SAFETY OF BLOOD AND PLASMA DERIVATIVES: PATHOGEN REDUCING TECHNOLOGIES

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ABSTRACT

Human blood and blood products is the source of a wide range of medicinal products used for the treatment and prevention of a variety of injuries and disease. Despite stringent routine measures and filters employed, residual pathogen infectivity remains an important challenge in the field of blood transfusion. In this article various measures and technologies that can be applied in order to reduce the residual risk are reviewed.

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INTRODUCTION

Human blood is the source of a wide variety of medicinal products used for treatment and prevention of various diseases.

Over the past two decades, blood transfusion in general and plasma fractionation in particular have developed into medical and scientific disciplines requiring skilled scientific expertise and advanced technology to prevent transmission of blood –borne infections.

Appropriate measures to prevent and reduce the risk of potential infections have been introduced with implementation of strict regulations for every step of production from donor selection right up to follow-up of the clinical use of end products.

In general, blood products are of two major categories: (a) the single donor products such as red cell concentrate, platelet concentrate, leucocytes and single donor fresh frozen plasma (FFP). (b) Plasma derivatives, which in contrast to single donor components are obtained from pooling thousands of plasma donations and require a manufacturing approach that includes specific measures to ensure the very high margin of safety demanded by society and regulatory bodies. Fig. 1 demonstrates the relative reduction of risk factor by taking various "safety initiatives". These plasma derivatives are comprised of an increasing variety of products including albumin, various coagulation factor concentrates, polyvalent or hyper-immune immunoglobulins, protease inhibitors, anticoagulants and fibrin sealants.

Viral safety of blood products relies mainly on the appropriate selection of donor population and on the quality of the viral screening procedures for each donation.

However stringent, these systems are not capable of excluding a unit of blood or plasma being collected from a donor in an infectious window period when routine screening tests are negative but viral infectious particles may be present at very low concentrations. This residual infectious risk which in developed countries is very low, becomes more significant when subsequent pooling is used to manufacture plasma products, because all resulting products from an infected pool may be infectious to varying degrees and potentially may infect a large population of recipients.

The diversity in the type of blood and plasma products illustrates the need for a "customized" strategy to ensure optimum viral safety for each product. It also underlines a complex sustaining regulatory process that governs blood and plasma derivative production worldwide. Fully mastering the relative risks of such diverse products remains a major challenge in this field.

This paper is aimed to provide an update and insight to the wide range of measures designed to enhance the safety of plasma products and to provide relative data and experience available in our country where applicable.

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Safety Initiatives

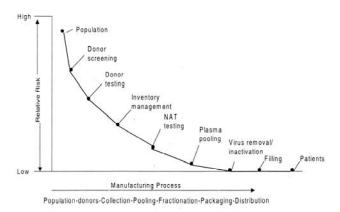**Fig 1..** Relative risk-reduction in blood products using current safety initiatives.

Infectious Risks

Human blood can be contaminated by various infectious agents, viruses, bacteria, protozoa and prions. The major viruses of concern are Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Human Immunodeficiency virus (HIV). Other important viruses are the Human T-cell leukemia virus (HTLV-I,II), Parvovirus B19, Herpes viruses including CMV and Hepatitis A virus (HAV).[1] More recently, a list of additional viruses such as GBV-c, West Nile Virus (WNV), TTV, etc…have emerged as potential threats.[2]

Despite continuous improvement of screening tests, transmission of viral infections by blood and products remains a major challenge and the risk is not totally excluded, since the current tests are still unable to detect infection in the window period today, in countries where donor selection and donor screening is implemented the residual risks are very low. Frequencies of 1 in 2.3 million for HIV and 1:620,000 and 1 in 400,000 for HCV and HBV has been reported for Europe.[3] Accordingly estimates of the frequency in France and in Germany is reported separately as 1 : 1 million for HIV, 1: 200,000 for HCV and 1:180,000for HBV.[4] Similar although slightly higher figures were reported in the U.S.A.[5,6] Evidently, in countries with a higher prevalence of blood borne viruses, and less rigorous donor exclusion, the risk rises and will be higher.

In Iran with prevalence rates of HCV at around 0.3%,[7] and HIV being close to the European countries and use of third generation Elisa techniques for screening, the residual risk factor for these infections should be close to that reported in Europe. However, in the case of HBV, the prevalence rate amongst blood donors in Iran is much higher than the European countries, an average 3% HBsAg positivity among the blood donors reported in 1994 is reduced to 1.2% in 2003[8] by employing more rigorous donor selection programmes, therefore the residual risk in Iran should be higher than that reported for the European countries.

Another important threat is bacterial contamination of cellular products. In some countries, bacterial sepsis today appears to be the most frequent infectious complication, especially of platelet transfusions, but the frequency of transfusion associated septic reactions varies widely in Europe up to six cases are reported annually in European countries and in Canada. In the USA one septic reaction is observed per 10000-20000 transfused units of platelet concentrate (PC) and fatal reactions are seen in one per 6 million units.[9]

Bacterial contamination of red blood cell concentrates (RBC) also occurs frequently.[10] In the period of 1986 to 1996 a total of 21 cases of sepsis associated with contaminated RBC were reported in the USA.[11]

Various parasitic diseases may also be transmitted by blood products, of greatest concern are post transfusion malaria and particularly Chagas disease.[1]

Finally, prions that is the agent of Creutzfeldt Jakob Disease (CJD) and its new variant (vCJD) is also a potential hazard which calls for concern. Citing two confirmed cases in the United Kingdom where v-CJD was transmitted from blood donors who were young and apparently healthy, is a warning for a new wave of infections of unknown magnitude around the world.[12]

As illustrated risk of transmission of bacterial and parasitic pathogens is limited to the single unit products, however in the case of plasma products due to having filtration steps using 2 μm filters these larger pathogens are removed but viruses and prions are of great concern.

The risk of contamination derives from the balance between the viral load in the starting plasma pool, the presence of any neutralizing antibodies, the ability of the manufacturing process to reduce the risks, and the patients own physiological condition.

In that respect, an extensive knowledge of the epidemiology of blood borne viruses in every society is of vital importance. For example although transmission of HAV through blood and its products is important in the Western societies where rate of exposure to this virus is not high in other countries such as Iran where 90% of adults have been exposed to the virus and carry neutralising antibodies to HAV[13], the high titre of antibody present in plasma products with local origin will neutralize any small residual risk of contamination with this virus, therefore lowering concern. In other words according to the endemicity of any infection specific safety strategies should be considered by the regulatory bodies in various countries.

In the past 20 years, more sophisticated production processes and advanced virus inactivation procedures have provided the means to reduce and prevent infections related to HBV, HCV, and HIV. More recently the progressive introduction of nucleic and amplification testing (NAT) for detection of viral genomes which closes the window period considerably provides a new dimension in the viral safety of blood and plasma derivatives.[14]

Use of viral inactivation procedures in blood safety

Specific viral inactivation treatments are the corner-stone in ensuring a sufficient margin of safety of plasma products. The major target of the antiviral treatments is the inactivation of the highly pathogenic plasma –borne enveloped viruses that is, HIV, HBV and HCV which cause chronic infection and disease. Additional targets are the less pathogenic non-enveloped viruses such as HAV and B19, which cause mainly acute illnesses. The various procedures applicable to plasma products is briefly described below. However, because of their cytotoxic properties, these methods cannot be applied to cellular products. The limited available procedures are discussed in a separate section.

I- Viral inactivation procedures of plasma derivatives

Pasteurization

Pasteurization is defined as the treatment of a liquid protein for 10h at 60ºC, the mechanism of viral inactivation using this procedure is heat denaturation of viral components, thereby inhibiting virus replication.

This procedure is generally performed in the presence of stabilizers, which are added to protect plasma protein biological function and limit molecular alterations. The stabilizers may have a protective effect that is specific to plasma proteins, this is the case for albumin where sodium caprylate and sodium tryptophanate bind to the protein and prevent its denaturation or aggregation during liquid heat treatment.[15,16]

Other stabilizers like sugars (e,g. sucrose), and polyols (e.g. sorbitol), have a more global protective effect on the biological activity of labile proteins, avoid the formation of protein aggregates and limit risk of precipitation.[17, 18] Amino acids (e.g. lysine, glycine, arginine, etc…) may also protect the functional activity of some plasma proteins.[18, 19, 20] Combination of sugars and amino acids has been successfully used for the pasteurization of Factor VIII [16,19,20, 21], Factor VII.[22]

Other plasma products that can be pasteurized are intravenous IgG[23], IgM–enriched IgG[24], where possibility of pasteurization in absence of stabilizers is reported, also anti-thrombin III and alpha-1 antitrypsin.[25,26]

Stabilisers when added prior to heat treatment can be subsequently removed by precipitation ultrafiltration, chromatography or a combination of those. Manufacturing processes must ensure consistent and homogenous application of the heating process, and avoid risk of subsequent contamination. Presently albumin is the only product which can withstand pasteurization in the final container, since the quantity of stabilizers used is compatible with direct clinical use and therefore alleviates the risk of downstream contamination.

Extensive validation studies has shown that pasteurization is capable of inactivating various viruses, whether enveloped or non-enveloped, including HIV, HBV, HCV and HAV.[21,26] The confidence in the efficacy of pasteurization for virus inactivation is linked to the remarkable record of safety for albumin.[27] More recently,

inactivation of WNV using this procedure has also been reported.[28]

Dry heat

During this treatment a lyophilized product in the final container is subject to heating for several minutes to several days at temperatures from 60 to 100ºC.[21, 26, 27] Initially, dry heat treatment at 60 to 68ºC for 72 to 96 h was applied to coagulation factors with objective of inactivating HIV. However, reliable inactivation of HCV in these concentrates is achieved only by more severe conditions at 80ºC for 3 days.[29] There are other reports where heat treatment at 100ºC for 30 min has been applied to Factor VIII concentrates in order to inactivate HAV, although, the transmission of B19 may not be eliminated.[30, 31]

The viral inactivation efficacy of heat is generally less than pasteurization due to lower moisture content of the product.

Steam heat

At equivalent temperatures, a higher level of virus inactivation can be achieved by the addition of steam and pressure, and a relatively high moisture content. The extent of viral inactivation is directly related to the temperature (60 to 80ºC), the pressure (1190 to 1375 mbar) and the duration of treatment (upto10h.).

Vapor heating was shown to inactivate HAV in plasma products[32], but cases of transmission of HCV through coagulation factor has been reported[33,34], although the preponderance of clinical data indicates safety with respect to hepatitis viruses and HIV.[35]

Solvent / detergent treatment

Organic solvent / detergent mixtures disrupt the lipid membrane of enveloped viruses. Once disrupted the virus no longer can bind to and infect host cells.

Non-enveloped viruses are, therefore, not inactivated using this procedure. The organic solvent, tri (n-butyl) phosphate (TNBP) is used at a concentration of 0.3% and 1% non ionic detergent, either Tween 80 or Triton x-100 at 24ºC for a minimum of 4h with Triton and 6h for Tween.[36, 37] The elimination of the viral inactivation agents is achieved by subsequent ultrafiltration, chromatography or precipitation techniques. Permitted residual levels of TNBP, Tween 80 and Triton x-100 typically are 3-25, 10-100 and 3-25 ppm, respectively.[21] The method has proven to be extremely robust and manufacturing processes have minimal effect on its efficiency.[38]

This method has been applied to coagulation factors VIII[39, 40], IX[41, 42], factor VII[43], immunoglobulins[44] although in the case of immunoglobulins a new alternative using caprylate has also been introduced.[45] S/D treatment has been shown to have unchallenged potential to inactivate enveloped viruses while maintaining plasma protein integrity and represented a breakthrough in the viral safety of plasma products. Enveloped viruses are typically killed in less than a few minutes. There is no report of protein neoantigen formation due to SD treatment and there is excellent protein recovery. The

method used to remove the SD agents may induce some protein loss. A drawback of the SD procedure is the failure to inactivate non-enveloped viruses.

To date, there has been no report of transmission of HIV, HCV or HBV by SD-treated plasma products in about twenty years of use. The efficacy of this procedure in inactivation of an emergent enveloped virus such as WNV has been demonstrated.[28] Cases of transmission of non-enveloped viruses such as HAV and B19 have been reported from SD-treated coagulation factor concentrates.[46, 47]

Acid pH

Some of the purification steps involved in the production of plasma derivatives have proved to act as viral inactivation steps. The case of pH 4 treatment of IgG which was developed initially to reduce anti-complementary activity and aggregation is a good example. Incubation of IgG at pH 4 and at temperatures between 30 to 37ºC for more than 20 hours inactivates substantial dose of enveloped virus.[48,49] Complete inactivation (over 5 to 8 log) of HIV and other enveloped viruses is achieved by contrast; non-enveloped viruses are more resistant.[21]

Specific viral elimination treatment: Nanofiltration

By contrast to fractionation technologies that remove viruses in a non-specific mechanism, nanofiltration is a new approach to eliminate microorganisms specifically. Nanofiltration is carried out on membranes of a few nanometers (15 to 45 nm) and works by the segregation of proteins and viruses on the basis of their size.[50, 51] Therefore, biological infectious agents such as viruses larger than the nominal filter porosity are retained on the membrane while smaller plasma proteins pass through.

Compared with other viral reduction means, nanofiltration may be the only method permitting efficient removal of enveloped as well as non-enveloped viruses, under conditions where 90-95% of protein activity is recovered.[52 ,53]

The technique is performed using commercially available cartridges and is reasonably simple. In fact, similar to aseptic filtration for removal of bacteria it can be implemented without the need for sophisticated equipment.

Viral validation studies have revealed that membranes with porosity in the range of 15 to 40 nm. can be used for all plasma products. As expected viral clearance of small non-enveloped viruses is greater with the 15 nmcartridge, although aggregates of these viruses have successfully been removed using 35 nm. Filters.[53] Preliminary data indicate that nanofiltration may also remove prions and is therefore becoming increasingly a routine step in manufacture of biopharmaceuticals.[54 ,55]

Application of nanofiltration has been reported for production of factor IX concentrates[41 ,56 ,57,58] ,intravenous immunoglobulins[59,60], factor VIII concentrates[61], and activated factor VII concentrate.[62]

II-Viral inactivation of single donor products

Most of viral inactivation procedures to date are limited to red cell concentrates, platelet concentrates and fresh frozen plasma (FFP). Since the addition of any compounds to these products may affect their functional properties, an assessment of their quality before and after pathogen inactivation is of crucial importance. In addition, their safety and efficacy have to be studied in vivo in animals and humans. Methods that are successfully applied in clinical medicine are as follows:

Solvent / detergent (S/D) treatment of FFP

As mentioned in the previous section, the lipid envelop of transfusion relevant viruses is destroyed by a combination of an organic solvent and a non-ionized detergent, whereas non-enveloped viruses are not affected. Few studies showed that structure and biological function of most plasma proteins were well preserved.[63, 64] However, these reports show that some sensitive proteins such as factor VIII, Protein S and alpha-2- antitrypsin may be slightly damaged. For the production of S/D treated FFP, the organic solvent TNBP is combined with Triton-X-100, at 30 Computer for 4h and the S/D reagents are then removed by extraction. The plasma is sterile-filtered and filled into plastic bags or glass vials in 200 mL units and at -30 C or lower.

Since 1991, S/D treated FFP has been clinically used in some countries and has good efficacy and safety record.[65] However, contamination by non-enveloped viruses may pose a problem. The S/D technology cannot be applied to cellular blood products, since the reagents would disintegrate the lipid bilayer of the cell membrane and destroy the cells.

Methylene blue treatment of FFP

The virus inactivating properties of Methylene Blue (MB) in combination with visible light have been known for a long time. Researchers at the German Red Cross Blood Transfusion Service have developed a procedure using this property of MB for inactivation of FFP. In this procedure 1 µmoL. of MB (320 ug/L) is added to single donor units of FFP, and illuminated with visible light.[66] This treatment inactivates enveloped viruses, HBV, HCV and HIV. Since 1992, this blood product has been used on large scale in Europe.[67] The Paul Ehrlich Institute has expressed concern about possible mutagenic properties of MB and removed approval in Germany.

Leucocyte depletion

A widely used approach for removing leucocytes is the" buffy coat" procedure, where the bulk of leucocytes (buffy coat) is segregated from the plasma and the cells and transferred into a special buffy coat bag. This method allows removal of 85% of the leucocytes.

The other method in common use is employing leucocyte filters. Development of several generations of leucocytes filters made it possible to reduce leucocytes to very low levels; leucocyte filters currently used are capable of reducing 5log of white cells in a one step procedure with a minimal loss of red cells. Leucocyte

depletion of platelet concentrates obtained by platelet apheresis from single donors may be achieved by centrifugation methods or by leucocyte reduction filter systems.

Leucocyte filtration may be performed at the bedside, that is, immediately before the transfusion. This method allows specific targeting of leukodepleted products to patients who need them.

In recent years, animal experiments have suggested that leucocytes may be involved in promoting v-CJD[68], thus in order to minimize a potential risk, some European countries have introduced universal leukodepletion of all labile products.[67]

Although appropriate prestorage leucocyte depletion by filtration was shown to prevent some cell-bound infectious agents such as CMV, studies demonstrating that this technology also excludes cell-bound HIV and HTLV-I,II infectivity are still lacking.

The high level of safety achieved using the very complicated and expensive technologies for plasma products should be an encouragement to seek further progress and indeed, new treatments continue to be developed. However, considering the high relative safety of blood in the industrialized world, simple, less expensive viral safety treatments of blood and plasma derivatives in the developing world appears to be urgent and challenging. To this date the only available methods of viral inactivation employed in the production of plasma derivatives in Iran has been pasteurization and mild heat treatment. The other more sophisticated methods such as severe heat treatment and solvent /detergent have been applied to such products only in laboratory scale. The technology for the proper application of these techniques to such products in industrial scale is not as yet available.

REFERENCES

1. Moor ACE, Dubbelman TMAR, et al: Transfusion transmitted disease, risk prevention and perspectives. European J Haematol 1999; 62,1-18.
2. Allain JP: NAT for emerging viruses. Transf. Med., 2002 , 12 , 275- 283.
3. Muller– Breitkreutz: Results of viral marker screening of unpaid blood donations and probability of window period donations. Vox Sang 2000,78,149- 157.
4. Pillonel J, Saura C, Courouce AM: Screening of viral markers for HIV, HBVand HCV infections in blood donors in France and residual risk of viral transmission by blood transfusion. Eurosurveillance 1998; 3: 76-79.
5. Aubochon JP, Birkmeyer JD, Busch MP: Safety of the blood supply in U.S. Annals of Intern. Med 1997;125,904- 909.
6. Kleinman S: In: Rossi's Principles of Transfusion Medicine ,ed. 3 ,Lippincott Wilkins 2002 p.703
7. Rezvan H, Ahmadi J, Farhadi M: Prevalence of anti-HCV amongst healthy blood donors in Iran. Vox Sang 1994;67 (suppl.),100
8. Rezvan H: In: Viruses and blood transfusion. 2005 IBTO Research center publications.
9. Engelfried CP, Recsink HW: Bacterial contamination of blood components. Vox. Sang. 78,59-67,2000.
10. Blajchman MA: Bacterial contamination and proliferation during the storage of cellular products. Vox. Sang. 74 (suppl.2), 155-159, 1998.
11. Centers of disease control and prevention, Red blood cell transfusion contaminated with *Yersinia enterocolitica* in U.S. Morbidity and Mortality Weekly Reports 46,553-555,1997.
12. Prion Disease is transmitted by transfusion. Labmedica International, 22,1 ,5, 2005.
13. Malekzadeh R, Khatibian M, Rezvan H: Viral hepatitis in the world and Iran.J Irn Med Council 1997;15 183-200
14. Lee HH, Allain JP: Genomic screening for blood borne viruses in transfusion settings. Vox Sang 1998, 74,119-123.
15. Mousavi K, Rezvan H, Motallebi Z, et al: Study of heat trealed human albumin stabilization by caprylate and tryptophate Iran. Biomed J.6, 135-140, 2002.
16. Ng PK, Dobkin MB: Pasteurization of anti hemophilic factor and model virus inactivation studies. Thromb. Res,39: 439-447,1985.
17. Macleod AJ, Welch AG, Dickson IH, et al: Stabilization of proteins to heat. Res Disclosures 224: 380,1984.
18. Nowak T, Niedrig M, Bernhardt D, Hilfenhaus J, Inactivation of HIV, HBV, HCV related viruses in human plasma derivatives by pasteurization. Dev. Biol Stand 1993, 81 169- 176
19. Hilfenhous J, Herrman A. Mauler R, et al: Inactivation of the AIDS- causing retrovirus and other human viruses in anti-hemophilic products by pasteurization. Vox. Sang. 50, 208-211, 1986.
20. Horowitg B, et al. Inactivation of viruses in labile products. Physical methods. Transfusion 25:523-27, 1985.
21. Guidelines on viral inactivation of human Blood and plasma products. WHO publication BS/ 01/1941, 2001.
22. Rezvan H, Nasiri S, Mousavi K, et al: Inactivation of polio and HSV in human coagulation factor VII concentrate by pasteurization. Arch Irn Med 2001; 4, 10-13.
23. Bridonneaw P, Marcilly H, Vernois- Martin M, et al. Liquid pasteurization of an immunoglobulin preparation without stabilizer. Vox Sang 1996, 70, 203-209.
24. Mousavi K, Dinarvand R, Rezvan H, et al: Pasteurization of IgM-enriched immunoglobulin. Daru 2004, 12, 40-43
25. Burnouf T: Example of virus inactivation treatment in plasma derivatives, manufacturing of plasma proteins. Biotechnology 175, 373-382, 1989.
26. Burnouf T, Radoservich M: Reducing the risk of infection from plasma products. Blood Rev 14,94-110, 2000.
27. Tabor E. The epidemiology of virus transmission by plasma derivatives. Transfusion 39, 1160-1168, 1999.

28. Remington KM, Trejo SR, Buczynski GL, et al: Inactivation of West Nile virus, Vaccinia virus and surrogates for relevant and emergent viral pathogens in plasma derived products. Vox Sang 2004, 87, 10 -18.

29. Study group of the UK hemophilia centre. Effect of day heating of coagulation factor concentrates at 80ºC for 72h. Lancet ii: 1988,814- 816

30. Rubenstein AI, Rubenstein DB. Sequential dry heat treatments at sterilizing temperature (100 C) to produce factor VIII concentrates. Vox Sang 1989, 57, 272

31. Santogostino E, Mannucci PM, Gringeri A, et al: Transmission of parvovirus B19 by coagulation factor concentrates exposed to 100ºC after lyophilisation. Transfusion 1997, 37 517-522.

32. Barrett PN, Meyer H, Wachtel I, et al: Inactivation of HAV in plasma products by vapor heating. Transfusion 1997, 37, 215- 220.

33. Mannucci PM, Zanetti AR, Columbo M: Prospective study of hepatitis after FVIII exposed to hot vapor. Br J Haematol 1988 68, 427-430.

34. Mannucei, PM, Zanetti, AR, Columbo M, et al: Antibody to HCV after a vapor VIII concentrate. Thromb Haemostasis 1990; 64, 232-234.

35. Mannucci PM et al. Low risk of viral infection after administration of vapor-heated factor VIII concentrate. Transfusion, 1992, 32, 134-138.

36. Horowitz B, Wiebe ME, Lippin A, et al: Inactivation of viruses in labile blood derivatives, disruption of envelope. Transfusion, 1985, 25, 516-522.

37. Horowitz B, et al: Virus inactivation by solvent / detergent treatment and the manufacture of SD-plasma. Vox Sang 1998, 74, 203-206.

38. Robert PL, Dunkerley C: Effect of manufacturing process parameters on virus inactivation by S/D treatment in a high purity factor IX. Vox Sang 2003, 84, 170- 5.

39. Vossebeld PJ, Tissing MH ,van Den Berg HM, et al: In vivo recovery and safety of human factor VIII product AAFACT in patients with hemophilia A. Hemophilia 2003, 7, 157 -63.

40. Wolf DM, Rokicka –Milewska R, Lopaciuk S, et al: Clinical efficacy ,safety and pharmacokinetic properties of the factor VIII concentrate Haemoctin SDH in previously treated patients with haemophilia A. Haemophilia 2004, 10: 438- 48.

41. Jhonston A, Mc Gregor A, Borovec S, et al: Inactivation and clearance of viruses during the manufacture of high purity factor IX. Biologicals 2000 3, 129 -36.

42. Michalski Computer, Bal F, Burnouf T , Goudemand M: Large –scale production and properties of a S/D factor IX from human plasma. Vox Sang 1988, 55, 20-30.

43. Rezvan H, Nasiri S, Mousavi K, et al: A study on the application and efficacy of S/D treatment in the process of purifying factor VII. 2002, Med J Is Rep Ir 16,179-182

44. Mould GP, Sutton JA, Matejtschuck P, et al: Solvent /Detergent treatment does not alter the tolerance of uptake of human normal immunoglobulin for intra muscular injection. Vox Sang , 2001, 80, 151- 8.

45. Bussell JB, Eldor A, Kelton JG, Varon D, et al: IgIv-Computer, anvel intravenous immunoglobulin, evaluation of efficacy, safety, mechanisms of action and impact. Thromb Haemost 2004, 91, 771-8.

46. Soucie JM, Robertson BH, Bell BP, et al: Hepatitis A virus infections associated with clotting factor concentrate in the US. Transfusion 1998, 38, 573-579.

47. Prowse C, Ludlam CA, Yag PL: Human parvovirus B19 and blood products. Vox Sang. 1997, 72, 1-10.

48. Bos OJ, Sang DG, Nieumeber CE, et al: Virus validation of pH 4-treated human immunoglobulin products produced by Cohn fractionation process. Biologicals 1998, 26, 267-276.

49. Hamalainen E, Suomela H, Ukkonen P: Virus inactivation during intravenous immunoglobulin production. Vox. Sang, 1992, 63, 6-11.

50. Burnouf T, Burnouf – Radosevich M: Strategy of virus removal/ inactivation of plasma derived products: Interest of nanofiltration as a new virus elimination method. In: Koba Yashi, et al., (eds),. Animal cell technology: Basic and applied aspects, Vol.6. Netherlands: Kluwer Academic Publishers, 1994, 17-27.

51. Burnouf T: Value of virus filtration as a method for improving the safety of plasma products. Vox Sang 1996, 70, 235-236.

52. Burnouf T, Radosevich M: Nanofiltration of plasma derived biopharmaceutical products. Haemophilia, 2003; 9 ,24-37.

53. Yokoyama T, Murai Kamran, Murozuka T, et al: Removal of small non-enveloped viruses by nanofiltration. Vox. Sang 2004 ,86, 225 -9.

54. Tateishi J, Kitamoto T, Ishikawa G, Manabe SI: Removal of causative agent of CJD through membrane filtration method. Membrane 1993, 18, 101.

55. Foster PR, Welch AG: Studies on the removal of abnormal prion protein by processes used in the manufacture of human plasma proteins. Vox Sang 2000; 78, 86 -95.

56. Schulman S, Wallensten R, White B, Smith OP: Efficacy of a high purity, chemically treated and nanofiltered factor IX concentrate for infusion in hemophilia patients. Haemophilia, 1999, 5, 96- 100.

57. Feldman P, Virus filtration of factor IX –an approach to process validation. PLANOVA Workshop, Oslo, Jun 27 ,1998.

58. Burnouf –Radosevich M, Appourchaux J, Huart JJ, Burnouf T: Nanofiltration, a new specific virus elimination method applied to high purity factor IX and factor XI concentrates. Vox Sang 1994; 67, 132-38.

59. Troccoli NM, Melver J Losikoff A, Poiley J: Removal of viruses from human Iv Ig by 35 nm. Nanofiltration Biologicals 1998; 26, 321- 29.

60. Von Holten RW, Ciavarella D, Oulundersen G: Incorporation of an additional viral clearance step into a human Ig manufacturing process. Vox Sang 2002; 83, 227 – 33.
61. Mauzurier M, Samor B, et al: In vitro study of a triple secured von Willebrand factor concentrate. Vox Sang 2004; 86; 100- 4.
62. Tomokyo K, Yamo H, Imamura M, et al. Large scale production and properties of human plasma derived activated factor VII concentrate. Vox Sang 2003; 84, 54- 84.
63. Williamson LM, Allain JP. Virally inactivated fresh frozen plasma. Vox Sang 1995; 69,159- 165.
64. Horowitz B., Bonomo R, Prince AM: S/D treated plasma. Blood 1992; 79, 826 831.
65. Klein HG, Dodd RY, Dzik WH, et al: Current status of S/D treated frozen plasma. Transfusion 1998; 39: 102-107.
66. Lambrecht B, Mohr A, Knuver –Hopf J, Schmidt H: Photoinactivation of viruses in human fresh plasma by phenothiazine dyes in combination with visible light. Vox Sang 1991; 60, 207-213.
67. Council of Europe expert committee in blood transfusion: Pathogen inactivation of labile products. Transf Med 2001; 11, 149- 175.
68. Wilson K, Code Ricketts MN: Risk of acquiring Creutzfledt – Jakob disease from blood transfusions. British Med J 2000; 321, 17- 19.