

**Comparative analysis of coagulation factors
FV, FVIII and fibrinogen activity in fresh
frozen plasma, frozen plasma,
cryoprecipitate and cryosupernatant.**

Thesis

Submitted For Partial Fulfillment of Master Degree
In Clinical and Chemical Pathology

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2018**



Acknowledgement

First of all, all gratitude is due to Allah almighty for blessing this work, until it has reached its end, as a part of his generous help, throughout my life.

Really I can hardly find the words to express my gratitude to Professor / Ibrahim Youssef Abdel-Messih, Professor of Clinical and Chemical Pathology, Faculty of Medicine - ASU, for his supervision, continuous help, encouragement throughout this work and tremendous effort he has done in the meticulous revision of the whole work. It is a great honor to work under his guidance and supervision.

I would like also to express my sincere appreciation and gratitude to Dr Lecturer/ Soha Ahmed Abuelela, Professor of Clinical and Chemical Pathology, Faculty of Medicine - ASU, for her continuous directions and support throughout the whole work.

Last but not least, I dedicate this work to my dear family, whom without their sincere support and love, pushing me forward this work would not have ever been completed.



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List of Abbreviations

ACD	: Acid citrate dextrose
aPTT	: Activated partial thromboplastin time
ADSOL	: Additive solutions (adenine, dextrose, sorbitol, sodium chloride and mannitol)
ATP	: Adenosine triphosphate
BC	: Buffy coat
CPD	: Citrate and nutrient phosphate and dextrose
ECV	: Extracorporeal volume
FFP	: Fresh frozen plasma
FP24	: Plasma frozen within 24 hours after phlebotomy
Hb	: Hemoglobin
MB	: Methylene blue
FNHTR	: Febrile non-haemolytic transfusion reactions
PRBCs	: Packed red blood cells
PLTC	: Platelet concentrates
PRP	: Platelet-rich plasma
PVC	: Polyvinyl chloride
RDP	: Random donor platelet
PRBCs	: Red blood cells
SAGM	: Saline, adenine, glucose and mannitol solution
SDP	: Single donor platelet
SD	: Solvent detergent
TPE	: Therapeutic plasma exchange
TBV	: Total blood volume
TRALI	: Transfusion-related acute lung injury
vWF	: Von Willebrand factor

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INTRODUCTION

Studies proved that storing whole blood overnight at 4°C resulted in a decrease in the activity of coagulation factor FVIII, without significant loss of activity of coagulation factors FV or fibrinogen. This study is conducted to compare the activity of labile factors V and VIII as well as fibrinogen level in FFP with that of FP24 and to assess their levels in cryoprecipitate and cryosupernatant bags as well.

Plasma is a crucial component of blood with albumin, coagulation factors and immunoglobulins being the most important components of plasma that can be transfused. There are many types of plasma such as fresh frozen plasma (FFP), plasma frozen within 24 h, single donor plasma, cryoprecipitate, cryoprecipitate - reduced plasma, pathogen inactivated plasma, and thawed plasma. FFP is human donor plasma frozen in a short period after the process of collection (often 8 h). Plasma frozen at later intervals (up to 24 h) after collection is referred to as frozen plasma (FP24) (*Stanworth and Tinmouth, 2009*).

Cryoprecipitate is a frozen blood product prepared from thawed FFP and contains fibrinogen, von Willebrand factor (vWF), FVIII, FXIII and fibronectin. It is used for treating patients with inherited or acquired hypo- or dysfibrinogenemias. It should no longer be the first choice in treating hemophilia A or von Willebrand disease given the widespread availability of recombinant or virally inactivated factors. Cryoprecipitate is prepared by thawing a unit of fresh-frozen plasma in 1 to 6°C and then the cryoprecipitated

material is separated from the liquid plasma. The cryoprecipitate is then frozen and stored at temperatures not exceeding -18°C for up to 1 year (**Franchini and Lippi, 2012**).

The current regimen for the preparation of FFP within 8 hours of whole blood collection was implemented to maintain the activity of coagulation factors. When whole blood is stored at 4°C for short time intervals, factor VIII significantly decreases in the extracted plasma, while other coagulation factors keep unchanged (**Cardigan et al., 2011**). A few studies have analyzed the stability of different coagulation factors when whole blood storage time is prolonged to 24 hours and compared this to FFP. The data available on the levels of coagulation factors (factor VIII, vWF, fibrinogen, and other proteins) in cryoprecipitate made from whole blood stored for 24 hours before component preparation is not enough (**Alhumaidan et al., 2010**).

The use of plasma frozen within 24 hours of phlebotomy (FP24) is currently preferred by many blood centers, since this not only offers operational flexibility and efficiency, but could indirectly enhance component safety, and would maximize the ability to use plasma from male donors to avoid the risk of HLA antibodies, and the potential occurrence of transfusion-related acute lung injury (TRALI) (**Triulzi et al., 2009**).

The aim of this study is to analyze and compare the activity of coagulation factor V, VIII and fibrinogen level in fresh frozen plasma, frozen plasma, cryoprecipitate and cryosupernatant as well.

AIM OF THE WORK

We aim to analyze and compare the activity of coagulation factor V, VIII and fibrinogen level in fresh frozen plasma, frozen plasma, cryoprecipitate and cryosupernatant as well.

Chapter 1:

Blood Components and Separation

Blood consists of a mixture of cells, colloids and crystalloids that can be separated into various components such as packed red blood cells (PRBCs), platelets, fresh frozen plasma (FFP), cryosupernatant and cryoprecipitate. Each blood component is used in certain conditions; therefore component separation has expanded the use of one whole blood unit (*Basu&Kulkarni, 2014*).

Different components of the blood have different relative density, sediment rate and size, thus they can be separated when various centrifugal forces are applied. Plasma has the lowest specific gravity after which platelets, leukocytes (Buffy Coat) and packed red blood cells respectively. Appropriate processing and storage is essential to maintain the functional efficiency of each component (*Hardwick, 2008*).

Preparation of each one of the previously mentioned components is done by centrifugation of one unit of whole blood. Another method of component separation is collecting the required component by apheresis procedure in blood donors. Different components need peculiar storage and temperature conditions for beneficial therapeutic effects. Hence, various equipments are needed to maintain suitable ambient conditions during storage and transportation. Maintaining blood supply to patients forms a major concern in blood banking especially concerning rare blood groups

and common blood groups as well during disasters. Cryopreservative techniques can extend the half-life of PRBCs up to several years. Blood components transfused to patients in need must be carefully examined, they may cause various adverse effects from mild up to inevitable allergic reactions. These complications can be reduced and even avoided by using blood products that are modified as leukoreduced (*Basu&Kulkarni, 2014*).

I- Blood Collection and Storage:

Blood units are collected by two main methods, either as whole blood into bags containing anticoagulant citrate and nutrient phosphate and dextrose (CPD) or by apheresis into bags containing acid citrate dextrose (ACD). Then, blood is centrifuged to bring down the red blood cells so they are separated from the rest of the blood. Separation is accomplished by two different ways; the first method involves draining the red blood cells out of the bag through a special port present in the bottom of the bag, leaving behind the buffy coat containing white cells, platelets and the plasma on top. This way is called the buffy coat method and the bags used in such method are called top and bottom bags. The other method is done by centrifuging the blood less hard in order to leave many of the platelets still suspended in the plasma. In this method, the platelet-rich plasma is squeezed off the top to leave the red cells, the buffy coat of white cells and some platelet-rich plasma behind. This is called the platelet-rich plasma method of component manufacture. Commonly, the concentrated red cells are then run through a

leukocyte reduction filter, this filter removes most white cells and platelets. An additive solution containing more nutrients is added to allow longer storage and dilute the viscosity of the units allowing their smooth flow during emergency administration (*Hess, 2010*).

Storage lesions are changes that alter the physiological properties of blood, it occurs in collected blood over time. Coagulation factor activity especially labile factors deteriorates rapidly in whole blood, particularly after the first 24 hours of storage. Therefore, it is not considered a suitable product to treat haemostatic disorders. Also, platelets in whole blood lose viability and functionality very quickly and are not considered a suitable source to treat patients requiring platelet therapy. Red cells increase their affinity for oxygen and lose some viability. Leucocytes deteriorate releasing leucocyte proteases. Microaggregates form and potassium is released from the red cells. Using anticoagulant-preservatives during collection of blood maintains component function and viability only if storage is done in the correct temperature range. Low temperature storage allows extending of the shelf life of the component by slowing glycolytic activity. Also it prevents bacterial proliferation if any contamination took place during the process of donation; either from the venipuncture site, the donor's circulation or any other source(*Hardwich,2008*).

II- Collection of Components by Apheresis

Apheresis is a method of blood separation in which the required component is collected and the remaining of blood

components are returned back to the donor. Numerous components can be collected using this method such as; single donor platelet (SDP), harvesting platelets, double unit red cell collection (red cells), plasmapheresis (collecting normal plasma) and therapeutic plasma, leukapheresis (harvesting granulocytes, peripheral blood haematopoietic stem cell) (**Maitta, 2014**).

The separation of blood by apheresis is done either by centrifugation (separation of components by different specific gravities) or by filtration (different size). The most commonly used principle in apheresis is the centrifugation principle giving leucodepleted products. In this method, fixed amount of blood is collected from the donor in a bolus called as extracorporeal volume (ECV) and the required blood component is collected in the collection bag and the rest of blood constituents are returned back to the donor. The main goal of the procedure is to maintain the ECV collection within 15% of total blood volume (TBV). Keeping the ECV within this volume is essential to avoid the occurrence of hypovolemia. Hence, ECV should not reduce beyond 20% of TBV at any point and the final product should not exceed 15% ECV of TBV (**Lu, 2008**).

There are two types of centrifugation apheresis equipments; ‘intermittent and continuous working’. The Intermittent equipment uses a single access of donor’s vein for both collection and return of blood. One cycle consists of one ECV whole blood collection in kit bowl, centrifugation of bowl to separate blood components, collection of the

required component (e.g. platelets) in the collection bag and return of other constituents to the donor. This cycle is repeated until the therapeutic dose is reached. On the other hand, there are two simultaneous phlebotomies in the continuous working equipment. One is for the collection and the other is for the return. The processes of centrifugation, component collection and return happen continuously and simultaneously (*Basu&Kulkarni, 2014*).

There are general guidelines for blood donation criteria to be met for apheresis donors. In addition to the usual criteria of general blood donation, additional criteria should be included such as presence of a prominent vein that is accessible and can withstand the apheresis procedure and the donor's weight must exceed 55 kg. A written consent should be signed by the donor consent in a language that he can understand after clear explanation of the procedure and the expected risks (*Hardwick, 2008*).

Complicated procedures such as therapeutic erythrocytapheresis and leukapheresis of small children for thalassemia and leukaemia can be performed in children weighing 11-25 kg safely without increased morbidity using continuous apheresis equipment despite the strict guidelines put for apheresis donors (*Maatta et al., 2014*).

Prior to the procedure, certain investigations must be done to make sure that all the parameters are within range before the apheresis procedure. These parameters include; complete blood count, same ABO grouping, total proteins, same Rh typing if needed and screening for transfusion

transmitted diseases. For double unit red cell collection, donors undergoing such procedure must weigh more than 65 kg and have hemoglobin level of 13.5 g/dl or more. The interval between the two procedures must be at least 16 weeks. Certain criteria should be met before plateletpheresis, donor's platelet count must be 150,000/mm³ or more. Moreover, total white cell count and differential count should be within normal ranges. Any donor with personal or family history of bleeding tendency should never undergo plateletpheresis (*Basu&Kulkarni, 2014*).

In candidates who have taken aspirin or similar antiplatelet drugs during the last 72 hours, plateletpheresis should be deferred for 3 full medication-free days. While donors taking clopidogrel or ticlopidine should stop their medications for 14 full medication-free days before undergoing plateletpheresis. Plateletpheresis can be repeated for the same donor after 48 hours, this should be restricted to a maximum of 24 procedures per year (*Elebute et al., 2009*).

III- Preparation of Blood Components from whole Blood:

A- Anticoagulant-preservative solutions:

Anticoagulants used for blood collection vary in their composition; the main aim of their use is to prevent clotting and to provide nutrients to maintain cell functionality and viability during storage. Sodium citrate is used as a calcium-chelating (binding) agent so it interferes with the calcium-dependent steps in the clotting cascade and stops coagulation.