INTRODUCTION

There are several types of plasma such as fresh frozen plasma (FFP) and the precipitate remaining after frozen plasma is thawed at 4˚C (cryoprecipitate), plasma frozen within 24 hrs of collection (PF24), liquid plasma, thawed plasma, cryopoor plasma, recently solvent/ detergent plasma (SDP) and donor retested plasma (DRP) ***(Spence, 2006)***.

Currently, the most commonly used product is FFP, which can be obtained from a plasmapheresis collection or from a whole blood donation. Plasma must be frozen within 8 hrs from the time of collection to be labeled as FFP. Thus, FFP should contain all pro- and anticoagulant factors at normal physiologic activity levels ***(Cardigan et al., 2005).***

Fresh-frozen plasma can be used to reverse a significant coagulopathy in a bleeding or perioperative patient, in plasma exchange procedures for various indications, to correct an isolated single factor deficiency for which a sterile or recombinant factor concentrate is not available or during massive transfusion situations ***(Yazar et al., 2008).***

On the other hand PF24 is plasma that has been stored in the refrigerator for up to 24 hrs before freezing, sometimes called (24 hrs plasma), contains stable coagulation factors at the same concentration as present in FFP ***(Novis et al., 2002).*** There is little difference in the levels of labile coagulation factors (i.e., Factor V and Factor VIII) between FFP and Plasma

frozen within 24 hrs. Some transfusion services use FFP and PF24 interchangeably for the first 24 hrs after thawing ***(Yazer et al., 2008).***

Recently, it has been strongly recommended that plasma be provided from predominantly male donors to mitigate transfusion-related acute lung injury (TRALI) risk. This recommendation places operational and logistic demands on production of plasma components for transfusion which could be partially relieved by freezing within 24 hrs of phlebotomy rather than 8 hrs ***(Sebok et al., 2007).***

AIM OF THE WORK

The aim of this work is to assess whether therapeutically adequate levels of labile coagulation factors FV and FVIII are maintained in thawed plasma frozen at 24 hrs as compared to thawed plasma derived from fresh frozen plasma during 5-days of storage.

TYPES OF PLASMA

Human plasma is a complex biological material composed of hundreds of biochemical entities, some of which have not yet been fully characterized. Among these are albumin, various classes of immunoglobulins, coagulation factors, anticoagulants, protease inhibitors, and growth factors ***(Wolfgang, 2007).***

Plasma is made up of 90% water, 7-8% soluble proteins (albumin maintains blood osmotic integrity), 1% electrolytes, and 1% elements in transit. Plasma also carries respiratory gases: O2 in small amounts (about 3%) and CO2 in large amounts (about 97%), various nutrients (glucose, fats), wastes of metabolic exchange (urea, ammonia), hormones, and vitamins (***WHO Recommendations for the production, control and regulation of human plasma for fractionation, 2005).***

Most plasma for transfusion is obtained by centrifugation of a unit of whole blood, which produces a unit of platelet rich plasma (PRP) and a unit of RBCs ***(Heal et al., 2005).***

The first step is the collection of whole blood into a bag that contains anticoagulant and has two satellites: one contains the additive solution and the other is empty. The whole blood is centrifuged and plasma or platelet rich plasma (depending on the time of spin) is expressed into the empty bag ***(Burnouf et al., 2003).***

Whole blood is separated by differential centrifugation based on the specific gravity (relative density) of the blood constituents. The relative density of the blood constituents ranges from most dense to least dense, as follows: RBCs, white blood cells (WBCs), platelets, and plasma. The degree of separation and component yield depend primarily on centrifuge rotor size, speed (g- force or rpm), and spin time. If a whole blood unit is intended for platelet production, the centrifuge temperature is set at 20°C, and a soft spin (low g-force) is used to separate the whole blood into platelet rich plasma and a unit of RBCs.

The PRP is further processed at 20°C, and a hard spin (high g-force) to separate the plasma from the platelets. The plasma is frozen and stored within 8 hrs as fresh frozen plasma. Plasma collection, processing and storage may affect plasma quality and recovery of labile protein such as FVIII ***(Burnouf et al., 2007).***

If a whole blood unit is not intended for platelet production, the unit is centrifuged at 4°C with a hard spin to separate the whole blood into a platelet poor plasma (PPP) layer, a buffy coat layer containing platelet and WBCs, and a packed RBCs layer ***(Shealynn et al., 2007).***

The main plasma volume obtained from whole blood donation is about 220mL, but varies depending upon the volume of collected whole blood (most often 400-450 mL) and donor's hematocrit ***(Burgstaler, 2003).***

Plasma can also be obtained using apheresis technology. The advantage of apheresis plasma technology is the ability to collect larger units, often called jumbo plasma. Larger units are desirable because fewer units are required per dose, which lessens the chance of infectious disease transmission and allergic reactions per recipient ***(Heal et al., 2005).***

Plasmaapheresis is a process that is used for collection of plasma for fractionation "apheresis plasma" or treatment of particular diseases "therapeutic plasmaapheresis" as in autoimmune disorder, where the rapid removal of autoantibodies from the circulation is required in addition to medical therapy ***(Burgstaler, 2003).***

Apheresis plasma, also called "source plasma", is collected from donors through a process where blood is removed from the donor, anticoagulated (generally with a 4% sodium citrate solution), and immediately separated by physical means (centrifugation or filtration, or a combination of both) into components. At minimum, the red cells are returned to the donor, while plasma is retained and collected in container (bag or plastic bottle) ***(Farrugia and Robert, 2006).*** The volume of plasma collected depends upon the duration of the plasmaapheresis procedure, which depends on the number of cycles, and lasts generally from 35 to 70 minutes. Apheresis plasma volume may range from 450 to 880 mL, depending upon the country’s regulations and collection protocol ***(Hellstern, 2001).*** Both recovered and apheresis plasmas are

suitable for the manufacture of the whole range of fractionated plasma products ***(Sward-Nilsson et al., 2006).***

There are several types of plasma such as Fresh Frozen plasma (FFP) and the precipitate remaining after frozen plasma is thawed at 4˚C (cryoprecipitate), plasma frozen within 24 hrs of collection, liquid plasma, thawed plasma, cryopoor plasma, recently solvent/detergent plasma (SDP) and donor retested plasma (DRP) (Table 1) ***(Spence, 2006)***.

Table (1): Plasma protein and coagulation factor contents of plasma products

Product	**Protein/Factors**
FFP	All plasma proteins and both stable and labile coagulation factors
FFP retested	All plasma proteins and both stable and labile coagulation factors
PF24	Same as FFP but reduced amounts of factor VIII and factor V
Thawed plasma	Same as FFP but reduced amounts of factor VIII and factor V
Liquid plasma	Same as FFP but reduced amounts of factor VIII and factor V
Cryoprecipitate-AHF	Factors VIII, XIII, vWF, fibrinogen and fibronectin
	Deficient in other plasma proteins
Cryoprecipitate reduced plasma	VWF metalloproteinase. deficient in factor VIII, vWF, fibrinogen, cryoglobulin and fibronectin
AHF, anti-hemophiliac factor; FFP, fresh frozen plasma; PF24, plasma frozen within 24h of phlebotomy; vWF, von Willebrand factor.	

(Spence, 2006)

I-Fresh Frozen Plasma

Fresh Frozen plasma (FFP) is prepared by separating citrated plasma from whole blood and freezing it within 8 hrs of collection or by freezing citrated apheresis plasma within 6 hrs of collection FFP. It may be stored at -18˚C or below for up to 1 year showing minimal loss of activity of the labile coagulation factors V and VIII. One mL of FFP contains approximately one unit of coagulation factors activity ***(Dazzi et al., 2000).***

Each unit of FFP prepared from whole blood contains approximately 200 ml of plasma. Apheresis plasma may be packaged into 200 or 400 ml bags. A typical dose of 10-15 mL/kg would constitute approximately 25 to 30% replacement therapy for coagulation factors (although there is lower recovery of some factors because of their diffusion into the extravascular space). It may have to be exceeded in massive bleeding. Therefore, the dose depends on the clinical situation and its monitoring ***(Hellstern and Haubelt, 2002).***

II-Plasma Frozen Within 24 hrs:

Plasma Frozen Within 24 hrs (PF24) is plasma that has been stored in the refrigerator for up to 24 hrs before freezing, sometime called (24 hrs plasma), contains stable coagulation factors at the same concentration as present in FFP ***(Novis et al., 2002)*** but there is little difference in the levels of labile coagulation factors (Factor V and Factor VIII) between FFP and PF24. However, some authors reported that factor V levels

essentially the same in plasma frozen at 8 hrs and at 24 hrs after collection ***(O'Neill et al., 1999 and Smith et al., 2000).***

III-Liquid plasma:

Liquid plasma is the plasma that is separated within 5 days after the expiration date of the whole blood. Levels of stable coagulation factors are similar to those in FFP, but levels of FV and FVIII are reduced significantly ***(Novis et al., 2002)***.

IV-Thawed plasma:

Thawed plasma is the plasma that has been stored in the refrigerator for more than 24 hrs after thawing ***(Porter et al., 2000)*** FFP and PF24 are thawed between 30 ˚C to 37˚ C in a water bath for approximately 30 minutes or rapidly thawed in an FDA-approved microwave device for approximately 6 minutes ***(Churchill et al., 2007).***

Although less expensive than microwave device, water baths contribute to a longer thaw time compared to microwave device, which can impact the immediate provision of thawed plasma in situations that require urgent need, particularly in the trauma setting. In addition, although the frozen unit is placed in a waterproof plastic overwrap bag prior to immersion in the water bath, the baths contain non-sterile water, which may contaminate the entry ports of the unit if the unit is not sufficiently protected. Although microwave devices are more sterile and result in more rapid turnaround time, these devices

are relatively expensive. Furthermore, if not properly maintained, microwave devices have the potential to produce temperature "hot spots" in the unit during thawing, which can damage plasma proteins ***(Suontaka et al., 2005).***

Thawed plasma should be kept at 4˚C if there is any delay in transfusion. Current UK guidelines ***(United Kingdom Blood Transfusion Services/National Institute for Biological Standards and Control, 2002),*** require transfusion within 4 hrs, whereas the ***American Association of Blood Banks (2002)*** allows a delay of up to 24 hrs. The FVIII activity in FFP will decline after 24 hrs at 4˚C by up to 28%, but all other factors remain stable for 5 days (Table 2).

In a study by ***Shehata et al. (2001)*** the storing of FFP for up to 72 hrs after thawing has been reported to cause about 40% of the FVIII activity to be lost. The activities of FII and FV in FFP were maintained up to 72 hrs after thawing. The authors recommended that FFP stored for up to 72 hrs after thawing can be used when FVIII replacement is not required**.**

Table (2): Hemostatic factor content of different types of plasma

	Levels when freshly thawed	**Levels at 24 h**	**Levels at 5 d**
Fibrinogen	2-67	2-25	2-25
FII	80	80	80
FV	80	75	66
FVII	90	80	72
FVIII	92	51	41
FIX	100		
FX	85	85	80
FXI	100		
FXII	83		
FXIII	100		
Antithrombin	100		
VWF	80*		

*With some loss of HMW multimers, particularly if SD-treated.

(BCSH, 2004)

V-Solvent/ detergent plasma:

Solvent/ detergent plasma (SDP) is a pooled plasma product that undergoes treatment with the solvent tri (n-butyl)-phosphate and the detergent X-100 to inactivate lipid enveloped viruses, such as hepatitis B (HBV), C (HCV) and HIV viruses.

The process is not effective against non-enveloped viruses, such as hepatitis A (HAV) and parvovirus ***(McLeod and Gregory, 2001).*** The SDP production process also eliminates bacteria, protozoa, cells, cellular fragments ***(Cai et al., 2005)***.

Solvent detergent plasma (SDP) treatment also results in some loss of integral plasma protein such as α2- antiplasmin and protein S. The α2- antiplasmin is crucial in maintaining hemostasis especially in patients with liver dysfunction. Decreased levels of the natural anticoagulant protein S can lead to a hypercoagulable state especially when massive SDP transfusions are used as major source of plasma replacement in massive traumas or therapeutic plasma exchanges ***(Hellstern, 2004).***

Recently, two new SDP treatment procedures have been developed for single unit or mini pools of 10 to 12 units of plasma that yield more than 90% mean recovery of coagulation factors, anticoagulants (including protein S), protease inhibitors (including α2- antiplasmin), total protein, albumin, and immunoglobulins ***(Burnouf et al., 2006).***

VI-Methylene blue plasma:

Methylene blue (MB) is a photoactive phenothiazine dye that has been used for pathogen inactivation of single units of plasma. Methylene blue has an affinity for nucleic acids and the surfaces of viruses. When MB-treated plasma is exposed to

ultraviolet (UV) light, most enveloped viruses are easily inactivated, however, nonenveloped viruses are more resistant to treatment ***(Bryant and Klein, 2007).***

Intracellular viruses are not inactivated by MB/UV light, but freezing and thawing plasma often disrupts the cell membrane of leukocytes; liberating the viral particles and leaving them susceptible to MB pathogen inactivation. Residual intact white blood containing viruses are removed by micropore filter. Neither protozoa nor bacteria are inactivated by MB treatment ***(Crettaz et al., 2004).***

Plasma proteins are moderately affected; fibrinogen and factor VIII activity is reduced by about 30%. Methylene blue treatment can be used for pathogen inactivation of single units of plasma, thus eliminating the risk of large plasma pools currently used to manufacture SDP ***(Wiliamson et al., 2003).***

A comparison of standard fresh-frozen plasma (FFP) with Methylene blue-treated FFP and solvent detergent-treated FFP were shown in Table 3.

Table (3): A comparison of standard fresh-frozen plasma (FFP) with methylene blue-treated FFP and solvent detergent-treated FFP

	Standard FFP	Methylene blue FFP	Solvent detergent FFP
Source	UK donors, all previously virus tested. Single unit format.	USA volunteers donors, all male. Single unit format.	Non-UK donors; pools of up to 380 1 (600-1500 ABO identical donations)
Donation tests			
Serology	HIV, HBV, HCV, HTLV	HIV, HBV, HCV, HTLV	HIV, HBV, HCV, HTLV
Genomic	HCV	HCV, HIV	HAV, HCV, B19, HIV, HBV
Virus risk			
HIV 1 + 2	1:10 million	No proven cases reported to date for HIV, HBV, HCV (one possible HCV transmission)	No reported transmissions to date of HIV, HBV, HCV in SDFFP or SD treated plasma products
Hepatitis C	1:50 million		
Hepatitis B	1:1.2 million		
Hepatitis A	Rare event		None reported
Parvovirus B19	Rare event	No greater than for standard FFP. None reported to date	Batch withdrawals due to possible B19 content. Seroconversion in patients no greater than with untreated FFP.
Volume	180-300 ml + 50 ml pediatric size.	235-305 ml + 50 ml pediatric size.	200 ml; no pediatric size.
Coagulation factor content	Variable between units. 75% units >0.7 IU/ml FVIII	Variable between units. 75% units FVIII >0.5 IU/Ml; all other factors >0.5 IU/ml; no reduction AT III, protein C, protein S. No coagulation factor complement activation.	Constant within batch. All factors > 0.5 IU/ml.
Cryoprecipitate/ cryosupernatant	Available	May become available	Not available
Residual additives	None	<0.3 μmol/1 MB. No toxicity seen or predicted at this level, even in premature neonates	<2 μg/ml TNBP**; <5 μg/ml Triton-X 100. Residual levels not toxic.
Allergic reactions	May be reduced by leucocyte depletion	Reactions attributable to cells would be expected to be reduced.	Probably less frequent than FFP.
Mild	1%	No data	
Severe	1%	No data	
Adverse reactions due to antibody		As for standard FFP	Pooling reduces all of these risks.
Red cell	Tested for high titre anti-A,B	Not tested for high titre anti-A,B	High titre anti-A, B not a problem since donations pooled.
TRALI	>20 cases/year (SHOT)	None reported to date.	Only one possible TRALI case reported
Thrombocytopenia	Very rare		
Cellular content	Leucocyte depleted; No need to RhD match	Leucocyte depleted; No need to RhD match	No intact cells or fragments; no need to RhD match
Product license	Not required	Medical device; CE marked	Licensed, batched product
Indications		As for FFP	As for FFP
Usage to date	300 000 units/year in UK	>1 000 000 units in Europe	3 000 000 units in Europe

** TNBP, tri-(N-butyl)-phosphate. ***(BCSH, 2004)***

VII-Donor retested plasma:

Donor retested plasma (DRP) refers to single units of FFP prepared from repeated blood donors. DRP is prepared by making FFP from a single unit of whole blood, then keeping the plasma in the frozen state then releasing the frozen plasma for use when the donor returns at least 112 days later and again has negative test results for HBV, HCV and HIV. The 112 daytime was chosen because it exceeds the window period interval during which viral transmission may occur despite negative test results for HBV, HCV and HIV ***(Friedman and Manitove, 2001*)**.

VIII-Cryopoor plasma (cryosupernatant plasma):

The material that remains after cryoprecipitate has been removed from FFP is a defined plasma product (plasma cryoprecipitate reduced) that is often called cryosupernatant plasma. It is relatively deficient in fibrinogen, FVIII and vWF multimers ***(Brecher, 2002).***

IX-Cryoprecipitate:

Cryoprecipitate is prepared by thawing FFP at 1 to 6 °C and recovering the precipitated material. The cold insoluble precipitate is then frozen and stored at -18 °C or colder for up to 1 year. Cryoprecipitate contains more than 150 mg of fibrinogen, more than 80 units of FVIII, significant amounts of vWF (including the high molecular weight multimers) and some fibronectin and FXIII in less than 15 mL volume ***(Erber and Preey, 2006)***.