..…

Telephone: ……..……..… E-mail: ……..…….

Are you married? Yes☐ No☐

Have you donated blood previously Yes☐ No☐ , If yes, how many times?.....

What is the date of the last donation:…… ……Where? ……

Did you have any discomfort during/after donation? Yes☐ No☐

BLOOD DONOR DECLARATION

I understand that I should not donate blood if:

- I consume drugs or use illegal intravenous drugs.
- I have HIV AIDS
- I have or have had sex with a partner of the same sex (Even using condom)
- My sexual partner has HIV/AIDS, hepatitis or any other Sexually Transmitted Infection.

I understand that I should wait for 6 months to donate blood if:

- I have had sex with a person who is not my spouse even using condoms
- I don't trust my partner even if I use condoms
- I have not got any HIV/AIDS test before marriage

QUESTIONS TO THE BLOOD DONOR (TICK THE APPROPRIATE ANSWER☒

Today

HAD YES NO

1	Are you feeling healthy and well?		
2	Are you taking any medication?		
3	Do you have any wound		

In the past 48 hours

1	Have you taken Aspirin?		

In the past 3 months

1	Have you had Malaria?		

In the past 6 months have you had

1	Weight loss?		
2	Repeated diarrhea?		

DO YOU HAVE OR HAVE YOU EVER

1	Heart Disease?		
2	Kidney Disease?		
3	Cancer?		
4	Epilepsy?		
5	Asthma?		
6	Diabetes?		
7	Tuberculosis?		
8	Abnormal bleeding tendency?		
9	Hepatitis B?		
10	Hepatitis C?		

3	Swollen glands?		
4	Continuous low – grade fever?		
5	Tattooing?		
6	Ear piercing?		
7	Dental Extraction?		
8	Surgery or circumcision?		
9	Blood transfusion?		
10	Endoscopy?		

11	HIV/AIDS		
12	Syphilis?		
13	Gonorrhea?		

For female blood donors only

1	Are you pregnant?		
2	Do you have a child who is under 12 months		
3	Have you had abortion in the last 6 months?		

I am not donating to receive an HIV test:

I agree to have my blood tested for HIV, Hepatitis B& C, and Syphilis, and haemostatic tests (APTT, PT, and Coagulation factor and inhibitors levels) and any other test that is required to ensure my blood is safe to give to another person and for research purpose. I know that someone may be infected and have a negative test. I understand that I was be notified of my test results whatever they are.

I have carefully read education materials and answered all the questions truthfully. I understand that not being honest while answering questions on this form is a serious matter and a lie could harm another person. I understand the eventual side effects of my donation.

I agree that the National Center for Blood Transfusion may use my blood as needed. I have had an opportunity to ask questions and all my questions were answered.

_____ _____ _____

Date Blood donor Signature Name and Signature of the Nurse

MINI-PHYSICAL EXAMINATION (Filled by Investigator):

	Value	Initials
Scale Wt		
Pulse		
Hb		
BP		
T°		

		Initials
Start time		
Stop time		
Quantity to bedrawn		
Blood donnor reaction		
Reaction management		

Comments :_____
_____
_____

Eligible ☐ Non-éligible ☐ Reason_____

Appendix 2: French; blood donor medical questionnaire

BLOOD DONOR NUMBER	DONATION NUMBER

QUESTIONNAIRE MEDICAL CONFIDENTIEL

Cochez où c'est nécessaire ☒

Répondez honnêtement aux questions suivantes. Cela vous protégera ainsi que le patient qui recevra votre sang. Merci beaucoup !

Noms:……..

Sexe: Masculin : ☐ Féminin : ☐

Date de naissance:…… Lieu de naissance (District-

Secteur):……..

Profession : …….....

Lieu de résidence

District: …….…..…….

Cellule(Akagari): ……

Secteur:……..……..

Village(Umudugudu): ……..…….

Téléphone: …….

E-mail: ……..……

Etes-vous marié(e) Oui ☐ Non ☐

Avez-vous déjà donné du sang? Oui ☐ Non ☐ si oui, combien de fois?.....

Quand avez-vous donné la dernière fois ?

……Où ?.....

Le dernier don de sang s'est-il bien déroulé? Oui ☐ Non ☐

DECLARATION DU DONNEUR

J'ai compris que je ne suis pas éligible au don de sang si:

- Je consomme de la drogue ou je prends de la drogue par voie veineuse

- J'ai le virus du VIH /SIDA
- Je fais ou j'ai fait des rapports sexuels avec une personne de même sexe que moi (même en utilisant le préservatif)
- Mon partenaire sexuel a le VIH/SIDA, l'hépatite virale ou autre Infections Sexuellement Transmissibles.

J'ai compris que je dois attendre 6 mois avant de donner du sang si:

- Je fais ou j'ai fait des rapports sexuels avec une personne qui n'est pas mon mari ou mon épouse même en utilisant des préservatifs.
- Mon partenaire sexuel a des comportements douteux même si j'utilise des préservatifs.
- Je n'ai pas fait de test VIH/SIDA avant le mariage.

COCHEZ LA REPONSE APPROPRIEE :

	Aujourd'hui	**OUI**	**NON**
1	Vous sentez-vous bien, en bonne santé ?		
2	Avez-vous pris les médicaments ?		
3	Avez-vous une blessure ?		
	Durant ces 48 dernières heures		
1	Avez-vous pris de l'Aspirine ?		
	Durant ces 3 derniers mois		
1	Avez-vous souffert de la Malaria		
	Durant ces 6 derniers mois avez-vous eu		
1	Une perte de poids inexpliquée ?		
2	Diarrhées à répétition ?		
3	Adénopathies ?		
4	Fièvre au long cours ?		

	Dans votre vie Avez-vous eu:	**OUI**	**NON**
1	Les troubles cardiaques		
2	Une maladie des reins		
3	Un Cancer		
4	Des crises d'épilepsie		
5	Des crises d'asthme		
6	Le Diabète		
7	La Tuberculose		
8	Une tendance anormale aux saignements ?		
9	Une maladie due à l'Hépatites B		
10	Une maladie due à l'Hépatites C		
11	Le SIDA		
12	La Syphilis		

5	Un tatouage ?		
6	Un perçage d'oreille ou d'une autre partie du corps ?		
7	Extraction dentaire		
8	Chirurgie ou circoncision ?		
9	Transfusion ?		
10	Endoscopie ?		

13	La Gonorrhée		

Pour les femmes ou les filles

1	Êtes-vous enceinte?		
2	Avez-vous un enfant âgé de moins d'1 an ?		
3	Avez-vous présenté une fausse couche (6 derniers mois)?		

Je ne donne pas du sang pour Juste connaitre mon statut sérologique (VIH) :

Je sais que mon sang sera soumis à un test de dépistage du VIH/SIDA et d'autres marqueurs de maladies transmissibles par le sang et des tests hémostatiques. Mon sang fera l'objet d'une validation biologique et de recherche. Je sais que quelqu'un peut être infecté et avoir un test négatif.

En outre, Je comprends que je serai avisé des résultats des tests effectués sur mon don quels qu'ils soient.

J'ai lu attentivement toutes les informations relatives au don du sang et répondu à toutes les questions honnêtement. Les renseignements que j'ai fournis sont, pour autant que je puisse en juger, exacts et complets. Je comprends que ne pas être honnête, tout en répondant à des questions sur ce formulaire est une affaire sérieuse et un mensonge pourrait nuire à une autre personne, même moi-même.

J'ai compris la procédure du don du sang et les effets secondaires éventuels de mon don. Je donne l'autorisation au Centre National de Transfusion Sanguine(CNTS) d'utiliser mon sang. J'ai eu l'occasion de poser des questions et toutes mes questions ont été répondues.

_____ _____ _____

Date Signature du donneur Initiales et Signature de l'examinateur

MINI-EXAMEN PHYSIQUE (Rempli par le chercheur)

	Valeur	Time	Initials
Poids			
Pouls			
Hb			
TA			
T°			

		Initials
Heure début prélèvement		
Heure fin prélèvement		
Quantité à prélever		
Réactions post don		
Conduite tenue		

Commentaires :……
……
……

Eligible : ☐ Non-éligible : ☐aison_____

Appendix 3: Kinyarwanda; blood donor medical questionnaire

BLOOD DONOR NUMBER	DONATION NUMBER

IBIBAZO BIGENEWE UTANGA AMARASO

Shyiraakamenyetsokogukubaahahuyen'igisubizocyawe. ☒

Subizanezaibibazobakubazahasi.Ibibizakurindakandibirinden'uzahabwaamarasoyawe.

Urakozecyane!

Amazina: ……....

Igitsina: Gal☐ G☐

Igihewavukiye: …… Ahowavukiye(Akarere-Umurenge):

….....

Icyoukora: .....

<u>Ahoutuye</u>:

Akarere: ..... Akagari: .....

Umurenge:..... Umudugudu: .....

Telefone: ..... E-mail: .....

Warashatse?YegoOya ☐ ☐

Wigezegutangaamaraso ?YegoOy☐ baariye☐ wayatanzekangahe?……

Itarikiyanyumawayatanze ni ryari ?..... Wayatangiyehe? ……

Ubwouherukagutangaamarasohariikibazowagize? YegoOya☐ ☐

AMABWIRIZA AREBANA N'UMUNTU USHAKA GUTANGA AMARASO:

Nzi neza ko ntakwiriyegutangaamarasoniba:

- Nywaibiyobyabwengecyangwambyitera mu mitsi
- Mbana n'ubwandubw'agakokogatera SIDA

- Narakoranyecyangwankoranaimibonanompuzabitsinan'uwoduhujeigitsina(nkoreshacyangwantakoreshaag akingirizo)
- Uwodukoranaimibonanompuzabitsinaarwaye SIDA, indwara y'umwijimacyangwaindindwarayandurira mu mibonanompuzabitsina.

Nzi neza ko nkwiriyegutegerezaamezi 6 mbereyogutangaamarasoniba:

- Narakoranyecyangwankoranaimibonanompuzabitsina n'uwotutashakanyeniyonabankoreshaagakingirizo
- Ntizeyeuwodukoranaimibonanompuzabitsinaniyonabankoreshaagakingirizo
- Narashyingiwentipimishijeagakokogatera SIDA

IBIBAZO BIGENEWE UTANGA AMARASO

HITAMO IGISUBIZO GIKWIRIYE UKORESHEJE AKAMENYETSO ☒ O GUKUBA

UyumunsiWabaurwayecyangwawarigezekurwaraYEGO ☐ YA ☐

1	Urumvaumezeneza ?		
2	Hari imitiurimoufata ?		
3	Hariahantuwabaufiteigikomere?		

Mu masaha 48 ashize

1	WabawafasheAspirine		

Muri aya mezi 3 ashize

1	WabawararwayeMalariya		

Mu mezi 6 ashizewaba :

1	Waratakajeibiro		
2	Ugiraimpiswiikiraikongeraikagaruka		
3	Ujyaugirainturugunyu		
4	Ujyaugiraumurirouhoraho		
5	Warisharatujekuruhu, waraciweimanzicyangwaindasago		
6	Waritobojeamatwi		
7	Warikujeiryinyo		
8	Warabazwecyangwawarisiramuje		

1	Umutima		
2	Impyiko		
3	Kanseri		
4	Igicuri		
5	Asima		
6	Diyabete		
7	Igituntu		
8	Gukomerekaamarasontakame		
9	Umwijimawo mu bwokobwa B		
10	Umwijimawo mu bwokobwa C		
11	SIDA		
12	Mburugu		
13	Imitezi		

Mugorecyangwamukobwawaba

1	Utwite?		
2	Ufiteumwanaurimunsi y'amezi 12 ?		

9	Warateweamaraso		
10	Warapimweigifuhakoreshejweumupira(andoskopi)		

3	Waragizeikibazocyokuvamo kw' inda mu mezi 6 ashize?		

Ntabwonjegutangaamarasongamijekwipimishaagakokogatera SIDA:

Nemeyekoamarasongiyegutangaazapimwamoagakokogateraubwandubwa SIDA, Umwijimawomubwokobwa B na C, mburugucyangwaibindibizaminibyangombwakugirangoamarasontanzeabentakibazoyaterauzayahabwa, ibizaminibyokurebainganoy'imisemburoitumaamarasoahagararakuva.Nzinezakoumuntuashoborakubayaranduye maze ibizaminintibigaragazeuburwayi.Nemeyekandikonzakiraibisubizonubwobyababigaragazakomfiteubwandubwa SIDA cyangwa se izindindwarazandurira mu maraso.

Nasomyenezainyigishokandindemezarwosekonasubijenezaibibazonabajijwentahombeshyenagatokukonzikokutavugis haukuribishoborakugiraingarukambikumuntuwahabwaamarasoyanjyenanjyeubwanjye.
NemeyekoIkigocy'IgihugucyoGutangaAmarasocyakoreshaamarasoyanjyenkukobikwiye.
Ibibazonarimfitenabibajijekandinabisubijebyose.

Itariki **Izinan'umukonobyamuganga**

ISUZUMA (Byuzuzwan'umushakashatsigusa):

	Valeur	Time	Initials
Poids			
Pouls			
Hb			
TA			
T°			

		Initial
Heure début prélèvement		
Heure fin prélèvement		
Quantité à prélever		
Réactions post don		
Conduite tenue		

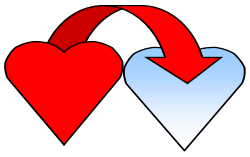
Icyitonderwa : _____

Wujujibisabwa ☐ Utujujibisabwa ☐ Impamvu_____

Appendix 3: Whole blood collection standard operating procedure

RWANDA BIOMEDICAL CENTER

A Healthy People. A Wealthy Nation



MEDICAL PRODUCTION AND PROCUREMENT | NATIONAL CENTRE FOR BLOOD TRANSFUSION (MPP/NCBT)

STANDARD OPERATING PROCEDURE (SOP)

WHOLE BLOOD COLLECTION

Authors:

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- **UTAMULIZA Clarisse, Donor Selection Officer**
- **MUJIJIMA Chantal, Phlebotomist**
- **NAMAHORO Yvette, Donor Selection Officer**
- **HAKIZIMANA Dieudonné, Phlebotomist**

Effective Date:	**Copy №:**

Approvals and Authorization				
Approved By	**Title**	**Names**	**Signature**	**Date**
	Process owner/Head of Department:			
	Quality Officer:			
	Medical Director:			

Authorized By	Head of NCBT Division:			

Document history

Version	Date	Author(s)	Main Changes
1.0	18-03-2011	- DUSHIME Gratia - UMURAZA Laetitia	N/A
2.0	09-07-2012	- NAMAHORO Yvette - MUJIJIMA Chantal - GAHONGAYIRE Félicité - HAKIZIMANA Dieudonne - UTAMULIZA Clarisse	- Format change - Addition of steps in the procedure

Purpose	This procedure provides guidance on how to collect whole blood units from a donor while providing a safe and comfortable environment for the donor during the donation process.
Scope	This procedure is applicable to all NCBT centers collecting blood. It is applicable to the fixed site as well as in mobile session. All Donor selection Officers and Phlebotomists in NCBT must follow this SOP.
Related procedures/ documents	1. COL_SOP_15_Use and maintenance of the collection scale/mixer 2. FRM_DON_SOP_01A_V02.0 (DMQ Kinyarwanda) 3. FRM_DON_SOP_01B_V02.0 (DMQ English) 4. FRM_DON_SOP_01C_V02.0 (DMQ French) 5. COL_SOP_06_Disinfection of the Venipuncture site 6. DON_SOP_05_Management of donor reactions 7. COL_SOP_09_Post donation care 8. COL_SOP_08_Packaging Blood Units and Samples for transport
Materials and reagents needed	1. Clips 2. Plastic hemostat 3. Scissors 4. Test tube racks 5. Containers for sharps (safety box) 6. Waste bin for non-sharp biohazard wastes 7. Scale/Mixer 8. Adjustable automatic donor scales 9. Beds for phlebotomy 10. Adhesive tapes 11. Labeled blood bags and tubes 12. Hand gripper 13. Hand sealer clips 14. Tube stripper 15. Pens 16. Gauzes 17. Gauzes containers 18. Tray 19. Trolley

Safety requirements	Refer to the safety plan
Sample preparation requirements	N/A
Reagent preparation requirements	N/A
Sample preparation	N/A

Donor reception	**Step**	**Action**
	1	Greet the donor cordially
	2	Tell the donor to lie down on the collection bed
	3	Ask the donor his/her names
	4	Compare the initials on the blood bag and tubes with the donor names. If the donation number and names are non-concordant, consult the donor registration officer for correction.
	5	Briefly explain to the donor the donation process by emphasizing on the following: • The venipuncture action (not harm) • The volume to be collected
	6	Reconcile the numbers and donor names on the tubes, bags, DMQ
Verification of the integrity of collection bags and tubes	**Step**	**Action**
	1	Verify the expiration date of the bags and the tubes
	2	Inspect the bag for any defects and discoloration.
	3	Apply pressure to check for leaks
	4	Verify the integrity of the bag and the appearance of the solution.
	5	If the bags appearance is abnormal, find a new bag from the donor registration officer and make sure for the same identification as previous one.
	6	Put the bag and its satellite bags to the scale mixer or Adjustable automatic donor scale in accordance with the procedure of use of the scale,
Selection of the	**Step**	**Action**

vein	1	Perform arm inspection and choose the arm on which the venipuncture was be performed Consider the followings: a) Best position for venipuncture b) Observation from arm inspection (Scar, malformation, skin diseases) c) Donor preference d) Availability and accessibility of the vein
	2	Apply and fix the tourniquet approximately 5-10 cm above antecubital area
	3	Ask donor to squeeze hand squeezer.
	4	Palpate donor's veins
	5	Select the best vein in order of preference: a) 1st choice: Median cubital b) 2nd choice: Cephalic c) 3rd choice: Basilic
	6	Repeat Step 1-5 above on another arm if the vein is not found on the first arm.
	7	Consult experienced staff if a suitable vein cannot be identified.
Disinfection of the venipuncture site	**Step**	**Action**
	1	Disinfect the venipuncture site in accordance with the procedure for disinfection of the venipuncture site (COL_SOP_06_ Disinfection of the Venipuncture site).
Performing phlebotomy using blood bag without diversion pouch	**Step**	**Action**
	1	Clamp the tubing of the bag at 10-15 cm next to the needle of the bag by a plastic hemostat
	2	Remove the needle protective cover and check the status of the needle before use
	3	Anchor the vein by holding the arm below the prepared area
	4	Point the needle, bevel up, in the direction of the vein. **Note**: Perform the venipuncture as soon as possible to prevent contamination of donor arm and material.
	5	Firmly pierce the skin at about a 15 – 20 degree angle and advance the needle horizontally into the vein.
	6	Open hemostat and verify blood is flowing into the tubing. If not flowing adequately. Refer to section below on Troubleshooting
	7	Stabilize the needle by holding the tubing to the arm of the donor and secure using an adhesive tape.
	8	Cover the venipuncture site with sterile gauze square and fix it with an adhesive tape.

	9	Record immediately the start collection time on the Donor Medical Questionnaire (DMQ)
	10	Loosen tourniquet slightly.
	11	Ask the donor to open and close hand every 10 to 12 seconds during collection.
	12	If no mixer is being used, gently mix the blood and anticoagulant by inverting the bag at least three times as it flows into the bag and periodically (approximately every 1 minute) throughout the collection process. A complete end-to-end mixing of the bag is required.
	13	Monitor donor and blood flow: For slow running units, assess troubleshooting needs. Refer to Troubleshooting section.
	14	If donor appears to have a reaction, refer to Management of Donor Reactions SOP. **Note:** Keep the donor under observation throughout the donation process. The donor should never be left unattended during or immediately after donation
Ending the phlebotomy when using blood bag without diversion pouch	**Step**	**Action**
	1	Note: The scale mixer end collection if the drawn quantity is achieved Clamp tubing near the diversion using a clip and hand sealer clips
	2	Record the end time on the DMQ
	3	Clamp the tubing using a hemostat and cut using scissors
	4	Draw sample for 2 tubes (one EDTA tube and one plain tube) from the tubing in donor vein. Mix the EDTA tube by gently inverting it at least 3 times.
	5	Release the tourniquet and remove the previously fixed gauze and tape from the venipuncture site.
	6	Find gauze, hold it on the donor arm above the venipuncture site and remove the needle.
	7	Ask the donor to raise the arm and hold the gauze firmly with the other hand
	8	Put the safety protective cover to the needle gently
	9	Don't recover the needle with the removed cover
	10	Discard the remaining tubing into the Biohazard container for sharps
	11	Immediately after discontinuing the phlebotomy, strip the tubing on the blood unit as completely as possible (at least twice) into the bag, starting at seal.

	12	Mix the blood in the collected unit by inverting bag several times to mix thoroughly and perform this step at least 3 times.
	13	Keep the collected units at room temperature for at least 2 hours to allow blood to cool. Do not put blood units directly into the containers with ice packs
Performing Phlebotomy using blood bag with diversion pouch	**Step**	**Action**
	1	Close white pinch clamp for 2 tubules of the needle and diversion pouch of the bag by a plastic hemostat provided with the bag
	2	Remove needle cover and check the status of the needle before use.
	3	Anchor the vein by holding the arm below the prepared area.
	4	Point the needle, bevel up, in the direction of the vein.
	5	Firmly pierce the skin at about a 15-20 degree angle and advance the needle horizontally into the vein.
	6	Open hemostat of the diversion pouch to let the first 10 mL to flow into the diversion pouch.
	7	Verify blood is flowing into the tubing-If not flowing adequately, refer to section below on Troubleshooting.
	8	Stabilize the needle by holding the tubing to the arm of the donor and secure using an adhesive tape.
	9	Cover the venipuncture site with sterile gauze square and fix it with an adhesive tape.
	10	Record immediately the start collection time
	11	Loosen tourniquet slightly.
	12	When the Diversion pouch is full, close white pinch clamp and tighten the loose knot in the tubing between the sample collection pouch and the intersection with the main tubing.
	13	Break snap-open closure completely to allow blood to flow to the primary collection bag by: a) Holding the snap-open closure with both hands b) Bending in two directions. An audible click was be heard in each direction.
	14	Take 2 samples by attaching the vacutainer tube to adapter of the Diversion pouch and mix sample thoroughly if EDTA tube is used.

	15	If not using a scale mixer, gently mix the blood and anticoagulant as it flows into the bag and periodically (approximately every 1minute) throughout the collection. A complete end-to-end mixing of the bag is required.
	16	Ask the donor to open and close hand every 10 to 12 seconds during collection.
	17	Monitor donor and blood flow: For slow running units, assess troubleshooting needs. Refer to Troubleshooting section.
	18	If donor appears to have a reaction, refer to Management of Donor Reactions SOP. **Note:** Keep the donor under observation throughout the donation process. The donor should never be left unattended during or immediately after donation
Ending the phlebotomy when using blood bag with diversion pouch	**Step**	**Action**
	1	End the phlebotomy following the steps 1 to 13 above for ending the phlebotomy when using blood bag without diversion pouch except step 4 for drawing sample for 2 tubes from the tubing in donor vein.
Discontinue Phlebotomy **in Emergency**	**Step**	**Action**
	1	Note: Discontinue a donor collection procedure in an emergency situation, i.e., donor has a donor reaction during collection. Clamp tubing near needle hub.
	2	Release and remove the tourniquet.
	3	Fold gauze in half and place over phlebotomy site.
	4	Remove the adhesive tape from tubing and hold the tubing near the needle steady.
	5	Remove needle from donor's arm
	6	Immediately apply pressure to site and react according the situation
	7	Apply a bandage to the puncture site arm and give post donation instructions
	8	Refer to the appendix "COL_SOP_07_Whole Blood Collection_A04_V02.0(Phlebotomy Exceptions)
	9	Notify the team leader or designee.

Inspection of the Venipuncture site and Application of Bandage

SITUATION	ACTION
If bleeding has stopped	Place the tape to the used gauze if no bleeding occurred.
If bleeding occurred,	Change the gauze before applying the tape
If bleeding continues,	1. Elevate arm again for approximately 1 minute, 2. Apply pressure to phlebotomy site, 3. Apply new gauze and tape once bleeding stops.
If bleeding persists after arm is wrapped and verbal donor consent for treatment is obtained,	1. Apply ice pack , 2. Allow donor to hold ice on the phlebotomy site, 3. Remove ice after approximately 2 minutes and check donor's arm to verify bleeding has stopped, 4. Continue ice pack until bleeding stops, Note: Ice should never be "wrapped" onto donor's arm,
If a hematoma develops,	Refer to the procedure for management of donor reactions

Post Phlebotomy Instructions

Step	Action
1	Keep the donor on donor bed for at least 5 minutes after donation
2	Instruct the donor to keep the bandage on for 2 hours once bleeding stops.
3	Instruct the donor to refrain from strenuous activities such as mountain climbing, running marathons and flying an airplane for at least 72 hours following the donation
4	Advise the donor that smoking or alcohol intake within 6 hours after donating may cause dizziness
5	Advise the donor that If He/ She get dizzy, to sit down and put the head between his/her knees or lie down with his/her legs elevated
6	Instruct the donor to eat well at the next meal
7	Instruct the donor to drink extra fluids, twice the normal intake
8	Advise the donor that normal activities may be resumed when He/She is finished with the refreshments
9	Advise the donor that If He/ She develops fever, chills or diarrhea within 24 hours, after donating please to call the NCBT "Hotline" number at 1011
10	Thank the donor and direct the donor in the refreshment area(Refer to Post donation care procedure)

Limitations (or Notes), if applicable	1. Blood collection must only be done using sterile, single use pyrogen free blood collection bags and using a closed system. If the collection bag sterility is questionable do not use it. 2. For phlebotomy exceptions Refer to the Phlebotomy Exceptions Appendix. 3. Routinely gloves are not used during phlebotomy, if you have got any skin scratches make sure to wear gloves while performing phlebotomy and change them between donors. 4. Use hand sanitizer in accordance with procedure.
References	AABB Technical manual, 17th Edition AABB standards for Blood Banks and Transfusion Services, 27th edition

Appendix 4: Standard operating procedure for fresh frozen plasma preparation

RWANDA BIOMEDICAL CENTER

A Healthy People. A Wealthy Nation

STANDARD OPERATING PROCEDURE (SOP)

FRESH FROZEN PLASMA PREPARATION

Authors:

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Effective Date:		**Copy №:**		
Approvals and Authorization				
	Title	**Names**	**Signature**	**Date**
Approved By	Process owner/Head of Department:			
	Quality Officer:			
	Medical Director			
Authorized By	Head of NCBT Division:			

Document history

Version	Date	Author	Main Changes
1.0	18-03-2011	- UWINKINDI Domitille - MUKARUZAMBA Laurence - UWISANGA Agnes	N/A
2.0	09-07-2012	- UWINKINDI Domitille - NDAHIRIWE Olivier - UWISANGA Agnès	Format change Title included on the first page Changes in steps of the procedure

Purpose This SOP provides guidance on preparation of Fresh Frozen Plasma (FFP) from whole blood units.

Scope This SOP applies to all CNTS Centers which prepare FFP. All staff in components preparation must follow this SOP

Related documents

1. COM_SOP_10_Visual inspection of blood and blood components
2. COM_SOP_15_ Electronic separation of blood components in eProgesa
3. COM_SOP_16_ Quarantine and release of blood components procedure
4. COM_SOP_01_Fresh Frozen Plasma preparation_A01_V02.0 (Conversion Tables)
5. COM_SOP_09_Scale validation procedure

Materials And reagents needed

1. Scale
2. Centrifuge
3. Plasma Separator
4. Hanger (Pendoir)
5. Plastic Hemostat
6. Carriers for blood bags
7. Freezer
8. Heat sealer
9. Gloves
10. Biohazard waste container
11. Hand Gripper (Stripeuse)
12. Plastic basket for blood units
13. Small parts of plastic bags
14. Markers

Safety requirements	Refer to the safety plan

Preparation for **Centrifugation**	**Step**	**Action**
	1	Switch on the centrifuge by rising up the switcher on the right middle corner of the centrifuge.
	2	Choose the program according to the product which is to be prepared at settings listed on the Centrifugation Data Sheet (posted on centrifuge).
	3	Open and control the centrifuge, to check that there is no liquid in the buckets of the rotor; also check that there is no waste in the hull of the rotor. Close the centrifuge lid
	4	Visually verify that the RPM, temperature and spin time of the centrifuge is set correctly. The RPM is 3800, spin time: 10, temperature:4°C
	5	Run the centrifuge before placing in the blood bags to reach the required temperature
Weighing of blood bags for acceptability	**Step**	**Action**
	1	Weigh all whole blood units and convert weight into Volume using the weight/Volume conversion table. Note: The whole blood to use must be in the range of 450 ml +/-10% (405-495 ml).
	2	Enter the data into eProgesa per SOP.
	3	Proceed to the steps bellow.

Preparation of blood bags for centrifugation	**Step**	**Action**
	1	Verify and control that the blood donation numbers are the same on the primary bag as well as on satellite bags.
	2	Weigh the blood bags with satellite bags and document the weight on the blood bags.
	3	Place the bags into the plastic basket for blood units and equilibrate their weights using small parts of plastic bags
	4	Open and place the basket bags into the centrifuge and make sure they are equilibrated: **Notes:**

		a. Each bag must be placed to be immobilized and without contact with materials that should cause leakage. b. Each "nacelle" must be balanced to 10 gr with the opposite nacelle. c. Each "nacelle" must be filled most symmetrically possible. d. Check the mobility and the good fixing of the "nacelles" on the pivots. f. There should not be tubing which exceed "nacelles".
Performing Centrifugation	**Step**	**Action**
	1	Close the centrifuge correctly
	2	Start centrifugation and control centrifugation until the right speed is reached.
	3	In case of imbalance the centrifuge makes signal 'RING', Press Enter Key. The centrifuge speed will progressively decrease to reach Zero. Open and resolve the problem immediately.
	4	At the end of the centrifugation, open the centrifuge and delicately remove the "baskets" with blood bags and avoid mixing up the components again
Plasma extraction	**Step**	**Action**
	1	Open the plasma extractor by shifting down the extractor spring
	2	Place the primary bag containing centrifuged blood on plasma extractor
	3	Release the spring, allowing the plate of the presser to contact the bag.
	4	Break the junction which links primary bag to satellite bag to allow plasma flow
	5	Entirely extract plasma
	6	Apply the plastic hemostat to avoid contamination of the plasma product by red cells
	7	Open the plasma extractor to remove the bag
	8	Detach the plasma bag using heat sealer

Weighing Plasma and acceptability	**Step**	**Action**
	1	Weigh the plasma and document weight on the bag label. Tare weight of bag is 30g,
	2	Convert the weight into volume referring to the conversion table.

		If the volume is < 200ml or the plasma contains red cells, discard the product manually and electronically per SOP.
Separation in eProgesa and storage	**Step**	**Action**
	1	Perform electronic separation in eProgesa per electronic separation of blood components in eProgesa procedure (COM_SOP_16_ Electronic separation of blood components in eProgesa)
	2	Place the prepared Plasma at -18 C or colder immediately in the freezer for unlabeled FFP.
Limitations (or Notes), if applicable	1. The preparation of FFP must be done on blood donation that has not exceeded 8 hours after collection, and the centrifugation should not be performed before 1 hour after collection. 2. Blood bags must provide ABO information, whether a donor is new, regular or irregular (ND for new donor, RD: for regular donor or ID for irregular donor) and ID # for Donor Identification Number. 3. Draw time must be placed on all whole blood units 4. **Outdated Plasma** – Plasma from blood not processed within 8 hours of donation is classified as outdated plasma and shall be discarded.	
References:	Brecher ME et *al.* Technical manual.14 ed. Bethesda: American association of blood banks.2008:908-909	

Appendix 5: Laboratory methods and principles

1. Actually methods and principles

Actually manual turbidimetric clot method is used for suspected coagulopathy samples, this method evaluate prothrombin time and activated partial thrombin time, clot formation is observed manual; it does not give exact amount of coagulation factor in plasma sample. Substitution test were done on samples with prolonged PT and APTT, to confirm if the cause of prolongation is due to factor deficiency or inhibitor. This test was done mixing equal volume of normal plasma and test plasma, and repeat PT and APTT test; it corrected to almost normal confirm is due to factor deficiency if not corrected think for inhibitor, still it is not exact, Because it does not give the activity or level of coagulation factor, It is a qualitative test. (Mwanda *et al.,* 2012)

- **Actually method for PT:** Blood is collected into 3.2% sodium citrate, centrifuged; 0.1ml plasma is putted into another tube, 0.1 ml brain thromboplastin is added; 0.1 ml of $CaCl_2$ is added and a stop watch started simultaneously. The tube is tilted and examined for the formation of a fibrin clot. When this occurs the watch is stopped and the prothrombin time noted.(Mwanda *et al.,* 2012)
- **Principle of PT:** To demonstrate deficiency of prothrombin, factor V, VII and X. A potent tissue thromboplastin is added to the test plasma and $CaCl_{2\ is}$ then added. The time between the additional of thromboplastin and formation of a fibrin clot is the "Prothrombin Time." (Mwanda *et al.,* 2012)
- **Actually method for APTT:** Place tube containing calcium chloride at 37^{o}C for 5 minutes prior to use. Pipette 0.1 ml plasma into a glass test tube;add 0.1 ml of phospholipids and mix. Incubate for five minutes at 37^{o}C, add 0.1 ml of $CaCl_2$to the mixture and simultaneously start a stopwatch. Examine for clot formation and note the time in seconds. (Mwanda *et al.,* 2012)

- **Principle of APTT:** Incubation of plasma with the optimal quantity of phospholipids and a surface activator leads to activation of the intrinsic coagulation system. The addition of calcium chloride triggers the coagulation process. (Mwanda *et al.,* 2012).
- **Actually method for Transmissible infectious disease:** Blood donations in EDTA or plain tubes are tested for TID, i.e. HIV, Anti-HCV, HBs Ag, and Syphilis. This was be done using CMIA by Architect machine.

  Estimate the quantity of the samples to be tested. Verify that the machine is in Ready status. Verify the status of onboard reagents and supplies. Verify that the quantities of reagents/Supplies on board are sufficient.

  If not sufficient, load additional reagents in accordance with the procedure for loading and unloading reagents. Replenish supplies as needed. Prepare the samples; place them in the carriers, run controls with samples (Abbott ARCHITECT, 2007).

2. **Principle of CMIA Architect machine:** this is a two steps immunoassay to determine the presence of antigen or antibodies in serum or plasma sample (Abbott ARCHITECT, 2007).
3. **Actually method for ABO & Rhesus blood grouping:** Arrange samples in Racks by ordering them following their identification numbers-Start by samples having the lowest ID number by considering the starting character Number to the highest (E.g. 0048928, 1048927, 0048945, 1048936, someone should arrange the samples as follow: 0048928,0048945,1048927,1048936, For each sample, properly label four test tubes with Greek letters α, β, αβ and *D* and with the donation number, Place one drop of anti-A, anti-B, anti-AB and Anti-D into the labeled test tube respectively, Add one drop of the 2 % to 5 % cell suspension to be tested to each of the tubes. Incubate at room temperature for at least one hour or mix and spin at 1000 RPM for 1 minute. After the incubation time/or centrifugation, shake the tubes and observe any agglutination/Hemolysis with a naked eye or Rhesus scope if required. Grade the reaction

as Zero or -, W, +1, +2, +3, +4, H referring to interpretation in the notes section. If Agglutination/Hemolysis is present then Record as positive and grade if No agglutination is present then Record as Negative (Brecher *et al.,* 2008)

4. **Principle of ABO & Rhesus blood grouping:** ABO& Rhesus Blood grouping is determined by the presence or absence of A or B and D antigens on the red cells and by the presence or absence of anti-A or anti-B and anti-D in the serum or plasma. (Brecher *et al.,* 2008).

Appendix 6: Ethical approval

REPUBLIC OF RWANDA/REPUBLIQUE DU RWANDA

NATIONAL ETHICS COMMITTEE / COMITE NATIONAL D'ETHIQUE

Telephone: (250) 2 55 10 78 84
E-mail: info@rnecrwanda.org
Web site: www.rnecrwanda.org

Ministry of Health
P.O. Box. 84
Kigali, Rwanda.

FWA Assurance No. 00001973

IRB 00001497 of IORG0001100

October 16, 2013
No.651/RNEC/ 2013

Schifra UWAMUNGU
Principal Investigator
Masters student

Your Project title: **"COAGULATION FACTORS LEVELS IN FRESH FROZEN PLASMA IN RWANDA"** has been evaluated by the Rwanda National Ethics committee.

		Involved in the decision		
			No (Reason)	
Name	Institute	Yes	Absent	Withdrawn from the proceeding
Dr.Jean-Baptiste MAZARATI	Biomedical Services (BIOS)	X		
Prof. Eugène RUTEMBESA	National University of Rwanda	X		
Dr.Laetitia NYIRAZINYOYE	National University of Rwanda(school of public Health)	X		
Prof.Alexandre LYAMBABAJE	National University of Rwanda		X	
Ms.Françoise UWINGABIYE	Lawyer at Musanze	X		
Dr. Egide KAYITARE	National University of Rwanda	X		
Sr.Domitilla MUKANTABANA	Kabgayi Nursing and Midwife school	X		
Mr. David K. TUMUSIIME	Kigali Health institute	X		
Dr. Lisine TUYISENGE	Kigali Teaching Hospital	X		
Dr. Claude MUVUNYI	Biomedical Services (BIOS)	X		

After reviewing your protocol during the RNEC meeting of 14 September 2013 where quorum was met, and revisions made on the advice of the RNEC submitted on 16 October 2013, **Approval letter has been granted to your study.**
Please note that approval of the protocol and consent form is valid for **12 months.**
You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrollment of participants
3. All consent forms signed by subjects should be retained on file. The RNIEC may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the RNEC in a timely fashion and before expiry of this approval.
5. Failure to submit a continuing review application will result in termination of the study.
6. Notify the Rwanda National Ethics committee once the study is finished.

Sincerely,

Dr. Jean- Baptiste MAZARATI
Chairperson, Rwanda National Ethics Committee.

Date of Approval: October 16, 2013
Expiration date: October 15, 2014

C.C.
- Hon. Minister of Health.
- The Permanent Secretary, Ministry of Health.

Appendix 7: Permission to carry out the study

REPUBLIC OF RWANDA

Kigali, 04/11/2013
Ref: 2203..../12.00/2013

MINISTRY OF EDUCATION
P.O BOX 622 KIGALI

Re: Permission to carry out research in Rwanda - No: MINEDUC/S&T/0168/2013

Permission is hereby granted to Ms. UWAMUNGU Schifra, a Master's student from Jomo Kenyatta University of Agriculture and Technology, to carry out research on: **"Coagulation factors levels in fresh frozen plasma in Rwanda".**

She will conduct research in Nyarugenge, Muhoza, Ngoma, Kigabiro and Bwishyura sector in Nyarugenge, Musanze, Huye, Rwamagana and Karongi Districts, in all provinces. She will interview Minister of Health, Director General of RBC, Head of RBC/NCBT and Head of BLS department, KHI. She will need access to the Blood Donor Medical questionnaires, to the eProgesa software, SOPs of TTI screening, Blood grouping and FFP production, Storage and use, SOPs& manual of ACL7000 coagulation machine.

The period of research is **from 1st November 2013 to 31st, November, 2014**. This period may be renewed if necessary, in which case a new permission will be sought by the researcher.

Please provide Miss. UWAMUNGU Schifra any support she may require in the course of conducting this research.

Yours sincerely,

Dr.Marie Christine GASINGIRWA
Director General,
Science, Technology and Research
Ministry of Education