Health Technology Assessment of pathogen reduction technologies applied to plasma for clinical use

Americo Cicchetti[1], Alexandra Berrino[2], Marina Casini[3], Paola Codella[1], Silvia Coretti[1], Giuseppina Facco[4], Alessandra Fiore[1], Giuseppe Marano[4], Marco Marchetti[2], Emanuela Midolo[3], Roberta Minacori[3], Pietro Refolo[3], Federica Romano[1], Matteo Ruggeri[1], Dario Sacchini[3], Antonio G. Spagnolo[3], Irene Urbina[2], Stefania Vaglio[4], Giuliano Grazzini[4], Giancarlo M. Liumbruno[4]

[1]Postgraduate School of Health Economics and Management (Altems), Catholic University of the Sacred Heart, Rome; [2]Health Technology Assessment Unit of "Gemelli" Teaching Hospital, Catholic University of the Sacred Heart, Rome; [3]Institute of Bioethics, Catholic University of the Sacred Heart, Rome; [4]Italian National Blood Centre, National Institute of Health, Rome, Italy

Abstract

Although existing clinical evidence shows that the transfusion of blood components is becoming increasingly safe, the risk of transmission of known and unknown pathogens, new pathogens or re-emerging pathogens still persists. Pathogen reduction technologies may offer a new approach to increase blood safety. The study is the output of collaboration between the Italian National Blood Centre and the Post-Graduate School of Health Economics and Management, Catholic University of the Sacred Heart, Rome, Italy. A large, multidisciplinary team was created and divided into six groups, each of which addressed one or more HTA domains.

Plasma treated with amotosalen + UV light, riboflavin + UV light, methylene blue or a solvent/detergent process was compared to fresh-frozen plasma with regards to current use, technical features, effectiveness, safety, economic and organisational impact, and ethical, social and legal implications. The available evidence is not sufficient to state which of the techniques compared is superior in terms of efficacy, safety and cost-effectiveness. Evidence on efficacy is only available for the solvent/detergent method, which proved to be non-inferior to untreated fresh-frozen plasma in the treatment of a wide range of congenital and acquired bleeding disorders. With regards to safety, the solvent/detergent technique apparently has the most favourable risk-benefit profile. Further research is needed to provide a comprehensive overview of the cost-effectiveness profile of the different pathogen-reduction techniques. The wide heterogeneity of results and the lack of comparative evidence are reasons why more comparative studies need to be performed.

Keywords: health technology assessment, pathogen reduction, pathogen inactivation, fresh-frozen plasma, transfusion safety.

The health problem and current use of the technologies

Human plasma accounts for about 55% of the body's total blood volume[1]. Plasma is a complex mixture of water, inorganic salts, nutrients (such as carbohydrates, lipids and amino acids), organic waste products, peptides, enzymes, hormones, and more than 1,000 proteins[1,2]. There are four major categories of plasma proteins[3]: (i) *albumins*; (ii) *globulins;* (iii) *fibrinogen*; and (iv) *coagulation factors* (factor [F] II, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII, antithrombin, von Willebrand factor [VWF], plasmin inhibitor, protein C and free protein S). Since the separation of plasma from whole blood became standard practice, the amount of frozen plasma used started to increase steadily[4,5]. Currently, human plasma may be used as a therapeutic product, known as clinical plasma or fresh-frozen plasma (FFP), or as source material for the production of pharmaceutical fractionated products (also called plasma products or plasma-derived medicinal products)[6,7]. Plasma units for transfusion may be obtained by separation of the plasma from collected whole blood or by apheresis procedures[2]. Plasma units contain many biologically active molecules[8]. Among these, labile coagulations factors, especially FV and FVIII, are unstable molecules because of their short half-lives[9]. To achieve the maximum yield of FVIII, plasma should be frozen to −30 °C or below[10].

Several plasma products are available for transfusion or for industrial fractionation: four frozen plasma products and two liquid-state plasma products.

The frozen plasma products are: (i) FFP, (ii) frozen plasma (FP), frozen within 24 hours of phlebotomy, (iii) cryoprecipitate-reduced plasma and (iv) cryoprecipitate. FFP and FP differ with regards to the time before freezing (6-8 hours -depending on the plasma source and manufacturer- for the former, 24 hours for the latter). Both products have a shelf-life of 24 hours

Blood Transfus 2016; **14:** *287-386* DOI 10.2450/2016.0065-16

once thawed[5,8]. In FP, the levels of FVIII, alone, are lower (65-80% respect to FFP), although adequate for haemostasis[8]. Cryoprecipitate-reduced plasma is also known as "cryo-poor plasma" and "cryosupernatant". It is prepared by centrifuging frozen plasma after thawing. Supernatant plasma (cryoprecipitate-reduced plasma) must be removed and the remaining plasma is refrozen as cryoprecipitate at −20 °C. The cryoprecipitate must be refrozen within 1 hour of the initial thawing. As a result, all plasma proteins remain in the cryoprecipitate and FVIII, VWF and fibrinogen are concentrated[2]. The cold-insoluble portion of plasma that precipitates when plasma is thawed between 1 and 6 °C is referred to as cryoprecipitate in European countries and as cryoprecipitated antihaemophilic factor in North American States[11].

The two liquid-state plasma products are: (i) thawed plasma and (ii) liquid plasma[5,8]. These products differ because thawed plasma is made from a frozen product (FFP or FP) and has a short shelf-life, when stored at refrigerated temperatures (1 to 6 °C), of 5 days, whereas liquid plasma is never frozen and has a refrigerated shelf-life (26 or 40 days) that is determined by the manufacturing process[5,8].

Indications

Plasma

Plasma has been available for transfusion since the 1940s and was initially often used as volume replacement. With the availability of "plasma expanders" (e.g. crystalloids and colloids) and purified albumin, its use as a volume expander has progressively declined[8]. It is now usually indicated for the treatment of congenital and acquired coagulation factor deficiencies, coagulopathy resulting from liver disease, massive blood loss, and thrombotic thrombocytopenic purpura (TTP or Moschowitz syndrome). In addition, plasma may be used to prepare cryoprecipitate for fibrinogen replacement and the treatment of von Willebrand's disease. The typical therapeutic use of plasma for correction of coagulopathy involves transfusion of approximately 10 to 20 mL/kg per transfusion, resulting in exposure to multiple donors. Plasma exchange with fluid replacement is the most effective treatment for acute idiopathic TTP[12-14].

Cryoprecipitate

Cryoprecipitate is an allogeneic blood product prepared from human plasma. It contains FVIII, VWF, fibrinogen, fibronectin and FXIII. Its use was first reported in the 1960s for the treatment of patients with FVIII deficiency. Nowadays cryoprecipitate is used to replace fibrinogen in patients with acquired fibrinogen deficiency[11].

Inappropriate indications

Plasma should not be used[13,15]: (i) to expand the circulatory volume; (ii) to correct hypoproteinaemia; (iii) to correct an immune deficiency; (iv) for nutritional purposes; (v) to correct congenital or acquired deficiencies of clotting factors in the absence of haemorrhage, or for the correction of disorders of haemostasis in patients with chronic liver disease who are not bleeding.

Adverse reactions to plasma transfusion

Adverse reactions to plasma transfusion can be classified as follows[13,14]: (i) allergic reactions (from mild urticaria to severe anaphylaxis); (ii) transfusion-related acute lung injury (TRALI); (iii) febrile reactions; (iv) citrate toxicity; (v) transfusion-transmitted infections (TTI); (vi) transfusion-associated circulatory overload (TACO); and (vii) formation of inhibitors against a deficient coagulation factor.

Target condition

The history of Transfusion Medicine is essentially that of a continuous and relentless pursuit of procedures to make blood transfusion and its products safe and effective. Safety is based on the prevention of the two main risks associated with allogeneic transfusion, which are infectious and immunological risks. Despite various approaches to blood safety (more stringent donor screening, introduction of new exclusion criteria, registries of previously deferred donors, various specific serological and nucleic acid tests [NAT], traceability and haemovigilance procedures and appropriate clinical use of blood), TTI still occur[16]. In the last decades blood banks, as well as the plasma manufacturing industry, have aggressively pursued strategies to reduce the risks of TTI. The continuous advances in procedures for the collection, preparation and storage of blood products have led to a constant improvement of the quality of blood components, resulting in more effective transfusion therapy[17]. Indeed, as shown by studies on incidence, transfusion-related transmission of pathogens has decreased significantly in the past years[18,19].

Table I reports the relative risks of the most common TTI.

Although the risk of TTI from the blood supply is currently low, processes to inactivate contaminating viruses, bacteria and protozoa may improve the safety of transfusion even further[20]. Nowadays, human plasma proteins, clotting factors in particular, have a high margin of viral safety as a result of three complementary approaches: (i) the selection of safe blood donors on the basis of their history and clinical evaluation; (ii) systematic screening of units of blood for transfusion; and (iii) the treatment of blood and blood products to

Table I - Relative risk of the most frequent transfusion-transmitted infections in Europe.

Pathogen	Risk in Europe
Viruses	
Human immunodeficiency virus	1 in 909,000-5,500,000
Hepatitis C virus	1 in 2,000,000-4,400,000
Hepatitis B virus	1 in 72,000-1,100,000
Bacteria	
Bacterial contamination	1 in 38,500 for red blood cells
	1 in 5,000 for platelets

Source: modified from reference 18.

inactivate infectious agents that, for various reasons, have escaped screening[21].

Donor selection is of paramount importance, but its limits are well known, even if conducted in accordance with the most stringent criteria; in fact, infected asymptomatic individuals and people who are not aware of adopting risk behaviours serve as an asymptomatic reservoir and a potential source of transmission of pathogens to blood recipients[22].

The traditional serological tests for the detection of donor carriers of antigens of infectious agents, and/or their respective antibodies, have been progressively improved over years and especially in the last decade[16]. In 2009, the World Health Organization (WHO) strongly recommended pre-transfusion blood tests for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis[23]. Nonetheless, in some cases these tests may not detect the marker of infection, usually because a blood donation is made during the potentially infectious "window period", early in the course of the infection. NAT have contributed to the reduction of the risk of TTI since NAT technologies have the potential to detect viraemia earlier than serological methods, which are based on seroconversion. Since viraemia precedes seroconversion by several days in the case of HIV and several weeks in the case of HCV, tests that detect viral nucleic acids are considered a significant technological advance and an additional step in achieving the goal of zero risk for blood transfusion recipients[24]. Viral NAT are meant to overcome the still existing limits of serological screening of units for transfusion, namely: (i) lack of a positive correlation between the "surrogate" marker (for example, alanine aminotransferase levels and anti-HBc for HBV) and the presence of the virus in the donor; (ii) employment of insensitive tests; (iii) no or low antibody response; and (iv) presence of mutant forms of hepatitis B surface antigen (HBsAg)[25]. Despite the progress made in screening for blood-transmitted diseases, both the post-infection diagnostic window and the appearance of novel infective agents remain critical issues for the safety of blood transfusions[26]. Furthermore, many pathogens that have the potential to invade the blood supply are not yet screened for because of the low prevalence of the disease in the general population, the unknown transmission rate of infection through transfusion, or the lack of a readily available test for the agent. In order to overcome the deficiencies of the above-mentioned tests, other procedures for the prevention of viral infection risk have been proposed and implemented. One of these consists in storing the donated plasma unit for a longer time than the window period for HIV, HBV and HCV, until the donor is confirmed to be negative for these TTI. This procedure, referred to as "quarantine" (Q), can reveal viral agents that escaped previous screening performed during the window period. Adopted in Germany since 1995 and approved by the American Food and Drug Administration (FDA) in 1998, it is applied to the plasma obtained by decomposition of whole blood, but is also suitable for productive plasmapheresis, at shorter intervals than the whole blood donations. The most appropriate name for the plasma that, regardless of the collection procedure is used only after repetition of the investigation of the donor serological tests for viruses is "donor-retested plasma" or "Q plasma"[27]. Q plasma, which is not submitted to any chemical treatment, retains the protein content and therapeutic efficacy of standard plasma[28]. Although Q plasma could potentially eliminate the window period by repeated donor testing within 4 to 6 months, it still carries the risk of transmitting blood-borne infections that are not detected by screening methods and the safety of the approach depends on test sensitivity. Furthermore, the risk of emerging infections is currently challenging the use of this approach. Finally, an important contribution to the prevention of infectious (and immunological) risks is offered by leucodepletion of whole blood units, especially if universal pre-storage leucodepletion is the method applied[29]. The importance of this procedure derives not only from the possibility of reducing the risk of transmitting viruses that are carried by leucocytes (cytomegalovirus [CMV], Epstein-Barr virus [EBV] and human T-lymphotropic virus [HTLV] 1 or 2), but also of reducing the risk of transmitting bacteria. Although these synergic actions (recruitment of donors, testing, storage, quarantine and leucodepletion) have increased the safety of blood components prepared for transfusions, the risk of transmitting pathogens by transfusion persists[30]. Pathogens such as West Nile virus (WNV) and Chikungunya virus, or variant Creuzfeldt-Jakob disease (vCJD) prion have recently emerged as transfusion-transmissible infectious agents, demonstrating that new infectious agents continue to enter the donor population and may pose significant risks to recipients before they can be completely identified and avoided by sensitive tests[31-34].

In this framework, pathogen reduction technologies (PRT) may offer a new approach to increase blood safety. In fact, these technologies are able to reduce the risk of known and unknown pathogens. The major pathogen reduction methods currently available for plasma involve the use of a solvent/detergent (SD) treatment, methylene blue (MB), and an amotosalen (A) or riboflavin (R) based photochemical process[35-41]. Recently, a new method for pathogen reduction, which is based on ultraviolet C (UVC) light without the use of any photoactive substance for the inactivation of pathogens and leucocytes in human platelet concentrates, has been introduced. This technology, called Theraflex® UV-Platelets system, was developed by the Research Foundation of the German Red Cross Blood Services (*Forschungsgemeinschaft der DRK-Blutspendedienste e.V.*) in cooperation with MacoPharma International GmbH. As the procedure for UVC irradiation applied to plasma units has not been fully validated and its clinical use is under preliminary evaluation, this method is not discussed in this HTA[42].

"Pathogen reduction" and "pathogen inactivation" are terms commonly used interchangeably in the scientific literature. The greatest advantage of these technologies is their proactive nature with the potential to eliminate the threat deriving from unknown pathogens[40]. The principle of all PRT is to use an inactivating agent capable of destroying or reducing the burden of pathogens in the blood components. These pathogens may be intracellular or extracellular. Inactivation techniques must also avoid causing any significant chemical or biological alterations to the therapeutic products. Thus, the efficiency of a technique is based on the destruction of a targeted functional element of the pathogen; this element is not present or not involved in the functionality of the blood component[39]. Most PRT use agents that target membranes or envelopes of the pathogens or their nucleic acids. Agents that destroy membranes or coverings, such as detergents and organic solvents, may be used for cell-free products. They are more effective against non-enveloped viruses. The agents with a mechanism of action targeting nucleic acids, such as photosensitive chemicals and alkylating agents, have a potentially broader spectrum of use. Functional synthesis, as well as microbial proliferation, are stopped by blocking transcription and translation without affecting the cell membranes of therapeutic products. Thus, most pathogens, including viruses, bacteria, fungi, parasites and donor leukocytes, may be destroyed[39].

A more evidence-based approach to the assessment of the safety and efficacy of these new pathogen-reduced plasma products is required[2]. The guidelines and instructions for the use of pathogen-inactivated plasma are currently the same as those for FFP, since there are no dedicated guidelines at present[43].

Utilisation

Some PRT for plasma have been licensed and are being used in Europe and elsewhere. In Europe, the opinions concerning PRT are discordant; some consider these technologies safer than bacterial screening, others claim that they can be risky and do not recommend their use until further research shows a favourable risk-benefit profile, in light of the single patient's health state. The utilisation of these technologies must be appraised country by country in relation to the different approaches to reduce the risks from pathogens in transfused blood components and the use of products made "in house". In general, European countries have totally or partially adopted at least one PRT. The situation is more complex in developing countries. In these countries, the principal transfused component is whole blood and there is a lack of data on their real capacities to adopt PRT[44].

The results of a survey carried out in 2010, which investigated the use of PRT in 51 countries, including 47 members of the Council of Europe, showed that 16 member states of the Council of Europe employed PRT for plasma. Nine of these countries used a mixture of quarantine and PRT: Austria, Germany, Greece, Italy, Poland, Portugal, Spain, Switzerland and the United Kingdom. Seven countries used PRT alone: Belgium, Finland, France, Ireland, Luxembourg, Norway and Sweden. MB plasma was used in Russia in 2009 and SD plasma has been used in Turkey since 1995. The most frequent PRT were solvent/detergent treatment and exposure to MB[44].

Pathogen reduction technologies for plasma for clinical use in Europe

Austria

Austrian health authorities decided to switch from Q plasma to MB technology for pathogen-inactivation of plasma. PRT are currently applied to plasma (SD treatment and MB treatment) and are being evaluated for platelets[44].

Belgium

In Belgium, pathogen reduction of plasma became mandatory in 1994, with the adoption of the SD method. Because of the risk of vCJD, the application of PRT to individual plasma units has been preferred since 2002 and became mandatory in 2003. In 2004, MB treatment was approved. The use of A plasma was introduced in the Blood Transfusion Centre of Mont Godinne in October 2003, and was then used for plasma apheresis in 2007, followed by buffy coat platelets in 2009[45].

Finland

Finland started to use SD plasma in 2007 and since 2013 all units administered are pathogen-reduced by SD

treatment. SD plasma has been transfused to all groups of patients and has also been used to prepare reconstituted blood for exchange transfusion in neonates[44].

France

In France, three types of plasma are authorised by the *Agence Nationale de Sécurité du Médicament et des Produits de Santé* and issued by the *Établissement Français du Sang* for clinical use[46]:

- Q plasma;
- SD plasma;
- A plasma.

A haemovigilance survey of 2005-2009 raised concerns because of the high rates of allergic reactions following transfusion of MB plasma. Consequently, the French regulatory authority decided to discontinue the use of MB plasma and prohibited its transfusion from March 2012[47-50]. However, when haemovigilance data from a French region over a 10-year period were considered, statistical analysis of the updated 2010 data did not confirm a significantly higher incidence of severe allergic reactions with MB plasma than with other types of plasma[49]. A recent analysis, including the 2007-2009 haemovigilance data, revealed a trend, with borderline significance, towards a higher incidence of severe allergic reactions with MB plasma[50]. The variability of these data demonstrates that differences in utilisation rates and slight variations in incidences of statistical outcomes need to be appraised cautiously when interpreting data on very rare events. Amotosalen treatment was introduced in 2006 for apheresis and buffy coat platelets, then used for plasma in 2007[44].

Germany

MB treatment to inactivate viruses in plasma was originally developed by the German Red Cross Blood Centre, Springe, Germany and MB plasma was first produced routinely for clinical use in 1992[51]. The MB system allows treatment of individual units of plasma. Over the years since it was introduced, the process has been modified and the current system (Theraflex MB® plasma system, MacoPharma, Tourcoing, France) has a number of improved features and is used routinely by blood centres in several countries[52]. In 2007, the German Red Cross Blood Centre also received approval from the German authorities to use plasma prepared with the Theraflex MB® plasma system[52]. Germany is waiting for the outcomes of ongoing research on the effectiveness, safety and cost-effectiveness of the amotosalen/riboflavin photochemical processes before introducing these technologies.

Greece

The Greek National Authority approved PRT for 40% of plasma, although only 11.7% is pathogen-reduced with MB. Greece has 5 years of experience with MB plasma: during this period 8,500 units of MB plasma were compared with 54,435 units of untreated plasma given to patients with a similar range of clinical conditions[53]. The Blood Transfusion Committee Implementation of Pathogen Reduction Technologies for Blood Components has strongly recommended the implementation of PRT for plasma and platelet concentrates[44].

Ireland

Since 2002, over 99% of FFP transfused in Ireland has been subjected to SD pathogen reduction; all the plasma for this product used in Ireland comes from the South Texas Blood and Tissue Centre in San Antonio (TX, USA)[54].

Italy

In Italy, SD plasma (Plasmasafe®), introduced in 2005 by Kedrion S.p.A (Castelvecchio Pascoli - Barga, Lucca, Italy), can be acquired as a "service": Blood Transfusion Units give the raw material, FFP, to the manufacturers, who return the pathogen-inactivated product charging the Blood Transfusion Units for the cost of the processing. This treatment is carried out by Kedrion S.p.A using an industrial process involving two filtration phases (1 μm and 0.22 μm)[55]. Only in some Italian regions have specific indications for the use of SD plasma been given, taking into account the cost faced by the Blood Transfusion Units for this activity[55]. In contrast, since 2002, MB treatment of plasma can be performed "in-house", in single Blood Transfusion Units that acquire all the necessary equipment, reagents and materials[55,56]. With respect to the use of photochemical processes (involving amotosalen or riboflavin), the Italian experience is limited to studies on platelet concentrates and the use of these processes for plasma is limited to a few centres or to clinical studies[15].

Norway

Norway has used SD plasma since 1993, even in children. Since 2005, the Health Authorities have also accepted single donor plasma treated with PRT and Q plasma[44].

Spain

In Spain, the Health Authorities of each of the 17 Autonomous Regions can choose between Q plasma and MB plasma as the preferred method for reducing the risk of virus transmission by plasma transfusion. Hospitals that are not allowed to arrange the supply of blood products within their Autonomous Region of competence, involve blood banks outside their jurisdiction. For this

reason, clinical teams have a very limited influence on the kind of plasma they use. Until 2009, plasma inactivated with MB was used in approximately 61% of the autonomous communities, whereas Q plasma was used in the remaining ones[57]. Currently, at a national level around 250,000 units of plasma are transfused annually and MB plasma (introduced in 1999) accounts for 65% of transfused plasma, while Q plasma accounts for the remaining 35%[51]. R plasma and A plasma are also used by customers in Spain[58,59].

United Kingdom

Imported plasma for internal use is currently pathogen-reduced with MB technology. SD plasma is also sourced from other countries. Since 2004, the United Kingdom Blood Services have been importing FFP from the USA for all neonates and children born after 1 January 1996. The decision is a precautionary approach to the risk of transfusion-transmitted vCJD[44].

Switzerland

Switzerland is moving towards nationwide application of PRT to plasma and platelets. SD treatment of plasma for clinical use has been licensed and is currently being used[60]. MB-treated plasma has been registered since 2007[51]. Swissmedic has decided that the implementation in each blood centre will depend on the centres' resources and has approved amotosalen treatment for platelets and plasma[44]. No patients are excluded, so the products are used for all patients including neonates, children, pregnant women, patients undergoing stem cell transplantations and patients receiving very strong chemotherapeutical treatment[61].

Pathogen reduction technologies for plasma for clinical use outside of Europe

PRT are less implemented in developed countries outside Europe[44].

Table II presents the use of pathogen-inactivated plasma in different countries.

Australia and New Zealand

In Australia and New Zealand, pathogen reduction/inactivation strategies employed by the American Red Cross Blood Services include epidemiological control of donors and leucocyte depletion, complemented by routine serological and NAT of the final product. MB plasma, R plasma and A plasma are currently not used in either country[62].

Canada

In Canada, plasma for transfusion is available as either FP, frozen within 24 hours of collection or FFP, frozen within 6-8 hours of collection. FP and FFP can be used interchangeably. There is no SD plasma currently available in Canada. A particular type of SD plasma, named PLAS+SD®, which was manufactured by V.I. Technologies, Inc. (Vitex, Watertown, MA, USA) was once available, but it is no longer made. The European SD plasma named Octaplas® (Octapharma, Vienna, Austria) was introduced into Canada in 1992. The Canadian Health Authority recently licensed Octaplas® SD plasma, which can be considered as an alternative to standard plasma for certain indications[63].

United States of America

In January 2013, the FDA approved the use of the SD plasma named Octaplas® in the USA for plasma exchange in patients with TTP and for the replacement of multiple clotting factors in patients with acquired deficiencies[64]. In December 2014, the use of A plasma was also approved[65]. This is the first step towards the resolution of an unmet need. The USA regulatory agencies have set an extremely high threshold for the approval of any new plasma product for the American market, including even minor changes to existing manufacturing conditions[2]. The SD plasma named PLAS+SD® (Vitex, Watertown, MA, USA), was licensed in the USA in 1998 but was subsequently withdrawn from the market. In spite of the fact that both products are treated by similar SD methods, significant product differences, which can be ascribed to the production methods and type of plasma used, have been reported. PLAS+SD®, along with a number of thromboembolic adverse reactions that had not been observed with European SD plasma, resulted in the interruption of SD plasma production in the USA in 2002-2003[66].

Current management of blood safety

There are two main strategies to ensure the safety of the blood supply: (i) procedures based on rigorous selection of donors and on screening each unit of blood or blood component, and (ii) elimination procedures, which aim to inactivate pathogens and reduce pathogen load[67]. The WHO, the International Federation of Red Cross and Red Crescent Societies, the Council of Europe, the International Society of Blood Transfusion, the International Federation of Blood Donor Organizations and a number of other international and national organisations have defined voluntary non-remunerated blood donation as a founding and guiding principle[68]. Additional measures that minimise the risk of TTI include: selection of low-risk donors through a comprehensive system of donor health and behavioural screening, with consequent temporary or permanent deferral of high-risk donors; skin disinfection procedures and use of diversion pouches to prevent contamination

Table II - Use of pathogen-inactivated plasma in different countries.

Country	Pathogen-inactivated plasma			
	A-plasma	*R-plasma*	*MB-plasma*	*SD-plasma*
Australia				x
Austria			x	x
Azerbaijan				x
Bahrain				x
Belarus		x	x	
Belgium	x	x	x	x
Canada				x
Chile	x			
Czech Republic		x		x
Finland				x
France	x			
Germany	x		x	x
Greece	x	x	x	
Hong Kong			x	x
Hungary				
Ireland				x
Italy	x	x	x	x
Kazakhstan	x	x	x	
Kuwait				x
Luxemburg		x		x
Malaysia			x	x
Malta				x
Mexico				x
Netherlands				x
New Zealand				x
Norway		x		x
Oman				x
Poland		x	x	
Portugal				x
Qatar		x		x
Romania				x
Russia	x	x	x	
Saudi Arabia				x
Serbia		x		
Singapore			x	
Slovakia				x
Spain	x	x	x	
Sweden				x
Switzerland				x
Turkey	x			
Ukraine	x		x	
United Arab Emirates	x			x
United Kingdom			x	x
United States of America	x*			x
Yemen		x		

* Approved by the FDA on 12/16/2014. A-plasma: amotosalen-treated plasma; R-plasma: riboflavin-treated plasma; MB-plasma: methylene blue-treated plasma; SD-plasma: solvent/detergent-treated plasma. Source: modified from reference 94.

from skin flora; routine serological screening for HBV, HCV, HIV 1-2, HTLV 1-2 and syphilis; routine NAT for HIV, HBV, and HCV; targeted testing for malaria and CMV; routine bacterial decontamination screening of platelet concentrates[23,69]; and the promotion of appropriate transfusion practices via education and clinical research to minimise patients' exposure to transfusions[70].

More recently, measures against potential TTI by prions (infectious proteins) have been introduced. In 2003, the first evidence of human-to-human transmission of vCJD via a blood transfusion was obtained in the United Kingdom, and two other cases were subsequently confirmed[71].

Early in 2004, the UK Department of Health, in addition to universal leucodepletion of all donated blood, excluded from blood donation all subjects known to have had blood transfusions since 1980[71]. Given the concerns about the possible transmission of vCJD and the unavailability of testing for this disease, individuals who lived in the United Kingdom for a total period of 6 or more months between 1980 and 1996 and individuals with a history of receiving transfusion in the United Kingdom after 1980 are ineligible to donate blood.

Many countries have adopted a system of leucodepletion at the point of collection, prior to storage (pre-storage filtration). Leucodepletion may reduce the risk of transmission of vCJD via the blood supply but there is some uncertainty regarding the efficacy of this method as infectious prions may reside in the plasma, as well as the white cell component of whole blood[72-74].

Gamma or X-irradiation of blood components, by validated systems, is already a well-established practice to prevent the development of transfusion-associated Graft-versus-Host disease (GvHD) and studies have been conducted to determine the virological and bacteriological safety of irradiated blood products and protein blood preparations[75]. Gamma irradiation can act by two different mechanisms. The first is the direct rupture of covalent bonds in target molecules, including both proteins and nucleic acids. The second is an indirect mechanism that uses water to produce reactive free radicals and other active radiolytic products, which in turn can react with a variety of macromolecules, including both proteins and nucleic acids. Indirect reactions can be reduced by adding radical scavengers, removing water by lyophilisation, and/or working at cold temperatures. More recently and for the same total dose of radiation, reducing the dose rate has been reported to improve the balance between protein recovery and virus inactivation. The kinetics of viral killing is typically linear in a semi-logarithmic plot of virus titre versus radiation dose, suggesting that inactivation occurs with a single hit of radiation that is absorbed directly by the nucleic acids. The principal challenge in using gamma irradiation is the inactivation of the desired quantity of virus while maintaining the structural and functional integrity of proteins[76].

As a result of these strategies, morbidity and mortality from TTI, including parasites, are exceedingly rare. There is still a residual risk of infections by pathogens for which no detection system currently exists (e.g. emerging pathogens) or for those with a short "window period", when blood levels of specific disease markers are too low for detection. NAT has markedly reduced but not completely eliminated this risk. Meanwhile, bacterial contamination, especially of platelet concentrates (because of their storage at ambient temperature allowing for bacterial proliferation), is recognised as the most common cause of TTI.

New screening tools are being developed not only to verify the functional integrity and quality of blood products, but also to assess the success of the pathogen reduction processes. Proteomics, the large-scale study of protein structure and function using technologies such as mass spectrometry, has the potential to play a crucial role in quality-control processes, but is not currently used in routine clinical practice because of its high costs and the lack of specialised personnel. High throughput systems, such as DNA microarrays, will also enable the simultaneous detection of a large number of pathogens; however, these systems currently rely on the amplification of target DNA and are not suitable for large-scale blood banking use[77]. Biosensors, nanoparticle-based DNA and protein bio-barcode amplification assays are some emerging diagnostic tools that may be used for screening donated blood[78,79].

Only plasma that is serologically and NAT negative for HIV-1, HBV and HCV is provided to industry for treatment. As regards the production of plasma-derived medicinal products, the FDA in the United States and the European Medicines Agency (EMA) in Europe have enforced the related regulations, leading to stricter[2] requirements for donor selection and donation screening since the 1970s, as well as for validated virus removal and/or inactivation during the production processes since the 1990s. In addition, since 2005, in Europe there is a requirement to report and assess the epidemiological data of the donor population in reference to the risk of infection[80].

In 2011, the EMA guidelines on epidemiological data assessment were enhanced. With regards to plasma fractionation, the assessment of epidemiological data in the framework of marketing authorisation for plasma-derived medicinal products is included in the Plasma Master File in Europe. In the United States, epidemiological data are evaluated, but out of the scope of marketing authorisation for plasma-derived medicinal products. There has been some harmonisation

of regulations in the USA, Europe, and Japan, through the International Conference of Harmonization, but further work is needed[81].

Current pathogen-reduction methods cannot, however, inactivate or destroy most spores and non-conventional agents such as prions[39]. That is why adoption of so-called "double viral elimination" procedures has been encouraged for plasma-derived medicinal products. Enhanced safety can be achieved through protein purification methods, such as antibody-mediated affinity chromatography, or the incorporation of "nanofilters" with a sufficiently small pore size so as to trap viruses while permitting the passage of the desired proteins, or the incorporation of a second viral inactivation step[41].

It must be ensured that the pathogen inactivation techniques affecting the integrity of nucleic acids do not cause toxicity in the transfused patients[43].

Methods of pathogen removal used by industry (but not by blood centres)

Pasteurisation

Pasteurisation by heating proteins in an aqueous stabilised solution at 60 °C for ~10 hours inactivates both lipid membrane-enveloped and a range of non-enveloped viruses. Since coagulation factors are heat sensitive, stabilisers (usually sugars, amino acids, or acetate) are added to preserve protein integrity and are removed after pathogen inactivation. The homogeneity of temperature throughout pasteurisation must be validated by temperature mapping techniques[76]. Studies on inactivation of HIV, hepatitis A virus (HAV), and parvovirus B19 (PVB19) by pasteurisation in a FVIII/VWF concentrate have demonstrated viral reduction of at least 6.4, 4.2, and at least 3.9 log, respectively. Additional studies have demonstrated that pasteurisation inactivates a wide range of enveloped and non-enveloped viruses, including bovine viral diarrhoea virus (BVDV; a specific model virus for HCV), pseudorabies virus (PRV; a non-specific model virus for HBV), herpes simplex virus-1, WNV, and poliovirus[82].

Dry heat treatment of lyophilised products

Most plasma-derived concentrates are lyophilised and, especially those treated with SD, subsequently treated with dry heat to inactivate non-enveloped viruses that resist the SD treatment. Lyophilisation inactivates viruses to a certain degree[83]; the moisture content of lyophilised products undergoing dry heat treatment should be kept low (typically <2%), as residual moisture may affect product stability, even though higher levels may enhance inactivation of some viruses[37]. Dry heat treatment of lyophilised products has been demonstrated to give favourable results regarding inactivation of relevant or model viruses of HAV, HBV, HCV, and HIV[38,83]. Dry heating of a lyophilised FVIII product at 80 °C for 72 hours inactivated a wide range of viruses; dry heating of FVIII or FIX concentrates reduced the risk of HCV transmission. An additional study demonstrated that terminal dry heating of a lyophilised FVIII concentrate at 100 °C for 30 minutes inactivated HAV and HIV to below detectable levels within 10 minutes, while retaining approximately 95% of FVIII activity; whereas heating a lyophilised FVIII and VWF concentrate at 100 °C for 120 minutes reduced a wide array of viruses (HIV, Sindbis virus, PRV, reovirus type-3, HAV, and PVB19) by more than 4 log[81,84]. Although PVB19 was reduced by dry heat in validation studies, the reduction factor may not be sufficient for complete inactivation of the virus load in the final product; indeed, asymptomatic PVB19 infection was detected in a patient who received FVIII concentrate treated at 80 °C for 72 hours[82].

Vapour heat

A drawback to lyophilisation, in addition to that of stabilising coagulation factors, is the removal of water that can also stabilise potentially infectious viruses in the product. By adding water vapour to lyophilised products before heating, higher levels of virus inactivation can be achieved at equivalent temperatures. Vapour heating of lyophilised products targets enveloped and non-enveloped viruses and has been demonstrated to inactivate hepatitis viruses and HIV. Lyophilisation and vapour heat at 60 °C inactivated approximately 6 log of HAV in spiked FVIII concentrates within 8 to 10 hours and similarly reduced HAV titres in FIX concentrates within 3 hours. Among 20 patients with haemophilia who received vapour-heated FIX infusions, none developed markers for infection with HCV or HIV during 6 to 15 months of follow-up, suggesting that vapour heat-treated concentrates may be associated with a low risk of viral infection. However, vapour heat at 60 °C may be insufficient to inactivate HBV completely, depending on the viral load in the starting material (batch-related) and the amount of product given to non-vaccinated patients against HBV; HBV infections have been reported in four non-vaccinated haemophiliacs who vapour-heated FVIII concentrate[82].

Iodine

Iodine is a strong oxidising agent and, therefore, a powerful microbicidal agent. However, it is not sufficiently selective in its free form. When bound to polymers such as polyvinylpyrrolidone, cross-linked starch, or dextran chromatographic medium, the virucidal action of iodine is more controlled. In these bound forms, the iodine is slowly released into the protein

solution and virus inactivation occurs over hours. Starch-bound iodine at a concentration of 1.05 mg/mL resulted in more than 7 $\log_{10}$ inactivation of model lipid-enveloped and non-enveloped viruses, while more than 70% of the activity of the clotting factors in plasma was retained. In another implementation, protein was passed through a bed of iodine-cross-linked dextran gel followed by a bed of cross-linked dextran gel used to trap and remove free iodine[76].

Partition processes: fractionation and chromatography

Besides being inactivated, viruses can be physically removed from plasma-derived products by fractionation (precipitation or chromatography) and filtration. Before the 1980s, plasma fractionation was mainly considered as a step in protein purification; no dedicated viral reduction step (except for pasteurisation of human albumin) was implemented during the manufacturing process. Although ethanol precipitation is the most widely used plasma fractionation method worldwide, it is not used for the production of coagulation factor concentrates, as this treatment denatures the desired proteins. After initial cryoprecipitation, many clotting factor concentrates are purified chromatographically[75]. Most viral reduction treatments are integrated with the protein fractionation process ("in-process" treatments), but some currently based on heat inactivation procedures are applied to products in their final container (terminal treatment)[43].

In-process viral inactivation treatments

SD treatment remains the most frequent viral inactivation procedure for plasma products. Typically, lipid-enveloped viruses are inactivated in a few minutes and the functional activity of even the most labile plasma proteins (with the possible exception of some serine protease inhibitors) is well preserved, but non-enveloped viruses are not inactivated. The SD agents are reduced to a level of a few parts per million, usually by chromatographic adsorption or specific precipitation of proteins, or selective adsorption on hydrophobic chromatographic support. Pasteurisation, another common viral inactivation procedure, is heat treatment of protein solutions for 10 hours at 60 °C. It denatures viral proteins and inhibits virus replication. Pasteurisation can inactivate both enveloped and non-enveloped viruses; however, the stabilisers, which are needed to limit the loss of protein functionality, may decrease the rate and the extent of viral inactivation. Stabilisers can be removed by ultrafiltration, protein precipitation, or chromatography. Vapour heat has also been used by one company; the extent of viral inactivation is influenced by the temperature, duration, and pressure during the treatment. The risk of neo-antigen formation, which can enhance protein immunogenicity, should be considered when using heat-based inactivation processes[82].

Low pH incubation, usually at pH 4, at 30 °C to 37 °C for more than 20 hours, was introduced in the early 1980s to allow the intravenous infusion of IgG. This form of treatment was subsequently found to inactivate most lipid-enveloped viruses. Caprylic (octanoic) acid precipitation/incubation at a pH below 6 is a recent treatment of human IgG that can inactivate lipid-enveloped viruses. Some products are further purified using affinity chromatography mediated by an antibody against the protein of interest. It is worth clarifying that when re-using chromatography columns, viruses that adhere to resins cannot be completely washed out. Equipment and materials must, therefore, be sanitised with validated chemical and/or physical treatments to inactivate and remove viruses to ensure no cross-contamination of subsequent batches of the product.

Terminal viral inactivation treatments

Heat treatment of lyophilised products (dry heat) is used in modern plasma fractionation; however, due to the limitations of this treatment, it is mainly utilised as a secondary viral inactivation step rather than the core inactivation method. The treatment is applied to some coagulation factor concentrates. Performed at 80 °C for 72 hours or at 100 °C for 30 minutes and generally in the presence of protein stabilisers, it provides added safety against HAV and other heat-sensitive viruses but may not be sufficient to exclude PVB19 transmission. Terminal (liquid) pasteurisation at 60 °C for 10 hours is the "gold standard" treatment of albumin preparations. The fatty acids, caprylate, and tryptophanate, which protect albumin from heat denaturation, are added at doses compatible with therapeutic use and, therefore, are not removed before product infusion[82].

Viral filtration

Nanofiltration is a specific viral filtration process applied to protein solutions. It involves multilayer membranes of 15 to 75 nm, or equivalent systems, to remove viruses mostly by a sieving mechanism. It was introduced in the early to mid-1990s and reached wide acceptance as a robust viral removal step for all products, apart from albumin. Nanofiltration is used to complement the core viral inactivation treatment and to provide enhanced safety against non-enveloped viruses or other resistant infectious agents. Viruses can also be removed incidentally during protein precipitation, chromatography, or filtration steps. These steps contribute to lowering the viral load from the protein production stream; they are difficult to monitor and do not, therefore, guarantee, as stand-alone procedures, a sufficient safety margin[82]. Viruses may be removed from clotting factor

concentrates by filtration, employing retentive filters with smaller pores than the diameter of the viruses[85]. Filtration may not be feasible for products containing proteins of a size comparable to or larger than the viruses or pores of the filter; thus, filtration is mainly limited to smaller-molecular-weight coagulation factors (e.g., FIX or FVIII). However, a VWF concentrate filtered through 35 nm pore membranes had a reduction of enveloped viruses (HIV, BVDV and PRV) of at least 5.0 log, although filtration removed large VWF multimers as well. Filtration of a FVIII concentrate using 35 nm or 15 nm sequential filtration resulted in removal of both enveloped viruses (HIV, BVDV, and PRV) and non-enveloped ones (HAV and PPV). Viral filtration alone resulted in virus reduction factors of at least 3.6 $\log_{10}$, while a combination of SD, chromatography and viral filtration yielded reduction factors of at least 5.1 log for all tested viruses. Viral filtration of a FXIII concentrate using a 20 nm filter resulted in a reduction of enveloped (HIV, BVDV, PRV) and non-enveloped (HAV) viruses by a factor of at least 5.5 log and a reduction of a non-enveloped parvovirus by 3.4 log. In a safety study of FIX concentrate, a final viral filtration step was added following fractionation, chromatography, and SD treatment. The 35- to 15-nm sequential filtration step resulted in a reduction of at least 6.8 log for HAV and at least 6.6 log for BVDV. Filtration of another FIX concentrate using two filters (20 nm mean pore size) in series removed enveloped and non-enveloped viruses very effectively[76].

Life cycle of the technologies

The use of PRT for labile blood components is increasing slowly but steadily. Concerns about reduced component quality and toxicity have, however, limited or prevented the routine use of PRT in many countries. In a variety of clinical situations, the supportive and often prophylactic nature of blood component therapy complicates the clinical evaluation of novel blood products. An increasing use and development of these novel technologies suggests that pathogen reduction has entered a stage of maturity that could further increase the safety margin in haemotherapy[86].

In Europe, PRT are now widely available with multiple CE-marked products to treat both plasma for transfusion (Intercept®, Mirasol®, Octaplas®, Octaplas LG®, Plasmasafe®, Theraflex®, Uniplas®) and platelets (Intercept®, Mirasol®).

Since the beginning of the 1990s, considerable progress has been made in the development of PRT and some of them are already employed in current clinical practice. Such PRT are based on photosensitisers that are added during processing and, after being activated, generate active oxygen species or utilise electron transfer processes, which are oxygen independent, to damage predominantly nucleic acids (photodynamic reactions). Another class of agents forms irreversible covalent cross-links in nucleic acids (photochemical reactions) to prevent transcription, translation, and proliferaton of the pathogen. The rationale of targeting nucleic acids is that pathogens and white blood cells require nucleic acid function that is not required for the therapeutic effects of platelets, plasma, and red blood cells. The reduction capacity should reach at least 4-6 $\log_{10}$. Currently, the most important substances with photodynamic properties studied for clinical application are the essential vitamin B2 (riboflavin), the phenothiazine derivative MB, psoralens such as S-59 (amotosalen) and S-303 or the inactive PEN 110. The latter compounds also interfere with nucleic acids by alkylation chemistry but become activated by mechanisms other than an external light source, i.e. upon pH shift, which is important for red blood cells, since their haemoglobin absorbs UV light strongly[87,88].

The use of MB technology for viral reduction of single plasma units was originally developed by the Blood Centre of the German Red Cross, Springe, Germany; and MB plasma was first produced routinely for clinical use in 1992. The emerging Theraflex® MB plasma system has a number of improved features and has been successfully and routinely used by blood centres in several countries[52]. After addition of MB to the plasma, the plasma is exposed to white-light to inactivate viruses and then, in more recent approaches, filtered to reduce the MB[89].

The SD method to inactivate enveloped viruses in plasma protein preparations was first developed in the early 1980s and as a pooled standardised pharmaceutical product with extensive in-process control was first described by Horowitz and Colleagues in 1992[90,91]. The SD process is the most widely used, best validated and robust pathogen inactivation technology currently known; many million doses of SD plasma proteins have been transfused[91].

Intercept®-treated plasma (Cerus Corporation, Concord, CA, USA) is a pathogen-reduced product, CE-marked in Europe, and gradually being adopted in blood centres in Spain, France, Belgium, Switzerland, and other European countries. A pathogen inactivation process employing the synthetic psoralen amotosalen HCl (S-59) and UVA light has been developed for the treatment of single units of plasma and apheresis or pooled platelet units[92] and is followed by the removal of the amotosalen and its breakdown products through the use of a filter compound absorption device[1]. Intercept® plasma was extensively studied before being released on the market and the published data on this product include *in vitro* and animal toxicology studies[93].

Mirasol®-treated plasma (CaridianBCT, Inc., Lakewood, CO, USA) has been available on the

European market for more than a decade, while the option of using Mirasol® to treat previously FP has been validated more recently and enables sites to treat products already in their inventory. Single units of apheresis or whole blood plasma are treated and frozen within 8 hours of collection. Although Mirasol®-treated plasma is being used for clinical purposes in some European countries, few data have been published on its clinical efficacy and safety[2].

Regulatory status of the technologies

Several PRT for plasma and platelets have been licensed and are in use in Europe and elsewhere. Some countries (e.g. Switzerland and Belgium) have adopted PRT nationwide, whereas other countries (e.g. France, Germany and Spain) only perform pathogen reduction in some regional blood transfusion services[86]. Blood components for transfusion are classified differently across European countries. In Germany, they are considered as pharmaceutical drugs and must meet the latest pharmaceutical research and development standards. Plasma production licenses are issued by local authorities and marketing authorisations by the competent federal authority. Therefore, major changes require that transfusion services apply for a new license. Similar systems are in place in other countries, such as Switzerland and France. Moreover, while European blood directives define quality and safety standards for the testing, processing, storage, and distribution of human blood and components, individual Member States are free to adopt higher standards than those set by the European Union directives. Therefore, as one might expect, stages of accreditation as well as opinions on the scientific/clinical, regulatory and economic aspects, vary widely across Europe. A recent survey revealed that 80% of European countries routinely use pathogen-inactivated plasma, while a further 50% plan to introduce it or to expand the current portfolio in the near future[94].

With regards to coming developments, the landscape of PRT in Europe is multifaceted, since regulation varies and decision makers have different scientific, medical and political viewpoints. Nevertheless, in principle the regulatory framework setting high standards for establishing pathogen inactivation and harmonising the procurement of safe blood in Europe is in progress.

In the early 1990s, SD plasma was considered a blood product in Germany and France. In most of the other European countries, it is treated as a medicinal product[95]. SD plasma was first introduced in the German Federal State of North Rhine-Westphalia in 1991. In Norway, SD plasma replaced plasma in 1993 following a successful clinical trial in 1992[96]. Currently, ordinary medicinal licensing is required for SD plasma in European countries except in France, where it is classified as a labelled blood product. Octaplas® is now a licensed biopharmaceutical product in 29 countries worldwide. Recently, the FDA approved the use of Octaplas® (ligand gel)[64], which is a SD-treated, blood group-specific, pooled human plasma product developed by Octapharma (Vienna, Austria). In several European countries and in Australia, OctaplasLG® has been marketed since 2009 and its previous versions since 1992. Confirmatory studies on the claimed capacity to remove prions have been requested by some countries and are currently approaching completion[60]. Octapharma has also developed Uniplas®/UniplasLG®, a non-blood group-specific SD plasma. Uniplas®/UniplasLG® differs from Octaplas®/OctaplasLG® only in that anti-A and anti-B antibodies are removed; thereby, making it universally transfusable. Uniplas®/UniplasLG® is not licensed for use in the USA or European Union[97].

Regulatory approval was long delayed in the USA compared to Europe. The FDA's attitude toward SD plasma began to change in 1990-1991. In 1998 VITEX (V.I. Technologies, Watertown, MA, USA) was selected as the sole distributor of PLAS+SD® plasma[60]. The introduction of PLAS+SD® with the American Red Cross logo and the fact that the American Red Cross was the exclusive US distributor provoked strong reactions from members of America's Blood Centres, who collect and distribute half of the nation's blood supply[59].

Additionally, the American Red Cross set the price for PLAS+SD® 30% higher than expected and remarkable differences between PLAS+SD® and the European SD plasma were demonstrated[2]. This issue, along with a number of thromboembolic adverse reactions that had not been observed with European SD plasma, resulted in the termination of SD plasma production in the USA in 2002-2003[60].

A promising development is the use of MB for viral reduction of single plasma units. This technology was originally developed by the German Red Cross Blood Centre in Springe, Germany and MB plasma was first produced routinely for clinical use in 1992[51]. The emerging Theraflex® MB plasma system (MacoPharma, Tourcoing, France) has a number of improved features and has been used routinely by blood centres in several countries. According to information from MacoPharma, the national authorities that have already given regulatory approval worldwide for the use of Theraflex® MB plasma are: Paul Ehrlich Institute (Germany), *Agence Française de Sécurité Sanitaire des Produits de Santé* (France), Swissmedic (Switzerland), *Administración Nacional de Medicamentos, Alimentos MacoPharma Tecnología Médica*-ANMAT (Argentina), *Ministerio de Salud Pública* (Uruguay), *Agence de la Santé Publique*

du Canada (Canada), Agency for Medicinal Products and Medical Devices (Croatia), *Agência Nacional de Vigilância Sanitária* (Brazil), and the Ministries of Health of Russia, Mexico, Kazakhstan, Belarus, Hong Kong, Singapore and Ukraine.

The current legislation in the United Kingdom does not require independent formal pathogen reduction validation other than occasional studies on individual pathogens. The UK Department of Health recommended that plasma given to neonates and children born on or after 1st January 1996 should be obtained from an area free of bovine spongiform encephalopathy and subjected to pathogen-reduction procedures. In 2012, this approach was extended to recipients aged 16 years or more by the Advisory Committee on the Safety of Blood, Tissues and Organs. Imported plasma is treated with MB as a pathogen reduction step and is currently recommended for use in all patients. Patients with TTP have high donor exposure and should be treated with non-UK virus-treated plasma and it is recommended that SD plasma is used[98].

In Spain approximately 250,000 units of plasma are transfused annually and 65% are MB plasma. The Springe method is currently used in Spain by Grifols (Parets del Vallés, Spain)[99]. The Intercept® Blood System gained the CE mark for platelets in 2002 and for plasma in 2006, allowing market distribution in Europe for both components. The Intercept® Blood System has been registered with a class III CE mark as a drug-device combination[59]. It has been approved by the French regulatory authority, Agence Francaise de Securite Sanitaire des Produits de Sante and is used for clinical purpose in four French regions: Ile de la Réunion, Martinique, Guadeloupe-Guyane and Alsace.

In Germany, the first blood centre marketing authorisation was approved by the Paul Ehrlich Institute for the Intercept® Blood System for Platelets in 2007 and for the System for Plasma in 2011. The approval by the Paul Ehrlich Institute allows the use of Intercept® as an alternative to gamma-irradiation and to omit CMV serology. The Intercept® Blood System for Platelets was approved by Swissmedic in 2009 and the Plasma System in 2010[61].

In the United States, the FDA approved the Intercept® Blood System for the preparation of plasma in order to reduce the risk of TTI[65].

The Mirasol® system for plasma for transfusion is currently approved and used in Europe for the treatment of platelets and plasma[100]. It received a CE mark in August 2008, after the initial CE-mark approval of the Mirasol PRT System® for Platelets in October 2007[101]. The Mirasol® system (mostly for platelet treatment) is currently used in more than 50 blood centres in 15 countries throughout Europe, the Middle East and Africa; it is not available for sale in the USA[102]. Mirasol®-treated plasma (R plasma) shows high overall protein retention under a broad range of blood banking conditions and meets the Council of Europe guidelines. The option of treating FP with Mirasol® has also been validated and enables sites to treat products already in their inventory[103].

Description and technical characteristics of the technologies

An effective method for pathogen reduction must fulfil at least three essential criteria: first of all, it must physically remove viruses and blood cells that contain viruses; secondly, it must penetrate selectively into blood cells without damaging them and finally, it must effectively inactivate free viruses, viruses attached to cells and intracellular viruses[35]. The SD method of treatment is standardised and is generally not carried out in individual sites but usually occurs in a centralised manufacturing centre in which plasma is processed as a large pool (500-2,500 donations). The other three procedures are used to treat single-donor units of plasma at the blood bank itself. The MB and A methods generally involve a final step in which the reactive agent is removed (such as the SD method). The R method is the only one in which removal of the active reagent is not required[35].

Solvent/detergent method

The SD method to inactivate enveloped viruses in plasma protein preparations was first developed in the early 1980s[90]. The method proved to be effective in the processing of coagulation factor concentrates by disrupting the membranes of lipid-enveloped viruses, cells and most protozoa, while leaving the labile coagulation factors intact. Its efficacy with respect to bacteria is generally more variable, and it is ineffective against non-lipid-enveloped viruses[91]. SD treatment is the only current technology for viral inactivation of pooled plasma[86]. The SD process is the most widely used, best validated and robust pathogen inactivation technology known today, and many million doses of SD-treated plasma proteins have been transfused without any report of transmission of enveloped viruses[91,104].

The procedure generally consists in incubating protein solutions for 1-6 hours with 0.3-1% of an organic solvent, tri(n-butyl) phosphate (TNBP), and one or several detergents, generally Tween-80, Triton X-100, or sodium deoxycholate[105]. The SD treatment is preceded by filtration with a 1 μm filter to remove cells and debris, followed by sterile filtration into bags or vials using a 0.2 μm filter and aseptic filling[106]. The SD treatment requires that plasma is thawed rapidly and

treated for 4 hours at ~30 °C with TNBP solvent and Triton X-100 (or Tween-80) detergent[107], both at 1%. The TNBP is then removed by extraction with ricin oil and the Triton X-100 by hydrophobic chromatography; these processes are followed by sterile filtration and packaging in units of 200 mL. Complete inactivation of an added virus inoculum typically occurs within the first 15 minutes of the 4-hour procedure[108]. A castor-oil extraction and separation phase are performed to remove TNBP, this is followed by a clear filtration.

Over the years, starting from the initial method proposed by Bernhard Horowitz in 1986-1987 the SD plasma method has undergone several changes. Octapharma introduced the process to its factory in Vienna and licensed it to the French Transfusion Service, which began the manufacture of SD plasma at its Regional Blood Transfusion Centre in Bordeaux in 1992[109,110]. In 1991-1992 manufacturing of SD plasma began, initially at Melville Biologics (a division of the New York Blood Center), using many of the same procedures pioneered in Europe by Octapharma.

The manufactured product was characterised in 1992, when Horowitz and his collaborators published their results[37,111]. Later, due to the lack of adequate resources at the New York Blood Center, manufacturing was transferred to a spin-off, V.I. Technologies, Inc. VITEX (Watertown, MA, USA), which subsequently produced the American SD plasma, PLAS+SD®. In the second half of the 1990s, the National Bioproducts Institute of Pinetown, South Africa, developed ABO-independent, universal plasma (Bioplasma FDP®) using the SD treatment manufacturing process licensed by Octapharma[60]. Biesert and Shartono subsequently validated the robustness of SD plasma treatment in their study of the viral safety of Octaplas® (Octapharma AG, Lachen, Switzerland)[112]. A universal SD plasma (Uniplas®) has been developed by Octapharma PPGmbH, Vienna, Austria. Uniplas® is obtained by optimal mixing of plasma of different blood groups in order to neutralise unwanted anti-A and anti-B antibodies (of both IgM and IgG classes). Kedrion (Castelvecchio Pascoli, Italy) introduced SD plasma (Plasmasafe®) in 2005[55], also using Octapharma's licensed manufacturing process. Production of SD plasma at the German Red Cross Center in Hagen ended in 2006[60]. Except for differences in the plasma pool size (60 L in France, 200 L in Germany and South Africa, 380 L in Austria and up to 650 L in the USA), SD plasma has so far been manufactured by similar, albeit not identical methods. Octapharma has launched OctaplasLG®, an SD plasma whose manufacture involves an additional chromatographic step combining a specific prion-binding ligand gel with SD treatment, time-reduced from 4 to 1-1.5 hours[113]. Recently, a SD plasma viral inactivation treatment for transfusion and minipool cryoprecipitate (5-10 L of plasma) was developed to address such viral transmission concerns[114]. This treatment uses 1% TNBP as the solvent and 1% Triton X-45 instead of the Triton X-100 or Tween-80 detergent widely used in the plasma fractionation industry. The SD treatment is then performed at 31 °C for 4 hours, followed by two or three oil extraction steps optionally followed by hydrophobic chromatography. For pathogen reduction by solvent only (2% TNBP), the incubation temperature is increased to 37 °C[115]. The data confirm that the treatment at 31 °C is effective at inactivating lipid-enveloped viruses in plasma and cryoprecipitate[115]. This "mini-pool" system was pioneered by Burnouf, in collaboration with Egyptian blood bankers, for use under Good Manufacturing Practice (GMP) conditions of blood banks in developing countries[116].

Table III shows a comparison of the main features of the various SD plasma products currently available.

In vitro studies have demonstrated that the SD method applied to plasma eliminates all lipid-enveloped viruses, including WNV, with high safety margins; the exception is vaccinia virus, which is relatively resistant to this inactivation treatment. The existing literature demonstrates the inactivation of several marker viruses (vesicular stomatitis virus, Sindbis virus, Sendai virus) and other viruses such as HIV and HBV. The SD plasma production process also eliminates bacteria, protozoa, cells and cellular fragments; however, it has no effect on non-lipid-enveloped viruses. Finally, laboratory, preclinical and clinical evidence indicates that FVIII and other proteins present in the preparation are unaffected by SD treatment[117]. Although SD treatment has no effect on non-lipid-coated viruses, it prevents transmission of HAV and PVB19. Moreover, it contributes, along with neutralising antibodies in the initial plasma pools, lowering the virus concentration by pooling, and the hydrophobic chromatography, to eliminating the residual risk of clinically apparent infections with PVB19 and HAV[118]. SD treatment can be preceded by nanofiltration to remove cells, cell fragments and membrane-associated viruses, and followed by sterile filtration and aseptic filling[119]. The production process for OctaplasLG®, Octapharma's second-generation SD plasma product, includes an affinity ligand chromatography step designed to bind prion agents at the level of prion infectivity, thus potentially reducing the risk of transmission of prion disease[120, 121].

Methylene blue method

The first procedure described for inactivating pathogens in plasma involved the use of MB.

MB is a positively charged phenothiazine derivative. It consists of a thiazine ring and two dimethylamino

Table III - Features of different solvent/detergent-treated plasma products.

SD plasma	Source	Pool size	Inactivation time and temperature
Octaplas® (Austria)	Apheresis or recovered plasma	380 litres (630 apheresis units or 1,520 recovered units)	4 h at 30 °C (1% TNBP + 1% Triton X-100)
Octaplas LG® (Austria)	Apheresis or recovered plasma	380 litres (630 apheresis units or 1,520 recovered units)	1-1.5 h at 30 °C (1% TNBP + 1% Triton X-100)
Uniplas® (Austria)	Apheresis or recovered plasma	380 litres of plasma of different blood groups (1,000 apheresis units or 1,520 recovered units)	1-1.5 h at 30 °C (1% TNBP + 1% Triton X-100)
Plasmasafe® (Italy)	Apheresis or recovered plasma	380 litres (630 apheresis units or 1,520 recovered units)	4 h at 30 °C (1% TNBP + 1% Triton X-100)
Plasma viro-atténué SD® (France)	Apheresis plasma	60 litres (100 apheresis units)	4 h at 30 °C (1% TNBP + 1% Triton X-100)
Bioplasma FDP® (South Africa)	Recovered plasma	200 litres	4 h at 30 °C (1% TNBP + 1% Triton X-100)
"Mini-pool" (for blood bank application in resource-limited countries)	Recovered plasma	5-10 litres	4 h at 31 °C (1% TNBP + 1% Triton X-45) or 4 h at 37 °C (2% TNBP)

SD: solvent/detergent; TNBP: tri(n-butyl)phosphate. Source: modified from reference 60.

groups. Its chemical name is: 3,7-bis(dimethylamino) phenothiazine-5-ylium chloride. At room temperature, MB is an odourless dark green powder and is soluble in cold water. The alternative colourless leucomethylene blue is readily oxidized into MB in the presence of oxygen[52]. The virucidal action of illuminated phenothiazines, such as MB, was recognised in the early 1930s[122]. MB intercalates with nucleic acid strands. Visible light excites the phenothiazine dye and, by energy transfer, produces highly reactive singlet oxygen. The latter oxidizes guanosine and thereby breaks nucleic strands, preventing pathogen replication[123].

An experiment on HIV showed that MB treatment also acts on other target sites such as the envelope, core proteins, and reverse transcriptase enzyme[124]. The MB system does not remove the viral nucleic acid that has been inactivated. As a result, assays that are based on NAT could remain positive, even though the treated plasma is no longer infectious. Similarly, assays based on specific antibodies could also detect inactivated viral particles. Consequently, infectivity assays using cell cultures or animal models are most suitable for assessing the pathogen inactivation capacity of the MB system[124]. Finally, the rationale for the use of phenothiazine for pathogen inactivation of blood components is based on the absence of nucleic acids in red cells or plasma; the potential for preferential binding of phenothiazine to viral nucleic acid over binding to lipids or proteins of plasma or red cells; and limited diffusion of reactive, oxidizing species produced by illuminated dyes[122].

In 1991, Lambrecht *et al.* described a method to inactivate virus in plasma using MB dye and visible light[125]. Two different methods for MB treatment of human plasma for viral reduction are currently in use. The original "Springe" method, developed by the Blood Centre of the German Red Cross Chapters of Lower Saxony, Saxony-Anhalt, Thuringia, Oldenburg and Bremen, Institute Springe and commercialised by Grifols S.A. (Barcelona, Spain) and the Theraflex® MB method originally designed by MacoPharma (Mouvaux, France).

The inactivating process can be divided into five steps: (i) intercalation of MB; (ii) excitation of the MB by visible light; (iii) formation of singlet oxygen; (iv) oxidation of guanosine; and (v) strand breakage.

The Springe process requires a 1 μM concentration of the photosensitiser and a red light (600-700 nm) fluency rate of approximately 10 $mWcm^{-2}$. The original protocol was in use for more than a decade and over one million MB plasma units were transfused before the Paul Ehrlich institute withdrew the license[126]. The original Springe MB plasma process, described by Lambrecht[51], used freezing and thawing of plasma units to disrupt leucocytes and thereby release intracellular viruses, because MB only partially penetrates cell membranes[38]. The recent introduction of blood collection packs that combine leucoreduction and addition of MB before inactivation of the plasma (Baxter Pathinact, Baxter Healthcare, Compton Newbury, Berkshire, UK, and MacoPharma Theraflex®, Middlesex, UK) has eliminated the need for freezing and thawing of the plasma[38].

There are also differences among the systems to add MB to plasma. With two of the systems (Springe and Baxter), a variable dose of MB solution was added to achieve a standard final concentration of 1 μM and, afterwards, the plasma bag was illuminated with fluorescent tubes on one side for 1 hour[127].

The efficiency of filtration is ensured by integrity testing during filter manufacturing and by routine monitoring of residual cell content and filtration time in the blood bank. The Blueflex® filter is acting as backup system for the PLAS4 filter, comprising an identical membrane[52]. In both Springe methods and the commercial system, the desired final MB concentration is 1 µM (~320 µg/L). In the Springe system this concentration is reached by addition of a MB stock solution (50 µM/L) calibrating the volume to the weight of the plasma pack to achieve precisely the same concentration of MB in every plasma pack[127]. The Theraflex® MB system, which is positioned in the tubing between the membrane filter and the illumination bag, incorporates an 85-µg (0.227 µM) pellet of MB hydrochloride per plasma unit: the concentration can, therefore, vary slightly (0.84-1.13 µM) depending on the plasma volume[38]. A plasma volume between 235 and 315 mL is acceptable, resulting in a final concentration of 0.72 to 0.97 µM/L MB plasma. After adding MB, the plasma bags are exposed to visible wavelengths of light to activate the dye. In the Springe procedure, the bag of plasma supplemented with MB used to be placed on a transparent glass plate illuminated with an array of fluorescent tubes from beneath to achieve an intensity of approximately 17×10^3 lux at a distance of about 8 cm for an illumination time of 60 minutes. This setup allowed already labelled units to be illuminated. In the Theraflex® MB system, the illumination device was equipped with high-intensity, low-pressure sodium lamps emitting yellow light at a wavelength of 590 nm, and both sides of the plasma pack were exposed to the illumination. A microprocessor-controlled device (Macotronic V; Macropharma) was developed for the illumination of the Theraflex® MB system. Positioning low-pressure sodium lamps above and below the area of the bags allowed the illumination time to be reduced to about 20 minutes only (180 J/cm^2)[52]. The latest version of the illumination device (Macotronic B; MacoPharma) exploits light-emitting diodes. The influence on the functional activity of plasma proteins is comparable to that seen after illumination with fluorescent tubes for 60 minutes[52].

The Theraflex® MB system also includes a Blueflex® filter to remove a substantial proportion of MB and its photo-degradation products (e.g. Azur B) from the MB plasma. This step results in a reduction of the final concentration of MB by approximately one $\log_{10}$ step. Illumination of the MB-supplemented plasma with visible light for about 20 minutes reduces the average concentration of MB from 890 nM/L plasma to 500 nM/L plasma at the end of the illumination, indicating that the photodynamic process causes a decomposition of approximately 45%. The filtration further decreases the MB content in the plasma to a final concentration of about 19 nM/L, which is less than 2% of the initial MB concentration. These PRT involve a sterile connection to a bag system equipped with a leucocyte-reduction filter, addition of MB and filters to remove the residual MB and its by-products[52].

The MacoPharma system is CE-marked and implemented under GMP in several European countries. However, there is still concern about the potential genotoxicity of MB. Indeed, various remaining intercalated proteins with phenothiazine-like dyes may have some long-term side effects[128].

The ability of MB to inactivate viruses is dependent on its binding to nucleic acids, being greater for double-stranded than single-stranded viruses, although viruses containing genomes of either type may be efficiently inactivated[129]. MB can also modify proteins and lipids, the relative rates depending on the concentrations of MB and local oxygen[122]. Virus-infected cells may be affected by reducing and detoxifying mechanisms. MB is not considered useful for inactivating intracellular viruses or for reducing bacteria and protozoa, even though it does penetrate cells. Its only application in transfusion has been to inactivate viruses in plasma, following cell removal by filtration or freeze-thaw lysis[130]. The reduction of enveloped viruses is at least 5 logs and the spectrum of affected viruses includes HIV, as well as model viruses for HBV and HCV. In addition, MB treatment is also effective against WNV[32,127]. For non-enveloped viruses, the efficacy is heterogeneous (e.g. approximately ≥4 logs for calicivirus and simian virus 40 and not effective against HAV or porcine parvovirus)[127]. Nevertheless, the MB plasma system has produced a reduction of ≥5 logs of PVB19 virus[131]. There is evidence that MB without illumination was also able to lower PVB19 levels by 1-2 log. Additional illumination enhances this by 3-4 log[132]. As for non-viral pathogens, a study on *Trypanosoma cruzi* using *in vitro* cultures and a mouse model showed that MB treatment provides a log reduction of >3.4 and 4.9-5.8, respectively, indicating that this pathogen reduction system could potentially prevent transfusion-transmitted Chagas disease[133]. Although the effect of the MB system on malaria, caused by an intracellular parasite, has not been characterised, leucocyte filtration and the freeze-thawing process remove and disrupt parasite-containing cells, thus preventing multiplication of the parasite. These benefits are also applicable to CMV and HTLV 1 and 2. There have been reports that phenothiazines might destroy prions but the actual effect of the MB system on this pathogen needs further investigation[134,135].

Amotosalen and light treatment

The Intercept® Blood System is a photodynamic viral inactivation technique for pathogen reduction. This PRT utilises amotosalen, a synthetic psoralen (formerly S-59-HCl), as the active compound and long-wavelength UVA to reduce pathgens in plasma and platelet concentrates[61]. Psoralens such as 8-methoxypsoralen and trimethylpsoralen are naturally occurring photoactive substances found in a number of plants such as limes, celery and parsnips[136]. Amotosalen, like all psoralens, is a tricyclic molecule consisting of a furan and a pyrone moiety. It can thus quickly cross cell membranes, bacterial walls or viral envelopes and readily interact with nucleic acids without interacting with proteins or cellular lipids. The chemical structure of amotosalen is not changed by its entry into the cell and it is, therefore, also exceptionally well suited for the inactivation of intracellular pathogens, unlike the other photoactive compounds currently in use. It readily intercalates into double-helical structures of DNA or RNA where covalent bonds between its reactive groups and pyrimidine bases are formed upon UVA illumination.

Even intrastrand reactions are possible. Thus, single-stranded nucleic acids are also targets. The chemical interaction between amotosalen and pyrimidine bases occurs with very high frequency. Therefore, low concentrations of nucleic acids will specifically react even in the presence of large amounts of other biological materials. Theoretically, the formation of one cross-link between the strands of nucleic acids is sufficient to prevent replication of the genome. The frequency of the interaction between amotosalen molecules and pyrimdines has been determined for different nucleic acids and organisms[137]. The reaction with nucleic acids does not depend on the generation of active oxygen species, which damage the cell, and is effective even in the absence of oxygen. The high frequency of interaction makes replication, the transcription of genes, impossible and inhibits DNA repair mechanisms. Amotosalen has no sequence specificity, thus it efficiently inactivates a broad spectrum of pathogens. In addition, amotosalen-nucleic acid cross-linking occurs in the presence of UVA light, but not in its absence. This feature represents another unique safety measure as the reaction can be tightly controlled *ex vivo* simply by turning the light source on or off[61].

The Intercept® technology for pathogen inactivation of platelets or plasma components is based on two major steps: the alkylating agent (150 µmol/L, final concentration) penetrates cellular and nuclear membranes and binds to double-stranded DNA and RNA. When activated by low-energy UVA light (320-400 nm), amotosalen cross-links nucleic acids, thus blocking DNA/RNA replication[86]. A plasma unit is sterilely connected to an Intercept® plasma disposable set using the TSCD-II device. All the plasma is allowed to flow through the amotosalen solution container into the illumination container. After heat sealing and separation of the amotosalen container along with the original plasma bag from the Intercept® illumination container, the plasma mixture is illuminated with a R4R008 illumination device (Cerus) at 3 J/cm for approximately 7 minutes. After illumination, the plasma is passed through a compound adsorption device by gravity into the final storage containers. The adsorption step takes approximately 20 minutes[138]. The method includes an additional step for the removal of the residual amotosalen and its metabolites using a compound adsorbing device for a period of 16 hours[139]. However, it is still debated whether the residual amotosalen and its degradation products, as well as the potential binding to proteins, could have some long-term side effects. The system has recently been CE-marked and it is supposed to be implemented under GMP conditions in Europe[66].

The mechanism of action of the Intercept® technology, especially the fact that the interaction between amotosalen and DNA or RNA molecules is not sequence-specific, theoretically allows the inactivation of unknown pathogens. The spectrum of inactivation capacity includes intracellular as well as cell-free enveloped viruses (e.g. HIV-1 and HIV-2). Other cell-associated viruses, such as HTLV and CMV, can be inactivated to a very marked extent[61,140]. The inactivation of intracellular viruses is becoming an increasingly important feature of the technology. Hepatitis viruses, including HBV and HCV, can be inactivated very effectively, as can HTLV-1 and HTLV-2. The broad spectrum of enveloped viruses for which very efficient inactivation has been demonstrated also includes duck HBV, BVDV, PRV, vaccinia virus and the influenza strains H1N1 and H5N1[141]. Most of the studies generating this information were performed as infectivity studies in cell cultures and animal models. The efficiency of inactivation of non-enveloped viruses varies greatly among the different viruses. While adenovirus 5 and bluetongue virus are very susceptible to the treatment, PVB19 shows an intermediate level of inactivation, HAV is not susceptible to inactivation and calicivirus is only poorly susceptible.

The capacity of the Intercept® technology to inactivate emerging and re-emerging pathogens is now of great interest. Highly effective inactivation has been demonstrated, for example, for a large panel of (re)-emerging viruses. These include the coronavirus causing SARS, WNV, the previously mentioned influenza strains H1N1 and H5N1 as well as Chikungunya virus and dengue virus. The versatility and efficiency of the Intercept® Blood System was demonstrated in 2006,

when a Chikungunya epidemic occurred in the French overseas department of Ile de La Reunion[142].

Besides (re)-emerging pathogens, bacteria are a major threat to blood safety, especially for platelet components. Any technology for pathogen inactivation must, therefore, achieve the highest level of inactivation. In order to test the capacity of the Intercept® technology to inactivate bacteria, studies with a broad spectrum of aerobic and anaerobic Gram-positive and Gram-negative bacteria as well as spirochaetes, have been performed. In recent years, the number of reports concerning infections with emerging protozoa has been increasing. The protozoa of greatest concern are *Plasmodium falciparum* (malaria), *Trypanosoma cruzi* (Chagas disease), *Leishmania* spp. and *Babesia microti*[143]. Recently, it has been demonstrated that Intercept® technology can also inactivate these protozoa.

Riboflavin and light-treated plasma

Mirasol® (Terumo BCT, Lakewood, CO, USA) is a riboflavin/UV light-based PRT system used for pathogen reduction of plasma and platelets[86]. This new technology uses riboflavin as a photosensitiser in combination with UV light[145]. Riboflavin, also called vitamin B2, is a dietary nutrient with a known pharmacokinetic and toxicology profile[145]. This essential natural vitamin absorbs both visible and UV light[86]. Shortwave UVC light (285-365 nm) interacts directly with nucleic acids, resulting in the formation of pyrimidine dimers that block the elongation of nucleic acid transcripts[146]. Through exposure to UVA and UVB light, it mediates selective damage to nucleic acids without binding to cells or proteins. The chemistry, toxicity and ability of riboflavin to interact with nucleic acids after photo activation have been studied extensively[86]. Riboflavin associates with nucleic acids and mediates oxygen-independent electron transfer, causing irreversible damage to nucleic acids[147]. The impairment of nucleic acids by direct electron transfer together with the production of singlet oxygen and generation of hydrogen peroxide with the formation of hydroxyl radicals damage the pathogen DNA/RNA in the absence of oxygen. Single units of apheresis or whole blood plasma are treated and frozen within 8 hours of collection[148]. This association of riboflavin/UV light has been used for a very long time as standard phototherapy of neonatal jaundice, without any reported problem[66]. Therefore, R plasma promises high efficacy and low protein damage with little toxicity. Since naturally occurring vitamin B2 and its photo-degradation products are considered to be non-toxic, the early prototype of the Mirasol® system is similar to that of Intercept®, with the difference that, in the former case, removal of residual by-products and metabolites is not necessary[86]. This new PRT facilitates processing and shortens the time of storage of plasma in the liquid state.

Riboflavin/UV light treatment has proven to be effective against a range of pathogens, including bacteria, enveloped viruses, protozoa (>4-6 log inactivation), leucocytes and some non-enveloped viruses[103,144,149]. On the other hand, HAV is resistant to inactivation and the evidence of bacterial inactivation depended on extremely low concentrations of organisms, at the time of treatment[2]. This PRT provides levels of viral reduction in excess of those required to successfully close the window period of transmission for HIV, HBV and HCV. Moreover, this method eliminates the viraemic period for WNV and possibly the chronic phase of transmission of human PVB19, bacteria and leucocytes associated with often fatal transfusion reactions and transfusion-associated GVHD, without substantial loss of plasma factors; features that make it suitable for both direct pathogen reduction for plasma and a useful supplement to SD plasma.

Clinical studies using the Mirasol® PRT for plasma are underway[66]. Data from some studies indicate that the Mirasol® PRT system can effectively be used to inactivate dengue and Chikungunya viruses in contaminated plasma and platelets. Combined with data previously obtained for other pathogens, this study reaffirms the efficacy of Mirasol® PRT as a potential means to enhance the protection of the blood supply from emerging infectious disease threats[34,150]. On the other hand, few data have been published on the clinical efficacy and safety of the Mirasol® product, although the product has been reported to be in clinical use in some European countries. Further information from clinical studies is necessary to evaluate the risk-benefit ratio of Mirasol® plasma better.

Table IV lists the infectious agents that can be inactivated using psoralen/UVA and riboflavin.

The different methods of pathogen inactivation in plasma are compared in Table V.

Investments and tools required to use the technologies

Several viral inactivation or removal methods for plasma for transfusion have been studied. MB illumination, psoralen-UVA and riboflavin-UV light are examples of treatments applicable to single plasma donations in a blood centre setting, whereas the SD plasma or pasteurisation processes have so far been developed for treating large plasma pools and require a plasma fractionation facility[35]. In 2006, Thierry Burnouf and co-workers developed a SD inactivation process for single-donor plasma or mini-pools of plasma to be used in blood establishments in developing countries[91,115].

Solvent/detergent method

SD treatment of plasma was the first method developed for the inactivation of viruses in plasma for transfusion. The current technology requires a plasma fractionation facility and is applied to large plasma pools, increasing the cost and risks of exposure to SD-resistant pathogens and lowering the content of protein S and α2-antiplasmin[115]. SD plasma is produced by incubating pools of hundreds or thousands of plasma donations with 1% TNBP and 1% Triton X-100 at 30 °C for 4 hours. The SD agents are removed by a process including oil extraction and hydrophobic interaction chromatography. The pooled

Table IV - Inactivation of infectious agents in plasma and platelets using psoralen/UVA and riboflavin.

Classification	Agents	Log reduction
Viruses (enveloped)	HIV-1/2, HTLV-I/II, HBV, CMV, DHBV, HCV, BVDV, WNV, SARS-CoV	>4.5 to >6.8
Viruses (non-enveloped)	PVB19, HAV, Human adenovirus-5	>5.1 to >6.8
Bacteria	Gram-positive and Gram-negative	>7.3
Spirochaetes	*Treponema pallidum, Borrelia burgdorferi*	>5.9 to >10.6
Protozoa	*Plasmodium falciparum, Trypanosoma cruzi, Babesia microti*	>5.0 to >6.9

UVA: ultraviolet A light; HIV: human immunodeficiency virus; HTLV: human T-lymphotropic virus; HBV: hepatitis B virus; CMV: cytomegalovirus; DHBV: duck hepatitis B virus; HCV: hepatitis C virus; BVDV: bovine virus diarrhoea virus; WNV: West Nile virus; SARS-CoV: severe acute respiratory syndrome coronavirus; PVB19: parvovirus B19; HAV: hepatitis A virus. Source: modified from reference 251.

Table V - A comparison of methods of pathogen inactivation in plasma.

	SD-FFP	MB-FFP	A-FFP plus UVA	R-FFP plus UV
Mechanism of action	Disruption of lipid membranes	MB intercalates into nucleic acids and mediates the formulation of singlet oxygen upon illumination	Amotosalen (S-59) intercalates into nucleic acids and induces covalent cross-linking upon UVA exposure	Riboflavin associates with nucleic acids and mediates an oxygen-independent electron transfer upon UV exposure
Blood components	Plasma	Plasma	Plasma and platelets	Plasma and platelets
Products	Octaplas®, OctaplasLG®, Uniplas® (Octapharma); Plasmasafe® (Kedrion); Plasmavitro-atténué solvent/ detergent (French Blood Service, EFS); Bioplasma FDP® (National Bioproducts Institute of Pinetown, South Africa); "Mini-pool" systems (V.I.P.S, SA; Switzerland)	Theraflex® MB (MacoPharma); Springe methods (Grifols)	Intercept® Blood system for plasma and platelets (Cerus)	Mirasol® PRT system for plasma and platelets (Terumo)
Product source	Made from apheresis plasma frozen within 4 h of collection	Whole blood-derived plasma as single unit and apheresis plasma (apheresis must be split if >315 mL)	Apheresis jumbo collections up to 635 mL treatable as single unit; whole blood-derived at least two plasma units must be pooled together	Whole blood-derived plasma as single unit and apheresis plasma (apheresis plasma must be split if >360 mL)
Shelf life	4 years at below –18 °C	2 years at –30 °C	2 years at below –25 °C; 1 year between –18 °C and –25 °C	2 years at –30 °C
Illumination time	Not applicable	15 min new Mecotronic B2; 20 min with older equipment	3-6 min for 2 bags	5-8 min for 1 bag
Primary target	Lipid membranes	Nucleic acids	Nucleic acids	Nucleic acids
Compound toxicity	Low	Low	High	None
Removal of active compounds	Yes, oil extraction and hydrophobic chromatography	Yes, by special filter (not universal)	Yes, by compound absorbing device	Not required
Residual level of active compounds	Undetectable or trace amounts far below the toxicity level	Very low levels of MB and photoproducts. Mutagenic effects improbable, but not excluded	Very low levels of free S-59 and photoproducts. Mutagenic effects improbable, but not excluded	Low levels of riboflavin and photoproducts are normally present in blood. Active compound not removed
Permanent binding to lipids and proteins in plasma	No binding to proteins or residual lipids	Proteins intercalated with phenothiazine-like dyes	Amotosalen bound to lipids and 2% to proteins	Does not appear to bind

SD-FFP: solvent/detergent-treated fresh-frozen plasma; MB-FFP: methylene blue-treated fresh-frozen plasma; A-FFP plus UVA: amotosalen-treated fresh-frozen plasma exposed to ultraviolet A light; R-FFP plus UV: riboflavin-treated fresh-frozen plasma exposed to ultraviolet light. Source: modified from reference 35.

SD plasma, which may be concentrated by ultrafiltration, is sterile-filtered and aseptically dispensed into its final containers[41]. Two SD treatment procedures for single donations or mini-pools of plasma have been developed with a single-use bag system[115, 151]:

- *2% TNBP treatment.* For TNBP treatment, a solution of pure TNBP (Prolabo, VWR, Fontenay-sous-bois, France) is carefully added to the plasma in a sterile syringe (at a ratio of 4 mL to 200 mL, respectively), over at least 20 minutes and with a constant mixing resulting in a final concentration (v/w) of 2%. Subsequently the plasma-TNBP mixture is shaken vigorously for 5 minutes to ensure dispersion of the TNBP in the plasma. The processing bag is then completely immerged into a water bath to warm the TNBP-plasma mixture at 37 °C and then treated for 4 hours under constant gentle stirring. The TNBP-plasma mixture is then transferred aseptically in a biological cabinet into a 300-mL bag filled with 15 mL of sterile castor oil (Fluka Chemie GmbH, Buchs, Switzerland). The TNBP-plasma/7.5% oil suspension is shaken vigorously for at least 1 minute and then put onto a shaker to ensure gentle stirring for at least 15 minutes. The bag is then suspended to ensure decantation between the oil phase (upper layer) and the plasma phase (lower layer). The plasma is transferred by gravity into a second bag filled with 15 mL of castor oil, and the extraction procedure is repeated twice. The plasma bag is then centrifuged in a reverse position in a centrifuge (Jouan, St-Herblain, France) at 3,800 *g* for 30 minutes at 4 °C to clarify the plasma. The plasma is separated from the lipid layer, transferred into a final plastic bag, and put in a freezer at −30 °C.
- *1% TNBP and 1% Triton X-45 treatment.* A 50-50% mixture of TNBP and Triton X-45 (Merck, Darmstadt, Germany) is used to fill a syringe. Four millilitres of the mixture are carefully and aseptically added to 200 mL of plasma to reach a final concentration (v/w) of 2% (1% TNBP and 1% Triton X-45). The procedure is then performed as described for the 2% TNBP treatment, with the exception that the temperature of treatment is 31 °C and the final centrifugation step is not performed.

The SD treatment can be implemented on single-donor units or minipools of plasma (e.g., an approximately 2 L pool of 10 donations). It has been found that the implementation of the processes by operators trained in blood establishment practices is straightforward; possible automation of some of the steps (e.g., oil decantation and separation) would contribute to increase the throughput and convenience of the processes. This small-scale and disposable-bag approach has some practical advantages that may counterbalance the caution required when dealing with large-pool SD plasma[115].

The introduction of novel technologies into manufacturing processes may have an impact on the quality of plasma-derived products. A new chromatographic step for the selective binding of scrapie prion protein (PrPSc) to an affinity ligand, which was developed and optimised for PrPSc capture and attached to synthetic resin particles (by Pathogen Removal and Diagnostic Technologies Inc., USA), was introduced into the manufacturing process of the SD-treated biopharmaceutical plasma Octaplas®[113].

The Octaplas® manufacturing process is illustrated in Figure 1.

Uniplas® (Octapharma) is a novel, universally applicable, blood group-independent, prion-depleted, SD-treated and human pooled plasma for infusion. Uniplas® can be given to all patients, irrespectively of their blood group, abolishing the risks and serious consequences that can result from a transfusion of an incompatible plasma unit. Uniplas® is obtained by optimal mixing of plasma of different blood groups in order to neutralise unwanted anti-A and anti-B antibodies (both IgM and IgG classes) by binding to free A and B substances[152]. Uniplas® and Octaplas®/OctaplasLG® plasma pool and final container samples are collected from routine batches manufactured at Octapharma PPGmbH. The Uniplas® manufacturing process is an exact copy of the OctaplasLG® process, except for the optimal mixing of plasma prior to SD treatment (i.e. universal plasma *vs* blood group-specific plasma). The differences between Uniplas®/OctaplasLG® and Octaplas® lie in the time of exposure to SD (i.e. 1-1.5 hours *vs* 4 hours) and in the prion removal step (i.e. with *vs* without prion removal)[155].

In Italy, SD virus-inactivated plasma is derived from pooled FFP (from up to 2,500 blood donors) by treatment with TNBP solvent and Triton X-100 detergent (PlasmaSafe®). The SD technique is an industrial process. Blood Transfusion Units give the plasma to the industry which returns the inactivated product after an industrial process involving two filtration phases (1 µ and 0.22 µ)[55].

Methylene blue method

One of the attractions of the MB technique is that it is applied to single plasma units, without the need to pool plasma donations. Commercial systems that can be set up in standard blood centre GMP conditions are available, without requiring the installation of specialised plants[127].

The Springe method is currently used in Spain by Grifols (Parets del Vallés, Spain). This MB procedure starts with a freeze-thawing step to disrupt the membranes of residual cells in the plasma and to release

their intracellular content. In the Springe method, the desired final MB concentration is 1 µmol (~320 µg/L), which is reached by the addition of a MB stock solution (50 µmol/L) calibrating the volume to the weight of the plasma in order to achieve precisely the same MB concentration in every plasma pack. After adding the MB, the plasma bags are exposed to visible wavelengths of light to activate the dye and placed on a transparent glass plate illuminated with an array of fluorescent tubes for 60 minutes[99].

A modified procedure has been marketed as the Theraflex® MB-Plasma System with a CE class III mark (MacoPharma, Tourcoing, France).

The Theraflex® MB-Plasma System includes an active compound, which is integrated into the bag system as a pill containing 85 µg of MB, which yields a MB concentration in the range of 0.8 to 1.2 µmol/L of plasma. The system is composed of a Macotronic® illumination device, MacoPharma flexible filters (Plasmaflex® and Blueflex®) and treatment steps. It is a pathogen-inactivation system capable of treating both single units and pools of many plasma units (Figure 2)[52].

Theraflex® MB Plasma sets are available either as fully integrated systems for use from donation through to blood product storage, or as a range of dockable filtration and pathogen reduction systems for use in the laboratory with any unit of plasma. This allows full flexibility in the choice of the processing method to easily integrate with the laboratory workflow. Theraflex® MB Plasma pathogen reduction can be carried out on demand or as an integral part of routine laboratory processing (*information supplied by the manufacturer*)[154].

Before treatment, the donation number is recorded by a barcode reader or optionally by radio frequency identification. When the same donation number is scanned for a second time, the software rejects illumination. Plasma units that have been treated are indicated by a barcode label comprising all essential information on the illumination process. After treatment, over 90% of the residual MB combined with its photo-activated products is removed by a specially designed filter (Blueflex®; MacoPharma). Thus, plasma is filtered twice (Plasmaflex PLAS4®; MacoPharma), resulting in virtually cell-free plasma[155].

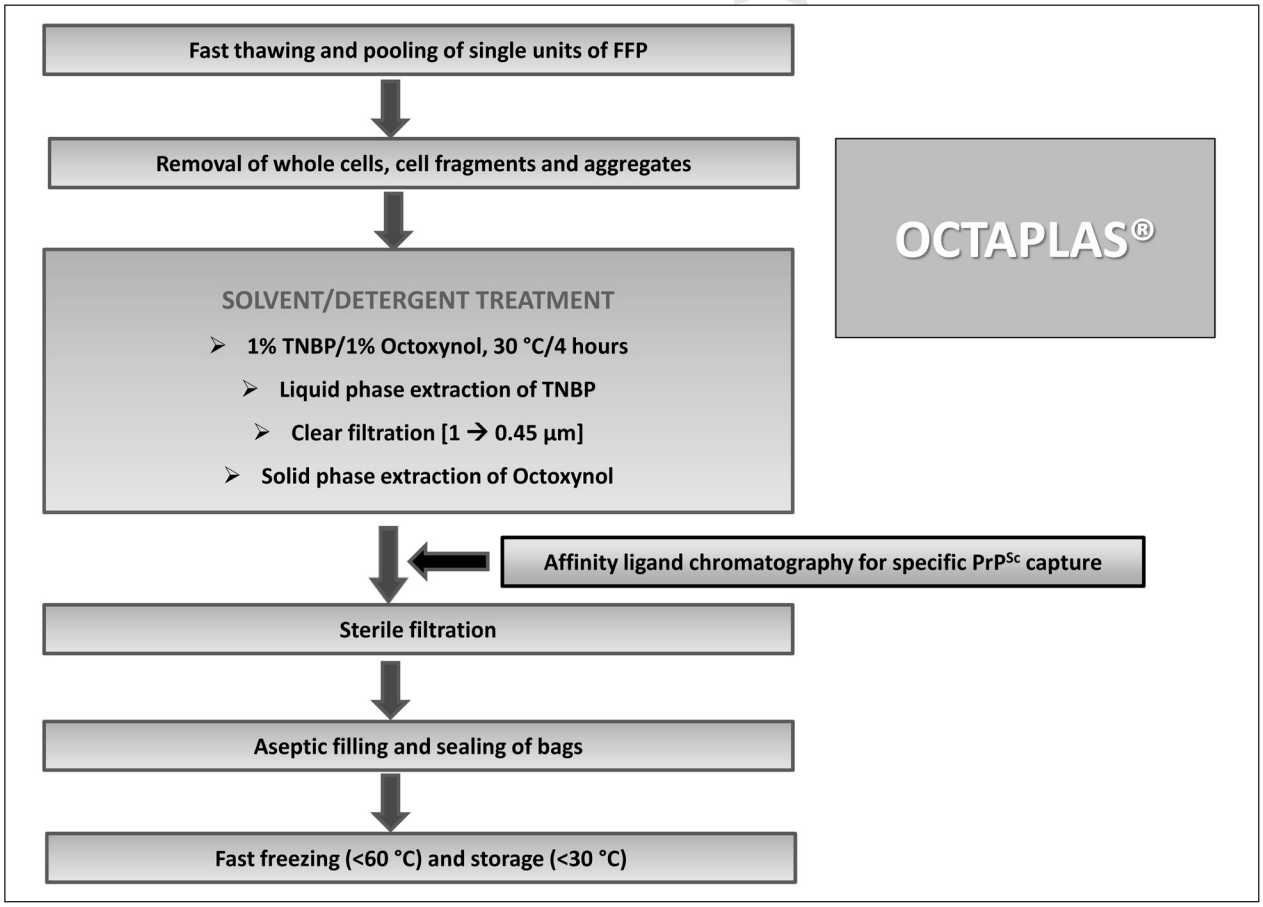

Figure 1 - Octaplas® manufacturing process.
FFP: fresh-frozen plasma; TNBP: tri(n-butyl) phosphate; PrP: prion protein.

The Blueflex® filter is simple and rapid to use, and available integrated with the Theraflex® MB Plasma System. Blueflex® reduces the concentration of MB and its derivatives in the treated plasma by more than 1 log. The combined use of MB and Blueflex® ensures minimal exposure to MB or its derivatives[154].

Many blood establishments around the world use other "in house" methods based on MB treatment, including that first developed by Mohr and Lambrecht (the Springe method). This system, however, is not currently marketed by MacoPharma (Theraflex®). The fundamental differences between the Theraflex® process and the Springe method are reported in Table VI[35].

Amotosalen and ultraviolet light treatment

Intercept® is the most widely used pathogen inactivation system capable of treating both platelets and plasma components on a single platform.

The Intercept® Blood System for Plasma includes an active compound, the illumination device, a sterile connecting device and treatment steps. Each Intercept® processing set can produce up to three 200 mL transfusion units with less than 10 minutes hands-on time per processing set. Up to 36 treated units can be produced per hour per Intercept® illuminator. Intercept® provides the flexibility to treat either freshly collected plasma or to treat FP that has been recently thawed. This option allows blood establishments to choose the most suitable processing timeline for their operations. *(This information was extracted from the characteristics reported by the manufacturer)*[156].

The Intercept® Blood System for Plasma is compatible with apheresis and whole blood plasma.

Riboflavin and ultraviolet light treatment

The innovative process of Mirasol® technology involves adding riboflavin to the platelet or plasma product, which is then exposed to UV light for a short period of time. The system consists of a Mirasol® illuminator, disposable sets, fully integrated data capture and storage software[157,158]. The characteristics reported by the manufacturer are[159]:

- a single system for platelets and plasma;
- minimal training time;
- limited bag transfers;
- less than 5 minutes hands-on time;
- less than 15 minutes total processing time;

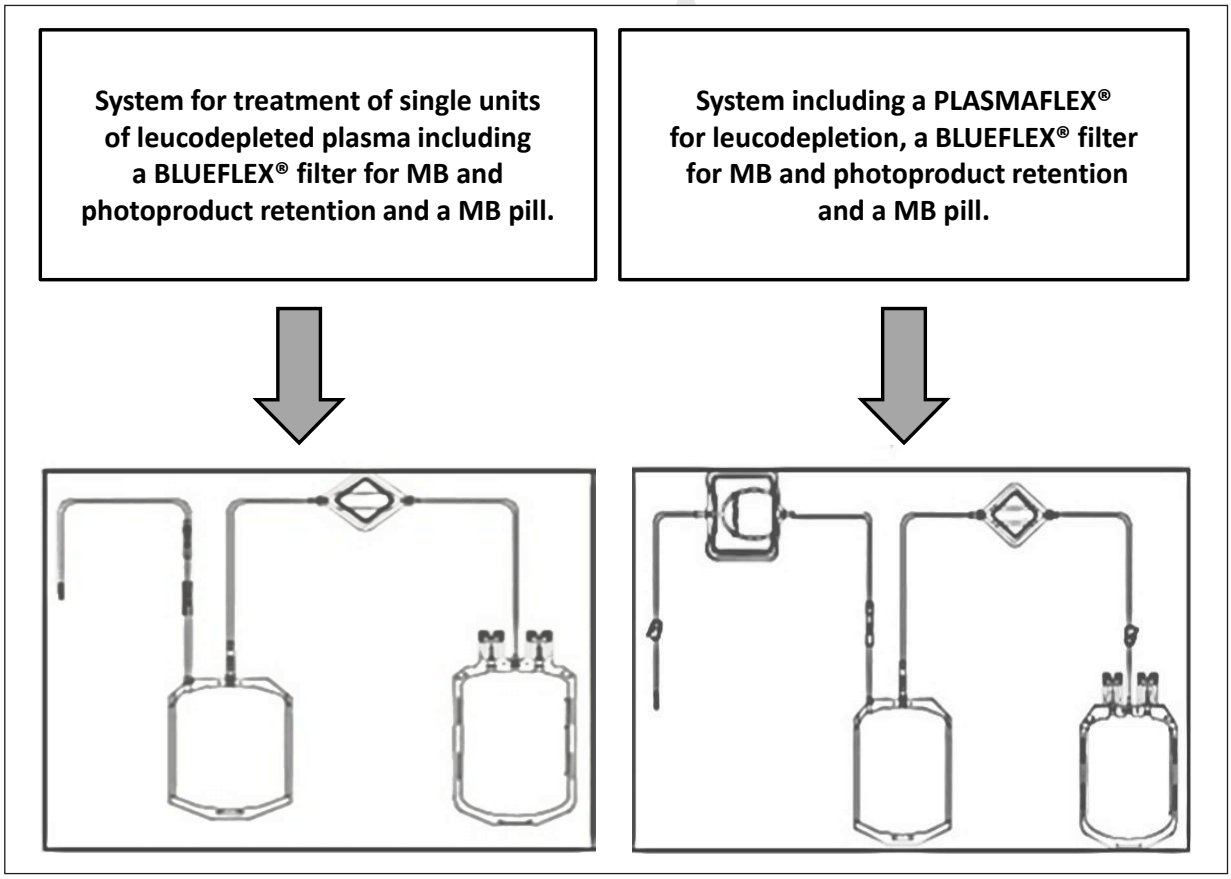

Figure 2 - Schematic representation of two different kits for Theraflex® MB-Plasma system for leucodepleted plasma (on the left side of the figure) and for non-leucodepleted plasma (on the right side of the figure). MB: methylene blue. Source: modified from reference 124.

- the system is adaptable, giving many options for implementing it into unique settings;
- intuitive, icon-based user interface;
- possibility of using one or several illuminators to support production requirements, on the basis of need;
- multiple illuminators can be set up in the same work area or distributed throughout a facility for optimal workflow and equipment layout;
- illuminators can be networked for centralised data capture.

As far as concerns plasma treatment specifically, the process:

- can be used on single-unit plasma volumes;
- can be applied to both apheresis and whole blood-derived plasma products;
- is validated for a variety of blood bank processing conditions for FFP;
- can treat previously FP, removing time constraints associated with plasma processing.

Future challenges

There is currently no universal pathogen reduction treatment for all different blood products. Consequently, if different inactivation techniques were to be introduced, this would have repercussions on both direct costs for equipment and materials, and indirect (logistical) costs. Apart from what the manufacturer would charge for the equipment and materials, the blood transfusion organisations would probably also charge for increased personnel costs and the expense of introducing the technique.

In the light of the above-mentioned considerations, clinical studies using prototype devices for whole blood pathogen reduction are ongoing.

Clinical efficacy

Introduction

Blood plays a crucial role in the human body. Blood transfusion is a medical practice that can be life-saving in patients affected by several diseases. Whole blood is fractionated into components such as plasma, platelets and red cell concentrates for replacement or prophylactic therapies. Plasma is a liquid suspension of several proteins, lipids and salts, and is the first labile blood component for which PRT were developed. Pre-clinical and clinical studies and experience from post-authorisation use of PRT have been used to investigated very relevant issues related to the clinical efficacy of these products. Four PRT use different methods of pathogen inactivation/reduction and, as described below, have been subjected to extensive study of their toxicological properties and clinical efficacy.

Methods

Search strategy and selection criteria

A domain-specific search for efficacy aspects was merged with a basic search on Medline to obtain efficacy data on technical approaches to pathogen inactivation in plasma for clinical use. The review was performed using grey literature and analysing previous HTA reports.

In detail, since the objective was an evaluation of clinical efficacy, the basic search was refined using the following key words: treatment outcomes, efficacy and clinical effectiveness. The search strategy is reported below: EFFICACY *Search (((((("Treatment Outcome"[Mesh]) OR Efficac*) OR Efficacy) OR "Clinical Effectiveness")).*

No publication date restrictions were applied and no safety-specific search was applied to the whole search

Table VI - Differences between the Springe method and the Theraflex® system for methylene blue treatment of plasma.

Container	Studies carried out in unspecified bags and cell culture flasks	MacoPharma bag
Amount of MB used	95 µg	85 µg
Form of MB	5:1 mL solution	Solid tablet form
Incubation time prior to treatment	60-90 min at 8-12 °C	None instructed
Light source	Broad-spectrum white light bulbs	Fluorescent bulbs or LED; 590 nm light
Energy dose	60-65 min at >45,000 Lux=25.7 J/cm^2	180 J/cm^2
Level of residual air in bag*	Not specified, likely considerable excess air in flask studies (only HIV study reported as being carried out in flasks)	Requires removal of residual air in IFU
Condition of plasma (pre-frozen)	Pre-frozen to break up white blood cells to allow better access	Relies on filtration and leucoreduction. This does not break up residual cells and thus some are intact
Use of filter	No	Yes - leucoreduction
Pre-treatment	No	Yes - MB removal
Post-treatment	-	-

* Methylene blue (MB) relies on oxidative chemistry to achieve pathogen reduction. If air is present in the viral reduction studies, it can lead to greater levels of reduction. Conversely, if absent in the plasma protein quality studies, results on maintenance of protein quality would be better, but the pathogen reduction under these conditions may also be less. For this reason, it is essential to measure both protein quality and pathogen reduction under the actual conditions of commercial use. Source: modified from reference 35.

strategy in the Cochrane and Call Detail Recording (CDR) databases. Some inclusion/exclusion criteria were defined:

- language: papers in languages other than English were excluded;
- relevance: studies focusing on safety and/or lacking clinical data were excluded;
- study design: literature reviews were excluded.

Results

The study selection process is illustrated in Figure 3.

On the basis of the "Efficacy" domain string, 124 articles were selected.

The 124 articles were 95 articles from PubMed, three HTA reports and 26 articles from grey literature. Articles from the Cochrane Library were not included because they were not relevant to the aim of the current analysis.

Forty-eight studies eventually met the inclusion criteria. Of these, 45 were clinical studies and three were HTA reports. The remaining 76 were excluded: five articles were excluded because they were written in a language other than English, 40 were not relevant, 24 were not clinical studies, five did not consider the efficacy domain, one was not available as a full text and, finally, one record was a duplicate. The level of evidence of the selected studies was appraised by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) scale.

Previous health technology assessments

Table VII summarises the previous HTA reports included in this analysis.

Pathogen-inactivated/reduced plasma vs *untreated plasma: clinical studies*

Three patients with acute TTP were studied by Evans *et al.* (1999)[160]. As primary treatment, they received SD-FFP plus prednisolone and all patients reached platelet counts of $>50\times10^9$/L. Serum creatinine was also corrected. With intensive plasma exchange with SD-FFP, neurological features slowly resolved in one patient, and developed again during plasma infusion in another with a rapid reversal. All patients maintained a stable remission 1 year later and tolerated the SD-FFP infusion well, without developing febrile or other reactions and without becoming HIV-, HCV- or HBV-positive.

In 1999, Williamson *et al.*[161] assessed the efficacy of SD-FFP in correcting the coagulopathy associated with liver disease and liver transplantation. The 49 patients with coagulation deficits due to liver disease requiring FFP for invasive procedures or liver transplantation were randomly assigned to receive either FFP or SD-FFP. They were assessed for side effects, correction of coagulopathy over 24 hours, and seroconversion for viral markers 6 to 18 months after treatment. Equal correction of clotting factors and partial thromboplastin time was observed with FFP and SD-FFP in both groups. The correction of the International Normalised Ratio in patients receiving SD-FFP was greater (p=0.037), but this group of patients had higher baseline values than recipients of FFP (p=0.024). The use of other blood components during transplantation was identical in the two treatment groups.

Flamholz *et al.* (2000)[162] described a reduction of protein S activity (about 0.5 units/mL), in three patients with TTP infused with SD-FFP as the replacement

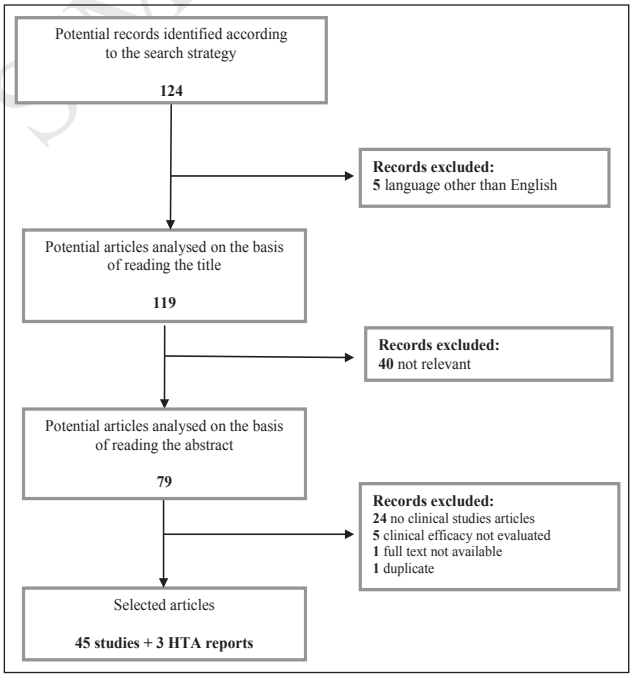

Figure 3 - Study selection process for clinical efficacy domain.

fluid during repetitive therapeutic plasma exchange, if compared with activity levels in those given FFP. Each patient developed one or more deep vein thrombosis while receiving 100% SD-FFP or 50% SD-FFP and 50% cryosupernatant plasma as replacement fluid. Their observations suggest that the use of SD-FFP alone or in 50% combination with cryosupernatant plasma may lead to difficulty in maintaining safe protein S activity levels.

A randomised, double-blinded study to assess the ability of SDP-FFP and FFP to reduce a prolonged prothrombin time to ≤15 seconds in patients with acquired coagulation deficits was performed by Lerner *et al.* (2000)[163]. Forty-five patients (22 given SD-FFP *vs* 23 given FFP) were treated with 71 infusions and laboratory and clinical outcomes were compared. There were no significant differences in the mean dose of plasma infused (7.8 mL/kg for SD-FFP *vs* 8.0 mL/kg for FFP, p=0.46), in the percentage of patients whose prothrombin time was to ≤15 seconds (32% for SD-FFP *vs* 26% for FFP, p=0.67), or in the percentage of patients whose bleeding ceased (27% for SD-FFP *vs* 22% for FFP). No clinical or statistically significant differences were observed after infusion of SD-FFP or FFP in patients with acquired coagulation deficiencies.

Atance *et al.* (2001)[164] compared the use of MB-FFP with FFP in 2,967 patients in different clinical settings. Most patients had undergone surgical procedures. The use of MB-FFP was associated with a 56% increase in the aggregated demand for plasma, whereas the transfusion of non-virus-inactivated cryoprecipitate doubled the first year and increased by three times the second year. The use of plasma increased in all the

Table VII - Previous health technology assessments.

Reference	Topic	Main results	Main references
Health Council of The Netherlands. Pathogen reduction in blood products. The Hague: Health Council of The Netherlands, 2003; publication n. 2003/16E.	*In-vitro* function of treated products	The quality of plasma treated with amotosalen or riboflavin was examined. Treatment resulted in a variable decline of between 0% and 30% in the activity of the plasma proteins. According to Goodrich, such a reduction is well within the accepted norm.	- Goodrich RP. The use of riboflavin for the inactivation of pathogens in blood products. Vox Sang 2000; **78** (Suppl 2): 211-5. - Alfonso R, Lin CY, Dupuis K. Inactivation of viruses with preservation of coagulation function in FFP. Blood 1996; **88** (Suppl 1): 2092.
	Clinical research	Phase II study in which the amount of coagulation factor VII in the blood of healthy volunteers was artificially lowered. The increase in factor VII achieved through administration of amotosalen-treated plasma was no different from the increase following administration of conventionally treated plasma. The first results of phase III research in a limited number of patients with hereditary abnormalities of blood coagulation have been published in abstract form. The authors concluded that the amotosalen treatment is an effective method for treating plasma.	- Hambleton J, Wages D, Radu-Radulescu L, *et al*. Pharmacokinetic study of FFP photochemically treated with amotosalen (S-59) and UV light compared to FFP in healthy volunteers anticoagulated with warfarin. Transfusion 2002; **42**: 1302-7. - de Alarcon P, Benjamin R, Shopnick R, *et al.* Hemostatic response in congenital coagulation factor-deficient patients transfused with FFP prepared by Helinx pathogen inactivation technology - the STEP CC trial. Transfusion 2001; **41** (Suppl): 89S.
Pathogen inactivation in donated blood. Health Technology Assessment Section, Medical Development Division, Ministry of Health Malaysia; publication n. 18/2010.	Effectiveness of Intercept®, Mirasol® and solvent/detergent processes for pathogen inactivation in plasma	i) *Psoralen light treatment (amotosalen-HCl)* Fair evidence on the effectiveness of the Intercept® blood system when used on platelets-plasma. ii) *Riboflavin light treatment* Poor evidence on the effectiveness of riboflavin light treatment technology when used on plasma. Clinical studies using Mirasol® (riboflavin) pathogen inactivation technology is still underway. iii) *Solvent-detergent treatment* Fair evidence on the effectiveness of solvent- detergent technology, when used for plasma and fresh-frozen plasma.	- Solheim BG, Seghatchian J. Update on pathogen reduction technology for therapeutic plasma: an overview. Transfus Apher Sci 2006; **35**: 83-90.
Pathogen reduction technologies for blood products: a review of the clinical effectiveness, cost-effectiveness, and guidelines. CADTH, October 13, 2009.	Intercept® blood system photochemical process	Overall, the available evidence indicates that the Intercept® blood system photochemical process (amotosalen + UVA light) does not adversely affect the clinical utility of the treated blood components, specifically plasma and platelet components. Intercept®-treated components were able to improve haemostasis in all of the included studies and had comparable adverse event profiles to those of conventionally-prepared plasma and platelet components.	- Membe SK, Coyle D, Husereau D, *et al.* Octaplas compared with FFP to reduce the risk of transmitting lipid-enveloped viruses: an economic analysis and budget impact analysis [Technology report]. Ottawa: Canadian Agency for Drugs and Technologies in Health; 2011.

categories studied. The consumption of MB-FFP was associated with a marked rise in the demand for plasma and cryoprecipitate, which was probably due to the low haemostatic quality of the new component.

In a study by De la Rubia *et al.* (2001)[165], 20 patients with TTP underwent plasma exchange using either standard FFP (group A, n=13) or MB-FFP (group B, n=7). Group A showed higher bilirubin values ($p<0.05$) and a higher complete remission rate (69% *vs* 57% in group B), while the mean number of procedures (21±7 *vs* 11±3, $p<0.01$) and the number of FFP units transfused were higher in group B and the mean stay in hospital was also longer (37±12 days *vs* 22±11 days; $p<0.01$). The use of MB-FFP to treat TTP seems to be associated with a higher number of plasma exchange sessions and greater transfusion requirements without improving clinical results.

Haubelt *et al.* (2002)[166] assessed the effect of SD-FFP and FFP on haemostasis and fibrinolysis in open-heart surgery coagulopathy through a prospective trial. Sixty-seven patients received 600 mL of SD-FFP (n=36) or 600 mL of FFP (n=31) at an infusion rate of 30 mL/min. Before treatment and 60 minutes after termination of the plasma infusion prothrombin time, activated partial thromboplastin time, fibrinogen, FVIII, antithrombin, protein C, and free protein S levels, protein S activity, prothrombin fragments F1+2, D-dimers, fibrinogen degradation products, plasmin-plasmin inhibitor complexes, plasminogen, protease inhibitor and α1-antitrypsin were measured. The rises in fibrinogen, FVIII, antithrombin, protein C, free protein, α-antitrypsin and plasminogen, and the decrease in prothrombin time and activated partial thromboplastin time did not differ significantly between the two study arms. However, although protein S activity did not increase after SD-FFP infusion, it was significantly elevated after the infusion of FFP. Protease inhibitor levels declined significantly after SD-FFP and remained uninfluenced by FFP. Neither SD-FFP nor FFP had any significant influence on prothrombin fragments F1+2, D-dimers or fibrinogen degradation products. Significant reductions in plasmin-plasmin inhibitor complex levels caused by both types of plasma can indicate a reduction in hyperfibrinolysis. There was no significant difference in clinical haemostasis between the two treatment regimens.

The study performed by Noddeland *et al.* (2002)[167] comprised 84 adult patients scheduled for elective open-heart surgery. Fifty-five of them received plasma transfusions, while 29 patients not given plasma transfusions functioned as controls. If plasma transfusion was indicated during the operation or following 2 days, patients were randomised 2:1 to receive Uniplas® or AB blood group Octaplas®. Activated clotting time, activated partial thromboplastin time and post-operative bleeding into chest drains were not significantly different between the transfused groups of patients. The median number of transfused units of Uniplas® was three (range: 1-23), while the median number of Octaplas® units was two (range: 1-11). These differences were not statistically significant. The authors concluded that Uniplas® has a similar effect as Octaplas® in the treatment of bleeding in patients undergoing open-heart surgery and may, therefore, substitute Octaplas® and reduce the risk of ABO-incompatibility.

A retrospective observational study (by de Jonge *et al.* 2002)[168] was performed to clarify an increased incidence of hyperfibrinolysis (75% *vs* 29%; p=0.005) observed during liver transplantation after the introduction of SD-treated plasma (ESDEP®) instead of FFP. Intraoperative plasma samples from patients treated with FFP or ESDEP® were analysed. During the anhepatic phase, plasma levels of D-dimer (6.58 *vs* 1.53 g/mL; p=0.02) and fibrinogen degradation products (60 *vs* 23 mg/L; p=0.018) were significantly higher in patients treated with ESDEP®. After reperfusion, the levels increased to 23.5 *vs* 4.7 g/mL (D-dimer, p=0.002) and 161 *vs* 57 mg/L (fibrinogen degradation products, p=0.001). The amount of plasma received per packed red blood cell concentrate, clotting tests, and levels of individual clotting factors were not significantly different between the groups. $Alpha_2$-antiplasmin levels were, however, significantly lower in patients receiving ESDEP® during the anhepatic phase (0.37 *vs* 0.65 IU/mL; $p<0.001$) and after reperfusion (0.27 *vs* 0.58 IU/mL; p=0.001). Analysis of $alpha_2$-antiplasmin levels in ESDEP® alone showed a reduction to 0.28 IU/mL (normal >0.95 IU/mL) because of the SD treatment.

In a single-blind crossover study, Hambleton *et al.* (2002)[92] compared post-infusion kinetics of FVII in photochemically treated FFP and standard FFP. Healthy volunteers donated plasma by apheresis: half of the collected plasma was treated with the psoralen amotosalen hydrochloride (A-FFP), and half was prepared as standard FFP. The same subjects received warfarin over 4 days to lower FVII levels then, on day 4, received 1 L of either pathogen inactivated or standard FFP. After 2 weeks, subjects underwent a regimen identical to that with the other type of FFP. After warfarin ingestion, the mean FVII concentration was 0.33 IU/mL. Both types of FFP exhibited comparable FVII kinetics, with a mean peak increment of 0.10 to 0.12 IU/mL occurring at the end of the infusion. The effect disappeared after 8 hours. Equivalent *in vivo* coagulation response was assessed. A 1 L dose of FFP in adults may provide an initial increment of FVII of 0.10 IU/mL. In the absence of bleeding, FVII levels return to baseline after 8 hours.

In a retrospective study, Alvarez-Larrán *et al.* (2004)[169] studied 56 episodes of TTP treated with MB-

FFP (27 episodes) as an alternative to FFP (29 episodes). Patients who received MB-FFP had an higher risk of death from progressive TTP (OR=31; 95% CI: 1.2 to >100), than those treated with FFP, a greater number of recurrences while on plasma exchange therapy (OR=4,6; 95% CI: 1.2-17), and a lower probability of remission within 9 days of starting plasma exchange (OR=5.2; 95% CI: 1.3-20). The authors concluded that MB-FFP seems to be less effective than FFP in the treatment of TTP and suggested avoiding the use of MB-FFP while waiting for demonstration of its therapeutic equivalency to FFP through randomised controlled trials.

An open-label, multicentre trial was conducted by de Alarcon *et al.* (2005)[170] to measure the kinetics of specific coagulation factors, haemostatic efficacy, and safety of photochemical treatment with amotosalen hydrochloride (S-59) FFP (A-FFP) in patients with congenital coagulation factor deficiencies (FI, FII, FV, FVII, FX, FXI, and FXIII and protein C). Post-transfusion prothrombin time, partial thromboplastin time and clinical haemostasis were evaluated before and after the A-FFP transfusions. Thirty-four patients received 107 transfusions of A-FFP for kinetic studies or therapeutic indications (mean dose, 12.8±8.5 mL/kg). Incremental factor recoveries ranged from 0.9 to 2.4 IU/dL per IU/kg (FII, FV, FVII, FX, FXI, and protein C). The mean pre-transfusion prothrombin time (20.7± 22.2 seconds) was corrected after A-FFP (13.8±2.4 seconds, p<0.001) as was the mean pre-transfusion partial thromboplastin time (51.2±29.3 seconds *vs* 32.0±5.1 seconds, p<0.001). A-FFP provided as effective haemostasis as FFP and was well tolerated.

Garcia-Noblejas *et al.* (2005)[171] reported a case of a patient who developed a pulmonary embolism during treatment with MB-FFP for a suspected haemarthrosis. The patient, without a family history of thrombosis, had homozygous inherited FV deficiency and two acquired factors predisposing to thrombosis: obesity and bed rest. He started anticoagulant treatment with low molecular weight heparin while the deficient factors were replaced with MB-FFP. After 8 days of treatment the patient developed severe respiratory failure and the anticoagulant therapy was suspended due to a suspicion of pulmonary haemorrhage, an inferior vena cava filter was placed and corticosteroid therapy was started. Spiral computed tomography and other investigations were performed, leading to a diagnosis of TRALI. The patient progressively improved, the inferior vena cava filter was removed 1 month later and after 5 months of follow up there were no visible sequelae. The authors consider that the infusion of MB-FFP was an important factor for the thrombosis (although other risk factors were also present), and to their knowledge this association had not been previously reported.

A randomised, controlled, double-blind phase III trial was conducted by Mintz *et al.* (2006)[172] with A-FFP or control FFP for therapeutic plasma exchange in patients with TTP. Patients were treated with FFP for a maximum of 35 days until remission was achieved plus an additional 5 days after remission. Remission within 30 days, the primary end-point of the study, was achieved by 14 of 17 (82%) A-FFP recipients and 16 of 18 (89%) control, FFP recipients (p=0.658). The 90% CI for treatment difference in remission rate between test and control patients was −0.291 to 0.163. There were no significant differences in time to remission, relapse rates, time to relapse, total volume and number of FFP units exchanged, and number of study plasma exchanges between the two groups. The improvements in VWF-clotting protease activity and inhibitors were similar in both groups. No antibodies directed against amotosalen neoantigens were detected. Given the rarity of this condition, the trial was not powered to demonstrate small differences between treatment groups, although the comparable results observed in the two treatment groups suggest that therapeutic plasma exchange with A-FFP is safe and effective for the treatment of TTP.

Santagostino *et al.* (2006)[173] studied the pharmacokinetics of deficient clotting factors and the haemostatic efficacy of SD-FFP treatment in 17 patients with recessively inherited coagulation disorders (1 with afibrinogenaemia, 4 with FV deficiency, 6 with combined FV and FVIII deficiencies, 1 with FX deficiency and 5 with FXI deficiency) in an open-label, multicentre trial. *In vivo* recovery of the deficient coagulation factor was determined in a non-bleeding state in all patients and the mean values for FV, FVIII, FX, FXI and fibrinogen were 1.3, 1.2, 1.5, 1.3 and 1.5 dL/kg, respectively. The mean plasma half-lives of FV, FVIII and FX were 18, 43 and 33 hours, respectively. All patients underwent replacement therapy for elective procedures at risk of bleeding, except one who was treated for a central nervous system surgical emergency. SD-FFP treatment was judged fully effective in 13/16 cases (81%). In the remaining three cases, mild bleeding occurred after major surgery in a FV-deficient patient with a factor level of 43%, in a FXI-deficient patient when factor levels were between 20% and 41%, and, after minor surgery, in a patient with FV and FVIII deficiencies when the levels of these factors were 41% and 18%, respectively. Bleeding was controlled by continuing or increasing treatment with SD plasma. The authors suggest that, even though the current absolute risk of blood-borne infections associated with FFP is relatively small, SD-FFP should be preferred in patients with recessively inherited coagulation disorders when virus-inactivated single-factor concentrates are not available.

Scully *et al.* (2007)[174] reviewed 50 acute TTP episodes in order to compare the efficacy and safety of

cryosupernatant and SD-FFP (Octaplas®). In 12 episodes only cryosupernatant was used, whereas in 15 episodes treatment was started with cryosupernatant and changed to Octaplas®. Once Octaplas® had been used, it was continued on further admissions. Cryosupernatant was used exclusively in 24% and Octaplas® exclusively in 42% of all episodes. With regards to the episodes treated only with cryosupernatant or SD-FFP, there was no significant difference in the median number of plasma exchanges to achieve remission: 7,0 (interquartile range 5.0-8.8) and 8.0 (interquartile range 6.5-22.0), respectively.

Politis *et al.* (2007)[53] described 5 years of experience of pathogen inactivation of FFP with the MB-FFP system in a blood centre in Athens. During the study period, 8,500 units of MB-FFP were issued for transfusion and 88 units were evaluated for coagulation factor activity and cytokine concentrations after pathogen inactivation. The losses after MB inactivation and leucoreduction of these 88 units were all statistically significant ($p<0.05$) and ranged from 18% to 22% for coagulation factors and from 58% to 68% for cytokine concentrations. Post-MB treatment values of fibrinogen and FV and FVIII appeared to be correlated with their pre-MB values. For fibrinogen (Pearson's correlation coefficient $r=-0.43$, $p<0.001$) and FV ($r=-0.24$, $p=0.025$) the correlation between pre- and post-MB treatment values was negative, suggesting that the loss was greater from the units with higher pre-MB values. For FVIII, however, this correlation was positive ($r=0.25$, $p=0.020$).

A multicentre cohort study was conducted by del Rio-Garma *et al.* (2008)[28] to compare MB-FFP with Q-FFP in the treatment of TTP. MB-FFP was used in 63 and Q-FFP in 39 of 102 episodes of idiopathic TTP. The treatment programme comprised daily plasma exchange and corticosteroids; the main end-point was remission status on day 8. Patients treated with MB-FFP required more plasma exchanges (median: 11 *vs* 5, $p=0.002$) and a larger volume of plasma (median: 485 mL/kg *vs* 216 mL/kg, $p=0.007$) to achieve a remission, and had more recurrences while on plasma-exchange therapy than those receiving Q-FFP (29 of 63 *vs* 8 of 39, $p=0.02$). After adjustment for possible confounding factors, the use of MB-FFP was associated with a lower likelihood of remission on day 8 (OR: 0.17; 95% CI: 0.06-0.47) and a higher risk of recurrence while on treatment (OR: 4.2; 95% CI: 1.6-10.8).

A study performed by Mikkola *et al.* (2012)[175] suggested that when the various blood product transfusions instead of transfused units were included in the multivariable analysis, SD-FFP (Octaplas®) transfusion (OR: 2.149; 95% CI: 1.141-4.047), but not red blood cell transfusion, was significantly associated with post-operative stroke after coronary artery bypass surgery. Use of blood products ranging from no transfusion (stroke rate 1.6%) to combined transfusion of red blood cells, platelets and Octaplas® was associated with a significant increase in post-operative stroke incidence (6.6%, adjusted analysis: OR: 1.727; 95% CI: 1.350-2.209). Patients who received more than two units of red blood cells, more than four units of Octaplas® and more than eight units of platelets had the highest stroke rate of 21%. Classification and regression tree analysis showed that increasing amount of transfused Octaplas®, platelets and history of extra-cardiac arteriopathy were significantly associated with post-operative stroke.

The same author, in another study[176], found that blood product transfusion is independently associated with all-cause mortality (relative risk [RR])=1.678; 95% CI: 1.087-2.590) after coronary artery bypass surgery. Among various blood products, SD-FFP (Octaplas®) was the only one associated with an increased risk of all-cause mortality (RR=1.692; 95% CI: 1.222-2.344). Among blood products, perioperative use of FFP or Octaplas® seemed to be the main determinant of mortality.

A prospective, randomised controlled trial including 60 patients who received transfusions of plasma was conducted by Stanojkovic *et al.* (2012)[177]. The experimental group (30 patients) were treated with Mirasol®-inactivated FFP and the control group (30 patients) were transfused with non-inactivated FFP. Pre-transfusion and post-transfusion values and improvement in International Normalised Ratio in patients' plasma per one unit transfused were evaluated. A total of 68 units of FFP were transfused to patients in the control group (2.24±0.83 units/patient), while the experimental group received 84 units of Mirasol®-inactivated plasma (i.e. 2.80±1.19 units/patient). The experimental group required a significantly higher number of plasma transfusions to normalise coagulation parameters compared to the control group ($p=0.039$). There was a significant improvement of International Normalised Ratio after every administration of a plasma unit application ($p=0.046$) and the relationship between pre-transfusion and post-transfusion International Normalised Ratio was linear ($r=0.97$; $p<0.001$). Plasma treated with riboflavin and UV light is haemostatically competent, but larger volumes are required compared to those necessary when using non-inactivated FFP.

Bindi *et al.* (2013)[178] evaluated the efficacy of SD-FFP in patients undergoing orthotopic liver transplantation, comparing it with the efficacy of FFP. The 63 patients undergoing liver transplantation were divided into two groups depending on whether they were transfused with FFP or SD-FFP. Both groups achieved the thromboelastography goals but with a lower number of transfusions in the SD plasma group

($p<0.0001$). At the end of surgery, FV, FXII, and protein S levels, International Normalised Ratio, activated partial thromboplastin time and antithrombin III levels were lower in the SD plasma recipients. The authors observed that in cirrhotic patients undergoing orthotopic liver transplantation, SD-FFP provides the same clinical results as FFP, but with a significant reduction in the amount of plasma transfused.

In 2013, Bartelmaos *et al.*[179] performed a randomised, blinded trial in four French liver transplantation centres. They compared Q-FFP, MB-FFP and SD-FFP. The outcomes examined were the volume of plasma transfused during transplantation, intraoperative blood loss, correction of haemostasis variables, and adverse events. One hundred patients were randomly assigned to receive MB-FFP, 96 to receive SD-FFP, and 97 to receive Q-FFP. The median volumes of MB-FFP, SD-FFP, and Q-FFP transfused were 2,254, 1,905, and 1,798 mL, respectively, while the median numbers of transfused plasma units were 10, 10, and 8 units. After adjustment for bleeding risk factors, the difference between groups decreased and the excess plasma volume transfused with MB-FFP compared to Q-FFP was reduced from 24 to 14%. Blood loss and coagulation factor corrections were not significantly different between the three study groups. These results suggest that the use of MB-FFP was associated with a moderate increase in volume transfused, partly explained by a difference in unit volume and bleeding risk factors, with respect to Q-FFP and SD-FFP. Fewer units of Q-FFP were transfused than either SD-FFP or MB-FFP.

Table VIII summarises the clinical studies comparing pathogen-inactivated/reduced plasma to FFP.

Pathogen-inactivated/reduced plasma* vs *untreated plasma:* in vitro *studies

In 1998, 12 plasma pools, 12 batches of SD-treated plasma, and 12 units of Q-FFP were extensively analysed by Beeck and Hellstern[180]. The stability of fibrinogen, FV, FVII, and FVIII after thawing, storage at room temperature and storage at +4 °C was also examined. Plasma pools before and after SD treatment were appraised for parameters of coagulation and fibrinolysis, as were quarantined single-donor plasma units. After the SD-FFP treatment, all clotting factor activities and the activities of most inhibitors and other plasma proteins were in the normal range in all batches, Protein S and plasmin inhibitor activities decreased by 35 and 76%, respectively; FVII resulted partially activated. However, markers of activated haemostasis did not increase significantly. The inter-individual variations of all proteins analysed were significantly lower in the SD-FFP than in the single-donor plasma units. After 8 hours of storage, a marked decrease of FVIII activity was found without significant variations of fibrinogen, FV and FVII. No significant reductions of the activities of clotting factors and important plasma proteins due to the SD treatment were observed. The authors concluded that although the activities of protein S and plasmin inhibitor were reduced, the efficacy and safety of SD-FFP were maintained because the reductions of the activities of clotting factors and inhibitors were not critical. On the other hand, more standardised SD-FFP units would allow simplified dosage calculations and evaluation of clinical responses.

Aznar *et al.* (1999)[181] studied the effects of viral inactivation by MB on the FVIII/VWF molecular complex, FXIII, and fibrinogen. FVIII function or activity, VWF activity, VWF antigen, VWF multimeric structure, fibrinogen, and FXIII were analysed in paired samples of control fresh (untreated) plasma and of the same fresh plasma treated with MB. Treated plasma was filtered (pore size 0.8-1.2 μm), mixed with a MB solution (300 μg/L), and illuminated at 50,000 lux for 30 minutes on both sides. The average loss of biological activity of the coagulation factors studied was 25% (FVIII function, 29%; fibrinogen, 39%; FXIII, 16%; and VWF activity, 18%). The reduction of VWF activity was significantly lower than that of FVIII function ($p<0.05$), and the VWF multimeric structure did not show alterations. MB-treated plasma and cryoprecipitates may be successful for replacement therapy in von Willebrand disease and deficiencies of FXIII and fibrinogen. The authors suggest that clinical studies are needed to confirm this.

Doyle *et al.* (2003)[182] investigated the loss of clotting factors in SD-FFP by measuring procoagulant factors (fibrinogen, FV and FVIII), anticoagulant factors (protein C and protein S), prothrombin time and activated partial thromboplastin time in 48 single-donor units of FFP and in 16 units of SD plasma (Octaplas®). Prothrombin time, activated partial thromboplastin time, FVII and protein C levels were within the normal reference range for both SD plasma and FFP. FV (31%), FVIII (28%) and protein S (50%) levels were significantly reduced in SD plasma. Nevertheless, the difference in coagulation factor levels may have been further exacerbated by the smaller volume of the SD plasma units (200 mL) compared with the units of standard FFP (250 mL). These differences can play a critical role in treatment efficacy, particularly in patients with liver disease, constitutional FV deficiency and congenital or acquired protein S deficiency.

In a paired study (11 subjects/arm) by Garwood *et al.* (2003)[38], plasma was frozen within 8 hours of collection, thawed, MB photo-inactivated, and then filtered using one of two MB removal filters. Fresh plasma (16 units) and plasma leucoreduced before freezing (19 units) were MB inactivated. Freeze-thawing resulted in a loss

Table VIII - A summary of studies comparing technologies for the inactivation/reduction of pathogens in fresh-frozen plasma.

First author, year	Population	N. of patients	Outcomes/results	Conclusions/recommendations	Level of evidence
Evans G, 1999	Patients with TTP	3	100% of patients achieved complete remission. All patients were in stable remission 1 year later.	Plasma exchange with SD-FFP is effective as the primary treatment in acute TTP.	Very low
Williamson LM, 1999	Patients with coagulopathy associated with liver disease and liver transplantation	Total 52: 24 treated with FFP *vs* 25 treated with SD-FFP	Equal correction of clotting factors and partial thromboplastin time was seen with FFP and SD-FFP.	SD-FFP is an efficacious source of coagulation factors for patients with liver disease who are undergoing biopsy or transplantation.	Moderate
Flamholz R, 2000	Patients with TTP	3	Three patients developed deep vein thrombosis during plasma exchange with SD-FFP for TTP: reduction of free protein S.	Use of SD-FFP alone or in 50% combination with cryosupernatant as replacement fluid in plasma exchange for TTP may lead to difficulty in maintaining safe free protein S levels.	Very low
Lerner RG, 2000	Patients with acquired coagulation deficits	Total 45 22 treated with SD-FFP *vs* 23 treated with FFP	Ability to reduce a prolonged prothrombin time to ≤15 s. Mean dose of plasma infused. Percentage of patients whose bleeding ceased. Clotting factors levels (II, V, VII, VIII, IX, X, XI).	No clinical or statistically significant differences were observed after infusion with SD-FFP or FFP in patients with acquired coagulation deficits.	Moderate/ high
Atance R, 2001	Patients with different diseases (cardiac valve surgery, liver transplant, wounds and traumatic injuries, and bowel surgery)	2,967	Demand for plasma and cryoprecipitate.	The use of MB-FFP was associated with a marked increase in the demand for plasma and cryoprecipitate, which was probably due to the low haemostatic quality of the new component.	Low/ moderate
de la Rubia J, 2001	Patients with TTP	Total 20 13 patients received standard FFP (group A) 7 patients received MB-FFP (group B)	Bilirubin values were higher in group A ($p<0.05$ *vs* MB-FFP). Mean number of procedures was higher in group B (21±7 *vs* 11±3, $p<0.01$). Mean duration of hospitalisation was also longer (37±12 days *vs* 22±11 days ($p<0.01$) Remission rate was higher in group A than B (69% *vs* 57%).	The use of methylene blue-treated FFP to treat TTP is associated with a higher number of plasma exchanges and greater transfusion requirements without improving clinical results.	Moderate
de Jonge J, 2002	Patients undergoing orthotopic liver transplantation	20 FFP *vs* 21 solvent/detergent-treated plasma (ESDEP®)	Anhepatic phase: D-dimer, 6.58 *vs* 1.53 mg/mL for ESDEP *vs* FFP (p=0.02); Fibrinogen degradation products, 60 *vs* 23 mg/L for ESDEP *vs* FFP (p=0.018). After reperfusion: D-dimer, 23.5 *vs* 4.7 mg/mL for ESDEP *vs* FFP (p=0.002); Fibrinogen degradation products, 161 *vs* 57 mg/L for ESDEP *vs* FFP (p=0.001); $Alpha_2$-antiplasmin levels: significantly lower in patients receiving ESDEP during the anhepatic phase (0.37 *vs* 0.65 IU/mL; $p<0.001$) and after reperfusion (0.27 *vs* 0.58 IU/mL; p=0.001)	Clotting analysis of the patients revealed low $alpha_2$-antiplasmin concentrations because of the SD process.	Moderate
Hambleton J, 2002	Healthy volunteers anticoagulated with warfarin	Total 54: 27 treated with A-FFP *vs* 27 treated with FFP	Parameters: - t_{50} (h); - clearance; - recovery; - volume of distribution No statistically significant differences.	This study demonstrated that the kinetic properties of the short-lived factor VII remained the same in FFP prepared with a method for inactivation of infectious pathogens as in standard FFP.	Low

SD-FFP: solvent/detergent-treated fresh-frozen plasma; MB-FFP: methylene blue-treated fresh-frozen plasma; A-FFP plus UVA: amotosalen-treated fresh-frozen plasma exposed to ultraviolet A light; R-FFP plus UV: riboflavin-treated fresh-frozen plasma exposed to ultraviolet light; A-FFP: amotosalen-treated fresh-frozen plasma; R-FFP: riboflavin-treated fresh-frozen plasma; Q-FFP: quarantined fresh-frozen plasma; 95% CI: 95% confidence interval; INR: International Normalised Ratio; IQR: interquartile range; OR: odds ratio; RR: relative risk; TTP: thrombotic thrombocytopenic purpura.

continued on next page

Table VIII - A summary of studies comparing technologies for the inactivation/reduction of pathogens in fresh-frozen plasma. *(continued from previous page)*

First author, year	Population	N. of patients	Outcomes/results	Conclusions/recommendations	Level of evidence
Noddeland H, 2002	Patients scheduled for elective open-heart surgery	Total 84: group 1 (n=25), Uniplas® group 2 (n=11), Uniplas® group 3 (n=19), Octaplas® group 4 (n=25), control	Plasma transfusions: group 4 fewer (p<0.005) Bypass time (min): group 4 shorter (p<0.005)	Uniplas® has a similar effect as Octaplas® in the treatment of bleeding in patients undergoing open-heart surgery.	Low
Alvarez-Larrán A, 2004	Patients with TTP	Total 56 MB-FFP was used for fluid replacement in 27 episodes and FFP in 29	Recurrence under treatment: 14 (52%) *vs* 5 (17%) Adjusted OR (95% CI): 4.6 (1.2-17) Lack of remission by the 9th day of treatment: 18 (62%) *vs* 11 (38%) Adjusted OR (95% CI): 5.2 (1.3-20) Death from unresponsive disease: 4 (15%) *vs* 1 (3%) Adjusted OR (95% CI): 31 (1.2-100)	MB-FFP seems to be less effective than FFP in the treatment of TTP. It is therefore prudent to avoid MB-FFP until therapeutic equivalency to FFP has been established by randomised controlled trials.	Moderate
Haubelt H, 2002	Adult patients undergoing open-heart surgery	Total 67: - SD-FFP (n=36) - FFP (n=31)	Protein S (PS) and plasmin inhibitor (PI) plasma levels following administration of SD-FFP or FFP were significantly different (p=0.0002). The levels of plasmin-PI complexes dropped as a result of treatment (SD-FFP, p=0.001; FFP, p=0.0002). The difference in mortality between groups was statistically significant (p=0.040).	With the exception of PS and PI, SD-FFP and FFP improved haemostasis and fibrinolysis to similar degrees. The clinical significance of these findings needs to be determined in patients with severe acquired PS and PI deficiency requiring plasma transfusions.	High
Garcia-Noblejas A, 2005	Patients with severe congenital deficiency of factor V	Case report	Pulmonary embolism in a patient with severe congenital deficiency of factor V during treatment with MB-FFP	The infusion of MB-FFP was considered to have been an important factor in the development of embolism (although other risk factors were also present). This appears to the first report of such an association.	Very low
de Alarcon P, 2005	Patients with congenital coagulation factor deficiencies	34	Factor (F) II, FV, FVII, and FX replacement: p<0.001 Mean pre-transfusion prothrombin time (20.7±22.2 sec) corrected after A-FFP (13.8±2.4 sec, p<0.001). Mean pre-transfusion partial thromboplastin time (51.2±29.3 sec) corrected after A-FFP (32.0±5.1 sec, p<0.001).	The kinetics and therapeutic efficacy of the replacement coagulation factors in A-FFP were consistent with those of conventional FFP.	Moderate
Mintz PD, 2006	Patients with TTP	Total 35: - 17 treated with A-FFP - 18 treated with control FFP	Remission within 30 days (p=0.658). Time to remission, relapse rates, time to relapse, total volume and number of FFP units exchanged, and number of study therapeutic plasma exchanges were not significantly different between groups.	The comparable results between treatment groups observed in this small trial suggest that therapeutic plasma exchange with A-FFP is safe and effective for the treatment of TTP.	Moderate/ high
Santagostino E, 2006	Patients with recessively inherited coagulation disorders	17	SD-plasma was effective in 13/16 cases (81%).	SD plasma should be preferred in patients with recessively inherited coagulation disorders who need replacement therapy when virus-inactivated single-factor concentrates are not available.	Moderate/ low
Politis C, 2007	MB-FFP units	88 units out of a total of 8,500 MB-FFP units produced and issued for transfusion	Losses after MB inactivation and leucoreduction (p<0.05). Fibrinogen (Pearson's correlation coefficient r=−0.43, p<0.001) and factor V (r=−0.24, p=0.025). The factor VIII correlation was positive (r=0.25, p=0.020).	MB-FFP is safer than the untreated product even in patients who require large quantities of plasma transfusion.	Moderate

SD-FFP: solvent/detergent-treated fresh-frozen plasma; MB-FFP: methylene blue-treated fresh-frozen plasma; A-FFP plus UVA: amotosalen-treated fresh-frozen plasma exposed to ultraviolet A light; R-FFP plus UV: riboflavin-treated fresh-frozen plasma exposed to ultraviolet light; A-FFP: amotosalen-treated fresh-frozen plasma; R-FFP: riboflavin-treated fresh-frozen plasma; Q-FFP: quarantined fresh-frozen plasma; 95% CI: 95% confidence interval; INR: International Normalised Ratio; IQR: interquartile range; OR: odds ratio; RR: relative risk; TTP: thrombotic thrombocytopenic purpura.

continued on next page

Blood Transfus 2016; **14:** 287-386 DOI 10.2450/2016.0065-16

Table VIII - A summary of studies comparing technologies for the inactivation/reduction of pathogens in fresh-frozen plasma. *(continued from previous page)*

First author, year	Population	N. of patients	Outcomes/results	Conclusions/recommendations	Level of evidence
Scully M, 2007	Patients with TTP	32 patients (50 episodes; 681 procedures: 172 cryosupernatant (median 1.5 per admission, IQR 0.0-6.2) and 509 with Octaplas® (median 8.0 per admission, IQR 0.8-16.5).	Complete clinical remission: (p=0.06, Mann-Whitney U-test)	The use of cryosupernatant and SD-FFP was equally efficacious in this cohort of patients.	Moderate/ low
del Rio-Garma J, 2008	Patients with TTP	Total of 102 TTP episodes. MB-FFP (n=63), Q-FFP (n=39)	Remission on day 8 of treatment (69% Q-FFP *vs* 38% MB-FFP, p=0.002). Recrudescence, 29 MB-FFP (46%) *vs* 8 (20%) Q-FFP (p=0.02). N. of plasma exchanges to remission 11 (2-52) MB-FFP *vs* 5 (2-25) Q-FFP (p=0.002). Plasma infused (mL/kg) to remission, 485 (93–3108) MB-FFP *vs* Q-FFP 216 (75-1224) (p=0.007). Adjusted odds ratio: Remission on day 8 of plasma exchange MB-FFP 0.17 (0.06-0.47) (p=0.001) Non-initial episode 7.8 (2.0-30.1) (p=0.003) Recrudescence under treatment MB-FFP 4.2 (1.6-10.8) (p=0.003)	MB-FFP is associated with a worse outcome in TTP patients as it requires more plasma exchange sessions, greater plasma usage, and entails a higher patient exposure to the non-infectious risks of plasma transfusion.	Moderate/ high
Mikkola R, 2012	Patients after coronary artery bypass surgery	2,226	Risk of stroke after coronary artery bypass surgery was significantly increased with SD-FFP (when adjusted for pre-operative haemoglobin and post-operative blood loss: OR 1.727, 95% CI 1.350-2.209, p<0.0001).	Use of blood products ranging from no transfusion (stroke rate 1.6%) to combined transfusion of red blood cells, platelets and Octaplas® was associated with a significant increase in post-operative stroke incidence.	Moderate
Stanojkovic Z, 2012	Haematological patients	Total 60: 30 patients received R-FFP, 30 received FFP	The mean improvement in pre-transfusion *vs* post-transfusion INR per transfusion of two plasma units was 0.66 (range, 0.33-1.68) for R-FFP and 0.83 (range, 0.32-2.40) for FFP. There was a significant difference (p<0.001) in the increase of INR values in R-FFP *vs* FFP.	Mirasol-inactivated FFP has good clinical effectiveness in comparison to conventional or non-inactivated plasma.	Low/ moderate
Mikkola R, 2013	Patients after coronary artery bypass surgery	2001	All-cause mortality: FFP/Octaplas® (p<0.0001). Cardiac mortality: (p<0.0001, RR=2,125, 95% CI: 1.414-3.194).	FFP/Octaplas® was the only blood product associated with increased risk of all-cause cardiac mortality.	Moderate/ high
Bindi ML, 2013	Cirrhotic patients undergoing orthotopic liver transplantation	Total 63: 30 patients received SD-FFP and 33 received FFP	Plasma transfused (mL): FFP *vs* SD-plasma, 2,617±1,297 *vs* 1,187±560 (p<0.0001). At the end of surgery, factors V (p=0.001 *vs* FFP) and XII (p=0.02 *vs* FFP) and protein S (p<0.0001 *vs* FFP) blood levels were lower in the SD-plasma patients who also showed lower INR (p=0.007 *vs* FFP), activated partial thromboplastin time (p=0.002 *vs* FFP) and antithrombin III levels (p=0.01 *vs* FFP).	In cirrhotic patients undergoing orthotopic liver transplantation, the use of SD-plasma provided the same clinical results (thromboelastography goals) but with a significant reduction in the amount of plasma transfusions.	Moderate/ high
Bartelmaos T, 2013	Patients undergoing combined liver-transplant	Total 408: 100 in the MB-FFP group, 96 in the SD-FFP group, and 97 in the Q-FFP group	Median (IQR) total volume of plasma transfused (mL): - MB-FFP: 2,254 (1,446-4,148) - SD-FFP 1,905 (1,400-3,259) - Q-FFP 1,798 (1,289-3,468) Median (IQR) amount of haemoglobin lost (g/L): - MB-FFP: 340 (226-592) - SD-FFP 359 (234-609) - Q-FFP 329 (238-475) After the first plasma transfusion, and after the end of the transplant procedure, no significant differences were found in the changes in fibrinogen, factor V, and prothrombin time among the three arms.	Statistical difference in disfavor of MB-FFP, suggesting that a greater volume of plasma needs to be used when compared to Q-FFP and SD-FFP for a similar clinical efficacy. However, differences in conditioning volume per plasma unit as well as imbalance in bleeding risk factors preclude definitive conclusions on a differential efficacy between MB-FFP, SD-FFP, and Q-FFP in the setting of liver transplantation.	Moderate/ high

SD-FFP: solvent/detergent-treated fresh-frozen plasma; MB-FFP: methylene blue-treated fresh-frozen plasma; A-FFP plus UVA: amotosalen-treated fresh-frozen plasma exposed to ultraviolet A light; R-FFP plus UV: riboflavin-treated fresh-frozen plasma exposed to ultraviolet light; A-FFP: amotosalen-treated fresh-frozen plasma; R-FFP: riboflavin-treated fresh-frozen plasma; Q-FFP: quarantined fresh-frozen plasma; 95% CI: 95% confidence interval; INR: International Normalised Ratio; IQR: interquartile range; OR: odds ratio; RR: relative risk; TTP: thrombotic thrombocytopenic purpura.

of activity of FXII and VWF of 0.06 and 0.04 units/mL, respectively, without significant loss of activity of FII through to FXI or fibrinogen. After MB treatment, there was loss of activity of FII (0.07 IU/mL), FV (0.11 U/mL), FVII (0.08 IU/mL), FVIII (0.28 IU/mL), FIX (0.12 IU/mL), FX (0.16 IU/mL), FXI (0.28 U/mL), FXII (0.15 U/mL), VWF antigen (0.05 IU/mL), VWF activity (0.06 U/mL), and fibrinogen (0.79 g/L). Losses due to this step were significantly (5-10%) less in fresh plasma than in frozen-thawed plasma. Neither MB removal filter resulted in significant loss of activity of any factor studied.

Depasse *et al.* (2005)[183] studied the influence of MB treatment on fibrinogen activity and fibrinogen polymerisation indices. They split ten apheresis plasma bags into two equal aliquots, both of which were leucofiltered and virally inactivated in a standardised way, using the Theraflex® MB-Plasma photodynamic viral inactivation MacoPharma method. One was subsequently processed, using a Blueflex® MB removal filter to remove residual MB and its by-products. Control plasma samples were obtained after filtration but before MB light treatment. All samples were analysed for fibrinogen activity and antigen, thrombin clotting time, and alterations in fibrin polymerisation indices. After MB light treatment, the mean fibrinogen concentration increased slightly (2%) whereas functional activity decreased by 31%, as compared to that in control samples. Mean thrombin clotting time increased by 6 seconds after MB light treatment, with no further changes after filtration to remove MB. A similar trend was observed when measuring reptilase time. Compared to the findings in control samples, both thrombin- and reptilase-triggered fibrin polymerisation was delayed and the polymerisation curve slopes were decreased slightly, with concomitant changes in fibrin opacity in samples subjected to MB light treatment and MB removal by filtration.

The aim of Heger *et al.* (2005)[184] was to study the quality of SD-FFP, Octaplas®, and single-donor FFP units during 48 hours of storage after thawing. Octaplas® bags of different blood groups and individual FFP units were thawed and stored at either +4 °C or at room temperature for 48 hours. Coagulation factor and protease inhibitor activities were investigated using standard coagulation and chromogenic assays. The generation of FVIIa was considered as a marker of coagulation factor activation. All investigated coagulation factors and protease inhibitors were stable for at least 8 hours during storage of Octaplas® at +4 °C. FVIII levels started to decline earlier in FFP than in Octaplas® at both storage temperatures. The optical density values of stored Octaplas® were more stable during the storage period than those of FFP, whereas VWF multimeric patterns were comparably stable in both types of plasma. The study results suggest that Octaplas® maintains high quality levels for 8 hours when stored at +4 °C and for 6 hours when stored at room temperature. In general, there was more variability in coagulation factor levels among FFP units than among different Octaplas® batches.

In 2007, Heger *et al.*[152] compared plasma derived from different sources. Twenty-four Octaplas® and three Uniplas® batches were selected for the study. ADAMTS13 activity was measured by fluorescence resonance energy transfer assay, ADAMTS13 antigen levels were measured using an enzyme-linked immunosorbent assay test kit, while factor H antigen levels were determined using a radial immunodiffusion method. In addition, VWF multimers were analysed. ADAMTS13 antigen and activity levels as well as factor H concentrations were normal in all the plasma samples studied, without significant differences. Furthermore Octaplas® and Uniplas® had VWF multimeric patterns similar to that of normal plasma and contained normal levels of ADAMTS13 with low variation among batches. The authors concluded that both products can substitute the missing or neutralised protease activity in TTP patients and thus limit VWF-dependent (platelet-related) thrombosis. Furthermore both products can be used efficiently in the treatment of patients with haemolytic-uraemic syndrome, given their normal content of factor H.

The study by Singh *et al.* (2006)[185] evaluated the pathogen-inactivation efficacy of A-FFP and the effect of photochemical treatment with the Intercept® Blood System on plasma function. Plasma function was evaluated through measurement of coagulation factor and antithrombotic protein activities. Factor activity after photochemical treatment, expressed as a proportion of pre-treatment (baseline) activity, was 72 to 73% of baseline fibrinogen and FVIII activity and 78 to 98% for FII, FV, FVII, F IX, FX, FXI, FXIII, protein C, protein S, antithrombin, and α_2-antiplasmin. Levels of inactivation expressed as log-reduction were: cell-free HIV-1, greater than 6.8; cell-associated HIV-1, greater than 6.4; HTLV-I, 4.5; HTLV-II, greater than 5.7; HBV and HCV, greater than 4.5; duck HBV, 4.4 to 4.5; BVDV, 6,0; severe acute respiratory syndrome coronavirus, 5.5; WNV, 6.8; bluetongue virus, 5.1; human adenovirus 5, 6.8; *Klebsiella pneumoniae*, greater than 7.4; *Staphylococcus epidermidis* and *Yersinia enterocolitica*, greater than 7.3; *Treponema pallidum*, greater than 5.9; *Borrelia burgdorferi*, greater than 10.6; *Plasmodium falciparum*, 6.9; *Trypanosoma cruzi*, greater than 5.0; and *Babesia microti*, greater than 5.3.

Pock *et al.* (2007)[89] found impaired thrombin generation in single-donor FFP units inactivated by MB

dye and subsequently exposed to white light compared to that in non-MB-treated single-donor FFP, the licensed plasma product Octaplas®, and a product under development (Uniplas) independently of the recipients' blood group. Thrombin generation was more impaired following the use of local ("in-house") MB inactivation systems in blood banks than following industrial MB treatment. Supplementation of functional fibrinogen to physiological levels did not normalise the altered thrombin generation capacity in MB-FFP, whereas addition of Octaplas® did. No clear-cut correlation between coagulation factor levels in MB-FFP and impaired thrombin generation capacity was found, suggesting a composite effect.

In their study Cid *et al.* (2008)[138] analysed 36 FFP units obtained from whole blood donations that were used for 12 replicate experiments. For each replicate experiment, three ABO-matched FFP units were pooled and divided into three units containing different volumes of identical plasma. One unit was used as control FFP, one was treated with MB and one was treated with amotosalen and UVA light. In this study, the coagulation factor levels were better maintained in untreated FFP than in photochemically treated plasma. However, coagulation factor levels of MB or treated plasma met or exceeded the European Pharmacopeia requirements for therapeutic plasma.

del Rio-Garma *et al.* (2008)[186] measured ADAMTS-13 activity levels and VWF antigen in plasma before and after MB treatment by either the Springe method or a commercial "in-house" system and also in cryoprecipitate-poor plasma and FFP (20 units each). Levels of ADAMTS-13 activity in MB-FFP processed by the Springe method or the commercial "in-house" system were comparable to one another and did not differ significantly from levels found in FFP (median [range]: 114% [57-139%], 99% [74-123%], and 106% [70-130%], respectively). ADAMTS-13 activity was significantly lower in cryosupernatant [median (range): 87% (70-107%)] than in FFP ($p<0.05$). Levels of VWF antigen decreased after photo-oxidation by both methods. *In vitro* ADAMTS-13 activity was conserved in MB-FFP processed by the two photo-oxidation methods and did not differ significantly from the activity found in FFP.

In a study performed by Lawrie *et al.* (2010)[187], coagulation factors levels, physiological protease inhibitors, markers of activation and procoagulant microparticles were assessed in four production batches of standard Octaplas® and six of Octaplas LG®. Global haemostasis was assessed by a thrombin generation test and rotational thromboelastometry (ROTEM). Mean levels of factors II, V, VII, IX, X, XI, XII and XIII, VWF antigen, antithrombin, protein C and free protein S were all >75 U/dL. ADAMTS-13 activity levels were normal. FVIII and VWF ristocetin cofactor levels were >55 U/dL. Results of the thrombin generation test and ROTEM were comparable in both preparations, and microparticles were present at insignificant levels. The levels of D-dimer and thrombin-antithrombin complexes were normal in all batches, two units of OctaplasLG® only had slightly elevated levels of prothrombin fragments. This study suggests that the affinity chromatography procedure used in OctaplasLG® does not adversely affect the haemostatic quality of Octaplas®.

Theusinger *et al.* (2011)[188] compared the concentrations of coagulation factors and cytokines in SD-FFP and FFP. Concentrations of the following parameters were measured in 25 SD-FFP and FFP samples: fibrinogen, FII, FV, FVII, FVIII, FIX, FX, FXIII, VWF, D-dimers, ADAMTS-13 protease, tumour necrosis factor-α, interleukin (IL)-1b, IL-6, IL-8, and IL-10. Mean fibrinogen concentrations in SD-FFP and FFP were similar, but the range was larger in FFP than in SD-FFP ($p=0.01$). Mean FII, FVII, FVIII, FIX, and FXIII levels did not differ significantly. Higher concentrations of FV ($p<0.01$), FX ($p<0.05$), VWF ($p<0.01$), and ADAMTS-13 ($p<0.01$) were found in FFP. With the exception of FVIII and FIX, the ranges of concentrations for all of these factors were smaller in SD-FFP than in FFP ($p<0.05$). Concentrations of tumour necrosis factor-α, IL-8, and IL-10 were higher in FFP than in SD-FFP (all $p<0.01$), again with greater variability and thus larger ranges ($p<0.01$). Coagulation factor contents in SD-FFP and FFP were similar, with the exceptions of less FV, VWF, and ADAMTS-13 in SD-FFP. Cytokine concentrations (tumour necrosis factor-α, IL-8, and IL-10) were significantly higher in FFP. The clinical relevance of these findings needs to be established in outcome studies.

Ettinger *et al.* (2012)[189] evaluated the *in-vitro* protein quality of apheresis-derived plasma products processed between 8 and 8.5 hours after collection and stored at −18 °C for 6 months and 1 year. Plasma (205±5 mL) was combined with 35±5 mL of 500 μM riboflavin, and exposed to UV light (6.24 J/mL). Overall, 14 plasma units were analysed using standard coagulation assays immediately following treatment, and after 6 months and 1 year of storage at −18 °C. Fibrinogen, total protein, FII, FVIII, FIX, FX and FXII levels were modestly affected by storage for 1 year at −18 °C. The average percent protein retention in R-FFP samples after 1 year of storage in comparison to control samples held under similar conditions were: total protein, 103%; FII, 88%; FVII and FXI, 62%; FV 60%; FVIII and FIX, 77%; fibrinogen, 75%; FX, 85%; and FXII, 82%. The results after 6 months (double the European Union guidelines' recommended storage

time) and 1 year of storage at −18 °C (four times longer than the European Union guidelines' recommended storage time), demonstrated that all proteins were well preserved. The values in treated products stored under these conditions were the same as those reported for other pathogen-inactivated plasma products.

Ettinger *et al.* (2012)[190] evaluated the possibility of making pathogen-reduced cryoprecipitate from riboflavin and UV light-treated plasma that meets the quality requirements specified by United Kingdom and European guidelines for untreated cryoprecipitate. Plasma units were thawed over a 20-hour period at 4 °C, and variable centrifugation settings (from 654 *g* for 2 minutes to 5,316 *g* for 6 minutes) were applied to identify the optimal centrifugation condition. Neither the centrifugation speed nor its time appeared to have an effect on the quality of the final cryoprecipitate product; however, the initial solubilisation of the cryoprecipitate product was found to be easier at the lower centrifugation setting (654 *g* for 2 min). A smaller quantity of protein was found in the cryoprecipitate units prepared from R-FFP than from untreated products, but this was on average 93 IU/unit, 262 mg/unit and 250 IU/unit for FVIII, fibrinogen and VWF ristocetin cofactor activity, respectively. The study shows that cryoprecipitate products prepared from Mirasol®-treated plasma using an optimised centrifugation method contain levels of fibrinogen, FVIII and VWF ristocetin cofactor activity which meet both the European and the UK guidelines for untreated cryoprecipitate.

Keller *et al.* (2012)[191] evaluated the stability of clotting factors and inhibitors in thawed SD-plasma stored at 4 °C for 6 days. Clotting factor levels and bacterial contamination were investigated using 20 units of SD plasma. Fibrinogen, FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII, antithrombin, VWF antigen, plasmin inhibitor, protein C and free protein S were analysed over time. After 6 days of storage the results were as follows: fibrinogen 270 mg/dL (−10mg/dL, p=0.0204), FII 75% (−5%, p<0.0001), FV 88% (−14%, p<0.0001), FVII 81% (−24%, p<0.0001), FVIII 70% (−16%, p<0.0001), FIX 96% (−8, p<0.0001), FX 92% (−1%, p<0.0001), FXI 119% (−4%, p=0.3666), FXII 94% (−2%, p=0.3602), FXIII 89% (−1%, p=0.0019), free protein S 76% (−4%, p<0.0001), protein C 96% (+1%, p=0.0371), antithrombin 92% (−3%, p<0.0001), plasmin inhibitor 29% (−4%, p<0.0299) and VWF antigen 137% (+2%, p=0.2205). FVII and FVIII showed a critical drop of more than 20% or approached the lowest quality assurance threshold after storage for more than 24 hours. No bacterial contamination was found in SD plasma. All clotting factors in thawed SD plasma remained stable for up to 24 hours when stored at 4 °C. Storage of thawed SD plasma may improve the availability of this product in emergency situations.

Cid *et al.* (2013)[192] analysed the quantity and quality of coagulation factors in cryoprecipitate prepared from A-FFP. Fibrinogen, FVIII, VWF antigen and activity, VWF cleavage protease activity (ADAMTS-13), and the multimeric structure of VWF were analysed. Fibrinogen, FVIII, and ADAMTS-13 content was lower in cryoprecipitates prepared from amotosalen-treated plasma than in cryoprecipitates prepared from untreated plasma (35, 40, and 18% loss, respectively). The quantity and quality of VWF, as well as VWF multimer patterns were not affected by the inactivation method. Cryoprecipitates prepared from A-FFP contained significantly reduced levels of fibrinogen, FVIII, and ADAMTS-13. However, VWF quantity and quality were well preserved. The authors concluded that VWF integrity was much better preserved when using A-FFP than when using either the original SD method or the MB method.

Table IX summarises the above-described *in vitro studies*, comparing technologies for the inactivation/reduction of pathogens in FFP.

Amotosalen-treated plasma* vs *plasma treated with other pathogen-reduction technologies

Osselaer *et al.* (2008)[45] compared plasma units made from a pool of two ABO-matched fresh apheresis units treated with either A-FFP or MB-FFP. A total of 12 paired samples were evaluated. Plasma coagulation function was assessed immediately after treatment, after 30 days of frozen storage, and after an additional 24 hours at 4 °C after thawing. The A-FFP and MB-FFP parameters were compared using paired t-tests and p-values less than 0.05 were considered statistically significant. The mean levels of FII, FXII, FXIII, VWF antigen, ADAMTS-13, D-dimers, and protein C were statistically equivalent in A-FFP and MB-FFP at all three time points. Mean prothrombin time, activated partial thromboplastin time (two time points), and thrombin time of A-FFP were shorter than those of MB-FFP and A-FFP contained higher mean levels of fibrinogen, FXI, and protein S than MB-FFP. The retention of FV, FVII, FVIII, FX, and VWF activity in A-FFP was either equivalent to or greater than that in MB-FFP. MB-FFP contained higher mean levels of plasminogen, antithrombin, and plasmin inhibitor than A-FFP. Retention of FIX in MB-FFP was greater than that in A-FFP only after the 4 °C storage after thawing. There is adequate preservation of therapeutic coagulation factor activities in both A-FFP and MB-FFP. Overall coagulation factor levels and stability were preserved better in A-FFP than in MB-FFP. Details of this study are provided in Table X.

Solvent/detergent-treated plasma* vs *cryosupernatant plasma

Rock *et al.* (2013)[193] reported on a series of TTP patients treated with SD plasma or cryosupernatant plasma and analysed the correlations between the patients' presentation, clinical response and outcome with the levels of ADAMTS-13, the inhibitor and VWF multimers. Plasma exchange was carried out in patients with a clinical diagnosis of acquired idiopathic TTP. ADAMTS-13 enzyme activity and inhibitor levels, VWF multimers and platelet count were analysed in correlation with patients' outcome. This randomised, controlled trial was intended to compare outcome in 280 patients treated with either cryosupernatant or SD plasma. The primary end-point was survival at 6 months. The study was closed prematurely due to removal of one of the interventional products from the market. ADAMTS-13 enzyme activity and inhibitor levels varied considerably among patients. At baseline, only 12/49 (24.5%) had ≤10% enzyme activity and 20/49 (41%) had levels ≥80%, whereas 16/49 had ≥80% inhibitors, 19/49 had ≤10% inhibitors and 18/49 (37%) had no inhibitors. No unusually large VWF multimers were identified in any of the patients at presentation. The 6-month, all-cause mortality rates for patients randomised to receive cryosupernatant plasma *vs* SD plasma were 3/34 (9%; 95% CI: 3-23%) and 1/27 (4%; 95% CI: 1-18%), respectively, with a difference of 5% (95% CI: –11-20%). ADAMTS-13 activity and inhibitor level at baseline could not differentiate the response of TTP to plasma exchange therapy. Details of this study are provided in Table XI.

Solvent/detergent-treated plasma: Uniplas LG® vs Octaplas LG®

Jilma-Stohlawetz *et al.* (2011)[194] evaluated the safety of a universal, virus-inactivated and prion-depleted, pharmaceutical-quality plasma through a randomised, double-blind, active-controlled, crossover, phase I trial. The 30 healthy adult volunteers (blood group A, B, or AB) were randomly assigned to transfusion of 1,200 mL of Uniplas LG® or 1,200 mL of Octaplas LG® or vice versa. In both periods, plasmapheresis (600 mL) preceded the infusion. Blood samples were drawn before and after plasmapheresis and 15 minutes, 2 hours, 24 hours, and 7 days after the plasma transfusion, to assess safety and efficacy. The primary safety outcome was variation in haemoglobin concentration while the secondary outcomes were direct antiglobulin test, complement activation, free haemoglobin, haptoglobin, and indirect bilirubin which could reflect haemolytic transfusion reactions. Efficacy was assessed by evaluating coagulation variables. Variations of haemoglobin concentration were comparable between treatments and within the normal range; the 90% CI was within predefined limits of equivalence. No subject had a positive direct antiglobulin test and most adverse events were mild. By eliminating the risk of ABO incompatibility, the universal plasma may represent a progress over blood group-specific plasma. Details of this study are provided in Table XII.

Solvent/detergent-treated plasma: Octaplas LG® vs Octaplas®

Jilma-Stohlawetz *et al.* (2013)[195] also compared the safety and tolerability of Octaplas LG® *vs* Octaplas®. In this comparative, block-randomised, open-label, active-controlled, crossover phase I trial, 60 healthy adult volunteers received single transfusions of 1,200 mL of the parent product (Octaplas®) in period 1 and of the LG plasma product (Octaplas LG®) in period 2 or vice versa. In both periods, plasmapheresis (600 mL) preceded the transfusion. Blood samples were drawn before and after apheresis and 15 minutes, 2 hours, 24 hours, and 7 days after the end of plasma transfusion, to assess recovery, safety, and tolerability. The primary efficacy end-points were changes in coagulation factors and haemostatic variables compared to baseline; their relative recovery was computed in the per-protocol analysis (n=43). Safety and tolerability were assessed (n=60). Variations in coagulation factors and haemostatic variables over time were within the normal range and similar with the two treatments; 90% CI for the derived recovery data were within predefined limits of equivalence. Plasmin inhibitor concentrations increased significantly after transfusion *in vivo*. Octaplas LG® was bioequivalent to its predecessor with respect to recovery of clotting factors and demonstrated comparable safety and tolerability in healthy volunteers. Both products compensated well for the loss of clotting factors after apheresis. Details of this study are provided in Table XIII.

Studies of the clinical efficacy of pathogen reduction technologies without a comparator

In their study, De Silvestro *et al.* (2007)[55] evaluated the clinical effectiveness of Plasmasafe® in 490 patients (879 transfusion events); performed pre- and post-treatment monitoring of indices of coagulation (prothrombin time, activated partial thromboplastin time, fibrinogen, proteins S and C, FVIII) in 15 patients; and treated three TTP patients undergoing plasma-exchange. The prothrombin time ranged from 50-120%, the activated partial thromboplastin time from 24-41 seconds and the fibrinogen concentration from 1.42-6.84 g/L. Seventy-six percent of the patients responded to the plasma administration. Moreover, two out of 15 patients in whom protein S was assayed showed no increase in the concentration of this haemostatic protein. The TTP patients responded to plasma exchange treatment following four sessions of apheresis. During the observation period 8,422 PlasmaSafe® units were transfused and no adverse reactions were recorded. The

Table IX - Technologies for the inactivation/reduction of pathogens in fresh-frozen plasma: *in vitro* studies.

First author, year	Population	N. of plasma samples	Outcomes/results	Conclusions/recommendations	Level of evidence
Beeck H, 1998	Plasma units	Total 36: (12 plasma pools, 12 batches of SD-FFP produced from these respective plasma pools, and 12 units of Q-FFP)	Decrease or increase in coagulation factor (%): fibrinogen 7.7, factor II 5.7, factor V 9.9, factor IX 3.8, factor XI 5.8, factor XIII 4.9, VWF activity 5.8 ($p<0.05$); factor VII 25.2, factor X 16.6, factor VIII 21.9, factor XII 13.4 ($p<0.01$)	There were no critical reductions of the activities of clotting factors, inhibitors, or other important plasma proteins due to the SD treatment. The efficacy and safety of SD plasma is not impaired by reduced activities of protein S and plasmin inhibitor.	Moderate
Aznar JA, 1999	Blood donations	26 units pre- and post-treatment with MB	Loss of clotting factors: factor VIII function (%) 29±7 ($p<0.001$); vWF activity (%) 18±15 ($p<0.001$).	MB-treated plasma could turn the plasma itself and the cryoprecipitates obtained from it into a useful therapeutic option for the treatment of von Willebrand disease, as well as deficiencies of factor XIII or fibrinogen.	Low/moderate
Garwood M, 2003	Plasma units	24	Loss of clotting factors: before *vs* after MB removal ($p<0.01$) for: PT (ratio), fibrinogen, factor (F) V, FVII, FX, FXIIa, C3a; ($p<0.05$) for aPTT (ratio), FII. Before *vs* after Bluflex removal ($p<0.01$) for: PT (ratio), aPTT (ratio), FXII.	MB removal, by either of the available filters, has little impact on the coagulation factor content of plasma, but freezing plasma before MB treatment resulted in a futrher small loss.	Moderate
Heger A, 2005	Plasma units	7 Octaplas® batches *vs* 6 FFP units	FFP *vs* SD-FFP: factor V 1.03±0.06 *vs* 0.91±0.07 ($p<0.05$); protein S 0.97±0.14 *vs* 0.560±0.04 ($p<0.005$); plasmin inhibitor 0.97±0.04 *vs* 0.27±0.03 ($p<0.005$).	Thawed Octaplas® maintains its high quality, even with a time safety margin, for 8 h at +4 °C and for 6 h at room temperature.	Low
Depasse F, 2005	Apheresis plasma	10	Fibrinogen activity: decreased significantly after MB inactivation ($p<0.0001$) but remained unchanged after MB-filtration. Fibrinogen antigen: increased by 2% after MB inactivation ($p=0.0058$) and remained stable after MB-filtration.	A decrease in functional activity of fibrinogen occurred subsequent to combined MB light treatment and MB filtration, affecting the overall changes in fibrinogen activity states and in fibrin polymerisation indices.	Low/moderate
Heger A, 2006	Plasma batches	24 Octaplas® 3 Uniplas® 20 FFP	ADAMTS13 activity: Octaplas® 0.96±0.06. ADAMTS13 antigen: Octaplas® 0.89±0.07.	Octaplas® and Uniplas® contain normal levels of ADAMTS13 with low batch-to-batch variations.	Low
Singh Y, 2006	Apheresis-derived plasma units	Not specified	Retention of coagulation factor activity was: 72 to 73% of baseline fibrinogen and factor (F)VIII activity and 78 to 98% for FII, FV, FVII, FIX, FX, FXI, FXIII, protein C, protein S, antithrombin, and α_2-antiplasmin.	The study confirmed maintenance of adequate plasma coagulation and antithrombotic protein function after inactivation.	Low
Pock K, 2007	Plasma units	Total 75: 18 FFP, 18 Octaplas®, 18 MB-FFP I and 18 MB-FFP II, 3 Uniplas®	FFP *vs* Octaplas® (A), FFP *vs* MB-FFP I (B), FFP *vs* MB-FFP II (C), Octaplas® *vs* MB-FFP I (D), Octaplas® *vs* MB-FFP II (E), MB-FFP I *vs* II (F). Peak thrombin: A: n.s., B: $p<0.00000001$, C: $p<0.005$, D: $p<0.00000001$, E: $p<0.000005$, F: $p<0.05$ Area under the curve: A: n.s., B: $p<0.00000001$, C: n.s., D: $p<0.00000001$, E: $p<0.05$, F: $p<0.00005$. Lag time: A: n.s, B: $p<0.000001$, C: $p<0.01$, D: $p<0.00000001$, E: $p<0.0005$, F: $p<0.005$.	A thorough elucidation and evaluation regarding the possible clinical impact of these findings seems prudent.	Moderate/low

SD-FFP: solvent/detergent-treated fresh-frozen plasma; Q-FFP: quarantined fresh-frozen plasma; VWF: von Willebrand factor; MB-FFP: methylene blue-treated fresh-frozen plasma; VWF: von Willebrand factor; PT: prothrombin time; aPTT: activated partial thromboplastin time; A-FFP: amotosalen-treated fresh-frozen plasma.

Blood Transfus 2016; 14: 287-386 DOI 10.2450/2016.0065-16

Table IX - Technologies for the inactivation/reduction of pathogens in fresh-frozen plasma: *in vitro* studies. *(continued from previous page)*

First author, year	Population	N. of plasma samples	Outcomes/results	Conclusions/recommendations	Level of evidence
Ettinger A, 2012	Apheresis-derived plasma units	14	Protein retention in treated plasma samples after 1 year of storage: total protein, 103%, factor II, 88%, factors VII and XI, 62%, factor V 60%, factors VIII and IX, 77%, fibrinogen, 75%, factor X, 85% and factor XII, 82%.	All proteins were well preserved during storage at –18 °C for 1 year.	Low
Keller MK, 2012	SD-FFP bags	20	Protein and clotting factor levels after 6 days of storage at 4 °C: fibrinogen 270 mg/dL (–10 mg/dL, p=0.0204), factor (F) II 75% (−5%, p<0.0001), FV 88% (–14%, p<0.0001), FVII 81% (−24%, p<0.0001), FVIII 70% (−16%, p<0.0001), FIX 96% (−8%, p<0.0001), FX 92% (–1%, p<0.0001), FXI 119% (−4%, p=0.3666), FXII 94% (−2%, p=0.3602), FXIII 89% (−1%, p=0.0019), free protein S 76% (−4%, p<0.0001), protein C 96% (+1%, p=0.0371), antithrombin 92% (−3%, p<0.0001), plasmin inhibitor 29% (−4%, p<0.0299), VWF-antigen 137% (+2%, p=0.2205).	All clotting factors in thawed SD-FFP remained stable for up to 24 hours when stored at 4 °C. Storage of thawed SD-FFP may improve the availability of this product in emergency situations.	Low/ moderate
	Octaplas® plasma bags	20	From 6 to 24 hours after thawing; Slight decreases were shown for factor (F) II, FV, FVII, FVIII, FX, FXIII, free protein S and plasmin inhibitor (p<0.0001). From 24 hours to 6 days after thawing FII, FV, FX and plasmin inhibitor levels changed significantly (p<0.0001). From immediately after thawing to 6 days after thawing; fibrinogen, FII, FV, FVII, FVIII as well as FIX, FX, FXIII, protein C, antithrombin, plasmin inhibitor and free protein S decreased significantly over time while protein C and VWF-antigen levels were slightly elevated after 6 days of storage (p<0.0001).	Clotting factors remain stable in thawed SD-FFP stored for 24 hours at 4 °C. Longer storage of plasma for up to 6 days results in limited quality.	Low/ moderate
Ettinger A, 2012		74	Fibrinogen: 295±102 control, 283±54 treated conditioned, 367±76 treated non-conditioned (p<0.04).	Cryoprecipitate products prepared from Mirasol®-treated plasma using a centrifugation method contain levels of fibrinogen, factor VIII and von Willebrand ristocetin cofactor activity that meet both the European and UK guidelines for untreated cryoprecipitate.	Moderate
Cid J, 2013	FFP units	15	Contents of cryoprecipitates prepared from FFP *vs* A-FFP: fibrinogen: 558±151 *vs* 362±120 (p<0.001); factor VIII: 203±60 *vs* 12±27 (p<0.001); ADAMTS-13: 77±9 *vs* 62±10 (p=0.02).	Cryoprecipitates prepared from A-FFP contained significantly reduced levels of fibrinogen, factor VIII, and ADAMTS-13. However, VWF quantity and quality were well preserved.	Low/ moderate

SD-FFP: solvent/detergent-treated fresh-frozen plasma; Q-FFP: quarantined fresh-frozen plasma; VWF: von Willebrand factor; MB-FFP: methylene blue-treated fresh-frozen plasma; VWF: von Willebrand factor; PT: prothrombin time; aPTT: activated partial thromboplastin time; A-FFP: amotosalen-treated fresh-frozen plasma.

authors concluded that PlasmaSafe® is a pharmaceutical-like product with a standardised content of coagulation factors and that it is effective in correcting coagulation defects and in treating patients with TTP. Details of this study are provided in Table XIV.

Discussion

The ever-present threat of emerging and re-emerging infectious diseases, as well as the risk of clinical conditions related to the transfusion of blood products, drives the need for innovation of new technologies.

PRT offer a new approach to increase blood safety and they have been adopted gradually but steadily over the past decade. The numerous technologies and mechanisms discussed in this paper reflect the complexity of finding a balance between effective pathogen inactivation in blood products and maintaining acceptable quality and functionality of haemostatic components.

The evidence-based indications for the use of plasma and PRT-treated plasma are few and specific. PRT-treated plasma has, in fact, been in clinical use

Table X - Photochemical treatment of plasma with amotosalen (A-FFP) *vs* methylene blue (MB-FFP).

First author, year	Population	N. of plasma samples	Outcomes/results	Recommendation/ conclusions	Level of evidence
Osselaer JC, 2008	Apheresis derived plasma units	Total 24: 12 A-FFP *vs* 12 MB-FFP	- Effect of pathogen inactivation on global coagulation assays: the mean thrombin time was shorter for A-FFP than for MB-FFP ($p<0.05$). - Effect of pathogen inactivation on coagulation factor activity: factor (F) XI higher in A-FFP than in MB-FFP ($p<0.05$). The mean retention of FV, FVII, FVIII, FX, and VWF:CoR in A-FFP was either equivalent or higher ($p<0.05$). MB-FFP exhibited higher FIX retention than the A-FFP after 24 hours of storage at 4 °C after thawing ($p<0.05$). - Effect of pathogen inactivation on inhibitors of coagulation: the mean retention of protein S was higher in A-FFP than in MB-FFP ($p<0.05$).	There is adequate preservation of therapeutic coagulation factor activities in both A-FFP and MB-FFP. The overall coagulation factor levels and stability of A-FFP were better preserved than those of MB-FFP.	Moderate

VWF:CoR: von Willebrand factor activity.

Table XI - Solvent/detergent-treated plasma (SD-FFP) *vs* cryosupernatant plasma.

First author, year	Population	N. of patients	Outcomes/results	Recommendations/conclusions	Level of evidence
Rock G, 2013	Patients diagnosed with primary idiopathic TTP	89 (41 given SD-FFP *vs* 48 given cryosupernatant plasma)	Survival at 6 months not significantly different between groups. Protein S, ADAMTS-13, the inhibitor and VWF multimers levels were not significantly different between groups.	Data suggest that ADAMTS-13 activity and inhibitor level at baseline cannot differentiate TTP patients' response to plasma exchange therapy. The study was closed prematurely due to removal of one of the interventional products from the market.	Low/ moderate

TTP: thrombotic thrombocytopenic purpura; SD-FFP: solvent/detergent-treated fresh-frozen plasma; VWF: von Willebrand factor.

Table XII - Solvent/detergent-treated plasma: Uniplas LG® *vs* Octaplas LG®.

First author, year	Population	N. of subjects	Outcomes/results	Recommendations/conclusions	Level of evidence
Jilma-Stohlawetz P, 2011	Healthy adult volunteers	30 subjects received single transfusions of 1,200 mL Uniplas LG® and Octaplas LG® or vice versa.	The primary safety outcome was change in haemoglobin (Hb) concentration; secondary safety outcomes were evaluation of the direct antiglobulin test, complement activation, free Hb, haptoglobin, and indirect bilirubin: no differences. Mean values of individual coagulation variables were within the normal range and variations in their levels were generally similar between treatment groups.	The universal plasma is a safe alternative and represents an advance over the currently available blood group-specific plasma transfusion products.	Low

for several years and has proven effective in a variety of therapeutic settings, but each PRT system has some weakness or shortfall in terms of efficacy and each of the major methods under development has a specific set of potential limits. The supportive and often prophylactic nature of blood component therapy in a variety of clinical situations complicates the clinical evaluation of novel blood products. Most clinical efficacy studies designed to compare PRT-treated and untreated plasma involve only small numbers of patients and are not, therefore, adequately powered to detect statistically significant minor differences. In addition, they assess increments in coagulation factor levels and changes in *in-vitro* coagulation blood tests rather than clinically meaningful outcomes. Concerns about low levels of plasmin inhibitor, protein S, or antitrypsin activity in PRT-treated plasma have not been confirmed in clinical studies, and claims of thrombosis or hyperfibrinolytic bleeding triggered by reduced protein S or low plasmin inhibitor potencies have not withstood critical review. Extensive clinical experience has shown that reduced levels of coagulation factors, as a result of PRT treatment, do not significantly impair the clinical efficacy or tolerance of plasma.

The first developed method to inactivate enveloped viruses in plasma protein preparations was SD treatment. SD-FFP has proven to be non-inferior to untreated FFP in the management of a wide range of congenital and acquired bleeding disorders. Furthermore, the major advantages of SD plasma over FFP and the other pathogen-inactivated plasmas are its extreme safety with respect to TRALI and the significantly lower likelihood of allergic reactions. Both advantages are interpreted as results of the dilutional effect of pooling.

While MB-treated plasma was associated with apparently fewer allergic reactions than plasma, a single-centre, retrospective haemovigilance investigation in France did not confirm this finding.

Two other technologies, based on photochemical processes exploiting amotosalen (a psoralen) and riboflavin, both plus light treatment, have been developed more recently. There are few studies available so far on these technologies and the levels of evidence is low or moderate.

Potential long-term side effects of the photosensitisers and their photoproducts are still a matter of debate, and, before these technologies are implemented widely, it must be shown that the photosensitisers and their photoproducts are absolutely safe, robust in daily routine practice, cost-effective and have controllable efficacy.

Clinical experience with PRT-treated plasma has substantiated the product's safety; nevertheless, work in this field is in an early stage and the full potential of these technologies has yet to be determined.

Safety

Introduction

The quest for maximum safety in transfusion has long been directed towards preventing the transmission of infections, particularly HBV, HCV and HIV. The procedures used for this purpose are highly effective, but a zero-risk level has not been reached. Nevertheless, a better understanding of donors' medical histories and the performance of blood tests to screen for infectious diseases have decreased the risk of transmitting infections by transfusion of blood products. Improvements in the sensitivity of tests, such as NAT, have contributed significantly to decreasing this risk. Systematic surveillance of transfusion-related adverse events and adverse reactions encompasses the entire transfusion chain and is aimed at increasing the safety of the transfusion process[196].

With regard to the safety of the blood supply, pathogen reduction might be an additional step. Table XV shows prospective safety levels of plasma inactivation methods for different pathogens[15,55,197,198].

Table XIII - Solvent/detergent-treated plasma: Octaplas LG® *vs* Octaplas®.

First author, year	Population	N. of subjects	Outcomes/results	Recommendations/conclusions	Level of evidence
Jilma-Stohlawetz P, 2013	Healthy adult volunteers	60 subjects received single transfusions of 1,200 mL of Octaplas® (in period 1) and the LG plasma product (in period 2) or vice versa.	Efficacy in terms of recovery of coagulation ractors and haemostatic variables (primary end-point): all 90% confidence intervals were within the equivalency interval (−10% to 10%). The concentration of plasmin inhibitor (secondary endpoint): significantly higher α_2-antiplasmin levels were found at 15 minutes (p=0.001) and 2 hours (p=0.019) after transfusion of the LG product compared to its predecessor	Recovery is indicative of the efficacy of the studied products to restore clotting factors and inhibitors. Values and variation of PT, aPTT, and protein C plasma concentrations were similar between treatments throughout the study period. In addition, the relative changes for PT, aPTT, and protein C were similar between treatments in the per protocol population.	Low/ moderate

PT: prothrombin time; aPTT: activated partial thromboplastin time.

Table XIV - Clinical efficacy of a pathogen-reduced plasma product (PlasmaSafe®) evaluated without comparator.

First author, year	Population	N. of patients	Outcomes/results	Recommendations/conclusions	Level of evidence
De Silvestro G, 2007	Patients with TTP	490 (879 transfusion events)	PT ranged from 50-120%. aPTT ranged from 24-41 seconds. Fibrinogen concentration ranged from 1.42-6.84 g/L. No increase of protein S.	PlasmaSafe® is a pharmaceutical-like product with a standardised content of coagulation factors, effective in correcting coagulation defects and in treating TTP.	Low/ moderate

TTP: thrombotic thrombocytopenic purpura; PT: prothrombin time; aPTT: activated partial thromboplastin time.

The clinical events are classified into three macro-classes: non-infective, infective and mixed (Table XVI)[198].

In literature, most of the studies report TRALI, infections and acute transfusion reactions (ATR).

TRALI is characterised by acute hypoxaemia and non-cardiogenic pulmonary oedema during or within 6 hours of transfusion. In 2002, the incidence of TRALI associated with plasma transfusions was 1:66,600 in Germany, which is comparable to the incidence of 1;60,000 recorded by the French haemovigilance system and with the English finding of 1:65,000[198].

National haemovigilance programmes and surveillance of transfusion reactions in some countries (including the UK, USA, Canada and The Netherlands) have corroborated the association between TRALI and the presence of HLA in women, resulting in a transition to a strategy of predominantly male donors for plasma transfusion. There are many published reports claiming that the risk of TRALI is five to seven times higher for transfusions containing relatively large quantities of plasma (250-300 mL) compared with those containing smaller volumes of plasma (<30 mL)[199].

The infectious risks are associated with the transmission of bacteria, viruses and prions. Bacterial contamination of plasma is generally rare because of the frozen storage, although cases are still described in the literature. Five cases of bacterial contamination of FFP were reported between 2002 and 2003 in Canada and between 1997 and 2007 in Germany[191,200]. In general, pathogen inactivation/reduction is not effective against prions, and currently there are no donor tests for prion screening[200].

As described in haemovigilance reports, ATR have increased in the past few years. The rates of ATR associated with FFP in two retrospective studies were 1:591 and 1:2.184 plasma units transfused[200].

Other common causes of adverse events associated with the transfusion of plasma are errors. For example, transfusion of an incorrect component or of one that does not meet the patient's specific requirements.

According to a cumulative analysis of all SHOT reports, and considering only plasma transfusions, the most common unpredictable transfusion reactions are ATR (allergic, severe febrile or anaphylactic). Adverse events can be classified into:

- transfusion reactions that are not preventable: TTI, transfusion-associated dyspnoea and ATR;
- possibly preventable transfusion reactions (by improved practice and monitoring): TACO, TRALI;
- adverse events caused by error: incorrect blood component transfused[196].

The mechanisms of action and spectra of pathogen inactivation of the various PRT differ greatly from one another. Table XVII reports the features of the pathogen inactivation methods applied to plasma[15,66,201-203].

Table XV - Comparison of prospective safety of different types of pathogen-inactivated plasma.

	SD-FFP	MB-FFP	A-FFP	R-FFP
Hepatitis B virus	+++	+	+	N/A
Hepatitis C virus	+++	+++	+++	N/A
HIV	+++	++	+++	+++
Parvovirus B19	– (NAT)	–	(+)	N/A
Hepatitis A virus	– (NAT)	+	–	N/A
Encapsulated viruses	+++	++	++	N/A
Non-encapsulated viruses	–	+	+	N/A
Bacteria	+++	–	+++	++ (data are limited to certain types of bacteria)
Protozoa	+++	–/+	+++	+++ (data are limited to certain types of protozoa)

SD-FFP: solvent/detergent-treated fresh-frozen plasma; MB-FFP: methylene blue-treated fresh-frozen plasma; A-FFP: amotasalen-treated fresh-frozen plasma; R-FFP: riboflavin-treated fresh-frozen plasma; HIV: human immunodeficiency virus; NAT: nucleic acid amplification testing; N/A: not applicable. Source: 15, modified from 55, 197, 198.

Table XVI - The most frequent adverse events occurring during transfusion of plasma.

Non-infective	Transfusion-related acute lung injury
	Transfusion-associated circulatory overload
	Acute transfusion reactions
Infective	Infections
Mixed	Leucocyte-associated risks
	Physicochemical reactions

Methods

Literature search strategy

A domain-specific search for safety issues was derived from the main literature strategy on MEDLINE. Safety data on technical approaches to pathogen inactivation in FFP for transfusion use were obtained. The review was performed using grey literature and previous HTA reports. Since the objective was an evaluation of the clinical and processing safety, the initial search was refined using the following keywords: adverse effects, adverse event, near miss, blood safety, safety management and haemovigilance.

The sub-strategy utilised to gather information on the safety domain is shown below.

SAFETY *(Search (((((("adverse effects" [Subheading]) OR "adverse effect" OR "adverse event" OR "near miss")) OR ("Drug Toxicity/classification"[Mesh] OR "Drug Toxicity/complications"[Mesh] OR "Drug Toxicity/prevention and control"[Mesh] OR "Drug Toxicity/transmission"[Mesh] OR "Drug Toxicity/virology"[Mesh] OR "drug toxicity")) OR ("complications" [Subheading]) OR complication*)) OR ("Blood Safety"[Mesh] OR "Safety-Based Medical Device Withdrawals"[Mesh] OR "Safety Management"[Mesh] OR "Blood safety" OR "Safety Management" OR "transfusion safety")) OR haemovigilance OR hemovigilance).*

No safety-specific search was performed in the Cochrane and CDR databases. No publication date, language or search field restrictions were applied.

A manual search of the references of selected literature, documents released by monitoring authorities (haemovigilance) and grey literature, were included in the safety research.

The websites of the following agencies were searched for guidelines: National Institute for Health and Care Excellence (NICE), UK; Scottish Intercollegiate Guidelines Network (SIGN), Scotland; Agency for Healthcare Research and Quality (AHRQ), USA; *Agence Nationale d'Accréditation et d'Evaluation en Santé* (ANAES), France; HTA Programme, England; Clinical Practice Guidelines, CMA Infobase, Canada; Canadian Task Force on Preventive Health Care (CTFPHC), Canada; MJA Clinical Guidelines, Australia; National Guidelines Clearinghouse, USA; The National Heart, Lung, and Blood Institute (NHLBI), USA; PRODIGY, National Health Service Department of Health, England; National Guideline Programme (PNLG), Italy; British Committee for Standards in Haematology; Italian Haematology Society, and the Italian Society for Transfusion Medicine and Immunohaematology (SIMTI).

The list of European haemovigilance systems was obtained from the web-site of the International Haemovigilance Network. Grey literature was searched using the "Google Scholar" search engine. A number of elements in the reference lists of selected articles were also evaluated.

Selection criteria

Eligible studies were required to report on one or more of the safety outcomes identified, including frequency of relevant events and reactions.

The definitions of clinical and processing safety outcomes are reported in Table XVIII.

The following exclusion criteria were defined and studies not eligible on the basis of these criteria were not included in the analysis:

- language: papers in languages other than English;
- relevance: studies focusing on efficacy data;
- study design: *in vitro* studies.

The following inclusion criteria were defined to identify potentially relevant studies:

- study design: randomised controlled trial, comparative or non-comparative observational study or case report;
- outcome: studies that assessed at least one outcome defined in Table XVIII[204].

Results

The study selection process is illustrated in Figure 4.

The literature search revealed 497 potentially relevant citations for the safety domain evaluation. Nine studies were excluded because they were not in English and four because they were duplicates. Overall, 484 records were analysed based on the study title and 382 were excluded because not relevant to the research question. The 102 studies included were then screened by reading their abstracts and full texts. A total of 64 further studies were excluded because the alternatives compared, the outcomes or the study design were not consistent with the aim of this analysis. A total of 17 studies of the primary literature search were eventually included in the analysis.

The secondary literature search, which included grey literature (such as haemovigilance reports) and references of the studies included in the primary search, led to the detection 35 further studies.

The level of evidence of the studies was appraised by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) scale.

Table XVII - Features of the pathogen-inactivation methods for plasma.

	Plasma product	Methods	Features
FFP	Single unit pathogen-reduced plasma	Non-photochemical treatment	Widely used Good viral safety if quarantined No standard product No guarantee regarding emerging viruses
SD-FFP	Pooled plasma	Industrial method	Inactivates enveloped viruses Does not inactivate non-enveloped viruses (HAV and parvovirus B19) Does not inactivate prions Lower risk of TRALI Less use of units transfused Dilution of antibodies (anti-HLA) Inactivation of prions
MB-FFP	Single unit pathogen-reduced plasma	Photochemical treatment; "in house"	Partially inactivates enveloped viruses Incomplete removal of the reagent Does not inactivate prions Possible removal of leucocytes
A-FFP	Single unit pathogen-reduced plasma	Photochemical treatment; "in house"	Inactivation of enveloped viruses Inactivation of non-enveloped viruses Inactivation of lymphocytes Incomplete removal of inactivating agent and its products
R-FFP	Single unit pathogen reduced plasma	Photochemical treatment; "in house"	Not inactivation of emerging viruses Effect regarding prion inactivation is not known

FFP: fresh-frozen plasma; SD-FFP: solvent/detergent-treated FFP; MB-FFP: methylene blue-treated FFP; A-FFP: amotosalen-treated FFP; R-FFP: riboflavin-treated FFP; HAV: hepatitis A virus; TRALI: transfusion-related acute lung injury. Source: 15, modified from: 66, 201, 202.

Safety of solvent/detergent-treated plasma* vs *untreated plasma

A systematic review[205] by the Canadian Agency for Drugs and Technology in Health (CADTH) explored the safety of SD-FFP as an alternative to FFP or FP. Safety was assessed for all indications identified through a systematic literature search conducted on electronic databases including Medline, EMBASE and the CDR database.

Safety outcomes included the frequency of relevant events such as TRALI, TACO and seroconversion for HTLV, HBV, HCV, HIV, PVB19 and CMV infections.

Table XIX lists the safety outcomes investigated in eight out of the 12 studies included in the CADTH review. The remaining four studies were not considered because they did not meet our inclusion criteria.

No cases of TRALI or TACO were observed in any of the studies considered. Adverse events associated with SD-FFP were rare and were, specifically, a rash, an urticarial reaction and a moderate anaphylactic reaction. No seroconversion for HIV, HBV or HCV was observed in 201 patients transfused with SD-FFP or in 55 patients transfused with FFP. One patient in the FFP group showed seroconversion for PVB19 but no patient transfused with SD-FFP had this complication. Overall, the adverse events and safety of SD-FFP were comparable to those of FFP, although comparative data for SD-FFP and FFP are limited and the authors of one study concluded that the impact of the two alternatives on the Canadian Health Service could not be determined.

The CADTH updated this systematic review in 2010[206], including another three studies dealing with the safety of SD-FFP as an alternative product to FP or FFP. One of these studies (Flesland, 2007[207]) is also included in our primary literature search and another (Scully, 2007[174]) is not compatible with this report. Table XX shows the results of the only additional study meeting the inclusion criteria.

Safety of methylene blue-treated plasma* vs *untreated plasma

Politis *et al.* (2007)[53] reported their 5-year experience of pathogen inactivation of MB-FFP in a multicentre observational study. Overall, 8,500 units of FFP treated with MB and 54,435 untreated units of FFP were transfused in four Greek hospitals between 2000 and 2005. The only adverse reactions related to MB-FFP transfusion was one allergic reaction, not classified as serious, observed in a patient with TTP. However, 25 adverse reactions, five of which were classified as serious, were recorded in patients treated with FFP. The serious adverse reactions were:

- two cases of anaphylactic shock in patients not previously known to be IgA deficient;
- two bacterial infections (staphylococcal infections);
- one case of endotoxic shock.

Table XXI reports the incidence of adverse reactions to FFP and MB-FFP.

No TRALI or seroconversion was reported in relation to either MB-FFP or FFP. The incidence of

Table XVIII - Descriptions of clinical and processing safety outcomes.

Outcome	Description	Sub-description
	Clinical outcomes	
Transfusion-related acute lung injury (TRALI)	Acute dyspnoea with hypoxia and bilateral pulmonary infiltrates during or within 6 hours of transfusion, not due to circulatory overload or other likely causes.	
Transfusion-associated circulatory overload (TACO)	Based on the presence of any four of the following symptoms occurring within 6 hours of transfusion: - acute respiratory distress - tachycardia - increased blood pressure - acute or worsening pulmonary oedema - evidence of positive fluid balance.	
Acute transfusion reactions (ATR)	A reaction occurring at any time up to 24 hours following a plasma transfusion, excluding cases of acute reactions due to ncorrect blood component being transfused, haemolytic transfusion reaction, TRALI, TACO, TAD or those due to bacterial contamination of the component.	Febrile type reaction Allergic-type reaction (urticaria, pruritus, flushing) Reaction with both allergic and febrile features Hypotensive reaction Febrile non-haemolytic transfusion reaction Anaphylactic reaction
Transfusion-associated dyspnoea (TAD)	This is characterised by respiratory distress within 24 hours of transfusion that does not meet the criteria of TRALI, TACO or ATR. The respiratory distress should not be explained by the patient's underlying condition or any other known cause.	
Transfusion-transmitted infections	An infection is classified as transfusion-transmitted if, following investigation: - the recipient had evidence of infection following transfusion with blood components and there was no evidence of infection prior to transfusion and no evidence of an alternative source of infection; - at least one component received by the infected recipient was donated by a donor who had evidence of the same transmissible infection or: - at least one component received by the infected recipient was shown to contain the agent of infection.	Bacteria Viruses (HBV, HCV, HIV-1/2) Other plasma-borne viruses (cytomegalovirus, parvovirus-B19) Emerging viruses (WNV, HGV, HEV, TTV) Protozoal parasites (malaria, Chagas' disease) Prions (Creutzfeldt-Jakob disease)
Transfusion-associated Graft-versus-Host disease	Generally fatal immunological complication of transfusion involving the engraftment and clonal expansion of viable donor lymphocytes contained in blood components in a susceptible host. It is characterised by fever, rash, liver dysfunction, diarrhoea, pancytopenia and bone marrow hypoplasia occurring within 30 days following transfusion.	
Physicochemical reactions		Citrate toxicity
Unclassifiable complication of transfusion	Occurrence of an adverse effect or reaction temporally related to transfusion, which cannot be classified.	

HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; WNV: West Nile virus; HGV: hepatitis G virus; HEV: hepatitis E virus; TTV: transfusion-transmitted virus. Source: 204.

continued on next page

Table XVIII - Descriptions of clinical and processing safety outcomes. *(continued from previous page)*

Outcome	Description	Sub-description
	Processing outcomes	
Incorrect blood component transfused (IBCT) (clinical and laboratory errors)	This is confirmed when a patient is transfused with plasma that was intended for another patient or which, in terms of its specification, was incorrect.	
Right blood - right patient (RBRP)	Incidents in which a patient was transfused correctly despite one or more serious errors that in other circumstances might have led to an incorrect blood component being transfused.	Administration with incorrect or incomplete/missing patient's details on the label Transposition of labels between units that are all intended for the same patient Absence of a patient's identification wristband Transfusion of a blood component that was intended for the patient, but was not formally prescribed/ authorised
Handling and storage errors	Incident in which the patient was transfused with a blood component intended for that patient, but during the transfusion process, the handling and storage may have rendered the component less safe for transfusion.	
Avoidable, delayed or under-transfusion	Incident in which: - the intended transfusion is carried out, and the blood component itself is suitable for transfusion and compatible with the patient, but the decision leading to the transfusion is flawed, - a transfusion of blood was clinically indicated but was not undertaken or was significantly delayed, - there was avoidable use of emergency O RhD negative blood when group-specific or crossmatched blood was readily available for the patient	

HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; WNV: West Nile virus; HGV: hepatitis G virus; HEV: hepatitis E virus; TTV: transfusion-transmitted virus. Source: 204.

Blood Transfus 2016; 14: 287-386 DOI 10.2450/2016.0065-16

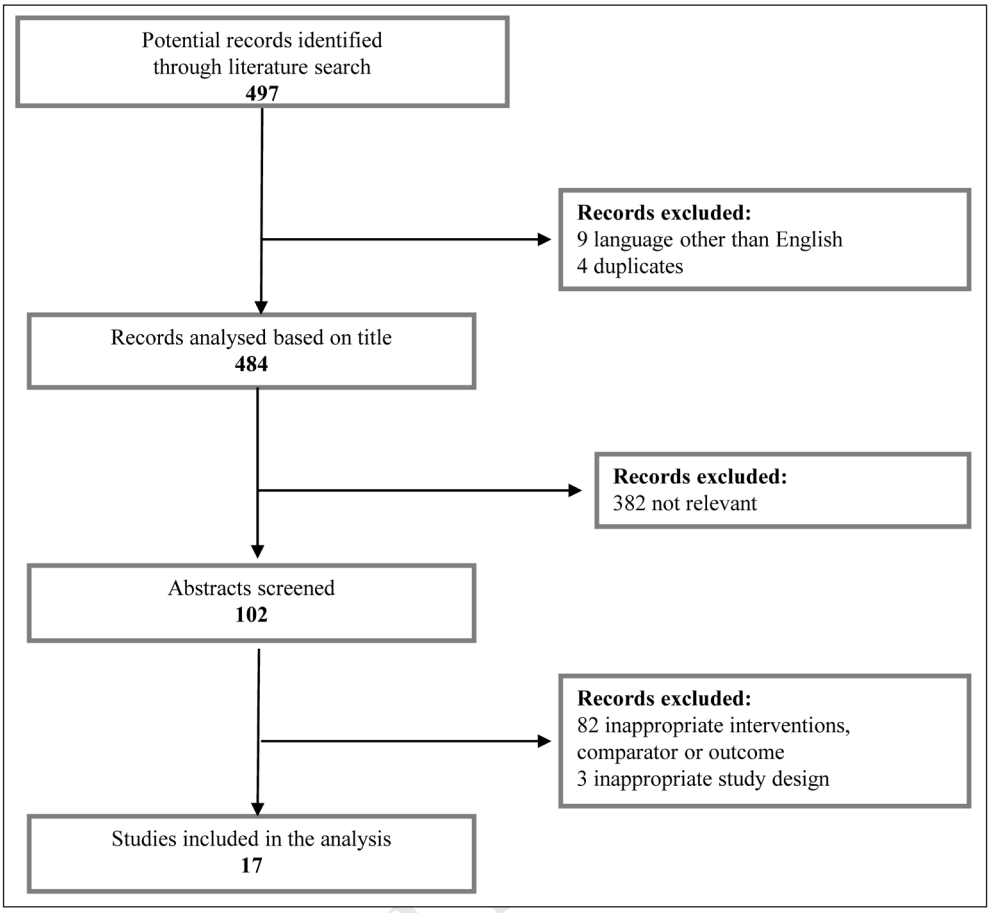

Figure 4 - Study selection process for safety domain.

serious allergic reactions was zero with MB-FPP, and 1:10,877 with FFP. The differences were not, however, statistically significant (p=0.17 and p=0.62 respectively). The authors concluded that MB-FFP is safer than the untreated product, even in patients who require the transfusion of large quantities of plasma (Table XXII).

Safety of methylene blue-treated plasma vs *photochemically treated plasma*

A study by Adelaide Health Technology Assessment (AHTA)[62] compared the safety of MB-FFP (Intercept® Blood System, Cerus Corporation, USA) and R-FFP (Mirasol® Pathogen Reduction Technology, Caridian-BCT Biotechnologies, USA). The medical literature up to May 2011 was investigated through major databases. HTA reports were also analysed.

Three studies assessed plasma transfusion, two were non-comparative studies and one was a comparative analysis between MB-FFP and untreated FFP and was included in our primary literature search (Politis, 2007)[53].

de Alarcon *et al.* (2005)[170], in a case series of 34 patients with congenital coagulopathies recruited from a haemophilia research registry, related the incidence of acute reactions to the use of A-FFP. Thirteen patients received 77 transfusions of pathogen-inactivated plasma during surgery. The reactions were classified as mild if there were no changes in the patient's capability to carrying out activities, moderate if some change occurred, and severe in the case that important impairments to the performance of usual activities developed. One case of nausea was considered to be serious, resulting in a medical emergency. The authors reported that no patient resulted positive to A-FFP antibodies. Table XXIII shows the number of mild and moderated acute reactions associated with transfusions of A-FFP.

Cazenave *et al.* (2010)[208] reported the adverse events that occurred in a series of 3,232 patients who received at least one transfusion of A plasma in two University hospitals/blood centres in Belgium and France. Overall, 7,483 plasma units were transfused to patients with haematological disorders. Table XXIV shows the adverse events that occurred. The total number of adverse events was very low and the authors concluded that their relevance was dubious.

A systematic review[209] compared the safety of MB-FFP (Intercept® Blood System) to A-FFP (Mirasol® Pathogen Reduction Technology). A limited literature search was conducted on major databases, including

Table XIX - Summary of studies on solvent/detergent-treated fresh-frozen plasma (SD-FFP) *vs* untreated fresh-frozen plasma (FFP).

Authors and year	Type of study	Intervention	N. of patients		Results
			SD-FFP (Octaplas®)	*Comparator*	
Williamson LM, 1999	RCT	Liver transplantation/ disease	25	FFP	No seroconversion for HIV, HBV or HCV. One patient in the FFP group showed seroconversion for human parvovirus B19.
Chekrizova V, 2006	Multicentre observational study	Liver transplantation/ disease	32	N/A	No adverse events were observed.
Noddeland H, 2002	RCT	Cardiovascular/surgery	19	36 Uniplas® and 29 not transfused	No seroconversion for HTLA, CMV, parvovirus B19, HIV, HBV or HCV.
Haubelt H, 2002	Prospective cohort study	Cardiovascular/surgery	36	31 FFP	No adverse events were observed.
Solheim BG, 1993	Prospective cohort study	Cardiovascular/surgery	20	20 FFP, 26 no plasma	No seroconversion for HIV, HBV or HCV.
Santagostino E, 2006	Prospective cohort study	Coagulation disorder	17	N/A	One rash reaction was observed. No seroconversion for HTLA, parvovirus B19, HIV, HBV or HCV.
Inabal A, 1993	Prospective cohort study	Coagulation disorder	11	N/A	One urticarial reaction was observed A moderate anaphylactic reaction was observed in one patient. No seroconversion for, CMV, HIV, HBV or HCV.
Chekrizova V, 2006	Cohort study	Paediatric and gynaecological disease	41	N/A	No adverse events were observed.

RCT: randomised controlled trial; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; CMV: cytomegalovirus; HTLA: high-titre, low-avidity antibodies.

Table XX - Studies about SD-FFP *vs* untreated FFP: summary.

First author, year	Type of study	Intervention	Comparator	N. of transfusions	Results
Chekrizova V, 2006	Prospective cohort study	Unclear	FFP	2,621	No transfusion reactions were observed 19 allergic reactions (8 mild, 6 moderate and 5 severe)

SD-FFP: solvent/detergent-treated FFP; FFP: fresh-frozen plasma.

PubMed, the Cochrane Library, University of York Centre for Reviews and Dissemination databases, ECRI, EuroScan, international health technology agencies, and a focused search on the Internet was performed, searching for evidence until September 2009. One randomised controlled trial on the safety of A-FFP compared to that of FFP was also included. No comparative studies between the technologies analysed were included in the report.

Mintz *et al.* (2006)[172] compared A-FFP with conventional FFP in a randomised, controlled, double-blind phase III trial. The study included 35 patients with TTP, 17 of whom were treated with A-FFP and 18 with FFP. There were no significant differences in the patients' baseline characteristics. The overall incidence of adverse events was similar between the two groups (100% A-FFP *vs* 89% control; p=0.486). Most adverse events were mild or moderate in intensity. Three patients

Table XXI - Incidence of adverse reactions following transfusion of fresh-frozen plasma (FFP) or methylene blue-treated fresh-frozen plasma (MB-FFP).

	Incidence/total n. of units		Statistical significance
	FFP	*MB-FFP*	
Viral	0:54,435	0:8,500	p=NR
Bacterial	1:9,073	0:8,500	p=NR
Allergic	1:5,444	1:8,500	p=NR
Serious allergic	1:10,877	0:8,500	p=0.62
Anaphylactic	1:27,218	0:8,500	p=NR
FNHTR	1:9,073	0:8,500	p=NR
Other	1:54,435	0:8,500	p=NR
Total	1:2,177	1:8,500	p=0.17

FNHTR: febrile non-haemolytic transfusion reaction; NR: not reported.

Table XXII - Summary of safety studies on methylene blue-treated fresh-frozen plasma (MB-FFP) *vs* untreated fresh-frozen plasma (FFP).

First author, year	Type of study	Units of plasma transfused	Incidence of adverse reactions	Conclusions/ recommendations	Level of evidence
Politis C, 2007	Multicentre observational study	8,500 units of MB-FFP 54,435 units of FFP	MB-FFP Allergic reactions 1:8,500 Total 1:8,500 FFP Bacterial infection 1:9,073 AR 1:5,444 SAR 1:10,877 Anaphylactic reactions 1:27,218 FNHTR 1:9,073 Other reactions 1:54.435 Total 1:2,177 No TRALI or seroconversion was reported in relation to either MB-FFP or FFP.	MB-FFP is safer than FFP	Low/moderate

AR: allergic reactions; SAR: serious allergic reactions; FNHTR: febrile non-haemolytic transfusion reactions; TRALI: transfusion-related acute lung injury.

(18%) in the A-FFP group and five (28%) in the control FFP group experienced severe adverse events (p=0.691). For two patients in each treatment group, the observed severe adverse events were refractory or relapsed TTP. Adverse events were mainly due to multisystem diseases consequent to TTP. The authors concluded that the safety profile of A-FFP was not different from that of the control FFP. This study was funded by Cerus, the manufacturers of Intercept® (Table XXV).

Haemovigilance

A haemovigilance system requires traceability of blood and blood products from donors to transfused patients and is an integral part of quality management in a blood system. Transfusion is a multistep process involving several different professionals as well as donors and recipients. An effective haemovigilance system is essential for continuous improvement of the quality and safety of blood products and to increase the safety, efficacy and efficiency of blood transfusions. An effective haemovigilance system is bi-directional tracking that collects and reports transfusion-related adverse reactions and events for a rigorous management of the information related to the transfusion process. Not all haemovigilance programmes collect the same type of information. For example in the United Kingdom and in Ireland they only collect information on the most serious risks connected to transfusions, while in France they collect all haemovigilance data, irrespective of the severity of the reactions.

European haemovigilance data

According to European legislation, by the 30th June of each year Member States must submit an annual report to the Commission, notifying the serious adverse reactions and serious adverse events received by their competent authority.

Table XXIII - Acute reactions following transfusion of amotosalen-treated fresh-frozen plasma: classification into mild and moderate reactions.

Acute reactions	Mild (n.)	Moderate (n.)
Skin rash	7	0
Urticaria	16	3
Bronchospasm	3	0
Haemoglobinuria	0	1
Haemolysis	0	0
Chills	3	4
Nausea	1	2

Table XXIV - Acute transfusion reactions following the administration of 7,483 units of amotosalen-treated fresh-frozen plasma: classification into adverse events and severe adverse events.

Acute transfusion reactions	Adverse events (n.)	Severe adverse events (n.)
Skin rash	2	1
Urticaria	3	1
Bronchospasm	1	1
Chills	3	1
Nausea	2	1
Tachycardia	3	3
Hypotension	3	3
Itching	2	2
Facial oedema	2	1
Laryngeal oedema	1	1
Dyspnoea	1	1
Fever	1	0

Table XXV - Summary of studies on methylene blue-treated fresh-frozen plasma (MB-FFP) *vs* amotosalen-treated fresh-frozen plasma (A-FFP).

First author, year	Type of study	Population	Outcomes/results	Conclusions/recommendations
de Alarcon P, 2005	Case series	30 patients with congenital coagulopathies; 77 transfusions with A-FFP	Adverse events *Mild:* Skin rash 7 Urticaria 16 Bronchospasm 3 Chills 3 Nausea 1 *Moderate:* Urticaria 3 Haemoglobinuria 1 Chills 4 Nausea 2	N/A
Cazenave JP, 2010	Case series	3,232 transfusions with A-FFP	Adverse events Skin rash 2 Urticaria 3 Bronchospasm 1 Chills 3 Nausea 2 Tachycardia 3 Hypotension 3 Itching 2 Facial oedema 2 Laryngeal oedema 1 Dyspnoea 1 Fever 1 Serious adverse events Skin rash 1 Urticaria 1 Bronchospasm 1 Chills 1 Nausea 1 Tachycardia 3 Hypotension 3 Itching 2 Facial oedema 1 Laryngeal oedema 1 Dyspnoea 1	The total number of adverse events was very low and the authors concluded that the relevance of the treatment of A-FFP regarding these events is dubious.
Mintz PD, 2006	RCT	17 TTP patients treated with A-FFP 18 TTP patients treated with FFP	The incidence of adverse events was similar between groups (100% A-FFP *vs* 89% control; p=0.486).	The authors concluded that the safety profile of A-FFP was not different from that of control FFP.

N/A: not applicable; RCT: randomised controlled trial; TTP: thrombotic thrombocytopenic purpura.

The haemovigilance data received from Member States in 2012 (covering the period going from 1st January to 31st December) have been summarised by the Health and Consumers Directorate-General of the European Commission. All Member States, as well as Liechtenstein and Norway, submitted the template, but although all countries reported serious adverse reactions and serious adverse events, not all denominators were reported, raising questions on the availability/accuracy of these data.

Overall, data from all countries supplying information from at least 80% of their facilities (hospital blood banks or blood establishments) were included in the analyses.

In total, 13,351,948* units of blood components were reported to have been transfused by facilities in the European Union and European Economic Area countries and 15.2% of these (2,029,496) were plasma units.

In total, 1,813 serious adverse reactions likely or certainly attributable to the blood or blood component transfused were reported by the 28 Member States, Liechtenstein and Norway (13.6 serious adverse reactions per 100,000 units transfused or, conversely, 7,365 units transfused per serious adverse reaction), 16% of which (290 serious adverse reactions) were related to plasma. The report did not, however, specify whether the units of plasma were pathogen-inactivated and, if so, which method had been used for the inactivation. Furthermore, the serious adverse reactions were not split by type[210].

*The total number of units transfused refers to 20 countries (AT, BE, BG, CZ, DK, EE, EL, ES, HR, IE, IT, LI, LT, MT, NL, NO, PT, RO, SE, and UK).

German haemovigilance clinical data

German haemovigilance report

The Paul-Ehrlich-Institute[211], a German research institution and medical regulatory body, summarised data reported since 2010 and compared the data of the preceding 13 years (1997-2009) with the current haemovigilance data.

Table XXVI shows only the serious transfusion reactions to plasma transfusions.

Table XXVII reports the frequency of the serious transfusion reactions to plasma transfusion per 10^6 transfused units.

Haemovigilance data on serious adverse reactions to FFP in Germany during 1997-2010 show that these reactions decreased. In 2010 only TACO and ATR occurred. The trend in the incidence of ATR increased and in 2010 29 events were confirmed, 20 of which were classified as serious. In 2009 and 2010, no bacterial and viral infections were reported. As a consequence of measures aimed at reducing immunogenic TRALI, the number of TRALI events decreased in 2009 and 2010 compared to the number recorded in 1997-2008.

Primary literature studies

In an observational cohort study in 2010, Funk *et al.*[199] reported the frequency of TRALI related to plasma transfusions in Germany, during a period of 5 years (2006-2010). Data before and after the introduction of risk-minimisation measures (2008-2009) were compared. The risk minimisation strategies, limited to FFP, consisted of:

- use of female donors without a history of pregnancy for the production of FFP;
- use of female donors with a history of pregnancy but without detectable white blood cell antibodies for FFP production;
- testing of HLA class I-, HLA class II-, HNA 1a-, HNA 1b-, HNA 2a- and HNA 3a-antibodies.

During the period 2006-2010, 419 suspected cases of TRALI were reported and a total of 80 cases associated with plasma transfusions were confirmed (according to the International Haemovigilance Network criteria): 75% of these were immune-mediated and 25% were non-immune-mediated.

With regards to the rate of immune-mediated TRALI after administration of FFP, this decreased during the transitional period. The rate of TRALI per million units transfused was 12.7 in 2006/2007, 6.81 in 2008/2009 and no cases were reported in 2010.

Table XXVIII shows the absolute number of TRALI-induced deaths and cases of non-immune-mediated TRALI during the pre-implementation period (2006-

Table XXVI - FFP-associated serious transfusion reactions in Germany between 1997 and 2010.

	1997-2009	2010
Acute transfusion reactions	59	(grade I, 29; grade II, 8; grade III, 18; grade IV, 2)
Number of TRALI reports	586	60
Number of TRALI cases with antibody-positive FFP donors	89	0
TRALI-related fatalities due to FFP	16	0
Transfusion-transmitted bacterial infections	5	0
Transfusion-transmitted viral infections (HBV, HCV, HIV)	12	0
TACO	ND	2
FNHTR	ND	0

FFP: fresh-frozen plasma; TRALI: transfusion-related acute lung injury; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; TACO: transfusion-associated circulatory overload; FNHTR: febrile non-haemolytic transfusion reaction.

Table XXVII - Frequency of serious transfusion reactions per 10^6 transfused units of plasma.

	Frequency of transfused units (number of events)				
	1997-2000	*2001-2004*	*2005-2008*	*2009*	*2010*
Units of FFP transfused*	6.346×10^6	4.781×10^6	4.474×10^6	1.096×10^6	1.152×10^6
Serious acute (allergic) TR (grade III and IV)	0.47 (3)	2.51 (12)	2.91 (13)	13.69 (15)	17.36 (20)
Immune-mediated TRALI	1.42 (9)	5.23 (25)	11.18 (50)	4.56 (5)	0
TTI (bacterial)**	0.63 (4)	0	0.22 (1)	0	0
TTI (viral)	1.42 (9 HCV transmissions)	0.63 (3 HBV transmissions)	0	0	0

FFP: fresh-frozen plasma; TR: transfusion reaction; TRALI: transfusion-related acute lung injury; TTI: transfusion-transmitted infection; HCV: hepatitis C virus; HBV: hepatitis B virus. *Estimated consumption; **Results of microbiological analyses in confirmed cases of transfusion-transmitted bacterial infections: two agents with low (human)-pathogenicity and three agents with medium/high pathogenicity.

2007), the transitional period (2008-2009) and the post-implementation period (2010).

The comparison of the data from before and after implementation of the risk minimisation strategies revealed a significantly reduced risk of TRALI between 2006-2007 and 2008-2009 (OR 0,54; 95% CI: 0.29-0.98; p=0,0404) as well as between 2006-2007 and 2010 (p=0.0002, OR not determined because no cases were recorded in 2010). Accordingly, the reported rate of TRALI-induced fatalities decreased from 2.97 cases per million FFP units transfused in 2006-2007 to 2.13 cases per million FFP units transfused in 2008-2009, and no further cases in 2010. The authors concluded that a significant reduction of immune-mediated TRALI has been achieved through the implementation of risk minimisation measures.

United Kingdom haemovigilance clinical data

The UK haemovigilance report

SHOT is the United Kingdom's independent, professionally led haemovigilance scheme that collects and analyses anonymised information on adverse events and reactions to blood transfusion from all the healthcare organisations involved in transfusion of blood and blood components in the country.

This analysis examines the adverse events and adverse reactions in 2010, 2011, 2012 and 2013 published in the annual SHOT reports[204,212-214].

The SHOT reports analyse data sent on a voluntary basis by several structures of the National Health Service. Table XXIX shows the total issues of pathogen-inactivated plasma transfused in years 2010, 2011, 2012 and 2013.

Tables XXX, XXXI, XXXII and XXXIII show the number of cases of ATR, TACO, transfusion-associated dyspnoea and TTI due to plasma transfusions in the UK in the years 2010, 2011, 2012 and 2013, respectively.

Acute transfusion reactions

In 2010, an anaphylactic reaction associated with MB-FFP transfusion was recorded in a young female who developed a rash and angioedema.

In 2011, SD-FFP was implicated in three reactions: a severe reaction in an infant, a mild allergic reaction and an undefined reaction of low imputability. MB-FFP was implicated in a mild febrile reaction of low imputability in a neonate.

In 2012, there was a moderate allergic reaction associated with SD-FFP in a young woman undergoing plasma exchange for haemolytic uraemic syndrome.

In 2013 there were two reactions associated with MB-FFP: a severe allergic (but not anaphylactic) reaction and a moderate allergic reaction. The two reactions associated with SD-FFP both occurred in adult patients undergoing plasma exchange: one patient developed severe hypotension and was later treated with standard plasma with no further problems. A second patient experienced anaphylaxis.

The authors of the report underline that the SD-FFP-related transfusion reactions were less frequent and usually less severe than those related to FFP. This might be due to the dilution of donor allergens in a large pool of plasma, or to the denaturation of allergens through the SD process. The data reported in the SHOT 2010-2012 reports showed that the incidence of allergic reactions to plasma was 2:100,000 with SD-FFP compared to 11.5:100,000 with standard plasma (p<0.001). Although "standard" SD-FFP is still available, all new stocks ordered by hospitals will be treated to eliminate prions.

Transfusion-related acute lung injury

There were no reported cases of TRALI in 2010, 2011, 2012 or 2013. Overall, in the United Kingdom rates of TRALI remained lower than in 2003-2004, when TRALI risk reduction strategies were first initiated. These included a reduction in plasma components from female donors.

Transfusion-associated circulatory overload

In general, the risk of TACO is higher for volumes of FFP greater than >1.000 mL. No cases of TACO were associated with SD-FFP or MB-FFP in the United Kingdom during the period 2010-2013.

Table XXVIII - Absolute number of TRALI-induced fatalities and non-immune-mediated TRALI episodes related to FFP during 2006-2010 in Germany prior to risk minimisation strategies (2006-2007), during the transitional period (2008-2009) and in 2010.

	2006-2007	2008-2009	2010	2006-2010
FFP	30	16	0	46

FFP: fresh-frozen plasma; TRALI: transfusion-related acute lung injury.

Table XXIX - Total units of pathogen-inactivated plasma issued in the UK in years 2010, 2011, 2012 and 2013.

	FFP	SD-FFP	MB-FFP
2010	292,884	57,487*	ND
2011	288,242	63,214	14,960**
2012	282,721	77,669	15,894
2013	266,332	79,473	13,547

*Octaplas® manufactured by Octapharma Ltd.; **Paediatric/neonatal MB-FFP transfusions are expressed as single units; all other components are adult equivalent doses. FFP: fresh-frozen plasma; SD-FFP: solvent/detergent-treated FFP; MB-FFP: methylene blue-treated FFP.

Table XXX - Total acute transfusion reactions in the UK in the years 2010, 2011, 2012 and 2013.

	2010			2011			2012			2013		
	FFP	*SD-FFP*	*MB-FFP*	*FFP*	*SD- FFP*	*MB-FFP*	*FFP*	*SD- FFP*	*MB-FFP*	*FFP*	*SD- FFP*	*MB-FFP*
Unclassified	0	0	0	4	0	0	0	0	0	0	0	0
Hypotensive	3	0	0	2	0	0	2*	0	0	3	1	0
Mixed allergic/febrile	2	0	0	1	0	0	3	0	0	1	0	0
Anaphylactic	8	0	1	10	1	0	9**	0**	0**	11**	1**	1**
Allergic	26	1	0	25	1	0	13	1	0	11	0	1
Febrile	4	0	0	0	1	1	4	0	0	1	0	0
Total	43	1	1	42	3	3	31	1	0	27	2	2

*One of these is referred to as MB cryoprecipitate **Anaphylactic/severe allergic. FFP: fresh-frozen plasma; SD-FFP: solvent/detergent-treated FFP; MB-FFP: methylene blue-treated FFP.

Table XXXI - Total episodes of TACO in the UK in the years 2010, 2011, 2012 and 2013.

	2010	2011	2012	2013
	*FFP**	*FFP*	*FFP*	*FFP*
TACO	3	3	7	2

*Untreated fresh-frozen plasma (FFP); TACO: transfusion-associated circulatory overload.

Table XXXII - Total episodes of transfusion-associated dyspnoea (TAD) in the UK in the years 2010, 2011, 2012 and 2013.

	2010		2011	2012	2013
	FFP	*SD-FFP*	*FFP*	*FFP*	*FFP*
TAD	3	1*	2	1	2

*TAD associated with anaphylactic reactions; FFP: fresh-frozen plasma; SD-FFP: solvent/detergent-treated FFP.

Table XXXIII - Total transfusion-transmitted infections (TTI) associated with plasma in the UK in 2010, 2011, 2012 and 2013.

	2010	2011	2012	2013
	FFP	*FFP*	*FFP*	*FFP*
TTI	0	2	1	0

FFP: fresh-frozen plasma.

Transfusion-associated dyspnoea

Between 2011 and 2013 there were no cases of transfusion-associated dyspnoea associated with SD-FFP or MB-FFP.

Transfusion-transmitted infections

The imputabilities associated with HBV transmission were level 3 (certain).

In 4 years, only one TTI has been recorded in the United Kingdom. This is due to the introduction of a number of precautionary measures:

- leucodepletion of all blood components (1999);
- use of MB-FFP obtained outside the United Kingdom for children under 16 years of age (2002);
- importation of plasma for fractionation (1998);
- imported SD-FFP for adult patients with TTP (2006);
- exclusion of donors who have received a blood transfusion in the United Kingdom since 1980 (2004).

Transfusion-associated graft-versus-host disease

There were no confirmed cases of transfusion-associated GvHD in 2010, 2011, 2012 or 2013 in the United Kingdom. Only two cases have been reported since 1994, when universal pre-storage leucodepletion was introduced and no cases have been reported since 2001.

Overall, FFP is associated with more adverse events and adverse reactions than SD-FFP or MB-FFP. The greater number of alerts in the United Kingdom between 2010 and 2013 is related to ATR, even though these decreased in 2013 compared to 2010. The most frequent ATR are anaphylactic and allergic reactions and are uniquely associated with SD-FFP and MB-FFP.

Irish haemovigilance clinical data

The Irish haemovigilance report

The Irish National Haemovigilance Office defines a haemovigilance programme and identifies unexpected or undesirable effects of transfusion of blood components by ensuring that they are reported in a timely and reliable manner. This analysis relates to the adverse events and adverse reactions published in the bi-annual national report[215,216].

Table XXXIV shows the total issues of pathogen-inactivated plasma transfused in 2008, 2009, 2010 and 2011.

Table XXXV lists the adverse reactions and adverse events registered in Ireland in 2008, 2009, 2010 and 2011.

Since 2002, over 99% of all plasma transfused in Ireland has been subjected to a pathogen reduction process in the form of SD-FFP (Octaplas®), and the use of FFP has been restricted to patients undergoing liver transplantation. In Ireland, the use of SD-FFP is safe and the rare adverse reactions are ATR and TACO.

Swiss haemovigilance clinical data

The Swiss haemovigilance report

Swissmedical (the Swiss Agency for Therapeutic Products) defines a haemovigilance programme and records unexpected or undesirable effects of transfusion of blood components. The haemovigilance report is published every year and describe the adverse reactions and other events that took place[217-220].

Table XXXVI shows the total number of plasma transfusions in 2011, 2012 and 2013.

It is not specified whether the adverse reactions were associated with Q-FFP or SD-FFP. In 2011, the total number of FFP high imputability transfusion reactions was 26 (4% of total) of which 25 were allergic reactions and one a hypotensive event.

The total distribution of the high-imputability transfusion reactions to plasma increased in 2012 compared to 2011. In fact, out of 31 reactions, only one was associated with SD-FFP. The total risk of allergic transfusion reactions to plasma in 2012 was ~1:1,600. In 2013, there were 23 (3% of total) high-imputability reactions to plasma tranffusion and the total risk of allergic transfusion reactions to plasma had increased to ~1:2,000.

No cases of transfusion-transmitted bacterial infection from FFP transfusions were reported in 2011, 2012 or 2013. The risk of TRALI and transfusion-associated GvHD has been significantly lower in recent years (1:100,000 plasma components issued). This is due to the implementation of several product-specific measures, such as stricter donor selection, leucodepletion, irradiation, and restricting plasma donors to males and women who confirm that they have never been pregnant (to their knowledge).

French haemovigilance clinical data

The French haemovigilance report

The annual French haemovigilance report is published by the *Agence Nationale de Sécurité du Medicament et des Produits de Santé,* previously known as the *Agence Française de Sécurité Sanitaire des Produits de Santé*. The notification of adverse events of all degrees is mandatory, while near miss errors are reported on a voluntary basis.

Table XXXVII shows the amount of plasma transfused in 2008, 2009 and 2010[47,221,222].

Tables XXXVIII and XXXIX show the numbers of adverse events that occurred in 2008, 2009 and 2010, based on the strength of the recipient adverse reactions imputability and grade.

In the French Haemovigilance system (and in general), SD-FFP is considered to be associated with a lower risk of inducing TRALI compared to other types of plasma. Allergic reactions, which increased between 2008 and 2009, account for the greatest number of adverse events. In 2011, the French haemovigilance group phased out the production of MB-FFP because of the more frequent allergic reactions associated with this product compared to other types of pathogen-inactivated plasma (SD plasma or A plasma). MB plasma was withdrawn from the French market from 1st March, 2012.

Many retrospective studies conducted in Greece, UK, Spain and Austria report rare allergic events that can be explained by the continuous use of MB plasma[223].

In a retrospective study conducted in a single centre in Austria, Nussbaumer *et al.* (2012) found a significantly lower incidence of adverse events due to MB plasma than to Q-FFP.

Muñiz-Diaz E *et al*. (2014), analysing Catalan haemovigilance data, observed that the total number of severe allergic/anaphylactic reactions following transfusions of MB-FFP is very low and that the rate of severe reactions to plasma was always below 1 per 4,700[224].

The issue of allergic reactions to MB-FFP has been considered in detail by the Joint UK Blood Transfusion Services and National Institute of Biological Standards and Control Professional Advisory Committee. At present, there are no changes to the UK policy. SHOT data do not show significant differences in allergic reactions to MB-FFP or standard FFP.

The SHOT report published in 2011 describes only one reaction, a mild febrile one, connected with MB-FFP. Between 2007 and 2011, there were a total of eight reports of ATR to MB-FFP (1 including multiple components), of which five were severe reactions (3 anaphylactic and 2 hypotensive)[213].

Table XXXIV - Total units of FFP and SD-FFP distributed/issued in Ireland in the years 2008, 2009, 2010 and 2011.

	FFP	SD-FFP
2008	474	23,856
2009	475	23,401
2010	317	23,078
2011	264	22,890

FFP: fresh-frozen plasma; SD-FFP: solvent/detergent-treated FFP.

Table XXXV - Total plasma-related acute reactions and adverse events registered in Ireland in 2008, 2009, 2010 and 2011.

	2008		2009		2010		2011	
	FFP	*SD-FFP*	*FFP*	*SD-FFP*	*FFP*	*SD-FFP*	*FFP*	*SD-FFP*
FNHTR	0	0	0	0	0	0	0	0
Acute allergic and anaphylactic TR	0	1	0	1	1	0	0	0
Hypotensive TR	0	0	0	0	0	0	0	0
TACO	0	1	0	0	0	1	0	1
TAD	0	0	0	0	0	0	0	0
TRALI	0	0	0	0	0	0	0	0
TTI (bacterial)	0	0	0	0	0	0	0	0
TTI (viral)	0	0	0	0	0	0	0	0

FFP: fresh-frozen plasma; SD-FFP: solvent/detergent-treated FFP; FNHTR: febrile non-haemolytic transfusion reaction; TR: transfusion reaction; TACO: transfusion-associated circulatory overload; TAD: transfusion-associated dyspnoea; TRALI: transfusion-associated acute lung injury; TTI: transfusion-transmitted infection.

Primary literature studies

Six articles were selected from the primary literature search; two of them commented and analysed the adverse reactions to MB-FFP AR France.

In a letter to the editor published on the Journal of Allergy and Clinical Immunology in 2013, Mertes *et al.*[48] analysed the mechanisms underlying allergic reactions after transfusion of MB-FFP. Table XL shows the most frequent severe allergic reactions observed between 2005 and 2009, comparing MB-FFP to other types of treated plasma (Q-FFP and SD-FFP).

The authors investigated 34 reactions to MB-FFP and it was observed that: only one death could be related to an allergic reaction; in 19 of the 25 cases (75%), increased serum tryptase concentrations were recorded; in 12 of 18 cases (66%), histamine concentrations were increased; in three 3 of 17 cases MB skin tests were positive; in two cases sensitisation to MB, detected by positive flow cytometry basophil activation, was confirmed; in three cases an allergen that was not related to the transfusion was incriminated; in nine cases (26%) the responsibility of MB was ruled out by recent previous or subsequent uneventful exposure; in four cases a history of hypersensitivity reaction to a labile blood product was reported; finally, no cases of IgA deficiency were recorded.

The authors concluded that although rare, allergic reactions to MB have been reported in various clinical settings. The results confirm that in some cases sensitisation to MB could be responsible for the allergic reaction observed and that this increased risk of allergic reactions could be elucidated.

Seltsam *et al.*[49] replied to the comment by Mertes *et al.*, claiming that between 2005 and 2009, three times more units of Q-FFP and SD-FFP than MB-FFP had been used. In addition, MB-FFP has been introduced in France in 2008, and relevant numbers of MB-FFP units were used in 2009 for the first time. These imbalances in the use of the different types of FFP limit the validity of comparative analyses concerning the frequency of rare adverse events. The authors added the 2010 haemovigilance data to their evaluation as well as data concerning a third type of pathogen-inactivated FFP, A-FFP. Tables XLI and XLII show the frequency of severe allergic reactions between 2000 and 2005 and between 2008 and 2010 associated with the four methods of pathogen inactivation, on an imputability level from 2 to 4.

The authors showed that when the 2010 haemovigilance data were included in the analysis, there were no statistically significant differences in the incidences of severe allergic reactions associated with the different types of plasma. Overall, including the 2010 haemovigilance data, it was not possible to

Table XXXVI - Total number of plasma transfusions in Switzerland in 2011, 2012 and 2013.

	FFP (therapeutic units)*
2011	50,063
2012	49,832
2013	44,083

*FFP, Q-FFP or SD-FFP. FFP: fresh-frozen plasma; Q-FFP: quarantined FFP; SD-FFP: solvent/detergent-treated FFP.

Table XXXVII - Total number of plasma transfusions in France in the years 2008, 2009 and 2010.

Year	FFP	Virus-inactivated plasma
2008	117,140	211,422*
2009	ND	371,658 (including 142,533 SD-FFP, 22,933 A-FFP, 204,814 MB-FFP)
2010	ND	83,163 SD-FFP, 52,692 A-FFP, 246,460 MB-FFP

*Including SD-FFP, Q-FFP, A-FFP, MB-FFP. ND: no data; FFP: fresh-frozen plasma; SD-FFP: solvent/detergent-treated FFP; MB-FFP: methylene blue-treated FFP; Q-FFP: quarantined FFP; A-FFP: amotosalen-treated FFP.

Table XXXVIII - Number of recipient adverse reactions (all grades) in the years 2008, 2009 and 2010; imputability level 2-4.

	2008				*2009*				*2010*			
	FFP	*SD-FFP*	*MB-FFP*	*A-FFP*	*FFP*	*SD-FFP*	*MB-FFP*	*A-FFP*	*FFP*	*SD-FFP*	*MB-FFP*	*A-FFP*
FNHTR	3	2	0	1	0	3	8	4	1	2	0	1
Allergy	57	32	30	2	0	48	114	7	11	26	124	19
Unknown	11	3	2	0	0	1	5	1	0	3	0	1
TRALI	4	1	0	0	0	1	1	0	0	0	6	1
TACO	1	3	0	0	0	2	3	0	1	1	3	0

FFP: fresh-frozen plasma; SD-FFP: solvent/detergent-treated FFP; MB-FFP: methylene blue-treated FFP; A-FFP: amotosalen-treated FFP; FNHTR: febrile non-haemolytic transfusion reaction; TRALI: transfusion-associated acute lung injury; TACO: transfusion-associated circulatory overload.

Table XXXIX - Number of recipient adverse reactions, grade 3-4 in the years 2008, 2009 and 2010.

	*2008***				*2009***				*2010****			
	*FFPs**	*SD-FFP*	*MB-FFP*	*A-FFP*	*FFPs**	*SD-FFP*	*MB-FFP*	*A-FFP*	*FFPs**	*SD-FFP*	*MB-FFP*	*A-FFP*
Allergy	1	5	4	0	0	4	8	0	0	4	10	2
TRALI	2	0	0	0	0	0	1	0	0	0	0	0
TACO	0	0	0	0	0	0	2	0	0	1	0	0
Unknown	0	0	0	0	0	0	1	0	0	0	0	0

*Secured FFP; **Imputability 3-4; ***Imputability 2-3. FFP: fresh-frozen plasma; SD-FFP: solvent/detergent-treated FFP; MB-FFP: methylene blue-treated FFP; A-FFP: amotosalen-treated FFP; TRALI: transfusion-associated acute lung injury; TACO: transfusion-associated circulatory overload.

confirm a significantly higher incidence of serious adverse reactions for MB-FPP than for the other types of FFP. The authors concluded that the currently available haemovigilance data do not support the hypothesis of additional allergenic mechanisms specifically related to the MB/light pathogen inactivation process. The analysis of the 2011 haemovigilance data revealed that the incidence of severe reactions of imputability levels 3 and 4 was 1:15,926 for MB-FFP units and of 1:31,374 for SD-FFP units and that the total number of severe allergic reactions associated with MB plasma in the years 2008-2011 had been 40, considering an imputability level of 2-4, or 45, considering an imputability level of 3 or 4.

Bost *et al.* (2013)[225] analysed retrospective data on the four types of FFP: MB-FFP, Q-FFP, SD-FFP and A-FFP. These data were drawn from the national adverse event reporting database and the regional database system for blood deliveries in the Auvergne-Loire Region (France). They compared the frequency of adverse events due to any of the four types of FFP over a 10-year period.

Between January 2000 and October 2011, 105,964 FFP units were delivered (40,631 units of Q-FFP, 19,015 units of SD-FFP, 10,283 units of MB-FFP and 36,035 units of A-FFP). The authors classified the adverse events as possible, probable or certain. Table XXVII shows the adverse events associated with SD-FFP, MB-FFP and A-FFP, but not Q-FFP, because their analysis was beyond the aim of the study[225].

Statistical comparisons of adverse events did not reveal significant difference in adverse event rates between SD-FFP, MB-FFP and A-FFP. Finally, the authors concluded that FFP is safe and plasma inactivation with SD, MB or amotosalen can lead to fewer adverse events than those associated with Q-FFP. FFP was confirmed to be extremely safe in general, especially considering "severe" adverse events. All types of FFP were associated with extremely low incidences of adverse events. Moreover, the newly introduced A-FFP appears to be safe.

In 2010, Nubret *et al.*[226] described two anaphylactic shock reactions occurring during infusion of MB-TTP in patients undergoing cardiac surgery. Clinical symptoms

Table XL - The frequency of severe allergic reactions with imputability levels from 2 to 4 and 3 and 4 in France (2005-2009).

Imputability 2-4						**Imputability 3 and 4**					
MB-FFP		***Q-FFP***		***SD-FFP***		***MB-FFP***		***Q-FFP***		***SD-FFP***	
N. of cases	*Incidence*	*N. of cases*	*Incidence*	*N. of cases*	*Incidence*	*N. of cases*	*Incidence*	*N. of cases*	*Incidence*	*N. of cases*	*Incidence*
34	1/7,751	33	1/19,269	26	1/25,351	14	1/18,824*	15	1/42,279*	16	1/41,195*

*Significant differences between the different types of FFP ($p<0.001$). FFP: fresh-frozen plasma; MB-FFP: methylene blue-treated FFP; Q-FFP: quarantined FFP; SD-FFP: solvent/detergent-treated FFP.

exhibited by these two patients were consistent with grade III anaphylaxis to MB-FFP and the hypothesis was confirmed by histamine and tryptase levels in patient 1 and by *in vivo* or *in vitro* tests with the patent and MB in patient 2[226].

Other haemovigilance clinical data

Primary literature studies

In an observational study, Norda *et al.* (2006)[227], reported the potential adverse effects of transfusing FFP, drawing on data in the Danish, French, and Quebec haemovigilance systems. The potential adverse effects were categorised according to the "Optimal use of blood: summaries of component characteristics" and the frequency of each adverse effect was been recalculated per 10,000 transfusions (Table XLIII).

The authors also reported other adverse effects not included in the categories described above (Table XLIV).

The authors concluded that the adverse effects of particular relevance for patients are probably TRALI and sepsis, due to inadvertent bacterial contamination.

Politis *et al.* (2014)[223] analysed haemovigilance data in order to compare MB-FFP and Q-FFP, which have been in use for more than 11 years in Greece, focusing on safety and adverse events. The total clinical use of FFP in the study period 2001-2011 was 290,951 units. Of these, 73,778 (25.4%) were MB-FFP units. No deaths related to plasma transfusions were reported, nor were there any cases of transmission of HBV, HIV, HCV or WNV. Table XLV shows the adverse events associated with MB-FFP.

The rate per 10,000 units of MB-FFP was estimated to be 0.41 with a 95% CI of 0.10-1.06, and there were no serious adverse reactions (grade 2 or 3) to MB-FFP. The allergic reactions in three patients (one each with TTP, trauma and septicaemia) were classified as mild.

Overall, the incidence of adverse events was lower with MB-FFP (1:24,593) than with Q-FFP (1:3,620). Indeed, other adverse events, such as 18 febrile non-haemolytic transfusion reactions (incidence 1:12,065), 6 leucoreduced post-storage at the bedside (1:7,601) and three leucoreduced pre-storage (1:28,956) were reported for Q-FFP.

The authors concluded that the adverse event rate was lower with MB-treated plasma and that all adverse reactions experienced by the MB plasma recipients were mild.

In France, Dewachter *et al.* (2011)[228] reported the first immediate IgE-mediated allergic reaction in a 22-year old man admitted to an intensive care unit for severe haematemesis after the transfusion of MB-treated plasma. The MB-induced IgE-mediated anaphylaxis was confirmed clinically and with subsequent assessments of the allergic reaction, including skin tests and basophil activation tests. The authors concluded that allergic IgE-mediated reactions to MB are rare, whereas allergic reactions to patent blue V and isosulfan blue occur frequently. The frequency of the MB-associated allergic reactions is very low considering the widespread use of this blood product. Extensive investigation of all immediate reactions to MB-FFP transfusion is recommended.

Table XLI - The frequency of severe allergic reactions with imputability from 2 to 4 and 3 and 4 in France (2005-2010).

MB-FFP		Q-FFP		SD-FFP		A-FFP	
N. of cases	***Incidence***	***N. of cases***	***Incidence***	***N. of cases***	***Incidence***	***N. of cases***	***Incidence***
Frequency of severe allergic reactions with imputability level from 2 to 4 (2005-2010)							
N/A	N/A	33	1/19,274	N/A	N/A	3	1/3,137
Frequency of severe allergic reactions with imputability level 3 and 4 (2005-2010)							
24	1/21,250*	15	1/42,402*	20	1/37,114*	2	1/45,205

*There were no significant differences among Q-FFP, SD-FFP, and MB-FFP (Fisher's exact test, p=0.066; Pearson χ^2 test, p=0.057). A-FFP was not included in the analysis because of the relatively low number of units for this period. FFP: fresh-frozen plasma; MB-FFP: methylene blue-treated plasma; Q-FFP: quarantined fresh-frozen plasma; SD-FFP: solvent/detergent-treated FFP; A-FFP: amotosalen-treated FFP.

Table XLII - Frequency of severe allergic reactions with imputability from 2 to 4 and 3 and 4 in France (2008-2010).

MB/FFP		Q/FFP		SD-FFP		A-FFP	
N. of cases	***Incidence***	***N. of cases***	***Incidence***	***N. of cases***	***Incidence***	***N. of cases***	***Incidence***
Frequency of severe allergic reactions with imputability level from 2 to 4 (2008-2010)							
N/A	N/A	5	1/23,778	N/A	N/A	3	1/30,137
Frequency of severe allergic reactions with imputability level 3 and 4 (2008-2010)							
24	1/21,250°	1	1/18,890	13	1/27,942°	2	1/45,205

°There were no significant differences between SD-FFP and MB-FFP (Fisher's exact test, p=0.506; Pearson χ^2 test, p=0.425). Q-FFP and A-FFP were not included in the analysis because of the relatively low numbers of units for this period. FFP: fresh-frozen plasma; MB-FFP: methylene blue-treated plasma; Q-FFP: quarantined fresh-frozen plasma; SD-FFP: solvent/detergent-treated FFP; A-FFP: amotosalen-treated FFP.

Secondary literature

Flesland *et al.* (2007)[207] compared Norwegian haemovigilance data on complications with the corresponding data from Sweden, United Kingdom and Denmark. The outcomes considered were immunological transfusion reactions, in particular TRALI.

In 2004, there were 241 reports of transfusion complications in Norway, four of which were associated with SD plasma. The number of immunological transfusion reactions associated with the transfusion of SD plasma was two (5:100,000 transfusions), of which only one was classified as a serious complication (2.5:100,000 transfusions). Table XLVI shows the comparison of serious immunological transfusion reactions per implicated product per 100,000 plasma transfusions in Sweden, United Kingdom, Denmark and Norway. The data on Finnish immunological reactions were not published in the haemovigilance report.

The authors determined that the number of cases of TRALI in other countries that use SD-FFP method is greater than in Norway, where no cases of TRALI were associated with plasma-inactivated with SD.

Eder *et al.* (2010)[229] described suspected TRALI cases reported to the American Red Cross Hemovigilance Program in 2006, 2007 and 2008.

The American Red Cross found that a female white blood cell antibody-positive donor was implicated in 75% (18 of 24) of the cases of probable TRALI deaths associated with plasma component transfusion between 2003 and 2005. This article reported the number of TRALI cases after the American Red Cross began distributing plasma from male donors in 2006.

In total, the American Red Cross distributed 1,638,055 plasma components in 2006, 1,702,419 in 2007, and 1,729,128 in 2008. Table XLVII shows non-fatal TRALI cases associated with plasma transfusions.

With regards to donor investigations in non-fatal cases, the recipients' HLA antigen type was determined in nine out of 127 (7%) cases involving plasma transfusion were linked to a female, white blood cell antibody-positive donor in six cases in 2008 compared to 29 cases in 2006 (OR: 0,20; 95% CI: 0.07-0.45). These six cases of non-fatal TRALI in 2008 involved female donors with blood groups O (two cases), A (one case), B (one case), and AB (two cases). The number of probable TRALI cases among reported fatalities that involved only plasma transfusion decreased significantly in 2008 (0 cases) compared to 2006 (6 cases; p=0.01) and 2007 (5 cases; p=0.03). The recipient's HLA type was available in nine

Table XLIII - Frequency of adverse effects per 10,000 transfusions.

Non-haemolytic transfusion reaction	0.22 (Danish data); 0.35 (French data); 2.35 (Quebec data)
TACO	0.1 (Danish data); 2.06 (French data); 0.02 (Quebec data)
Sepsis due to inadvertent bacterial contamination	0.02 (French data); 0 (Quebec data)
TRALI	0.18 (Danish data); 0 (Quebec data)
Post-transfusion purpura	0.04 (French data)
TTI (viral)	0
Severe anaphylaxis with IgA deficiency and anti-IgA	0
Transfusion-associated GvHD	0
Citrate toxicity	NR
Transmission of other pathogens not tested	NR
Alloimmunisation against HLA-antigens	NR

TACO: transfusion-associated circulatory overload; TRALI: transfusion-associated acute lung injury; TTI: transfusion-transmitted infection; GvHD: Graft-versus-Host disease; NR: not reported.

Table XLIV - Frequency of other adverse effects per 10,000 transfusions.

Minor allergic reaction	16.2 (Quebec data)
Febrile non-haemolytic transfusion reaction	5.3 (Quebec data)
Allergic reaction (not specified)	2.6 (French data)
Immunological incompatibility	0.06 (French data); 2.9 (Quebec data)
Incorrect blood component issue	0.15 (Danish data)
Acute haemolytic transfusion reaction	0 (Danish data)
Unknown	1.3 (French data); 0.88 (Quebec data)
Other reaction	0.31 (French data); 0.9 (Quebec data)

Table XLV - Number of adverse events associated with methylene blue-treated fresh-frozen plasma.

	MB-FFP (73,778 units)						
	N	*Incidence*	*Incidence rate*		*Imputability levels*		
			Grade 1	*Grade 2-3*	*1*	*2*	*3*
Allergic/anaphylactic	3	1:24,593	3	0	0	1	2
FNHTR	0	0:73,778	0	0	0	0	0
TRALI	0	0:73,778	0	0	0	0	0
TACO	0	0:73,778	0	0	0	0	0
TAD	0	0:73,778	0	0	0	0	0
Bacterial	0	0:73,778	0	0	0	0	0
Other	0	0:73,778	0	0	0	0	0
Total	0	1:24,593	0	0	0	1	3

MB-FFP: methylene blue-treated fresh-frozen plasma; FNHTR: febrile non-haemolytic transfusion reaction; TRALI: transfusion-associated acute lung injury; TACO: transfusion-associated circulatory overload; TAD: transfusion-associated dyspnoea.

out of 38 (24%) cases. The authors concluded that the incidence of TRALI associated with plasma decreased by approximately 80% in 2008 with respect to 2006 (OR: 0.21; 95% CI: 0.08-0.45). The aim of the American Red Cross was to continue the assessment of the effectiveness of the male-predominant plasma donor strategy.

Emerging pathogens

There are always risks of HIV, HCV and HBV infections in relation to blood and plasma transfusions. However, these risks appear to be low in all developed countries.

Special attention has been paid to HHV-8, HGV and vCJD. Only tests for HIV, HCV and HBV are currently carried out on a stable basis, while other tests for the identification of pathogens such as HAV, HEV and WNV are facultative.

There are two case reports of HEV transmission in patients transfused with MB plasma and A plasma.

In 2014, Hauser *et al*. [230] reported two cases of HEV transmission by two units of Intercept®-treated plasma originating from the same donor. They concluded that HEV transmission through A-FFP establishes resistance of HEV and suggested HEV screening for all blood donations in France.

In reference to vCJD, in 2004 the UK Department of Health implemented a risk-reduction measure, aimed at decreasing the risk of transmission of the disease-causing prion. In particular, it "*recommended the use of fresh-frozen plasma (FFP) sourced from countries with a low bovine spongioform encephalopathy prevalence for children born after 1 January 1996. In 2005 this was extended to all children under 16 years*." Overall, *"the English National Blood Service (NBS) imports FFP from volunteer US donors and, because of the higher prevalence of viral markers, this is subjected to a viral inactivation process (methylene blue and white light). In line with current UK guidelines, the specification for imported FFP restricts donors to those who have had virology testing within the previous 24 months, thus excluding new or lapsed donors"*[13].

A summary of primary studies and grey literature studies about haemovigilance is presented in Table XLVIII.

A comparison of European haemovigilance clinical data

Table XLIX shows clinical haemovigilance data from various European countries. Only the last report published in the different countries has been considered.

Table XLVI - Comparison of serious immunological transfusion reactions (TR) and episodes of TRALI in Sweden, UK, Denmark, Norway and Finland.

Country	Period	N. of immunological TR	N. of serious immunological TR associated with plasma transfusion per 100,000 plasma transfusions	N. of reports of TRALI	Ratio per 100,000 plasma transfusions
Sweden	2004	36	ND (FFP)	5 (FFP)	4.1
United Kingdom	2004	100	3.7 (FFP)	6 (FFP)	1.6
Denmark	1999-2003	45	5.6 (FFP)	4 (FFP)	1.5
Norway	2004-2005	241	2.5 (SD-FFP)	9 (SD-FFP)	0
Finland	2004-2005	ND	ND	7 (FFP)	8.8

FFP: fresh-frozen plasma; SD-FFP: solvent/detergent-treated FFP; TRALI: transfusion-associated acute lung injury; ND: no data.

Table XLVII - Non-fatal TRALI cases associated with plasma transfusions in the USA.

Component	2006		2007		2008		2008 *vs* 2006 OR (95% CI)
	Cases	*Rate per 1,000,000*	*Cases*	*Rate per 1,000,000*	*Cases*	*Rate per 1,000,000*	
Plasma	26	19.5	12	7.0	7	4.0	0.26 (0.10-0.57)

TRALI: transfusion-associated acute lung injury; OR: odds ratio; 95% CI: 95% confidence interval.

The haemovigilance systems are very different from each other, so comparisons of the results obtained from different programmes need to be considered cautiously. Nonetheless, it can be concluded from the existing data that acute allergic and anaphylactic transfusion reactions are the most frequently occurring adverse events and that techniques to prevent TTI have a good safety and efficacy profile.

Fresh-frozen plasma *vs* solvent/detergent-treated plasma

In the United Kingdom, acute allergic and anaphylactic transfusion reactions were more frequent with FFP than with SD-FFP (respectively, 2.920 *vs* 0.079 per 10^6 transfusions), but the reverse was seen in France (0 *vs* 7.412 per 10^6 transfusions).

TACO, transfusion-associated dyspnoea and TRALI were more frequent with FFP, while only one case of TACO was connected to SD-FFP use in Ireland and only one case of TRALI in France.

Fresh-frozen plasma *vs* methylene blue-treated plasma

In the United Kingdom, acute allergic and anaphylactic transfusion reactions were more frequently associated with FFP than with SD-FFP (respectively, 2.920 *vs* 0.014 per 10^6 transfusions), but this was not the case in France (0 *vs* 33.384 per 10^6 transfusions).

Solvent/detergent-treated plasma *vs* methylene blue-treated plasma

More acute allergic and anaphylactic transfusion reactions have been recorded in association with MB-FFP in France. In the United Kingdom, only one acute allergic and anaphylactic transfusion reaction was associated with SD-FFP and one with MB-FFP; however, expressing these numbers in relation to a number of plasma transfusions, this type of reaction was shown to be more frequent with SD-FFP than with MB-FFP (respectively 0.079 *vs* 0.0135 per 10^6 transfusions);

In the United Kingdom, no TACO, transfusion-associated dyspnoea or TRALI was observed in association with the use of SD-FFP or MB-FFP, while in France one case was associated with SD-FFP and two with MB-FFP;

In France febrile non-haemolytic transfusion reactions were more common with MB-FFP than with SD-FFP (respectively, 0.998 *vs* 3.277 per 10^6 transfusions).

Amotosalen-treated plasma *vs* plasma prepared with all the other methods

It is not possible to compare data on A-FFP because this product is only available in France. However, considering only the French data, the method appears to have a safety profile comparable to that of other techniques.

Processing outcome

During the clinical transfusion process (the phases of which are pre-transfusion, testing, screening and management of plasma), adverse reactions and adverse events can occur and it is difficult predict them. According to the Directive 2002/98/EC of the European Parliament and of the Council of 27 January 2003, a serious adverse event is *"any untoward occurrence associated with the collection, testing, processing, storage and distribution of blood or blood components, that might lead to death or life-threatening, disabling or incapacitating conditions for patients, or which results in, or prolongs, hospitalization or morbidity"* and *"Blood Establishments/the person responsible for the management of a hospital blood bank ('hospital transfusion laboratory' is used in this report for consistency with other chapters) shall notify… any serious adverse events related to the collection, testing, processing, storage and distribution of blood or blood components by the Blood Establishment which may have an influence on their quality and safety"*.

Table L reports the number of serious adverse reactions recorded in the SHOT report in 2013.

According to the SHOT *"near miss events are defined as any error, which if undetected, could result in the determination of a wrong blood group or transfusion of an incorrect component, but was recognized before the transfusion took place"*[204].

No near misses were recorded in the 2013 SHOT reports.

Moreover, according to the definition proposed by the International Haemovigilance Network a *"near miss is an error or deviation from standard procedures or policies that is discovered before the start of the transfusion and that could have led to a wrongful transfusion or to a reaction in a recipient"*[231].

Table XLVIII - Summary of haemovigilance studies.

First author, year	Type of study	Population	Intervention	Country	Outcomes	Results	Conclusions/recommendations	Level of evidence
Funk MB, 2010	Observational cohort study	N/A	FFP	Germany	Frequency of TRALI events between 2006 and 2010. Comparison of the period before and after introduction of risk-minimisation measures, implemented in 2008/2009.	During the period 2006-2010 there were 80 cases of TRALI: - 75% immune-mediated; - 20% non-immune-mediated. The rate of immune-mediated TRALI decreased during the transitional period: - 12.7/1,000,000 in 2006/2007 - 6.81/1,000,000 in 2008/2009 No cases in 2010. Comparing 2006/2007 with 2008/2009: OR=0.54, 95% CI: 0.29-0.98; (p=0,0404). Comparing 2006/2007 with 2010 p=0.0002. Odds ratio not determined because no cases in 2010.	Risk minimization measures produced a significant reduction of immune-mediated TRALI.	Moderate
Mertes PM, 2013	Letters to the Editor of the Journal of Allergy and Clinical Immunology	N/A	MB-FFP, Q-FFP, SD-FFP	France	Frequent SAR observed between 2005 and 2009 with MB-FFP compared to other types of plasma (Q-FFP and SD-FFP).	Frequency of allergic reactions imputability level 2 to 4 (2005-2009). MB-FFP: 34 cases Q-FFP: 23 cases SD-FFP: 36 cases Frequency of SAR imputability level 3 and 4 (2005-2009) MB-FFP: 14 cases Q-FFP: 15 cases SD-FFP: 16 cases	SAR related to MB-FFP are rare. The results confirm that in some cases sensitisation to MB could be responsible for the allergic reactiond	Low
Seltsam A, 2014	Letters to the Editor of the Journal of Allergy and Clinical Immunology	N/A	MB-FFP, Q-FFP, SD-FFP, A-FFP	France	Frequent SAR observed between 2005 and 2010 with MB-FFP compared to other types of plasma treated (Q-FFP, SD-FFP, A-FFP).	Frequency of SAR of imputability level 2 to 4 (2005-2010) MB-FFP: N/A Q-FFP: 33 cases SD-FFP: N/A A-FFP: 3 cases Frequency of SAR of imputability level 3 and 4 (2005-2010) MB-FFP: 24 cases Q-FFP:15 cases SD-FFP: 20 cases A-FFP: 2 cases	Currently available haemovigilance data do not support the hypothesis of additional allergenic mechanisms specifically related to the MB/light pathogen-inactivation process	Low

FFP: fresh-frozen plasma; TRALI: transfusion-associated acute lung injury; OR: odds ratio; 95% CI: 95% confidence interval; MB-FFP: methylene blue-treated fresh-frozen plasma; Q-FFP: quarantined fresh-frozen plasma; SD-FFP: solvent/detergent-treated fresh-frozen plasma; N/A: not available; SAR: serious adverse reaction; TACO: transfusion-associated circulatory overload; HEV: hepatitis E virus.

continued on next page

Blood Transfus 2016; **14:** 287-386 DOI 10.2450/2016.0065-16

Table XLVIII - Summary of haemovigilance studies. *(continued from previous page)*

First author, year	Type of study	Population	Intervention	Country	Outcomes	Results	Conclusions/recommendations	Level of evidence
Bost V, 2013	Observational retrospective study	105,964 units of FFP transfused Q-FFP: 40,631 SD-FFP: 19,015 MB-FFP 10,283 A-FFP: 36,035	MB-FFP SD-FFP A-FFP	France	Comparison of the frequency of AR associated with the four types of FFP. The AR of Q-FFP were not analysed.	Possible, probable and certain AR MB-FFP 5 cases SD-FFP 2 cases A-FFP 15 cases Probable and certain AR MB-FFP 8 cases SD-FFP 1 cases A-FFP 3 cases No statistically significant difference in AR frequency	All types of FFP were associated with an extremely low frequency of AR.	Moderate/ low
Dewachter P, 2010	Case report	N/A	MB-FFP	France	N/A	The first case of an immediate allergic IgE-mediated reaction in a patient transfused with MB-FFP.	MB allergic IgE-mediated reactions are rare.	Very low
Nubert, 2010	Case report	N/A	MB-FFP	France	N/A	Two anaphylactic shock reactions occurred during MB-FFP infusion in patients after cardiac surgery.	The frequency of this type of allergic reaction is very low with respect to the widespread use of this blood product	Very low
Norda, 2006	Observational study	N/A	FFP	N/A	Potential AR of transfusing FFP in the Danish, French and Quebec haemovigilance systems.	AR per 10,000 transfusions: Non-haemolytic transfusion reactions 0.22 (Danish data); 0.35 (French data); 2.35 (Quebec data) TACO 0.1 (Danish data); 2.06 (French data); 0.02 (Quebec data) Sepsis due to inadvertent bacterial contamination 0.02 (French data); 0 (Quebec data) TRALI 0.18 (Danish data); 0 (Quebec data) Post-transfusion purpura 0.04 (French data)	AR of particular relevance for patients are probably TRALI as well as sepsis due to inadvertent bacterial contamination	Low

FFP: fresh-frozen plasma; TRALI: transfusion-associated acute lung injury; OR: odds ratio; 95% CI: 95% confidence interval; MB-FFP: methylene blue-treated fresh-frozen plasma; Q-FFP: quarantined fresh-frozen plasma; SD-FFP: solvent/detergent-treated fresh-frozen plasma; N/A: not available; AR: adverse reaction; TACO: transfusion-associated circulatory overload; HEV: hepatitis E virus.

continued on next page

Blood Transfus 2016; **14:** *287-386* DOI 10.2450/2016.0065-16

Table XLVIII - Summary of haemovigilance studies. *(continued from previous page).*

First author, year	Type of study	Population	Intervention	Country	Outcomes	Results	Conclusions/recommendations	Level of evidence
Flesland O, 2007	Observational study	N/A	FFP SD-FFP	Norway	Comparison of immunological transfusion reactions and, in particular, TRALI events in Norway with those in Sweden, UK Finland and Denmark.	Number of immunological transfusion reactions Sweden: 36 UK: 100 Denmark: 45 Norway: 241 Finland: ND Number of TRALI reports Sweden: 5 FFP UK: 6 FFP Denmark: 4 FFP Norway: 9 SD-FFP Finland: 7 FFP	The number of TRALI events following transfusion of FFP is greater than in Norway, where no case of TRALI has been associated with plasma inactivated with SD.	Low
Hauser L, 2014	Case report	N/A	A-FFP	France	N/A	Two cases of HEV transmission by two units of A-FFP.	HEV screening of all blood donations in France is under consideration.	Very low
Politis C, 2014	Observational study	73,778 units of MB-FFP transfused between 2001–2011	MB-FFP	Greece	AR associated with MB-FFP. in Greece between 2001 and 2011.	Only three allergic/anaphylactic reactions were observed. The incidence of serious adverse reactions (grade 2–3) was 0 with MB-FFP.	The authors concluded that the AR rate was lower with MB-treated plasma and all adverse reactions experienced by the MB plasma recipients were mild.	Moderate/ low
Eder AF, 2010	Observational study	Plasma components transfused: 1,638,055 in 2006, 1,702,419 in 2007, 1,729,128 in 2008.	N/A	USA	Suspected TRALI cases reported in calendar years 2006, 2007, and 2008.	Non-fatal TRALI cases 2006: 26 2007: 12 2008: 7	The authors concluded that TRALI cases associated with plasma decreased by approximately 80% in 2008 with respect to 2006.	Moderate

FFP: fresh-frozen plasma; TRALI: transfusion-associated acute lung injury; OR: odds ratio; 95% CI: 95% confidence interval; MB-FFP: methylene blue-treated fresh-frozen plasma; Q-FFP: quarantined fresh-frozen plasma; SD-FFP: solvent/detergent-treated fresh-frozen plasma; N/A: not available; AR: adverse reaction;; TACO: transfusion-associated circulatory overload; HEV: hepatitis E virus.

Table XLIX - Comparison of European haemovigilance data.

	Germany (2010)	UK (2013)		Ireland (2011)		Switzerland (2013)		France (2010)			Italy[1] (2012)	
	FFP	*SD-FFP*	*MB-FFP*	*FFP*	*SD-FFP**	*FFP (therapeutic units)°*	*FFPs°°*	*SD-FFP*	*MB-FFP*	*A-FFP*	*FFP*	*SD-FFP*
Units of FFP transfused	1,152**	266,332	79,473	13,547	264	22,890	44,083	ND	83,163	246,460	52,692	319,666	113,218
FNHTR	0	ND	ND	ND	0	0	ND	1	2	0	1		
Acute allergic and anaphylactic TR	59	11**	1**	1**	0	0	23	11	26	124	19		
Hypotensive TR	ND	1	0	0	0	0	0	ND	ND	ND	ND		
TACO	2	2	0	0	0	1	ND	1	1	0	0		
TAD	ND	2	0	0	0	0	ND	ND	ND	ND	ND		
TRALI	0	0	0	0	0	0	0	0	0	6	1		
TTI (bacterial)	0	0	0	0	0	0	0	ND	ND	ND	ND		
TTI (viral)	0	0	0	0	0	0	0	ND	ND	ND	ND		
Total	61	5	0	0	0	1	23	0	60	181	11	299	6

*99% of plasma transfused in Ireland, **Calculated consumption; °FFP, Q-FFP, SD-FFP; °°Secured fresh-frozen plasma; [1]The majority of adverse reactions to plasma transfusion, considering all levels of imputability and severity, were allergic. SD-FFP: solvent/detergent-treated fresh-frozen plasma; MB-FFP: methylene blue-treated fresh-frozen plasma; A-FFP: amotosalen-treated fresh-frozen plasma.

A potential transfusion error could be the unjustified use of plasma when there are therapeutic alternatives which are equally or more effective, and/or with lower costs/risks. Table LI describes the recommendations of "The Transfusion of Plasma in Adults" guidelines of the San Giovanni Hospital of Turin[201].

Interactions of the reagents used for plasma pathogen-inactivation with biological components

The quality of plasma depends on a number of factors such as its optimal freezing (core temperature of –30 °C within 60 minutes) and storage at <20 °C[232]. In addition, the processing features of the plasma inactivation method play a critical role in producing toxic effects and permanent binding with biological components. The residual toxicity and permanent binding between residual solvent or reagents used to inactivate plasma and lipids or proteins in the plasma are reported in Table LII.

The SD-method of inactivating pathogens in plasma generally has a low toxicity for biological components and low permanent binding. MB-FFP should be filtered in order to remove the MB.

Discussion

Complete safety of transfused blood components will never be reached because of an intrinsic risk due to the transmission of emerging pathogens. However, plasma pathogen-inactivation methods may be considered safe especially as regards the transmission of viruses and bacteria. Indeed, no transmission of HIV, HCV or HBV has been observed in the European countries analysed in the last years. As for the other clinical outcomes, there was a recent increase in the number of allergic reactions, while the actions taken by many countries to minimise the risk of TRALI have been appropriate. Overall, the number of cases of TRALI has dropped in recent years and is now tending to zero.

The results emerging from the literature search do not enable a direct comparison between the different pathogen-inactivation methods. Few studies compare the various techniques with each other or with the conventional method. Furthermore, haemovigilance data are conflicting across European countries, in part because alerts are reported on a voluntary basis in some countries. However, according to the data reported above, the SD-FFP technique seems to have the best safety profile, even if the evidence supporting this statement is limited and the conclusion should be considered cautiously. The current review highlights the need to perform more comparative studies in order to assess the safety profiles of the various methods directly.

Economic domain

Introduction

The Italian Society of Transfusion Medicine and Immunohaematology (SIMTI) recommends the transfusion of plasma to correct deficiencies of clotting factors, for which a specific concentrate is not available, in patients with active bleeding[14]. The most commonly utilised product is FFP, a blood component prepared from whole blood or collected by apheresis, frozen within time limits and at a temperature such as to preserve the labile clotting factors adequately[14]. The risk of transmitting viruses such as HIV, HBV, HCV, HAV and PVB19 through blood transfusions, along with causing TRALI and other serious adverse reactions, has driven the introduction of an increasing number of measures aimed at improving the safety of therapeutic transfusions[62]. So, currently there are many blood products available treated with the aim of reducing the risk of transmitting the abovementioned viruses and preventing TRALI and other serious adverse reactions. In detail these are plasma that has undergone viral inactivation with SD, MB, amotosalen and light or riboflavin and light. The widespread implementation of these approaches may have significant public health and economic implications. In publicly-funded health care systems, such as the Italian one, finite resources imply that not all possible technologies can be provided in any situation for all those who may need or want them. There is a conflict between what is technologically possible and what is economically feasible.

Table L - Number of serious adverse events recorded in the 2013 SHOT report.

IBCT	19 (FFP) of which:
ABO incompatible	3 (FFP)
ABO non-identical	1 (FFP)
Wrong component type	2 (FFP)
ABO identical	1 (FFP)
RBRP	9 (FFP)
HSE	9 (FFP)
ADU	11 (FFP)
Anti-D immunoglobulin - prescription, administration and sensitisation	0

IBCT: incorrect blood component transfused; FFP: fresh-frozen plasma; RBRP: right blood - right patient; HSE: handling and storage errors; ADU: avoidable, delayed or undertransfusion.

Table LI - Recommendations of "The Transfusion of Plasma in Adults" guidelines of "San Giovanni Hospital" (Turin, Italy).

Level of recommendation	Recommendation
E*	Plasma should not be used to correct deficiencies of coagulation factors when there are specific completely inactivated or recombinant factors.
E*	Plasma should not be used to correct hypoproteinaemia.
E*	Plasma should not be used as a means to expand the intravascular volume.
E*	Plasma should not be used in hypogammaglobulinaemia.
E*	Plasma should not be used as a replacement fluid in plasma-exchange procedures.

E*: strong recommendation.

Economic evaluations of health technologies should provide indications on how resources should be allocated or utilised to maximise health outcomes under economic constraints[233]. Thus, it is no longer sufficient to consider only aspects of safety, efficacy, clinical effectiveness and ethics in HTA reports; information on costs, cost-effectiveness, or opportunity costs from economic evaluations, is also needed[233]. Economic evaluation has been defined as a comparative analysis of alternative courses of action in terms of their costs and consequences[234]. The primary aim of this section is to provide information about the relative costs and cost-effectiveness of technologies for pathogen reduction in FFP. The economic domain is intended to summarise the economic evidence available when allocating resources between emerging, new and existing health technologies in order to inform value-for-money judgments[233,234].

Methods

Literature search

A literature search was conducted to obtain economic data on technical approaches to pathogen inactivation of FFP for transfusion use. The review was performed using the following databases: PubMed, the Cochrane Library and CRD database. Google and Google Scholar search engines were queried to search for additional material and information. References and abstracts of key papers and conference proceedings were also reviewed.

The following keywords were used in the search: plasma, fresh-frozen plasma, blood component transfusion, blood component, therapeutic plasma, riboflavin, UV, UVA light, UVA, Mirasol, amotosalen, methylene blue, Theraflex® MB plasma, solvent/detergent, solvent/detergent-treated plasma, Octaplas®.

Since the objective was the evaluation of the economic aspects, the basic search was refined using the following key words: cost, cost-effectiveness, cost-utility, cost-benefit, economic evaluation, ICER (incremental cost-effectiveness ratio).

The search strategy was:

ECONOMIC DOMAIN *(((((((((Cost*) OR "Cost effectiveness") OR "Cost utility") OR "Cost benefit") OR "Economic evaluation") OR ICER) OR "Cost benefit analysis"[MeSH Terms]) OR ("Cost[MeSH Terms] AND Cost analysis"[MeSH Terms]))) AND (((((((Plasma[MeSH Terms]) OR "Fresh frozen plasma")*

Table LII - Interactions of the reagents used in different plasma inactivation methods with biological components.

	SD-FFP	MB-FFP	A-FFP	R-FFP
Compound toxicity	*Low*	*Low*	*High*	*None*
All active components removed	Yes	Yes (special filters)	Yes (by adsorbing device)	Not required
Residual level of active compounds	Undetectable or trace amount, far below toxicity level	Very low. Mutagenic effects improbable, but not excluded	Very low. Mutagenic effects improbable, but not excluded	Low levels of riboflavin are usually present in blood. Active component is removed
Permanent binding to lipids or proteins in plasma	No binding to proteins or residual lipids	Protein intercalated with phenothiazine-like dyes	Majority of amotosalen bound to lipids and 2% to proteins	Does not appear to bind

Source: 232.

OR "Blood component transfusion"[MeSH Term]) OR "Blood component") OR Therapeutic plasma))) AND (((((("Solvent/detergent") OR "Solvent/detergent-treated plasma") OR Octaplas)) OR ((("Methylene blue"[MeSH Terms]) OR "Methylene blue") OR Theraflex MB plasma)) OR ((((((Amotosalen[Supplementary Concept]) OR Amotosalen)) AND ((((((UV) OR "Ultraviolet light") OR "UV light") OR "Ultraviolet A")) OR Mirasol))) OR intercept)) OR (((((((UV) OR "Ultraviolet light") OR "UV light") OR "Ultraviolet A")) OR Mirasol)) AND Riboflavin))).

No publication date, language or search field restrictions were applied.

Selection criteria

The selection process was based on a set of inclusion criteria, reported in Table LIII.

Results

Results of the literature search

The literature search revealed 343 citations that were potentially relevant for the economic domain. All the records were analysed based on the study title and 316 were excluded because not relevant to the research question. In a second step, studies included were screened by reading their abstracts and the full texts, if necessary. Overall, 19 articles were excluded because the alternatives compared or the study design were not consistent with the aim of this analysis.

Eight studies eventually met the inclusion criteria. Of these, seven were economic evaluations and one was a HTA report.

The flowchart in Figure 5 depicts the various stages of the literature selection process.

General characteristics of the selected studies

Literature on cost-effectiveness was limited and only eight studies eventually met the inclusion criteria: one HTA report from the CADTH (2011)[63] and seven economic analyses.

No Italian studies were identified. Two out of the seven economic evaluations were conducted in the United Kingdom[235,236], three in the United States[237-239], one in Spain[240] and one in Canada[241]. Some of them (n=2) were cost-effectiveness analyses reporting results in terms of cost per life-year saved (LYS) and cost per death avoided. Three economic studies and the CADTH report[63] described both cost-effectiveness and cost-utility analyses, so the authors also reported results in terms of cost per quality-adjusted life year (QALY) gained[236,238,241]. Finally, two economic studies reported only cost-utility analyses. The CADTH[63] and Huisman *et al.*[241] also conducted a budget impact analysis.

All the selected economic analyses adopted the healthcare payer perspective, including only direct healthcare costs.

Comparators, cost drivers and outcome measures

The papers selected for analysis in the present review explored the cost-effectiveness of only one PRT to treat plasma, that is SD.

In more detail, four studies and the CADTH report[63] investigated the cost-effectiveness of SD-FFP *vs* FFP and three economic analyses considered the SD technique (without specifying the brand) *vs* FFP.

Studies included in the present analysis evaluated resource consumption associated with the intervention and comparators by considering different cost drivers, so results across the included studies are neither consistent nor comparable. The only cost driver considered in all the studies reviewed is the inactivation technique and FFP. Most of the studies[63,236,238,241] reported costs of treatment of infectious complications (HIV, HBV and HCV) and their sequelae (HBV and HCV-related compensated cirrhosis, HBV and HCV hepatocellular carcinoma, post liver transplant, etc.). Aubuchon *et al.*[239] and Riedler *et al.*[235] estimated costs related to the management of acute hepatitis, HIV infection and AIDS, but they did not take into account the sequelae of HCV or HBV infections. Instead, Pereira *et al.*[240] considered costs associated with the treatment of acute symptomatic hepatitis and chronic hepatitis, follow-up of chronic hepatitis, decompensated cirrhosis and AIDS.

With respect to non-infectious complications, which include TRALI, allergic reactions and TACO[63], van Eerd *et al.*[236] took into account all of these, while Huisman *et al.*[238,241] considered only TRALI and allergic reactions. Riedler *et al.*[235] and the CADTH[63] estimated only costs associated with TRALI, excluding other non-infectious complications. In the analysis by Huisman *et al.*[241], allergic reactions were the main cost driver of the model, while in the study by Riedler *et al.*[235], the most important driver of cost-effectiveness was TRALI, because of its relatively high incidence and associated mortality rate.

Huisman *et al.*[238,241] and van Eerd *et al.*[236] included infectious and non-infectious transfusion-related complications. In particular, they considered: TRALI, severe allergic reactions, HAV, HBV, HCV, HIV, prion disease, PVB19, bacterial infections and two emerging infections (one acute [WNV-like] and the other chronic [HIV-like]).

Table LIII - Selection criteria for literature on economic evaluations of pathogen-reduction technologies applied to plasma for transfusion.

Category	Inclusion criteria
Study design	Full or partial economic evaluations
Study population	Plasma transfusion recipients of all ages
Interventions and comparators	FFP, riboflavin-treated plasma, UV light-treated plasma, Mirasol®, amotosalen, methylene blue, Theraflex®-MB plasma, solvent/detergent-treated plasma, Octaplas® solvent/detergent-treated plasma
Outcome measures	Infectious and non-infectious complications, QALY, LYS, health utility
Cost measures	Direct costs (cost associated with treating infectious and non-infectious complications, intervention cost), social costs
Incremental cost-effectiveness ratio	Cost per QALY gained, Cost per LYS, ICER

FFP: fresh-frozen plasma; UV: ultraviolet; QALY: quality-adjusted life years; LYS: life years saved; ICER: incremental cost-effectiveness ratio.

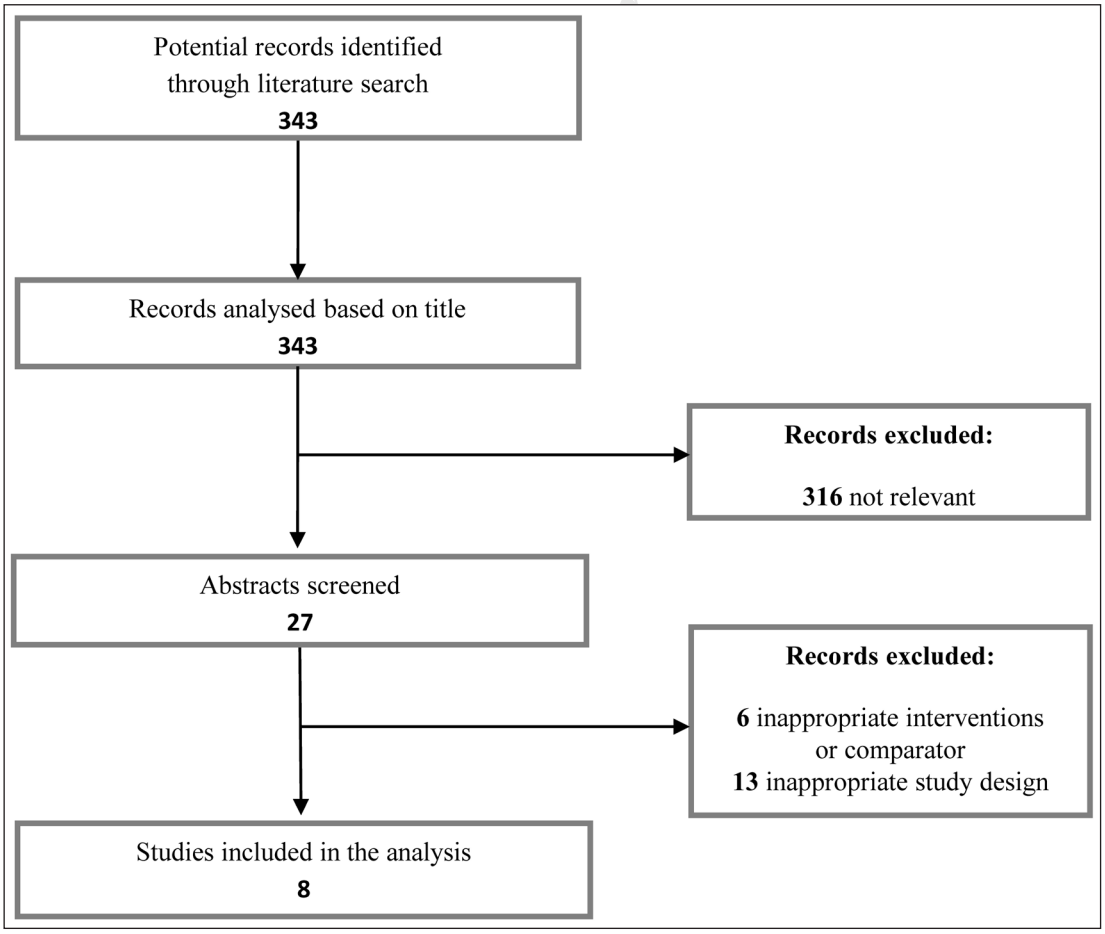

Figure 5 - Study selection process for economic domain.

One economic study[237] did not evaluate the costs of complications, but it compared screening costs, such as HIV, HCV and HBV antibody testing, with SD treatment costs.

QALY, deaths avoided and LYS were utilised by authors as outcome measures.

Cost-effectiveness of solvent/detergent-treated plasma vs untreated plasma: evidence from the literature

Most of the selected studies (n=8) estimated the cost-effectiveness of SD-FFP *vs* untreated plasma. Three of these papers[237,239,240] did not mention the particular brand of the SD, while the remaining five studies evaluated the cost-effectiveness of pooled SD-FFP *vs* untreated FFP.

AuBuchon *et al.* 1994[239] used a previously published Markov model to perform a cost-utility analysis for a hypothetical cohort of 61 patients receiving plasma transfusions. A unit of SD-FFP produced a net benefit of 35 minutes expressed in terms of quality-adjusted life expectancy at a cost of about $ 19. The ICER of SD-FFP was $ 289,300 per QALY, with an incremental cost of $ 142.5 million per 147 incremental QALY. So, SD-FFP was not recommended by the authors because it was very expensive and produced small benefits in terms of reducing the risk of viral infections.

In the study by Pereira (1999)[240], base case results showed that SD-FFP infusion prolonged the quality-adjusted survival by 1 hour and 11 minutes per patient, producing an incremental cost of $ 2,156,398 per QALY gained, with an average net incremental cost of $ 39.66 per unit of virus-inactivated plasma. Cost-effectiveness was very sensitive to the cost of the virus-inactivation procedure, the patients' age and the rate of short-term mortality due to underlying diseases. The authors concluded that virus-inactivated plasma produced benefits but at a very high cost, because of the advanced age of plasma recipients, the high cost of virus-inactivation procedures, the very low risk of infection by transfusion-transmitted viruses, and the poor prognosis of the patients' underlying diseases.

In the study by Blumberg *et al.* 2000[237] the cost-effectiveness of three approaches to improve blood transfusion safety was evaluated. These approaches were: apheresis platelets *vs* random platelets, SD-treated plasma *vs* untreated plasma, and leucocyte-reduced *vs* unmodified transfusions in cardiac surgery. In the analysis of SD-FFP, the estimated risk of a fatal HIV-1, HBV or HCV infection was approximately 4.6% per year, implying the occurrence of an event every 22 years, on average. The authors assumed that switching completely to SD-FFP, transmission of HIV-1, HBV or HCV by this route should not occur. The cost increment of using SD-FFP instead of FFP was $ 75.00 per unit, which is equivalent to $ 750,000 annually, considering the case mix of the specific centre. The cost per death prevented was approximately $17,000,000 dollars. SD plasma was a comparatively expensive approach to reduce mortality from transfusion complications.

The cost effectiveness of Octaplas® was evaluated in one HTA report[63] and in four economic analyses[235,236,238,241].

In the analysis by the CADTH[63] a Markov decision model was constructed to represent six possible transfusion-related complications (infections by HAV, HIV, HBV, HCV, or PVB19, and TRALI) in a hypothetical cohort of patients (with a 1-year mortality rate of 20%) receiving an average of four units of SD-FFP *vs* FFP. SD-FFP resulted more costly and more effective than FFP. Treating a 50-year old patient, the incremental cost per QALY gained was $ 934,000 and the incremental cost per life-year gained was $ 1.3 million. The probability of cost-effectiveness for SD-FFP was 0% for any cost-effectiveness threshold less than $ 100,000 and only 6.3% if a $ 500,000/QALY threshold was adopted. In the budget impact analysis, potential savings resulting from the replacement of FFP, FP, apheresis plasma and cryosupernatant plasma with Octaplas® were calculated. The results showed that if 100% of the demand of all forms of plasma was switched to Octaplas®, the additional cost would be about 16.5 million Canadian dollars (C$) per year. If 50% of the total demand of all forms of plasma was switched to Octaplas®, the additional cost would be about C$ 8,2 million per year. If Octaplas® replaced all forms of FFP, plasma from ongoing donations would go for fractionation, potentially enabling the health care system to save about C$ 3 million per year (in absolute terms) by increasing the volume of intravenous immunoglobulins and albumin. Even if the health care system would save C$ 3 million per year in absolute terms, in relative terms the yearly net loss would be C$ 13.5 million, because the incremental cost would be about C$ 16,5 million per year. The authors concluded that Octaplas® was not cost-effective because it is associated with only a minimal reduction in disease burden at a higher cost than FFP. The high incremental cost per QALY resulted from low transfusion-related risks for FP or FFP due to advanced safety measures of blood transfusion, such as testing, donor screening, and deferral. Thus, by switching to Octaplas®, the health care system would incur a net loss.

In their study, Riedler *et al.*[235] used a decision-tree model simulating a hypothetical cohort of patients aged up to 70 years. In their model a proportion of patients undergoing transfusion would experience adverse events, such as TRALI or HIV infection, and would therefore incur additional costs and mortality either immediately or over a period of several years. A sensitivity analysis was undertaken to evaluate the

robustness of the results. The discounted cost/LYS of SD-FFP in the UK increased with age. Indeed, in neonates it amounted to £ 22,728 while in the population ≥70 years old it was £ 98,465. These results considered post-transfusion mortality. The authors also considered the scenario without post-transfusion mortality. In this case ICER values were lower than those of the base case. Indeed, in neonates the discounted cost/LYS amounted to £ 12,335 and in people ≥70 years old the ratio was £ 61,692. In the sensitivity analysis the cost/LYS was very sensitive to variations in incidence of TRALI, while it was less sensitive to variations in the cost of treating complications. The study demonstrated that SD-FFP was most cost-effective in children, as a result of their long life expectancy. The cost-effectiveness ratios calculated in this study were lower than the ones previously reported[238,239], which ignored non-infectious events such as TRALI. This analytical model showed that when non-infectious transfusion-related complications were taken into account, SD-FFP had a cost/LYS of £ 22,700-98,500 in the UK (depending on the age of the recipient). If a value of £ 50,000/LYS is taken as an "acceptable" cost-effectiveness threshold, the model suggests that SD-FFP is a cost-effective treatment for all patients up to the age of 48, and for older patients with a good clinical prognosis.

The model by van Eerd *et al.* 2010[236] explored the cost-effectiveness of Octaplas® in the base case and in two alternative scenarios: (i) reduced risk of TRALI from FFP transfusions through the use of male plasma only; (ii) risk of TRALI from FFP based on SHOT[242]. In the base case analysis the incremental costs per LYS and QALY were £ 949 ($ 1,504) and £ 1,030 ($ 1,632), respectively. The total average cost per patient transfused with Octaplas®-inactivated plasma was £ 280.02 ($ 443.81), which is £ 27.97 ($ 44.33) more than the total cost of FFP transfusion. In the first scenario costs per LYS and QALY were higher than in the base case. The costs per life year and per QALY gained were £ 4,836 ($ 7,665) and £ 5,246 ($ 8,315), respectively. This means that in this scenario, too, the use of Octaplas® was cost-effective adopting a threshold of £ 30,000 ($ 47,548) per QALY. A second scenario analysis, performed using SHOT data more conservatively, demonstrated that the total costs associated with FFP were reduced (compared to the base case) when the TRALI incidence was lowered. In this scenario, Octaplas® was more effective and more costly. The costs per life year and QALY gained were £ 968,909 ($ 1,535,654) and £ 868,172 ($ 1,375,993), respectively. Since TRALI is underreported in SHOT[242], these results need to be interpreted with caution. The study suggested that Octaplas® represents good value for money *vs* FFP, which is considered the main comparator for critically ill patients who need a plasma transfusion.

Huisman *et al.* carried out two different cost-effectiveness analyses of Octaplas® in 2014: one in the United States[238] and one in Canada[241].

The former[238] was conducted assuming a hypothetical cohort of 50-year old patients receiving plasma transfusions in the United States. Acute and long-term complications of plasma transfusions were modelled in a decision tree followed by a Markov model. The analysis involved a base case and two more scenarios: one excluding the risk of unknown emerging viruses and one using a historic (lower) price of FFP.

In the base case analysis, the costs arising from transfusion-related complications were lower for Octaplas® than for FFP. Despite the higher product cost of Octaplas®, its overall cost for treatment was lower ($ 525.86) than that of FFP ($ 549.73). Over a lifetime horizon, patients receiving Octaplas® accumulated slightly more life years (0.00613) and QALY (0.023) at lower cost than those treated with FFP. When the risk of unknown emerging viruses was excluded from the analysis, the incremental cost per QALY was $ 16,159 and that of LYS was $ 289,633. In such a scenario, the incremental benefit of using Octaplas® rather than FFP was reduced as the risk of this complication was zero for both products. Since the costs for treatment of unknown emerging viruses were an important driver of the model, excluding these costs had a large impact on the total costs of FFP in the alternative scenario in which unknown emerging viruses were excluded.

The second scenario analysis using a historic (lower) price of FFP (from 2008) showed that Octaplas® would have been cost-effective in comparison to FFP even if the latter had not undergone a price increase over the last 5 years. The authors concluded that Octaplas® can be considered the dominant treatment option over FFP because it is expected to produce cost savings while improving QALY.

Huisman *et al.* 2014[241] carried out four different analyses in order to investigate the cost-effectiveness of Octaplas® *vs* FFP in 50-year old patients. The base case analysis showed that Octaplas® was dominant compared to FFP because it yielded an increase in QALY at lower costs (C$ 612.91 *vs* C$ 916.05), resulting in 595.74 complications prevented per 10,000 transfused patients. FFP produced an average 16.571 QALY, corresponding to 16.588 life years while Octaplas® resulted in 0.005 incremental life years (95% CI: 0.001-0.012) and 0.021 incremental QALY (95% CI: 0.011-0.040). Transfusion costs were the main cost drivers (C$ 588.00) for Octaplas®, while for FFP treatment costs (C$ 384.00) and the costs of allergic reactions (C$ 373.86) were the most influential cost items. The probability that Octaplas® is cost-effective adopting a willingness to pay threshold of C$ 30,000 per QALY is greater than 98%. Octaplas®

was also dominant in the second analysis, excluding the risk of unknown emerging viruses, yielding 0.0007 additional life years (95% CI: 0.0003-0.0012) and 0.013 additional QALY (95% CI: 0.007-0.019) compared to FFP at a lower cost (C$ 148.09 *vs* C$ 761.00). Octaplas® can still be considered the dominant treatment option over FFP when lowering the cost of allergic reactions or reducing the risk of allergic reactions by 50%. Finally, in the last analysis, reducing the risk of allergic reactions by 90%, Octaplas® had an ICER of C$ 2,551 per life year and C$ 1,085 per QALY gained, far below the generally accepted cost-effectiveness threshold. The authors also performed a budget impact analysis. In 2017, switching to Octaplas® would allow a saving of C$ 6,785,396 for the Canadian health care system, relative to the current scenario in which only FFP is used.

Discussion

The results of the studies included in this analysis are summarised in Table LIV.

This review was aimed at identifying economic evaluations of different PRT. There was a very limited number of studies evaluating the cost-effectiveness of techniques for pathogen inactivation. All the studies included focused on the cost-effectiveness of SD-treated plasma compared to FFP.

Studies with very different and sometimes incomparable results were included in the current review. One reason is that, given the complexity of transfusion risks and practices, economic evaluations are often based on assumptions and simplifications and only focus on specific aspects. The innovative techniques to prevent TTI by pathogen reduction make transfusion safety a moving target[86].

If we consider the threshold commonly adopted as acceptable by the NICE in the UK (£ 20,000-£ 30,000/QALY), four articles suggest that Octaplas® can be considered a cost-effective option, while the same PRT did not result cost-effective in the remaining studies. Some studies assessed the cost-effectiveness of SD-FFP, producing a wide range of costs per QALY estimates ($ 289,000[239], $ 2,156,398[240], $ 934,000/QALY[63]) suggesting that the increased safety of SD-FFP does not justify its additional cost. Conversely, other studies showed that SD-FFP is the dominant alternative, producing more QALY or LYS at lower cost compared to FFP[236,238,241] or that it can be considered cost-effective in specific cohorts of patients[235].

Results of the selected studies revealed that the ICER of these technologies is very sensitive to several factors. The economic analyses reviewed considered different categories of patients and different cost drivers. The cost-effectiveness of Octaplas® *vs* FFP was analysed in the UK, in Canada and in the USA in a total of four articles as well as in the Canadian HTA report. Octaplas® proved to be the dominant treatment option over FFP in Canada[241] and in the USA[238], while it resulted in an ICER of £ 1,030 (corresponding to $ 1,640) compared with FFP in the UK[236]. These results conflict with those obtained by the CADTH[63]. The difference in results between the British analysis and the American and Canadian ones, may be due to differences in the populations of patients described. This is because a critically ill population with a reduced life expectancy was considered in the British model. When life expectancy is short, any long-term effects of chronic conditions (implying costs and poorer quality of life) diminish. In addition, the risk of experiencing allergic reactions per plasma transfusion was higher in the analysis by Huisman *et al.*[238], since it concerned patients who received plasma transfusions on a regular basis. Other important factors that affect the results of the studies are transfusion-related complications and the costs considered. Studies reporting a very high ICER considered only rare infectious complications (HIV, HBV and HCV infections) and disregarded several relatively common non-infective events, such as TRALI[237]. However, in the CADTH analysis[63], in which TRALI costs were included, the authors concluded that Octaplas® is not cost-effective using a threshold of C$ 30,000 per QALY (while the studies by Huisman *et al.*[238,241] showed that Octaplas® was the dominant treatment compared to FFP). The remarkable variability in the studies' results may be due to differences in the models' assumptions and sources of data. The CADTH model[63] assumed that the risk of transmitting HAV or PVB19 is the same for Octaplas® and FFP, while in the study by Riedler *et al.*[235] the risk of transmitting HAV or PVB19 in the Octaplas® arm was set at zero. Although there are no large epidemiological studies evaluating this risk, the CADTH[63] itself reported that no HAV or PVB19 infections have been published for Octaplas® using the current manufacturing and testing procedures. Moreover, the analysis by Riedler *et al.*[235] also included the risk of severe allergic reactions, which were excluded from the CADTH analysis[63] because of lack of evidence, given the minimal rate of severe or minor allergic reactions associated with Octaplas®.

Several studies showed a significant correlation of ICER with patients' age and prognosis[235,240]. The higher age of plasma recipients and their poor short-term prognosis due to their underlying diseases are important factors that negatively influence the cost-effectiveness of virus-inactivated plasma. In the study by Riedler *et al.*[235], the cost-effectiveness ratios for SD-FFP were significantly lower for patients with a good short-term prognosis who were likely to survive for as long as would be normally expected at their

Table LIV - Summary of the results of selected economic studies.

First author, year	Country, funding	Population (P)/ intervention (I)/ comparator (C)	Type of assessment (study endpoint)	Cost items	Study results	Conclusion
AuBuchon JP, 1994	USA, N/A	P: hypothetical patients (age not reported) I: SD-FFP C: untreated FFP	CUA (cost/ QALY gained)	Evaluation and management of: acute hepatitis, HIV, AIDS	ICER: $ 289,300/QALY	SD-FFP produces limited incremental benefits and high incremental costs
Pereira A, 1999	Spain, Ministry of Health	P: hypothetical patients aged up to 70 I: SD-FFP C: untreated FFP	CUA (cost/ QALY gained)	Treatment of acute symptomatic hepatitis interferon treatment and follow-up of chronic hepatitis, decompensated cirrhosis, AIDS unit cost of SD-FFP and FFP	ICER: $ 2,156,398/QALY	Virus-inactivated plasma produces little incremental benefit and very high incremental cost due to low current risk of infection with transfusion-transmitted viruses, the advanced age and poor short-term prognosis of most plasma recipients
Blumberg N, 2000	USA, Baxter, Pall Biomedical, Ortho Biotech	P: N/A I: SD-FFP C: untreated FFP	CEA (cost/death avoided)	HIV, HCV, HBV antibody testing HIV antigen testing	ICER: $ 17 million/death avoided	SD-FFP was a comparatively expensive approach to reduce mortality from transfusion complications
CADTH, 2011	Canada, Not relevant	P: hypothetical 50-year old transfused patients I: Octaplas® C: FFP	CUA (cost/QALY gained) CEA (cost/LY gained)	Treatment of TRALI, HAV, HBV, HCV, HIV and their sequelae, prion disease, PVB19, cost per unit Octaplas® infusion FFP	ICER=$ 934,000 per QALY and $ 1.3 million per LYS	Octaplas® is not cost-effective, being associated with only a minimal reduction in disease burden at a higher cost than FFP
Riedler GF, 2003	UK, Octapharma UK	P: hypothetical patients aged up to 70 years I: Octaplas® C: FFP	CEA (cost/LY gained)	Treatment of TRALI, HIV, HCV, HBV unit cost of SD-FPP and FFP	ICER=£ 22,728/LY (neonates) ICER=£ 98,465/LY (70-year-old patients) ICER=£ 82,000/LY (65-year-old patients) Cost/LY <£ 50,000 (<48-year-old patients) Cost/LY <£ 30,000 (<21-year-old patients)	Inclusion of non-infectious complications suggests that SD-FPP is cost-effective in <48-year-old patients and in older patients with good clinical prognosis
Huisman EL, 2014	USA, Octapharma AG	P: hypothetical cohort of 50-year old transfused patients I: Octaplas® C: FFP	CUA (cost/QALY gained) CEA (cost/LY gained)	Treatment of TRALI, HAV, HBV, HCV, HIV and their sequelae Treatment of prion disease, PVB19, (severe) allergic reactions, bacterial infections and emerging infections unit cost of Octaplas® and FFP	Octaplas® dominant (base case) ICER=$ 16,159/QALY and $ 289,633/LY (excluding the risk of unknown emerging viruses) ICER=$ 771/QALY and $ 2,925/LY (using the historic price of FFP)	Octaplas® is dominant over FFP, producing cost savings and increasing QALY.
Huisman EL, 2014	Canada, Octapharma AG	P: Hypothetical cohort of 50-year-old transfused patients I: Octaplas® C: FFP	CUA (cost/QALY gained) CEA (cost/LY gained)	Treatment of TRALI, HAV, HBV, HCV, HIV and their sequelae Treatment of prion disease, PVB19, (severe) allergic reactions, bacterial infections and emerging infections unit cost of Octaplas® and FFP	Octaplas® dominant (base case, excluding unknown viruses even reducing allergic reaction by 50%) ICER=C$ 1.085/QALY and C$ 2.551/LY (reducing allergic reactions by 90%)	Octaplas® is dominant over FFP, producing cost savings and increasing QALY
van Eerd MC, 2010	UK, Octapharma UK	P: hypothetical cohort of critically ill transfused patients (age not reported) I: Octaplas® C: FFP	CUA (cost/QALY gained) CEA (cost/LY gained)	Treatment of severe allergic/anaphylactic reactions, TACO, TRALI, HIV, HCV, HBV, HAV and their sequelae unit cost of Octaplas® and FFP	ICER=£ 949/QALY and £ 1,030/LY (base case) ICER=£ 4,836/LY and £ 5.246/QALY (TRALI risk of FFP corrected for the use of male plasma only) ICER= £ 968,909/LY and £ 868,172/QALY (TRALI risk of FFP is based on SHOT)	Octaplas® represents good value for money versus FFP, which is considered the main comparator for critically ill patients who need a plasma transfusion

N/A: not available; FFP: fresh-frozen plasma; CUA: Cost–utility analysis; CEA: Cost-effectiveness analysis; QALY: quality-adjusted life years; LYS: life years saved; ICER: incremental cost-effectiveness ratio; TRALI: transfusion-related acute lung injury; TACO: transfusion-associated circulatory overload; AIDS: acquired immunodeficiency syndrome; HIV: human immunodeficiency virus; HBV: hepatitis B virus; HCV: hepatitis C virus; PVB19: parvovirus B19; HAV: hepatitis A virus; SHOT: Serious Hazards of Transfusion.

Blood Transfus 2016; **14:** 287-386 DOI 10.2450/2016.0065-16

age ($p<0.01$). Finally, Pereira[240] showed that the cost-effectiveness ratio is very sensitive to the cost of the virus-inactivation procedure.

It was not possible to perform a meta-analysis to increase the reliability of the results of this review because of the relevant differences in the intervention strategies considered and in the designs of the studies.

Organisational domain

Introduction

Methods for pathogen inactivation of plasma can improve the safety of transfusions, reducing the risk of possible transmissible infections, even those for which there are currently no screening methods available.

In the organisational domain, the implications of findings retrieved for the other aspects included in this assessment (i.e., efficacy and effectiveness, safety, economics, ethics, and social and legal/regulatory issues) were analysed, in terms of mobilisation and organisation of human and material resources and changes in processes and health care delivery.

Three different levels of analysis were taken into account: (i) within each blood centre, hospital blood bank, or industrial facility, (ii) among these structures, and (iii) at the level of the health care system (regional or national).

Health care systems and transfusion structures are all complex and different from each other. For this reason, organisational issues might vary depending on the context. Possible scenarios retrieved from experience in various settings that might be relevant in the Italian health care system are discussed in this section.

The aim of this section is to discuss the impact of the implementation of any kind of PRT for plasma (SD-FFP, MB-FFP, A-FFP, R-FFP) on organisational aspects (i.e. workflow, work process, patient flow, centralisation or decentralisation, human resources, training, collaboration, communication, teamwork, management), compared with the use of conventional FFP.

Methods

Sources

The organisational domain analysis drew on information from scientific literature retrieved from:

- Electronic databases:
  - MEDLINE via PubMed;
  - CRD databases:
    - DARE (Database of Abstracts of Reviews and Effects);
    - HTAi database;
    - NHS EED (National Institute for Health Research/Economic Evaluation Database);
    - Cochrane Library;
    - GIN (Guideline International Network);
    - NICE (National Institute for Health and Care Excellence);
    - TRIP (Transfusion and Transplantation Reactions in Patients).
- Other sources:
  - manual searches in references from selected literature;
  - regulatory institutions' or authorities' websites;
  - grey literature;
  - manufacturers' websites;
  - experts' opinion (clinician: Dr. Maria Bianchi, Blood Transfusion Department, "A. Gemelli" Teaching Hospital, Rome, Italy; industry: Kedrion S.p.A, Tuscany, Italy).

Search terms

The terms sought in all databases to refine the original search are specified below. No limits of publication date, language or study type were applied to the search strategy:

ORGANISATIONAL DOMAIN *"Organisational aspects", "organizational aspects", staff, staffing, personnel, manpower, human resource*, competenc*, skill*, train*, collaborate, collaboration, communication, teamwork, "team work", multiprofessional, "Health Care Facilities", manpower, services, "Health Personnel", "Delivery of Health Care", centralis*, centraliz*, decentralis*, decentraliz*, regionalis*, regionaliz*, "work process*", "work flow", workflow, management, managerial, "Patient Care", "Patient Care Team", "Patient Care Management", "patient flow".*

Inclusion criteria

Publications reporting any kind of information about organisational aspects were included.

Duplicates and publications considered not relevant based on the abstracts were excluded. Following the review of full texts, publications meeting inclusion criteria were considered for the analysis of this domain.

Quality appraisal

The main sources of information for the analysis were documents released by regulatory authorities such as Italian national/regional guidelines, European directives and Italian laws. This might entail a risk of bias, and can be considered a limitation of this review. Furthermore, information from experts (from clinical and industry backgrounds) might be limited by positions, knowledge asymmetry and time constraints. A quality assessment of the retrieved studies was not conducted.

Analysis

We identified relevant data through a qualitative review of included evidence. Secondary research was prioritised. The methodology used to summarise the retrieved evidence is a narrative review.

The structure of the analysis was based on clustering the four technologies under study and the comparator into three categories based on similarities and differences regarding their organisational impact: (i) FFP, (ii) "in-house" pathogen-inactivation methods, and (iii) industrial pathogen-inactivation methods.

Results

Results of the literature search

A total of 110 records were retrieved using the search process. Following review, three of these scientific papers were eventually included in the present analysis. The study selection process is illustrated in Figure 6. Further scientific papers and different types of literature were retrieved from other sources specified in the methods section and included according to inclusion criteria.

Additional scientific papers were retrieved from a manual search of the reference lists of the included studies. Further literature was taken from the TRIP and NICE databases, while no records were found in the GIN database. Legislative decrees and European Union directives were searched for in the websites of regulatory institutions or authorities. A total of 19 scientific documents were selected from these sources and 20 documents were retrieved from the websites of manufacturers or regulatory institutions.

Table LV lists the scientific papers included in the current review and indicates the respective topic[13,35,43,58,66,86,95,112,198,232,243-251].

Delivery process

The transfusion chain is a process that covers the whole pathway of plasma, from its extraction from the donor, to the actual transfusion and follow-up (Figure 7). The steps of this process that are common to all FFP, pathogen-inactivated or not, are the following: (i) collection; (ii) testing and other procedures to minimise risk of infections; (iii) preparation of FFP; (iv) storage; (v) distribution; and (vi) transfusion.

Collection

Whole blood collection and/or plasmapheresis are carried out in blood collection facilities.

Testing and other procedures to minimise the risk of infections

Blood donors are selected based on their history and clinical evaluation. The limits of selecting donors are well known, including those concerning the recognition of donors at risk of transmission of prions responsible for Creutzfeldt-Jakob disease (CJD) and, especially, its variant (vCJD), which appeared in recent years in some European countries[13]. Donors and blood units undergo systematic screening tests for a limited number of markers of transmissible diseases. Quarantine programmes allow the identification of viral agents possibly missed during initial screening. These encompass the preservation of frozen plasma for a period of at least 112 days (16 weeks), to be used only after receiving confirmation of the negative serological tests at the following donation[76].

Preparation of fresh-frozen plasma

Whole blood is fractionated by high speed centrifugation (preferably within 6 hours of collection and no later than 18 hours for cool blood units). Plasma is separated from the other blood components, and all products are packed separately in pre-connected bags. Collected plasma is rapidly frozen to –30 °C, which is the recommended temperature for storage. These procedures must be designed to avoid contamination and are performed either within hospital blood centres or in regional blood centres[86].

Companies authorised to fractionate blood collected in Italy and the locations of their respective industrial establishments are indicated in Table LVI[252].

Additional methods for pathogen inactivation of FFP can be implemented during the FFP preparation phase to enhance the prevention of infectious risks.

- The MB, amotosalen and riboflavin techniques are so-called "in-house" methods. Each single unit of FFP is thawed, the agent (MB, riboflavin or amotosalen) is added and, after incubation, each unit is subjected to light irradiation which activates the agent resulting in the inactivation of the pathogens. The product is then packed in the final bag and refrozen; its composition is comparable to that of FFP. Staff involved in the inactivation of plasma through any of these systems must be qualified laboratory technicians, who should process each bag in a maximum time of 15 minutes. This process can be carried out industrially or in blood bank facilities[86].
- Additional steps are involved in the SD method of pathogen inactivation; plasma bags from single donors must be sent from the facilities where the FFP is prepared to the industrial plant where the inactivation is performed. In Italy, bags from the southern and northern regions of Italy are initially stored in two intermediate industrial deposits, whereas bags from central regions and Sardinia go directly to the plant. Once the bags of plasma have

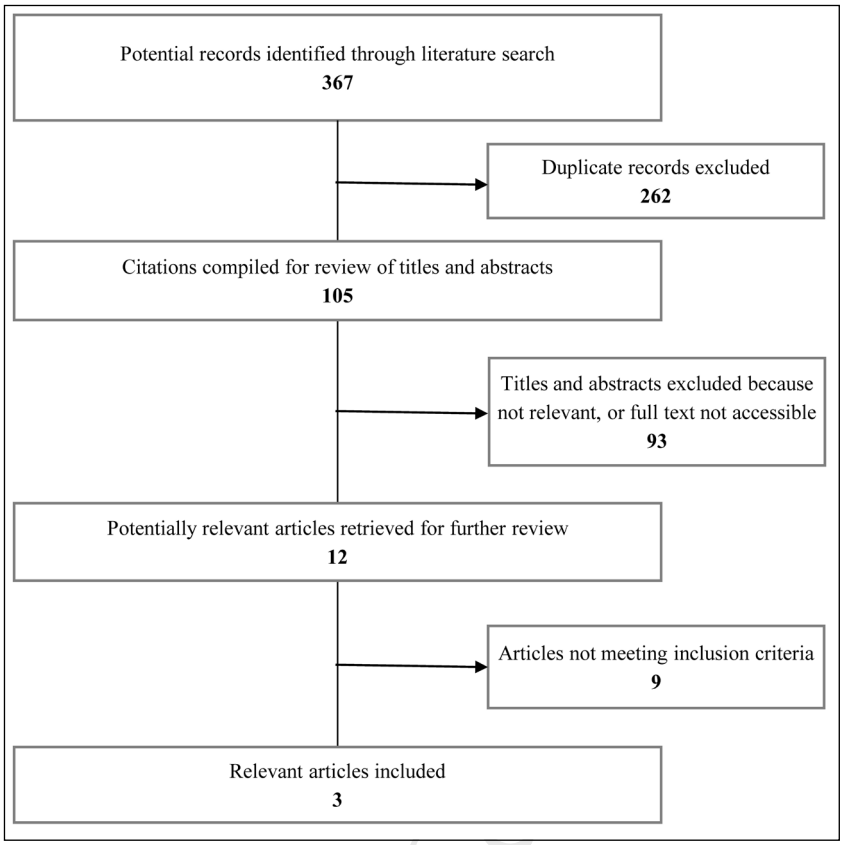

Figure 6 - Study selection process for organisational domain.

Table LV - Papers included in the organisational domain analysis and related topics.

First Author, year	Country	Related topic
British Committee for Standards in Haematology, 2004[13]	United Kingdom	Delivery process
MacLennan S, 2006[198]	United Kingdom	Delivery process/culture
Jimenez-Marco T, 2014[58]	Spain	Delivery process
Seltsam A, 2013[86]	Germany	Delivery process
Rock G, 2011[35]	Canada	Delivery process/structure
Ofosu FA, 2008[243]	Canada	Delivery process
Liumbruno GM, 2011[244]	Italy	Delivery process/structure
Biesert L, 1998[112]	Switzerland	Process-related costs
Hellstern P, 2004[245]	Germany	Process-related costs
Sharma AD, 2000[246]	USA	Process-related costs
Solheim BG, 2006[66]	Norway	Process-related costs
Koenigbauer UF, 2000[247]	USA	Process-related costs
Ruggeri M, 2014[248]	Italy	Process-related costs
Pamphilon D, 2000[95]	United Kingdom	Management
Solheim BG, 2008[232]	Norway	Management
Vamvakas EC, 2010[249]	USA	Management
Klein HG, 2007[43]	USA	Culture
Klein HG, 2009[250]	USA	Culture
Alter HJ, 2008[251]	USA	Culture

arrived at the industrial facilities, they undergo a physical/visual quality check and document control. Batches of 60-380 L of plasma are then pooled to create a standardised biopharmaceutical product with uniform plasma protein concentrations, through a process that requires 180 minutes. A sample of the pool is tested for infections and the inactivation process is applied to the whole pool. The plasma is then packed and refrozen[86].

All industrial processes must respect quality requirements set by the European GMP and must obtain Plasma Master File certification[253-255].

Storage

FFP inactivated by "in-house" methods is kept at all times in hospital blood banks, whereas industrially pathogen-inactivated FFP units are stored in interim warehouses belonging to the company before being distributed to hospital blood banks.

The storage temperature of all FFP, inactivated by any method, is maintained at all times at –20 ºC or lower in accordance with WHO directions[76,256,257]. Temperature conditions are monitored and recorded throughout the supply chain.

In addition, FFP undergoing SD treatment is stored and transported in compliance with the European

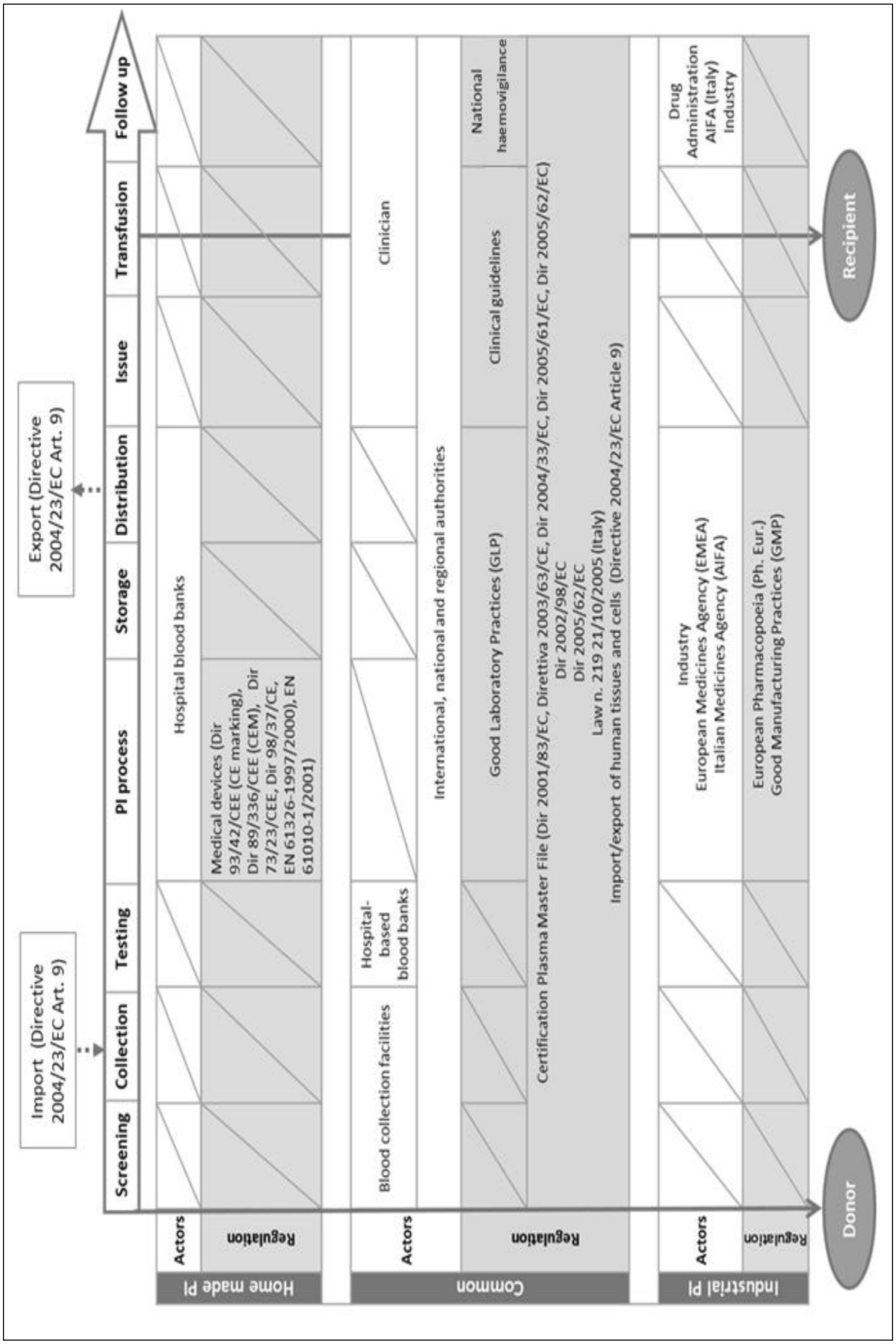

Figure 7 - Transfusion Chain: differences between PI methods.
PI: plasma inactivatioon.

Pharmacopoeia monograph "Human Plasma for Fractionation" (0853).

Distribution

In Italy, SD-FFP plasma units are distributed by the industry in an on-demand basis to hospital pharmacies/ blood transfusion departments, or delivered to regional pharmaceutical depots, depending on the type of distribution model (decentralised or centralised, respectively). In both cases, the same amount of plasma collected is returned, excluding bags that might have not met quality checks. In order to respect the cold chain, the plasma is transported in certified vehicles at a temperature of –20 °C or below[76,256,257].

"In-house" inactivated plasma, on the other hand, does not require transportation between facilities, since the pathogen inactivation process is carried out within the hospital blood centre.

Transfusion

The decision to perform a transfusion must be based on clinical practice guidelines and determined by the treating clinician[13], who requests blood units from the hospital's transfusion medicine department.

A qualified specialist in transfusion medicine supervises the entire process and decides which blood product to use (SD-FFP or "in-house" inactivated FFP). Frozen plasma may be thawed only when it is absolutely certain that the transfusion will be performed. Each unit must be immersed in a water bath at a maximum temperature of +37 ºC or it may be kept for a maximum of 6 hours at +4 ºC before transfusion if it is intended to improve a coagulopathy[256].

However, not all patients requiring transfusion are eligible for all types of inactivated plasma. MB-FFP is contraindicated in pregnant and nursing women, premature babies and for intrauterine transfusions, patients with severe renal failure, patients with methaemoglobinaemia associated with glucose-6-phosphate dehydrogenase deficiency, and patients with congenital deficiency of glucose-6-phosphate dehydrogenase[16]. A-FFP is contraindicated in patients with a history of allergy to amotosalen or psoralens. The contraindications to R-FFP and SD-FFP are the same as those to FFP[58,86]. SD-FFP has been recommended by clinical guidelines for a series of patients, including those with TTP and other thrombotic microangiopathies, and patients with acquired coagulation factor deficiencies, probably because of the greater standardisation of its content of these factors[232].

FFP units that have been issued but not used must be disposed of by the hospital transfusion laboratory; they must not be refrozen or reissued to another patient[256].

Throughout the transfusion chain, many stakeholders cooperate and interact in the delivery process of FFP:

- international, national and regional authorities;
- blood establishments; i.e., any structure or body that is responsible for any aspect of the collection and testing of human blood or blood components, whatever their intended purpose, and their processing, storage, and distribution when intended for transfusion;
- hospital blood banks; i.e., a hospital unit which stores, distributes and may perform compatibility tests on blood and blood components exclusively for use within hospital facilities, including hospital-based transfusion activities;
- hospital transfusion medicine departments;
- companies and drug authorities involved in industrial pathogen-inactivation of plasma (Table LVII).

Quality assurance

Quality must be assured and monitored throughout the process at the different levels (health care system, within and between blood establishments, blood banks, transfusion medicine departments, and industry) following national and international standards.

All plasma and plasma products

In Europe, Directive 2002/98/EC[254,257-263] of the European Parliament and of the Council of 27th January 2003, amending Directive 2001/83/EC[253], sets standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components. The Directive 2005/61/EC[267] of 30th September 2005 implements Directive 2002/98/EC[254] with respect to Community standards and specifications relating to a quality system for blood establishments.

Activities related to blood components and the production and transfusion of plasma derivatives are regulated in Italy by Law n. 219 of 21st October 2005, "New legislation on transfusion activities and national production of blood derivatives"[265].

Plasma suppliers are monitored in compliance with the agreement "Accordo Stato-Regioni" dated December 16th 2010 (Legislative Decree 281/1997, article 4[266]),

Table LVI - Companies authorised to fractionate blood collected in Italy and the locations of their industrial plants.

Company	Location
Baxter Manufacturing S.p.A	Rieti, Italy
CSL Behring S.p.A.	Bern, Switzerland
Grifols Italia S.p.A	Barcelona, Spain
Kedrion S.p.A.	Bolognana, Gallicano and Lucca, Italy
Octapharma Italy S.p.A	Stockholm, Sweden

Legislative Decree 261/2007[259] and Legislative Decree 191/2005[258] (implementing Directive 2002/98/EC). Eligibility criteria for blood/plasma donors are determined in compliance with the Decree of the Ministry of Health of 3rd March 2005 "Protocols for determining the suitability of blood and blood components donors"[267] (implementing Directive 2004/33/EC), with the Legislative Decree of 20th December 2007, n. 261[259] (which revokes Legislative Decree of 19th August 2005, n. 191[258]), and the Guide to the preparation, use and quality assurance of blood components of the European Council[10].

GMP, denoting minimum organisational, structural and technological requirements for health care institutions that offer blood transfusion and/or blood collection services (blood establishments), are also determined by the above-mentioned agreement[266]. In accordance with the stipulations, each Italian Region, as a competent authority, assesses blood establishments and hospital blood banks, and grants or denies them authorisation to operate. Moreover, the Regions carry out periodic inspections in their blood establishments and blood banks at least every 2 years[277] (see Box 1).

As far as concerns the risk of vCJD, donor exclusion criteria are specified in the "Protocols for determining the suitability of blood and blood components donors"[267] as determined by the Italian Ministerial Order dated 29th March 2004[268]. Furthermore, the Italian Health Institutions' Position Paper "Management of reports of CJD infected donors"[269] and the Committee for Medicinal Products for Human Use Position Statement on Creutzfeldt-Jakob disease and plasma-derived products (EMA)[270] do not allow the clinical use of plasma and plasma-derived medicinal products after donation reports of CJD.

"In-house" pathogen-inactivated plasma

Pathogen inactivation by "in-house" methods is performed in accordance with the European Directive 93/42/EEC[271]. Equipment, kits and reagents are CE-labelled and are, therefore, subject to the regulations for medical devices entering the market. Illumination equipment must conform with Directives 89/336/EEC (EMC), 73/23/EEC, and 98/37/EC and norms EN 61326-1:1997/2000, EN 61010-1:2001[272-276] (security rules for electrical apparatus for measuring, regulation and the laboratory).

Industrially pathogen-inactivated plasma

In Europe, plasma must comply with European Pharmacopoeia Monographs and Good Manufacturing/ Laboratory Practices.

Individual donations are tested for infective agents by biological qualification laboratories in accordance with the European Pharmacopoeia monograph on Human Plasma for Fractionation (0853). Inspection and approval according to GMP are required for the processing, storage and transport of blood and blood components, including plasma, to be used for manufacturing medicinal products such as SD-FFP[278,279].

Table LVII - Companies and reference markets involved in pathogen-inactivation.

Pathogens	Companies	Methods	Marketing approval
Viruses Bacteria Parasites	Cerus	Amotosalen + UV	CE
	Caridian	Riboflavin + UV	CE
	MacoPharma	Methylene blue	CE
	Baxter	Methylene blue	CE
	OctaPharma	Solvent/detergent	AIC (Italy)
	Kedrion	Solvent/detergent	AIC (Italy)

The plasma product requires Plasma Master File certification, EMA/CPMP/BWP/4663/03. The Plasma Master File contains common information on plasma, from collection to the plasma pool, relevant to the manufacture of all intermediate fractions including cryoprecipitate, all constituents of the excipient and all active substance(s), which are part of medicinal products or medical devices, for which such a file is applicable.

Structure

As stated in the previous section, the process of donor recruitment and screening, and collection and preparation of blood units are performed in a primary care setting, i.e., blood establishments or hospital blood banks.

Infectious disease tests are performed in blood establishments where "in-house" pathogen inactivation procedures are performed[35].

The SD-FFP method for pathogen inactivation is standardised and carried out at a centralised manufacturing centre where plasma is processed as a large pool (500-2,500 donations) and provided to users frozen in 200 mL bags[35].

The settings in which "in-house" technologies can be used are different from those for the SD process, which can only be carried out in industrial establishments. The procedure for obtaining SD-FFP involves plasma inactivation in centralised laboratories with storage in centralised depots, allowing economies of scale and streamlining of the activity of laboratories in hospital blood banks.

The most important drivers of economies of scale are:

- centralisation of large plasma stocks in a single storage site, thanks to the possibility of long-term conservation of plasma (2 years);
- the cost of training production chain staff, who are specialised in the activities of the pathogen

Box 1 - The entry into force of the State-Regions Agreement of 16 December 2010.

Law n. 10 of 26th February 2011 determined the procedures for plasma and all other blood products to be compliant with European standards, to be adopted before 31st December 2014 (later extended to 30th June 2015), according to Law n. 219 of 21st October 2005, which resolved fundamental guidance regulating the production of pharmaceutical products derived from plasma collected within the Italian territory, as well as import and export of blood and blood products.

The agreement between the Italian State and the Regions defined the "Structural, technological and organizational requirements for health care institutions that offer blood transfusion and/or blood collection services" and the "Model for monitoring visits of blood establishments", as disposed by Legislative Decree n. 261 of 20th December 2007 (Art. 5), incorporated the European Directive 2002/98/EC.

Along with decree n. 261/2007, the State-Regions agreement stipulates that certification of compliance with European structural, technological and organisational requirements be achieved through regional processes of authorisation and accreditation. It is thus mandatory that all regions and autonomous provinces check facilities on-site at least every 2 years, by means of a regional monitoring team consisting of at least one registered evaluator approved by the National Blood Centre).

With Legislative Decree n. 216 of 29th December 2011, the Ministry of Health:

(i) established the national list of evaluators for the transfusion system, through the National Blood Centre, who can perform the tasks required by Legislative Decree n. 261 of 20th December 2007 (Art. 5).

(ii) defined, for the issuance of the Ministerial Decree stipulated by Law n. 96 of 4th June 2010 (Art. 40, Par. 4), submission procedures to be used by blood establishments and pharmaceutical companies for obtaining assessment by the Italian Medicines Agency (AIFA)

(iii) decided, pending the complete implementation of the State-Regions agreement to be ready by 31st June 2015, the procedures for AIFA to assure the marketing of pharmaceutical blood products obtained from plasma collected in the Italian territory, as well as the export of these products for processing within European Union countries, while the National Institute of Health ensures state monitoring.

inactivation procedure in the industrial setting;
- the cost of acquisition of sophisticated technologies;
- all overhead costs that are incurred in the production chain.

In addition, a centralised storage site enables a more streamlined system of governance of the plasma, in which production, consumption and distribution are planned and programmed at the governmental level.

As stated in the previous paragraphs, the centralisation of pathogen inactivation also involves standardisation of procedures that reduce associated risks and facilitate control[255,278].

Process-related costs

The most important cost drivers in all processes are related to adverse events derived from plasma transfusion and the concentration of coagulation factors and other plasma proteins in plasma.

Many studies have demonstrated that the dilution and neutralisation of antibodies and allergens during the industrial process of SD plasma pooling can reduce the incidence of allergic reactions and TRALI in recipients. It has been reported that the transition from FFP to SD-treated plasma significantly reduces the incidence of adverse reactions, and subsequently involves significant savings. A study by Ruggeri *et al.* (2014)[248] demonstrated that the savings per patient arising from the use of SD-FFP (*vs* FFP) were between € 2,800 and € 3,200 in the case of a mild reaction, between € 2,500 and € 4,600 in the case of a moderate reaction and between € 34,000 and € 44,000 if resuscitation procedures were needed. We did not identify studies investigating this aspect in relation to "in-house" methods.

SD-FFP generates better standardisation of the content of clotting factors and greater effectiveness on coagulation tests compared to FFP and MB-FFP, eliminating the biological variability across single units of FFP[13,280]; therefore, the use of SD-FFP is particularly recommended in special populations such as TTP patients and liver transplantation candidates.

Management

The key areas in which to identify critical management issues and opportunities of each of the five methods are transfusion chain management and appropriate staff training in withdrawal/collection of plasma, pathogen inactivation (in either blood centres or industrial plants), transport and cold chain management, thawing and patient management for the transfusion.

Our analysis shows that the organisational model and network generated around SD-FFP creates a better structured and controllable organisational environment, due to centralisation in industries managing the main steps of the transfusion chain (processing, storage and distribution). This greater control is of benefit not only to the safety of the final product (industrial pathogen inactivation is guaranteed by GMP authorisation from national health authorities), but also to the planning of regional and national governmental bodies that have the goal of ensuring plasma self-sufficiency[13,241,245].

Culture

A series of concerns within the medical community limited the acceptance and diffusion of pathogen inactivation when it was an emerging technology. First, there were doubts about blood safety including risks

resulting from the presence of residual chemicals in the final product. The limits of the technologies (inability to inactivate agents such as spores, prions, and certain small non-encapsulated viruses, and non-existence of a method able to treat whole blood or all components) led to the value and cost-effectiveness of PRT being questioned. So did the success of the control of emerging pathogens with the routine strategies of surveillance and screening. PRT were not recommended back then on the evidence available alone[43].

It was important to the scientific community to balance the introduction of PRT against the risk of giving rise to new unwanted effects such as loss of coagulation factors, toxicity and neoantigenicity[251].

Even after licensing in Europe and in other countries, and given delayed regulatory approval in the USA and Canada, the medical community agreed on the importance of post-marketing surveillance studies, recommended by regulatory authorities and supported by manufacturers and/or blood suppliers. A system of surveillance for adverse reactions linked to the national haemovigilance systems, as well as further research in terms of development of novel PRT, seem to be necessary to improve blood safety[250,281,282].

Nonetheless, there was a demand for a transition to a proactive approach to the prevention of TTI, given their continued emergence or re-emergence and the fact that the interval between the first recognition that a disease is transfusion-transmitted and the eventual implementation of a donor-screening test to prevent that transmission, had historically been long. This perception suggested that the risk-benefit balance and cost-effectiveness could be positive in the long run[283].

A large amount of evidence on the safety and efficacy of PRT has since been accrued. The gradual introduction of PRT into guidelines worldwide and their consequent regulation can be taken as a measure of the adoption and diffusion of these technologies.

Discussion

The analysis of organisational aspects was carried out by dividing infectious risk prevention procedures into three groups: traditional FFP, "in-house" pathogen inactivation procedures (grouped together and assumed to have the same organisational impact) and the SD-FFP industrial method.

Few studies have investigated organisational aspects of PRT; in fact, most of the information was retrieved from documents released by monitoring authorities, grey literature, and manufacturers' handbooks. This creates a risk of bias due to insufficient or selective inclusion of information sources and data, and can be considered as a limitation of our review. Organisational changes emerging from the adoption of PRT occur mainly in the stages of processing, storage and distribution of plasma.

"In-house" pathogen inactivation procedures are performed in blood establishments on single donor bags, blood units are stored in the hospital blood bank, and transport between facilities is not, therefore, required but enough storage space must be available. On the other hand, industrial pathogen inactivation procedures require that collected blood is transported to and from the manufacturer's facilities implementing logistics for the maintenance of the cold chain, while pathogen inactivated blood units are stored in interim depots before being distributed to hospital blood banks.

The strengths of PRT linked to these differences are that plasma undergoing "in-house" pathogen inactivation procedures is not pooled, thereby lowering the risk of a whole plasma pool being contaminated by a single infected bag of plasma, whereas the industrial method guarantees standardisation of the final product.

With regards to quality control, SD-FFP is a pharmaceutical product, whereas "in-house" procedures use medical devices and the resulting plasma is not considered a drug. This fact underlies the difference between the regulatory systems for the two groups of technologies.

In Europe, quality control and validation of procedures for producing pathogen-inactivated FFP are guided by the guidelines of the European Community and the EMA, under State Laboratory control and marketing authorisation issued by national health authorities. This explains its wide and growing use in transfusion practice in Europe and the USA as an alternative to FFP that has not undergone pathogen inactivation.

Italian transfusion services can currently cover the supply of pathogen-inactivated FFP in three ways: (i) purchasing it from private manufacturing companies which produce and distribute such FFP in various European countries; (ii) sending plasma collected in hospital blood banks/blood establishments to a private company with appropriate facilities for SD treatment, which then returns the product to hospital blood banks for clinical use; (iii) producing the FFP in an accredited hospital blood bank laboratory with specific kits and machines using "in-house" methods.

Ethical domain

Introduction

The ethical domain has been identified as a key element of HTA since its conception in the 1970s. This domain aims to analyse the ethical questions raised by the use of a given technology: since technologies are always introduced into societies or organisations with their own set of values, the implementation of the technologies can have ethical consequences.

The second purpose of the ethical domain is to analyse in detail the ethical issues that are inherent in the HTA process. In this sense, an ethical analysis should not be considered as a "one session" task, but rather as a support through the whole HTA process, from the prioritisation of topics, to the definition of research questions, and to the choice of methodologies to summarise results and draw conclusions.

The present study does not include this analysis. However, the working group on ethics oversaw the whole HTA process, particularly checking that no conflicts of interests were present and that the choice of both end-points and comparators was "ethically" justified (see Introduction).

Methods

A considerable number of models and frameworks can be used to conduct ethical assessments in HTA[289-292]. The EUnetHTA model was chosen from among several possibilities[233]. This model is based on six different topics, which together cover nineteen issues. The issues stem from the general values of the population, the aims of the health care system and the values arising from the use of a technology. This ethical evaluation was conducted by a team including four bioethicists (one with a background in philosophy and three with medical training).

Each issue was first clarified, and then its relevance considered, information sources were identified, and the literature was searched. Not all issues were considered relevant. The information was gathered from the following sources: the ongoing assessment of safety, efficacy and cost-implications; the literature; and philosophical analysis of the logic and coherence of the argumentations.

The results of the analysis do not explicitly include the set of issues. The answers to the set of questions have been summarised and organised into paragraphs.

Literature search

A three-part literature search was applied: (i) all participants in the HTA project were requested to notify the ethics group if they found contributions discussing ethical issues. One article was notified; (ii) a search was made for HTA reports on the topic in online databases. Fifteen references were found, of which ten were available; and (iii) PubMed and the Cochrane Library were searched for articles on ethical topics relating to PRT for plasma.

The PubMed query was based on the following scheme: ETHICAL DOMAIN *(((((((ethics[MeSH Terms]) OR ethics[MeSH Subheading]) OR "ethics, istitutional"[MeSH Terms]) OR "ethical aspects") OR "ethical issue") OR "ethical consideration") OR ethic*))*.

Overall, the search yielded 916 contributions from scientific journals: four focused searches relating to specific issues were found in PubMed. Sixty-four articles were considered of possible interest.

Information selection

The contributions notified by all participants in the HTA project were considered not relevant to the purpose of the ethical domain. Instead, the contents of ten HTA reports were scrutinised. This activity revealed that none, except for the contribution from the Health Council of The Netherlands[288], contained a section on the ethical domain. However, even the contribution of the Health Council of The Netherlands was considered not relevant.

Concerning the articles in PubMed and the Cochrane Library, titles and abstracts of the 916 were screened by two independent reviewers to verify whether they contained information on "ethical" topics relating to PRT for plasma. No reference was considered relevant.

Results

Two specific contributions on ethical issues relating to use of PRT for plasma were found but were considered not relevant. Seventeen articles were considered as useful but not specific[289-305]. The ethical assessment was, therefore, essentially based on discussion of the issues under question, which relied on information from the other sections of the current report.

The morally relevant issues identified were first summarised and then organised into paragraphs.

Pathogen reduction technologies for plasma

Human plasma is used as a therapeutic product or as source material for the production of pharmaceutical fractionated products. An optimal plasma product should not only have a satisfactory content of coagulation and antithrombotic factors, but also be as safe as possible, reducing the risk of transfusion reactions and minimising the transmission of infectious agents.

The development of increasingly sensitive laboratory screening methods and restrictive donor criteria have greatly decreased the risk of transmission of many pathogens through blood transfusion. In this context, the issue of transfusion-associated infections with HIV, HBV and HCV seems to be adequately managed.

Residual risks do, however, always remain. These particularly concern pathogens for which no detection method exists (i.e. emerging viruses) and the so-called "window period", in which blood levels of a disease marker are too low for detection[88]. In other words, the risk of transmission is not static, as new pathogens continue to emerge, old ones change their properties and epidemiological patterns, and new information and technologies become available to change our understanding of the risks.

Since the beginning of the 1990s, considerable progress has been made in reducing or preventing the transmission of blood-borne pathogens in plasma by implementing different methods, i.e. PRT. These seem to be a promising alternative to expanded testing of FFP and donor deferral.

The present assessment aims to analyse the ethical questions raised by the use of the four PRT currently available for plasma, i.e. SD, MB, amotosalen and riboflavin.

Beneficence/non-maleficence

The first step in conducting an ethical analysis concerning health technologies consists in considering risks (principle of non-maleficence) and benefits (principle of beneficence) for patients and the society. The term "risk" refers to the possibility that harm may occur, while the term "benefit" refers to something of positive value related to health or welfare. In other words, people are treated in an ethical manner by protecting them from harm, but also by making efforts to secure their well-being. In some cases, the calculation and weighting of risks and benefits can be difficult.

With respect to the risk-benefit ratio of using SD, MB, amotosalen and riboflavin, the major concern regards their impact on the integrity of blood components and the toxicity of the chemicals utilised. Ideally, the active substances in PRT should be selectively toxic to a broad range of pathogens but, at the same time, non-toxic to blood cells/proteins and transfusion recipients.

PRT have been subjected to preclinical and clinical testing (see the sections on efficacy and safety). Available data suggest that they provide adequate safety margins: in particular, the safety of the techniques in relation to TTI is high and the number of other adverse events associated with the various methods is low. Nevertheless, long-term side effects still remain a matter of debate[88] and studies are particularly difficult to perform in this case.

Furthermore, PRT seem to be effective overall in increasing blood safety, although more robust data would be desirable.

In the context of further studies on the safety and efficacy of PRT for plasma particular attention should be paid to haemovigilance monitoring and controlled post-marketing studies. In fact, only the latter are able to demonstrate the added value of these technologies and, in the near future, support the identification of the safest and most effective PRT.

Every effort should be made to comply with the European Union Directive 2002/98/EC, which states that: "*it is important to introduce a set of organised surveillance procedures to collect and evaluate information on the adverse or unexpected events or reactions resulting from the collection of blood or blood components in order to prevent similar or equivalent events or reactions from occurring thereby improving the security of transfusion by adequate measures. To this end a common system of notification of serious adverse events and reactions linked to the collection, processing, testing, storage, and distribution of blood and blood components should be established in Member States*".

To sum up, although the conclusion that the risk-benefit ratio of using PRT is favourable should be consolidated through additional data, it seems that the clinical use of these technologies can be considered justified from an ethical point of view.

Autonomy

The second step in conducting ethical assessments concerning health technologies regards the patient's freedom and responsible choice. This aspect is connected to the issue of informed consent, an essential prerequisite to the beginning of any medical intervention.

Informed consent is generally considered as a process by which the health care provider discloses appropriate information to a competent patient, so that the patient can make a voluntary choice to accept or refuse the treatment. In this sense, informed consent is connected with the principles of autonomy and the issue of self-determination.

In order to be valid, informed consent requires that the individual has the capacity to make the decision, that the choice is voluntary, that the patient is provided with appropriate information, in a format that he or she can understand, regarding the benefits, risks, consequences and alternatives to the proposed treatment, and that the individual's decision is correctly documented[289-293].

In the field of transfusion medicine, there is lively debate on whether separate informed consent should be obtained from patients for transfusion of blood/blood components and plasma-derived medicinal products[294-302]. However, it is generally agreed that the minimum elements to be discussed with a patient before requesting his or her consent to a transfusion should include[303]:

- clarification of the reason for the transfusion, i.e. the purpose and anticipated benefit of the transfusion, and the consequences of not having the transfusion;
- the alternatives to transfusion, which may include the possibility of an autologous transfusion;
- clarification of the risks associated with transfusion;
- patients should also be given the chance to ask questions and should be informed of their right to refuse a transfusion.

In Italy, written informed consent or dissent is requested for transfusion of blood or plasma-derived medicinal products[306].

As noted, before a patient is asked to consent to a transfusion, the risks associated with the transfusion must be clarified. Therefore, patients should always be informed that more "equivalent" methods exist for pathogen reduction and which method is used. Furthermore, patients should be provided with appropriate information, in a format that they can understand, regarding the potential benefits and risks associated with the method (including the potential long-term side effects). Finally, they should be informed that transfusion of pathogen-reduced blood products is safe, even if a residual risk always remains.

Justice and equity

In health care systems based on the idea of solidarity (i.e. National Health Services), one of the main principles for the provision of care is guaranteeing equal access to effective care for those in need. A scarcity of health care resources does, therefore, necessarily lead to cost-effectiveness considerations and the prioritisation of health care policies. In this sense, the use of a certain technology may influence the fairness of the health care system, or require special considerations in order to ensure that justice is not compromised. Moreover, in order to achieve maximum uniformity in health care policy-making and to guarantee equal access to effective care, harmonisation would seem to be desirable when making decisions about the use or non-use of a certain technology.

The available cost-effectiveness studies on different pathogen inactivation approaches show contradictory and sometimes incomparable results. Most of these studies are, in fact, limited to the comparison of only two different PRT, i.e. SD and riboflavin. Given that the generalisability of available evidence is limited, it is not possible to determine whether the implementation of PRT fulfils the criteria of equal resource allocation, or, in other words, to state whether broad implementation of any of the technologies is acceptable in terms of justice. Thus, cost-effectiveness studies on PRT for plasma need to be performed and new data should be collected. Only such studies will be able to demonstrate the added value in economic terms of these technologies and, potentially, identify the most cost-effective one.

Discussion

From the ethical point of view, important points to be considered during decision-making about the use of PRT for plasma are that: although the risk-benefit ratio of using these technologies seems favourable, this conclusion should be consolidated through additional data; before a transfusion of pathogen-reduced blood products, patients should always be informed about the method used and its potential benefits and risks, clarifying that a minimal risk always remains: and it is still uncertain whether the implementation of PRT fulfils the criteria of equal (just) resource allocation.

Legal domain

Introduction

The legal domain aims to analyse the juridical questions and aspects raised by the use of a certain technology, in this case those technologies concerning blood use. Since technologies are always ruled by European Directives and National Decrees with a set of norms, their implementation has legal implications. Some issues are directly related to the patient and his or her basic rights, such as autonomy, informed consent, protection of data and quality management. Other issues are linked to the technology, such as authorisations, equality in health care access, acquisition processes, high standard settings, product safety, guarantees, control and harmonisation of PRT for plasma. However, some of these elements were considered not relevant to this research and are not analysed here.

The analysis of legal aspects was carried out at national and European levels. In Europe blood directives define quality and safety standards for the testing, processing, storage and distribution of human blood and blood components. Thus, various regulations and documents of the European Union and the Council of Europe were taken into account.

Methods

Information sources

Legal aspects of HTA concerning PRT for plasma were examined through an analysis of legal texts of the European Union.

Our work was based on finding and reviewing the main European regulations. These belong to a large, complex, heterogeneous set of biojuridical material generated within the European Union and the Council of Europe. This documentation is diversified in terms of force (some documents are binding, others simply steering) and origin (the bodies of enactment differ). However, despite different degrees of detail, we found common elements in the discipline of information and consent, protection of privacy, and access to treatment.

A considerable number of models and frameworks can be used to conduct legal assessments within the HTA framework[284,287]. Among several possibilities, the EUnetHTA Core Model was chosen[233]. This model is based on seven different topics.

The legal evaluation was conducted by a group composed of three bioethicists, two of whom are jurists and one a physician.

Information selection

Different European and national legislative documents were analysed. In principle, the European Union regulatory framework, which sets high standards for establishing pathogen reduction and harmonising the procurement of safe blood in Europe, is still under development. Since the early 1990s, SD-FFP has been considered as a blood product in Germany and in France. In most of the other European countries it used to be considered as a medicinal product, and it was often permitted during the registration process on the basis of its obvious viral safety, although no efficacy against non-enveloped viruses had been found.

SD-FFP was first introduced in 1991 in the German Federal State of North Rhine-Westphalia, and replaced FFP in 1993 following a successful clinical trial in 1992 in Norway. Today, ordinary medicinal licensing is required for SD-FFP in European countries except for France[307], where it is classified as a labelled blood product. Octaplas® is now a licensed biopharmaceutical product in 29 countries worldwide. Octapharma has also developed Uniplas®/UniplasLG®, which differs from Octaplas®/OctaplasLG® only by the fact that anti-A and anti-B are removed, meaning that it can be universally transfused to patients of any blood group. Uniplas®/UniplasLG® is not licensed in the USA or the European Union.

Several European countries have established national programmes to monitor the occurrence of different adverse events. Unfortunately, in countries in which different methods for safeguarding plasma are used in parallel, adverse events are not always differentiated with respect to the type of plasma used, making it difficult to interpret and compare data accurately. The French haemovigilance report of 2009 described a relatively higher incidence of allergic events connected to MB-FFP than to Q-FFP. The authors of a French case report found evidence of potential cross-reactivity between patent blue V, a food colourant widely used in Europe, and MB, which may explain such allergic reactions to MB-FFP. This is in contrast with previous publications. Recently, allergic reactions in patients receiving blood products have become a special focus of interest in the French haemovigilance programme. It should be specified that this focus has emerged from sentence n. 357463 of the *Conseil d'Etat*, between MacoPharma and the *Agence Française de Sécurité Sanitaire des Produits de Santé*[308]. The available information from Belgium, indicating no difference between the safety profiles of MB-treated and untreated plasma, is the most promising.

Results

Authorisation and safety

Numerous legislative acts regarding the quality, control, safety and traceability of technologies have been produced at national and European levels.

In the European Union, Directive 2002/98/EC outlines particular requirements for the processing of blood supplies and sets out standards for blood establishments in reference to responsibilities, quality management, documentation, record keeping, traceability, adverse events, storage and transport. This Directive states that *"in order to safeguard public health and to prevent the transmission of infectious diseases, all precautionary measures during their collection processing, distribution and use need to be taken making appropriate use of scientific progress in the detention and inactivation and elimination of transfusion transmissible pathogenic agents"*[254].

In addition, Directive 2001/83/EC[253] of the European Parliament and of the Council of 6th November 2001 on the Community code relating to medicinal products for human use and European Union Directive 2004/33[263] set out technical requirements for blood and blood products in all Member States and defined some of the aforementioned standards. The treatment of human blood is regulated in Directive 61/2005/CE[264], adopted in Italy through Legislative Decree n. 207 of 9th November 2007[260] (published in the Official Journal of the Italian Republic n. 261 of 9th November 2007). Directive n. 61/2005/CE[264] had, in turn, implemented the previous Directive 2002/98/CE[254], concerning quality and safety standards of the collection, testing, processing, storage and distribution of human blood and blood components. Moreover, Directive n. 62/2005/CE[262] was adopted by the Italian Legislative Decree n. 208 of 9th November 2007[261] (Official Journal of the Italian Republic n. 261 of 9th November 2007 - Ordinary Supplement n. 228). Directive 62/2005/CE[262] implements the previous Directive 2002/98/CE[254] regarding the quality and safety of whole blood and blood components.

The quality and safety of technologies are regulated by articles 20 and 25 of the Italian Legislative Decree 261/2007[259] and, at the European level, by Directive 2002/98/CE[254]. In particular, specific guidelines on the quality and safety of blood components (such as tests to be performed; information provided and obtained from donors; information regarding the suitability of blood and plasma donors and screening of donated blood) are provided by articles 16-23 of Directive 2002/98/CE[254]. Articles 11-13 define a quality system for blood establishments and the requirement to keep a record of the previous year's activity.

Indeed, in accordance with Annex II of Directive 2002/98/CE[254], 22 Member States receive annual

reports from blood establishments on the previous year's activities. Italian Law n. 219/ 2005[265] defines, in clause 5, the basic package of health care services to be provided in the field of transfusion activities, relying on specific agreements within the Permanent Conference for Relations between the State, the Regions and the Autonomous Provinces of Trento and Bolzano on the respective responsibilities for the uniform delivery of transfusion services included in the basic package, across the country (Clause 6 Law 219/2005)[265].

Clause 5 of the Italian Legislative Decree 261/2007[259] states that "*The Regions and the Autonomous Provinces, in order to implement the legislation in force, shall organise inspections and appropriate control measures in blood establishments and collection units, with the aim of verifying their compliance with the requirements*".

Moreover, additional obligations for Member States are established by clauses 5-8 of Directive 2002/98/EC[254]. Clause 5 states that "*Each Member State must ensure that activities relating to the collection and testing of human blood and blood components, whatever the intended purpose, and to their preparation, storage and distribution when intended for transfusion, are undertaken only by the competent authority for that purpose*".

Clause 6 regulates hospital blood banks, while clause 8 defines the inspection and control measures. Each *"Member State must ensure that the competent authority organises inspections and appropriate control measures in blood establishments to check that the requirements of Directive 2002/98 are complied with"*.

With regards to the need for national and European registers on PRT, it is worth clarifying that Medical Device Directives require a European databank for medical devices, which has been developed under the name of "EUDAMED" (EUropean DAtabase on MEdical Devices). The aims of EUDAMED are to strengthen market surveillance and transparency in the field of medical devices by providing the competent authorities of Member States with fast access to information on manufacturers and authorised representatives, devices, certificates, vigilance and clinical investigation data, as well as to contribute to a uniform application of the Directives, in particular in relation to registration requirements.

EUDAMED contains registration data of manufacturers, authorised representatives and devices; data relating to certificates issued, modified, supplemented, suspended, withdrawn or refused; data obtained in accordance with the vigilance procedure; and data on clinical investigations.

In Italy, SISTRA (*Sistema Informativo dei Servizi Trasfusionali*) is the information system established by Italian Ministry of Health Decree of 21st December 2007[309], developed as a strategic support for the achievement of the objectives set out in Law 219/2005[265]: "*self-sufficiency of blood and blood products, transfusion safety, basic levels of care and uniform development of transfusion medicine*".

SISTRA enhances the flow of information between the Italian Ministry of Health, the Regions and the Autonomous Provinces of Trento and Bolzano and the National Blood Centre, encouraging regional and national interactions, as well as a detailed analysis of consumption and production data.

Informed consent, information and data protection

It is important to provide information on technologies in such a way that the patient can truly understand it. The principle of informed consent consists in the right of patients to obtain all necessary information in order to make a well-informed and responsible decision and to have greater participation in their treatment. According to clause 5 of the Oviedo Convention report[310] the information must be appropriate with respect to the goals, the nature of the intervention and the potential consequences and risks for the patient.

Regarding FFP, another salient regulation is the *"Additional Protocol to the Convention on Human Rights and Biomedicine concerning Biomedical Research"*[311], in which clauses 13 and 14 regulate the information to be provided to patients, as well as their informed consent. The Declaration of Helsinki is another important document concerning informed consent[317].

Informed consent is an essential prerequisite for the patient's free and responsible choice to begin any medical intervention. Indeed, informed consent is generally considered as a process by which the treating health care provider discloses appropriate information to a competent patient, so that the patient may make a voluntary choice to accept or refuse treatment. In order to be valid, informed consent requires that the individual should have the capacity to make the decision, that the choice should be voluntary, that the individual be provided with appropriate information, in a format that he or she can understand, regarding the benefits, risks, consequences of and alternatives to the proposed treatment; and that the decision is made carefully[289,293].

Whether separate informed consent for blood transfusions should be required is the object of debate[294,302]. However, there is general agreement that before being asked to consent to a transfusion, there should be discussion with the patient about the reason for the transfusion, i.e. the purpose and anticipated benefit of the transfusion, including the consequences of not performing the transfusion, the alternatives to transfusion, which may include the possibility of an autologous transfusion, and clarification of the risks

associated with transfusion. Patients should also be given the opportunity to ask questions and should be informed of their right to refuse a transfusion[303].

In Italy, informed consent to blood transfusion is required by the Ministerial Decree of 3rd March 2005 "*Characteristics and modalities for blood and blood by-products donation*". It is worth mentioning clause 11 ("Informed consent of the recipient") which specifies that the recipient of blood products should be previously informed that such procedures can never be completely risk-free, and that he or she must express written consent or dissent to the transfusion[306].

Nevertheless, the practice of obtaining a valid consent for blood transfusion is still highly heterogeneous across the country and should be conformed as soon as possible. As noted, an element to be discussed with patients requiring a transfusion concerns its risks. Patients should be informed of potential damages due to the PRT. Moreover, they should be informed of residual risks concerning pathogens for which no detection method exists (i.e. emerging viruses) or the "window period", in which blood levels of specific disease markers are too low for detection.

To achieve this goal, some requirements have to be fulfilled. Particular attention must be paid to the statement within Directive 2002/98/EC: "*It is important to introduce a set of organised surveillance procedures to collect and evaluate information on the adverse or unexpected events or reactions resulting from the collection of blood or blood components in order to prevent similar or equivalent events or reactions from occurring thereby improving the security of transfusion by adequate measures. To this end a common system of notification of serious adverse events and reactions linked to the collection, processing, testing, storage, and distribution of blood and blood components should be established in Member States*"[254]. For this reason, haemovigilance programmes must be implemented as extensively as possible. The first haemovigilance programme in Italy was presented in a document from the Italian National Health Service in 2004/2005. Since 2009, all haemovigilance data are collected on SISTRA and are available on the websites of both the Italian National Health Service and the National Blood Centre.

In the same vein, Directive 2002/98/EC states, in clauses 14-15, that *"Member States must ensure that blood establishments implement a system for identifying each single blood donation and each single blood unit and components thereof, enabling full traceability to the donor as well as to the transfusion and the recipient thereof"*[254].

In brief, data on potential harm due to PRT for plasma must be collected and patients should be informed of them. As a consequence, informed consent forms must be updated from time to time. The right to have access to full information and to safeguard one's privacy imposes on authorities the duty to guarantee proper, safe access to personal data, as affirmed in Directive 95/46/EC of the European Parliament and of the Council[313]. In more detail, this Directive establishes that:

1) Member States shall prohibit the processing of personal data revealing racial or ethnic origin, political opinions, religious or philosophical beliefs, trade-union membership, and the processing of data concerning health or sexual habits.
2) Paragraph 1 shall not apply where: (i) the patient has given his or her explicit consent to the processing of those data, unless the laws of the Member State explicitly claim that the prohibition referred to in paragraph 1 may not be lifted even in the presence of the patient's consent; (ii) processing is necessary to carry out the obligations and specific rights of the controller in the fulfilment of law, when it is authorised by national law providing for adequate safeguards; (iii) processing is necessary to protect the vital interests of the patient or of another person who is physically or legally incapable of giving his or her consent; (iv) processing is carried out in the course of its legitimate activities with appropriate guarantees by a foundation, association or any other non-profit-seeking body with a political, philosophical, religious or trade-union aim and if the processing relates solely to the members of the body or to persons who have regular contact with it, in connection with its purposes and if data are not disclosed to a third party without the consent of the data subjects; or (v) the processing relates to data which are manifestly made public by the data subject or are necessary for the establishment, exercise or defence of legal claims.
3) Paragraph 1 shall not apply where processing of the data is required for the purposes of preventive medicine, medical diagnosis, provision of care or treatment or management of health-care services, and where those data are processed by a health professional under national law or rules established by national competent bodies, under the obligation of professional secrecy or by another person also subject to an equivalent obligation of secrecy.
4) Subject to the provision of suitable safeguards, Member States may, for reasons of substantial public interest, lay down exemptions in addition to those laid down in paragraph 2 either by national law or by decision of the supervisory authority.
5) Processing of data relating to offences, criminal convictions or security measures may be carried out only under the control of an official authority, or if suitable specific safeguards are provided under national

law, subject to derogations which may be granted by the Member State under national regulations providing suitable safeguards. However, a complete register of criminal convictions may be kept only under the control of an official authority. Member States may establish that data relating to administrative sanctions or judgments in civil cases shall also be processed under the control of an official authority.

6) Derogations from paragraph 1 provided in paragraphs 4 and 5 shall be notified to the Commission.
7) Member States shall determine the conditions under which a national identification number or any other identifier of general application may be processed; moreover, clause 8 of the European Convention on Human Rights "Right to respect for private and family life" claims that[314]:
   - Everyone has the right to respect for his or her private and family life, home and correspondence.
   - There should not be any interference by a public authority with the exercise of this right, except for cases regulated by law and if it is necessary in the interests of national security, public safety or the economic well-being of the country, for the prevention of disorder or crime, for the protection of health or morals, or for the protection of the rights and freedoms of others.

Finally, we recall the Sentences of the European Court of Justice of the 20th May 2003 C-465/00, C-138/01, C-139/01[315] and the Working Document on research on biological materials of human origin by the Committee on Bioethics (DH-BIO) of 18th March 2014[316].

In addition, patients should be given some time to think about their treatment and take a decision, especially if this involves assimilating complex technical information or a tough weighting of risks and benefits of the procedure. It should be assessed beforehand if a given technology allows such time for consideration.

Unfortunately, European dispositions do not establish time limits for the provision of informed consent. Effective informed consent implies allowing the patient the time necessary to consider alternatives. We believe that sufficient time is implied in an adequate and appropriate informed consent. It is also true that informed consent may require more time for specific patients[310-313].

However, there are situations in which a patient's health state requires immediate action. In emergency situations it is unlikely that such pre-transfusion discussion can take place. This could be, for example, because the patient does not have the capacity to understand the situation. In these cases, since prior consent for blood transfusion is usually considered necessary, then we must consider that such prior consent should also be required for all the other potential interventions that might be required during a medical or surgical procedure, such as, for example, magnetic resonance imaging, X-rays and anaesthesia[317]. With regards to emergency situations and informed consent issues we could cite clause 9 of the Oviedo Convention and clauses 15 and 16 of the *"Additional Protocol to the Convention on Human Rights and Biomedicine concerning Biomedical Research"*. Moreover, implementing a requirement to obtain and document specific consent for blood transfusions could require deeper insight into the patient's role within medical decision-making processes. It is, however, crucial to remember that the consent process is not merely a form-filling exercise in the physician-patient relation.

Justice and equity

As mentioned above, guaranteeing equal access to effective care for those in need is one of the main principles for the provision of health care in systems based on solidarity (i.e. National Health Services). Limited health care resources do, therefore compel cost-effectiveness considerations and prioritisation of health care measures. In this sense, the use of technologies may influence the fairness of healthcare systems, or require special considerations in order to ensure that justice is not compromised.

The European Union has produced many documents on healthcare matters within Member States, such as: Decision n. 1350/2007/EC of the European Parliament and of the Council, establishing a second programme of Community Action in the field of health (2008-2013)[318]; the White paper of the European Commission "Together for Health: A Strategic Approach for the EU 2008-2013"[319]; the Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions e-Health - making healthcare better for European citizens: an action plan for a European e-Health Area[320]. With regard to cross-border healthcare assistance in Europe the most relevant documents include the 2011/24/UE Directive of the European Parliament and the Council, concerning the application of patients' rights in cross-border healthcare[321]; Recommendation Rec (2006)18 of the Committee of Ministers to Member States on health services in a multicultural society[322]; and the Council of the European Union Directive on cross-border healthcare adopted on 28th February 2011[323]. It is also worth recalling the judgment of the Court of Justice of the European Union issued on 28th April 1998 (C-120/95)[324].

Finally, regarding the possibility of infringing some intellectual property right, at the European level the reference standard for the patentability of biotechnological inventions is Directive 98/44/EC of the European Parliament and of the Council[325].

Discussion

We feel that the respect of safety, quality, and security as well as of consent, information- and data- protection is fundamental. In Italy, the current quality standards of transfusion medicine activities and the quality standards and safety levels of therapeutic blood and blood products are good, especially regarding the lack of TTI. Haemovigilance and clinical risk management systems contribute to the evolution of comprehensive blood and blood product safety. Nevertheless, the continuous improvement and optimisation required by the *"Good Practice Guidelines for Blood Establishments and Hospital Blood Banks"* must be pursued in order to align our country's standards to the European Union's higher standards.

The application of guidelines/criteria for the appropriate management and clinical utilisation of blood resources is of paramount importance as it represents a fundamental "parallel tool" for blood and blood product self-sufficiency, as well as being a safety measure *per se*. However, greater legislative uniformity among regions and specific provision for the application of the best PRT are still crucial.

In conclusion, although many documents concerning blood management have been produced, there is still a relevant lack of homogeneous guidelines in Italy.

Social domain

Introduction

The social domain is one of the basic areas in a HTA. This domain "takes the patient as a point of departure in its analysis of the manifold social implications of health technology. The focus of the domain is on the diverse social arenas where the patient lives and acts during the period of sickness and treatment"[326]. These "social places", in which health technologies are put in use, include hospitals, out-hospital clinics, home care, general practitioners' offices, daily life, homes, schools, and workplaces. People deal with health technologies with feelings (hope, fear, uncertainty, etc.), experiences, and meanings, in personal and social ethical frameworks. In other words, the social analysis is concerned with all those aspects that involve the human being and his or her role in different arenas (as a citizen, employee, consumer, family member, etc.) in the society.

Moreover, the social domain, just as the ethical analysis, can support the HTA process as a whole (e.g., by prioritising topics/issues, defining/framing research questions). For practical reasons, in our HTA process the social assessment was conducted as a separate section of the HTA process.

Methods

Two kinds of issues should be addressed in a social analysis of PRT for plasma from the individual's point of view (patient first, but also health professionals involved in plasma management): (i) the resources required for the health technology in question, aimed at producing satisfactory results (first of all, safety and clinical benefit) [327] through the use and management of the health technology itself; (ii) experiences, feelings (e.g. hopes, fears), actions/reactions and social changes/consequences (e.g. working capacity, relationships) produced by the given health technology.

Furthermore, the social analysis addresses aspects that are also important for all the other domains of the HTA[328].

There are several theoretical frameworks for carrying out a social analysis[326,329-338] Of these, we chose and applied the EUnetHTA model which considers three different topics (major life issues; individual issues; communication) covering nine issues (Table LVIII).

Each issue was first defined, and then its relevance considered, information sources identified, and literature searches performed. All issues were considered relevant to the technology in question. The results of the analysis explicitly include the set of issues, with the topics organised into two main paragraphs.

The social assessment was carried out by a team of four ethicists (three of them with social research skills/experience).

The main sources of information for the social analysis were literature identified in international databases (PubMed, Cochrane Library, CRD) and the specific assessments provided by other HTA domains in this process.

Literature search

A three-step literature search was applied: (i) all participants in the HTA project were asked to notify the social analysis group if they found specific contributions discussing social issues. No contributions were notified; (ii) a search was made for HTA reports on the topic in online databases. Fifteen references were found, but five of them were not available; and (iii) PubMed and the Cochrane Library were searched for clauses on social topics relating to PRT for FFP.

The PubMed query was based on the following scheme:
SOCIAL ASPECTS *("family" OR "caregiver" OR "daily life" OR "communication" OR "informed consent" OR "return to work" OR "hospital stay" OR "perceptions" OR "preference" OR "expectation" OR "quality of life").*

Twenty-six contributions of possible interest for the social domain were identified.

Information selection

The contents of the ten HTA reports available were scrutinised. None of them contained a specific section on the social domain.

As far as concerns articles in PubMed and the Cochrane Library: the titles and abstracts of the 916 papers were screened by two independent reviewers to verify whether they contained specific information on "social" topics concerning PRT for plasma. Of the 916 screed articles, 29 were of possible relevance for the social domain. Their titles and abstracts were scrutinised and four were considered to contribute slightly to answering the set of EUnetHTA social domain questions, although none explicitly addressed the social issues related to PRT for plasma[50,121,223,339].

Finally, socially relevant data gathered from other HTA domains were considered.

Results

Four PRT for plasma (SD, MB, amotosalen and riboflavin) are the object of the present analysis. These PRT have been developed in different times to reduce/prevent the transmission of blood-borne pathogens in plasma and, consequently, to increase the safety of plasma products for individuals and for society as a whole.

The socially relevant issues identified were first summarised and then organised into two main paragraphs, according to the EUnetHTA social domain table of issues.

Major life areas/individual issues

In general, social areas concerning the patient (family life, hospital/home care, work, leisure time, lifestyle, cultural activities) are directly influenced to variable extents, by the technologies in question, because the availability of plasma that is as safe as possible implies a longer and better quality life for the patient, without neglecting the value connected to clinical benefits (cf. efficacy, safety, legal and ethical domains). This could allow patients to maintain/pursue their roles with regards to functioning and relations in a socially appropriate way in major life areas, because their physical and psychological status could improve (cf. ethical, efficacy and safety domains).

Safe plasma is also relevant to health professionals involved in different levels of the health care service in which daily clinical routine requires the use of blood components, to employees of plasma product companies, and to the social environment (namely work, family and friends) of the patient.

Furthermore, human, technical, economic, cultural, physical, emotional, personal, social, juridical, and organisational support and resources are needed, in general, to assure the following satisfactory results: fair availability of safe blood components, irrespective of regional contexts within a country; safe blood management in daily clinical/organisational routines; and an increasingly safe production of plasma (cf. organisational and legal domains).

In particular, adequate juridical/technical regulations should be considered as a resource for helping patients to benefit fully from a technology: for example, the European Union Directive 2002/98/EC states the need to set up organised surveillance procedures to collect/evaluate information on the adverse/unexpected events/reactions resulting from the collection and transfusion of blood or blood components. Likewise, an appropriate quality control and organisational framework for plasma delivery should be considered as a resource. Furthermore, it is socially relevant to address the matter of which economic/financial resources the patient can (or cannot) count on, to activate the use of the technology. Moreover, depending on personal orientations, patients may react to a technology in a positive or negative way, for example with preferences/perceptions (such as a critical attitude towards "scientific" medicine in favour of "alternative" medicine) and existential experiences/feelings (e.g.: the person's values as a human being, or depending on his/her social status; changes in self-conception/esteem) (cf. also efficacy, safety, legal and ethical domains).

Finally, although a free and responsible choice is needed to make sure that the patient has acted adequately

Table LVIII - Issues considered in the EUnetHTA social domain assessment.

Topic	Issue
Major life areas	H0001 - Which social areas does the use of the technology influence?
	H0002 - Who are the important others that the use of the technology may affect in addition to the patient?
	H0003 - What kind of support and resources are needed or might be released as the technology is put to use?
	H0004 - What kinds of changes does the use of the technology generate in the patient's role in the major life areas?
	H0005 - What kind of changes does the implementation and use of the technology mean for the patient's physical and psychological functioning in his or her major life areas?
Individual	H0006 - How do patients and important others react and act upon the technology?
Communication	H0007 - What is patients' and important others' knowledge and understanding of the technology?
	H0008 - How is the information regarding the use of the technology processed and exchanged?
	H0009 - What are the consequences in decision making?

towards the technology, this should not be reduced to a mere signature on an informed consent form (cf. legal and ethical domains).

Communication

Personal and social knowledge and understanding about PRT-FFP are crucial both for an adequate perception of the technology in question and for the socially accepted cost-effectiveness ratio in the light of the core values of the welfare-based Italian National/ Regional Health Service (centrality of the person's health; universal, fair and humanitarian access to health care provisions, economic/organisational sustainability of the health service).

Nowadays, traditional media (radio, television, newspapers, magazines) and web-based media (social networks, blogs, institutional/patients'/citizens' websites, etc.) offer a large amount of information, which is not, however, synonymous with providing full, real knowledge, understanding and awareness of all the aspects of PRT-FFP, which include the real clinical benefits gained through plasma inactivation technologies, but also the fact that the risk of transmission of pathogens is very low but not absent, and that close haemovigilance and post-marketing surveillance programmes are needed in order to continue to collect information on risks and benefits of the technology (cf. also current use of technology, safety and ethical domains); furthermore, continuous updated cost-effectiveness analyses on the technology in question are needed to understand the financial outlay required from the National Health Service (i.e.: from citizens), but also to identify and share a possible, defined economic threshold for the reimbursement of these technologies. At present, the available cost-effectiveness analyses (cf. economic domain) seem to suggest that the best option for plasma pathogen inactivation is the SD method. It is crucial, from the social point of view, to define how the information regarding the use of the technology is processed and exchanged. The qualitative and quantitative multidisciplinary adequacy of the information about the technology in question must, therefore, be continuously verified[340]. From the qualitative point of view, institutional information strategies, shared by the Ministry of Health and citizens/patients' organisations, should be designed and updated in the light of biomedical advances. From the quantitative point of view, every possible effort must be made in order to provide citizens with clear and understandable information, and to avoid forms of triumphalism and dramatization or demonization of the technology. Furthermore, information should be free from conflicts of interest (cf. also organisational domain). Social information campaigns should be aimed at avoiding public mistrust towards the technology or towards medicine in general. Finally, patients should be involved and empowered in decision-making processes, also through citizens/patients' associations (cf. also organisational and ethical domains)[341-349].

Discussion

The ever-present threat of emerging and re-emerging infectious diseases and the risk of clinical repercussions from the transfusion of blood products drive the need for new technologies.

PRT offer a new approach to improve blood safety and their adoption has been gradual, but steady over the past decade. The technologies and methods discussed in this HTA report highlight on the complexity of finding a balance between effective pathogen inactivation in blood products and acceptable quality and functionality of the haemostatic components.

Overall, the assessment did not reveal a dominant strategy for pathogen inactivation of FFP. The vast majority of the studies considered investigated the safety, efficacy and economic aspects of SD-FFP, MB-FFP, R-FFP and A-FFP, but comparative studies among the pathogen inactivation technologies were limited.

The evidence-based indications for the use of plasma and PRT-treated plasma are few and specific. PRT-treated plasma has, in fact, been in clinical use for several years and proven effective in a variety of therapeutic settings, but each PRT system has some weakness or gap in terms of efficacy and each of the major methods in development has a specific set of potential concerns unique to the method. The supportive and often prophylactic nature of blood component therapy in a variety of clinical situations complicates the clinical evaluation of these novel blood products.

Efficacy

Most clinical efficacy studies designed to compare PRT-treated and untreated plasma involve only small numbers of patients and are not, therefore, adequately powered to detect statistically significant minor differences. In addition, they assess increments in coagulation factor levels and changes in *in-vitro* coagulation blood tests rather than clinically meaningful outcomes. Concerns about the low levels of plasmin inhibitor, protein S, or antitrypsin activity in PRT-treated plasma have not been confirmed in clinical studies, and claims of thrombosis or hyperfibrinolytic bleeding triggered by reduced protein S or low plasmin inhibitor potencies have not withstood critical review. Extensive clinical experience has shown that reduced levels of coagulation factors, as a result of PRT, do not significantly impair the clinical efficacy or tolerance of the treated plasma.

The first method developed to inactivate enveloped viruses in plasma protein preparations was SD treatment. SD-FFP has proven to be non-inferior to untreated FFP in the management of a wide range of congenital and acquired bleeding disorders. Furthermore, the major advantages of SD plasma over FFP and the other types of pathogen-inactivated plasma are its extreme safety with respect to TRALI and the significantly lower likelihood of provoking allergic reactions. Both advantages are interpreted as results of the dilution effect of pooling.

While MB-treated plasma was associated with apparently fewer allergic reactions than plasma, a single-centre retrospective haemovigilance investigation in France did not confirm this finding.

The difficulty of comparing the frequency of adverse events recorded in different haemovigilance reports, because of different reporting procedures, has been confirmed.

Two other technologies, based on a photochemical process involving amotosalen (a psoralen) or riboflavin, both plus light treatment, have been developed more recently. There are few studies available on these technologies and the level of evidence they provide is low or moderate.

Potential long-term side effects of photosensitisers and their photoproducts still remain a matter of debate, and, before broad implementation of these photoactive techniques, it must be shown that they are safe, robust in daily routine, and cost-effective and that their efficacy is controllable.

Clinical experience with PRT-treated plasma has substantiated that the products are safe, although this work is in an early stage of exploration and the full potential of these technologies has yet to be determined.

Safety domain

Blood component transfusions will never be completely safe because of an intrinsic risk of transmitting emerging pathogens to the recipients. However, plasma pathogen inactivation methods may be considered safe especially as regards the transmission of viruses and bacteria. No cases of HIV, HCV or HBV transmission have been observed in the European countries analysed in the last few years. As far as concerns other clinical outcomes, there has recently been an increase in allergic reactions, while the actions taken by many countries to minimise the risk of TRALI have been appropriate. Overall, the number of cases of TRALI has been very low in recent years and tended to zero in the past year.

The results of the literature search did not enable a direct comparison between the different pathogen-reduction methods. Overall, there are few studies that compare the various techniques with each other or with the conventional method. Furthermore, haemovigilance data are conflicting across European countries because the alerts are reported on a voluntary basis in some countries. However, with these caveats, according to the data collected, the SD-FFP technique seems to have the best safety profile, even if evidence supporting this statement is limited and this conclusion should be considered cautiously. It became clear from this review that more comparative studies need to be performed in order to assess the safety profile of the various methods directly.

Economic domain

Cost-effectiveness studies included in this literature review are limited to the comparison of SD-FFP *vs* FFP. Some articles took into account the SD technique, while others examined the branded product (Octaplas®). The results of the studies are conflicting and not comparable. The variability of the results depends on differences in study designs, study populations and assumptions. The evidence included in this assessment primarily indicates that the cost-effectiveness of the SD-FFP technique compared to FFP depends on several factors, such as the patients' age and therefore life expectancy, the health status of patients undergoing plasma transfusion and the related transfusion complications considered. Evidence on cost-effectiveness is very limited: a good cost-effectiveness profile was observed only in certain subgroups of patients. Thus, criteria for denying the new component should be established and would be acceptable from an ethical point of view. The wide heterogeneity of results and the lack of comparative evidence for the SD technique make the cost-effectiveness of this method uncertain. Further research is needed to investigate the cost-effectiveness of other PRT and to obtain more reliable results. Organisational analyses investigating "real practice" issues should be carried out in order to identify relevant and common cost drivers of the various approaches to pathogen inactivation for future cost-effectiveness studies. Moreover, different outcome measures should be considered to improve the effectiveness estimates and to carry out evaluations from a social perspective too, in order to present a broader overview of the impact of alternative plasma products on patients and on the health care system. Finally, value-specific thresholds for interventions to improve blood safety should be defined also for clinical outcome measures, since the cost-utility approach can be insufficient in this clinical area.

Organisational domain

The analysis of organisational aspects was carried out by dividing infectious risk prevention procedures into three groups: traditional FFP, "in-house" pathogen

inactivation procedures (grouped together and assumed to have the same organisational impact) and the SD-FFP industrial method.

Few studies have investigated organisational aspects of PRT; in fact, most of the information was retrieved from documents released by monitoring authorities, grey literature, manufacturers' handbooks and experts' opinions, which creates a risk of bias due to insufficient or selective inclusion of information and data, and can be considered a limitation of our review. Furthermore, data from experts were limited by their positions, knowledge asymmetry and time constraints, but the information included was analysed critically.

Organisational changes resulting from the adoption of PRT occur mainly in the stages of processing, storage and distribution of plasma. "In-house" pathogen-inactivation procedures are performed in hospital blood centres on single donor bags, blood units are stored in the hospital blood bank and transport between facilities is not, therefore, required but enough storage space must be available. On the other hand, industrial pathogen inactivation procedures require collected blood to be transported to and from the manufacturer's facilities, involving logistics for the maintenance of the cold chain, while pathogen inactivated blood units are stored in interim depots before being distributed to hospital blood banks. The strengths of these different PRT are that plasma prepared with "in-house" procedures is not pooled, thus reducing the risk of a whole plasma pool being contaminated due to a single infected bag, while plasma prepared with the industrial method requires EMA Plasma Master File certification, which guarantees full traceability of the plasma, simplification and standardisation of the transfusion chain and the quality of information at the European level.

With regards to quality control, SD-FFP is a pharmaceutical product, whereas "in-house" procedures use medical devices and the resulting plasma is not considered as a drug. This fact underlies the difference between the regulatory systems for the two groups of technologies. In Europe, quality control and validation of SD-FFP are guided by the guidelines of the European Council and the EMA, under State Laboratory control and marketing authorisation is issued by national health authorities.

Ethical domain

From the ethical point of view, important points to consider during decision-making about SD-FFP, MB-FFP, A-FFP and R-FFP are that the risk-benefit ratio of using these PRT seems to be favourable, although this conclusion should be consolidated by additional data, and before a transfusion of pathogen-reduced blood products, patients should always be informed about the PRT used and its potential benefits and risks, clarifying that a minimal risk always remains.

It is still uncertain whether the use of SD-FFP, MB-FFP, A-FFP and R-FFP fulfils the criteria of just resource allocation.

Legal domain

The legal domain descriptive analysis at national and European levels considered the directives and laws which define quality, and safety standards for the testing, processing, storage and distribution of human blood and blood components. Within the documentation studied, we found common elements concerning the discipline of information, consent, protection of privacy and access to treatment. There are no *ad hoc* directives on pathogen reduction at the European level and a set of homogeneous guidelines is also lacking in the Italian scenario.

Social domain

The analysis of the social domain revealed the need for further analyses to safeguard the uniform access to blood services for patients. Economic evaluations should play a critical role for this purpose.

The Authors declare no conflicts of interest.

References

1) Psychogios N, Hau DD, Peng J, et al. The human serum metabolome. PLoS One 2011; **16**: e16957.
2) Benjamin RJ, McLaughlin LS. Plasma components: properties, differences, and uses. Transfusion 2012; **52**: 9S-19S.
3) Sherwood L. *Human Physiology: From Cells to Systems*. 8th ed. Belmont, CA: Brooks/Cole-Cengage Learning; 2013.
4) Strumia MM, McGraw JJ. The development of plasma preparations for transfusions. Ann Intern Med 1941; **15**: 80-8.
5) Gosselin RC, Marshall C, Dwyre DM, et al. Coagulation profile of liquid-state plasma. Transfusion 2013; **53**: 579-90.
6) Grazzini G, Mannucci PM, Oleari F. Plasma-derived medicinal products: demand and clinical use. Blood Transfus 2013; **11**: s2-5.
7) Burnouf T. Plasma fractionation. ISBT Science Series 2012; **7**: 62-7.
8) Labarinas S, Arni D, Karam O. Plasma in the PICU: why and when should we transfuse? Ann Intensive Care 2013; **3**: 16.
9) Alhumaidan H, Cheves T, Holme S, Sweeney J. Stability of coagulation factors in plasma prepared after a 24-hour room temperature hold. Transfusion 2010; **50**: 1934-42.
10) Council of Europe. *Guide to the Preparation, Use and Quality Assurance of Blood Components. Recommendation No R (95) 15 on the Preparation, Use and Quality Assurance of Blood Components*. 17th ed. Strasbourg: Council of Europe Press; 2013.
11) Yang L, Stanworth S, Baglin T. Cryoprecipitate: an outmoded treatment? Transfus Med 2012; **22**: 315-20.
12) Kappler S, Ronan-Bentle S, Graham A. Thrombotic microangiopathies (TTP, HUS, HELLP). Emerg Med Clin North Am 2014; **32**: 649-71.
13) O'Shaughnessy DF, Atterbury C, Bolton Maggs P, et al.; British Committee for Standards in Haematology, Blood Transfusion Task Force. Guidelines for the use of fresh-frozen

plasma, cryoprecipitate and cryosupernatant. Br J Haematol 2004; **126**: 11-28.
14) Liumbruno G, Bennardello F, Lattanzio A, et al; Italian Society of Transfusion Medicine and Immunohaematology (SIMTI) Work Group. Recommendations for the transfusion of plasma and platelets. Blood Transfus 2009; **7**: 132-50.
15) Italian National Institute of Health. Giampaolo A, Barca A, Catalano L, Hassan HJ. Proceedings of national meeting on appropriate use of blood, Rome, February 25-26, 2003. In: *Rapporti ISTISAN 04/10*. Italian National Institute of Health 2004; ix: 1-186.
16) Seghatchian J, de Sousa G. Pathogen-reduction systems for blood components: the current position and future trends. Transfus Apher Sci 2006; **35**: 189-96.
17) Moor AC, Dubbelman TM, VanSteveninck J, Brand A. Transfusion-transmitted diseases: risks, prevention and perspectives. Eur J Haematol 1999; **62**: 1-18.
18) Bihl F, Castelli D, Marincola F, et al. Transfusion-transmitted infections. J Transl Med 2007; **5**: 25.
19) Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion-transmitted viral infections. The Retrovirus Epidemiology Donor Study. N Engl J Med 1996; **334**: 1685-90.
20) AuBuchon JP, Dodd RY. Inactivation of microbial contaminants of blood components. Clin Lab Med 1992; **12**: 787-803.
21) Molaro GL. La prevenzione del rischio trasfusionale infettivo e immunologico con il plasma fresco congelato trattato con il metodo del solvente/detergente. Blood Transf 2001; **46**: 1-9.
22) Friedman C, Newsom W. IFIC Basic Concepts of Infection Control. 2nd ed. - Revised 2011. International Federation of Infection Control; 2011.
23) World Health Organization. Screening Donated Bloods for Transfusion-Transmissible-Infections. Available at: http://www.who.int/bloodsafety/ScreeningTTI.pdf. Accessed on 10/12/2014.
24) Leparc GF. Nucleic acid testing for screening donor blood. Infect Med 2000; **17**.
25) Jongerius JM, Wester M, Cuypers HT, et al. New hepatitis B virus mutant form in a blood donor that is undetectable in several hepatitis B surface antigen screening assays. Transfusion 1998; **38**: 56-9.
26) Jerome KR. *Lennette's Laboratory Diagnosis of Viral Infections.* 4th ed. New York: Informa Healthcare USA, Inc; 2010.
27) Bianco C. Choice of human plasma preparations for transfusion. Transfus Med Rev 1999; **13**: 84-8.
28) del Río-Garma J, Alvarez-Larrán A, Martínez C, et al. Methylene blue-photoinactivated plasma versus quarantine fresh frozen plasma in thrombotic thrombocytopenic purpura: a multicentric, prospective cohort study. Br J Haematol 2008; **143**: 39-45.
29) Molaro GL. [Universal leucodepletion. Part one]. Il Servizio Trasfusionale 2000; **29**. [In Italian.]
30) Dodd RY, Notari EP 4th, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. Transfusion 2002; **42**: 975-9.
31) Dodd RY, Leiby DA. Emerging infectious threats to the blood supply. Annu Rev Med 2004; **55**: 191-207.
32) Mohr H, Knüver-Hopf J, Gravemann U, et al. West Nile virus in plasma is highly sensitive to methylene blue-light treatment. Transfusion 2004; **44**: 886-90.
33) Leydold SM, Farcet MR, Kindermann J, et al. Chikungunya virus and the safety of plasma products. Transfusion 2012; **52**: 2122-30.
34) Vanlandingham DL, Keil SD, Horne KM, et al. Photochemical inactivation of chikungunya virus in plasma and platelets using the Mirasol pathogen reduction technology system. Transfusion 2013; **53**: 284-90.
35) Rock G. A comparison of methods of pathogen inactivation of FFP. Vox Sang 2011; **100**: 169-78.
36) Mundt JM, Rouse L, Van den Bossche J, Goodrich RP. Chemical and biological mechanisms of pathogen reduction technologies. Photochem Photobiol 2014; **90**: 957-64.
37) Horowitz B, Bonomo R, Prince AM, et al. Solvent/detergent-treated plasma: a virus-inactivated substitute for fresh frozen plasma. Blood 1992; **79**: 826-31.
38) Garwood M, Cardigan RA, Drummond O, et al. The effect of methylene blue photoinactivation and methylene blue removal on the quality of fresh-frozen plasma. Transfusion 2003; **43**: 1238-47.
39) Naegelen C, Isola H, Dernis D, et al. Evolution of techniques for preparation of labile blood products (LBP): pathogen inactivation in LBP. Transfus Clin Biol 2009; **16**: 179-89.
40) Webert KE, Cserti CM, Hannon J, et al. Proceedings of a Consensus Conference: pathogen inactivation - making decisions about new technologies. Transfus Med Rev 2008; **22**: 1-34.
41) Horowitz B, Lazo A, Grossberg H, et al. Virus inactivation by solvent/detergent treatment and the manufacture of SD plasma. Vox Sang 1998; **74**: 203-6.
42) Seltsam A, Müller TH. UVC irradiation for pathogen reduction of platelet concentrates and plasma. Transfus Med Hemother 2011; **38**: 43-54.
43) Klein HG, Anderson D, Bernardi MJ, et al. Pathogen inactivation: making decisions about new technologies. Report of a consensus conference. Transfusion 2007; **47**: 2338-47.
44) European Directorate for the Quality of Medicines & HealthCare. Symposium on Implementation of Pathogen Reduction Technologies for Blood Components. Executive Summary. Strasbourg, France; 2010.
45) Osselaer JC, Debry C, Goffaux M, et al. Coagulation function in fresh-frozen plasma prepared with two photochemical treatment methods: methylene blue and amotosalen. Transfusion 2008; **48**: 108-17.
46) French Ministry of Health. [Decision of 20 October 2010 establishing the list and the characteristics of blood components.] JORF 28 November 2010. [In French.]
47) Agence Française de Sécurité Sanitaire des produits de Santé (AFSSAPS). Haemovigilance Annual report 2010. Available at: http://ansm.sante.fr/var/ansm_site/storage/original/application/365a9 7e590280fb1192c05a838cb97bb.pdf. Accessed on 02/12/2014.
48) Mertes PM, Demoly P, Alperovitch A, et al. Methylene blue-treated plasma: an increased allergy risk? J Allergy Clin Immunol 2012; **130**: 808-12.
49) Seltsam A, Mueller TH. Updated hemovigilance data do not show an increased risk of allergic reactions to methylene blue-treated plasma. J Allergy Clin Immunol 2013; **131**: 1253-4.
50) Ozier Y, Muller JY, Mertes PM, et al. Transfusion-related acute lung injury: reports to the French Hemovigilance Network 2007 through 2008. Transfusion 2011; **51**: 2102-10.
51) Lambrecht B, Mohr H, Knüver-Hopf J, Schmitt H. Photoinactivation of viruses in human fresh plasma by phenothiazine dyes in combination with visible light. Vox Sang 1991; **60**: 207-13.
52) Seghatchian J, Struff WG, Reichenberg S. Main properties of the THERAFLEX MB plasma system for pathogen reduction. Transfus Med Hemother 2011; **38**: 55-64.
53) Politis C, Kavallierou L, Hantziara S, et al. Quality and safety of fresh-frozen plasma inactivated and leucoreduced with the Theraflex methylene blue system including the Blueflex filter: 5 years' experience. Vox Sang 2007; **92**: 319-26.
54) Murphy WG. Pathogen reduction: state of reflection in Ireland. Transfus Clin Biol 2011; **18**: 488-90.

55) De Silvestro G, Bagatella P, Tison T, et al. Virus-inactivated plasma - Plasmasafe: a one-year experience. Blood Transfus 2007; **5**: 134-42.
56) Equitani F, Mistretta G, Mele L, et al. Methylene blue-photoinactivated fresh frozen plasma is an effective and safe treatment for inherited and acquired coagulopathies and bleeding disorders. Blood 2003; **102**: 138b.
57) Navarro L, Lozano M, Puig L, Almazan C. Amotosalen (Intercept®) for the Inactivation of Pathogens for Transfusion Therapy. Catalan Agency for Health Information, Assessment and Quality. INAHTA Briefs Issue 2009/103.
58) Jimenez-Marco T, Ruiz-Alderton D, Bautista-Gili AM, Girona-Llobera E. Role of riboflavin- and UV light-treated plasma in prevention of transfusion-related acute lung injury. Transfus Med Hemother 2014; **41**: 172-5.
59) INTERCEPT Use Overview. 2014 Cerus Corporation. Available at: http://www.interceptbloodsystem.com/intercept-in-use/overview. Accessed on 10/12/2014.
60) Hellstern P, Solheim BG. The use of solvent/detergent treatment in pathogen reduction of plasma. Transfus Med Hemother 2011; **38**: 65-70.
61) Irsch J, Lin L. Pathogen inactivation of platelet and plasma blood components for transfusion using the INTERCEPT Blood System™. Transfus Med Hemother 2011; **38**: 19-31.
62) Adelaide Health Technology Assessment (AHTA) Technology Report, July 2011. Technologies for the inactivation/reduction of pathogens in blood products.
63) Canadian Agency for Drugs and Technologies in Health, CADHT Technology Report, March 2011. Octaplas compared with fresh frozen plasma to reduce the risk of transmitting lipid-enveloped viruses: an economic analysis and budget impact analysis.
64) FDA News Release. FDA approves Octaplas to treat patients with blood clotting disorders. January 17, 2013. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm336009.htm. Accessed on 02/12/2014.
65) FDA News Release. FDA approves first pathogen reduction system to treat plasma. December 16, 2014. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm427111.htm. Accessed on 20/12/2014.
66) Solheim BG, Seghatchian J. Update on pathogen reduction technology for therapeutic plasma: an overview. Transfus Apher Sci 2006; **35**: 83-90.
67) World Health Organization. Fact sheet N° 279. Blood safety and availability. Updated June 2014. Available at: http://www.who.int/mediacentre/factsheets/fs279/en/. Accessed on 02/12/2014.
68) World Health Organization. WHO Library Cataloguing-in-Publication Data: Towards 100% voluntary blood donation: a global framework for action. Available at: www.who.int/bloodsafety/voluntary_donation/en/. Accessed on 02/12/2014.
69) Polizzotto MN, Wood EM, Ingham H, Keller AJ; Australian Red Cross Blood Service Donor and Product Safety Team. Reducing the risk of transfusion-transmissible viral infection through blood donor selection: the Australian experience 2000 through 2006. Transfusion 2008; **48**: 55-63.
70) Special Issue: The evolving paradigm of patient blood management. Transfusion 2014; **54**: 2587-790.
71) Clarke P, Will RG, Ghani AC. Is there the potential for an epidemic of variant Creutzfeldt-Jakob disease via blood transfusion in the UK? J R Soc Interface 2007; **4**: 675-84.
72) European Centre for Disease Prevention and Control. Questions on variant Creutzfeldt-Jakob disease and blood transfusion; 2011. Available at: http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?ID=477&List=4f55ad51-4aed-4d32-b960-af70113dbb90. Accessed on 02/12/2014.
73) Food and Drug Administration. Guidance for Industry. Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products. Available at: http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/GuidanceSDefault.htm. Accessed on 02/12/2014.
74) Blajchman MA. The clinical benefits of the leukoreduction of blood products. J Trauma 2006; **60**: S83-90.
75) International Atomic Energy Agency. *Effects of ionizing radiation on blood and blood components: A survey.* Vienna: IAEA-TECDOC-934; 1997.
76) World Health Organization. Annex 4 Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products. WHO Technical Report, Series No. **924**, 2004.
77) D'Alessandro A, Zolla L. Proteomics for quality-control processes in transfusion medicine. Anal Bioanal Chem 2010; **398**: 111-24.
78) Fournier-Wirth C, Coste J. Fitting new technologies into the safety paradigm: use of microarrays in transfusion. Dev Biol (Basel) 2007; **127**: 61-70.
79) Fournier-Wirth C, Jaffrezic-Renault N, Coste J. Detection of blood-transmissible agents: can screening be miniaturized? Transfusion 2010; **50**: 2032-45.
80) Velthove KJ, Over J, Abbink K, Janssen MP. Viral safety of human plasma-derived medicinal products: impact of regulation requirements. Transfus Med Rev 2013; **27**: 179-83.
81) Burnouf T. Modern plasma fractionation. Transfus Med Rev 2007; **21**: 101-17.
82) Klamroth R, Gröner A, Simon TL. Pathogen inactivation and removal methods for plasma-derived clotting factor concentrates. Transfusion 2014; **54**: 1406-17.
83) Unger U, Poelsler G, Modrof J, Kreil TR. Virus inactivation during the freeze-drying processes as used for the manufacture of plasma-derived medicinal products. Transfusion 2009; **49**: 1924-30.
84) Stadler M, Gruber G, Kannicht C, et al. Characterisation of a novel high-purity, double virus inactivated von Willebrand factor and factor VIII concentrate (Wilate). Biologicals 2006; **34**: 281-8.
85) Burnouf T, Radosevich M. Nanofiltration of plasma-derived biopharmaceutical products. Haemophilia 2003; **9**: 24-37.
86) Seltsam A, Müller TH. Update on the use of pathogen-reduced human plasma and platelet concentrates. Br J Haematol 2013; **162**: 442-54.
87) Picker SM. Current methods for the reduction of blood-borne pathogens: a comprehensive literature review. Blood Transfus 2013; **11**: 343-8.
88) Picker SM. Pathogen reduction technologies: the best solution for safer blood? J Blood Disorders Transf 2012; **3**: 5.
89) Pock K, Heger A, Janisch S, et al. Thrombin generation capacity is impaired in methylene-blue treated plasma compared to normal levels in single-donor fresh-frozen plasma, a licensed solvent/detergent-treated plasma (Octaplas) and a development product (Uniplas). Transfus Apher Sci 2007; **37**: 223-31.
90) Prince AM, Horowitz B, Brotman B. Sterilisation of hepatitis and HTLV-III viruses by exposure to tri(n-butyl)phosphate and sodium cholate. Lancet 1986; **1**: 706-10.
91) Liumbruno GM, Franchini M. Solvent/detergent plasma: pharmaceutical characteristics and clinical experience. J Thromb Thrombolysis 2015; **39**: 118-28.
92) Hambleton J, Wages D, Radu-Radulescu L, et al. Pharmacokinetic study of FFP photochemically treated with amotosalen (S-59) and UV light compared to FFP in healthy volunteers anticoagulated with warfarin. Transfusion 2002; **42**: 1302-7.

93) Ciaravino V, Hanover J, Lin L, et al. Assessment of safety in neonates for transfusion of platelets and plasma prepared with amotosalen photochemical pathogen inactivation treatment by a 1-month intravenous toxicity study in neonatal rats. Transfusion 2009; **49**: 985-94.
94) Seifried E, Henschler R, Müller MM. European perspectives on implementation. In: AuBuchon JP, Prowse C, editors: *Pathogen Inactivation: The Penultimate Paradigm Shift.* Bethesda: AABB Press; 2010. p. 261-78.
95) Pamphilon D. Viral inactivation of fresh frozen plasma. Br J Haematol 2000; **109**: 680-93.
96) Solheim BG, Svennevig JL, Mohr B, et al. The use of Octaplas in patients undergoing open heart surgery. In: Müller-Berghaus G, editor. *DIC: Pathogenesis, Diagnosis and Therapy of Disseminated Intravascular Fibrin Formation*. Amsterdam: Elsevier; 1993. p. 253-62.
97) FDA Blood Products Advisory Committee 104th Meeting, September 20, 2012. Issue Summary - Topic II: Safety and efficacy of Octaplas LG, solvent/detergent, ligand gel affinity chromatography treated plasma. Available at: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/BloodProductsAdvisoryCommittee/UCM319773.pdf. Accessed on 02/12/2014.
98) Department of Health measures currently in place in the UK to reduce the potential risk of vCJD transmission via blood. April 2013. Available at: https://www.gov.uk/government/news/measures-currently-in-place-in-the-uk-to-reduce-the-potential-risk-of-vcjd-transmission-via-blood. Accessed on 02/12/2014.
99) Lozano M, Cid J, Müller TH. Plasma treated with methylene blue and light: clinical efficacy and safety profile. Transfus Med Rev 2013; **27**: 235-40.
100) Castrillo A, Cardoso M, Rouse L. Treatment of buffy coat platelets in platelet additive solution with the Mirasol® pathogen reduction technology system. Transfus Med Hemother 2013; **40**: 44-8.
101) Meddeviceonline. CaridianBCT Receives CE Mark For Mirasol Pathogen Reduction Technology System For Plasma. Available at: http://www.meddeviceonline.com/doc/caridianbct-receives-ce-mark-for-mirasol-0001.Accessed on 02/12/2014.
102) Edwin Poots, MLA Tours Terumo BCT Northern Ireland, Manufacturing Facility. 2015 TERUMO BCT. Available at: https://www.terumobct.com/location/japan/about-terumobct/press-room/Pages/21-AUG,-2014---Edwin-Poots,-MLA-Tours-Terumo-BCT-Northern-Ireland,-Manufacturing-Facility.aspx. Accessed on 02/12/2014.
103) Marschner S, Goodrich R. Pathogen reduction technology treatment of platelets, plasma and whole blood using riboflavin and UV light. Transfus Med Hemother 2011; **38**: 8-18.
104) Dichtelmüller HO, Biesert L, Fabbrizzi F, et al. Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion 2009; **49**: 1931-43.
105) Burnouf T, Goubran HA, Radosevich M, et al. Impact of Triton X-100 on alpha 2-antiplasmin (SERPINF2) activity in solvent/detergent-treated plasma. Biologicals 2007; **35**: 349-53.
106) Horowitz B: United States: solvent/detergent plasma. In: AuBuchon JP, Prowse CV, editors. *Pathogen Inactivation: The Penultimate Paradigm Shift.* Bethesda: AABB Press; 2010. p. 217-28.
107) Roberts P, Sims G. Use of vegetable-derived Tween 80 for virus inactivation by solvent/detergent treatment. Biologicals 1999; **27**: 263-4.
108) Klein HG, Dodd RY, Dzik WH, et al. Current status of solvent/detergent-treated frozen plasma. Transfusion 1998; **38**: 102-7.
109) Piquet Y, Janvier G, Selosse P, et al. Virus inactivation of fresh frozen plasma by a solvent detergent procedure: biological results. Vox Sang 1992; **63**: 251-6.
110) Andreu G: France: solvent/detergent plasma. In: AuBuchon JP, Prowse CV, editors. *Pathogen Inactivation: The Penultimate Paradigm Shift.* Bethesda: AABB Press; 2010. p. 137-51.
111) Hellstern P, Sachse H, Schwinn H, Oberfrank K. Manufacture and in-vitro characterization of solvent/detergent-treated plasma. Vox Sang 1992; **63**: 178-85.
112) Biesert L, Suhartono H. Solvent/detergent treatment of human plasma--a very robust method for virus inactivation. Validated virus safety of OCTAPLAS. Vox Sang 1998; **74**: 207-12.
113) Heger A, Svae T-E, Neisser-Svae A, et al. Biochemical quality of the pharmaceutical licensed plasma OctaplasLG after implementation of a novel prion protein (PrPSc) removal technology and reduction of the solvent/detergent (SD) process time. Vox Sang 2009; **97**: 219-25.
114) Ekiaby M, Sayed MA, Caron C, et al. Solvent-detergent filtered (SD-F) fresh frozen plasma and cryoprecipitate minipools prepared in a newly designed integral disposable processing bag system. Transfus Med 2010; **20**: 48-61.
115) El-Burnouf T, Goubran HA, Radosevich M, et al. A process for solvent/detergent treatment of plasma for transfusion at blood centres that use a disposable bag system. Transfusion 2006; **46**: 2100-8.
116) El-Ekiaby M, Radosevich M, Goubran H, et al. New methods of plasma fractionation - a presentation of the 'mini-pool' fractionation procedure developed in Egypt. ISBT Science Series 2009; **4**: 99-106.
117) Horowitz B, Prince AM, Hamman J, Watklevicz C. Viral safety of solvent/detergent-treated blood products. Blood Coagul Fibrinolysis 1994; **5**: S21-8; discussion S29-30.
118) Solheim BG, Rollag H, Svennevig JL, et al. Viral safety of solvent/detergent-treated plasma. Transfusion 2000; **40**: 84-90.
119) Pelletier JP, Transue S, Snyder EL. Pathogen inactivation techniques. Best Pract Res Clin Haematol 2006; **19**: 205-42.
120) Heger A, Bailey A, Neisser-Svae A, et al. Removal of prion infectivity by affinity ligand chromatography during OctaplasLG® manufacturing--results from animal bioassay studies. Vox Sang 2012; **102**: 294-301.
121) Svae TE, Neisser-Svae A, Bailey A, et al. Prion safety of transfusion plasma and plasma-derivatives typically used for prophylactic treatment. Transfus Apher Sci 2008; **39**: 59-67.
122) Agner SJ. Virus inactivation in blood components by photoactive phenothiazine dyes. Transfus Med Rev 2002; **16**: 61-6.
123) Seghatchian J, Krailadsiri P. What's happening? The quality of methylene blue treated FFP and cryo. Transfus Apher Sci 2001; **25**: 227-31.
124) Seghatchian J, Walker WH, Reichenberg S. Updates on pathogen inactivation of plasma using Theraflex methylene blue system. Transfus Apher Sci 2008; **38**: 271-80.
125) Lozano M, Cid J. Pathogen inactivation: coming of age. Curr Opin Hematol 2013; **20**: 540-5.
126) Mohr H, Lambrecht B, Seltz A. Photodynamic virus inactivation of blood components. Immunol Invest 1995; **24**: 73-85.
127) Williamson LM, Cardigan R, Prowse CV. Methylene blue-treated fresh-frozen plasma: what is its contribution to blood safety? Transfusion 2003; **43**: 1322-9.
128) Mohr H. Methylene blue and thionine in pathogen inactivation of plasma and platelet concentrates. Transfus Apher Sci 2001; **25**: 183-4.
129) Wainwright M, Mohr H, Walker WH. Phenothiazinium derivatives for pathogen inactivation in blood products. J Photochem Photobiol B 2007; **86**: 45-58.

130) Abe H, Yamada-Ohnishi Y, Hirayama J, et al. Elimination of both cell-free and cell-associated HIV infectivity in plasma by a filtration/methylene blue photoinactivation system. Transfusion 2000; 40: 1081-7.
131) Knuver-Hopf J, Mohr H. Parvovirus B19 is sensitive to photodynamic and photochemical treatment. Abstract Book VIII European Congress of the ISBT; 2003; 94.
132) Knuver-Hopf J, Schaefer W, Groener A, et al. Human parvovirus in plasma is highly sensitive to methylene blue/light treatment. Vox Sang 2010; **99**: 1-516.
133) Gironés N, Bueno JL, Carrión J, et al. The efficacy of photochemical treatment with methylene blue and light for the reduction of Trypanosoma cruzi in infected plasma. Vox Sang 2006; **91**: 285-91.
134) Achour A. Phenothiazines and prion diseases: a potential mechanism of action towards oxidative stress. Int J Antimicrob Agents 2002; **20**: 305-6.
135) Amaral L, Kristiansen JE. Phenothiazines: potential management of Creutzfeldt-Jacob disease and its variants. Int J Antimicrob Agents 2001; **18**: 411-7.
136) Wagstaff DJ. Dietary exposure to furocoumarins. Regul Toxicol Pharmacol 1991; **14**: 261-72.
137) Lin L, Cook DN, Wiesehahn GP, et al. Photochemical inactivation of viruses and bacteria in platelet concentrates by use of a novel psoralen and long-wavelength ultraviolet light. Transfusion 1997; ***37***: 423-35.
138) Cid J, Ramiro L, Martínez N, et al. Quantitative and qualitative analysis of proteins in fresh frozen plasma obtained from whole blood donations and prepared with two photochemical treatments. Transfus Apher Sci 2008; **39**: 115-21.
139) Cazenave JP. Photochemical inactivation of pathogens in platelets and plasma: five years of clinical use in routine and hemovigilance. Towards a change of paradigm in transfusion safety. Transfus Clin Biol 2011; **18**: 53-61.
140) Roback JD, Conlan M, Drew WL, et al. The role of photochemical treatment with amotosalen and UV-A light in the prevention of transfusion-transmitted cytomegalovirus infections. Transfus Med Rev 2006; **20**: 45-56.
141) Lin L, Hanson CV, Alter HJ, et al. Inactivation of viruses in platelet concentrates by photochemical treatment with amotosalen and long-wavelength ultraviolet light. Transfusion 2005; **45**: 580-90.
142) Cid J, Escolar G, Lozano M. Therapeutic efficacy of platelet components treated with amotosalen and ultraviolet A pathogen inactivation method: results of a meta-analysis of randomized controlled trials. Vox Sang 2012; **103**: 322-30.
143) Grellier P, Benach J, Labaied M, et al. Photochemical inactivation with amotosalen and long-wavelength ultraviolet light of Plasmodium and Babesia in platelet and plasma components. Transfusion 2008; **48**: 1676-84.
144) Goodrich RP, Edrich RA, Li J, Seghatchian J. The Mirasol PRT system for pathogen reduction of platelets and plasma: an overview of current status and future trends. Transfus Apher Sci 2006; **35**: 5-17.
145) Reikvam H, Marschner S, Apelseth TO, et al. The Mirasol® pathogen reduction technology system and quality of platelets stored in platelet additive solution. Blood Transfus 2010; **8**: 186-92.
146) Douki T, Laporte G, Cadet J. Inter-strand photoproducts are produced in high yield within A-DNA exposed to UVC radiation. Nucleic Acids Res 2003; **31**: 3134-42.
147) Kumar V, Lockerbie O, Keil SD, et al. Riboflavin and UV-light based pathogen reduction: extent and consequence of DNA damage at the molecular level. Photochem Photobiol 2004; **80**: 15-21.
148) Silliman CC, Khan SY, Ball JB, et al. Mirasol pathogen reduction technology treatment does not affect acute lung injury in a two-event in vivo model caused by stored blood components. Vox Sang 2010; **98**: 525-30.
149) Allain JP, Bianco C, Blajchman MA, et al. Protecting the blood supply from emerging pathogens: the role of pathogen inactivation. Transfus Med Rev 2005; **19**: 110-26.
150) Kiang Tan L, Lam S, Low S, et al. Evaluation of pathogen reduction systems to inactivate Dengue and Chikungunya viruses in apheresis platelets suspended in plasma. Advances in Infectious Diseases 2013; **3**: 1-9.
151) Burnouf T, Chou ML, Cheng LH, et al. Dengue virus inactivation by minipool TnBP/Triton X-45 treatment of plasma and cryoprecipitate. Vox Sang 2013; **104**: 1-6.
152) Heger A, Kannicht C, Römisch J, Svae TE. Normal levels of ADAMTS13 and factor H are present in the pharmaceutically licensed plasma for transfusion (Octaplas®) and in the universally applicable plasma (Uniplas) in development. Vox Sang 2007; [92]: 206-12.
153) Heger A, Brandstätter H, Prager B, et al. Universal pooled plasma (Uniplas®) does not induce complement-mediated hemolysis of human red blood cells in vitro. Transfus Apher Sci 2015; **52**: 128-35.
154) Macopharma. Blood Safety Website. Available at: http://www.macopharma.com/en/theraflex-methylene-blue-MB plasma. Accessed on 02/12/2014.
155) Steinmann E, Gravemann U, Friesland M, et al. Two pathogen reduction technologies--methylene blue plus light and shortwave ultraviolet light--effectively inactivate hepatitis C virus in blood products. Transfusion 2013; **53**: 1010-8.
156) INTERCEPT eNews delivers product news & information updates. Available at: http://www.interceptbloodsystem.com/product-overview/intercept-plasma. Accessed on 02/12/2014.
157) de Cock P et al. The Mirasol Evaluation Program: use of Mirasol pathogen reduction technology for platelets in routine clinical practice. Transfusion 2008; 48: 156A.
158) Custer B. Update on pathogen reduction technology. ISBT Science Series 2013; **8**: 80-84
159) Mirasol® pathogen reduction technology (PRT) system. A simple, proven system with built-in flexibility. Available at: https://www.terumobct.com/location/asia-pacific/Documents/306690275A-MiraSimplicity-web.pdf Accessed on 02/12/2014.
160) Evans G, Llewelyn C, Luddington R, et al. Solvent/detergent fresh frozen plasma as primary treatment of acute thrombotic thrombocytopenic purpura. Clin Lab Haematol 1999; **21**: 119-23.
161) Williamson LM, Llewelyn CA, Fisher NC, et al. A randomized trial of solvent/detergent-treated and standard fresh-frozen plasma in the coagulopathy of liver disease and liver transplantation. Transfusion. 1999; **39**: 1227-34.
162) Flamholz R, Jeon HR, Baron JM, Baron BW. Study of three patients with thrombotic thrombocytopenic purpura exchanged with solvent/detergent-treated plasma: is its decreased protein S activity clinically related to their development of deep venous thromboses? J Clin Apher 2000; **15**: 169-72.
163) Lerner RG, Nelson J, Sorcia E, et al. Evaluation of solvent/detergent-treated plasma in patients with a prolonged prothrombin time. Vox Sang 2000; **79**:161-7.
164) Atance R, Pereira A, Ramírez B. Transfusing methylene blue-photoinactivated plasma instead of FFP is associated with an increased demand for plasma and cryoprecipitate. Transfusion 2001; **41**: 1548-52.
165) de la Rubia J, Arriaga F, Linares D, et al. Role of methylene blue-treated or fresh-frozen plasma in the response to plasma exchange in patients with thrombotic thrombocytopenic purpura. Br J Haematol 2001; **114**: 721-3.
166) Haubelt H, Blome M, Kiessling AH, et al. Effects of solvent/detergent-treated plasma and fresh-frozen plasma on haemostasis and fibrinolysis in complex coagulopathy following open-heart surgery. Vox Sang 2002; **82**: 9-14.

167) Noddeland H, Töllöfsrud S, Svennevig J, et al. Universal solvent/detergent-treated fresh frozen plasma (Uniplas--rationale and clinical properties. Thromb Res 2002; **107**: S33-7.
168) de Jonge J, Groenland TH, Metselaar HJ, et al. Fibrinolysis during liver transplantation is enhanced by using solvent/detergent virus-inactivated plasma (ESDEP). Anesth Analg 2002; **94**: 1127-31.
169) Alvarez-Larrán A, Del Río J, Ramírez C, et al. Methylene blue-photoinactivated plasma vs. fresh-frozen plasma as replacement fluid for plasma exchange in thrombotic thrombocytopenic purpura. Vox Sang 2004; **86**: 246-51.
170) de Alarcon P, Benjamin R, Dugdale M, et al. Fresh frozen plasma prepared with amotosalen HCl (S-59) photochemical pathogen inactivation: transfusion of patients with congenital coagulation factor deficiencies. Transfusion 2005; **45**: 1362-72.
171) García-Noblejas A, Osorio S, Durán AI, et al. Pulmonary embolism in a patient with severe congenital deficiency for factor V during treatment with fresh frozen plasma. Haemophilia 2005; **11**: 276-9.
172) Mintz PD, Neff A, MacKenzie M, et al. A randomized, controlled phase III trial of therapeutic plasma exchange with fresh-frozen plasma (FFP) prepared with amotosalen and ultraviolet A light compared to untreated FFP in thrombotic thrombocytopenic purpura. Transfusion 2006; **46**: 1693-704.
173) Santagostino E, Mancuso ME, Morfini M, et al. Solvent/detergent plasma for prevention of bleeding in recessively inherited coagulation disorders: dosing, pharmacokinetics and clinical efficacy. Haematologica 2006; **91**: 634-9.
174) Scully M, Longair I, Flynn M, et al. Cryosupernatant and solvent detergent fresh-frozen plasma (Octaplas) usage at a single centre in acute thrombotic thrombocytopenic purpura. Vox Sang 2007; **93**: 154-8.
175) Mikkola R, Gunn J, Heikkinen J, et al. Use of blood products and risk of stroke after coronary artery bypass surgery. Blood Transfus 2012; **10**: 490-501.
176) Mikkola R, Heikkinen J, Lahtinen J, et al. Does blood transfusion affect intermediate survival after coronary artery bypass surgery? Scand J Surg 2013; **102**: 110-6.
177) Stanojkovic Z, Balint B, Antic A, et al. Clinical efficacy of riboflavin and ultraviolet light inactivated fresh frozen plasma evaluated with INR-quantification. Transfus Apher Sci 2012; **47**: 33-7.
178) Bindi ML, Miccoli M, Marietta M, et al. Solvent detergent vs. fresh frozen plasma in cirrhotic patients undergoing liver transplant surgery: a prospective randomized control study. Vox Sang 2013; **105**: 137-43.
179) Bartelmaos T, Chabanel A, Léger J, et al. Plasma transfusion in liver transplantation: a randomized, double-blind, multicenter clinical comparison of three virally secured plasmas. Transfusion 2013; **53**: 1335-45.
180) Beeck H, Hellstern P. In vitro characterization of solvent/detergent-treated human plasma and of quarantine fresh frozen plasma. Vox Sang 1998; **74**: S219-23.
181) Aznar JA, Molina R, Montoro JM. Factor VIII/von Willebrand factor complex in methylene blue-treated fresh plasma. Transfusion 1999; **39**: 748-50.
182) Doyle S, O'Brien P, Murphy K, et al. Coagulation factor content of solvent/detergent plasma compared with fresh frozen plasma. Blood Coagul Fibrinolysis 2003; **14**: 283-7.
183) Depasse F, Sensebé L, Seghatchian J, et al. The influence of methylene blue light treatment and methylene blue removal filter on fibrinogen activity states and fibrin polymerisation indices. Transfus Apher Sci 2005; **33**: 63-9.
184) Heger A, Römisch J, Svae TE. Stability of solvent/detergent-treated plasma and single-donor fresh-frozen plasma during 48 h after thawing. Transfus Apher Sci 2005; **33**: 57-67.
185) Singh Y, Sawyer LS, Pinkoski LS, et al. Photochemical treatment of plasma with amotosalen and long-wavelength ultraviolet light inactivates pathogens while retaining coagulation function. Transfusion 2006; **46**: 1168-77.
186) del Río-Garma J, Pereira A, Arroyo JL, et al; Spanish Group of Apheresis (Grupo Español de AféresisGEA). ADAMTS-13 activity and von Willebrand factor levels in methylene-blue photo-inactivated plasma processed by either the Springe method or an 'in house' system. Vox Sang 2008; **95**: 101-5.
187) Lawrie AS, Green L, Canciani MT, et al. The effect of prion reduction in solvent/detergent-treated plasma on haemostatic variables. Vox Sang 2010; **99**: 232-8.
188) Theusinger OM, Baulig W, Seifert B, et al. Relative concentrations of haemostatic factors and cytokines in solvent/detergent-treated and fresh-frozen plasma. Br J Anaesth 2011; **106**: 505-11.
189) Ettinger A, Miklauz MM, Hendrix BK, et al. Quality of proteins in riboflavin and UV light-treated FFP during 1 year of storage at −18°C. Transfus Apher Sci 2012; **46**: 15-8.
190) Ettinger A, Miklauz MM, Bihm DJ, et al. Preparation of cryoprecipitate from riboflavin and UV light-treated plasma. Transfus Apher Sci 2012; 46: 153-8.
191) Keller MK, Krebs M, Spies C, et al. Clotting factor activity in thawed Octaplas® LG during storage at 2-6°C for 6 days from a quality assurance point of view. Transfus Apher Sci 2012; **46**: 129-36.
192) Cid J, Caballo C, Pino M, et al. Quantitative and qualitative analysis of coagulation factors in cryoprecipitate prepared from fresh-frozen plasma inactivated with amotosalen and ultraviolet A light. Transfusion 2013; **53**: 600-5.
193) Rock G, Clark WF, Anderson D, et al; Members of the Canadian Apheresis Group. ADAMTS-13 may not predict disease or outcome in patients with thrombotic thrombocytopenic purpura. Thromb Res 2013; **131**: 308-12.
194) Jilma-Stohlawetz P, Kursten FW, Walasek C, et al. Safety of a universal, virus-inactivated and prion-depleted, pharmaceutical-quality plasma: a randomized, double-blind, clinical trial in healthy volunteers. Transfusion 2011; **51**: 1228-40.
195) Jilma-Stohlawetz P, Kursten FW, et al. Recovery, safety, and tolerability of a solvent/detergent-treated and prion-safeguarded transfusion plasma in a randomized, crossover, clinical trial in healthy volunteers. Transfusion 2013; **53**: 1906-17.
196) Bolton-Maggs PH, Cohen H. Serious Hazards of Transfusion (SHOT) haemovigilance and progress is improving transfusion safety. Br J Haematol 2013; **163**: 303-14.
197) Stramer SL, Hollinger FB, Katz LM, et al. Emerging infectious disease agents and their potential threat to transfusion safety. Transfusion 2009; **49**: 1S-29S.
198) MacLennan S, Barbara JA. Risks and side effects of therapy with plasma and plasma fractions. Best Pract Res Clin Haematol 2006; **19**: 169-89.
199) Funk MB, Guenay S, Lohmann A, et al. Benefit of transfusion-related acute lung injury risk-minimization measures--German haemovigilance data (2006-2010). Vox Sang 2012; **102**: 317-23.
200) Pandey S, Vyas GN. Adverse effects of plasma transfusion. Transfusion 2012; **52**: 65S-79S.
201) Evidence-Based Medicine Group. San Giovanni Battista Hospital, Turin, Italy. [The transfusion of plasma in adults.] November 2006. Available at: http://old.cpo.it/lineeguida/lgplasma.pdf. Accessed on 10/12/2014. [In Italian.]
202) McCullough J. Pathogen inactivation: a new paradigm for preventing transfusion-transmitted infections. Am J Clin Pathol 2007; **128**: 945-55.
203) Neisser-Svae A, Bailey A, Gregori L, et al. Prion removal effect of a specific affinity ligand introduced into the manufacturing process of the pharmaceutical quality solvent/detergent (SD)-treated plasma OctaplasLG. Vox Sang 2009; **97**: 226-33.

204) Bolton-Maggs PH, Poles D, Watt A, Thomas D; on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2013 Annual SHOT Report (2014).
205) Canadian Agency for Drugs and Technologies in Health, CADHT Technology report, 2007. A systematic review of clinical, laboratory and safety outcomes associated with use of Octaplas in multiple clinical indications.
206) Canadian Agency for Drugs and Technologies in Health, CADHT Technology report, 2010. Update. A systematic review of clinical, laboratory and safety outcomes associated with use of Octaplas in multiple clinical indications.
207) Flesland O. A comparison of complication rates based on published haemovigilance data. Intensive Care Med 2007; **33**: S17-21.
208) Cazenave JP, Waller C, Kientz D, et al. An active hemovigilance program characterizing the safety profile of 7483 transfusions with plasma components prepared with amotosalen and UVA photochemical treatment. Transfusion 2010; **50**: 1210-9.
209) Canadian Agency for Drugs and Technologies in Health, CADHT Systematic Review, 2010. Pathogen Reduction Technologies for Blood Products: A Review of the Clinical Effectiveness, Cost-Effectiveness, and Guidelines.
210) European Commission, Health and Consumers Directorate-General, 2013. Summary of the 2013 annual reporting of serious adverse events and reactions (SARE) for blood and blood components. Available at: http://ec.europa.eu/health/blood_tissues_organs/docs/blood_sare_2013_en.pdf. Accessed on 01/12/2014.
211) The Paul-Ehrlich-Institut. Assessment of the Reports of Serious Adverse Transfusion Reactions pursuant to Section 63 c AMG (Arzneimittelgesetz, German Medicinal Products Act), 2010. Available at: http://www.pei.de/SharedDocs/Downloads/vigilanz/haemovigilanz/publikationen/haemovigilance-report-2010.pdf?__blob=publicationFile&v=5. Accessed on 01/12/2014.
212) Knowles S, Cohen H; on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2010 Annual SHOT Report (2011). Available at: http://www.shotuk.org/shot-reports/. Accessed on 01/12/2014.
213) Bolton-Maggs PH, Cohen H; on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2011 Annual SHOT Report (2012). Available at: http://www.shotuk.org/shot-reports/. Accessed on 01/12/2014.
214) Bolton-Maggs PH, Poles D, Watt A; on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2012 Annual SHOT Report (2013). Available at: http://www.shotuk.org/shot-reports/. Accessed on 01/12/2014.
215) Irish National Haemovigilance Office. NHO REPORT 2008/2009. Available at: http://www.giveblood.ie/Clinical_Services/Haemovigilance/Publications/. Accessed on 01/12/2014.
216) Irish National Haemovigilance Office. NHO REPORT 2010/2011. Available at: http://www.giveblood.ie/Clinical_Services/Haemovigilance/Publications/. Accessed on 01/12/2014.
217) Swissmedical. Haemovigilance Annual report 2010. Available at: https://www.swissmedic.ch/marktueberwachung/00138/00188/index.html?lang=en. Accessed on 02/12/2014.
218) Swissmedical. Haemovigilance Annual report 2011. Available at: https://www.swissmedic.ch/marktueberwachung/00138/00188/index.html?lang=en. Accessed on 02/12/2014.
219) Swissmedical. Haemovigilance Annual report 2012. Available at: https://www.swissmedic.ch/marktueberwachung/00138/00188/index.html?lang=en. Accessed on 02/12/2014.
220) Swissmedical. Haemovigilance Annual report 2013. Available at: https://www.swissmedic.ch/marktueberwachung/00138/00188/index.html?lang=en. Accessed on 02/12/2014.
221) Agence Française de Sécurité Sanitaire des produits de Santé (AFSSAPS). Haemovigilance Annual report 2008. Available at: http://ansm.sante.fr/Mediatheque/Publications/Information-in-English. Accessed on 02/12/2014.
222) Agence Française de Sécurité Sanitaire des produits de Santé (AFSSAPS). Haemovigilance Annual report 2009. Available at: http://ansm.sante.fr/Mediatheque/Publications/Information-in-English. Accessed on 02/12/2014.
223) Politis C, Kavallierou L, Hantziara S, et al. Haemovigilance data on the use of methylene blue virally inactivated fresh frozen plasma with the Theraflex MB plasma System in comparison to quarantine plasma: 11 years' experience. Transfus Med 2014; **24**: 316-20.
224) Muñiz-Diaz E, Puig L. Allergic and anaphylactic reactions to methylene-blue-treated plasma in Catalonia in the period 2008-2013. Blood Transfus 2014; **12**: 628-30.
225) Bost V, Odent-Malaure H, Chavarin P, et al. A regional haemovigilance retrospective study of four types of therapeutic plasma in a ten-year survey period in France. Vox Sang 2013; **104**: 337-41.
226) Nubret K, Delhoume M, Orsel I, et al. Anaphylactic shock to fresh-frozen plasma inactivated with methylene blue. Transfusion 2011; **51**: 125-8.
227) Norda R, Tynell E, Akerblom O. Cumulative risks of early fresh frozen plasma, cryoprecipitate and platelet transfusion in Europe. J Trauma 2006; **60**: S41-5.
228) Dewachter P, Castro S, Nicaise-Roland P, et al. Anaphylactic reaction after methylene blue-treated plasma transfusion. Br J Anaesth 2011; **106**: 687-9.
229) Eder AF, Herron RM Jr, Strupp A, et al. Effective reduction of transfusion-related acute lung injury risk with male-predominant plasma strategy in the American Red Cross (2006-2008). Transfusion 2010; **50**: 1732-42.
230) Hauser L, Roque-Afonso AM, Beylouné A, et al. Hepatitis E transmission by transfusion of Intercept blood system-treated plasma. Blood 2014; **123**: 796-7.
231) International Haemovigilance Network. Proposed Standard Definitions For Surveillance Of Non Infectious Adverse Transfusion Reactions; 2011. Available at: http://www.ihn-org.com/wp-content/uploads/2011/06/ISBT-definitions-for-non-infectious-transfusion-reactions.pdf. Accessed on 01/12/2014.
232) Solheim BG. Pathogen reduction of blood components. Transfus Apher Sci 2008; **39**: 75-82.
233) EUnetHTA Joint Action 2, Work Package 8. HTA Core Model® version 2.0; 2013. Available at: http://www.corehta.info/BrowseModel.aspx. Accessed on 10/12/2014.
234) Drummond MF, Sculpher MJ, Torrance GW, et al. *Methods for the economic evaluation of health care programmes*. 3rd ed. Oxford: Oxford University Press; 2005.
235) Riedler GF, Haycox AR, Duggan AK, Dakin HA. Cost-effectiveness of solvent/detergent-treated fresh frozen plasma. Vox Sang 2003; **85**: 88-95.
236) Van Eerd MC, Ouwens JN, De Peuter MA. Cost-effectiveness study comparing pharmaceutically licensed plasma for transfusions (OctaplasLG®) versus fresh frozen plasma (FFP) in critically ill patients in the UK. Transfus Apher Sci 2010; **3**: 251-9.
237) Blumberg N, Heal JM. Mortality risks, costs, and decision making in transfusion medicine. Am J Clin Pathol 2000; **114**: 934-7.
238) Huisman EL, de Silva SU, de Peuter MA. Economic evaluation of pooled solvent/detergent treated plasma versus single donor fresh-frozen plasma in patients receiving plasma transfusions in the United States. Transfus Apher Sci 2014; **51**: 17-24.

239) AuBuchon JP, Birkmeyer JD. Safety and cost-effectiveness of solvent-detergent-treated plasma. In search of a zero-risk blood supply. JAMA 1994; **272**: 1210-4.
240) Pereira A. Cost-effectiveness of transfusing virus-inactivated plasma instead of standard plasma. Transfusion 1999; **39**: 479-87.
241) Huisman EL, van Eerd MC, Ouwens JN, de Peuter MA. Cost-effectiveness and budget impact study of solvent/detergent (SD) treated plasma (OctaplasLG®) versus fresh-frozen plasma (FFP) in any patient receiving transfusion in Canada. Transfus Apher Sci 2014; **51**: 25-34.
242) Taylor C, Cohen H, Mold D, Jones H; on behalf of the Serious Hazards of Transfusion (SHOT) steering group. The 2008 Annual SHOT Report; 2009. Available at: http://www.shotuk.org/shot-reports/report-and-summary-2009/. Accessed on 01/12/2014.
243) Ofosu FA, Freedman J, Semple JW. Plasma-derived biological medicines used to promote haemostasis. Thromb Haemost 2008; **99**: 851-62.
244) Liumbruno GM, Panetta V, Bonini R, et al. Institutional authorization and accreditation of Transfusion Services and Blood Donation Sites: results of a national survey. Blood Transfus 2011; **9**: 436-54.
245) Hellstern P. Solvent/detergent-treated plasma: composition, efficacy, and safety. Curr Opin Hematol 2004; **11**: 346-50.
246) Sharma AD, Sreeram G, Erb T, Grocott HP. Solvent-detergent-treated fresh frozen plasma: a superior alternative to standard fresh frozen plasma? J Cardiothorac Vasc Anesth 2000; **14**: 712-7.
247) Koenigbauer UF, Eastlund T, Day JW. Clinical illness due to parvovirus B19 infection after infusion of solvent/detergenttreated pooled plasma. Transfusion 2000; **40**: 1203-6.
248) Ruggeri M, Marietta M, Krusius T. Plasma S/D: le ragioni di una scelta. J Health Sci 2014; **17**.
249) Vamvakas EC, Blajchman MA. Blood still kills: six strategies to further reduce allogeneic blood transfusion-related mortality. Transfus Med Rev 2010; **24**: 77-124.
250) Klein HG, Glynn SA, Ness PM, et al. Research opportunities for pathogen reduction/inactivation of blood components: summary of an NHLBI workshop. Transfusion 2009; **49**: 1262-8.
251) Alter HJ. Pathogen reduction: a precautionary principle paradigm. Transf Med Rev 2008; **22**: 97-102.
252) Official Journal of Italian Republic n. 80, 7 April 2015. Decreto Ministeriale 5 dicembre 2014. "Individuazione dei centri e aziende di frazionamento e di produzione di emoderivati autorizzati alla stipula delle convenzioni con le regioni e le province autonome per la lavorazione del plasma raccolto sul territorio nazionale".
253) Official Journal of the European Union n. L 311, 28 November 2001. Directive 2001/83/CE on the Community code relating to medicinal products for human use.
254) Official Journal of the European Union n. L 33, 8 February 2003. Directive 2002/98/EC of the European Parliament and of the Council of 27 January 2003 setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components and amending Directive 2001/83/EC
255) Official Journal of the European Union n. L 262, 14 October 2003. Commission Directive 2003/94/EC of 8 October 2003 "laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use".
256) World Health Organization. Safe Blood and Blood Products: Manual on the Management, Maintenance and Use of Blood Cold Chain Equipment. Geneva: WHO; 2005. Available at: http://www.who.int/bloodsafety/Manual_on_Management,Maintenance_and_Use_of_Blood_Cold_Chain_Equipment.pdf. Accessed on 01/12/2014.
257) Di Fazio C. Studio sulla validazione del congelamento del plasma. Undergraduate dissertation. Biomedical engineering, Engineering school. Università degli studi di Padova. 2012.
258) Official Journal of Italian Republic n. 233, 6 October 2005. Decreto Legislativo 19 agosto 2005 n. 191. "Attuazione della direttiva 2002/98/CE che stabilisce norme di qualità e di sicurezza per la raccolta, il controllo, la lavorazione, la conservazione e la distribuzione del sangue umano e dei suoi componenti".
259) Official Journal of Italian Republic n. 19, 23 January 2008. Decreto Legislativo 20 dicembre 2007, n. 261. "Revisione del decreto legislativo 19 agosto 2005, n. 191, recante attuazione della direttiva 2002/98/CE che stabilisce norme di qualità e di sicurezza per la raccolta, il controllo, la lavorazione, la conservazione e la distribuzione del sangue umano e dei suoi componenti".
260) Official Journal of Italian Republic n. 261, 9 November 2007. Decreto Legislativo 9 novembre 2007, n. 207. "Attuazione della direttiva 2005/61/CE che applica la direttiva 2002/98/CE per quanto riguarda la prescrizione in tema di rintracciabilità del sangue e degli emocomponenti destinati a trasfusioni e la notifica di effetti indesiderati ed incidenti gravi".
261) Official Journal of Italian Republic n. 261, 9 November 2007. Decreto Legislativo 9 novembre 2007, n. 208. "Attuazione della direttiva 2005/62/CE che applica la direttiva 2002/98/CE per quanto riguarda le norme e le specifiche comunitarie relative ad un sistema di qualità per i servizi trasfusionali".
262) Official Journal of the European Union n. L 256, 1 ottobre 2005. Commission Directive 2005/62/CE of 30 September 2005 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards Community standards and specifications relating to a quality system for blood establishments.
263) Official Journal of the European Union n. L 91, 30 March 2004. Commission Directive 2004/33/CE of 22 March 2004 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components.
264) Official Journal of the European Union n. L 256, 1 October 2005. Commission Directive 2005/61/EC of 30 September 2005 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards traceability requirements and notification of serious adverse reactions and events.
265) Official Journal of Italian Republic n. 251, 27 October 2005. Legge 21 Ottobre 2005, n. 219. "Nuova disciplina delle attività trasfusionali e della produzione nazionale degli emoderivati"
266) Official Journal of Italian Republic n. 113, 17 May 2011. Conferenza permanente per i rapporti tra lo Stato, le Regioni e le Province Autonome di Trento e Bolzano. Accordo 16 dicembre 2010. Accordo, ai sensi dell'articolo 4 del decreto legislativo 28 agosto 1997, n. 281, tra il Governo, le Regioni e le Province autonome di Trento e Bolzano sui requisiti minimi organizzativi, strutturali e tecnologici delle attività sanitarie dei servizi trasfusionali e delle unita' di raccolta e sul modello per le visite di verifica. (Rep. Atti n. 242/CSR del 16 dicembre 2010).
267) Official Journal of Italian Republic n. 85, 13 April 2005. Decreto del Ministro della Salute 3 marzo 2005: Protocolli per l'accertamento della idoneità del donatore di sangue e di emocomponenti.
268) Ordinanza 29 marzo 2004 Ministero della Salute. Non idoneità alla donazione di sangue di coloro che hanno soggiornato nel Regno Unito. Gazzetta Ufficiale della Repubblica Italiana, n. 102, 3 maggio 2004.

269) Centro Nazionale Sangue, Agenzia Italiana del Farmaco e Istituto Superiore di Sanità. Gestione delle segnalazioni di donatori con malattia di Creutzfeldt-Jakob (post donation information). Position paper 2012.
270) CHMP Position statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products. European Medicines Agency (EMA); London: Jun 23, 2011. EMA/CHMP/BWP/303353/2010.
271) Official Journal of the European Union n. L 169, 12 July 1993. Directive 93/42/CEE of the Council of 14 June 1993, concerning medical devices.
272) Official Journal of the European Union n. L 139 del 23 May 1989. Directive 89/336/CEE of the Council of 3 May 1989 on the approximation of the laws of the Member States relating to electromagnetic compatibility.
273) Official Journal of the European Union n. L 077, 26 March 1973. Directive 73/23/CEE of the Council of 19 February 1973 on the harmonisation of the laws of Member States relating to electrical equipment designed for use within certain voltage limits.
274) Official Journal of the European Union n. L 331, 07 December 1998. Directive 98/37/EC of the European Parliament and of the Council of 22 June 1998 on the approximation of the laws of the Member States relating to machinery.
275) European HAS (Harmonised standards): EN 61326:1997. Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 1: General requirements.
276) International standards: EN 61010-1:2001 (General requirements) Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements.
277) European Commission Health and Consumer Protection Directorate General Public Health and Risk Assessment Directorate. European Blood Inspection System. Common European Standards and Criteria for the Inspection of Blood Establishments Reflecting European good practice within the area addressing the quality and safety of blood. 2003-2008.
278) Official Journal of the European Union L 102, 7 April 2004. Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells.
279) Official Journal of the European Union n. L 159, 26 June 2003. Commission Directive 2003/63/EC of 25 June 2003 amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use.
280) Sheffield WP, Bhakta V, Talbot K, et al. Quality of frozen transfusable plasma prepared from whole blood donations in Canada: an update. Transfus Apher Sci 2013; **49**: 440-6.
281) AuBuchon JP, Birkmeyer JD, Busch MP. Safety of the blood supply in the United States: opportunities and controversies. Ann Intern Med 1997; **127**: 904-9.
282) Bacterial contamination of blood components: risks, strategies, and regulation: joint ASH and AABB educational session in transfusion medicine. Hematology/the American Society of Hematology Education Program. 2003.
283) Jorquera JI. Safety procedures of coagulation factors. Hemophilia 2007; **13**: 41-6.
284) Van der Wilt GJ, Reuzel R, Banta HD. The ethics of assessing health technologies. Theor Med Bioeth 2000; **21**: 103-15.
285) Hofmann B. Why ethics should be part of health technology assessment. Int J Technol Assess Health Care 2008; **24**: 423-9.
286) Sacchini D, Virdis A, Refolo P, et al. Health technology assessment (HTA): Ethical aspects. Med Health Care Philos 2009; **12**: 453-7.
287) Assasi N, Schwartz L, Tarride JE, et al. Methodological guidance documents for evaluation of ethical considerations in health technology assessment: a systematic review. Expert Rev Pharmacoecon Outcomes Res 2014; **14**: 203-20.
288) Health Council of the Netherlands. Pathogen reduction in blood products. The Hague: Health Council of the Netherlands, 2003; publication no. 2003/16E.
289) Refolo P, Sacchini D, Minacori R, Spagnolo AG. [Internet use in clinical trials]. Clinica Terapeutica 2014; **165**: e79-85. [In Italian.]
290) Berto D, Peroni M, Milleri S, Spagnolo AG. Evaluation of the readability of information sheets for healthy volunteers in phase-I trials. Eur J Clin Pharmacol 2000; **56**: 371-4.
291) Refolo P, Minacori R, Mele V, et al. Patient-reported outcomes (PROs): the significance of using humanistic measures in clinical trial and clinical practice. Eur Rev Med Pharmacol Sci 2012; **16**: 1319-23.
292) Minacori R, Sacchini D, Cicerone M, Spagnolo AG. Insurance and clinical trials. Contemp Clin Trials 2012; **33**: 573.
293) Refolo P, Mele V, Minacori R, Spagnolo AG. [Patient-reported outcomes (PRO): Historical profile, definitions, classifications and problems.] Clinica Terapeutica 2012; **163**: 39-45. [In Italian.]
294) National Advisory Committee on Bioethics. Department of Health. Specific Informed Consent for Blood Transfusion: The Ethical Considerations; 2013. Avaialble at: http://www.dohc.ie/working_groups/Current/nacb/SpecificConsent_Blood%20Transfusion.pdf?direct=1. Accessed on 10/12/2014.
295) Rodriguez del Pozo P. Paying donors and the ethics of blood supply. Med Ethics 1994; **20**: 31-5.
296) Buyx AM. Blood donation, payment, and non-cash incentives: classical questions drawing renewed interest. Transfus Med Hemother 2009; **36**: 329-39.
297) McQuillen MP. Ethical lessons learned from the use of therapeutic plasma exchange in neurologic disease. Ther Apher 2000; **4**: 190-4.
298) Sazama K. The ethics of blood management. Vox Sang 2007; **92**: 95-102.
299) Sazama K. Managing infectious or untested autologous blood components: the ethical dilemma of private rights versus public safety. Arch Pathol Lab Med 2005; **129**: 1212-3.
300) Farrugia A1, Penrod J, Bult JM. Payment, compensation and replacement--the ethics and motivation of blood and plasma donation. Vox Sang 2010; **99**: 202-11.
301) Weiskopf RB. The ethics of blood management. Vox Sang 2007; **93**: 91.
302) Sacchini D, Liumbruno GM, Bruno G, et al. Ethical and deontological issues in Transfusion Medicine. Blood Transfus 2013; **11**: 14-23.
303) Farrell AM, Brazier M. Patients should consent to blood transfusion. Br Med J 2010; **341**: c4336.
304) Holland PV. Consent for transfusion: is it informed? Transfus Med Rev 1997; **11**: 274-85.
305) Court EL, Robinson A, Hocken DB. Informed consent and patient understanding of blood transfusion. Transf Med 2011; **21**: 183-9.
306) Official Journal of Italian Republic n. 85, April 13th, 2005. Decreto del Ministro della Salute 3 marzo 2005: "Caratteristiche e modalità per la donazione del sangue e di emocomponenti".
307) Judgment of the Court (First Chamber) 13 March 2014, (Approximation of laws - Directive 2001/83/EC - Directive 2002/98/EC - Scope - Labile blood product - Plasma prepared by means of an industrial process - Simultaneous or exclusive application of the directives - Option for a Member State to provide for a more rigorous regime for plasma than for medicinal products) - (C 512/12).

308) Conseil d'Etat sentence no. 357463 between MacoPharma and the Agence Française de Sécurité des Produits de Santé, France. Available at: https://www.legifrance.gouv.fr/affichJuriAdmin.do?oldAction=rechJuriAdmin&idTexte=CETATEXT000029288214&fastReqId=233964865&fastPos=1. Accessed on 10/12/2014.
309) Official Journal of Italian Republic n. 3, 16 January 2008. Italian Ministerial Decree 21 December 2007. New legislation on transfusions and national production of blood products.
310) Council of Europe, Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine, Oviedo, 4-IV-1997.
311) Additional Protocol to the Convention on Human Rights and Biomedicine, concerning Biomedical Research, Strasbourg, 25.I.2005
312) World Medical Association. WMA Publishes its Revised Declaration of Helsinki, 2013. Available at: http://www.wma.net/en/40news/20archives/2013/2013_28/. Accessed on 10/12/2014.
313) Official Journal of the European Union n. L 281, 23 November 1995. European Parliament And Council, Directive 95/46/EC of 24 October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data.
314) European Convention For The Protection Of Human Rights And Fundamental Freedoms, Rome 4.XI.1950.
315) Court Of Justice Of European Union, Judgment 20 May 2003, Joined Case C-465/00, C-138/01, C-139/01.
316) Committee On Bioethics (DH-BIO) Working document on research on biological materials of human origin, DH-BIO/INF (2014) 3, Strasbourg, 18 March 2014.
317) National Advisory Committee on Bioethics - Department of Health. Specific Informed Consent for Blood Transfusion: The Ethical Considerations; January 2013. Available at: http://health.gov.ie/wp-content/uploads/2014/07/SpecificConsent_Blood-Transfusion1.pdf. Accessed on 10/12/2014.
318) Repealed by Decision No 1350/2007/EC of the European Parliament and of the Council of 23 October 2007 establishing a second programme of Community action in the field of health (2008-13). Official Journal L 301 of 20.11.2007.
319) Commission, White Paper "Together for Health: A Strategic Approach for the EU 2008-2013, COM (2007) 630
320) Commission of the European Communities, Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions e-health - making healthcare better for European citizens: an Action plan for a European e-Health area, {SEC(2004)539}, Brussels, 30.4.2004, COM (2004) 356 final.
321) European Parliament - Council of the European Union. 2011/24/UE Directive of the 9 March 2011 concerning the application of patients' rights in cross-border healthcare.
322) Council of Europe, Committee of Ministers. Recommendation Rec(2006)18 on health services in a multicultural society (adopted by the Committee of Ministers on 8 November 2006 at the 979th meeting of the Ministers' Deputies).
323) Council of the European Union. Directive on cross-border healthcare, 28/02/2011.
324) Court of Justice of the European Union 28/04/1998 (C-120/95). Judgment of the Court of 28 April 1998. Nicolas Decker v Caisse de maladie des employés privés. Reference for a preliminary ruling: Conseil arbitral des assurances sociales - Grand Duchy of Luxemburg. Free movement of goods - Articles 30 and 36 of the EC Treaty - Reimbursement of medical expenses incurred in another Member State - Prior authorisation of the competent institution - Purchase of spectacles.
325) Official Journal of the European Union L 213, 30 July 1998. Directive 98/44/EC of the European Parliament and of the Council of 6 July 1998 on the legal protection of biotechnological inventions.
326) EUnetHTA, Work Package 4 (2008). The HTA Core Model, HTA Core Model for Medical and Surgical Interventions (v 1.0r). Available at: http://www.eunethta.eu/outputs/hta-core-model-diagnostic-technologies-10r. Accessed on 11/07/2014.
327) Ovretveit J. The contribution of new social science research to patient safety. Soc Sci Med 2009; **69**: 1780-3.
328) Lehoux P, Williams-Jones B. Mapping the integration of social and ethical issues in Health Technology Assessment. Int J Technol Assess Health Care 2007; **23**: 9-16.
329) Whyte SR. *Questioning misfortune the pragmatics of uncertainty in Eastern Uganda*. Cambridge: Cambridge University Press; 1997.
330) Bech U. *Risk society. Towards a new modernity*. New Delhi: Sage; 1992.
331) Bourdieu P. Social being, time and the sense of existence. In: Bourdieu P, editor. *Pascalian Meditations*. New York: Polity Press; 2000. p. 206-45.
332) Good BJ. *Medicine, rationality, and experience an anthropological perspective*. Cambridge: Cambridge University Press; 1994.
333) Foucault M, Martin LH, Gutman H, Hutton PH. *Technologies of the self: a seminar with Michel Foucault*. Amherst: University of Massachusetts Press; 1988.
334) Jackson M. *Minima ethnographic intersubjectivity and the anthropological project*. Chicago: University of Chicago Press; 1998.
335) Latour B. *'Constitution' I: we have never been modern*. London: Prentice Hall; 1993.
336) Kleinman A. Introduction: medical Anthropology as intellectual career. In: Kleinman A, editor. *Writing at the Margin. Discourse between Anthropology and Medicine*. Berkeley/Los Angeles/London: University of California Press; 1995. p. 1-20.
337) Bijker WE, Law J., editors. *Shaping technology/building society. Studies in sociotechnical change*. Cambridge, MA: MIT Press; 1992.
338) Harbers H. I*nside the politics of technology: agency and normativity in the co-production of technology and society*. Amsterdam: Amsterdam University Press; 2005.
339) Reid S, Johnson L, Woodland N, Marks DC. Pathogen reduction treatment of buffy coat platelet concentrates in additive solution induces proapoptotic signaling. Transfusion 2012; **52**: 2094-103.
340) Denis J-L, Lehoux P, Champagne F. Knowledge utilization in health care: From fine-tuning dissemination to contexualizing knowledge. In: Lemieux-Charles L, Champagne F, editors. *Using knowledge and evidence in health care: multidisciplinary perspectives*. Toronto: University of Toronto Press; 2004. p. 18-40.
341) Ross SE, Lavis JN, Rodriguez C, et al. Partnership experiences: Involving decision-makers in the research process. J Health Serv Res Policy 2003; **8**: 26-34.
342) Bastian H. Speaking up for ourselves. The evolution of consumer advocacy in health care. Int J Technol Assess Health Care 1998; **14**: 3-23.
343) Mitton C, Smith N, Peacock S, et al. Public participation in health care priority setting: A scoping review. Health Policy 2009; **91**: 219-28.
344) Gauvin F-P, Abelson J, Giacomni M. Conceptualizing public involvement in the context of health technology assessment agencies. Soc Sci Med 2010; **70**: 1518-26.

345) Lehoux P, Daudelin G, Abelson J. The unbearable lightness of citizens within public deliberation processes. Soc Sci Med 2012; **74**: 1843-50.
346) Lehoux P, Blume SS. Technology assessment and the sociopolitics of health technologies. J Health Polit Policy Law 2000; **25**: 1083-120.
347) Lehoux P, Saint-Arnaud J, Richard L. The use of technology at home. What patient manuals say and sell vs. what patients face and fear. Sociol Health Illn 2004; **26**: 617-44.
348) Lehoux P, Daudelin G, Demers-Payette O, Boivin A. Fostering deliberations about health innovations: what do we want to know from publics? Soc Sci Med 2009; **68**: 2002-9.
349) Mooney G. "Communitarian claims" as an ethical basis for allocating health care resources. Soc Sci Med 1998; **47**: 1171-80.

Correspondence: Stefania Vaglio
Italian National Blood Centre
Via Giano della Bella 27
00162 Rome, Italy
e-mail: ricerca.cns@iss.it

Appendix I

Working team

The current report results from a joint effort between the Postgraduate School of Health Economics and Management (Altems) - *Università Cattolica del Sacro Cuore* (UCSC) and the Italian National Blood Centre (INBC). This research project, led by Professor Americo Cicchetti, Director of Altems, involved Dr. Giancarlo M. Liumbruno, Director of the INBC, four clinical experts from the INBC, a biomedical engineer from Altems, three health economists from Altems, a health economist and two clinical experts from the Health Technology Assessment Unit of "Gemelli" Teaching Hospital - UCSC, four bioethicists from the Institute of Bioethics of the UCSC, three with a clinical background and one with a background in philosophy and, finally, two jurists from the Institute of Bioethics of UCSC. Authorship of each section is reported below:

1 - The health problem and current use of the technologies: Giuseppina Facco, Stefania Vaglio, Giuseppe Marano, Giuliano Grazzini (INBC).
2 - Description and technical characteristics of the technologies: Giuseppina Facco, Stefania Vaglio, Giuseppe Marano, Giuliano Grazzini (INBC).
3 - Clinical efficacy: Giuseppina Facco, Stefania Vaglio, Giuseppe Marano, Giuliano Grazzini (INBC).
4 - Safety: Alessandra Fiore (Altems).
5 - Economic domain: Matteo Ruggeri, Federica Romano, Paola Codella (Altems).
6 - Organisational domain: Marco Marchetti, Alexandra Berrino, Irene Urbina (Health Technology Assessment Unit of "Gemelli" Teaching Hospital-UCSC).
7 - Ethical domain: Pietro Refolo, Dario Sacchini, Roberta Minacori, Antonio G. Spagnolo (Institute of Bioethics of the UCSC).
8 - Social domain: Dario Sacchini, Pietro Refolo, Roberta Minacori, Antonio G. Spagnolo (Institute of Bioethics of the UCSC).
9 - Legal domain: Emanuela Midolo, Marina Casini, Antonio G. Spagnolo (Institute of Bioethics of the UCSC).