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Plasma fractionation programmes for developing countries

Technical aspects and infrastructural requirements

WORLD HEALTH ORGANIZATION
Regional Office for the Eastern Mediterranean

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Foreword

One of the challenges facing the blood services of developing countries is the provision of blood components, instead of whole blood. The transfusion medicine practitioner is well aware of the desirability of using a specific component and the attendant improvement in safety, as well as the husbandry of a human resource in limited supply. Frustration results when the desired derivative cannot be supplied because of the unavailability of a fractionation programme. This can be due to the lack of physical and financial resources, or the prohibitive expense of importing foreign product. A further compounding of this situation can occur when misguided bureaucrats advocate the purchase (at a donor country's expense) of an inappropriate, state-of-the-art fractionation plant, when the country's blood system needs radical upgrading before fractionation can be considered.

The World Health Organization's Regional Office for the Eastern Mediterranean has shown itself alert to the transfusion scene in developing countries within its own region, and other regions, as evidenced in recent years by a series of relevant workshops, and the publication of an excellent book on transfusion microbiology practice.[1] This latest monograph addresses in a simple, but not simplistic manner, the fractionation needs of developing countries. It has been prepared through the collaboration of a senior transfusionist and an experienced plasma fractionator, and represents the distillation of a unique experience in both developing and developed countries.

In this publication the importance of a competent transfusion infrastructure in the establishment of a fractionation programme is emphasized; a mature blood collection organization must always precede any consideration of blood component or plasma derivative production. The importance of donor selection, recruitment and retention is properly dealt with in detail. Conventional transfusion medicine texts tend to emphasize the clinical use of blood products, and pay little attention to the mechanics of the fractionation process. This monograph is well balanced, in that it analyses and compares, in an objective manner, the various fractionation methodologies available and the issues which must be considered when a choice is to be made. The importance of economic factors in this decision process is properly emphasized. An Annex contains a series of six

[1] Kitchen A.D., El-Nageh M.M. *Transfusion microbiology practice,* Alexandria, World Health Organization Regional Office for the Eastern Mediterranean, 1997 (WHO Regional Publications, Eastern Mediterranean Series 16).

commentaries on the theme of plasma derivative production in developing countries. These contributions, published originally in *Transfusion today*, provide authoritative and perceptive views from around the world.

This is an eminently readable and worthwhile publication which, deservedly, will have a much wider readership than the developing countries for which it is principally intended. The authors and WHO are warmly to be commended.

Professor Robert Beal
President, International Society of
Blood Transfusion

Preface

This book is targeted at national health authorities, blood transfusion professionals and managers of blood transfusion services. Its origin lies in a repeated expression of interest by blood transfusion service directors and Department of Health officials from a variety of countries, mainly, but not exclusively, in the Eastern Mediterranean Region, in establishing their own national plasma fractionation facilities. In turn this complex and interesting issue was submitted to the International Society of Blood Transfusion membership, through the publication of the following editorial in *Transfusion today*, the quarterly newsletter of the Society:

> A number of sufficiently populous and prosperous countries (as well as many which are neither), are anxious to establish their own plasma fractionation facilities, either on their own, or in collaboration with other neighbouring countries. Unfortunately, many of these countries do not yet possess the fundamental infrastructural attributes which must exist before fractionation can be contemplated:
>
> - well organized, centralized national blood transfusion services
> - sufficient numbers of safe, established voluntary blood donors
> - an adequate volume of high quality plasma production to attain self-sufficiency and cost-effectiveness
> - a truly functional quality assurance/(GMP) system
> - a well trained technical and scientific cadre
> - a sufficiently developed level of demand and sophistication in the clinical sector, together with the availability of diagnostic facilities for congenital bleeding disorders, clinical immunology defects, etc., which will determine the level of clinical need for fractionated plasma products.
>
> What are the pros and cons which must be weighed up before these countries create their own fractionation facilities, even if the prerequisites referred to earlier are fulfilled?
>
> Can the relatively large capital expenditure, efforts and training programmes required be justified at the current evolutionary stage in the "state of the art", where recombinant products, monoclonal antibodies and specific human proteins generated by transgenic animals are either already available, or imminent and when international regulatory standards are so stringent?
>
> What alternative options should be evaluated before embarking upon such a demanding venture? Options, for instance, such as: consummating a contract-fractionation agreement with a reputable western public or private sector establishment; a fixed-term technology transfer agreement which will yield both

early availability of reliable, locally-sourced fractionated products and ultimate national self-sufficiency, in the longer term; or finally, a reluctant but pragmatic acceptance of the need for continued reliance on imported, increasingly expensive finished products.

Naturally, it is difficult to generalize in response to these questions. Developing economies differ enormously in myriad significant respects other than gross national product, numbers of acute hospital beds, etc. In considering how successfully technology transfer will become engrafted one must also consider the wider human "ecology" of a given country, the degree of evolution of biological sciences, true clinical demand and the availability of basic technical expertise.

An additional closely related issue affecting national blood services in developing countries and relevant health authorities, is their serious concerns about the safety of plasma products. In many countries, national authorities remain suspicious and confused, not to say somewhat paranoid when they are faced with issues related to transfusion and blood products. How should an appropriate control of imported or locally made products be conducted? What is the relevance of tests like anti-HCV or PCR tests of end-products? How should a national plasma fractionation programme be implemented in a safe, professional, and cost effective manner? What are the current reliable fractionation and viral inactivation technologies?

The various key parameters and requirements to be met in initiating a sound national plasma fractionation programme, include:

- the establishment of an appropriate blood donor base;
- the appropriate collection and testing practices of blood and plasma;
- a well-conceived quality assurance programme;
- the rational and reasonable use of blood products;
- the convenient manufacture of plasma derivatives.

It is the objective of this book to review the significance and application of each of these major issues which need to be considered before embarking upon a national plasma fractionation programme.

Part 1 is a broad review of plasma fractionation history, the place of plasma products in clinical medicine, a description of the different fractionation technologies and various viral inactivation methods, together with a consideration of some of the economic and organizational issues which must be taken into account in approaching the establishment of plasma fractionation programmes or plasma fractionation facilities.

In Part 2, the infrastructural prerequisites which must be in place before fractionation is contemplated (whether by local manufacture, or contract fractionation), are described in some detail. These fundamental issues range from the creation of a coordinated national blood service, the selection of safe, regular, voluntary donors in adequate numbers,

appropriate viral screening procedures for every donation and the establishment of credible quality management systems, to the appropriate clinical use of blood components. In addition, a survey is included of the component laboratory production strategies which may be adopted to ensure that the best use is made of blood resources and that national requirements for both labile blood components and plasma fractions are adequately met through a judiciously organized whole blood programme.

The basic questions posed at beginning of this Introduction were put to seven experienced plasma fractionators and transfusion specialists in various countries. Their commentaries (previously published in the ISBT quarterly newsletter, *Transfusion today*, No. 34 and 35) are reproduced in Annex 1.

The text of section 7.4 was contributed by J.G. Watt, former Director of the Plasma Fractionation Centre of the Scottish National Blood Transfusion Service.

Abbreviations

AIDS	acquired immunodeficiency syndrome
Alpha 1 AT	alpha 1 protease inhibitor (or alpha 1 antitrypsin)
ATIII	antithrombin III
CMV	cytomegalovirus
DEAE	diethylaminoethyl cellulose
F	factor
FFP	frozen fresh plasma
GMP	good manufacturing practice
HAV	hepatitis A virus
HBc	hepatitis B core
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HTLV	human T-cell lymphotropic virus
IgG	immunoglobulin class G
IgM	immunoglobulin class M
IM	intramuscular
IU	international unit
IV	intravenous
min	minutes
PCC	prothrombin complex concentrate
PCR	polymerase chain reaction
PEG	polyethylene glycol
PKA	cAMP-dependent protein kinase
SD	solvent–detergent
UV	ultraviolet
vWF	von Willebrand factor

Part 1

Rationale, technical requirements and clinical applications of plasma products

Chapter 1

Considerations for the implementation of a national programme of plasma fractionation

Plasma products are an important part of the human therapeutic arsenal and it is expected that they will continue to fulfil increasing worldwide needs in spite of the development of biotechnical substitutes obtained by recombinant technologies.

Today in Europe and the United States of America (USA), where the development of plasma fractionation was initiated 50 years ago, the plasma fractionation industry continues to grow, as improved products or new clinical indications are found and new markets developed. Due to a number of reasons such as the present shortage of intravenous immunoglobulin which has been particularly acute in the USA, significant plasma product shortages are currently being experienced in some countries.

In some countries of Asia, eastern/central Europe, south America and the Middle East, needs for plasma products are high, especially for albumin, IgG, F VIII, and to a lesser extent, prothrombin complex or F IX, and in many situations local patients cannot receive appropriate treatment due to a lack of sufficient product supply or because imported product costs are too great. It is likely that the demand for additional plasma products, such as hyperimmune IgG, antithrombin III (ATIII) and fibrin sealant, will rise in the next few years.

As the economies of some emerging countries improve, cost-recovery policies for basic medical care are being established and awareness of the clinical benefits of plasma products is developing. As a result health authorities and blood collection organizations are seeking to establish local production of plasma products. The goal is to ensure, step by step, a level of self-sufficiency that would provide for long-term supply and allow better control of the various production phases of such critical health products.

This trend is supported by the view that the fractionation of plasma collected locally, with strong quality links and supervision extending from collection to fractionation, would reduce risks of exposure to infectious agents by products manufactured from the plasma collected from other parts of the world. The possibility of controlling and limiting the size of the plasma batches within reason (e.g. from 1000 to 4000) would also provide better control over the spread of emerging infectious risks that could more quickly be transmitted

to a large population of patients by products made from very large plasma batches. In addition, medical evidence shows significantly better clinical benefit due to products made from plasma collected among local blood/plasma donors. For example, WHO has advocated the use of IgG preparations made from local plasma as a means for dealing with indigenous bacterial or viral infections more effectively, since the antibody specificities of such preparations would reflect the prevalence of these pathogens in the community. In addition, the World Federation of Haemophilia continues to remind us that 80% of the world haemophilic population is not receiving adequate treatment and therefore it would support local manufacture as long as it is performed in accordance with adequate standards.[2]

It is becoming clear that, for the reasons mentioned above, the development of local plasma processing may represent a modern approach to handling the complex issues related to plasma product manufacture. Such an approach may coexist with the "giant" plasma fractionation industries, as both have distinct missions which can be complementary. A local fractionator may only have the continuous need for (and the expertise to produce) a limited range of "main-line" products. The giants can cater cost-effectively for a more extended range of uncommon clinical conditions and more sophisticated products. What is essential however, is the development of a mature blood collection organization that provides the basis and education for the success of a plasma fractionation programme.

Quite understandably, some countries hesitate to initiate such a programme due to the technical complexity of the goal and the difficulty and constraints in designing and running a "state-of-the-art", economically feasible programme. This fear is often exacerbated by the fact that plasma fractionation would often represent these countries' first large-scale venture into the biotechnology area and that local expertise might be insufficient to ensure success.

These countries must consider issues such as:

- the establishment of a centralized blood/plasma collection organization;
- testing strategies relevant to plasma for fractionation;
- selection of a suitable plasma fractionation strategy (contract fractionation, local processing, combined approaches);
- the choice of plasma fractionation technologies and viral inactivation treatments;
- application of current good manufacturing practices;
- personnel recruitment and training.

These questions will be addressed in Part 2.

[2] Jones P. Haemophilia, a global challenge, *Haemophilia,* 1995, 1: 11–13.

Finally, one important aspect underlying the development of a national plasma fractionation programme is the establishment of a sound and efficient regulatory system. The complexity of blood transfusion and plasma fractionation, the diversity of products made from human blood and plasma, as well as the diversity in purification technologies and viral inactivation treatments currently used by plasma fractionators all over the world, make the task of plasma fractionation managers and regulators particularly difficult. Ensuring an appropriate decision-making process as well as regulations which are suitable for all products made from human blood is a significant challenge. Table 1.1 lists the various types of products currently made from human blood or under development, specifies those derived from single donations or produced from pooled donations and states whether or not a viral reduction step is performed during processing. When the implementation of a new viral screening test intended to improve the viral safety of some blood products is being considered, in view of the wide diversity of products made from human blood, one must also take into account its effect upon the viral safety of others. For instance, the anti-HBc screening of whole blood donations (without complementary anti-HBs screening) to improve the viral safety of cellular components may have a negative

TABLE 1.1 **Current viral reduction methods for blood and plasma products**

Blood product type	Approved viral inactivation step
Single donor products	No[a]
Red cell concentrates	No[a]
Platelet concentrates	No[a]
Stem cell	No[a] / yes (methylene blue)
Clinical FFP	No[a]
Cryoprecipitate	
Small pool product (< 10 donations)	
Platelet concentrates	No[a]
Large pool, unfractionated plasma product	
Clinical FFP	Yes (solvent–detergent)
Large pool, purified haemoglobin	Yes
Large pool, purified leukocyte derived product	
Alpha interferon	Yes
Large pool, fractionated plasma products	
Albumin preparations	Yes
Immunoglobulin preparations	Yes
Clotting factors concentrates	Yes
Protease inhibitor concentrates	Yes
Anticoagulants	Yes
Other	Yes

[a] Leukoreduction by filtration (with benefit in removal of large viruses like CMV and HTLV) is increasingly performed. Viral inactivation treatments are under development.

impact by eliminating plasma donations also containing neutralizing anti-HBs antibodies. The overall effect of this may play a role in the viral safety of plasma products. Similarly, the implementation of anti-HCV testing removes antibodies that may foster the viral safety of IgG preparation with regard to HCV transmission.

Chapter 2
Diversity of plasma proteins and clinical use of plasma products

2.1 General

Human plasma represents a unique, complex biological material comprising more than 200 biochemical entities that have not yet been fully characterized. Among these are important glycoproteins and proteins like albumin, various types of immunoglobulins, coagulation factors, anticoagulants, protease inhibitors, and growth factors. The complexity of plasma, as illustrated in Table 2.1, is qualitative as well as quantitative, since concentrations of the various protein components vary from about 40 g/L (albumin) down to just 1 pg/L (growth factor). Similarly, plasma protein molecular mass varies from several million daltons (the von Willebrand multimer complex) to only a few thousand daltons.

The cardinal functions of proteins derived from blood or plasma, or produced by other manufacturing techniques in clinical medicine are:

- to replace a congenital or acquired deficiency of these proteins in patients, such as the use of factor VIII:C in haemophilia A;
- to mitigate or prevent a disease process, which is frequently immune-mediated, such as the prophylactic use of specific anti-D immunoglobulin to prevent maternal sensitization to fetal Rh D positive red cells and haemolytic disease of the newborn, or the administration of high-dose intravenous immunoglobulin in idiopathic thrombocytopenic purpura.

The leading plasma derivatives are F VIII:C, albumin and immunoglobulins, whether for intramuscular or intravenous use. In recent years, transfusion medicine in the developed world has progressively evolved towards more challenging therapeutic frontiers where the clinical use of plasma products has started to be used in preventive medicine (prophylactic treatment). They are also being used to target more complex clinical situations like disseminated intravascular coagulation or sepsis and shock, where a variety of physiological events, largely associated with an imbalance in the biochemical status and concentration of several plasma proteins, is seen in affected patients. The clinical benefits of plasma products in some of these domains still warrants further investigation and validation.

TABLE 2.1 **Characteristics of plasma proteins**

Major proteins	Daltons	mg/L
Albumin	68 000	40 000
IgG	150 000	12 500
Protease inhibitors		
Alpha 2 macroglobulin	815 000	2 600
Alpha 1 antitrypsin	52 000	1 500
C1-inhibitor	104 000	170
Antithrombin III	58 000	100
Heparin cofactor II	65 000	100
Antiplasmin	69 000	70
Protease nexin 1	44 000	< 1
Fibrinolytic proteins		
Plasminogen	92 000	200
Histidine-rich glycoprotein	75 000	100
Urokinase-type plasminogen activator	5	0.02
Coagulation factors and anti-coagulant proteins		
Fibrinogen	340 000	3 000
Fibronectin	250 000	300
Prothrombin	72 000	150
Factor XIII	320 000	30
Protein S	69 000	29
von Willebrand factor (monomer)	220 000	10
Factor X	59 000	10
Factor V	286 000	7
Factor XI	80 000	5
Factor IX	57 000	5
Protein C	57 000	4
Factor VII	50 000	0.5
Factor VIII	330 000	0.3
Cytokines		
G-CSF	20 000	< 30 pg/mL
Erythropoietin	34 000	0.3 ng/mL

2.2 Coagulation factors

2.2.1 Blood coagulation

Clot formation, comprising fibrin, platelets and trapped red cells, involves the interaction of some 20 plasma glycoproteins. *In vivo*, damage to the vascular endothelium triggers a number of interlinked events which lead to the formation of a platelet plug, the development of a fibrin clot on the platelet plug, endothelial cell growth and clot

dissolution. Once the platelet plug is formed, a cascade of serine proteases is activated on the phospholipid surface of the plug in a progressively amplified series of reactions.

Conventionally, coagulation has been divided into intrinsic and extrinsic pathways. The extrinsic system requires a lipoprotein co-factor, a tissue factor, which is not normally present in the blood. Both pathways are required to bring about haemostasis and F VIII:C is a crucial non-enzymic, intrinsic co-factor in the coagulation cascade. Deficiency of other factors, such as the precursor proteases F VII, IX, X, II and co-factor V also lead to bleeding, but apart from F IX deficiency (haemophilia B), these are often less clinically severe and considerably less common. In consequence, they are less important industrial products of plasma fractionation.

2.2.2 Factor VIII:C

2.2.2.1 Haemophilia A and factor VIII:C

Haemophilia A is a congenital bleeding disorder affecting between 7 and 10 males per 100 000 males at birth, or between 3 and 5 individuals per 100 000 population. The incidence appears to be remarkably constant in all the ethnic groups and geographical locations surveyed so far. Prevalence, of course, depends upon survival and does vary according to the availability of diagnostic facilities and specific medical care. In the words of W. Legg (1872)[3] "the number of cases in a given country depends upon the previous education of the medical men, and the interest which is taken in the disease".

Like haemophilia B, this common congenital coagulation disorder is inherited in a sex-linked, recessive pattern. Males are therefore affected and females are carriers of the trait, although in rare families, carrier females may also have clinical disease. The genetic defect may be caused by deletional or missense mutations, although most cases have a family history of abnormal bleeding, if they are carefully questioned. The F VIII:C gene has a high mutation rate so that some 20% of congenital cases are due to *de novo* mutation. This is sufficient to ensure that prevalence figures remain fairly constant, despite occurrence of fatalities due to transfusion-transmitted AIDS and haemorrhages. The majority of affected individuals suffer from the severe form of the disease, with factor VIII:C levels <1% and an obvious history of spontaneous haemarthroses, haematomas and a susceptibility to intracranial or other bleeding after minor trauma. The intermediate or mild cases of haemophilia A (F VIII:C >1%) are, if anything, more dangerous in that they usually do not have a history of spontaneous haemorrhage and may therefore present with grossly abnormal bleeding following surgery or trauma.

[3] Legg W. *A treatise on haemophilia,* London, H.K. Lewis, 1872.

Very occasionally, an acquired, immune-mediated F VIII:C deficiency may arise in adult life due to the development of auto-antibodies associated with disseminated lupus erythematosus, rheumatoid arthritis, pregnancy, penicillin or methyldopa therapy.

2.2.2.2 Principles of replacement therapy with factor VIII:C

If no preventive measures are taken, patients with severe haemophilia A are likely to develop significant muscle haematomas or haemarthroses as many as 30 to 35 times a year. Haemarthroses occur primarily in the weight-bearing joints, such as the knees and ankles, almost as soon as affected boys start to walk. It is these repeated joint haemorrhages which cause chronic synovitis and progressive destructive arthropathy, ultimately leading to joint deformity and instability, muscle atrophy and crippling, together with considerable psychosocial disability and financial cost to society.

The availability of clotting factor concentrates, which have become progressively more potent and purified since they were first produced in the mid-1950s, has revolutionized treatment of the condition and profoundly altered the natural history and management of haemophilia and haemophilic arthritis.

The policy adopted for managing haemophilia will naturally depend upon how comprehensive and well-organized haemophilia care is in the relevant country, how much financial support is provided and the nature and quantity of concentrates that are available. The amount of F VIII:C administered depends upon the circumstances and severity of each bleeding episode but the aim is to achieve a circulating level of 30% to 50% with daily or twice daily doses sufficient to raise F VIII:C to 15% to 25% for the required duration. It has been estimated that clotting factor concentrates alone represent between 50% and 80% of the total direct cost of haemophilia care.

a) Treatment of bleeding episodes in hospital

Replacement therapy may be confined to treating haemorrhagic episodes when they occur, exclusively at the hospital haemophilia centre. This is more convenient for medical and nursing staff and ensures that supplies of concentrate are tightly controlled. Although patients are well documented and monitored it is inconvenient for them to receive therapy in this way and treatment of bleeds is often delayed. If resources are available, a less passive approach may be adopted which will aim to prevent bleeding, or at least, to treat bleeding as early as possible in order to shorten the period of immobilization and hasten resolution.

b) Home therapy

Home therapy is a clinical management policy which has been widely applied in up to 80% of cases in countries with well-developed haemophilia services, starting as early as

the age of 3 years. It only became truly accessible with the advent of lyophilized concentrates in the early 1970s. This strategy shifts the primary responsibility for care from the hospital to the family setting. Not all families feel able to cope with this task of course, but those that can undoubtedly benefit in a variety of ways: treatment with concentrates is instituted early, the sense of utter dependency upon the hospital centre is reduced and self-reliance is fostered among patients.

Treatment is usually on demand, as soon as there is the earliest evidence of bleeding. In selected cases, a prophylactic treatment regime is instituted in the short term, to prevent anticipated bleeding in a primary mode, before joint complications occur, or as a secondary regime, to prevent further damage when recurrent bleeding occurs. Naturally, patients must be carefully monitored for F VIII:C inhibitors at regular intervals, and they must be well trained in keeping a meticulous record of bleeding episodes and self-administered therapy. Although home therapy does lead to more liberal use of concentrates, and appears therefore, to be more expensive at first encounter, it is ultimately cost-beneficial in this life-long disorder. The cost–benefits come through a reduction or delay in the development of crippling arthropathy and the loss of potentially effective members of the work force, and through a diminished drain on hospital resources.

c) Long-term prophylaxis

Long-term prophylaxis is another clinical management policy option which is increasingly used in developed countries. Its aim is to convert severe haemophilia into the moderate form of the disorder. The evidence so far suggests that if prophylaxis is started at 2 to 3 years of age, infusions of 25 to 40 IU F VIII:C per kilogram of body weight (amounting to approximately 3000 IU/kg per year), 2 to 3 times a week can prevent, rather than merely delay, the onset and inexorable progression of joint lesions, so that objective orthopaedic and radiological joint scores (from 0 to 15 and 0 to 13 respectively) remain at 0/0, even after a prolonged 6-year follow-up. In addition, there is no evidence of an increased incidence of F VIII:C inhibitor formation with this regime. Indeed, it is theoretically possible that a tolerizing effect may be exerted in low-titre inhibitor cases. Needless to say, the major problems associated with this option are retention of adequate venous access and a four-fold increase in clotting factor utilization.

d) Continuous infusion regimes

Finally, there is the ultimate form of therapy (short of a cure through liver transplantation or, in the future, gene therapy) which avoids the high peaks and correspondingly low troughs of intermittent single-bolus therapy, by giving F VIII:C by continuous infusion, either in hospital, or even, in the ambulant patient, through the use of an infusion mini-pump. The objective is to maintain a constant blood level over 1% F VIII:C at all times. The pharmacokinetics of F VIII:C and F IX are such that a

progressive decline in clearance is obtained which, together with the elimination of unnecessary peaks, exercises savings of concentrate—between 50% and 75% in post-operative cases. The limiting factor here is the stability of reconstituted F VIII:C or F IX concentrates, which must retain their activity over the 10 to 12 hours of continuous infusion. It does appear however, that many high-purity preparations do retain their potency remarkably well in the undiluted form.

Various adjuvant treatment options are also available which can assist in minimizing the unnecessary use of expensive F VIII:C concentrates. Examples include desmopressin (DDAVP or 1-desamino, 8-D-arginine vasopressin) given intravenously, subcutaneously, or through a nasal spray. DDAVP provides a rapid two- to six-fold increase in plasma concentrations of F VIII:C and von Willebrand factor, as well as tissue plasminogen activator. Even though repeated administration of desmopressin leads to a progressively diminishing response (tachyphylaxis), this seldom causes significant clinical problems. Other agents, such as anti-fibrinolytic drugs like tranexamic acid, can also be effective in reducing the requirements for specific replacement therapy, in mucosal bleeding, or following tooth extractions.

2.2.2.3 Factor VIII:C inhibitors

One of the dreaded complications of over 20% of severe haemophilia cases is the development of IgG antibodies to human F VIII:C, which usually make their appearance early in the therapeutic history of patients, sometimes even after a single dose of any of the factor concentrates. Contrary to earlier assumptions, it now appears that it is not the type or quantity of F VIII:C concentrate employed which solely determines the appearance of inhibitors. This is a genetically determined propensity that is commonly associated with gene deletions.

Many of these inhibitors are mild, low-titre antibodies that are relatively easily overcome by high doses of specific concentrates. Indeed, as many as a third may remit spontaneously. High-titre inhibitors however, are sometimes refractory to even the largest doses and an anamnestic response rapidly puts the attainment of a haemostatic level of F VIII:C out of reach. In addition, future treatment is naturally compromised as well. The treatment modalities currently available to deal with these cases are clinically demanding and costly. The induction of immune tolerance, for instance, has been successful, employing an initial 10 000 to 15 000 IU bolus of F VIII:C, followed by the infusion of 1000 IU per hour. Attempts to remove the inhibitor by apheresis or extracorporeal adsorption, together with cytotoxic immune suppression, have also met with some success. An alternative strategy is to use materials that bypass factor VIII—the so-called factor VIII inhibitor bypassing agents, where activated prothrombin complex concentrates (PCCs), or recombinant factor VIIa are used, leading to the generation of thrombin at the site of injury. Finally, purified animal F VIII:C concentrates, obtained by polyelectrolyte

fractionation can be used, provided that the patient's antibody does not cross-react very actively with animal as well as human F VIII:C.

The choice of therapy will depend upon the severity of the haemorrhagic episode and whether the patient has a low-titre (under 5 Bethesda units), or a high-titre (over 10 BU) antibody. In the most challenging cases, a combination of these treatment strategies may be needed to overcome the inhibitor. Few countries are sufficiently prosperous or willing to support a "carte blanche" policy which will defray the heavy cost of inducing immune tolerance, or the other regimes used in dealing with this minority group of patients with high-titre inhibitors. Further objective longitudinal studies demonstrating long-term cost benefit will be required to make a strong enough case to funding authorities.

2.2.2.4 Sources of therapeutic factor VIII:C

Considering the meagre therapeutic options at his disposal, the Russian monk Rasputin did as well as he could for the little Tsarevich Alexis (who inherited his severe haemophilia indirectly from Queen Victoria) by using hypnosis and exerting a calming influence on the boy during bleeding crises. Today, we have a wealth of materials at our disposal to treat or prevent these episodes and their sequelae.

If the relative specific activity of fresh plasma is 1, cryoprecipitate is 30, and high purity F VIII:C is 300 000. Even though good quality frozen or lyophilized cryoprecipitate can provide sufficient F VIII:C to cover acute bleeding and even major surgery, it is only barely adequate. Despite the attractions of low cost and the relative safety of a small-pool product, it has been overtaken, in most developed economies, by industrially manufactured large-pool products which are now rendered safe by viral inactivation procedures. These fractions can yield intermediate or high purity products with levels of purity (expressed in terms of units of activity per milligram of total protein) ranging between 10 and 50 IU/mg of protein for intermediate purity F VIII:C; 50 and 200 IU/mg for high purity products, obtained by anion exchange chromatography which captures the vWF/F VIII complex; and over 2000 IU/mg for F VIII:C using monoclonal antibodies in an affinity chromatography system. Since the pure factor VIII has to be stabilized by adding albumin however, the final specific activity is less than 10 IU/mg.

The public pressure in the developed countries for ever increasing purity was given a new impetus following the onset of the AIDS epidemic, which revealed immune suppression among affected haemophilic patients. Further investigation however, showed that similar abnormalities of cell-mediated immunity could also be discerned in uninfected haemophiliacs as follows:

- inhibition of T-lymphocyte proliferation and IL-2 production;
- down-regulation of monocyte and natural killer-cell function;
- anergy to dermal recall antigens;

- lower CD4 helper T-cell counts and higher CD8 T-cell suppressor cells;
- raised markers of B-cell and T-cell activation.

It has been postulated that these *in vitro* aberrations are caused by the regular administration of contaminating proteins in F VIII:C concentrates of lower purity. Despite all the efforts to demonstrate that these laboratory phenomena are clinically significant however, the case has remained unproven. Indeed clinical comparisons between those exclusively treated with intermediate purity concentrates are entirely inconclusive.

Unfortunately, quite apart from the escalation of costs incurred with increased processing and purity, as well as aggressive viral inactivation procedures, there is an even more significant penalty to pay in terms of diminishing yields of F VIII:C from a given volume of plasma. In consequence, no more than some 100 to 180 IU of factor VIII per kilogram of starting plasma can be expected when latter-day separation techniques are employed to manufacture high purity products, while a yield of 200 to 250 IU per kilogram of plasma can be achieved for intermediate purity factor VIII.

Finally, recombinant F VIII:C produced in various cell lines by biotechnology would appear to provide a solution to many of the disadvantages of plasma-derived concentrates: an unlimited supply of pure product and elimination of the risk of viral transmission from one infected donor in the "window" period, among 25 000 or more contributors to a plasma pool. These recombinant products have now been extensively studied in clinical trials and they have proved to be effective and to have a normal circulating half-life. However, there still remains a suspicion that they are more likely to induce the development of F VIII:C inhibitors than plasma-derived materials. In addition, since they are lacking in von Willebrand Factor, plasma-derived albumin must also be added as a carrier protein. This apparently retrograde step has been criticized as a potential source of infection despite the outstanding safety record of human albumin fractions and this measure reduces the activity per milligram of protein to between 1 and 10 IU/mg in the final product. Newer, second generation recombinant factor VIII is now becoming available however, where the B-domain of the molecule, which is entirely dispensable in terms of haemostatic function, is deleted. Specific activity is extremely high at 15 000 IU VIII:C/mg protein and the formulation does not require albumin, as it appears to be quite stable when reconstituted.

Needless to say, these products of biotechnology still carry a very heavy cost. Even though they are likely to become a major source of F VIII:C in developed countries and despite the considerable safety of current virally inactivated, plasma-derived products, government funding authorities in even the most highly developed countries will have to ensure that the overall supply of therapeutic material is not compromised by a disproportionate expenditure on high-tech products which have yet to establish their cost–benefit credentials.

2.2.2.5 Requirements for F VIII:C—estimating needs

The various therapeutic and clinical management options available in dealing with haemophilia and its complications have been described in some detail, not so much to provide the reader with a textbook in haemophilia care (which is not the primary object of this chapter) but rather to use haemophilia as a model to illustrate principles going far beyond coagulant plasma fractions alone. The object is to convey the variety of options which are currently available and the consequences that will flow from the choice of each of these options in terms of: the volume and quality of plasma required for fractionation; the level of risk which is tolerable; and in turn, the viral inactivation measures which will be applied; and even the technical blood component manufacturing policies which must be selected to make optimal use of donor blood and provide sufficient plasma for fractionation, as well as an adequate provision of red cells, platelets and frozen fresh plasma to satisfy clinical demand (see Chapter 11).

There is a great deal of variation in the use of F VIII:C concentrates, as well as the type of material which is employed, even among the highly developed economies of western Europe. In a few countries, no more than 1.5 to 2 IU F VIII:C per inhabitant is used, while in others, up to 6 IU are consumed. In some countries, freeze-dried cryoprecipitate is still employed in the bulk of cases, while in the majority of countries, high purity, plasma-derived, or recombinant F VIII:C are used in most cases. Prophylactic F VIII:C infusion is available to all haemophiliacs in a very small number of countries, while elsewhere this option is only provided for a highly selected few cases.

As examples, the consequences of two different treatment regimes for two different types of concentrate, one relatively high yield, and the other low, are illustrated in Table 2.2. It will be seen that, at one extreme, where 2 IU of F VIII:C per inhabitant are used, in a hypothetical country with a population of 5 million and no access to apheresis facilities, 40 whole blood donations per 1000 inhabitants will yield sufficient plasma, if all the donations are red cell concentrates. Many more donations would be required in reality, of

TABLE 2.2 **Comparison of the consequences of two treatment regimes for two types of concentrate**

Concentrate	Yield of FVIII:C per kg plasma (IU)	Use of FVIII:C per inhabitant	Plasma required (kg/1000 inhabitants)	Whole blood donations required (per 1000 inhabitants)
Intermediate purity F VIII:C	250	2	8	40
High purity F VIII:C	150	2	13	65
Intermediate purity F VIII:C	250	6	24	120
High purity F VIII:C	150	6	40	200

course, because clinicians may be reluctant to accept an exclusive diet of packed cells alone. The example has been simplified in the interests of clarity. At the other extreme, if 6 IU high purity F VIII:C per inhabitant are required, a virtually impossible 200 donations per 1000 inhabitants would be required—a five-fold difference.

2.2.3 Factor IX

Congenital deficiency of factor IX (F IX) is the cause of haemophilia B, or Christmas Disease, which is clinically indistinguishable from haemophilia A and has a similar sex-linked recessive inheritance pattern, although it is considerably less prevalent (haemophilia A constitutes 85% while haemophilia B constitutes 15% of cases). In consequence, even though the principles of clinical management and the use of F IX concentrates for patients with haemophilia B are the same as those applied in haemophilia A, there is considerably less difficulty in procuring adequate supplies of specific material for treatment. The concentrates used for the treatment of these two coagulation disorders share the same drawbacks; viral transmission and immune modulation. Hitherto, F IX concentrates of low purity (prothrombin complex concentrate or factor IX complex concentrate) have been associated with a hypercoagulable state and thromboembolism, especially when they are administered in large doses following major surgery. Thrombogenicity is thought to be due to contamination of these preparations with activated forms of the other vitamin-K dependent serine proteases, factors IIa, VIIa and Xa. This particular problem, which is associated with both the four-factor concentrate PPSB and the triple factor concentrate PCC (deficient in factor VII), has now been resolved by the advent of the new high-purity "factor IX only" concentrates which are no longer thrombogenic.

Apart from their role in the treatment of haemophilia B, prothrombin complex concentrates are also used for haemophilia A patients who have developed F VIII:C inhibitors and they can also be employed to good effect in treating acquired vitamin K-dependent clotting factor deficiencies due to warfarin overdosage, intestinal malabsorption, or chronic liver disease. The addition of F VII is also necessary when the triple factor concentrates are used for this purpose, however. Clearly, the newer high-purity F IX preparations are not appropriate in these conditions and should be used exclusively for the treatment of factor IX deficiency.

2.2.4 von Willebrand factor

The overall prevalence of the many different forms of von Willebrand disease has been found to be considerably greater than it was thought to be in the 1960s and 1970s. Indeed, it is now regarded as the most common inherited bleeding disorder, with a world prevalence which may be as high as 1%. This very heterogeneous group of disorders is

essentially due to a quantitative and/or qualitative defect in the von Willebrand factor (vWF) protein. The different sub-types of the disease fall into four categories:

- reduced synthesis of the protein;
- abnormal formation or stability of vWF polymers;
- synthesis of dysfunctional molecules;
- impaired release from storage sites.

vWf is a large protein produced in endothelial cells and megakaryocytes. It is essential for platelet adhesion to sites of endothelial damage, for platelet aggregation and as a carrier protein for F VIII:C. The normal protein exists in a large multimeric form and when it is absent or deficient, patients with the commonest Type I disease, constituting some 70% to 80% of all cases, develop defects of primary haemostasis with mucosal bleeding, menorrhagia and easy bruising or bleeding following minor trauma. Inheritance follows an autosomal dominant character (Types I and II) and females are therefore affected in the same way as males. Although severe forms of the disease (Type III autosomal recessive) are generally uncommon, they do occur more frequently in countries where inter-marriage and consanguinity are prevalent. These individuals may develop spontaneous internal, muscle and joint haemorrhages which are similar to haemophilia.

The common Type I von Willebrand disease cases synthesize much reduced, but nevertheless functional, multimeric forms of the molecule. Therefore because a number of F VIII:C concentrates (particularly the less highly purified products) happen to contain a certain amount of vWF, these patients can be successfully treated with some of the intermediate purity factor VIII preparations and they may not require treatment with the new, specific vWF concentrates which have recently become available. Those specific concentrates, however, present the advantages of being standardized, specifically manufactured and assayed for the treatment of von Willebrand disease. The treatment of choice for Type I cases however, is the synthetic analog of vasopressin or desmopressin (DDAVP) whereas, in Type 2 and other forms of the disease where there is a qualitative defect, factor VIII concentrates are required.

2.3 Albumin

2.3.1 General

This is the most abundant protein in plasma and is the principal contributor to maintenance of blood osmolarity. Albumin is synthesized in the liver and approximately 40% of the exchangeable albumin pool is within the vascular compartment, at a concentration of 40 mg per ml.

Currently, more than 200 t of albumin per year are used throughout the world over but there is enormous variation in usage between countries, within countries and indeed,

between different hospitals within countries, without any clear evidence of a corresponding difference in morbidity or mortality. For instance, while hospitals in Japan may use up to 1000 kg of albumin per million inhabitants and Germany or Belgium consume 500 kg or more, the mean clinical use of albumin in the United Kingdom is only 150 to 200 kg per million. Admittedly, this may be caused by variable availability or financial considerations but it is also undoubtedly due, in part, to uncertainty regarding the exact clinical indications and requirements for this plasma fraction.

Albumin is available in preparations of either 4.5% to 5%, or 20% and 25%. The plasma quality requirements for the manufacture of this fraction, as well as the immunoglobulins, are relatively less stringent than they are for the labile coagulant fractions, F VIII:C and F IX. In consequence, recovered plasma separated from whole blood donations which does not meet the criteria for frozen fresh plasma, is quite suitable for albumin production. Albumin, which is prepared from large pools of donor plasma and is pasteurized in the final container at 60 °C for 10 h, has a virtually unblemished record of viral safety in nearly half a century of clinical use. The sole adverse effect of any significance ascribed to this product in the past was the presence of contaminating pre-kallikrein activator, which caused hypotensive reactions in some patients, when given in large doses. This problem has long been resolved. Injudiciously rapid or high volume infusion of the fraction, most particularly the 20% preparation, can cause fluid overload and congestive cardiac failure, of course, but this is more a failure of clinical judgement, than an inherent shortcoming of the therapeutic material itself.

2.3.2 Clinical indications for use of albumin

The clinical indications of albumin remain controversial, but the oncotic role of the protein still remains the leading attribute which is utilized in clinical practice, just as it did during the Second World War. Albumin solutions are mostly employed in acute and chronic hypovolaemia, septic shock and obligatory sequestration of the extravascular fluid, as well as a replacement fluid in therapeutic plasma exchange. The low viscosity of albumin solutions is exploited in priming pumps for haemodialysis and cardiopulmonary bypass. For this same reason, some paediatricians also prefer to use albumin, in place of plasma where fibrinogen is a major contributor to viscosity, in the treatment of hyperviscosity states. The binding and transport of bilirubin by albumin, in the treatment of haemolytic disease of the newborn is another, relatively minor, application of these preparations. Although one of the major indications for albumin cited in the literature is in the treatment of shock accompanying severe burns, there is some uncertainty about the evidence to support this long-hallowed application. Fluid resuscitation with large volumes of buffered crystalloid solutions is certainly essential in the earliest stages of treatment when micro-circulatory hyper-permeability is a major problem. However, while it is usually recommended that the infusion of colloid solutions, such as albumin is initiated

within 48 to 72 hours after burn injury, when capillary permeability is beginning to return to normal, the clinical evidence from a number of controlled trials has failed to show any clear advantage to its use and some authors have even suggested that it may be deleterious.[4] In the diuretic-resistant oedema which can occur in severely hypoproteinaemic patients, the infusion of 20% albumin, together with a loop diuretic and aldosterone antagonists can produce diuresis. In these cases, of course, it is important to ensure that the albumin salt content is low.

There is a general consensus that in chronic protein-losing enteropathy and nephropathy; chronic ascites, or in cases of malnutrition, the use of albumin solutions is contraindicated and wasteful. This is particularly true in the latter condition because albumin is slowly metabolized and contains very few of the essential amino acids. A very recent application of albumin has been as a stabilizing additive to recombinant and monoclonal antibody purified F VIII:C.

2.3.3 Albumin in the plasma fractionation equation

Albumin utilization in any given country with a fractionation industry bears a very intimate economic relationship to the production methodology and clinical demand for F VIII:C concentrates. The current annual worldwide demand for albumin is roughly 250 t, whereas factor VIII demand could be satisfied by only 2–3 kg of specific protein. It has been emphasized in section 2.2.2.4 that the increasing demands for greater purity have caused yields of F VIII:C to decline and the application of viral inactivation procedures has reduced yields by up to a further 20%. At the same time, the adoption of more liberal haemophilia treatment and prophylaxis regimes has meant that F VIII:C is the main driving force in the plasma fractionation industry of some developed countries. This has led to the demand for a growing input of starting plasma (which now is close to 20 million L of plasma per year) to satisfy clinical demand for F VIII concentrates. This grossly exceeds the volume of plasma required to satisfy world demand for albumin, or for that matter, F IX and immunoglobulin. Therefore, it is only when albumin utilization is very high that large quantities of plasma-derived F VIII:C can be manufactured economically and in a balanced, reciprocal relationship with the other major products of the fractionation equation. When one takes into account the uncertainties surrounding clinical indications for albumin, and the possibility that the most profligate users of this fraction are likely to reduce their demand in the future, it is clear that the drive to replace human plasma as a source of therapeutic fractions in the developed countries owes as much to economics as it does to fear of transfusion-transmitted infections.

[4]Among others: Cochrane Injuries Group Albumin Reviewers. Human albumin administration in critically ill patients: systematic review of randomised controlled trials. *British medical journal,* 1998, 317:235–240.

2.4 Immunoglobulins

2.4.1 General

The immunoglobulins are therapeutic preparations of pooled immunoglobulin G (IgG), which are available in the original intramuscular form or, more recently, since the problems associated with their anti-complementary aggregates have been resolved, in the increasingly popular intravenous preparations. They are both also available as either polyvalent or specific immunoglobulins. Polyvalent or multispecific IgG contains antibody specificities for the whole spectrum of infections and antigens that donors contributing to the starting plasma pool have been exposed to, which may also include anti-idiotypic specificities important in providing effective therapy. The World Health Organization criteria for minimum standards in IgG preparation[5] specify that they should:

- be prepared from a pool of >1000 individual donations;
- contain >90% intact IgG and the lowest possible IgA concentration;
- be free of fragments and aggregates as far as possible;
- be modified biochemically as little as possible;
- possess good Fc function, opsonizing, complement-fixing and other biological functions;
- contain all IgG subclasses in naturally occurring proportions;
- be free of cAMP-dependent protein kinase, kinins, plasminogens, etc.;
- have an ascertained level of antibodies against at least one viral and one bacterial antigen;
- be free from the risk of viral transmission;
- be well tolerated clinically.

The original prime indications for immunoglobulins were the prevention of infections in congenital or acquired immune deficiency conditions, such as common variable immunodeficiency, X-linked agammaglobulinaemia and other severe primary immunodeficiencies (Box 2.1). Intramuscular IgG is painful however, particularly in children with a small muscle mass and this also imposes limitations upon the maximum dose administered. In addition to delays of up to 6 or 7 days in the development of peak circulating IgG levels, as much as 40% to 50% of the dose may be destroyed at the site of injection. The very welcome advent of intravenous IgG overcame these drawbacks and intramuscular preparations have now generally been relegated to the prophylaxis of hepatitis A, hepatitis B (although an IV form is also available) rubella, tetanus, rabies.

[5] Appropriate uses of human immunoglobulin in clinical practice: memorandum from an IUIS/WHO meeting. *Bulletin of the World Health Organization*. 1982, 60(1): 43–47.

BOX 2.1 Primary immunodeficiency disorders

- X-linked agammaglobulinaemia
- Di-George anomaly
- Severe combined immunodeficiency
- X-linked hyper-IgM syndrome
- Wiskott Aldrich syndrome
- Leukocyte adhesion defects (Types 1 and 2)
- Chronic granulomatous disease

A more recent, highly significant use for IV Ig, quite apart from its role in the replacement therapy of primary or secondary immunodeficiencies (Box 2.2) and hypogammaglobulinaemia, is in the treatment of autoimmune and systemic inflammatory disorders. The initial observations, in the early 1980s, that idiopathic thrombocytopenic purpura (ITP), particularly among children, can be successfully treated with large doses of IV Ig, has spurred experimental studies and clinical trials which are greatly extending their range of proven or probable benefit. Some of these conditions are: autoimmune acquired factor VIII inhibitors, Guillain Barré syndrome, Kawasaki disease, polymyositis, and many others (Box 2.3).

ITP is caused by the rapid splenic removal of platelets opsonized by auto-antibodies. In many forms of the disease, platelet surface glycoproteins GP IIb/IIIa and GP Ib/IX are the target antigens for these auto-antibodies so that, in addition to thrombocytopenic bleeding, platelet dysfunction may also exacerbate the clinical condition. The disease often follows an acute respiratory tract infection in children, although no such association exists among adults. It is acute and self-limiting in about 80% of children but in only 10% to 20% of adults. IV Ig is recommended in patients with a platelet count below 30 000/dl who present with serious bleeding or who are at risk before surgery. Over 60% of patients achieve a remission following infusion of IV Ig (0.4 to 1.0 g/kg body weight for 1 to 2

BOX 2.2 Secondary immunodeficiencies

- Multiple myeloma and chronic lymphocytic leukaemia
- Protein-losing enteropathy (with low gammaglobulin)
- Nephrotic syndrome (with low gammaglobulin)
- Post bone marrow transplantation

BOX 2.3 **Non-infectious uses of intravenous Ig**

Proven benefit	**Probable benefit**
• Kawasaki disease	• Post-infectious thrombocytopenic purpura
• Immune thrombocytopenic purpura	• Immune neutropenia
• Guillain-Barré syndrome	• Autoimmune haemolytic anaemia
• Dermatomyositis	• Myasthenia gravis
• Chronic inflammatory demyelinating polyneuropathy	• Multi-focal motor neuropathy
	• Anti-factor VIII autoimmune disease

days). In chronic ITP among adults however, a long-term response to IV Ig therapy is only occasionally observed, although brief remissions are often achieved, which can be exploited in preparing patients for surgery.

As in other peripheral autoimmune cytopenias, the short-term effect of IV Ig in acute ITP has been attributed to a transient blockade of splenic macrophage Fc receptor function and to consequent decrease in splenic clearance of antibody-coated platelets.

A consensus development conference held by the National Institutes of Health, USA, in 1990 concluded that the proven indications for IV Ig administration were confined to:

- primary humoral immune deficiencies;
- treatment of children with acute ITP, and adults in preparation for surgery;
- Kawasaki syndrome, together with aspirin;
- Guillain-Barré syndrome;
- prevention of infection in immunosuppressed bone-marrow transplant recipients, where acute graft-versus-host disease may be modulated as well;
- reduction of infections in chronic lymphocytic leukaemia, even though survival appears to be unaffected.

It was also concluded that these preparations were of uncertain utility in preventing infection among premature, underweight neonates, and in cases of paediatric AIDS.

2.4.2 Specific IgG preparations

Specific, hyperimmune immunoglobulins are available for the treatment or prevention of a number of clinical diseases. They are derived from pools of highly selected blood

donors with a high titre of particular antibodies. These donors are usually able to give plasma by apheresis up to once or twice weekly without adverse effects. Examples of the most effective specific IgGs derived from human plasma are:

- anti-RhD Ig, which is very widely used to prevent haemolytic disease of the newborn and Rhesus sensitization in other circumstances but may well be the first plasma-derived therapeutic IgG to be displaced by biotechnology.
- anti-hepatitis B, which is employed to prevent infection following accidental exposure to HBV infection, such as cases of needle-stick injury, or else to prevent maternal-fetal transmission and re-infection of a transplanted liver.
- anti-varicella zoster to prevent infection in susceptible individuals such as the newborn or patients immunosuppressed by chemotherapy; anti-tetanus, and anti-rabies.

Other useful specific Ig preparations will undoubtedly become available in the near future.

2.4.3 Viral safety of intravenous IgG

Even though intramuscular preparations of Cohn Fraction II, produced by cold-ethanol fractionation have an excellent record of safety, the same has not been entirely true of the intravenous form. There have been a number of sporadic episodes of HCV transmission by IV Ig which have recently been documented, although it is still not clear whether these have been the result of GMP failures, bypassing cold-ethanol fractionation, which appears to have an important role in enhancing safety, or the absence of neutralizing anti-HCV antibodies due to the introduction of donor screening. Certainly, the current consensus is that all IV Ig production methods should now incorporate specific virucidal steps, not only capable of dealing with the erroneous inclusion of overtly infected or window period donations in plasma pools but also to prevent transmission of new viruses not yet tested for.

2.5 Appropriate clinical use of plasma products

Table 2.3 summarizes the current clinical indications for plasma products. Considering the controversies in the clinical use of some plasma products, one important criterion underlying the sizing of a national plasma fractionation programme is the determination of the level of clinically-appropriate local need. In the industrialized countries, the clinical consumption of plasma products varies widely, depending upon clinical habits as shown in Table 2.4 for albumin, IgG and factor VIII.

Extrapolating from these 1991 consumption figures as well as from further clinical experience with use of plasma products since then, it may be speculated that the average

TABLE 2.3 Clinical application of plasma products

Product	Main indication
Albumin	
Human serum albumin	Protein and volume replacement
Plasma protein fraction[a]	Volume replacement
Coagulation factors[b]	Bleeding disorders
Factor VIII	Haemophilia A
Factor IX complex (PCC/PPSB)	Haemophilia B; haemophilia A with inhibitors; complex liver diseases; warfarin reversal
Factor IX	Haemophilia B
Factor VII	Factor VII deficiency
von Willebrand factor	von Willebrand factor deficiency
Factor XI	Haemophilia C
Fibrinogen	Fibrinogen deficiency
Factor XIII	Factor XIII deficiency
Activated factor VII (FVIIa); activated PCC	Haemophilia with inhibitors
Protease inhibitors	
Antithrombin III	Antithrombin III deficiency – thrombosis
Alpha 1 antitrypsin[b]	Pulmonary emphysema; cystic fibrosis
C1-inhibitor[b]	Angioedema
Anti-coagulants	
Protein C[b]	Protein C deficiency – thrombosis
(Activated protein C)	(Thrombotic states)
(Protein S)	(Protein S deficiency)
Fibrin glue (fibrin sealant)	Topical haemostatic/healing/sealing agent (surgical adjunct)
Intramuscular immunoglobulins G	
Normal (polyvalent)	Prevention of hepatitis A, rubella, and other infections
Anti-hepatitis B	Prevention of hepatitis B
Anti-tetanus	Treatment or prevention of tetanus infection
Anti-rho(D)	Prevention of haemolytic disease of the newborn
Anti-hepatitis A	Prevention of hepatitis A
Anti-rabies	Prevention of rabies infection
Anti-varicella/zoster	Prevention of chicken-pox infection
Anti-pertussis	Prevention of whooping cough infection
Intravenous immunoglobulins G[c]	
Normal (polyvalent)	Immune deficiency states – immune disorders
Anti-cytomegalovirus (CMV)	Prevention of CMV infection (e.g. bone marrow transplant)
Anti-HBs	Prevention of HBV infection (e.g. liver transplant)
Anti-rho (D)	Prevention of haemolytic disease of the newborn
Intravenous immunoglobulins M	Septic shock; binding of endotoxins
Immunoglobulin A (oral use)	Gastro-intestinal infections of the newborn (rotavirus)[a]

[a] Product similar to albumin, but with a lower purity level.

[b] Used mostly for substitutive therapy to restore normal circulating levels and stop symptoms in patients.

[c] Other hyperimmune preparations are under development.

use of those plasma products in the late 1990s in Europe and the USA is as follows, per million inhabitants:

- albumin: from 250 to 500 kg;
- IV IgG: 20 to 40 kg;
- F VIII: 2 to 5.0 IU $\times 10^6$.

While the use of albumin may decrease in countries where usage is high, the use of IV IgG and factor VIII may increase due to the introduction of immunomodulation therapy and prophylaxis, respectively.

TABLE 2.4 **Plasma product use per 10^6 population 1990–1991**

Country	Albumin (kg)	IgG IV (kg)	Factor VIII (IU $\times 10^6$)
Belgium	601	9.4	2.21
Denmark	320	3.8	3.54
England and Wales	160	4.1	1.99
France	508	17.1	1.74
Germany	596	17.4	3.63
Italy	497	19.0	1.36
Japan	614	79.0	2.67
The Netherlands	392	6.8	2.58
Norway	297	1.8	1.76
Portugal	99	2.9	1.31
Scotland	245	5.7	2.02
Spain	158	4.2	1.51
Sweden	243	5.4	4.23
Switzerland	536	16.1	2.11
USA	521	35.3	2.54
Average	386	15.2	2.35

Source: Foster PR. Blood plasma fractionation. In: Kirk-Othmer *Encyclopedia of chemical technology*, 4th ed. Vol. 11. New York, John Wiley and Sons, 1994, 990–1021.

Chapter 3
Basic technical requirements for plasma fractionation

3.1 Background

Plasma fractionation has emerged as one of the most scientifically demanding fields in the biopharmaceutical manufacturing area. Producing safe plasma derivatives implies the development, use and proper understanding of sensitive testing technologies to detect markers of infection in the starting plasma. It also requires the implementation of carefully selected, non-denaturing, efficient plasma protein purification and viral reduction technologies which do not alter the physiological functions and clinical potential of plasma proteins. Manufacture must be carried out with great care and in very clean environmental conditions. Plasma product manufacture requires complex multi-step production procedures (Table 3.1) including precipitation, centrifugation, chromatographic

TABLE 3.1 **Typical process steps and know-how required in the making of plasma products**

Step	Purpose
Precipitation	Protein purification and fractionation
Large-scale, low speed centrifugation	Solid/liquid separation
Large-scale filtration (e.g. depth filtration)	Solid/liquid separation
Ultrafiltration (e.g. on 30–100 kda membranes)	Removal of low molecular weight components (ethanol; salts) and concentration of proteins
Dialysis	Buffer equilibration
Aseptic filtration on 0.22 mm filters	Removal of bacteria
Chromatography (e.g. ion exchange; affinity; size-exclusion)	Protein purification and fractionation
Viral inactivation treatment (e.g. chemical incubation or heat treatment)	Destruction of virus infectivity
Nanofiltration (filtration on nm-membranes)	Removal of viral infectivity
Aseptic filling	Dispensing of sterile-filtered product into final glass bottles or syringes
Freeze-drying	Protein stabilization by desiccation
Autoclaving	Sterilization of equipment

purification, viral inactivation and elimination treatments, filtration, ultrafiltration, aseptic filling, and freeze-drying.

Furthermore, in contrast to most other parenteral products, plasma products cannot be sterilized in their final container e.g. by autoclaving, therefore requiring very clean manufacturing conditions, and appropriate in-process aseptic filtration steps to ensure absence of pyrogenic substances generated by endotoxins.

The discipline and high standards imposed by plasma fractionation are among the most rigorous examples to be found in the entire pharmaceutical industry and can rightly be regarded as an excellent first step and training school for countries considering the introduction of biotechnology industries. In addition to the usual skills required for the pharmaceutical industry, plasma fractionation also calls for specialists in the following areas: virology, protein chemistry, protein downstream processes, bacteriology.

3.2 Modern plasma fractionation technologies and product profile

Plasma fractionation, based on an ethanol precipitation process in the cold, was developed in the 1940s to extract albumin and immunoglobulins G. Recent refinements in protein separation techniques have played a major role, adding new dimensions to the plasma fractionators' tool kit. Indeed, in the past two decades, plasma fractionators have improved the plasma extraction and purification processes, greatly enhancing product yield, viral safety and purity. These improvements have been achieved by refining the traditional ethanol fractionation process and implementing specific chromatographic production steps, and have resulted in provision of new therapeutic products and increased purity of existing preparations (factors VIII and IX, von Willebrand factor, immunoglobulins G, albumin). These developments have led to a more rational and optimal use of human plasma, while improving the overall cost–benefit of plasma fractionation. More importantly, newly developed technologies have become relatively easy to adjust to small-scale local processing, making them available for the use of countries wishing to establish their own plasma fractionation programme.

If ethanol fractionation has been retained as the backbone of plasma processing for the extraction of albumin and IgG in the vast majority of centres, chromatography has already brought about a significant impact in the large-scale fractionation of human plasma and the purification scheme of several essential therapeutic plasma products. The major benefits of chromatography have been seen in the design of new purification methods, yielding plasma products with significantly improved purity and safety, as well as recovery.

Based on today's scientific understanding, one may therefore consider that currently available plasma-derived F VIII, F IX and protease inhibitor preparations produced by chromatography (e.g. ion exchange or affinity) have reached an appropriate level of purity to provide a standardized, efficient treatment of patients without the side effects (e.g. thrombogenicity, haemolysis, immunosuppression) associated with low purity preparations. The technology required to make these products can relatively easily be implemented within an appropriately professional environment.

Considering current product purity profiles, it is unlikely that major changes in purification technology will be seen in the next few years unless the elimination of newly identified plasma-borne infectious agents requires the implementation of different production processes. While the occurrence of new infectious agents is always a possibility, the requirement for a sudden dramatic change in plasma fractionation technology to eliminate those agents is unlikely. Consequently, it appears that plasma fractionation technologies have now reached a mature level of development and are unlikely to change in the foreseeable future. This should be seen as a reassuring factor in the decision-making process for initiating new plasma fractionation programmes. In other words, technical and technological investments which have already been made are unlikely to undergo early outdating.

Table 3.2 summarizes the advantages of current new plasma fractionation technologies based on the combination of ethanol fractionation to perform bulk extraction of albumin and IgG and chromatography to purify the other products.

TABLE 3.2 **Advantages of current plasma fractionation technology trends**

Advantage	Results in
Products with a higher purity level and fewer side effects	Reduced thrombogenicity: high-purity F IX Reduced risk of haemolysis: high-purity F VIII Reduced immunosuppressive effects: high-purity F VIII
Products with a better safety profile	Reduced potential virus load following extended purification steps
More products can be made from each plasma batch	Higher cost–effectiveness of the plasma fractionation process Optimal use of plasma
New products can be made: development of selective haemotherapy	Products specific to each plasma protein deficiency state Patients not overloaded with unnecessary plasma components
Making a wider range of products	Self-sufficiency attained for the treatment of a variety of diseases

Table 3.3 lists some points to consider in selecting the purification technology that will constitute the backbone fractionation process.

TABLE 3.3 **Points to consider in selecting plasma fractionation technologies**

Fractionation means	Advantages	Considerations for product quality
Ethanol fractionation	Large batch processes Well-suited for "bulk" protein production (albumin, IgG) Relatively easy to control Ethanol: low toxicity, relatively low cost, large supply Viral reduction potential (e.g. HIV) Established since late 1940s for albumin and IgG	Not adapted for trace proteins and labile proteins Ethanol precipitation may induce protein aggregation and denaturation
Chromatography	Excellent for trace and labile proteins Excellent tool for purifying therapeutic products Choice of commercial chromatographic gels available High binding capacity for trace plasma proteins allowing "miniaturized" purification step Viral reduction potential Easy to scale-up Established technology for coagulation factors and protease inhibitors since mid 1980s	Elution conditions should be mild to avoid protein alteration Careful control of buffer production and use to limit risks of bacterial contamination

Chapter 4
Technology for start-up of plasma fractionation

4.1 General

Selecting the appropriate technology as the initial backbone for the plasma fractionation process is of critical importance and the choice must be carefully assessed based on established safety and quality of the technology.

4.2 Albumin

Most current albumin preparations are obtained by ethanol plasma fractionation processes following either the Cohn-Oncley method 6+9 developed in the USA or the Kistler-Nitschmann method developed in Switzerland. Most manufacturers have introduced "in-house" modifications to these initial processes by modifying parameters like pH, osmolarity, or contact time with ethanol at various steps of the production process. Other changes have included the co-precipitation of fraction I together with fraction II+III and replacement of the final precipitation of fraction V by direct ultrafiltration of fraction IV-4. Most of these changes were implemented as a means for increasing albumin recovery, improving productivity or facilitating working schedules (e.g. to avoid or limit night shifts or time-consuming, manual production steps over the week-end), without altering product quality.

Another significant aspect of the process design used is the choice of the methodology for separating the precipitates formed during the fractionation process. This has a significant impact on yield. When centrifugation is used (e.g. to remove precipitate I + II + III, precipitate II + III or precipitate IV-4), it is not possible to recover the albumin solution entrapped in the precipitate. Depending upon the type of centrifuge used, the precipitate may have a relatively high humidity level and carry significant amounts of albumin. The total amount of albumin "lost" by entrapment in the various precipitates generated during the fractionation process may amount to 2 to 5 g per litre of plasma, corresponding to a loss of up to 10% to 25% of the starting plasma content. Using filtration instead of centrifugation makes it possible, under certain conditions, to recover part of the albumin solution entrapped in the precipitates and to obtain a cleaner separation, while requiring less processing time. In the filtration separation method, precipitates can be

"washed" by a buffer solution thus "eluting" albumin from the precipitate. Further drying of the "protein cake" flushes out and recovers the remaining protein solution still entrapped in the precipitate. In order to avoid clogging of the suspension on the filters, the addition of filter aids to the ethanol-plasma mixture is required. Care should be taken in the selection of the filter aids as some filter aid types may release significant quantities of aluminum. However, aluminum can be eliminated from the albumin solution by properly designed ultrafiltration cycles.

Long-term experience indicates that the impact of properly validated and controlled variations in the manufacturing process on the specifications and safety of albumin is marginal. There do not seem to be any limitations to selecting ethanol fractionation which will allow optimal recovery of albumin. Under the best conditions, a mean production-scale yield of 26 to 28 g of albumin per litre of plasma is therefore achievable, corresponding to 70% to 80 % overall recovery of albumin from the starting plasma. Since the availability of albumin is usually limited in developing countries and fresh frozen plasma is still widely used instead, there is a strong rationale for developing economies intending to develop plasma fractionation to select a plasma fractionation technology ensuring an optimal recovery of albumin.

A modified production technique, combining ethanol plasma fractionation up to the II + III precipitation step and a three-step chromatographic purification method has been developed and is now used on a large scale by one established plasma fractionator. The purity of the resulting albumin is excellent. It appears that carefully controlled conditions must be used to limit albumin loss during chromatography, and to ensure that the final product is stable enough to withstand terminal pasteurization in its final container.

All current albumin products are virally inactivated by a pasteurization step (heat-treatment at 60 °C for 10 h) which should be performed in the final container, following aseptic filling.

4.3 Immunoglobulin G products

Most IgG preparations are obtained by the original ethanol fractionation process that leads to fraction II (crude IgG fraction). Some products are purified by a process combining ethanol fractionation and chromatography. Most products have an IgG purity which is above 98%. However, preparations can be distinguished from each other, by the nature and quantity of some protein contaminants, like IgA. A major difference in IgG products depends upon the kind of treatment performed to ensure well tolerated, non-reactive intravenous infusion, as well as on the type of viral reduction process used. Although some processes yield 2.5 to 3 g of IgG per litre of plasma with today's fractionation equipment and technology, carefully conducted large-scale fractionation procedures make it possible to recover up to 3.5 to 5.5 g of IgG per litre.

Developing countries starting their own plasma fractionation should take advantage of the considerable evolution and refinement in production processes which have occurred within the past two decades and which can now yield native IgG molecules with a high recovery. This implies that harsh enzymatic (high concentration papain or pepsin treatment) or chemical (e.g. sulfonation) treatment to eliminate aggregates or reduce anti-complementary activity should be avoided. A combination of ethanol fractionation and chromatography, as well as low pH, or low pH/mild pepsin treatments avoid IgG alteration. In terms of viral safety, solvent–detergent (SD), low pH (with or without pepsin) and beta-propiolactone treatments appear to ensure the currently required safety level. More recently, pasteurization alone has also been applied to IgG preparations, while nanofiltration using 35 nm or 15 nm membranes has been used as an additional viral safety step for products already subjected to SD, beta-propiolactone, or pH 4/pepsin procedures. In selecting a viral reduction process for IgG, it should be remembered that owing to the potential presence of neutralizing antibodies (like anti-HAV, anti-B19, or anti-HBs) the viral safety profile of the IgG concentrate may differ from that of the other products (e.g. coagulation factors) obtained from the same plasma pool.

In addition to polyvalent IgG products, the production of hyperimmune IgG preparations, such as anti-RhD, anti-HBs, anti-HAV, anti-tetanus, or anti-rabies, should be considered, after taking into account the characteristics of the plasma donor population as well as the local epidemiology and clinical needs. Basically, processes for manufacturing hyperimmune IgG preparations do not differ from that of intramuscular or intravenous IgG, although the starting plasma pool volume may be much smaller due to the lower availability of hyperimmune starting plasma.

4.4 Factor VIII

All currently used plasma derived factor VIII products, regardless of their degree of purity, are prepared by processing steps starting from cryoprecipitate. Cryoprecipitate is obtained by thawing plasma under controlled conditions of temperature. All industrial-scale plasma fractionators perform cryoprecipitation in bulk, comprising several hundreds or thousands of litres. Prior to the opening of the bags, plasma donations may be placed in a cold room at a temperature between 0 and 4 °C, in order to warm the bags up to about −7 °C, so that the frozen plasma will break away from the plastic (skin-thawing). This facilitates the subsequent operation of opening the bags. Alternative procedures consist in placing plasma bags in a liquid nitrogen bath for a few seconds. The shattered plastic bags are stripped from the plasma. After opening the bags, the frozen plasma donations are expelled from their plastic containers and plasma is pooled and allowed to thaw at between 1.5 and 3.5 °C, using jacketed stainless steel thawing tanks. In the past (and even today in small facilities) cryoprecipitation was performed in the individual plasma donation bags; after thawing in the cold, the bags were centrifuged and the cryoprecipitate was recovered

by manual procedures carried out under aseptic conditions. The colder the temperature, the higher the quantity of cryoprecipitate recovered. Average recovery of cryoprecipitate is from 6 to 10 g per litre. A higher quantity of paste recovered often means that more fibrinogen has been precipitated but does not necessarily imply that the cryoprecipitate will contain more factor VIII. The cryoprecipitate may be frozen at –30 °C or below and stored in a freezer or immediately processed into factor VIII concentrate. In a typical manufacturing process of factor VIII cryoprecipitate is subjected to:

- an adsorption step (e.g. in the presence of aluminium hydroxide) or precipitation (e.g. with polyethylene glycol, at a temperature between 10 and 15 °C) to remove traces of vitamin K-dependent coagulation factors entrapped in the cryoprecipitate;
- a specific viral inactivation treatment (e.g. by SD or pasteurization);
- a chromatographic step using anion-exchanger, size-exclusion, or a combination of immunoaffinity (using murine anti-F VIII or anti-vWF antibodies) and anion-exchange to remove the bulk cryoproteins (like fibrinogen, residual IgG, or fibronectin) and therefore purify F VIII;
- formulation, sterile-filtration on a 0.22 mm membrane, dispensing into vials and freeze-drying.

There has been a prolonged scientific and medical debate (largely stimulated by commercial interests) as to whether or not immuno-affinity purified F VIII products have a definite clinical advantages over other types of high-purity F VIII products in reducing their immune modulating effects in severe haemophiliacs. However, today with the benefit of 10 years of clinical experience, there do not seem to be significantly different clinical outcomes between such high-purity products. High purity preparations have the advantage over low-purity products, of being essentially free of IgG, IgM, fibrinogen and fibronectin; as a consequence, they do not contain blood group iso-haemagglutinins, and do not induce the haemolytic side-effects of low purity products, even in large doses. In addition, high-purity products have been shown to cause fewer febrile and allergic reactions. High-purity F VIII concentrates which still contain vWF (which may mask F VIII epitopes), have the advantage of not requiring the addition of albumin as a stabilizer, and may induce a lower incidence of inhibitors in patients. Therefore in selecting a F VIII production process, the viral safety profile of the preparation should be considered first (does the process include a strong, non-protein denaturing, viral inactivation treatment? Does it include also a second viral reduction treatment?) and as a secondary consideration, the level of purity.

Recovery of F VIII depends greatly upon the quality of the starting plasma, as well as on the choice of the viral reduction treatments. Average current recoveries range between 130 and 200 IU F VIII per litre of plasma. Figure 4.1 shows a production scheme for F VIII concentrates.

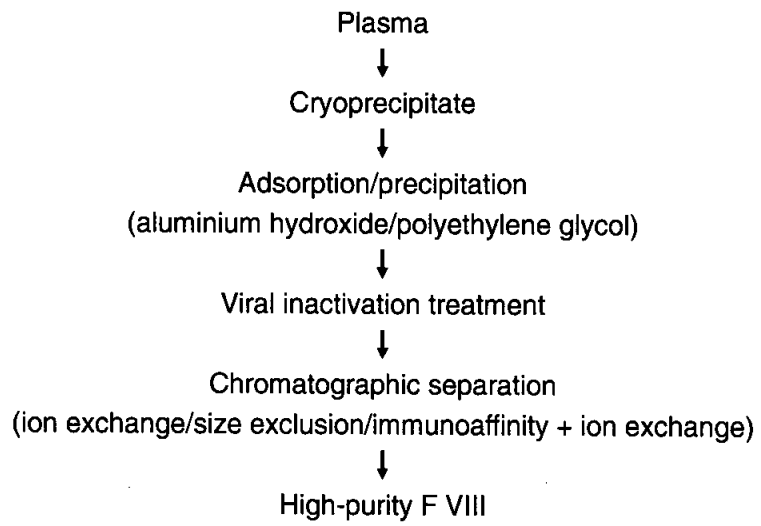


FIGURE 4.1 **Production scheme for F VIII concentrates**

4.5 Factor IX preparations

The majority of factor IX preparations are obtained by the following manufacturing steps.

- Cryo-poor plasma is absorbed onto an anion-exchange matrix (typically DEAE-Sephadex A-50 or DEAE-cellulose) under conditions where vitamin K dependent proteins as well as other proteins like protein C and protein S are bound;
- The gel is then carefully "washed" with various buffer solutions to recover the unbound proteins (e.g. albumin, IgG, ATIII, alpha 1 AT and many others), elute proteins, contaminants and then the prothrombin complex (PCC) fraction;
- PCC is further processed to ensure inactivation of potential viruses (possibly including a chromatographic elimination of the virus inactivating agents);
- The final PCC is formulated, sterile-filtered, dispensed aseptically into vials and freeze-dried.

When high-purity F IX is produced, the general production process starts from virally inactivated PCC which is further processed by additional chromatographic purification steps combining, for example, DEAE-Sepharose and immobilized Sepharose, or various types of cation-exchangers. These steps successively remove F VII, F X, F II, as well as protein C, protein S and other proteins like inter-alpha inhibitor. The final F IX is formulated, sterile-filtered, dispensed aseptically into vials and freeze-dried.

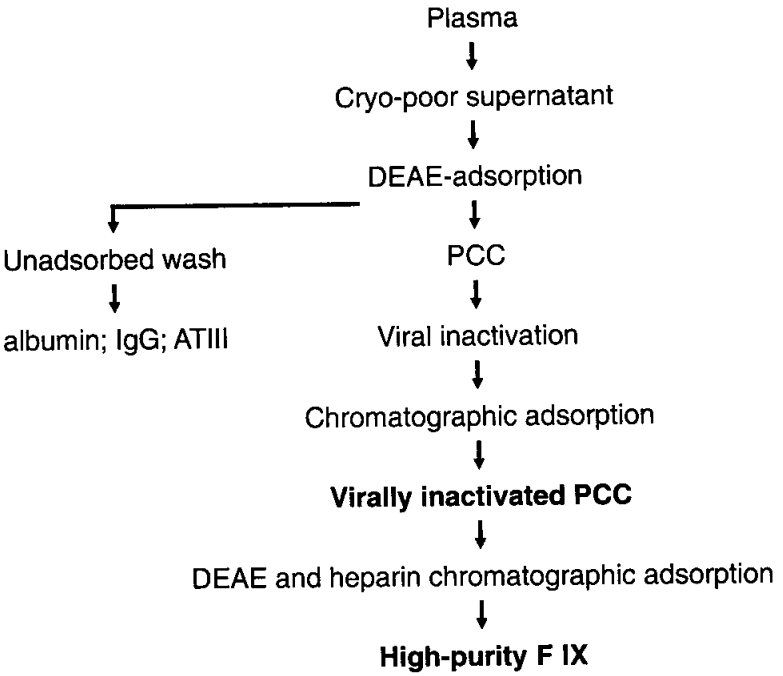


FIGURE 4.2 **Production scheme for PCC and F IX concentrates**

Recovery of PCC is in a range from 300 to 500 IU/L of plasma (expressed in terms of F IX content), that of F IX from 200 to 350 IU/L. Figure 4.2 shows a production scheme for PCC and F IX concentrates.

4.6 Other protein fractions

A number of additional plasma products can also be made. It would be reasonable to develop these as by-products of the four or five major plasma fractions, by designing further specific purification steps from already available pre-purified fractions, rather than trying to design a completely new purification process from cryoprecipitate or cryo-poor plasma. As an example, fibrinogen can be purified from a discarded fraction generated during the purification process of F VIII. Another example is the purification of protein C as a by-product of the F IX production scheme. Table 4.1 gives examples of such an approach.

TABLE 4.1 **Use of intermediate plasma protein fractions for new product development**

Starting plasma fractions	End products
Cryoprecipitate	Factor VIII
	Fibrinogen
	von Willebrand factor
	Fibronectin
Paste I	Fibrinogen for fibrin glue
Cryo-poor plasma (cryo-supernatant)	Factor IX
	Prothrombin complex
	Thrombin for fibrin glue
	Protein C
	Factor XI
	Factor VII
	C1-inhibitor
Heparin-adsorbed cryo-poor plasma	Antithrombin III
Precipitate II + III	Alpha 1 antitrypsin
Precipitate IV	Alpha 1 antitrypsin
Precipitate II	Intravenous polyvalent IgG
	Intravenous anti-HBs IgG and other hyperimmune IgG
	Intramuscular polyvalent IgG
	Intramuscular anti-HBs IgG and other hyperimmune IgG
Precipitate V	Albumin

Following such a strategy provides numerous industrial advantages which include:

- common validation steps, possibly including a viral inactivation treatment. For example, it is possible to perform a solvent-detergent step upstream of a fractionation procedure, thus ensuring that all by-products fractionated downstream have been subjected to an identical viral reduction treatment;
- reduction in development costs;
- reduction in the production costs, including human resources and in-process quality control, as parts of the fractionation process which are common to several downstream sub-products;
- reduction in the production space requirements for the additional products.

Chapter 5
Viral elimination or inactivation methods

5.1 General

As purification processes *per se*, have proved to be inadequate to the task of providing sufficient viral safety, specific and efficient viral reduction techniques are required during the fractionation process of each plasma derivative. The primary target is the inactivation of highly pathogenic plasma-borne lipid-enveloped viruses (HIV, HBV and HCV); an appropriate level of safety against these viruses can be achieved by the introduction of techniques involving the use of solvent-detergent or pasteurization. Thus, when plasma products are manufactured under strict GMP conditions with careful monitoring of process steps, the risk of transmitting such viruses is now essentially nil. Again, under appropriate technical supervision, managing these technologies is feasible even for newly established fractionation centres.

The viral safety of all blood and plasma products has made significant progress over the last few years. This progress originates from the implementation of efficient measures, especially the improved selection of safer donors, the increased sophistication and specificity of viral screening of each blood or plasma donation, the implementation of new specialized test strategies (mini-pool testing) and improved manufacturing practices, including, for plasma products, the implementation of efficient viral inactivation treatments. The last few years have also seen blood transfusion, as a whole, being subjected to increasingly stringent regulatory procedures and controls which have largely increased awareness of good laboratory and manufacturing practices.

5.2 Viral safety of plasma products

To be as safe as possible, plasma products require a specific series of safety nets. The viral safety concerns specific to plasma products, as compared to single donor blood components or frozen fresh plasma, relate to the fact that they are extracted from a pool of several thousand plasma donations. As a consequence, a single infectious unit may contaminate the whole plasma pool and the end-products derived from the pool may themselves be infectious. Fortunately, considerable progress has been made in the past

few years to make plasma products safer than before. Product safety results from the complementary benefits of several crucial measures:

- careful selection of blood/plasma donors;
- appropriate screening of each donation;
- PCR testing of mini-plasma pools;
- control of plasma pools for the absence of the major viral markers (anti-HIV; anti-HCV; HBsAg);
- efficient and validated viral reduction steps for each plasma product;
- good manufacturing practices to avoid the risks of downstream or adventitious microbial contamination.

Historical perspectives indicate however that, over the years, the most significant improvements in plasma product safety against the major plasma-borne viruses have resulted from:

- a better selection and screening process of donors and plasma donations using more sensitive and specific viral screening tests; and
- the choice of an appropriate manufacturing strategy (particularly the type of viral reduction treatments) which, through appropriate validation, must have been shown to offer a margin of safety exceeding the potential infectivity of the starting plasma.

5.3 Specific viral reduction treatments

5.3.1 Rationale

Specific viral reduction treatments are designed to destroy or eliminate viral elements which are essential for viral replication. These include:

- structural or functional protein binding to cell receptors required for viral replication (e.g. DNA polymerase, reverse transcriptase);
- lipids of the envelope which are essential to the structural integrity of the virus;
- the nucleic acids essential to virus replication or whose alteration may lead to mutations that inhibit transcription.

As an example, viral inactivation techniques based on heat treatment aim at denaturing proteins of the viruses and, to a lesser extent, nucleic acids so that the aggregate alteration achieved renders the virus unable to infect cells or replicate. The solvent–detergent method destroys the lipid components of the envelope, while irradiation or beta-propiolactone treatment degrades nucleic acids. An important criterion which must be taken into consideration in selecting a viral inactivation technology, is ensuring the

minimum risk of plasma protein neoantigen formation, as these could induce the formation of inhibitors in patients receiving the preparation.

During the manufacture of plasma products, several production steps may contribute to viral reduction. Their effect may be more or less pronounced, as indicated in Table 5.1 and Box 5.1.

The main features of plasma product viral reduction treatments are listed in Table 5.2.

TABLE 5.1 **Effects of the various parameters of viral reduction treatments**

Parameter	Effect	
	Non-specific partitioning	Specific inactivation or elimination
Reduction factor	Low	High[a]
Reproducibility	Medium	High
Control of parameters	Medium	High
Scale-down potential	Difficult	Easy
Spectrum of virucidal efficacy	Limited	Wide

[a]Typically more than 4 log

BOX 5.1 **Effect of the various viral reduction treatments**

Weakly effective

- Precipitation/centrifugation, e.g.
  - ethanol fractionation
  - polyethylene glycol
  - caprylic acid
  - ammonium sulfate
- Chromatography, e.g.
  - immunoaffinity
  - ion exchange
- Adsorption, e.g.
  - aluminium hydroxide
- Micrometer filtration/ultrafiltration

Strongly effective

- Solvent–detergent
- Heat treatment
- Pasteurization
- Dry heat treatment
- Steam treatment
- Nanometer filtration (nanofiltration)
- Beta-propiolactone

TABLE 5.2 **Characteristics of the main viral reduction treatments in use for plasma products**

Treatment	Technical features	Where applied in the production process?	Remarks on relative degree of technological complexity[a]	Patent protection/ proprietary process
Solvent–detergent	Incubation in the presence of tri (*n*-butyl) phosphate and a detergent[b]	In process	Simple	Yes (licence available)
Pasteurization	Heat treatment in solution for 10 hours at 60 °C	Usually in process (albumin: final container)	Simple	Often
Dry heat treatment	Heat treatment of dry product[c]	Final container	Control of important moisture content is important	Limited protection
Steam treatment	Heat treatment of dry product in the presence of steam and under pressure	In process	Not fully described	Proprietary process
Beta-propiolactone/ UV	Incubation with beta-propiolactone followed by UV irradiation	In process	Special equipment	Yes
Nanofiltration	Filtration on nanometer pore membrane	In process	Simple; control pressure and flow rate during filtration	Nanofiltration devices commercially available

[a] Control of homogeneity of the treatment and validated parameters (temperature, duration, etc.) is essential
[b] Tween 80; triton X-100; sodium deoxycholate;
[c] Duration, time and temperature are product specific.

5.3.2 Physical methods (Heat treatment)

5.3.2.1 Pasteurization

Pasteurization is defined as the treatment of a liquid protein fraction for 10 hours at 60 °C. Pasteurization is generally performed in the presence of stabilizers which are added to protect plasma proteins functionality. For labile proteins (e.g. coagulation factors) a loss of 10% to 30% of the biological activity may be encountered during pasteurization. The stabilizers used may have a protective effect specific to certain plasma proteins; this is the case for the pasteurization of albumin in the presence of sodium caprylate and sodium tryptophanate, which bind to albumin and avoid its denaturation or aggregation during liquid heat treatment. Other stabilizers, like sugars (e.g. glucose or sucrose) and

polyols (e.g. mannitol, sorbitol) have a more global protective effect on the biological activity of labile proteins or avoid the formation of protein aggregates or polymers (e.g. IgG) or limit risks of precipitation. Amino acids (e.g. lysine, glycine, arginine, etc.) may also protect the functional activity of some plasma proteins.

Pasteurization is often carried out during the plasma fractionation process itself. Stabilizers, when added prior to the heat treatment, can be subsequently removed by ultrafiltration or chromatography, or a combination of both. Good manufacturing practices must be applied to ensure appropriate application of the heating process (e.g. homogeneity of the treatment) and avoid the risks of viral contamination subsequent to the viral inactivation step. To date, only albumin preparations can withstand a pasteurization treatment in the final container subsequent to the aseptic filling step. This is because the quantity of caprylate (and tryptophanate) is relatively small and remains compatible with direct clinical use.

Although stabilizers may protect viruses from heat denaturation, viral validation studies show that pasteurization has the potential to inactivate viruses of different types, whether enveloped or non-enveloped and particularly HIV (typically more than 5 log are inactivated in less than 15 to 30 min). There are only a few published data on the inactivation of some resistant plasma-borne non-enveloped viruses like parvovirus B19 or some model viruses like SV 40 or reovirus type 3. In some instances, inactivation of such viruses may be incomplete (less than 3 to 5 logs).

Points to consider in the application of pasteurization to plasma products are listed below:

- What is the recovery of labile factors?
- Can the process generate protein neoantigens?
- Are the heating conditions inducing protein aggregates?
- Can the stabilizers used protect viruses from inactivation?
- Is there a risk of biochemical reactions (e.g. alteration of glycosylation) between the stabilizers and the plasma proteins?
- How can the stabilizers be eliminated following pasteurization?

5.3.2.2 Dry heat

Dry heat treatment is defined as the technology of subjecting a lyophilized product under a defined residual moisture content to a heating process of several minutes to several days at temperatures varying from 60 to 100 °C. In most cases, the heat treatment is applied to the product in its final container, following the terminal freeze-drying step. Less often, the treatment can be performed on an intermediate raw material during the manufacturing process. Initially, dry heat treatment at 60 to 68 °C for up to 72 or 96 hours has been applied to coagulation factor concentrates as a means of inactivating HIV.

However, the less drastic conditions were not optimal for the inactivation of HBV and HCV. Inactivation of HCV in F VIII and F IX complex concentrates requires so called "severe" dry heat treatment conditions at 80 °C for 3 days. Even then, risks of transmission of parvovirus B19 are not totally eliminated. More recently, heat treatment at 100 °C for 30 min has been applied to high-purity F VIII concentrates in order to inactivate HAV. Again such conditions do not seem to eliminate the transmission of parvovirus B19 from these products.

It is clear that the efficacy of dry heat treatment is less than that of pasteurization because the products are heated at a lower humidity content. However, most dry heat treatments have the advantage of being applied to the final product, thus avoiding the risk of down-stream contamination and they do not require the addition of excessive quantities of stabilizers, so that the product can be administered to patients without further processing steps. The common stabilizers used include limited amounts of sucrose or amino acids such as lysine, arginine, or glycine. When implementing a dry heat treatment it is important to ensure perfect temperature control of the oven, to make sure that all vials will be subjected to the validated heat conditions. Also, the composition of the final product (total protein content, precise formulation) as well as the residual moisture content of the product have to be very well monitored as they influence both the efficacy of the viral inactivation as well as the recovery of protein biological activity. In general the drier a product, the higher the protein recovery and the lower the degree of viral inactivation will be. Due to its limitations, dry heat treatment is now more often used as a second viral inactivation step, to improve viral safety against non-resistant, non-enveloped viruses (e.g. HAV) and as a complement to solvent detergent treatment.

Points for consideration in applying terminal dry heat treatment are largely similar to those applicable to pasteurization.

5.3.2.3 Other heat treatments

One manufacturer has developed proprietary variations, by performing dry heat treatment of intermediate raw materials in an oxygen-free atmosphere, in the presence of steam and pressure ("steam treatment") and at a relatively high moisture content. The extent of viral inactivation is directly related to the temperature (60 to 80 °C), the pressure (1190 to 1375 mbar), and the duration of the treatment (generally up to 10 hours). Although under the same conditions of temperature and duration, steam treatment may be more virucidal than dry heat, cases of transmission of hepatitis have been reported.

Heat treatment at 60 °C for 20 hours in suspension in N-heptane (20 hours) or for 72 hours in chloroform (72 hours), have been applied to coagulation factors but have now been abandoned due to incomplete inactivation of HCV.

Both heat treatment variations required relatively complex equipment.

5.3.3 Chemical inactivation treatment

5.3.3.1 Solvent–detergent

Solvent–detergent (SD) methodology consists in incubating a plasma protein solution in the presence of an organic solvent and a detergent. The organic solvent used is tri-(*n*-butyl) phosphate at a concentration of 0.3%. Tween-80, triton X-100 and more rarely nowadays, sodium cholate are the detergents used. The treatment is generally performed in closed stainless steel containers, at temperatures between 24 °C and 30 °C over a maximum of 6 hours and under mild stirring. The elimination of the viral inactivation agents is often achieved, when feasible, by a subsequent chromatographic adsorption step of the proteins of interest, or by ultrafiltration, or by precipitating the proteins and eliminating the chemicals in the supernatant.

This method has been shown to have unchallenged potential to inactivate lipid-enveloped viruses. These viruses are typically killed in a few minutes. Another advantage in terms of avoidance of protein neoantigen formation is the absence of protein alteration, which ensures excellent protein recovery during the SD treatment step. However, the method used to remove the SD agent may induce some protein loss. The drawback of the SD procedure is the failure to inactivate non-enveloped viruses. Also, as the treatment is necessarily performed on intermediate plasma fractions (to allow removal of the chemicals), precautions must be taken to avoid the risks of recontamination of the SD-treated fraction during the downstream production process.

Implementation of the SD treatment does not require sophisticated equipment. Care must be taken to ensure homogenous incubation of the whole protein solution and appropriate recording of temperature and duration of treatment must be made.

Some points for consideration in applying SD treatment are:

- how to eliminate the solvent and detergent following treatment;
- how to integrate the SD treatment into the overall fractionation process.

Elimination of the SD agents is usually ensured by a subsequent chromatographic adsorption step designed to bind the protein(s) of interest (e.g. coagulation factors) while the virus sterilizing agents are removed in the chromatographic breakthrough fraction. Another approach is to precipitate the protein(s) of interest (e.g. fibrinogen) while the SD agents remain in the supernatant and are discarded.

The advantages of the SD treatment can be summarized in the following manner:

- efficient inactivation of enveloped viruses;
- no impact on protein characteristics and biological activity;
- no formation of protein neoantigens during the treatment;
- relatively easy to control and record;
- relatively straightforward to implement at the production scale.

5.3.3.2 Acid pH incubation

Some treatments developed in the course of the production of plasma derivatives have also turned out to be efficient viral reduction steps. This is the case for immunoglobulin G preparations which require particular treatment steps to avoid the side-effects usually associated with the intravenous infusion of intramuscular immunoglobulin preparations. These specific production steps for IV IgG include:

1. enzymatic treatment such as incubation in the presence of pepsin, papain or plasmin;
2. incubation at pH 4 in the presence of traces of pepsin; treatment with beta-propiolactone; sulfonation; reduction/alkylation;
3. physical treatment step: precipitation in the presence of polyethylene glycol.

Some of these treatments have been shown to inactivate viruses, particularly the incubation at low pH in the presence (or absence) of pepsin, at temperatures between 30 and 37 °C for 20 hours or more. Validatory studies of the virucidal power of pH 4 treatment show complete inactivation (more than 5 to 8 log) of various enveloped viruses used as models, such as HIV, Semliki forest virus, herpes simplex virus type 1, vesicular stomatitis virus, CMV. By contrast, poliovirus, a non-enveloped virus, seems more resistant.

As for any specific viral inactivation step, pH treatment must be performed under strict manufacturing conditions to ensure homogeneity of the acid protein solution and the absence of any risk of downstream contamination.

5.3.4 Neutralization by antibodies

Plasma pools contain a large variety of antibodies, some of which possess viral neutralizing activity. This may explain why non-virally inactivated IM and IV immunoglobulin preparations have not been associated with the transmission of diseases such as HBV (particularly after the implementation of HBsAg screening), HAV, parvovirus B19, and possibly also HCV (prior to the implementation of anti-HCV screening). Low purity F VIII concentrates may not have transmitted HAV due to the residual presence of anti-HAV neutralizing antibodies. For the same reasons, in the past, prior to the development of efficient viral reduction treatments, some plasma fractionators actually added anti-HBs immunoglobulins to coagulation factor concentrates to avoid the risks of transmission of HBV. HIV and HCV differ however, in that they appear to foster fewer neutralizing antibodies than HAV or HBV infections. The incorporation of specific virucidal procedures is therefore particularly important to guard against the transmission of these viruses.

5.3.5 Physico-chemical methods

5.3.5.1 Beta-propiolactone combined with UV irradiation

This proprietary "cold sterilization" procedure combines chemical and physical means to achieve deleterious alteration of viral nucleic acids and loss of virus infectivity. The plasma protein fraction is first incubated with beta-propiolactone, then irradiated for a short period of time with ultraviolet light, using a specifically designed irradiator. Viral validation and clinical experiences have demonstrated considerable efficacy against HIV, HBV, HCV and HAV.

However, this procedure causes significant loss of biological activity of some coagulation factors, especially F VIII, and is therefore not applicable to labile preparations.

5.3.5.2 Beta-propiolactone without UV irradiation

Some polyvalent or hyperimmune IgG products obtained by ethanol fractionation are further subjected to beta-propiolactone treatment to decrease the anti-complementary activity. This treatment also contributes to reducing the viral infectious risks, the extent of inactivation achieved being more than 4.5 log and 3.5 log for HIV and HCV, respectively. This treatment is also applied to an IgG preparation enriched in IgM.

5.4 Non-specific viral elimination methods

5.4.1 Fractionation by precipitation ethanol

The major plasma proteins, albumin, immunoglobulins G as well as some fibrinogen concentrates are purified by a fractionation process which is based on a succession of precipitation steps under precise conditions of ethanol concentration, pH, osmolarity, and temperature (below 0 °C). The ethanol concentration varies from 8% (for fibrinogen) to 25% (IgG) or 40% (albumin). The precipitation steps last for up to several hours at a pH that may be as low as 4.5.

It has been established that the infectivity of non-resistant viruses can decrease significantly during the fractionation process of IgG and albumin, due to a combination of viral elimination and viral inactivation. During the Cohn-Oncley fractionation process, as well as during the Kistler-Nitschman process, the cumulative reduction of infectivity linked to HIV reaches over 10 to 15 log. It is considered that the reduction of infectivity for HBV or HCV is much lower. The reduction factor for HCV, as defined by polymerase chain reaction assay, is probably less than 5 log. It has been suggested that for HCV, viral elimination during ethanol fractionation may be dependent upon the presence of neutralizing antibodies which may combine with viruses and favour their elimination in discarded fractions, through a partitioning effect.

Experience indicates that ethanol fractionation *per se*, is not sufficient to ensure the elimination of the infectivity of resistant viruses. For example, non-pasteurized albumin preparations have transmitted hepatitis B (at the time when the starting plasma was not screened for HBsAg). In addition, after the implementation of second generation anti-HCV screening, an IV IgG preparation that was not subjected to a strong specific viral inactivation treatment has been found to transmit HCV.

5.4.2 Other precipitation methods

Industrial ethanol fractionation starts by thawing plasma at 1 to 3 °C to isolate the cryoprecipitate which contains factor VIII, von Willebrand Factor, fibrinogen and fibronectin. It has been found that this step does not contribute to viral reduction. For example, for HIV, infectivity is only about 1 log lower in the cryo-poor plasma as compared to whole plasma.

Polyethylene glycol at a low ionic strength, is used during IV IgG production, to remove aggregated IgG and immune complexes. It has been found that polyethylene glycol precipitation can also eliminate more than 5 log of HIV, vesicular stomatitis virus, Sindbis, and HBV (as determined by HBsAg assay).

5.4.3 Chromatographic methods

The highest viral safety profile of current plasma derivatives may be associated to some extent, with their higher purity afforded by the implementation of more refined purification techniques. Indeed progress made in plasma protein separation has led to the development of new generations of plasma derivatives, generally prepared by procedures which involve one or several adsorption chromatographic steps. As an example, current high-purity F VIII and F IX concentrates which are obtained by adsorption chromatography are 100-fold to 1000-fold (2 to 3 log) more pure than earlier products available in the 1980s, corresponding to an overall purification factor from whole plasma of more than 10 000. This level of purification is typical of what can now be achieved for other preparations like F XI, thrombin, von Willebrand factor, etc. In addition to providing 2 to 3 log enhancement of purification, chromatography may improve the safety of new plasma derivatives by contributing to the removal of plasma-borne viruses. Viral validation studies show that the improvement in purity is often associated with a reduction in viral load. Indeed, validation studies have demonstrated that immunoaffinity, ion exchange, or other types of adsorption chromatography can eliminate 1 to 5 log of HIV-1, or of several model viruses, enveloped or non-enveloped, such as Sindbis virus, pseudorabies virus, vesicular stomatitis virus, Reovirus 3 or simian virus 40.

When chromatography is used as a basic fractionation method involving the elution of the chromatographic medium by several buffers, thus generating additional side-fractions, clearance factors are especially high as chances to separate viruses increase. In

addition, a process combining several chromatographic purification steps may enhance the viral clearance factors.

Several parameters may influence the chromatographic behaviour of viruses, including the size, shape, symmetry and membrane structures of virions. In addition, buffer conditions that may induce aggregation of viruses and change their apparent size and surface properties, as well as chromatography flow-rate and packing material characteristics are among the important parameters potentially influencing binding of viruses on chromatographic resins. Reuse of the chromatographic gels, possibly associated with discrete changes in the gel chemistry may also change virus/gel interactions. Due to the complexity of the phenomena potentially influencing the chromatographic behaviour of viruses and because these are not well understood, it is important to design chromatographic production processes for plasma derivatives and to perform validation studies following a rigorous scientific approach. It is also becoming clearer that, compared to specific viral reduction treatments, chromatography must be considered as only one means for marginally enhancing the viral safety of plasma products.

In addition, one should also consider the situation where an improved level of purity may also tend to remove antibodies, and this may have been the case where highly purified F VIII preparations devoid of neutralizing anti-HAV have transmitted hepatitis A.

5.5 Nanofiltration

Nanofiltration is a new specific viral elimination approach which presents the potential to extend the concept of sterility from bacteria to viruses, and is the approach discussed here. Pasteurization and SD treatment are excellent current methods for eliminating the risks of transmitting the most pathogenic plasma-borne viruses, HIV, HBV, and HCV. Nevertheless, some plasma products, especially coagulation factors, still seem to be involved in the transmission of non-enveloped viruses, such as HAV and parvovirus B19. Although those viruses may have limited pathogenicity in most patients, the potential for their transmission indicates that additional measures must be taken to further enhance the viral safety of plasma derivatives. In addition, the possibility of new infectious agents contaminating the human plasma supply always exists. It is therefore wise for new plasma fractionation programmes to take this potential risk into consideration and adopt measures that will ensure an optimal safety profile for local plasma products. One approach for attaining such a goal is the use of new viral filtration procedures, known as nanofiltration technology.

The nanofiltration technology principle is based on the segregation of proteins and viruses on the basis of their size. Biological entities larger than the nominal filter porosity (including viruses or other infectious agents) are retained on the membrane while smaller ones (e.g. plasma proteins) pass through the membrane. The technique is performed using commercially available cartridges and is reasonably simple. It can generally be

implemented without the need for sophisticated, costly equipment. However, it does require great care to ensure that appropriate filtration conditions and control of the integrity of the nanofilters are always present. Major parameters to consider in nanofiltration include a constant protein solution volume/filter surface area ratio, control of flow rate and pressure during filtration. Integrity tests for the commercial filters currently available, include a leakage test (bubble test) as well as a control of the filter porosity using a calibrated gold particle suspension, followed by spectrophotometric determination of the adsorption of the filter eluate.

Viral validation performed with coagulation factor concentrates, as well as IgG preparations, has revealed that 15 nm and 35 nm membranes are able, under the experimental conditions used, to remove more than 4 to 6 log of a wide range of viruses on the basis of their size, including non-enveloped viruses. The extent of viral clearance of the smallest non-enveloped viruses (e.g. poliovirus, encephalomyocarditis virus, HAV with a size lower than 30 nm) is greater with the 15 nm cartridge, than with the 35 nm cartridge, unless the conditions used induce some degree of virus aggregation. Both pore-size cartridges have been shown to be excellent at removing larger non-enveloped model viruses like Reovirus 3 and simian virus 40, which are both resistant to most viral inactivation treatments.

The preliminary data showing that nanofilters with a porosity of 35 nm or less could remove the Creutzfeldt-Jakob disease (CJD) agent from brain extract have recently been evaluated in an albumin solution deliberately contaminated by this prion. Removal was complete (over 6 log) when the albumin solution was nanofiltered on a 15 nm membrane and a partial 4.5 log removal was achieved when using the 35 nm cartridge. Removal of the prion appears to be closely related to its state of aggregation and the presence of a detergent (e.g. Tween 80) appears to impair the capacity of the 35 nm membrane to remove the CJD agent. Nevertheless, nanofiltration appears to be the sole possibility of eliminating or reducing infectious risks related to prions, should there be any demonstrated danger of transmission by plasma-derived products. The mechanism of prion removal by filtration is not yet established and it is likely that experimental conditions will have to be carefully controlled.

A major advantage of nanofiltration techniques lies in the fact that they can be performed under mild conditions of pH, temperature, osmolarity and pressure, that, by contrast to some harsh viral inactivation treatments, preserve plasma protein integrity and biological activity. Consequently, the recovery in plasma protein biological activity is usually over 90% and the risk of generating neoantigens is minimal. A second advantage is that nanofiltration can be carried out at the end of a purification scheme, prior to the aseptic filling step, allowing one to free the final product from residual pathogenic agents, while limiting the risk of downstream contamination. A drawback is that the filters can be relatively expensive but as they are commercially available there is no patent charge associated with the use of the nanofilters, making the technology available to all plasma

fractionators. In addition there are no royalty payments associated with their application in the manufacture of plasma derivatives.

The advantages of nanofiltration are summarized below:

- elimination of viruses based on size, regardless of structure or resistance;
- no change in product yield and properties;
- unlikely to induce protein neoantigenic structural changes;
- integrity of filters and virus removability can be validated after use of each filter cartridge;
- require only a minor process variation.

The most up-to-date viral inactivation treatments applied to plasma products are summarized in Table 5.3.

TABLE 5.3 **Examples of viral reduction treatments currently applied for therapeutic concentrates**

Product	Treatment						
	Solvent–detergent	Pasteurization	Steam	Dry heat	pH 4	Beta-propiolactone	Nano-filtration
Albumin		+++					
IgG IM + IV	++	++			+++	+	+
Factor VIII	+++	+	+	++			+
Factor IX	+++	+	+	+			++
vWF	+	+		+			
PCC	+		+	+		+	+
Factor VII	+		+				
Factor XI	+						+
Fibrinogen for IV use	+	+					
Fibrinogen for fibrin glue	++	+	+	+			+
Thrombin for fibrin glue	++	+		+			+
Antithrombin III	+	++					+
Alpha 1 antitrypsin		++					
C1-inhibitor	+	+					+
Protein C	+						+

+, ++, +++ indicate relative frequency of use

5.6 Conclusion

Current viral reduction treatments have advantages and limitations. Table 5.4 summarizes the main characteristics of viral inactivation and elimination treatments.

So far, there is no procedure which will ensure inactivation of all known plasma-borne viruses, HIV, HBV, HCV, HAV and parvovirus B19. It is therefore important when setting up plasma fractionation programmes to make a judicious choice with regard to the

TABLE 5.4 **Advantages and limitations of current viral reduction treatments applied to plasma products**

Treatment	Advantages	Limits
Solvent-detergent	Excellent inactivation of lipid enveloped viruses Relatively easy to implement Know-how available from New York Blood Center	Viral inactivation agents must be removed (toxic) Does not inactivate NE viruses License required; payment of royalties
Pasteurization	May inactivate most E and NE viruses	Often requires large amounts of protein stabilizers which may stabilize viruses Recovery of labile proteins may be low Poor treatment design may lead to protein alterations (neoantigens)
Steam/ pressure	May inactivate most E and NE viruses	Limited efficacy against HCV and resistant viruses Proprietary process Relatively complex Risks of protein alterations
Dry heat	May inactivate HAV No risk of downstream contamination	May not inactivate B19 Possible loss of 10% to 20% in coagulation factor activity Requires careful control of the residual moisture content
Acid pH	Good efficacy against E viruses and some NE viruses	Only applicable to IgG Long process (>22 h)
Nanofiltration on 15–35 nm membranes	Generic viral elimination process May remove resistant NE viruses and prions Mild process: low risks of protein alteration Usually at the end of production process: limit risk of downstream contamination Commercially available No patent rights	Larger pore-size membrane may not totally retain small HAV or B19 In-process validation of the filters (integrity test) is a delicate control step

E: enveloped NE: non-enveloped

viral reduction technologies which are to be adopted. Ensuring unambiguous inactivation of the major infectious plasma-borne viruses (HIV, HBV, and HCV) by implementing validated technologies, such as SD or pasteurization, is a key point. Making sure that plasma products will also attain the optimal viral safety level by the selection of an appropriate second viral reduction step is also essential. The goal is to eliminate or reduce the risks associated with less pathogenic agents (such as HAV or parvovirus B19) not inactivated during the first inactivation phase, while ensuring some degree of safety against potential future infectious agents.

An additional measure to further guarantee the quality of source plasma is the polymerase chain reaction (PCR), or nucleic acid test (NAT) of mini-pools of plasma, which is currently under implementation by plasma fractionators in Europe. Under this test regime, samples of plasma donations (mini-pool of 100 to 500 donations) intended to

BOX 5.2 **Elements influencing the viral safety of pooled plasma products**

- Establishment of a base of regular donors provides long-term clinical data on the donor population
- Careful selection eliminates donors susceptible to known (HIV, HBV, HCV, HAV) or emerging infectious agents
- Refined viral testing procedures of each donation eliminate, within the sensitivity limits of the test used, positive donations for known infectious agents
- Mini-pool PCR testing using sensitive nucleic acid tests eliminates donations positive for relevant virus
- Large plasma pool tests for absence of anti-HIV, anti-HCV, and HbsAg guarantee that virus load in the starting plasma pool is within certain limits
- Antibodies in the pool may neutralize viruses (e.g. HBV, B19, HAV)
- Application of one specific viral inactivation step (e.g. SD) must ensure complete killing of major viruses (HIV, HCV, HBV)
- Application of a second viral reduction step (e.g. nanofiltration, dry heat) eliminates other potential infectious agents
- Application of stringent manufacturing practices avoids contamination with infectious agents during processing
- Purification/fractionation techniques contribute to virus elimination
- Final product testing of IgG preparation for absence of anti-HIV, anti-HCV gives some evidence of absence of contamination of the starting plasma pool

be mixed in a production pool are controlled for the absence of nucleic acids of selected relevant plasma-borne infectious viruses. The test allows elimination of the window period donations that are ELISA-negative, or antigen-test- negative but PCR-positive. Since the beginning of 1999 all plasma products distributed in western Europe are required to be made from HCV PCR-negative plasma donations.

The major elements influencing the viral safety of pooled plasma products obtained by industrial plasma fractionation are summarized in Box 5.2.

Chapter 6

Quality assurance and good manufacturing practices in plasma fractionation

Plasma product manufacture must be carried out with great care and under very clean conditions. Plasma products are usually obtained following complex multi-step production procedures including precipitation, centrifugation, chromatographic purification, viral inactivation and elimination treatments, filtration, ultrafiltration, aseptic filling and freeze-drying. Product sterility results from clean manufacturing steps and appropriate in-process aseptic filtration steps to ensure the absence of endotoxin contamination. The manufacturing process of plasma products must fulfil the requirements of any pharmaceutical products designed for human clinical use. However, the safety of plasma products is more specifically related to the infectious risk of the starting plasma material; to the manufacturing steps involved in the preparation of the products (purification process; viral reduction steps) and to the fact that these protein products cannot withstand sterilization in the final container. Examples of good manufacturing practices especially relevant to plasma product quality and safety are listed in Table 6.1.

It is therefore important that the design of the plant takes into consideration all aspects specific to plasma products. The major production areas of the plasma fractionation facility will usually include the following:

- warehouse for the storage of the reagents used for production and control;
- plasma storage area which should be large enough to allow potential quarantine of plasma;
- the various clean room areas for cryoprecipitation, ethanol fractionation, albumin processing and formulation, IgG processing and formulation, coagulation factor purification and formulation, etc.;
- aseptic filling area and freeze-drying;
- albumin pasteurization area;
- product quarantine zone;
- product packaging and shipment zone;

- utility areas (water production, steam production, compressed air, air cooling system, air conditioning, etc.);
- quality control laboratories (possibly including an animal house).

TABLE 6.1 **Good manufacturing practice criteria for plasma products**

Measures	Purpose
Clean room environment	Limits risks of bacterial contamination during critical in-process purification and viral reduction steps
Segregated production areas	Avoid cross-contamination between products and production steps, e.g. prior to and after viral reduction
Specific viral reduction treatments	Apply on a large scale the parameters and conditions validated to ensure expected viral inactivation or removal
Segregated operations	Avoid cross-contamination between production steps
Separate air handling system	Segregates production areas especially prior to and after viral reduction steps
Buffer sterilization	Avoids contamination with endotoxins Avoids hypothetical risks of viral contamination from raw materials
Segregated flow of product, operators, waste	Avoids risks of downstream and cross-contamination
Validated equipment sterilization procedures	Avoids batch-to-batch contamination from reusable equipment
Traceability/documentation	Allows identification of all constituents (plasma donations and raw materials) and ingredients used during each product batch

Chapter 7
Economic and organizational aspects

7.1 Approaches to implementation

One crucial criterion when considering the establishment of a plasma fractionation project is its economic viability. There are two approaches to the implementation of a plasma fractionation programme and these may be complementary: contract fractionation performed with an established plasma fractionator and/or operation of a local plasma fractionation facility.

7.2 Contract fractionation

If a contract fractionation project is considered, the selling (reimbursement) price of the plasma products should cover:

- the collection cost or purchasing price of plasma;
- the test procedures carried out on single donations;
- the cost for mini-pool PCR testing which may be included in the overall fractionation charge;
- storage of plasma under appropriate conditions;
- transportation (usually by air or ship) of plasma to the fractionation plant;
- the processing charge per unit of product (e.g. per gram of albumin and IgG, and per international unit of F VIII and F IX), or per litre of plasma fractionated with a minimum guaranteed yield;
- shipment of the products to the country of origin;
- distribution costs;
- clinical and marketing/sales expenses;
- miscellaneous expenses;
- administration and overhead charges.

Usually, the price of plasma and processing fees represent the two single major components of the overall cost of end-products. The cost of distribution and marketing depends upon the organization in question (e.g. whether or not the blood transfusion network is involved in distribution).

Additional criteria in selecting a contract plasma fractionator are listed in Box 7.1.

BOX 7.1 **Criteria to consider in the selection of a contract plasma fractionator**

- Plasma and product batch size at the contract fractionation facility is amenable to the low plasma volume that may be available from the country of origin
- Guarantees should be provided that plasma sent for contract fractionation will be processed in separate batches and will not be mixed with plasma from other sources which may have different quality criteria
- Products made have established records of safety and efficacy
- Recovery of products is acceptable
- Contract fractionation cost is acceptable
- All products made will be returned to the country of origin of the plasma, unless some alternative agreement is made
- The contract fractionator is the owner of the production technologies or is free to use these technologies to perform contract fractionation
- Foreign plasma can be imported into the country of the contract fractionator, provided that the quality requirements set by the health authorities can be met
- Excess products can be distributed/provided outside of the country of origin of the plasma
- If combined with a phased plant construction, transfer of technology can be provided including the training of local operators, on acceptable financial terms

7.3 Local plasma fractionation plant

When a facility is established and local manufacture is implemented, the other major parameters influencing the manufacturing cost, in addition to cost of the starting plasma material, are:

- the batch size of starting plasma pool and of end-products;
- the yield achieved for each individual products;
- the number of products made from plasma.

The degree of complexity of the processing steps (e.g. freeze-drying is a relatively expensive component of processing costs) as well as the level of royalties to be paid for the imported technologies (for viral inactivation and/or purification technology) are additional parameters in the evaluation of the economical feasibility of the project.

The analysis of the economical feasibility of the project must take on board the overall cost of the construction of the plant and the depreciation of the equipment (average

amortization time is 5 to 10 years) . The average cost of a state-of-the-art GMP facility to fractionate the five major plasma derivatives from 100 000 L of plasma can be estimated to be between US$ 20 million and 35 million (cost for building and equipment) depending upon the technologies selected, the degree of automation of the equipment and the potential for creating common production areas, e.g. for the coagulation factor concentrates (which would decrease the production room area required).

Criteria to consider prior to initiating construction of a plasma fractionation plant include those listed in Box 7.2.

BOX 7.2 **Criteria to consider in the establishment of a local plasma fractionation plant**

- Supply of high-quality plasma for fractionation is ensured at an annual capacity of at least 50 000 to 75 000 L
- Technologies acquired allow the manufacture of products with an established record of safety and efficacy. Similar products are registered in at least one recognized country in the world
- Yield of products is acceptable
- Range of products made initially include at least four or five products including albumin, intravenous IgG, factor VIII, factor IX, or prothrombin complex
- Additional plasma products will be made in a second phase, e.g. hyperimmune IgG, antithrombin III, fibrinogen, fibrin glue, etc.
- Technology transfer contract leaves the potential to export excess products if required
- There is political/public support, as well as support from the medical community for the establishment of a local facility
- There is a strong local regulatory body, well-informed about the specificity of blood products
- There is enough qualified personnel to ensure professional management of the facility and to start research and pilot-scale product development
- There are university or research institutions available for scientific studies on plasma products
- Maintenance of equipment can be ensured when the plant starts operating
- The environmental conditions are favourable (water supply, air quality, temperature)
- Long-term assistance from the provider of technology and consultation with groups experienced in plasma fractionation, is available

7.4 Practical issues which must be considered in planning a local plant

7.4.1 Feasibility study

Making a feasibility study is an essential part of the planning process, because it allows for committing mistakes on paper before they are irretrievably made on a more permanent basis after the planning phase is activated.

The study should review and confirm the plasma supply situation, including all extrapolations which may be required. It should also review the need for plasma fractions and reconcile differences between these and plasma availability. This may require the setting out of a programme of priorities, matching supply of plasma of appropriate quality with the clinical need. Thus, the brief description of the project would be able to define accurately the work-load for the plant, both in terms of raw material and the expected final products. This is important because the scale of all parts of the plant will depend upon this information.

The study should address the technology required and its availability, as well as the availability of staff skilled, at least, in pharmaceutical practice. The ideal is that such staff have experience of production biologics, but most important of all is a basic understanding and acceptance of the concepts underlying Good Manufacturing Practice (GMP).

7.4.2 Schedule of accommodation

This may be included as part of the feasibility study, or may be a separate stage of the project development. However, as the study has defined the extent of the work for the project there may be merit in combining the two activities. The design of the schedule of accommodation has a major influence on the architectural design of the finished fractionation plant. Thus, at this vital stage it is necessary that due care is given to defining surface finishes, air movement barriers, product movement tracks, lines for supply of raw materials used in process and staff movements, so that the building is conducive to incorporation of GMP from the start.

It is true that GMP can be installed in any plant at any time, but it will be found to be safest if the building is designed in a manner which makes it easy for staff to adopt the discipline and concepts of GMP as part of the standard operating procedures documents.

Broadly, the schedule should define four main areas:

- the process area
- the services area
- the laboratory area
- staff amenities and administration areas.

The first two areas, in particular, deserve some definition.

The process area

This area may be difficult to delineate in some cases, because it will depend upon the constraints of the chosen site. Nevertheless, the key provisions can be defined.

a) Plasma reception and storage areas

These areas should include a space where vehicles can be accepted under cover and free from external atmosphere, so that by virtue of an airlock the vehicle can be unloaded without contamination of the building as a whole. There must be an area set aside for documentation of the arriving material, checking of the assay status and quality definition. These should lead to cold-rooms in which the plasma can be quarantined for validation before release to the main storage area. Normally, plasma should be stored at minus 35 ± 5 °C. Such areas are difficult to manage but the technology is well understood. However, specialist care is needed during design and construction. Faults in construction of plasma storage areas are major contributors to unsuccessful ventures.

b) Fractionation areas

These areas will vary, depending upon the technology chosen but they need to contain some form of clear physical barrier to define and separate materials in-process, which may be capable of viral transfer, from those that have been rendered safe by inactivation. Equipment, staff and supply lines serving on each side of the barrier should be defined and separated with strict rigour. Care is needed in the choice of technology, since extensive cold rooms as fractionation halls are expensive and not well suited to tropical or sub-tropical climates.

c) Equipment wash and preparation areas

Entirely separate arrangements should be provided for maintenance and servicing of equipment on both sides of the safety barrier, so that cross-contamination is not possible.

d) Dispensing and product quarantine areas

These areas require special consideration because of the need for tight environment control. Ideally, there should be separate areas for cleaning and preparation of equipment and containers, so that contamination from outside the plant cannot transgress into fractionation areas and there is no possibility of transfusion-transmitted infection transfer to final dispensed products from earlier in-process stages of fractionation. This should mean that there is a double barrier between potentially contaminated raw material (plasma) and the final dispensed products.

e) Packing areas

These areas tend to be dusty, often contaminated by moulds and fungi. They should therefore be separated from all other areas so far as ventilation is concerned.

f) Dispatch areas

These areas should be separate from raw material reception areas but should be treated in a similar fashion, so that the contamination of the working environment by external air is minimized.

The service area

The activities in this area tend to be dirty and noisy, so it is advisable that barriers between this section and the main plant area are well maintained. The services required by a fractionation plant are quite demanding, requiring high levels of maintenance. In consequence, they should ideally be operated on a planned preventive maintenance schedule, so that one can be confident that when any production process is initiated all services are in a validated state. Although initially very expensive, there is great merit in arranging that each service is divided between three units, each capable of supplying 50% of the actual requirement. This means that any two units can provide for the needs of the plant, while one is held in reserve for repair, maintenance, or stand-by function. The main services required are as follows.

a) Water purification

There is much truth in the observation that a factory which cannot produce reliable water will be unsuccessful in the fractionation of plasma. The quality of the water brought to the plant is of major importance and should be monitored regularly. Town water is normally treated for bacterial contamination by chlorination, but this may be inadequate, particularly if the water is delivered in steel pipelines, since steel removes free chlorine and the water may be recontaminated and pyrogenic. Water extracted from deep artesian systems may cause fewer difficulties but the problem of increasing contamination of deep aquifers, particularly in urban or intensively farmed areas requires that such water sources are treated with special vigilance.

The validation of water of varying purity is a major aspect of the quality assurance activity in a fractionation plant. Indeed, many such plants now use water which has been purified, at least in part, after arrival on site. There are many well documented systems for processing of pharmaceutical water, such as demineralized water, pyrogen-free water and water for injection. Regulatory requirements in some countries insist that water for injection must be produced by distillation, but this is becoming more unusual.

It is recommended that pipelines carrying purified water, or water for injection, should be constructed of stainless steel, with all joints welded by orbital welding. However, there

is a need for realism since this technology is not available in many countries, so the lines may be jointed. This means that particular care is required in selecting the type of joints and their installation and testing, to avoid the danger of contamination.

In some situations, where water costs are high, waste water (e.g. water discarded from reverse osmosis systems) can be used for domestic purposes, so that the more valuable purified water may be used more generally in the plant environment. It may seem wasteful and expensive to wash floors with demineralized water but the advantage is in validation and predictability of the results.

Where water is scarce, it may be necessary to consider recovery from waste water. Much of the water discarded from a plant was originally demineralized but was then contaminated with protein and detergent. Such water can be recovered and used again, if a standard waste water treatment unit is combined with systems for final "polishing" to domestic use standards. In any event, waste water from a fractionation plant should be handled carefully since the combination of traces of protein and alcohol produces an effluent with a high biological oxygen demand and may be destructive of water courses, unless treated before discharge.

b) Steam generation

Steam may be used for many purposes, but these can be divided into two categories: general domestic use and process use. For the latter, it is normal to use clean steam. This is usually generated from pyrogen-free demineralized water and can be provided by a central supply with piped distribution to the point of use. However, development and installation of such a distribution system is costly and it is more usual for the steam to be generated close to the point of use, with the water delivered to the generator by a ring main, constructed of stainless steel. There are many standard generators of this type available, which can use electricity, domestic steam or gas as the source of energy. Most can be sited within the clean work area and some may be sited within clean room suites, although this should be avoided, if possible, as it complicates maintenance.

c) Ventilation

It is now unusual for any part of a fractionation plant to be ventilated naturally and barriers are required to ensure that unfiltered air is kept out of the building. This means that entries for staff, delivery of raw materials, or dispatch of products, should be air-locked so that external air is removed and replaced with clean, filtered air of appropriate quality. Within the work areas sensitive activities, where product contamination could occur, are provided with air of a quality suited to the activity. In most cases this will be air bearing the ASTMS Class 100 quality grade, or an equivalent national grade. The areas of different air quality should be operated at different pressures, so that the cleaner areas are at higher pressures. Careful attention is required to achieve a balance between all areas of the building and the discipline of door-handling needs to be defined closely. Much of

the final GMP success will be dependent upon the design of air handling at the planning stage.

It is a frequent mistake that the ducts and channels bearing air are concealed within the fabric of the building where access is difficult, if not impossible. Maintenance of air handling equipment and validation of duct-work is a major aspect of engineering services, so comfortable access to all parts of the system is necessary. In some cases, this may require the insertion of a complete interstitial floor in the building structure, to provide good horizontal and vertical access to the entire system. The same facility, of course, may be planned to give access to all other services. It should be well lit and equipped for the general working comfort of engineers, who may have to spend many hours in such places. It should be considered also within the ventilated "envelope" of the plant and be easily cleaned to reduce dust or insect burden.

A further common problem is encountered with the ducts themselves. In normal domestic ventilation the ducts are sealed only in a nominal manner, but a pharmaceutical plant requires that the ducts are sealed so as to withstand pressure testing, leaving no gaps through which unvalidated air can be aspirated.

d) Refrigeration

There is a massive need for refrigeration in plasma fractionation: for maintenance of reaction temperatures; for procedures such as centrifugation or lyophilization and for storage of raw material and materials in process. These needs can all be supplied by direct primary refrigeration, but capital costs then become excessive and operating costs can be prohibitive. In some cases, such as lyophilization, the simplest installation is a complete console unit, with integral refrigeration, which may be air-cooled or water-cooled depending mostly upon size.

Secondary systems work by the cooling of large volumes of an aqueous solution. This may be a solution of ethylene glycol, or of methyl or ethyl alcohol. The alcohols are more awkward to manage, but when chilled the liquid is much less viscous and requires less energy for pumping. A major advantage of the use of secondary cooling is that the system can be chilled over a period, so that there is capacity to spare when sudden demands are placed for major heat transfer. Thus smaller equipment, working over longer periods, can have the capacity for instant availability. This reduces both capital and operating costs.

For air-conditioning systems the use of secondary cooling permits reserves to be built up during the cooler times of day, so that there is no heavy power drain at the peak time of solar gain. In some climates, the secondary coolant of choice for air-conditioning is chilled water. This has the advantage that a cold bank of ice can make the system more efficient.

e) Central clean-in-place systems

The value of a clean-in-place (CIP) system, especially for large vessels and other fixed equipment, is that the system can be validated and shown to be safe. It therefore brings considerable uniformity to the problem of equipment cleaning. It always requires strict validation to be certain that it remains in control, but this also can be reduced to a routine. There are several mobile systems available from suppliers but these are less attractive, because they should be re-validated each time new connections are made, to be entirely safe.

By creating a central system it is also possible to have the main equipment in a service area outside the clean work areas, so that the manipulations associated with preparing reagents for the cleaning cycle can be carried out in a less vulnerable zone.

f) Alcohol storage and reclamation

The ethyl alcohol preferred for organic solvent fractionation systems is synthetic spirit, derived from the petrochemical industry, and which has been twice rectified. Such material is more uniform, with less lot-to-lot variation than, for example, grain or sugar cane alcohol. It is best to arrange for storage in a securely enclosed tank storage area, at least 30 m from any building, with strictly controlled access. This is for safety against ignition. If it is decided that it will be economical to reclaim waste spirit (usually possible at the 1000 tonnes per year level), then the recovery plant can be sited in the same area for similar safety reasons. Standard recovery systems are available, but most of these depend, at the terminal stage, on a distillation step to bring the purity level to 96%, or thereabouts.

g) Emergency power generation

All fractionation plants will be rendered vulnerable from time to time, due to failures of the public electricity supply. It is necessary to examine the plant to determine which of the services are essential and must be kept functional. A power generator with the capacity to meet this need should then be installed. It should have a fast start procedure so that loss of power does not cause failure of certain vital systems. Lyophilization equipment is especially vulnerable, as are the ventilation systems in clean areas. Normally it will be found that a generator capable of supplying around 40% of the full power needs of the plant will be sufficient, but in areas where power failures are frequent or prolonged it may be prudent to provide emergency generation up to 100% of need. Routines should be established for ensuring safety of product-in-process, in the event of failure and for bringing it to a safe stage where storage can be effected.

Where an emergency generator plant is installed there should be a routine for checking the functionality of the equipment by regular switch-over, to test that the system starts quickly and smoothly. In colder climates this may mean keeping the lubricating oil of a diesel engine warm at all times.

Part 2

The blood transfusion service: Implications for plasma fractionation

Chapter 8
Selection of safe blood and plasma donors

8.1 General

One of the fundamental prerequisites for providing a safe and adequate supply of plasma for fractionation for direct clinical use, together with sufficient labile blood components, such as red cells, platelets and clinical frozen fresh plasma is an established, voluntary blood donor base, carefully selected from the low-risk segments of society.

A growing number of viral infectious agents are known to be transmitted to recipients by transfusion: hepatitis B and C, HIV 1 and 2, human T-cell lymphotropic viruses type I and II (HTLVI/II). Further transmissible agents posing a lesser, albeit significant threat, are CMV, human parvovirus B19, HAV, human herpes virus type 6 and so-called transfusion-transmitted virus (TTV). Other emerging, or newly identified infectious agents, such as hepatitis G virus (HGV) and human herpes virus type 8 (HHV-8, associated with Kaposi's sarcoma in Senegal and the Central African Republic) may also prove to be significant transmissible viruses in the future. In addition, various Gram-negative (*Yersinia enterocolitica* and pseudomonads) and Gram-positive bacteria can infect blood components, more particularly platelet concentrates stored at room temperature, in the case of the latter. Plasmodia, such as malaria and trypanosomes (Chagas disease), may also be passed on by the transfusion of labile blood products. As a consequence of increasing population mobility and travel, risk of contracting these infections is no longer confined to endemic regions. Finally, the theoretical risks of transmitting prion disease by plasma fractions derived from pooled plasma, leading to new variant Creutzfeldt-Jakob Disease (nvCJD) among recipients, is currently causing a great deal of concern, even though there have not yet been any reports of such transmission. Figure 8.1 shows some of the comparative risks of transfusion.

Various strategies are applied by well developed transfusion centres to improve the safety of the blood supply.

- One of the key elements fostering safety is the application to all blood donations of ever more sophisticated and sensitive screening assays for the major known microbial agents. Molecular testing assays are on the horizon and where the funding and expertise are available they will undoubtedly reduce "window-period" donations even further.

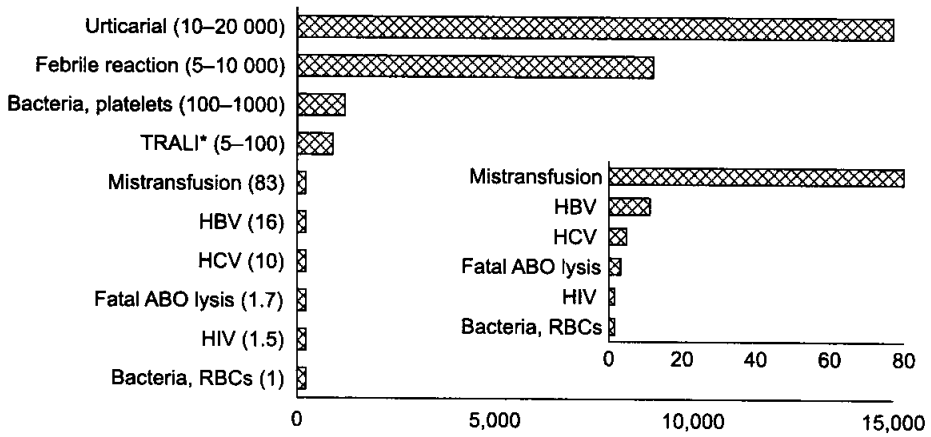


FIGURE 8.1 **Comparative risks of transfusion**

However, as testing with microbial assays approaches the limits of sensitivity, increasing expenditure will inevitably bring diminishing returns and increasing complexity, which may be counter-productive. For instance, the introduction of HIV p24 antigen testing costs the USA over US$ 24 million per year, but only four p24 positive, HIV-antibody negative donations have been detected in the 2 years since this policy was initiated. Needless to say, adopting test strategies such as this does not improve the safety of plasma products subjected to viral inactivation procedures.

- An important step in ensuring the safety of plasma fractions has been the introduction of viral inactivation procedures. Similarly, physical and photo-chemical techniques for inactivation of viruses and bacteria will soon also be applied to labile cellular blood components.
- A sound infrastructural quality assurance/GMP system, which must also include good organization and management information, is also essential in providing documentary evidence that all specified operational standards throughout the production process, from donor to recipient, have been adhered to.
- Another element of importance is a well developed transfusion medicine system capable of influencing clinical blood utilization, so that the profligate usage of the past is replaced by a more rational, evidence-based approach to the transfusion of blood products. The risks (and costs) entailed are always balanced against the potential benefits of clinical transfusion practice.
- Among all these concurrent policies, however, the cornerstone of a safe blood supply, and almost certainly the most cost-effective strategy, involves the exclusion of high-risk blood donors by the targeted recruitment of volunteers with well defined

behavioural, demographic and geographic characteristics, together with the deliberate application of donor selection criteria, employing direct, face-to-face verbal interviewing techniques. Risk occurs when the incidence of transmissible viral infection in the population is high. Since not even the most sensitive viral screening assays are capable of excluding all such early, "window-period" donations the focus of efforts must be shifted to the key role of donor selection in preventing the entry of these undetectable, yet potentially dangerous donations into the blood supply.

There is ample evidence from studies in both developed, but more particularly in developing economies, to show that voluntary, nonremunerated blood donors are significantly less likely to carry markers for viral infection, than paid donors. This is also true when comparing repeat, voluntary donors, with so-called "family replacement", or directed donors. For instance, in Greece, India, Singapore, and Zimbabwe, "replacement" donors have been found to have an approximately three-fold greater risk of carrying disease markers, than non-remunerated donors. In addition, first-time voluntary donors are between two and three times more likely to show evidence of past, or active viral infection, than repeat, or established blood donors. Therefore while a successful programme starts with the identification and recruitment of donors with characteristics which class them in the category of greater safety, a major and parallel programme of donor retention is absolutely essential. Established, voluntary donors, with a well characterized history, viral serology profile and incidentally, a well defined red-cell, platelet and white-cell antigenic make-up, are the safest category of donors, whose retention must be ensured by every possible means.

8.2 Defining characteristics of voluntary, nonremunerated donors

There is a good deal of confusion surrounding the term "voluntary nonremunerated", and a number of European countries which ostensibly subscribe to the principles of voluntarism do reimburse donors for their "expenses", which is in reality a covert form of remuneration.

Voluntary donors are those who volunteer on their own initiative, when asked to give blood by the transfusion service, by a colleague, friend or relative. Individuals who should not be considered to be volunteers are:

- prisoners, or those who cannot comprehend what they are agreeing to;
- those who may be coerced into giving blood because they are put under emotional pressure as close relatives of the patient;
- soldiers who are ordered to "volunteer";
- youths who must give blood to obtain a driver's license, or register for a university degree;

- all those who are rewarded by means of incentives which are exchangeable for cash, such as lottery tickets, discounts on merchandise and rebates on mortgages or taxes.

Even minor inducements may strongly motivate certain individuals, causing them to conceal information about a risk activity or history.

8.3 Selection of safe donors

The selection of safe donors starts with the targeted recruitment of donors from the safer sections of society. The organizational efforts expended in the recruitment of volunteers need to be specifically aimed at those demographic, geographic and behavioural segments of society which have been shown to be safer, more reliable and more likely to become established, repeat donors. This requires the implementation of sentinel, or point-prevalence studies for disease markers in various geographical areas of the country, among different age-groups, occupations and sexes, in order to delineate the profile of the safer sectors of the population. For instance, studies in southern Africa have shown that secondary school students have the lowest prevalence and incidence of HIV infection. Prevalence is only 1%, while it is 27.8% in those who are aged between 26 and 30 years. Indeed, up to the age of 22 years, seroprevalence was found to be below 6%, whereas, over the age of 22.5 years, it was 21%. It was also found that disease markers were highest among long-distance drivers, in high-density urban populations and among migrant workers who lived away from their families. Similarly, a recent study in the USA found age, sex, education and ethnic origin to be relevant in seroprevalence and incidence of disease markers. Naturally, caution must be exercised in applying any of these criteria to populations elsewhere and it is essential that they are based upon a knowledge of local epidemiology.

8.4 Public education and information

Public education is an important means of motivating blood donors and for excluding those who may be at increased risk for "window-period" infections. This is particularly true of HIV infections of course, whereas in the case of hepatitis C carriers over 40% may not have any obvious history of exposure to risks, such as intravenous drug abuse, previous transfusion, skin-piercing, or other known factors associated with HCV infection.

The diffusion of a continuous background of information through the media is a valuable means of increasing public awareness of the need for blood, as well as the risks of disease transmission to potential blood recipients. Needless to say, "test-seeking" behaviour, where individuals who are aware that they have put themselves at risk of transfusion transmissible infections donate blood only in order to find out about their viral

status, must be avoided by ensuring that easily accessible alternative test sites are available, that they are known to the public and receptive to requests for testing.

Although public education must be aimed at the entire population, young people—the donors of the future—should be the focus of particular educational and motivational effort. The young are the most gratuitously generous and enthusiastic segment of society, with an idealistic urge to contribute to the improvement of the community and they are therefore ideal partners in blood collection programmes, often playing a leadership role in organizing recruitment drives among their peers, in schools and universities.

The support and participation of prominent people, such as leading politicians, film actors or pop stars and sporting heroes, can do a great deal to raise the profile of the transfusion service and emphasize the need for everyone to contribute to their community. The spiritual dimension of giving for the benefit of others must also be remembered and respected religious leaders must also be drawn into supporting recruitment programmes, or even better, actually donating blood themselves.

Naturally, public appeals for more donations must be used sparingly, for their repeated use through the public media can be counter-productive, demonstrating weakness in what should be a steady, year-round organization of the donor motivation and recruitment programme. Although helpful, publicity programmes can only reinforce the effects of a properly organized recruitment process. Newspaper announcements, posters and audio-visual media campaigns by themselves do not recruit donors.

The crucial importance of retaining known blood donors to ensure the safety of the blood supply has already been mentioned and an active programme, and adequate staff devoted to this aspect of recruitment, is essential. There is some evidence that attitudes to blood donation and motives for giving blood are different for first-time donors in comparison with repeat or established donors. Therefore, each category of donors deserves a different approach. A recent European survey found that only 32% of first-time donors return to give blood, while nearly 60% of repeat donors do so within the year.

Inhibitory factors include fear of the process and particularly frustration due to the inconvenience of waiting at busy sessions; anxiety about physical reactions to the process, such as fainting, pain, feeling "weak" after giving blood; ill-health; advancing age; and fear of contracting viral infection as a result of giving blood. Inadequate follow-up by the blood transfusion service (BTS) indicating the continued need for blood and how much each individual donor is valued, are also among the factors causing established donors to lapse.

Not all of these factors (such as the ageing population European services must deal with) can be countered by even the best organized BTS. Nevertheless, the basic principles a successful donor retention programme must embrace are:

- an adequate donor recruitment and blood collection staff which is carefully selected for qualities such as good communication skills and an out-going personality;
- a rigorous, continuing training programme for recruitment and collection staff to improve their understanding of blood and blood transfusion and the importance of "customer satisfaction";
- attention to making the donation experience as pleasant, quick and efficient as possible;
- ensuring that the current searching, and often very personal, questions put to donors every time they volunteer do not alienate them and are tactfully phrased;
- provision of sufficient privacy during verbal screening interviews carried out prior to donation;
- reliable, up-to-date and accurate records maintained in a secure, confidential form, whether they are computerized or manual. No blood service can afford to lose track of its regular donors. In addition, good records provide the opportunity for statistical analysis, early detection of trends, future planning, linking viral screening results with demographic and geographic donor profiles, identification of lapsed donors for special attention and exclusion of "black-listed" donors who seek to donate again.

8.5 Exclusion of unsuitable donors

Blood donation must be safe for both donor and recipient. The blood service must ensure that donors are in good health to minimize the possibility that donation may cause adverse effects.

Examples of factors which should lead to exclusion because a donor may be harmed, include a history of cardiovascular disease, pregnancy, epileptic seizures, anaemia or recent surgery. Donors must be large enough to part with a full 450 mL donation and they must be neither too young nor too old. Latterly, a number of transfusion services have adopted more flexible age limits, which take into account the greatly improved health and life-expectancy of well nourished populations in Europe and the USA, as well as the earlier maturation of young people. In consequence, many countries have delayed retirement from the donor panel from the age of 60 or 65 to 70, provided that the donor is certified to be in good health. Similarly, some countries have set a minimum age for starting donation below the conventional age of 18, at 17, or even 16 years of age. Individual countries must naturally determine the appropriate age cut-off locally.

Infectious agents which may harm potential blood recipients have already been discussed, including viruses, spirochetes, plasmodia and bacteria. Other conditions which should lead to donor exclusion are diseases of uncertain origin which may possibly pose a risk to recipients, such as malignant disease, and chronic immune-mediated disorders (rheumatoid arthritis, Crohn disease, sarcoidosis, and systemic lupus). Close questioning, if properly conducted, should elicit behavioural factors which may cause risks and should

lead to exclusion: male homosexual activity; males or females who have consorted with prostitutes, as well as prostitutes of either sex; males or females given to indiscriminate sexual activity; users of intravenous drugs of addiction who may share injection equipment; frequently transfused individuals; and the sexual partners of all these categories. In addition, individuals with a history of accidental (needle-stick injury), or deliberate, unregulated (acupuncture, tattoos) skin-piercing, are unsuitable donors who must be excluded. Some of the donor selection criteria employed in the USA and the United Kingdom are compared in Table 8.1.

TABLE 8.1 **Differences in donor selection**

Variable	United Kingdom	USA
Age	18–65 years Up to 70 years with medical certificate	At least 17 years Written consent for <17years acceptable
Frequency	Usually twice a year, however a minimum 16 week interval is acceptable, with an absolute minimum of 12 weeks	No more than 8 week intervals
Volume	13% of blood volume if >50 kg Usually 450 mL	525 mL if >50 kg
Haemoglobin	13.5 g males/12.5 females	12.5 g for all
Blood pressure	Defer if diastolic >100 mm	Not >180/100
Pregnancy	1 year post-delivery	6 weeks post-delivery
Aspirin	4 days	3 days
Vaccination		
Killed and toxoid	48 hours	Immediate
Live virus	21 days	2 weeks (rubella 4 weeks)
Hepatitis B	Accept 1 year after recovery if HbsAg and anti-HBc negative	Do not accept if hepatitis was after 11th birthday or HbsAg +/anti-HBc +
Defer for 1 year	Tattoo	Tattoo
	Acupuncture	Mucous membrane exposed to blood
	Electrolysis	Non-sterile skin piercing with contaminated instruments
	Blood transfusion	Close hepatitis contact
Malaria		
Previous history	Accept if well after 6 months and ELISA test negative	Plasma only for 3 years
Prophylaxis	Immaterial	Plasma for 3 years
Travel to malarial area	Accept if ELISA negative	6 months if afebrile and not on prophylaxis

Clearly, a distinction must be made between those who are permanently disqualified, for whatever reason, and individuals with a temporary disqualifying condition, who may be encouraged to return to regular donation after a suitable interval. In all these cases, it is essential to inform donors of the reasons for their temporary or permanent exclusion, in a sympathetic manner, and, if necessary, to refer them for further investigation and advice to an appropriate specialist centre. Although this may be an onerous task, it will ensure that the reputation of the blood transfusion service as a caring organization with integrity will be enhanced.

8.6 Organizational infrastructure

The error-free organization of all these functions presupposes the existence of a first-class infrastructure with a coordinated national blood programme, a well documented national donor database and excellent communications.

There are a number of organizational models for transfusion services which have proved to be successful in various parts of the world and there can be no single model which is universally valid in every instance, or in every country. It must nevertheless be emphasized that whether blood services are organized on a national or provincial basis, whether they are run by a non-profit or profit-making agency, by the military, or by the Red Cross/Red Crescent Society, or through an entirely decentralized, hospital-based system, the need for some form of central coordination is a paramount consideration, if the following core functions are to be effectively managed.

- long-term planning, financing, and organizational accountability;
- establishment of uniform regulatory and quality standards, together with a system of inspection or audit, ensuring that these standards are adhered to;
- delivery of a coherent message to the potential blood donor public from a single source;
- recruitment and retention of the safest available donors and the documentation required to organize this activity successfully;
- coordination of blood component availability;
- coordination of the policy for providing plasma for fractionation, whether a national fractionation capability exists or not;
- coordination of plasma fraction availability;
- training of scientific, technical, and other personnel.

8.7 Key performance indicators for donor selection and blood collection

Establishing accurate indices of performance is an essential part of effective quality systems. This is most particularly relevant to the recruitment, selection and retention of blood donors. Key performance factors may be divided into three broad categories: those which relate to organization and management functions; indices of successful donor selection functions; and measures of operational functions.

a) Organization and management perspective

The following criteria should be satisfied.

- Sufficient donor blood to meet the needs, by blood-group specificity. This requires accurate estimates of hospital usage, a panel of voluntary, established, low-risk donors large enough to meet seasonal variation in demand, as well as emergencies or disasters.
- Minimal outdating of blood of all blood group specificities. This requires that stocks are appropriate to cater for both day-to-day needs, as well as unexpected demand without undue excess. Relatively less used blood groups, such as A+ or AB+, are not outdated in excess. Donor call-up should not be indiscriminate.
- Good donor retention which allows the new donor requirement to be no more than 10% to 15%.
- The age profile and sex ratio of the established donor panel is well balanced.
- Donor complaints are rare.
- Donor adverse "incidents" are uncommon (fainting, haematomas, arterial punctures, nerve injuries, etc.).

b) Donor selection perspective

The following criteria should be satisfied.

- Few viral seropositive donations are collected (against the known background of prevalence and incidence in the population).
- Few donors have been bled who were ineligible (e.g. anaemic, sick, history of drug abuse).
- Few donors were rejected for trivial reasons. This requirement involves the standardization of selection criteria and good training of doctors, nurses, and donor attendants in blood collection teams.
- Haemoglobin testing is well standardized to avoid false negatives and false positives.

c) Operational perspective

The following criteria should be satisfied.

- Bacterial contamination of platelets and red cells is very rare. This requirement involves a well validated skin cleansing protocol, and a good technique.
- Errors in blood bag labelling are very rare.
- The temperature profile of blood units at the collection session and during transport back to the blood centre was appropriate.
- Burst or leaking blood bags are low in number. This requirement involves selective blood bag purchasing; good bag storage and handling.
- Staff needle-stick injuries are rare.
- The BTS blood issue department can cater for unusual combinations of red cell antigens. This requirement involves a well established, highly characterized blood donor panel and rapid access to the donor database.

Finally, how can these levels of performance be achieved? Through:

- careful staff selection;
- good staff induction, training, and continuing education;
- good staff morale and motivation, pride in their work and a sense of participation;
- achieving a good public image and a reputation for integrity and high standards.

Chapter 9
Quality management in the blood transfusion services

9.1 Background

There was a time when it was acceptable for blood programmes to be based upon a quasi-charitable health service model and the safety and quality of these life-saving services were largely taken for granted. While this conceptual basis remained appropriate for many years, the imperatives and expectations of the community today demand the application of standards which have been practised in the pharmaceutical industry for several decades. Thus, even though transfusion is still a public service it is increasingly regarded as the manufacturer of a range of industrial products requiring stringent levels of quality, accountability, and transparency. The shift in culture and thinking which this change demands of transfusion services is difficult to encompass but it must take place in order to maintain their credibility and retain the confidence of the public they serve.

When the concepts of quality were first applied to industry in the early 1930s, they involved the separation of manufacture and control and the centralization of quality control(QC), which was seen as a monopoly of the management. QC was originally confined to end-product testing, in an analytical and retrospective manner, where deficiencies were remedied by a curative approach.

Quality systems have progressively evolved over the intervening decades. Today, auto-control is exercised by all those involved in manufacture and responsibility for quality is decentralized, so that everyone must participate in its assurance. Above all, the concept of quality assurance has been introduced—a preventive, prospective approach which ensures conformity with pre-established product (or service) specifications. In other words, process validation and process control and all the conceivable elements which may affect the nature of finished products have, ideally, been so meticulously controlled that quality can be predicted with confidence.

Thus, achieving quality is not solely dependent upon good retrospective quality control of the manufactured product. A quality programme must embrace every aspect of the process, from dissemination of public information about blood donation and the selection of blood donors, to the processing, screening and delivery of blood which is predictably as safe as possible, all the way to the patient's bedside. Quality assurance

cannot be guaranteed, of course, by merely following the principles of current good manufacturing practices (cGMP), which define the minimum standards adhered to in the production of blood components and plasma. Comprehensive or total quality systems take in activities which go far beyond those involved in the physical "hands-on" aspects of manufacture, as GMPs do. They must comprise a culture of quality and continuous improvement influencing: organization and management, blood bank financial administration, training, occupational health and safety of donors and personnel alike, specialized reference testing, equipment calibration and maintenance, supplier quality criteria, statistical process control and quality metrics, building design and maintenance, documentation and labelling, dealing with errors and accidents, as well as product recall and destruction procedures. Finally, audit, accreditation systems and haemovigilance, extending into the hospital and allowing for complete traceability from the patient back to the donor and vice versa, all form a part of the core quality systems required. The process of quality assurance cannot be allowed to end with the delivery of blood products to hospital blood banks or surgical theatres. The practice and monitoring of appropriate clinical transfusion medicine at the bedside must also be incorporated into modern quality systems, to complete the loop joining blood donor to blood recipient.

The attainment of these aims requires the commitment and leadership of the highest echelons of management, and the active participation of transfusion service staff in a process of continuous change.

9.2 Definitions

9.2.1 General

The terminology employed when quality is discussed is often confusing and ambiguous. It may therefore be worthwhile clarifying what is intended when the principles of quality assurance, good manufacturing practice and quality control, which are applicable to all activities of blood services, are mentioned. Fortunately, there has been considerable international coordination and agreement regarding the defining characteristics of the core elements which make up modern quality systems (most commonly based upon the matrix given in the ISO 9000 series of standards published by the International Organization for Standardization (ISO)). This can clearly be seen in the convergence of the rules and guidance provided by agencies such as WHO, the International Federation of Red Cross and Red Crescent Societies, the European Commission and the American Association of Blood Banks.

9.2.2 Quality management

It is management's responsibility to agree a quality policy for the blood centre which will be made known to all members of staff. In the implementation of this policy, a quality system should be drawn up to define a comprehensively designed system of quality assurance, incorporating GMP and quality control. All elements of this quality system must be recorded in a quality manual, which will set out interrelationships between the elements of the system, including computer systems. In the particular application of quality management to blood services, the "vein-to-vein" concept must be followed, such that the quality system covers all activities from recruitment of donors to the transfusion of components and follow-up of its clinical consequences (haemovigilance). The basic concepts of QA, GMP and QC are inevitably interrelated.

9.2.3 Quality assurance

QA is a wide-ranging concept covering all activities and materials which may affect the quality of the end-product. It is in fact the sum total of all systems designed to ensure that the end-product meets its specifications and thus incorporates GMP. The system of QA ensures that:

- the responsibilities of all personnel are clearly defined;
- quality principles are applied to recruitment and selection of donors, as well as to the supply of other starting material;
- the principles of GMP are applied to all production and control operations;
- all necessary validation and in-process controls are carried out;
- the finished component is subjected to defined processes and checked before release;
- storage, distribution and subsequent handling of components is such that quality is maintained throughout their shelf-life;
- a system of internal quality audit is in place which will regularly check the effectiveness of the QA system.

9.2.4 Good manufacturing practice

GMP is that part of QA which ensures that blood components are produced and controlled in a consistent manner, meeting the appropriate quality standards. GMP covers all activities concerned with the production of safe and effective blood components and their QC. It ensures provision for:

- appropriately qualified and trained personnel;
- suitable premises and space;
- adequate equipment and services;
- approved procedures and instructions;

- appropriate starting materials, containers and documentation;
- appropriate storage and transport.

9.2.5 Quality control

QC is that part of GMP which is concerned with ensuring that components meet their specifications by the testing of defined samples. It is also concerned with the sampling and testing of all materials and systems allied to the safety and effectiveness of the final component, beginning at the donor clinic and going through, where possible, to the bedside of the patient. It also ensures that no component is released for use until all quality requirements are satisfied. The basic requirements are:

- that adequate facilities, trained personnel and approved procedures are in place for all QC activities involving sampling, inspection and testing, including the monitoring of environmental conditions;
- that test methods are validated;
- that records are made of all QC activities, showing that all such required activity has been carried out and deviations fully recorded and investigated;
- that records are made to the effect that finished components and all materials used have been formally assessed against specification and that no component is released for use prior to certification that it meets the required specification;
- that provision is made for the retention of archive samples of serum from every donation, in a form which will permit repeat testing when necessary.

9.2.6 Documentation

Good documentation forms the heart of conformity with principles of GMP. Documents must be clearly written in unambiguous form. In its entirety, the documentation system of a blood centre should enable an audit trail to be established in either direction between donor and recipient. Specifications will describe in detail the requirements with which finished blood components will have to conform, a similar approach being taken to all materials used in the chain of events leading to their manufacture.

Acceptable standards for setting up appropriate quality systems which may also serve as the framework of the management's quality manual, are defined in terms of 10 core essentials:

- *Organization*— a well-defined organizational structure, quality goals and objectives;
- *Personnel*—qualifications required for each specific job, current job descriptions, a defined training programme, assessment of competence, and identification of retraining needs;

- *Equipment*—the commissioning and installation process/documentation, schedules for calibration, maintenance, performance and documentation, management of defective equipment;
- *Supplier issues*—qualification/performance of suppliers to meet the requirements, review of contracts, receipt, inspection and testing of incoming goods;
- *Process control*—final inspection and handling;
- *Documents and records*—document structure, defined SOP format, control of documents, annual revision of documents, storage, protection and retrieval;
- *Incidents, errors and accidents*—capture and classification of events, problem resolution, management review and reporting;
- *Assessments: internal and external audits*—follow-up of assessment findings;
- *Process improvement*—use of statistical tools;
- *Facilities and safety*—general health and safety, emergencies and disasters, adequate environmental conditions and control.

These headings defining the application of GMP to the blood transfusion centre setting are closely modelled on the 10 principles set out by the European Commission.

9.2.7 Quality systems in transfusion microbiology

Twenty years ago transfusion laboratories might have focused on blood group serology, or the accurate identification of unexpected red cell alloantibodies, as the salient aspect of transfusion technology calling for the most stringent quality standards. Currently, the nexus of attention in quality assurance is the prevention of transfusion-transmitted viral infection. In essence, the task is ensuring that all laboratory screening results are accurate and reported against the correct sample.

The core quality systems in transfusion microbiology are designed to make certain that tests are performed correctly, according to manufacturer's instructions. They must be performed in exactly the same way by every member of staff within a given centre and in a national setting, by staff in all regional or district transfusion laboratories. Quite apart from establishing a national standard of quality, this allows the results of different laboratories to be confidently compared with each other and provides vital prevalence and incidence data from different ethnic and socioeconomic strata and geographical locations within the country.

9.2.8 Standard operating procedures

A key element of these quality systems is carefully prepared standard operating procedures, preferably structured in three tiers, as policies, procedures and finally, detailed instruction documents. These are controlled documents, formally prepared, numbered and issued with appropriate authorization of the head of department and they must not be

altered except when they are formally and periodically revised and re-issued as new editions. Copies of the superseded editions are collected and, except for archive copies, are destroyed.

Laboratory forms must be treated in a similar, controlled manner, as formal standard documents.

Audits of the quality systems, whether independent external or internal auditing, rely heavily upon the integrity of the documentation system in place.

9.2.9 In-process control

In keeping with the proactive, prospective principles underlying quality assurance, in-process control is a means of detecting adverse trends at an early stage, before overt failure of test systems occurs. It is the provision of an integral, continual monitoring of assay performance in use, which enables operators to take corrective steps when required. Various tools are available for the implementation of in-process control of the variables affecting test performance at their most critical weak points (such as sample or reagent addition, incubation, microplate washing, or reading results) as follows:

- *Automated sample handling and assay processing*— provided that the equipment is carefully calibrated, more consistent results can be obtained than with a non-automated system and human error can be minimized. Positive sample identification together with bar-coded labels and bar-code readers also add to confidence in the integrity of the link between donor, blood bags, test samples, and results;
- *Setting performance criteria* e.g. expected optical density (OD) values for assay controls, or to check reagent addition;
- *Sample addition monitors* provide colour change upon addition of serum samples or the inclusion of dyed reagents which positively demonstrate the addition of solutions to test wells;
- *The use of QC samples* e.g. national working standards such as "go-no-go" samples to demonstrate assay validity, self-assessment or proficiency testing panels of samples, national positive control samples, etc;
- *Statistical process control (SPC)*—a tool which can be applied to any process or procedure in order to analyse screening data continuously, on a daily or weekly basis, on each test plate, or for each run. It can be used for instance to monitor initial and repeat reactive rates, QC sample data, or sample OD/cut-off ratio for specific QC samples. In brief, SPC demonstrates that a system which has some inherent variability, is under control or conversely, that it is going out of control. The visual display of SPC charts also provides a valuable retrospective documentary record of the prevailing standards of quality at a given point in time (see Annex 2).

Chapter 10

Appropriate use of blood components and principles of blood transfusion medicine

10.1 Problems in the use of blood components

The emergence of HIV within the past 15 years has had a profound, lasting impact upon attitudes towards blood transfusion in Europe and the USA, which has been compounded by the economic crisis in health care, as expenditure forms an ever more significant proportion of gross national product and political pressure for cost-containment grows. Patients are increasingly anxious about the safety of blood transfusion—concerns which are often needlessly exacerbated by the media. The passive patient attitudes of the past have been replaced by the new consumer, with greater expectations of perfection. There is greater pressure for accountability, a critical evaluation of all forms of medical treatment, including blood transfusion and the search for evidence of clinical benefit, as well as cost benefit. In addition, many countries are facing the limited availability of blood resources and are experiencing difficulties in meeting their objectives of self-sufficiency for blood products. The European Union is one example, where the problem is, at least in part, due to an ageing population, growing consumption and fewer eligible donors, although there are other countries where these insufficiencies are more closely related to the poor organization and management of their transfusion services.

Against this background, there is remarkably little information available regarding who is transfused, why and with what effect. A detailed prospective study of transfusion practices, carried out by the Sanguis group in 1994, examined data from 43 teaching hospitals (which might be expected to represent the best practice) in 10 European countries, with regard to six common elective operations: total hip replacement, transurethral prostatectomy, cholecystectomy, abdominal aortic artery repair, hemicolectomy and coronary artery bypass graft. The study underlines the impact of transfusion practices upon patient outcome, the cost of care and the need to conserve limited resources. It also has important implications for the education and training of our future medical practitioners.

The most striking finding of the Sanguis study group was the very wide variation among hospitals in the clinical use of all the blood products included in the survey: whole

blood, red cells, plasma, platelets, albumin and synthetic colloids. These differences were also found between hospitals in the same country, where the organization of health care, funding and access to products might be expected to be uniform.

Albumin, synthetic colloids and plasma. Some hospitals in the Sanguis study rarely used any of these products for the given surgical procedures. At the other extreme, some hospitals consistently used large quantities of albumin solutions, often in combination with both synthetic colloids and plasma. The three hospitals representing one country alone in the study collectively used nearly half of the total albumin consumed by all patients in the entire study, but provided only 12% of the study patients.

Frozen fresh plasma in surgical patients. In two countries studied where red cells were transfused, plasma was usually transfused at the same time, effectively reconstituting whole blood, negating the concept of component therapy and needlessly increasing patient risks, with no benefit. Frequently, there was no record of FFP having been given and in only 16% of cases receiving plasma was there any evidence of a coagulation test (PT or APTT) being carried out. In the minority who did have a test, the results were within normal haemostatic limits in 88% of cases.

Clinical records—why blood was ordered. Only 23% of all clinical records documented the reason for giving a transfusion. Proper documentation is essential to ensure continuity of care, to facilitate retrospective studies in cases of post-transfusion infection or other adverse events and to provide evidence in medico-legal investigations.

Red cell use. For hemicolectomy operations, for instance, the use of red cells varied between 0% and 79% of patients, and among the hospitals where red cells were given, the median utilization varied between 1 and 4 units. For total hip replacement the use of red cells ranged from 29% to 100% (Table 10.1).

Thus, it appears that the chances of receiving any given blood and blood products are largely determined by where the patient is operated on. What are the causes of these differences in practice? A number of studies carried out in the USA and the United Kingdom found the following:

- criteria for transfusion were poorly defined due to incomplete scientific knowledge; effective methods for translating available knowledge into clinical practice were lacking;
- there were differences in the application of established criteria for transfusion, or differences in the physicians' beliefs about the value of practices in meeting patient needs;
- practitioners tended to rely on their years of experience without necessarily keeping up-to-date in the field;
- practices differed between institutes, and within institutes between surgical teams.

TABLE 10.1 **Transfusions given to patients with hemicolectomy and total hip replacement, from the Sanguis project, 1994**

Product	Hemicolectomy	Total hip replacement
Red cells		
% patients	40.9 (0 to79)	80.8 (29 to 100)
Units per patient	2 (1 to 4)	3 (2 to 5)
Plasma		
% patients	10 (0 to 57)	6 (0 to 95)
Units per patient	4 (2 to 6)	2 (2 to 2)
Platelets		
% patients	0.2 (0 to 4)	0.2 (0 to 2)
Units per patient		
Albumin		
% patients	32 (0 to 98)	13 (0 to 76)
Grams per patient treated	62 (11 to 126)	26 (9 to 41)
Synthetic colloids		
% patients	36 (0 to 94)	49 (0 to 98)
Litres per patient treated	0.5 (0.5 to 1.0)	0.5 (0.5 to 2.5)

Clearly, further studies on the same lines are of considerable importance, particularly since there is very little information about clinical outcome in relation to different transfusion practices which can be used as a guide to a more effective, consistent approach to transfusion medicine. While over-transfusion, exposing patients to risk and wasting resources must be avoided, the notion that "less blood is better", should also not be assumed to be true for all circumstances. Under-transfusion or withholding transfusion may put certain categories of patients at even greater risk.

10.2 Overcoming these problems

First and foremost, there is a great need for efforts to educate surgeons, anaesthetists, nurses and junior doctors in the principles of transfusion medicine. Blood transfusion specialists have often been remote, behaving more like clinical pathologists, preoccupied with laboratory issues, rather than clinical practice.

Secondly, clinical audit by peers can do a great deal to foster the transfer of "best practice" from one surgical unit, or one hospital, to another and to overcome the hallowed traditions based upon the poorly informed prejudices of senior clinicians, rather than scientific evidence.

The wide availability of up-to-date clinical guidelines and consensus statements grounded in the best available scientific evidence of the indications for transfusion is essential. Admittedly so far, the publication of these reports and guidelines has had a

limited impact on transfusion practice, even in teaching hospitals. Nevertheless, through the medium of regular hospital transfusion committee meetings, with a widely representative membership from clinical specialities, nursing and laboratory staff and hospital management, the audit process, based upon simple indicators of performance and a continuous feed-back of information to clinicians, can succeed in modifying excessive pre-operative blood ordering and over-transfusion of red cells, plasma and albumin.

10.3 Current criteria determining the need for blood products

10.3.1 Red cell transfusion

There is general agreement that for adults, red cells should be transfused to increase the oxygen-carrying capacity of acutely anaemic patients. However, disparate views are offered by both clinicians and committees of "experts", regarding what constitutes anaemia of sufficient magnitude to require correction.

Because delivery of oxygen to tissues is essential for organ function, it is expected to be subject to the regulation of numerous mechanisms. A high degree of redundancy ensures tissue integrity but it also leads to complex interactions among the various levels of regulation.

Oxygen supply to tissues depends upon many factors: inspired oxygen concentration, ventilation, perfusion of the lungs, diffusion of oxygen into the blood, the metabolic characteristics of red cells (such as 2,3 BPG), the action of the heart, perfusion of tissue, diffusion of oxygen into tissue, oxygen consumption by tissue and its distribution in tissue etc.

It is difficult to develop a clinical or experimental model capable of considering all these points of regulation in a concerted, coherent manner, nor can clinicians measure or control all these variables. We must therefore look at a few key points which can be addressed.

a) Physiological considerations

Even though a haemoglobin level <10 g/dL has long been the unquestioned transfusion threshold employed by surgeons, a large number of patients with a level far from this arbitrary figure are operated on. Patients with renal failure frequently have haemoglobin level as low as 5 g/dL, and conversely, those with chronic obstructive pulmonary disease may be polycythaemic with a level of 17 g/dL. There is no universally safe value for all patients.

Haemoglobin is only one index of the oxygen transport system. It is more appropriate to consider oxygen delivery as the product of blood flow, or cardiac output and arterial oxygen content:

O_2 delivery = CO × $[O_2]_a$

where CO is cardiac output and $[O_2]_a$ is arterial oxygen content.

The equation predicts that a fall in the haemoglobin level will either lead to a fall in oxygen delivery, if the cardiac output does not change, or it will lead to an increased cardiac output, to maintain oxygen delivery

For anaemic patients with good cardiac function, this increase in cardiac output does occur and is the justification used by advocates of deliberate, iso-volaemic haemodilution. The ability of the cardiovascular system to compensate for significant reductions in haemoglobin is well documented. As long as the circulating blood volume remains normal, tissue oxygenation is well maintained at haematocrits as low as 20%. Clearly, the haemoglobin alone cannot be an adequate determinant of the need for red cell transfusion.

The important issue then becomes the adequacy of oxygen supply at any given haemoglobin level. The actual utilization of oxygen by tissues, or consumption, is the product of cardiac output and the differences in oxygen concentration between arterial and venous blood:

$$O_2 \text{ consumption} = CO \times ([O_2]_a - [O_2]_v)$$

where $[O_2]_v$ is venous oxygen content.

Tissue oxygen tension is the appropriate monitor of supply. The mixed venous pO_2 (pvO_2) is the oxygen tension of the venous blood when oxygen unloading has been completed and is the best indication of mean tissue oxygen tension. The pvO_2 therefore becomes a key measure of the adequacy of oxygen transport and whether an anaemic patient requires red cells. The normal value for pvO2 is 40 mmHg.

Another physiological indicator of transfusion need, is the oxygen extraction ratio (ER), which is the ratio of oxygen consumption to oxygen delivery:

$$ER = O_2 \text{ consumption}/O_2 \text{ delivery}.$$

Because the normal arterial oxygen is 20 volumes per cent, and the normal arterio-venous oxygen difference is 5% (in volumes), the normal extraction ratio is 25%. Available oxygen is therefore four times the oxygen normally consumed, leaving a huge oxygen reserve.

b) Silent myocardial ischaemia (SMI)

There is extensive data on the ability of animals to survive surgical manipulation with extreme normo-volaemic anaemia. Experiments on baboons in the late 1970s showed that they can survive at haematocrit levels as low as 5% and that oxygen consumption only drops when haematocrit falls below 10%. However, not everyone is as confident about the safety of allowing haematocrit to drop below 30% as these animal experiments suggest and some experts have expressed concern about coronary circulation in apparently healthy surgical or intensive care patients. New technology which permits the continuous

monitoring of the ECG reveals that up to 80% of all episodes of myocardial ischaemia remain silent. Oxygen tension in the myocardium itself falls with progressive anaemia, even when the coronary circulation is normal and despite an increased coronary blood flow. Thus, in patients with coronary insufficiency, a haematocrit below 30% may cause myocardial dysfunction and this also occurs in supposedly healthy patients. This dysfunction is attributed to "silent myocardial ischaemia", which may appear in up to 4% of completely asymptomatic, middle-aged males, whether or not they are subjected to the stress of surgery.

In patients over 40 years therefore, it is concluded that appropriate investigations should be carried out to exclude silent myocardial ischaemia when haemodilution to a haematocrit below 30% is contemplated. In summary, as a patient's "non-haemoglobin variables", such as cardiac output, oxygen demand, or mixed venous oxygen, deviate from their normal range, due, for instance, to sepsis, extensive tissue damage, respiratory or coronary insufficiency, or alkalosis, the patient becomes more dependent upon haematocrit or haemoglobin as a protective buffer against an oxygen deficit due to non-haemoglobin causes.

c) Severity of anaemia and mortality

The relationship of anaemia to morbidity or mortality was reported in the USA in a case control study of 125 surgical patients who refused blood transfusion for religious reasons.[6] Here, the operative mortality was inversely related to pre-operative haemoglobin levels, rising sharply from 7.1%, for those with haemoglobin above 10 g/dL, to 62% for those with haemoglobin levels below 6 g/dL. The mortality rates were also related to blood loss at surgery, increasing from 8% for losses under 500 mL, to 42.9% for those who lost over 2000 mL. The study corroborates other data suggesting that, although the risks of death do increase with more severe anaemia, the critical level in terms of decision-making appears to be nearer to 8 g/dL, the point at which cardiac output usually rises, than to 10 g/dL.

We can also draw upon the experience of clinicians in developing countries who, because of shortages of safe, screened blood, are forced to to undertake surgery with no allogenic transfusion. There is little systematic data, nor are there controlled studies comparing the effect of different pre-operative red cell regimes on surgical outcome. An almost unique study in Africa has provided valuable information on the survival of children in western Kenya, hospitalized with haemoglobin levels below 5 g/dL (over one in four of all admissions).[7] Children with haemoglobin under 3.9 g/dL who were transfused had a

[6] Carson JL et al. Severity of anaemia and operative mortality and morbidity. *Lancet*, 1988, 1:727–9.
[7] Lackritz EM et al. Effect of blood transfusion on survival among children in a Kenyan hospital. *Lancet*, 1992, 340:524–8.

lower mortality than those with a similar level who were not, provided that transfusion was given on the day of admission. In consequence, strategies were developed which reduced the frequency of transfusion, while enhancing survival, by directing blood only to children who might die without it, as early as possible after admission to hospital.

Clinical guidelines are almost universally based upon a consensus of experts, rather than upon data from controlled clinical trials. One of the rare prospective, properly randomized trials designed to evaluate red cell transfusion practices in intensive care units was carried out by the Canadian Critical Care Trials Group and reported in 1999. Over 800 patients were randomly assigned to either a liberal transfusion strategy, where haemoglobin levels were maintained between 10 and 12 g/dL, or a restrictive strategy of transfusion, in which haemoglobin levels were kept at between 7 and 9 g/dL. The results showed a significantly higher in-hospital mortality rate associated with liberal transfusion practices. In addition, the restrictive strategy resulted in a relative decrease of 54% in transfusion, and 33% of patients in this group received no red cell transfusion at all. In short, the conventional adage that maximizing tissue oxygen delivery in critically ill patients by any means (using red cells, albumin, or synthetic colloids) will improve survival, must now be questioned. Indeed, until these findings are refuted or confirmed by further studies, practitioners should be prudent in their use of both red cells and colloids in critically ill patients.

In conclusion, there is very wide variation in red cell transfusion practice. We have relatively good knowledge of the risks of transfusion but poor information about its benefits and patient outcomes. Although transfusion of red cells is rarely indicated at or above a haemoglobin level of 10 g/dL, it is usually necessary below a level of 7 g/dL. In the individual patient the oxygen extraction ratio and mixed venous oxygen tension may be valuable in deciding the need for red cell transfusion but there can be no substitute for a meticulous clinical assessment, as there cannot be a valid "transfusion trigger" which is applicable to all patients.

10.3.2 Indications for therapeutic platelet transfusion

a) Decreased platelet production

In the past, thrombocytopenic patients with bone-marrow failure due to leukaemia, hypoplasia or cytotoxic chemotherapy most frequently died of cerebral haemorrhage. Retrospective studies published in the 1960s showed a 50% or greater reduction in the mortality from haemorrhage in leukaemia patients after platelet transfusion became routinely available. The use of prophylactic platelet transfusions in this setting has become the norm, although the effectiveness, rationale and appropriate threshold are still controversial.

b) Immune platelet destruction

Patients with auto-immune thrombocytopenia (AITP) have relatively less bleeding at a given platelet count than those with failure of production. The primary modes of therapy for AITP are steroids, intravenous immunoglobulin (IV Ig) or splenectomy. Platelet transfusions are usually reserved for major life-threatening haemorrhage. Large doses of platelet concentrates are required to produce a therapeutic effect in the presence of auto-antibodies and there is some evidence that they can be transiently effective in serious emergencies.

In post-transfusion purpura, heparin-induced thrombocytopenia and thrombotic thrombocytopenic purpura, platelet transfusions are either ineffective, or positively contraindicated. It is cases of neonatal alloimmune thrombocytopenia, carrying a significant risk of intracranial haemorrhage, that benefit from platelets lacking the incriminating antigen (for instance HPA 1) and given either by serial intrauterine transfusions, prenatally, or directly to the newborn, postnatally.

c) Nonimmune platelet consumption

Massive transfusion produces thrombocytopenia proportional to the amount of blood transfused but clinically significant problems are unlikely to occur before the transfusion of 1.5 to 2.0 blood volumes, unless there is some accompanying degree of acquired platelet dysfunction as well. Current consensus guidelines suggest maintaining the platelet count between 50×10^9/L and 100×10^9/L when clinically abnormal bleeding is observed in traumatized or post-surgical patients who have received massive transfusion. Some 25% of such patients develop micro-vascular bleeding, manifested as a generalized oozing of blood and mucosal bleeding and suggesting a defect of primary haemostasis. This is usually due to either thrombocytopenia or platelet dysfunction, accompanying disseminated intravascular coagulation and these cases do benefit from platelet therapy.

Exposure to extracorporeal circuits also causes a bleeding tendency, particularly if the older bubble-oxygenators are employed. Factors contributing to this tendency include haemodilution, exposure to heparin or aspirin and hypothermia. The success of high-dose aprotinin and tranexamic acid in reducing blood loss associated with extracorporeal bypass surgery suggests that hyperfibrinolysis is a major factor. Certainly, controlled trials of routine prophylactic platelet transfusion in cardiac surgery have shown no evidence of benefit.

d) Disorders of platelet function

Platelet transfusions are effective in congenital disorders of platelet function such as Glanzmann thrombasthenia, Bernard-Soulier syndrome and storage pool disorders, as well as the very rare cases of von Willebrand disease with platelet dysfunction. Because of the risk of HLA-alloimmunization in these life-long disorders, platelet concentrates are usually leukocyte-depleted with specific filters.

10.3.3 Prophylactic platelet transfusion

The enormous increase in platelet utilization within the past 10 years (300% to 400%) has largely been due to the use of these concentrates in a prophylactic mode for patients undergoing aggressive chemotherapy for leukaemias or solid tumours.

The current levels of platelet concentrate utilization in Europe and the USA vary between 6 and 15 platelet equivalents per 1000 inhabitants/year (a platelet equivalent is taken to represent the platelets harvested from a single blood donation).

In the past, a threshold or "transfusion trigger" of 20×10^9/L or less, was generally accepted as the appropriate level calling for prophylactic platelet transfusion. The evidence for this tradition is highly questionable, however, because the early studies underpinning this policy were carried out on patients who were virtually all receiving aspirin as an antipyretic/analgesic, before its effects on platelet function were known. More recent studies in the 1960s and 1970s have shown that there is indeed a threshold below which serious haemorrhage is likely to occur but that this level is at or below 5×10^9/L, rather than 20×10^9/L. Many modern centres now accept that, unless patients are febrile, septic or actually bleeding, a rational threshold value for prophylaxis, should lie between 5 and 10×10^9/L (Table 10.2). Naturally, it is essential that automatic cell-counters or manual methods employed in carrying out platelet counts are properly standardized and validated, so that their accuracy is as reliable as possible. Clearly, adoption of this newer policy, has the potential for reducing donor exposure, treatment costs and possibly alloimmunization.

10.3.4 Patients refractory to platelet transfusion

The refractory state is the failure to achieve an expected platelet increment after two consecutive transfusions of platelet concentrates. Many formulae have been derived to establish failure, based upon platelet counts before and after transfusion, the platelet content of the transfusion and the surface area or blood volume of the patient. These include corrected count increment (CCI) and percentage recovery. A failed transfusion is

TABLE 10.2 **Platelet transfusion protocol**

Morning platelet count ($\times 10^9$/L)	**Prophylactic platelet transfusion on the same day indicated**
0.5	In every case
6 to 10	In the presence of fresh minor haemorrhagic manifestation Body temperature >38 °C
11 to 20	In the presence of coagulation disorders and/or heparin therapy Before bone marrow biopsy or lumbar puncture
>20	In the presence and until control of major bleeding complications Before minor surgical procedures

indicated by a CCI of <7.5 × 10^9/L at 20 to 24 hours after transfusion and a recovery of <15% to 25% at 1 hour after transfusion.

Refractoriness may be immunologically mediated or nonimmunological. Non-immune causes are much more common, including poor quality platelets, hypersplenism, fever, sepsis, disseminated intravascular coagulation, drugs (such as amphotericin-B), viral infections and graft versus host disease. The commonest cause of the immune-mediated refractory state is alloimmunization to HLA class I antigens, although ABO incompatible platelet transfusion and to a lesser extent, platelet-specific antibodies, may also contribute to this condition. Previous pregnancy and transfusion are major risk factors for refractoriness as they are frequently a cause of primary HLA alloimmunization. It must be emphasized, however, that HLA alloimmunization is not synonymous with immunological refractoriness. Some 30% to 70% of platelet-dependent patients develop HLA antibodies (lympho-cytotoxic antibodies) but only 8% to 40% actually develop a refractory state.

Prevention of alloimmunization by leukocyte depletion (to a maximum level of 5 × 10^6 leucocytes per transfusion) is undoubtedly the most effective way of avoiding primary sensitization and reducing the number of patients who fail to respond to random platelets, requiring instead expensive cross-matched or HLA Class I matched platelet concentrates.

It may be that the future use of UV-B irradiated platelets, or growth factors (such as thrombopoietin), as well as reduced exposure to platelets will further reduce the incidence of immune-mediated refractoriness.

10.3.5 Clinical use of frozen fresh plasma

10.3.5.1 General

Studies of the use of frozen fresh plasma (FFP) have shown that it is often misused, largely due to misconceptions regarding its haemostatic effectiveness and ignorance of the clinical situations in which its use is inappropriate. Consumption of FFP has increased up to ten-fold in Europe and the USA although there is no rational basis for its administration in a wide range of conditions for which it is so intensively utilized. Indications for its use are, nevertheless, well defined.

10.3.5.2 Indications for FFP

a) Replacement of single-factor deficiencies

More specific coagulation factor concentrates are becoming available for clinical use and, except for factors II, V, X, and XII, all the others can be obtained (albeit at some cost) as individual or combined preparations. For instance, concentrates combining factors II, IX and X, and sometimes also factor VII, are still available (despite the vogue for factor IX-only concentrates) and this will serve for replacement therapy of factors II and X.

Factor XII deficiency is rare in itself and replacement therapy for this condition is almost never required. Deficiency of von Willebrand factor can be treated either with cryoprecipitate, DDAVP (1-desamino, 8-D-arginine vasopressin), some intermediate purity preparations of factor VIII, or more recently, the specific vWF concentrates which have become available.

b) Immediate reversal of the oral anticoagulant effect (warfarin)

Patients taking oral anticoagulants have a functional deficiency of the pro-coagulant vitamin K-dependent factors II, VII, IX, X and anticoagulant factors, proteins C and S. This condition can be reversed, within 4 to 6 hours, by the parenteral administration of vitamin K. However, if life-threatening bleeding should occur in a poorly controlled patient, or if emergency surgery requires immediate reversal of the warfarin effect, plasma may be used in suitably large doses of 1 to 1.5 L If they are available, a combination of prothrombin complex concentrate and factor VII (if it lacks F VII) can be given instead, provided that they are used with caution, in the light of their potential thrombogenic effect.

c) Vitamin K deficiency

Haemorrhagic disease of the newborn and conditions interfering with vitamin K absorption, such as biliary obstruction or malabsorption syndrome, may cause a coagulation abnormality with overt bleeding which can be treated successfully with FFP.

d) Acute disseminated intravascular coagulation (DIC)

DIC can be associated with hypovolaemic shock, trauma and sepsis, resulting in variable deficiency of factors V and VIII, fibrinogen, fibronectin, ATIII and platelets due to activation of the coagulation and fibrinolytic systems. Treatment must first be focused on reversing the underlying cause of DIC, as there is no evidence that replacement therapy can be effective until this is achieved. In acute DIC with haemorrhage, the infusion of platelets and FFP constitute the first line of therapy, which must be accompanied by close laboratory and clinical monitoring. Chronic DIC, on the other hand, does not call for treatment merely in order to correct abnormal laboratory results, unless there is overt bleeding.

e) Thrombotic thrombocytopenic purpura

FFP or cryo-supernatant plasma are well-established forms of treatment which must be administered in large doses of at least 3 L per day.

f) Inherited deficiency of inhibitors of coagulation

Plasma can be used as a source of ATIII, protein C or protein S in patients with deficiencies who require surgery or heparin therapy for spontaneous thromboses. Concentrates of ATIII are now widely available and protein C/S preparations are also

obtainable in gradually improving quality, however and where they are affordable and accessible, FFP is no longer utilized.

g) C1 esterase inhibitor deficiency

FFP can be used for patients who develop angio-oedema, although specific concentrates have now become available for those who can afford them.

10.3.5.3 Conditional uses of FFP

The following uses are indicated only in the presence of bleeding and disturbed coagulation.

a) Massive transfusion

Massive transfusion is usually defined as the replacement of the patient's total blood volume with stored blood in under 24 hours. Even though dilution of clotting factors with stored blood, sometimes called the "wash-out" effect, is commonly taken to be the cause of haemostatic failure and microvascular bleeding in patients who require large volume blood transfusions, that is rarely the case.

More significant factors underlying bleeding are: any delay in fully restoring the circulating blood volume and tissue perfusion, together with consumption of platelets and coagulation factors due to shock, exacerbated by sepsis, pre-existing liver disease and platelet dysfunction. The latter is caused by fibrin degradation products, or by certain antibiotics. In addition, even circulating levels of coagulation factors (such as factors V and VIII) as low as 25 to 30%, are haemostatic. Therefore, the ritual prophylactic use of plasma, according to pre-established formulas, calling for the infusion of one unit of FFP for every 3 or 4 units of stored blood, is wasteful and inappropriate.

The initial use of platelet concentrates, in these cases, supplemented, as indicated by laboratory monitoring (prothrombin time and partial thromboplastin time $>1.5 \times$ control values, with a fibrinogen > 0.8 g/L), with cryoprecipitate or FFP, are more likely to be effective. When FFP is indicated, it must be given in an adequate dose of at least 1 L, in the adult.

b) Liver disease

Spontaneous bleeding seldom occurs in liver disease, unless there is a precipitating cause, such as surgery, including liver biopsy, or ruptured oesophageal varices. In practice, FFP is indicated for overt bleeding, or when surgery is proposed. Large volumes of FFP are required however, because these patients have water retention, with an expanded plasma volume and some of the coagulant factors have a very brief biological half-life. In consequence, complete correction of the coagulation deficit is difficult or impossible to achieve.

Prothrombin complex concentrates with factor VII have been used with success in emergencies or prior to surgery but they must be employed with great caution because of their intrinsic thrombogenic potential, which is aggravated by the impaired clearance of activated clotting factors and reduced ATIII levels of advanced liver disease.

c) Cardiopulmonary bypass surgery

The majority of these patients do not have major coagulation abnormalities and the routine peri-operative use of FFP for cardiopulmonary bypass surgery cases provides no known benefit, exposing patients to unnecessary risk. Platelet infusions usually form the best initial management when post-operative bleeding does occur. FFP should only be used where bleeding is associated with demonstrable abnormalities of coagulation other than a residual heparin effect. The widespread use of pharmacological agents, such as aprotinin, has considerably reduced the need for transfusion of blood components, including FFP.

d) Paediatric indications

Severe sepsis in the newborn may be complicated by DIC and the use of FFP and cryoprecipitate are useful in their supportive treatment. Small volume paediatric units of FFP are often made available by transfusion services, to avoid wasting an adult dose, where only 10 to 15 mL per kg of FFP is required.

10.3.5.4 No justification for the use of FFP

a) Hypovolaemia

Crystalloid and synthetic colloid solutions are safer, cheaper and more readily available. These may be combined with 4.5% or 5% human albumin, to better effect.

b) Following intensive plasma exchange

FFP should only be employed to correct coagulation abnormalities when bleeding occurs.

c) "Formula" replacement

There is no indication for the use of FFP according to pre-determined replacement schedules, during or after surgery.

d) Nutritional support for protein-losing conditions

In chronic cirrhosis with ascites, nephrosis, protein-losing enteropathy or chronic thoracic duct drainage, there is no justification for using FFP.

In summary, FFP is frequently misused and overused, exposing patients to risk with little or no benefit unnecessarily wasting blood resources and diverting plasma from

fractionation. In a well regulated programme, no more than 1 to 1.5 kg of FFP per 1000 inhabitants should be sufficient to provide for all clinically justifiable purposes.

10.4 Clinical audit

The establishment of hospital transfusion committees, together with regular peer review and clinical audit procedures, are the most effective mechanisms for enforcing high standards of transfusion practice, based upon published guidelines, scientific evidence and common sense.

Clinical audit highlights activities that are undertaken well, and exposes those that are not. Externally developed guidelines are not always applicable or acceptable locally. Local guidelines that are simple and concise are more likely to alter practice. Appropriate dissemination of guidelines to relevant staff, particularly junior doctors, laboratory and nursing staff, who provide day-to-day service, raises awareness to the expected standards, is educational and results in better use of resources.

Audits provide objective evidence of adherence to high standards, and reveal competence and good practice, thus raising the self-esteem of staff. Identification of noncompliance provides an opportunity to review staff selection methods, training programmes and standard operating procedures.

Hospital transfusion committees, with representation of relevant clinicians as well as nonmedical staff involved in administering transfusions, are a very powerful forum for improving transfusion practice. However, to be effective, such committees must be granted executive power to deal with noncompliance.

Chapter 11
Optimal use of blood resources

11.1 General

A whole range of options may be adopted in blood component manufacture which will have a direct influence upon the yield, quality and volume of plasma available for fractionation, as well as the availability and quality of labile blood components, such as red cells, platelet concentrates and frozen fresh plasma used in day-to-day blood transfusion practice. Whether these options are exercised or not will depend upon where national priorities lie and whether the requisite financial support and expertise for their implementation is available. For instance, should the major emphasis be directed towards harvesting maximal volumes of plasma for fractionation, or is it more important to improve the quality of plasma so that coagulant factor yield will be enhanced? How economically feasible is it to collect plasma and platelets by apheresis and how clinically relevant is it to provide leukocyte-depleted platelet concentrates? One can only begin to answer these questions after a careful consideration of local demand and the budgetary consequences of each of these policies.

In order to attain self-sufficiency for the whole spectrum of blood components, as well as plasma derivatives (whether fractions are manufactured within the country or through contract fractionation of local plasma by a foreign company) a balance must be achieved between component production and consumption on the one hand, and the production of plasma for fractionation on the other.

This equilibrium presupposes the establishment of certain supply targets and it is worth emphasizing that the need for blood products cannot be equated with clinical demands, which may be irrational. The inappropriate use of both plasma fractions and blood components is unsafe and costly.

In countries where the demand for plasma fractions outstrips the supply of "raw" plasma provided as a by-product of whole blood processing and component production, and exceeds red cell and platelet requirements, blood services have had to resort to various strategies to redress the resulting imbalance:

- over-collection of donor whole blood with destruction of surplus red cells, which is a wasteful, expensive solution, or their exportation to countries where blood is in short supply;

- large-scale plasmapheresis to obtain an adequate feed-stock of plasma for fractionation.

However, the judicious implementation of component preparation techniques and policies which have recently become available in routine use can now ensure that, while red cell needs remain the most important component of blood collection, an adequate supply of plasma for fractionation can also be catered for to satisfy all but the most excessive demands for plasma fractions These include coagulant factors, albumin and immunoglobulins. There can be little doubt that achieving such an equilibrium not only makes optimal use of the blood donor's gift, it also makes more economic sense.

11.2 Blood component utilization

11.2.1 General

Much of what follows assumes that component therapy is a well-established priority in the country and that the diagnosis of congenital disorders of coagulation, such as haemophilia, or of leukaemia, as well as the medical expertise for their treatment is available. It also presupposes that there is at least some demand for and familiarity with the clinical use of the major plasma fractions. It is also assumed that the frequently difficult phase of persuading medical colleagues, and surgeons in particular, that the insistence upon employing fresh whole blood is no longer a defensible clinical option, has long been replaced by an acceptance of the added value blood components bring to transfusion medicine.

11.2.2 Red cells

At an early stage in the development of component therapy, when demand for platelet concentrates and plasma first became pressing, packed red cells, with a haematocrit varying between 60% and 75%, presented difficulties in storage and infusion. This was due to excessive viscosity and poor viability, which could be explained, in part, by the fact that red cells were deprived of most of their metabolic nutrients as soon as the plasma was separated.

The development of partially packed cells in some countries represented an effort to mitigate these undesirable characteristics but this product proved to be an unsatisfactory interim compromise. These early efforts, in the 1960s and 1970s, nevertheless had the virtue of preventing the needless waste of platelets and plasma which could be better employed in the specific treatment of appropriate medical conditions, instead of merely acting as a passive, inevitable accompaniment to red cells. Thus, each unit of packed red cells yielded some 180 to 200 mL of plasma, either for direct clinical use, or for fractionation.

In an ingenious initiative, first conceived by Högman in the late 1970s, red cell additive solutions, such as saline adenine glucose mannitol (SAGM) followed by others, were developed. The objective was to uncouple the anticoagulant function provided by a citrated solution in the primary blood collection bag, from the nutrient function, necessary for the resuspension and longer-term storage of plasma-depleted red cells. These additive solutions, which progressively superseded the packed red cell method throughout the 1980s, offer a number of advantages:

- better flow characteristics and rheology, at any storage age;
- improved red cell adenosine triphosphate retention, metabolic viability and oxygen radical scavenging, resulting in better circulating survival;
- a more prolonged shelf-life (6 weeks), resulting in fewer time-expired blood units (under 3% in well managed centres);
- greatly reduced residual haemagglutinins, allowing more flexible use of donor/patient blood group specificities which could safely be compatible, without necessarily being identical (blood group O to A or B, A to AB etc.);
- above all, a greatly enhanced plasma harvest, of between 275 mL and 300 mL.

As a direct consequence of the widespread adoption of additive solutions in England and Wales, the persistent shortage of plasma for fractionation of the 1970s and the relatively high red cell outdating (over 10% at times) was rapidly resolved. Despite their initial reservations and fears that the dearth of coagulant factors or oncotic protein in these plasma-depleted red cell preparations would prove to be unsafe, surgeons, obstetricians and physicians quickly accepted resuspended red cells as the standard preparation, largely replacing both packed cells and whole blood. Whole blood currently forms under 1% of the blood supply in the United Kingdom and is almost confined to certain neonatal and exchange transfusions. Indeed, most Scandinavian countries have entirely discontinued the supply of whole blood. Clinical experience extending over almost two decades has now provided ample evidence that in both surgical and paediatric practice these policies are safe and effective.

It is therefore quite feasible and clinically acceptable to process at least 90% of the whole blood units collected into SAGM red cells and plasma. The added cost of the more complex blood bag assemblies is easily offset by the increased plasma harvest in those countries where this is a priority.

Newer nutrient additive solutions will soon become available, which will greatly improve red cell storage properties at room temperature, by maintaining ATP as well as 2,3 bis-phosphoglycerate (2,3 BPG) at near-normal levels for up to 4 weeks. Thus, the energy levels are retained and red cell oxygen affinity, which rises as 2,3 BPG declines, remains largely unimpaired, preserving the oxygen delivery capacity.

11.2.3 Volume of plasma recovered from a whole blood programme

Surveys of worldwide blood collection show an enormous variation between countries. For example at one extreme Switzerland over-collects whole blood at more than 100 donations per 1000 inhabitants to meet a very high demand for plasma as a source material for fractionation and Cyprus collects over 90 units per 1000, to satisfy the requirements of a very active thalassaemia hyper-transfusion programme. At the other extreme are countries with less highly developed health care and blood transfusion systems, which may collect less than one donation per 1000 inhabitants.

Countries with health care and transfusion services which are sufficiently diversified and which possess adequate economic strength to aspire to the establishment of their own plasma fractionation facilities will ultimately need between 40 and 50 blood collections per 1000 inhabitants per year, in order to satisfy demand for both labile blood components and plasma fractions.

For instance, a hypothetical national blood service, collects some 45 donations per 1000/year and retains only 10% of all blood units collected as whole blood. If the remaining 40 units are processed using an additive solution such as SAGM, each unit will yield between 275 and 300 mL of plasma, amounting to a total of approximately 11 to 12 kg of plasma per 1000 inhabitants. Setting aside a very generous 10% for direct clinical use as frozen fresh plasma this will leave 9.9 to 10.8 kg/1000, for use as the raw material for fractionation.

11.2.4 Yield of fractionated products from plasma

Although viral inactivation procedures, which are now an essential requirement for all plasma fractions take their toll on factor VIII:C yields, it is usually possible to obtain between 130 and 250 IU per kilogram of good quality plasma, depending upon whether an intermediate purity or very high purity product is sought. Referring once again to the model above, this means that, with 9.9 to 10.8 kg/1000 of plasma dedicated to plasma fractionation we can expect to supply haemophilic patients with approximately 2 IU per inhabitant, which is a reasonably acceptable level. Obviously, if the demand is greater and support is required for prophylactic or continuous-infusion regimes, then a greater input of plasma will be needed. It is here that a supplementary plasmapheresis programme may be of value.

Albumin yields are usually 25 to 27 g per kilogram of plasma and in applying this to our model, one would expect to obtain about 270 kg albumin per million population, a more than adequate level of supply. At some 2 g per kilogram of plasma, for immunoglobulin, one would also anticipate 20 to 22 g Ig/1000.

Naturally, apheresis may be employed to provide source plasma, instead of the plasma recovered from whole blood donation. The capacity of fit, well-nourished donors to provide plasma as often as once every 2 weeks, without detriment to their health, will mean that a little less than one donor will suffice to supply 10 kg of plasma per inhabitant. In a blood component programme however, at least 12 donors would be required to supply a similar volume of plasma. However, apheresis is demanding, in terms of both cost and donor time and the best value-for-money and optimal utilization of donor resources can undoubtedly be obtained by using a well-integrated whole blood programme as the main source of plasma, reserving apheresis as an adjunct, to supplement, rather than replace recovered plasma as required.

11.2.5 Platelets

When platelet concentrates are prepared using the traditional platelet-rich plasma (PRP-PC) technique, 50 to 60 mL of the original plasma remains in the platelet satellite bag as a nutrient storage medium. The mean plasma yield is therefore only 180 to 210 mL per unit (or 52% to 63% of the original plasma volume).

More recently, platelet concentrates have increasingly been prepared by using the platelet-rich buffy coat as a source of platelets, with the following distinct benefits:

- platelets suffer less trauma during processing, with a consequently lower level of platelet activation;
- a much reduced level of contaminating leukocytes is present, with lower cytokine secretion and fewer adverse reactions, together with fewer cases of alloimmunization to leukocyte antigens.

The buffy coat platelet concentrate (BC-PC) method of platelet preparation results in a somewhat greater plasma yield (67% to 74% of the original volume) but a striking improvement can be obtained when 4 to 6 buffy coat derived platelets are pooled to form an "adult dose" and resuspended in a specific platelet additive solution (analogous to red cell additives), retaining only some 10% plasma. A multicentre comparative study conducted in Europe by the ISBT Working Party on Biomedical Excellence for Safer Transfusion (BEST) in 1994 found that between 275 and 297 mL, or 85% to 89% of the original plasma volume can be retained using this technique (Table 11.1), which can be used to supplement the volume of recovered plasma available for fractionation. Provided that suitably gas-permeable platelet bags (PL 2209) and an additive solution containing acetate (which acts as a platelet fuel) are used, pooled, buffy-coat-derived platelets maintain a satisfactory metabolic profile, including blood gases and residual plasma bicarbonate.

TABLE 11.1 **Plasma yield in component preparation using platelet-rich plasma and buffy coat methods for platelet concentrate preparation**

Method	Plasma volume (ml)					Plasma yield (% of original content)
	No. of centres using the method	Total volume	Remaining in RBC	Collected in BC	Collected in plasma unit	
PRP-PC	2	336–347	64–77		181–210	52–63
BC-PC						
Single	5	282–327	14–45 (4–16%)	15– 80 (4–25%)	206–245	67–74
Pooled[a]	1	312	5 (2%)	69 (22%)	222	71
Pooled AS[b]	4	322–337	4–37 (1–11%)	23–34 (7–10%)	275–297	85–89

RBC: red blood cells PRP: platelet-rich plasma AS: additive solution
BC: buffy coat PC: platelet concentrate
[a] Prepared from a pool of 6 BCs with plasma as storage medium.
[b] Prepared from a pool of 4 or 6 BCs with additive solution and plasma as storage medium.
Note. Figures from 12 centres; mean yields from <100 routine PCs in each centre.
Source: ISBT Working Party on Biomedical Excellence for Safer Transfusion (BEST). Statistical analysis of BEST study No. 6. *Cellular harvest in blood components prepared with different methods,* November 1994.

11.2.6 Quality of red cell and platelet preparations

Leaving aside considerations of volume, the quality of red cell and platelet products can also be improved in two different ways: by leukocyte depletion and by enhanced storage conditions (see 11.2.7).

The removal of buffy coats from red cells can readily be carried out on a large scale and at relatively low cost by either manual methods or by automated equipment. Although overall leukocyte depletion will be limited to between 50% and 90%, most of the more immunologically significant mononuclear cells are removed by buffy coat depletion, leaving mainly granulocytes. In the best hands, this degree of depletion will suffice for the purposes of avoiding most adverse, non-haemolytic reactions in multi-transfused patients such as thalassaemics. Leukocyte filters, on the other hand, are much more effective, but they are correspondingly more expensive.

In the case of platelets, the same multicentre comparative investigation carried out by the ISBT BEST Study Group in 1994, showed that good platelet yields can be achieved by both PRP and pooled BC techniques (66 to 78 $\times$ 10^9, and 60 to 83 $\times$ 10^9 platelets per donation, respectively) (Table 11.2). The single unit BC technique yielded fewer platelets than the PRP method but leukocyte depletion was distinctly superior. However, both a high platelet yield (64 to 70 $\times$ 10^9 platelets per donation) and impressive leukocyte depletion (<5 $\times$ 10^6 per transfusion) was obtained when platelet concentrates prepared from a pool of buffy coats were subjected to pre-storage leukocyte filtration as an integral

TABLE 11.2 **Platelet yield in platelet concentrate preparation using platelet-rich plasma and buffy coat methods**

Method	No. of centres	Platelets/donation ($\times 10^9$)	Platelet yield (% of original)	WBC in PC ($\times 10^9$) per donation
PRP-PC	2	66–78	62–64	65–181
	2[a]	53–62	50–51	<5
BC-PC				
Single	4	52–56	42–54	3–12
	2[b]	37–66	33–56	41–59
Pooled-plasma	1[c]	72	62	6
Pooled-PAS-NF	2[d]	60–83	53–74	11–54
Pooled-PAS-F	2[e]	64–70	53–70	<5

WBC: white blood cells PAS: crystalloid platelet additive solution PC: platelet concentrate
NF: unfiltered F: filtered
PRP: platelet-rich plasma BC: buffy coat
[a] Bedside leukocyte filtration with 20% loss.
[b] From a single centre using two methods.
[c] Prepared from a pool of 6 BCs with plasma storage medium.
[d] Prepared from a pool of 4 or 6 BCs with additive solution/plasma storage; unfiltered.
[e] Prepared from a pool of 4 BCs with additive solution/plasma storage; filtered.
Note. Figures from 12 blood centres; mean yields from <100 routine PCs in each centre.
Source: BEST study No. 6, 1994. (see note Table 11.1)

part of the process. This latter technique therefore appears to provide an optimal yield of plasma and platelets, as well as good quality leukocyte depletion. Cytokine secretion and the possibility of bacterial contamination, or CMV (and probably HTLV) transmission are also much reduced, as an added benefit.

While it is true that apheresis methods, employing highly selective and efficient modern machines, are capable of providing a larger harvest of platelets or plasma from a single donor than the processing of whole blood in the components laboratory, they are considerably more expensive and time-consuming. Once again, careful local consideration of priorities, together with economic feasibility must be the final arbiter of choice.

11.2.7 Technical options determining blood component quality

Conventional operating procedures usually specify that plasma must be separated within 6 to 8 hours of blood collection and rapidly core-frozen in under one hour to qualify as frozen fresh plasma. This tradition was established in the light of the poor stability of factor VIII:C at room temperature, and its relatively rapid deterioration in these conditions, at a rate of about 1% per hour. Indeed before the enormous increase in platelet demand made room temperature storage a necessity at blood collection sessions and during transport to the blood processing laboratory, whole blood units were held, transported and

centrifuged at 2 to 4 °C, in order to preserve the plasma F VIII:C activity as well as possible. Needless to say, maintaining this cold chain from the blood collection site all the way to the final core-freezing of plasma was an onerous and costly task.

In fact, factor VIII:C is remarkably stable at room temperature in the presence of calcium. When calcium-chelating, citrated agents such as acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose (CPD) are used to anticoagulate donor blood, this metalloprotein becomes unstable unless the plasma is rapidly frozen. However, Prowse in Scotland and later, Högman in Sweden showed that when half-strength CPD is used as a whole blood anticoagulant, factor VIII:C stability can be significantly improved by some 10% at room temperature and that this enhanced level is carried through the fractionation process to finished F VIII.

In addition, earlier work in the 1970s suggesting that the factor VIII/vWF complex tends to precipitate when blood is stored at 2 to 4 °C was more recently confirmed when Dutch investigators devised a system for the overnight storage of freshly collected donor blood on butane-diol filled plates. Butane-1,4diol is a wax with a freezing temperature of 18 to 20 °C, which is maintained for at least 10 to 12 hours. Freshly collected blood stored in crates with inter-leaved cooling plates is rapidly cooled to a uniform temperature of 18 to 20 °C and held overnight, with the following advantages:

- F VIII:C is better preserved, providing a better yield on fractionation;
- white cell phagocytic activity and complement activation are well preserved, whereas they are inhibited below 17 °C; this allows whole blood to retain its self-sterilizing properties in the face of inadvertent bacterial contamination;
- night-shift work to separate and freeze plasma immediately is obviated, providing clear logistical and cost advantages;
- platelets in whole blood are maintained at a temperature which preserves their viability and function.

A very interesting illustration of the profound impact the choice of different therapeutic options and processing techniques can have upon national plasma requirements, blood collection policy and costs, can be found in the detailed analysis carried out by Högman, contrasting the situation in two Scandinavian countries: Norway and Sweden. These neighbouring countries, with very similar cultural and socioeconomic structures and virtually identical incidence of haemophilia, have adopted entirely different treatment policies, although these are not determined by any restrictions in supply or funding.

Sweden provides haemophiliacs with a regular prophylactic regime, supported by 5.4 IU factor VIII:C per inhabitant, while Norway does not, using only 2.4 IU. Norway achieves national self-sufficiency for all blood components and plasma fractions, at a relatively low level of whole blood collection and requires very little supplementary source

TABLE 11.3 **Comparison of blood component and plasma fraction utilization per 1000 inhabitants in two neighbouring countries with a similar socioeconomic structure, 1993–1994**

Components/fractions	Norway	Sweden
Blood donations for components	41.9	50.1
Red cell units transfused	40.6	46.1
Platelet equivalents	5.9	13.5
Plasma collected (kg)		
Total	12.8	25.1
Apheresis	2.5	11.9
Plasma transfused (ml)		
Inactivated	908	0
Native	0	2919
Factor VIII:C (IU)	2391	5406
Albumin (g)	280	430

plasma, or platelet concentrates derived from apheresis, due to their modest utilization targets and efficient component production policies (Table 11.3). Thus, although the difference between the two countries in red cell and platelet consumption is moderate, they differ radically in their use of albumin, factor VIII:C and frozen fresh plasma. As a consequence of its greater needs, Sweden is compelled to collect almost twice as much plasma (half of which is from plasmapheresis) in order to attain self-sufficiency.

In a further analysis of these differences (Table 11.4) it can be seen that red cell and platelet production generates only one-third of Swedish costs, while the remaining two-thirds, are attributable to plasma and plasma fractions. Indeed, among these it is the coagulation fractions, heavily dominated by F VIII, which are responsible for generating 40% of total national costs. For instance if Sweden were to convert to the Norwegian model for haemophilia management, its savings would amount to as much as US$ 2000 per 1000 inhabitants, or half the expenditure on red cells and platelets, clearly demonstrating the important economic consequences of different therapeutic approaches.

These data and costings refer to 1993 and 1994 however, and the use of intravenous immunoglobulins has greatly increased in the intervening years. There has been a corresponding increase in the use of recombinant products during this time, with a decline in plasma-derived F VIII:C. In consequence, it is likely that the total costs of fractionated products will rise, as the previous state of equilibrium is altered and the cost-effectiveness of conventional plasma-based fractionation is diminished.

Clearly, when decisions regarding the establishment of national self-sufficiency for plasma derivatives are being contemplated, plasma fractionation cannot be looked at in

TABLE 11.4 **Consumption and cost of blood components and plasma fractions in Sweden**

Item inhabitants	Per 1000 inhabitants	US$ per 1000 of total cost	Percentage
Red cell units	46	3333	27.7
Platelet equivalents	14	640	5.3
(From components	10	440)	
(From apheresis	4	220)	
Plasma (kg)			
For transfusion	3	453	3.8
For fractionation	22	2467	
(Recovered	10	880)	
(From apheresis	12	1587)	
Blood components, including plasma for fractionation		6893	
Blood components for clinical use		4426	36.8[a]
F VIII, F IX anti-inhibitor factor complex		4880	40.5
Inhibitors		227	1.9
Immunoglobulins		1 213	10.1
Albumin		1 293	10.7
Total plasma products		7 613	63.2[b]
Total blood components and plasma fractions		14 506	100

[a] i.e. approximately one-third of total costs.
[b] i.e. approximately two-thirds of total costs.

isolation and national authorities must carefully scrutinize every aspect of the chain of events and policies, extending from donor to recipient.

In summary, while policies can be employed to maximize the volume of plasma recovered in a well-balanced whole blood programme for fractionation, further technical strategies can also be implemented which will significantly improve the factor VIII yield of plasma as well, while the quality of red cells and platelets will benefit at the same time.

Chapter 12
Conclusion

The rapid development of recombinant DNA technology in the past 10 to 15 years is already creating a storm of change in the future prospects of therapeutic protein manufacture, which was hitherto derived from human plasma. Our objectives in this book have been to focus upon the salient issues involved in the fractionation of naturally occurring human blood plasma. Nevertheless, a few words must be devoted to the factors determining whether a given product is better prepared by molecular biotechnology, or from native plasma.

The foremost factor influencing this choice is cost. The development and licensing of a single recombinant therapeutic protein product may require the expenditure of hundreds of millions of dollars and the passage of more than 10 years. In addition, each individual scheme will usually yield only a single product, whereas conventional fractionation of human plasma has the potential for providing multiple valuable therapeutic derivatives.

Other factors which must be considered include the likely demand for a given product. Whereas only a few grams of factor VIII:C may be sufficient to satisfy the requirements of an entire population of haemophiliacs, albumin demands will range into tonnes of the material, which is clearly more difficult to achieve. A single dose of F VIII:C may be no more than 200 μg, where 1 g is worth about US$ 1.9 million in the market. Albumin, on the other hand, is usually administered as a dose of 10 to 12 g, worth only US$ 3.2 per gram.

Another consideration relates to the costs of purification. Recombinant products, unlike their plasma-derived homologues, contain residual non-human proteins and nucleic acids, from the culture cells or media. To consistently achieve and demonstrate the levels of purity and freedom from contaminants which is currently required may actually cost more than the production process itself. Naturally, the nature of the target protein will also influence production costs; the F VIII:C molecule is large and complex, whereas insulin, for instance, is small and simple. Whether mammalian cells are the obligate system for a given product (to achieve the correct glycosylation, for instance), or whether micro-organisms, such as *Escherichia coli*, or yeasts can provide an adequate expression system, will vastly affect costs.

Thus, the sheer magnitude of the potential costs, as well as the complexities involved in producing consistently safe, nonimmunogenic, effective products by biotechnology

will inevitably restrict their manufacture to a handful of financially muscular industrial firms in highly developed countries of the world. Similar caveats are also likely to apply to the use of genetically modified, or transgenic animals for the expression of human proteins, which has been shown to be technically feasible. Secretion of the requisite protein into the milk facilitates separation of the product from the synthesizing system, which would carry the advantage of being virtually identical to the native protein. Nevertheless, organizing production to comply with current pharmaceutical manufacturing requirements would be very problematical, and the limitations imposed by any system where expanding production from the original individual depends upon the reproductive cycle of the animal, or upon the ability to clone embryos, is also fraught with difficulty.

There is a theoretical rationale for supporting the development of a national plasma fractionation programme and achieving national self-sufficiency in keeping with World Health Organization recommendations.[8] However, such a task represents a major challenge, which requires the following infrastructural elements before it can be implemented with success:

- long-term political and public support, as well as the support of the medical community for the project;
- a well-organized, nationally coordinated blood transfusion service;
- establishment of a solid and safe regular, voluntary donor base;
- centralization of the blood/plasma collection system (see Annex 3);
- use of internationally recognized viral test kits for plasma screening;
- validation of the testing procedures;
- implementation of full quality standards and total quality management at the blood/plasma collection centres and throughout the national blood transfusion service;
- creation of an active, influential transfusion medicine programme which will promote the rational use of both labile blood components and fractionated plasma derivatives;
- appropriate conditions for local transportation and storage of plasma;
- appropriate selection of the plasma fractionation technologies, especially the backbone plasma fractionation process and the specific viral reduction steps, based on recognition by international regulatory bodies;
- evaluation of the benefits and limitations of a contract plasma fractionation phase prior to construction of a local facility, with the object of using this phase as a "forcing

[8] World Health Assembly Resolution, WHA28.72. *Blood and blood products,* WHO Official Records No. 226, 1975, page 110.

ground" for the development of technical skills and a sound quality programme (see Annex 3);

Note. Annex 3 gives two case histories which illustrate some of the advantages and disadvantages of the various strategic options discussed in the preceding chapters.

- appropriate recruitment of local managers and operators;
- a careful design of the local facility with due regard for good manufacturing practices, e.g. space required, product flow, zone segregation, water production techniques, personnel training, etc.;
- the introduction of a carefully designed, well-phased training programme and technology implementation;
- a research and development programme to maintain plasma products at their optimal level of safety, aiming at a constant commitment to building and improving, often step by step, the various safety nets already in place, from donor selection to quality control of end-products.

Annexes

Annex 1
Commentaries[9]

Plasma fractionation in emerging economies: a fiction or a possibility in a rapidly moving environment?

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Introduction

Several emerging countries have reached a stage where they must decide whether the procurement of plasma products should continue to depend entirely upon imports (usually from the USA or Europe), or whether programmes to develop local production should be implemented to increase the supply or provide self-sufficiency in these strategic products.

Procurement of products made from local plasma can be managed whether by organizing contract fractionation with established plasma fractionators, or by building and running a local fractionation plant. Is the establishment of a local plasma fractionation plant a viable choice at a time when cost constraints and demanding quality standards are an absolute requirement?

Environment

The blood collection/plasma processing environment in emerging economies in Asia, south America and eastern Europe, is heterogeneous but evolving rapidly. It can generally be characterized by several common factors, when compared to the USA, Europe or Japan:

- Irrespective of the size of the population, the volume of plasma collected locally is small.
- There are relatively few if any local plasma fractionators.
- The demand for plasma products is increasing, due to the greater degree of awareness of the clinical benefits of plasma fractions and the more favourable reimbursement policies provided by national social security systems.

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[9] Reproduced by kind permission of *Transfusion today* and the International Society of Blood Transfusion. The views expressed are those of the authors.

- Few foreign plasma fractionators are currently prepared to undertake contract fractionation due to technical difficulties (such as non-compliance with small pool processing) or regulatory constraints.
- Plasma product demand is generally met exclusively by imported material.

Challenges in a world of mutable technology

Plasma fractionation has witnessed dramatic technological improvements and regulatory mutations in the last few years, making this area complex and sophisticated. Therefore, if a decision to support local production of plasma products is implemented, programmes should be designed appropriately; the world plasma environment creates increasing economic pressure and cost constraints as well as requiring fulfilment of international quality standards. It would be unacceptable for local plasma fractionation programmes not to meet these standard quality criteria.

Some of the challenges which must therefore be faced include:

1. Establishing an appropriate and solid donor base to procure the volume and the quality of plasma required to meet clinical demand for plasma derivatives.
2. Implementing a viral testing strategy specific to the plasma for fractionation (which may differ from that which is already in place for cellular products and clinical frozen fresh plasma).
3. Addressing technical questions such as the capacity of the local facility, the size of plasma pools for fractionation, the appropriate choice of the fractionation process, especially protein purification techniques and viral inactivation procedures and the plasma fractionation plant design.
4. Implementing the appropriate logistic organization and total quality management, to ensure compliance with good manufacturing and laboratory practices and full traceability from donation to release of finished plasma products.
5. Ensuring appropriate public education, public perception and support being an important factor in the success of a national programme.

Establishing an integrated plasma collection/plasma fractionation system is therefore a challenging task.

Plasma collection aspects

In spite of the implementation of efficient viral inactivation steps during plasma product manufacturing, the selection of plasma donors, the establishment of a safe donor base comprising donors with a known medical history, the relevant exclusion of individuals carrying objective risk factors must never be underestimated. These remain an important means for ensuring a constant quality level of the starting plasma and represent a primary safeguard against emerging infectious agents.

Proper quality links and liaison between the organization in charge of collecting plasma and the agency responsible for the plasma fractionation process must be established. Indeed, ideally, plasma fractionation should be an integral part of national blood services to provide the necessary coordination and accountability. Plasma collection centre has obligations in terms of donor recruitment and selection, which must be of high quality and able to fulfil professional and ethical criteria, as well as reliability of plasma testing, while the plasma fractionator will have to audit collection sites.

Appropriate testing strategies

Single-donor testing

Single-donor testing is usually the responsibility of blood transfusion centres. The quality and safety of starting plasma for fractionation depends upon the appropriate implementations of screening tests performed on individual donations, just as it does in the case of cellular components. These tests contribute to ensuring a minimal load of the known infectious agents in the starting plasma pool. Nevertheless, a different, complementary testing strategy is now required for plasma destined for fractionation. Such additional tests are usually considered to be the responsibility of the fractionator.

Plasma pool testing

In large plasma pool testing, a sample of the plasma batch, obtained after the pooling process of hundreds of thousands of donations is taken to control, by traditional testing techniques, the absence of anti-HIV and anti-HCV antibodies, and of HbsAg. Although one can express justifiable doubts about the sensitivity of such assays due to the dilution effect, this strategy does at least contribute to ensuring the absence of a possible gross failure in the upstream screening process and it must be implemented until more sensitive and specific tests are developed.

PCR mini-pool testing

One such new test strategy, based upon a more sound scientific approach, is PCR testing performed on a mini-pool of plasma obtained by mixing samples from between 100 and 500 donations prior to the industrial pooling process. This strategy allows detection and elimination of individual donations positive for the virus markers tested. PCR mini-pool testing of plasma for fractionation has the advantage, over large-pool testing, of providing increased sensitivity and allowing identification of individual infectious donations prior to the pooling process.

Another advantage is that mini-pool testing uses assays which have a higher degree of sensitivity, thus opening a new frontier in establishing improved viral safety of the plasma pool for fractionation.

Considering the current technical difficulties in implementing and validating PCR assays, the choice of the viruses to be tested by PCR should be based upon scientific considerations and not irrelevant considerations from faraway countries, taken into account the local epidemiology as well as the virucidal potential of the treatment used during the manufacture of plasma derivatives.

Relevant choices

Policy makers must always make sure that validated tests are used for testing, but also that, when applicable, these tests are relevant to the specific epidemiology of the area, so that local variants or specific infectious agents which are potentially transmissible are detected. It is also important that decision makers and regulators, in their attempts to increase product safety exercise an appropriate balance of judgement based on objective scientific criteria, to selectively favour those measures or implement those regulations which will have a real impact on plasma product quality and safety, and are not carried out solely to please an uneducated, over-anxious public.

Plasma fractionation technologies

Protein purification

In terms of plasma fractionation technologies, significant progress has been achieved in the last few years. Considering the exceptional richness of plasma as a source of therapeutic products, it is essential that the initial 'backbone' plasma fractionation process is designed to permit the production of an appropriate range of plasma products when demand develops. To date, all established plasma fractionation processes have used cold-ethanol, sometimes combined with one or several chromatographic purification steps, to extract the main protein components of plasma, namely albumin and immune globulin, under satisfactory conditions for yield and purity. By combining ethanol fractionation with chromatographic techniques, it is possible to extract additional proteins like the coagulation factors F VIII and F IX, anticoagulant proteins such as protein C, or protease inhibitors like ATIII and alpha-1-AT. Making optimal use of human plasma as a source of therapeutic products and developing selective haemotherapy for patients with haematological and other disorders is a key element in the success of local progammes. Making four or five plasma products in place of only two or three, even using a small volume of plasma annually, is essential for the economical success of the project.

Current technology used to make plasma products is well characterized and can usually be transferred without significant technical difficulties. These technologies are easily amenable to small-scale operations. Recent techniques allow the development of new coagulation factors and protease inhibitor products possessing high purity and high potency. The purification process of such products also allows implementation of multiple viral reduction steps.

Viral reduction treatment

The appropriate choice of viral reduction regimes is an important decision for any plasma fractionation programme. There is no single definitive solution available to ensure the absolute safety of plasma products. What is required is that each individual product is subjected to a viral inactivation step ensuring unambiguous inactivation of known viruses like HIV, HCV and HBV. Processes based on solvent-detergent incubation and heat treatment generally provide a high viral safety margin for these viruses. Again, transferring such technologies to emerging countries is not difficult as long as the technical and human environment in which they are to be used is properly controlled.

Current recommendations include implementation of a second viral reduction treatment which is capable of providing efficient reduction of other relevant infectious agents. In Europe and the USA, emphasis has recently been placed on the reduction of non-enveloped viruses which may not have been inactivated during the first virucidal step. However, each plasma fractionation programme must assess the specific infectious risks linked to local epidemiology, so that additional viral reduction regimes are appropriately selected in accordance with an objective local assessment of the risks.

Good laboratory/manufacturing practices

Historical perspectives provide ample evidence demonstrating that product quality and safety depends upon the application of good manufacturing practices, for instance, the avoidance of crossed or downstream contamination or production errors linked to improperly sterilized equipment; appropriate design of new fractionation facilities and professional training of operators, which are all of crucial importance.

Conclusion

Plasma fractionation has become a highly scientific and technical field. Progress made in the last ten years in protein purification and viral inactivation treatments now makes it possible to manufacture well-tolerated plasma products with a high degree of confidence that they are safe from major plasma borne viruses. Implementing these technologies in emerging countries is reasonably straightforward as technologies are amenable to small-scale processing and fairly easy to scale up. Technical aspects may therefore not necessarily represent the major difficulties to be faced in establishing successful programmes, as long as transfer of technology includes appropriate training programmes and long-term technical assistance. Putting in place the relevant national organization, from collection of blood to distribution of products, may well engender more fundamental difficulties. Establishing a successful integrated plasma collection/ fractionation system often first requires an exceptional degree of commitment and vision from local blood

transfusion leaders, in order to assemble the various pieces of the complex puzzle described in this paper. The judicious support of the government and the public is usually essential.

Private industries, when they are involved, must understand the significance of plasma fractionation programmes to countries and they must not jeopardize quality in favour of short-term profits. In spite of these challenges, several emerging countries in the world are now reaching the stage where, on a step-by-step, and individual basis, they need to establish model national plasma fractionation programmes. These programmes may benefit from the past experience of Europe and the USA, in order to create and design today, what tomorrow's plasma fractionation world standard will be. The choice as to whether local procurement of plasma products should be based on a contract fractionation agreement with established, reliable foreign manufacturers, or on creating and running a local plant will depend on individual factors, such as, the availability of plasma in sufficient volume and quality, local expertise, and product demand.

A valued experience

J.G. Watt, Forfar, United Kingdom

National testing ability

It is important to differentiate between blood services and the national pharmaceutical regulatory services. In some countries these may be more or less the same but in most there is an agency, separate from the blood service or haemophilia treatment services, charged with the provision of safe biological products, whether made inside the country or imported. Often these were established in the heyday of antiserum or vaccine production and may not be much changed.

Frequently they occupy old and poorly equipped laboratories and are not staffed very well to cope with the demands of modem screening systems. Even more frequently they have rather inadequate budgets. For example, purchase of one domestic refrigerator may be the largest equipment expenditure for the year and purchase of one ELISA test kit for anti-HCV may equate to two months of salary for a pharmacist on the staff of the agency. They may well be aware of the advantages of PCR technology but can only dream of such luxuries. In many cases the assay ability is totally absent and they feel it necessary not to rely on the expertise of the blood service so that objectivity can be seen to have been maintained. One escape is to approach an academic department which just may possess the necessary equipment and expertise. Sadly, such facilities are rarely operated under conditions of good laboratory practice and little or no confidence can be placed on the results obtained. Also the chore is approached as an obligation and is fitted into the schedule of the laboratory when there is a spare moment which coincides with spare

reagents. Such processes take no account of clinical urgency and it may be difficult to get the assay done in a timely manner.

In many countries reliance is placed on assays made by the regulatory agency of the exporting country but it would be shaming to admit such dependency so it may be denied. There is an international camaraderie among regulators, especially at the laboratory and inspection levels so some help may come by this route. This is important because the personnel of some national laboratories feel under threat of loss of income if they admit that they are unable to carry out their function unaided. While it is now unusual to discover dumping of unsatisfactory materials into the developing world it was not always so nor is it now totally unknown so suspicion does exist and spurious or false positive test results add to this paranoia in some cases. It is wrong, also, to assume that all imported plasma fractions come to a developing country from the developed world. There is a significant and growing trade between developing countries and presents special problems for the regulators in the importing country.

They may not have great confidence in the quality of the approvals given in the exporting country but cannot refuse the material because it is available at lower cost than if it came from, say Europe or North America. Unlike products from developed country sources there may be major doubts on the provenance of the plasma fractionated and on the validation of any screening assays which may have been applied. This comes on top of their own testing deficiencies but they have to decide whether or not to accept the material. The judgement is not easy.

It is not always in the difficult or expensive test systems that problems come. For example, a regulatory laboratory may receive data showing that albumin has been tested successfully by accepted systems for particulate inclusions but they see visible particles in the supplied samples. These inclusions may be few in number but the finding is in apparent conflict with the data supplied. So they have the question; was the data wrong or is the product unstable? If there is no particle counter the question may be almost unanswerable and they have to make a decision. A similar laboratory in a developed country is never likely to face such difficulty and may not even recognize that such conflicts are possible.

Organizations doing the best they can do even though knowing it is not the best they should do are not helped particularly by authoritative statements unless these are geared to helping them improve their capabilities. ISBT and WHO have, in the past, counselled national governments to encourage voluntary blood donation and most developing countries subscribe to this ideal but some have.to take a realistic position so the voluntary and commercial systems have to co-exist.

It might be most helpful if these international bodies also gave counsel on the need for proper training and equipment for the regulatory agencies. In almost all countries, with a

few notable exceptions, there is sufficient money in the national budget to finance such relatively minor matters but it has to be recognized that, for many, the diversion of, for example, a new and even more splendid football stadium is politically more important than regulation of imported biological medicines. By comparison haemophilia care is an extremely expensive clinical service of benefit to only a few of the citizens. Health authorities in some developing countries, for example, barely admit that haemophilia exists as a problem because they know that such acknowledgement leads to identification of patients who will become an increasing major drain on their meagre resources so long as the patient may live.

Visitors from developed countries need to recognize that the poor conditions under which the staff of many regulatory agencies work is not due to lack of knowledge or lack of skill. Journals and textbooks may be scarce but they do filter through and nowadays many scientists in the developing world are adept users of the Internet with global access to technical knowledge. Their real lack always is investment or the political influence needed to make investment happen.

One sees occasionally gifts of equipment and reagents from multinational companies to national regulatory agencies. The officers of these companies know that if they wish their products to be available in a particular country it is necessary that import approval is given so they help the process by these gifts. If handled with sensitivity and without too many strings attached, such benevolence can assist more than international agencies with nothing but advice to be handed out. This seems to work best if the purchaser is independent of national government so is not associated directly with the regulatory agency.

Sadly, in several countries one sees another side where corrupt practice intervenes, often where the purchaser is also the regulator, and the main criterion of acceptance has little or nothing to do with the scientific or other technical merit of the product. In such circumstance the relevance of the assay to be applied is of no consequence.

HCV is not a new infection; it has been around for a long time under different guises. The only difference today is that its presence can be identified to some degree. Developing countries which have their own fractionation facility, usually insufficient to meet national need, may be obliged to operate on double standards. Does it matter if albumin preparations show the presence of HCV? If pasteurization has been done properly the risk is no greater than it was in 1987, 1977 or, indeed, 1947 when pasteurized albumin was introduced as a hepatitis-free product. Does it have to be demonstrated that the detectable antigen is: absent? For many developing countries almost all fractionation plasma may start contaminated and retain detectable characteristics, even when it has been rendered safe. Plasma destined to provide coagulation factor concentrates is a different matter and usually is recognized as such.

It has been experienced that medical opinion in many developing countries places a premium on the need for immunoglobulin for intravenous administration when the true clinical need is for the more mundane and less costly intramuscular product. To some degree this false appetite arises from commercial advertising literature and from envy of cutting edge publications from the developed world. That is not to say that the intravenous product is not needed but that it may not have the clinical priority it receives. In some cases physicians who work in both the public and private sectors are eager that the IV material be available in the country so that they may prescribe it in their private practice where it can be a conduit to enhanced earning capacity.

Intramuscular immunoglobulin derived from organic solvent fractionation systems has been around a long time and has an enviable history of clinical safety, especially so far as hepatitis transfer is concerned. Products subjected to chromatographic, purification techniques without benefit of virus inactivation have been less satisfactory in this respect. Provided the material is well made by the older systems it may still serve a great need in the developing world and does not need to be proven free from HCV contact; indeed may be more useful if it contains the neutralizing antibody.

There is no doubt that, in the best of all possible worlds, products proven free from all contact with all known transfusion transmissible infections are to be desired but the reality for the developing world is that the levels of safety deemed acceptable in the developed countries for the better part of the century may still be the best that can be afforded. Demands for more stringent testing may mean less clinical availability. Whilst it is desirable that the criteria for any medicine should be that it be stringently safe and efficacious the primary criterion may be that it be available.

This is not a plea for global double standards but seeks that people trying very hard and honourably to help their country improve health care provision are not presented with impossible hurdles at every step of the way.

I can recall things I did twenty or thirty years ago which make me shudder today, but I was doing my best with the knowledge available and the resources to hand at the time. On balance, I think I did much good and little harm and find it hard to judge or censure others who are working through the same process today. Providing, of course, that they are not reconciled to the existing system and do not seek to improve.

Establishment of fractionation facilities

In many countries, developed and undeveloped, the establishment of a fractionation facility has been the essential catalyst in creation of an organized and centralized national blood transfusion service with fully functional quality assurance programmes including GMP, system validation and a truly professional approach to the pharmaceutical aspects

of transfusion supply. Australia, France and the United Kingdom are good historical examples.

The most logical reason for state-supported fractionation development is the need for pasteurized albumin as a safe material for treatment of trauma. Albumin is the unique fraction of plasma which may be considered a national strategic resource. The other fractions may be of great clinical and emotional value but are not nearly so essential in national emergency. In many countries the coagulation factor concentrates might well be considered as orphan medicines were it not for their emotional connotations.

Paradoxically, it is often the need for these fractions which stimulates the haematologist community to demand or to create a processing facility for supply of materials to treat coagulation disorders. The interesting exception is China where the network of scrum and vaccine institutes, established in the early part of the century to combat infectious diseases, adopted small fractionation facilities primarily as a means of obtaining immunoglobulin for passive protection when it had been shown that heterologous antisera carried inherent dangers.

It can be argued that the appearance of biosynthetic materials, including the coagulation factors, will make plasma fractionation obsolete. The history of clinical use of plasma fractions has been spotted with accidents involving transfusion-transmitted infections (TTIs). Materials of cultural or transgenic origin should not carry such risks. Probably this argument is correct but not in the short term, particularly now that means for making products safer have been developed and in developing countries where the cost of synthetic materials remains prohibitive.

The argument is used that the individual physician should do all possible to aid the health of the individual patient but this is naive in a national context where health care budgets are constrained severely. If, by using the most expensive and safe material available at great cost for one patient, the physician places in hazard the health and welfare of all patients it might be argued that the decision is incorrect. That there may be serious side effects attached to the use of the more highly purified natural and biosynthetic materials makes the decision all the more questionable.

However, whilst there are some substitutes for products traditionally recovered from plasma this does not yet apply to human albumin, so the strategic aspect remains the most serious need. Until albumin is available at low cost from biosynthetic sources there will remain an unarguable case for plasma fractionation in all countries and especially so in countries which have poorly developed health care services in the public sector. Some may not wish, for reasons of security, that a strategic material like albumin be available only from sources outside the country.

The availability of by-products from recovery of albumin may be regarded as a bonus which reduces the cost of the essential provision. At least one country, now in course of

developing a state-owned but self-sustaining fractionation facility, is giving serious consideration to distributing a good intermediate factor VIII concentrate at no cost to the user. This will underwrite the cost of making a purified albumin and is barely worth the accounting cost. That decision may be logical but is probably wrong since everything has a perceived worth—that which you get for nothing is worth what you pay!

There is another very serious reason for the continuance of plasma fractionation in those countries where it exists and its creation in some where it does not. This is one of the few places where the medical profession comes into real contact with the pharmaceutical industry other than as a label on a bottle. That interface is important because the essential discipline of the pharmaceutical industry may have a beneficial impact on medical practice, particularly in developing countries. The concept of medical and surgical audit comes directly from this interface and has been shown of inestimable value. The philosophy for such self-critical analysis is often absent from medical practice in developing countries and needs to be encouraged, preferably from within the profession of the country concerned.

Constructive self-criticism is an essential part of the philosophy of quality assurance in the modern pharmaceutical industry and offers a useful example to physicians trained to avoid self-doubt. Since, most often, the need for plasma fractionation is noticed first by the haematologist wishing to treat bleeding disorders there is a common tendency for the fractionation facility to be driven by that need at the outset. That is often the start of the problems for the new plant since an outward-looking haematologist is not often also a good designer of pharmaceutical processing facilities. This is why many nascent plants are designed to resemble enlarged laboratories rather than effectively validated processing facilities. Sometimes, when government departments are involved, the initial step is to invite tenders for a plant and it is rare for this to be matched properly to the actual need of the country or to its ability to provide raw material. One such was scaled to operate at about 1000 kg plasma per week when the true plasma availability was around 100 kg per month with no plans in place to improve the supply. In other words, the customer gets what the supplier wishes to supply but the price may be the lowest offered. This may be construed as being dishonesty on the part of the supplying organization but is actually the result of a buyer who did not know or understand what was needed in the first place and was unable to judge the technology offered.

A modem fractionation facility needs to be in custom-built premises offering good separation of products which are free from viral contamination from those which are not and if it is designed properly the basic elements of good manufacturing practice will have been incorporated in the design. Even operators still learning the concepts of GMP will be encouraged by the design to operate the plant correctly in any case. Ventilation gradients and other services will be developed to ensure the safety of product and staff. Materials in process will flow smoothly through the process areas without opportunity for confusion

and all raw materials will arrive at the point of use, fully validated and shown fit for the intended purpose.

If a plant approaching this sort of performance is to be achieved the first stage must be the preparation of a good feasibility study which will examine first the needs of the country and the availability of plasma. A secondary programmer may be needed to develop plasma supply closer to the perceived need and this may require major investment in the 'existing transfusion services; or in a service resulting from rearrangement of existing facilities. A plant would be designed to accommodate a fractionation process at a level, which may well be lower that the optimum but in keeping with the expected rate of plasma supply. Such a decision would need to take note of the need for an expansion of the plant or for a second plant in due course.

In many developing countries, especially those with Hispanic connections, the existing transfusion service will be hospital-based and very dependent on the "Buddy System" where friends and family are constrained to provide blood and plasma. Such systems are not really able to support a fractionation facility because the donor population is not motivated in an altruistic sense and is not well enough organized to be useful as an organized service. In such cases the transfusion organization tends to employ many people rather inefficiently and these, naturally, are resistant to change because they see their employment under threat. In any event, the quality of plasma they can provide is usually rather poor and not suited to production of coagulation factor concentrates. Screening for TTIs is not usually controlled properly so little reliance can be placed in the result which means that the fractionator has to test again under better conditions and extra cost.

Expert help at this stage should result, in collaboration with local professionals, in the development of a sketch plan for the facility with a full schedule of accommodation and definition of the equipment and services required in each area. With this should be also the base plan for operation of the plant according to GMP requirements. If tenders are sought at this stage the purchaser should be in a position to make a serious assessment of the value of each tender in terms of expected performance as well as cost.

It is possible that, during the study, it becomes apparent that the projected plant will be too small to be viable or the development of plasma collection will require more time. In such case the best advice may be to seek contract fractionation from a plant already in operation in a neighbouring country or from a commercial plant in a developed country. If the latter is to be the case it will be necessary to demonstrate that the quality and safety of the plasma meets the minimum standards of the country in which processing is to be carried out. This, for many countries, may be close to impossible due to TTIs endemic in the population. Then sharing with a neighbour is one option and the other is to build the plant, whether or not it is to be cost-effective. Sharing works if one country already has a plant and the neighbour contracts a fractionation service. It tends not to work if neither has a facility since there comes to be disagreement on where the plant may be sited.

During the construction and commissioning phases it is important that plant and process engineers with pharmaceutical experience are available to the project. It should also be possible to discover pharmacists with industrial experience although, in many countries, these are scarce and expensive. It is not likely that these will have experience of protein manipulation but if they start with some basis they can build on their experience. Few countries have no pharmaceutical industry and many have in-country facilities that are part of a multinational venture. Thus some, at least, of the needed GMP concepts should be known and can be obtained without expensive training.

Possibly the most important requirement is to establish a strong board of directors for the new venture. Some at least should be drawn from industry within the country as a pool of commercial experience. The board should not be dominated by bureaucrats or academics. It may be advisable also to establish an advisory group drawn from academia and the medical community but it is not commonly possible to find such people also with real industrial management experience. They will need a budget to cover the capital cost and the commissioning cost. The complexity of the latter phase is most often underestimated and under-funded.

One important function for the advisory group is to introduce the physician community to the best practice in use of plasma fractions. Some may have some experience of some fractions but not all will be equally skilled and wasteful uses will occur. For example, the use of albumin to correct chronic malnutrition or protein-losing conditions is often seen in developing countries and needs to be controlled. The choice of the correct innnunoglobulin preparation and when it should be used is a difficult problem since many physicians desire to use only the intravenous variant, frequently without logical reason.

It is quite common to send staff to established fractionation plants in other countries for training but in most cases this hardly justifies the expense. It tends to be senior graduate staff who are sent when it might be more useful if it were technologists; provided they have a good grasp of the language of the training site and are able to get true, hands-on experience. These should be contracted to work for the venture for a minimum period after return since, in many developing countries, such people are much sought after for other projects.

The actual technology of fractionation, even today with the complexity of viral inactivation added, is quite simple but the application and the development of good housekeeping practices, about 85% of the task, is very hard to establish and usually takes much more time than is realized at the outset. This arises in part from a general desire to set the science to work rather than get the more simple and mundane activities started first. The development of good validation practices and the consequent development of documentation take much longer and are more costly than appreciated. Establishment of standard operating protocols and the documentation these generate also is costly in terms of time at the outset. Scientists who are unused to manufacturing discipline are often

unwilling to accept this at the start and need to learn that they should be part of a continuing assessment programmer to ensure that the error of all assays and measurements is known and up-to-date. Some never accept this discipline and are not useful for the venture.

Once the facility is commissioned with all systems operational it is natural that attention be paid first to the most desired fractions and these, most likely, will be factor VIII concentrate and factor IX, either as a separate concentrate or as a complex of several coagulation factors. However, the initial quality of plasma is probably more suitable for recovery of albumin with discard of all other fractions and it is best that albumin be the first product extracted from plasma; not least because the recovery is complex but relatively simple to control. It tests all aspects of the process system and can be achieved with plasma of doubtful quality.

Albuminoid products have a long history of safety and are relatively easy to be licensed for clinical use so the new venture is most likely to get useful product for distribution at the earliest time possible. This is important for the image the venture should have in public perception and for its commercial well-being. It is also extremely important for the staff of the new venture who need the encouragement of success at the earliest time possible to recompense them for the hard and apparently non-productive work they have done to get the venture operational. Even a venture financed by the state should know the commercial reality of its activity; if only in terms of import substitution.

As soon as a routine operation is established it will become apparent that not all of the system design is fitted to the strain of daily operation, and it will be necessary to redesign operating protocols in the light of experience.

Plasma fractionation—the Tunisian experience

K. Boukef, Tunis

Tunisia currently has a National Blood Service and a separate military centre. The latter also comprises a plasma fractionation unit which has been officially authorized to produce and market human albumin by the Ministry of Health since June 1995.

Since the introduction and widespread use of plastic blood bags in 1992, surplus plasma has been collected and stored frozen. Our first experience of contract-processing Tunisian plasma was carried out with the French National Transfusion Centre (Paris-Les Ulis). This first batch of some 800 L allowed us to procure albumin and intra-muscular immunoglobulin from our local plasma.

Following the French "blood scandal" and the mandatory introduction of anti-HCV screening, it has become increasing difficult for us to find a partner for the contract-fractionation of our plasma, however. Discussions have taken place with Octapharma (Austria), ZLB (Switzerland), ISI (Italy), with no satisfactory outcome. We persisted

nonetheless, and in March 1996, discussions were resumed with LFB (Lille), which recently despatched an audit team to the Tunisian National Transfusion Centre. Soon after this visit, a contract for processing Tunisian plasma was signed in July 1997. This contract may well become operational by the end of 1997, and is likely to involve about 4000 kg of plasma for fractionation to albumin and intravenous immune globulin.

At present, Tunisia does import stable plasma products such as albumin, polyvalent immune globulin, as well as fibrinogen, F VIII, etc. through the Pasteur Institute of Tunis. These products are controlled by both the Pasteur Institute and the National Central Laboratory for Drug Control. Each batch is controlled in accordance with European pharmacopoeial standards, and in the case of HCV in particular, an ELISA test is carried out. If this preliminary screen is positive, a supplementary genomic amplification assay (GAT) is performed. Positive or indeterminate reactivity by the GAT leads to discarding of the entire batch, while a negative result allows the batch to be issued and put on the market.

In conclusion, countries which have succeeded in developing national blood services in keeping with international standards are faced with the need to find some means for processing their excess plasma, particularly in those cases where the volume of plasma collected is not sufficiently great to justify the development of a local fractionation facility. In addition, the spectrum of products may have to exclude the coagulant fractions, unless the quality of plasma collected attains the requisite standards.

The main future problem for developing countries will be the increasing predominance of biotechnology products.

Plasma fractionation—a Finnish view

J. Leikola, Helsinki, Finland

The editors raise interesting issues. First, assays designed for screening donor serum or plasma are not directly applicable for finished plasma products. We have had tremendous problems at our Service when validating the use of serological tests for intermediate or finished plasma products, and still, I believe, we have a reasonable amount of both experience and expertise. I can just imagine a state control laboratory somewhere trying to check the "purity" of plasma proteins. Controls that do not measure accurately what they are supposed to measure are, of course, of no value and may be even harmful. It reminds me of a small local firm in Finland, importer of a serological test. They sent it to the Finnish Red Cross for evaluation, and we found that the test was not acceptable. They went ahead with marketing anyway, and the salespeople reportedly told the customers that "our kits have been tested by the Finnish Red Cross". This was true, but not necessarily all the truth. The second question is more difficult. In the past, the estimates for a minimum size of a fractionation laboratory to be profitable varied between 100 000

and 1 000 000 litres, and then it turned our that each well-informed source quoted his own fractionation capacity. Currently, our operations with approximately 100 000 litres break even, where Pharmacia with 250 000 litres makes losses.

When to construct your own laboratory and when to send the plasma abroad for contract fractionation? I was confronted with this question very recently when I evaluated the system in Estonia. My clear conclusion and recommendation was contract fractionation, but even there are difficulties. A reputed fractionator usually demands that the plasma sent in for contract fractionation fulfils all the requirements by the authorities of the country of fractionation. This entails good recording system, quality assurance, etc. included in the plasma master file. If you want to make just albumin, which is safe anyway, I think that in a developing country it would be a waste of time to build a western testing and QA system for just contract fractionation. On the other hand, manufacturing of safe products of good quality cannot be cost-effective in volumes of 10 000 litres.

Looking around what is going on in the world does not help you much in the reasoning. The French fractionation laboratories were reduced to barely two, and of the 5 German Red Cross fractionation centres that were members of EPFA at the time of its founding in 1990, only one survives (Springe), and even there we do not know for how long. On the other hand, the Czech government has decided to build a fractionation laboratory in Prague, the Lithuanians have serious plans to construct one, the Brazilian government has already allocated funds for 3 fractionation establishments and Ankara, once again, wants to build one for Turkey. The decision is eventually a political one. I think that e.g. BPL could easily handle all Scottish plasma if it would be politically possible.

I think the answer to the first question is: each test system must be validated under local conditions for the intended use before starting the routine. The second question is too difficult for me to tackle, and leave this question to other experts in field to be answered properly.

Quis custodiet ipsos custodes (Who will regulate the regulators)?

C.V. Prowse, Edinburgh, United Kingdom

I write to share the two concerns (Editorial theme, *Transfusion today,* 1997; 33:3) in relation to this field, and to invite comment from other readers.

In general

Regulations of blood products and blood services is currently a growth industry, for understandable reasons. Increasingly, many countries are requiring their own regulatory institutes to assess the potency and safety of blood-derived products prior to issue. Such

institutes vary in their funding, degree of independence and competence. While there are moves, for instant through the Committee for Proprietary Medical Products (CPMP) in Europe, to ease the pressure on producers by providing licensing arrangements that cover multiple countries, in practise these still require individual implementation at a national level.

First question

Firstly, the proliferation of regulatory activity brings with it an inevitable additional cost that has to be borne by the health budget of the countries involved, some of whom can ill afford it. As the blood product business becomes increasingly multinational, this will result in multiple testing of individual product batches, particularly for recombinant products. Given the number of regulatory institutes, the quality of assessment is bound to differ, if for no other reason than the fact that the levels of funding for such activity vary. Such differences are likely to lead to difficulty, particularly in those areas of technology that are at the forefront of technological development, such as genome amplification technology (GAT). All of this would seem to suggest room for mutual recognition procedures, whereby results from one regulatory institutes could be accepted in other countries, and could allow established centres to undertake assessment on behalf of countries less able, for whatever reason, to undertake their own.

Second question

Given the above, the second question (implied by my title) then arises. Who assesses the technical competence of regulatory institutes? At present there would seem to be little formal process for this. While individual institutes may participate in external quality schemes, such data is not overtly made public, and appears to be only audited internally. Although I am sure common sense usually prevails, perhaps it is a question worth posing to supra-national authorities, such as the CPMP, whether it is not time that some form of certification of competence of institutes of the kind described above, be considered. I am aware that schemes such as these operate in some countries, such as Germany, already.

The views expressed above are individual and do not represent, necessarily, those of the Scottish National Blood Transfusion Service.

A plasma fractionation adviser's perspective

J.K. Smith, Oxford, United Kingdom

The fractionation issue

It is true that many plasma proteins may now be prepared by various gene technologies, and indeed factor VIII and IX concentrates are now in licensed use.

Demonstration of the ability does not always guarantee industrial exploitation. Proteins important to people with rare deficiencies may not be produced economically in this way. One suspects that normal IgG will be difficult to prepare by recombinant methods. It is not clear that albumin may be better or more cheaply made in this way. Plasma-derived (PD) proteins are being made virtually safe from virus transmission by addition of new, non-invasive stages such as virus filtration.

Since any populous country with a thriving blood transfusion service will have a relatively cheap supply of plasma for the foreseeable future, the economics of recombinant protein production will have to improve if competition from PD proteins is to disappear. Some countries may see a 20–30 years future for plasma fractionation. Of these, some will be persuaded to invest in a complete "turn-key" plant from an industrially-developed country, with at least initial assistance in management and control of production. Production practices and product quality would inevitably converge on the standards existing in USA and Europe, but the country would be free to make high-yielding "no frills" products at least as safe as those currently imported.

It is less likely that we will see many new "national" plants being built by small countries wishing to have strategic independence in the supply of products. The initial costs are daunting, there may be difficulty in recruiting well-trained nationals to key posts and the industrial infrastructure may be developing too slowly. Above all, it has become very expensive to generate new products, license them and their associated plant, and keep both in compliance against rising international standards. The overhead costs of quality control, research and development and clinical trial programmes fall most heavily on laboratories with a small product base. I would regret the loss of any of the existing smaller fractionators, who have contributed disproportionately to high quality standards, novel processes and fresh thinking in the industry.

Some developing countries, especially the smaller ones, will be attracted to contract fractionation of their FFP by long-established companies, with secure return of the products from those plasma pools. In theory, there is excess capacity worldwide and it is sometimes possible to make a mutually satisfying long-term contract with a fractionation company. In practice, it is usual to invite tenders regularly for a short-term agreement.

It may be less attractive for a large country to commit all its plasma to a single commercial company, operating in a local plant or abroad, yet it becomes difficult to offer a single national product if it is being made in several plants under different national regulators.

A populous country producing a lot of plasma may, of course, find itself operating several of these options, simultaneously or sequentially. There is no optimum volume at which any single option becomes compelling without reference to the strength of local blood transfusion, the flow of resources and the expectations of clinicians who may have been using newer products. Any country which has imported a substantial proportion of

its plasma products will come under heavy marketing pressure to expand provision in the same way, rather than set up its own plant or attempt to control contract fractionation. However convenient, simple purchase of products is blind alley. There will be no advance in national understanding of the issues and the factual basis for important economic decisions. It may also undermine national investment in comprehensive blood transfusion services.

In order that local control be maintained in the balance between supply, price and quality under the different options outlined above, I would argue that the first institutions to be strengthened should be those of the national regulatory agency.

A cadre of scientists and pharmaceutical experts, trained in the regulatory institutions already controlling large companies, but responsive to their own national realities, should be recruited in advance of any fractionation undertaking and their independence secured by appropriate legislation.

The safety issue

A high proportion of plasma products for export are made by fractionators whose domestic products are batch-released by a national reference laboratory serving the exporter's regulatory agency. In many case, batches for export are also batch-released by the reference laboratory and if this were mandatory, many purchasers' fears might be assuaged. These laboratories may be relied upon to use the most sensitive and specific tests available, in line with WHO, FDA, EMEA or pharmacopoeial guidelines. The regulators must also be satisfied with the provenance and testing of plasma by the exporter, described in a lengthy Plasma Master File. Several firm guidelines require that processing includes virus-reduction stages and that the calculated residual risks of transmitting important viruses should be minimal.

European regulators now specify at least "2nd generation" testing for anti-HCV and, from January 1999, will require gene-amplification testing for HCV virus [1]. Such standards have applied for several years and few batches made prior to 2nd generation testing should now remain within their original dating period.

FDA was originally reluctant [2] to permit the use of anti-HCV negative ("1st generation") plasma for production only of IgG. Their hypothesis that some neutralizing antibody would be lost from the pool was tested experimentally and found invalid [3]. Ironically, the original hypothesis appears to have been vindicated, at least partially, by the later transmission of HCV by Gammagard IV Ig. I believe that this was associated with the introduction of 2nd generation testing, more effectively eliminating donations containing protective antibody, but still passing highly infective donations in the "window" period [4]

I do not wish to convey the impression that these difficulties in making and reviewing national decisions are confined to industrially-developing countries. They simply present themselves at a later point in the national growth of blood transfusion. Perhaps a later forum could address the fragmented and overlapping policies of non-profit fractionators in the allegedly developed world.

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Human plasma derivatives for developing countries

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The interaction between developing and the more developed countries regarding plasma derivatives has been difficult for the past 10 years. During the sombre period 1985–86, some fractionators from several continents attempted and, on occasions, succeeded in delivering products that had not been submitted to appropriate procedures of viral inactivation. Since the early eighties, several countries in the African continent have been offered various collaborations from government, non-profit and for-profit organizations that were often more interested in collecting data to publish and obtain headway over competitors, than in the pressing needs of the host countries. Although these practices have almost completely disappeared, a climate of suspicion has developed. The trust that takes years to build can very quickly vanish and takes many more years to rebuild.

In that context, developing countries who need to import plasma derivatives for their internal consumption tend to distrust the manufacturer's documentation accompanying lot release and the described methods utilized to prepare the products. As a consequence, some have instituted quality control tests to satisfy themselves with the quality of the goods they import. The means and experience of government agencies who perform these tests are limited and results may appear questionable to the wealthy providers. Enzyme immunoassays (EIA) for antibody screening have been developed and licenses for testing

in serum and plasma. The delicate balance of reagents and procedures developed to achieve optimal sensitivity and specificity is easily jeopardized when used in any other medium. This includes protein preparations of various degrees of purity and concentration like plasma derivatives. Dilutions in normal plasma would be effective to overcome the problem, but, when considering the extremely low risk of contamination by HIV, HBV or HCV in plasma pools made from over 10 000 donations, the dilution factor is such that only genomic amplification techniques (GAT) such as PCR have a chance to detect the agent associated with the diluted antibodies.

Safety of the blood supply has been a continuous concern of national and commercial organizations alike for blood components, source plasma and plasma derivatives. All developed countries have implemented screening for syphilis, HBsAg, anti-HIV-1, HIV-2 and HIV-O, and anti-HCV. Some of these countries also test blood donations for anti-HBc to reduce hepatitis B post-transfusion infections, HIV antigen to limit infections during the pre-seroconversion window period, and antibodies to HTLV-I and HTLV-II. Finally, some are still screening for elevated ALT, although the efficacy of this test has been serious questioned after the implementation of anti-HCV. To these extremely efficient EIAs, genomic detection of HIV, HCV and, to a lesser extent, HBV, will be added in the coming few years. The discussion is not to decide whether or not GAT should be used, but when, how and at what cost? GAT is expected to reduce and possibly eliminate the risk of viral transmission related to the window period when applied to individual blood or plasma donations. It is to be understood that, when screening assays become mandatory, all parties involved comply and control agencies verify the quality of testing before granting or renewing licences.

The second line of attack against viral transmission by plasma derivatives, is the many processes of viral inactivation applied during the fractionation or on the finished product. Heat treatment, pasteurization, viral membrane disruption by solvent detergent or other methods, are mandatory, and today are applied to a considerably reduced bioburden which makes these methods more effective than at the time of their development.

Some developing countries essentially need factor VIII to treat patients with haemophilia A. One should not forget that, for at least 10 years, developed countries relied on frozen cryoprecipitate which, though less practical than concentrates, provided perfectly adequate treatment. Cryoprecipitate can be prepared in small blood banks, in sealed blood bags, with minimal equipment, and used either as single units or in small pools of 2–12 units. In developing countries, the frozen material would require patients to be treated at the manufacturing site or the hospital, but could be freeze-dried, allowing local and even home treatment. Locally prepared frozen cryoprecipitate currently covers the needs of Zimbabwe.

Supplying developing countries with plasma derivatives can be approached from a totally different angle: the manufacturing products from local plasma processed under

contract. Some developed north American (Canada), European (Greece, Ireland, Norway, Poland) and Australasian countries (New Zealand) with a population or finances that do not justify the considerable investment of a fraction plant are testing and collecting their own plasma, which is shipped abroad to a non-profit or commercial manufacturer. Final products are then returned to the country of origin for a negotiated fee. This approach has already been considered and preliminarily implemented in some developing countries. In south-east Asia, Malaysia and Indonesia ship plasma to Australia for fractionation. In Africa, plasma from Zimbabwe and Namibia was or is processed in the relatively over-sized fractionation facilities of South Africa. The investment required for these ventures is essentially plastic containers, flash freezing cabinets and substantial storage facility at – 35 °C. In addition, the cost of plasma collection and testing procedures requested by the health authorities of the country where manufacturing is performed have to be met. Massive quantities of plasma can then be transported frozen by air or road to the fractionation plant. Depending upon the actual needs of the plasma collection country, some of the plasma fractions which are not in great local demand, can be left to the manufacturer as part of the compensation for the service. The primary demand varies according to countries: in some albumin; in others, factor VIII concentrates, or both. IgG, although rarely in demand, historically is of increasing therapeutic importance. Once the cost of preparation of adequate course plasma is covered, the plasma provider receives back the products it needs at nor or minimal cost.

One of the issues for the potential manufacturers is that they require that the standards used to select their own plasma be met by the country where plasma is collected. HBsAg and antibody to HIV are widely screened with adequate techniques, but the low prevalence of HCV antibody and the cost of screening did not encourage developing countries to implement a systematic screening they cannot afford. They rarely went beyond epidemiological studies to assess the HCV infection prevalence in their blood donor population. While international aid has provided substantial help to many developing countries to establish and support anti-HIV screening, such is not the case for anti-HCV. In countries or populations where HIV is endemic, the clinical impact of the window period in transfusion practice and plasma derivatives is quite considerable. Published and unpublished data from south-east Asia and Africa indicate that the HIV antigen test as a definite efficacy. However, the protection provided by this test is only partial, covering 25 to 50% of the infectious window period. The bioburden being higher than in plasma pools from countries where HIV prevalence in blood donors is often more than 100 times lower, the relative efficacy of the viral inactivation procedures might be less.

Developing countries may also, in time benefit from the integration of market forces in developed countries. Recombinant factor VIII is progressively increasing its market share; recombinant albumin will soon be commercialized in Japan and may follow suit. Polyvalent immunoglobulins already are, and will become, even more the driving product. Its cost will increase to cover manufacturers' expenses and profit margin. By the same

token, prices of albumin and factor VIII extracted from human plasma will decrease and may become more affordable by the less affluent countries.

Developing countries have limited resources for health care and these resources are often unequally distributed in the population. The choice of limited importation of expensive products for a few has to be put in balance with other ways to obtain for more patients more product at a lower cost. The co-operative approach suggested above would require strategic decisions for both the developing country governments and the potential manufacturers. With the development of recombinant clotting factors and other plasma proteins, one can anticipate that more spare capacity will become available and that plasma fractionators will welcome the opportunity to improve the return for their investment by contracting with countries who desperately need products and are prepared to embark in an efficient plasma collection strategy. Mutual benefit usually provides the underlying basis for the best collaborations.

Herrington's view

B. Herrington, Essendon, Australia

Thank you for your kind invitation to make comments on the subject of supply of fractionated plasma derivatives to "developing countries" (Editorial theme, *Transfusion today*, 1997, 33:4). The views expressed here are those of a private, although involved and concerned, individual.

Question 1

Testing of finished product for virus markers is an anachronism gradually being dismantled from the repertoire of prescribed tests by more forward-looking regulatory agencies such as the EMEA. There has been some backsliding by FDA, and I allude to that agency's requirement to test finished product immunoglobulin for HCV RNA. I think most transfusionists would now agree that reduction of risk of viral transmission by a plasma product has to rely on the quality of the starting plasma and the effectiveness of the production process to remove or inactivate virus. The concept of testing finished products for virus markers using test kits that are not designed, manufactured or validated for that purpose is flawed. It is essential that this message be conveyed by whatever means to regulatory authorities in developing countries. This could take place through direct contact between regulatory bodies or via the transfusion specialists of WHO, or groups of national fractionators such as EPFA.

To allow the national control authority (NCA) to have confidence in the safety of a plasma fraction, it must be able to independently assess crucial data with respect to the quality of the starting plasma used for that batch of product and the virucidal potential of the fractionation process. I will comment on these two aspects separately.

In Europe and in Australia, manufacturers are required to submit a document called Plasma Master File (PMF) to the licensing authority when applying to market a plasma product. The document is updated annually. This document describes in detail the methods used to select plasma donors (including epidemiological statistics on transmissible disease for the region where the donation centre is located), to collect plasma and to test plasma before use for manufacture. The document also contains a copy of the contract between the plasma supplier (collection centre) and manufacturer that describes the quality assurance carried out by the manufacturer to ensure that the collection centre meets the required standards. It is my belief that access to the PMF would give reassurance to the NCA on the general quality of plasma used. I will discuss individual batch testing below.

To obtain marketing approval in Australia or Europe, a plasma fractionator must submit a dossier which gives a details description of the virus safety profile of the product. This includes the validation of the process for reduction in load of actual blood-borne pathogens or acceptable marker viruses. The manufacturer is required to assess the potential level of contaminating pathogen in starting plasma pools using data from the PMF and the validated dilutional sensitivity of the tests he applies to plasma pools. Using the validated process reductions and the potential starting titre, the risk of transmission of blood borne pathogens is calculated. This risk number can also be used by the NCA to compare various manufacturers' claims.

Fractionators marketing products in Europe and Australia must meet certain requirements of the European Pharmacopoeia which describe the minimum requirements for starting plasma. Manufacturers must test the first homogeneous starting pool for virus markers using appropriately sensitive tests (the appropriate sensitivity of this pool test is being debated in Europe and Australia at present. The governing body of regulators in Europe, the CPMP, has stated that testing of pools for HCV RNA is to become mandatory. This test would possibly be beyond the means of laboratories in countries with developing economies). The validated dilutional sensitivity of the tests are described in the PMF. The results of these tests could be made available to the NCA on a batch by batch basis. In certain countries where the NCA's approval is required for batch release of plasma fractions, provision of these tests results is mandatory and the test is sometimes repeated by the NCA on samples of the starting plasma pool provided by the manufacturer. This avenue could form the third aspect of reassurance required by NCAs before supply of a plasma fraction is permitted.

Question 2

This is indeed a huge topic which you will agree has baffled many learned committees over the past few years, ever since the inevitability of the introduction of genetically engineered safe plasma proteins became obvious. I am not competent to draw any logical conclusion on such a complex issue, but rather I would like to suggest a few proposals for

consideration. I see the fundamental question as "Why would a national government or a private investor wish to invest some $ 200 million to build a national plasma fractionation plant?"

The private investor must see this as a profit-making venture; one would assume that most of the initial outcome would be from selling to the government. It therefore follows that the government is fully aware of costs and profit margin.

If the national government were investing, its capital outlay would need to be amortized against the savings made in not choosing alternatives such as contracted fractionation or purchase of "off the shelf" commercial product. Within a developing economy, it is unlikely that the government would be expecting the investment in plant to yield commercial profit (for example, by fractionation and sale of product from commercial plasma). It is more likely that profit could come from performing contract fractionation for other countries. This topic was raised for the south-east Asia region many years ago, when it was proposed that several countries invest in a plant. International agreements of this sort are notoriously difficult to obtain.

The government may have a deliberately protectionist policy on pharmaceutical manufacture, which is designed to boost the local economy. This will undoubtedly carry over into the area of pricing of imported pharmaceuticals. Such a policy may extend to differentiating between the permitted GMPs for locally manufactured goods compared with imported goods. The availability of hard currency to pay for the fractionation costs or for commercial product is also a major problem.

Perhaps the reason to invest is the belief in national self-sufficiency. Personally, I cannot see any difference between fractionated plasma products and other well-regulated pharmaceuticals. (This ignores the question of the quality and safety of starting plasma discussed previously.)

Having invested in an infrastructure that includes a group of well-trained transfusionists, scientists and engineers, where are the products? It is unlikely that such a group could develop even one product at today's standards, starting from scratch through pharmaceutical, pre-clinical and clinical development, within five years. The technology must be obtained through licensing and technology transfer agreements. Such technology is no longer freely given. The rapidly evolving state of the art and the legal necessity to stay at the cutting edge require an ongoing commitment to research and development: hence an additional investment cost to be borne in mind.

My personal opinion is that the entry-level costs are far too high compared with alternative options discussed below. What then are the alternatives to such a capital-intensive venture?

An obvious answer is to pursue one or more contract fractionation agreements. Contracts with reputable fractionators would require development of a transfusion service with plasma collection facilities that will meet the fractionator's requirements. On the other hand, the purchase of this contract service would be able to satisfy themselves and their NCA on the safety of the products being supplied. The plasma supplier is able to specify which products of the available range are to be supplied and the disposition of any product surplus to needs. There are many formulae for deriving the agreed price for a contract service. Here it is important for the plasma supplier to know the true cost of a litre of its plasma, so that the cost of the fractionation service can be compared with commercial "off the shelf" products. Another important determinant of pricing is the availability of surplus capacity from both national and commercial fractionators. As alluded to above, even national fractionators are being forced to be self-funding. An opportunity being pursued by many is to invest in additional capacity that can be sold for contract fractionation purposes.

Another alternative is not to fund the transfusion service for plasma collection, but to simply purchase the national supply of plasma fractions from commercial sources. Given the reassurances now available concerning virus safety, this must be an approach that has the potential to save healthcare dollars. The reasons for the inevitability of price rises in this sector should be understood. I see it as stemming from the Western consumer or his lawyer refusing to accept anything other than zero risk. As the supply of lower risk alternatives broadens, the fractionator is forced to spend more on testing plasma and enhancing the perceived virus safety of his product. What government of a self-respecting developing nation is going to accept products with lesser safeguards? We have recently seen the consequences of governments or their bureaucrats taking a more pragmatic approach.

Looking at the question from the point of view of a government authority concerned with maintaining a supply of safe blood products, neither approach is entirely satisfactory. A single fractionation contract puts supply at risk if that contractor fails to perform (lead-time to manufacture these types of product can be up to six months). A shared contract is satisfactory, but this does not overcome the problem if a disaster hits the transfusion service. An example could be the known contamination of multiple lots of product with plasma from a CJD-afflicted donor. The option of commercial "off the shelf supply" should be retained.

My personal opinion, in weighing up the merits of the arguments proposed above, comes down in favour of a contract fractionation policy that retains the option of open market supply.

Annex 2
Statistical process control

Test systems are expected to vary within a narrowly defined range (usually no greater than plus or minus 3 standard deviations [SD] of the mean expected value) calculated from historical data. Statistical process control (SPC) charts show the monitoring period in question on the *x*-axis, and test result values on the *y*-axis. The *y*-axis is marked with the mean value and the range of plus or minus 3 SD.

A system under control must meet certain criteria: all points must be within ±3 SD from the mean; two-thirds of all points must be within ±1 SD from the mean.

A system out of control is demonstrated by: runs of 7 or more consecutive points up or down in daily readings; runs of 7 or more consecutive points above or below the mean; a cyclical pattern. This "early warning system" allows problems to be identified and resolved before system failure occurs. Figures A2.1, A2.2, A2.3 and A2.4 illustrate examples of both in-control and out-of-control SPC patterns.

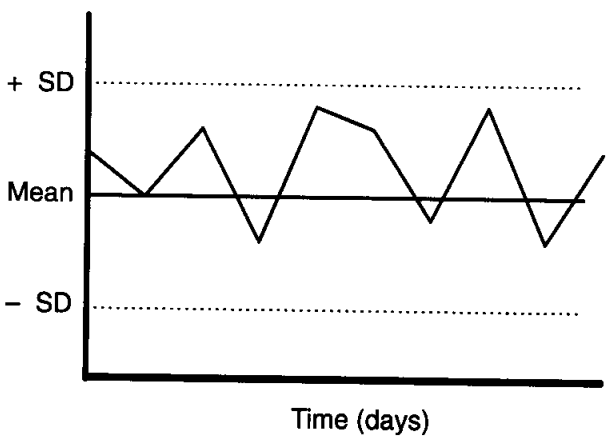


FIGURE A2.1 **Example of in-control SPC chart**

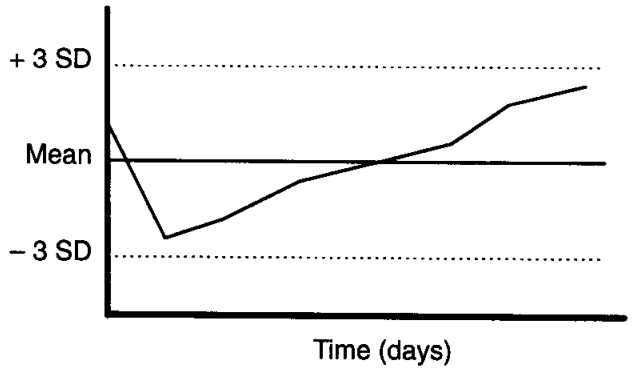


FIGURE A2.2 **Example of an SPC chart which shows an upwards run of 7 points**

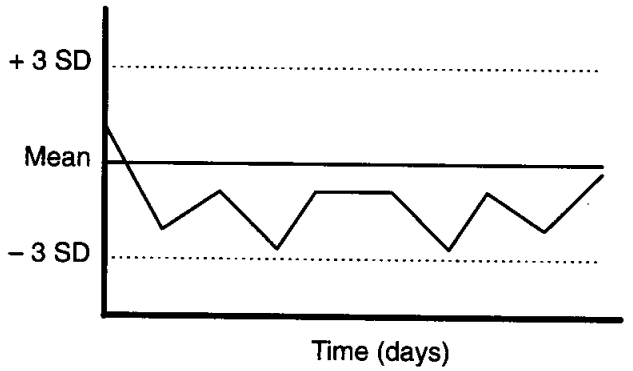


FIGURE A2.3 **Example of an SPC chart which shows a run of 7 points below the mean**

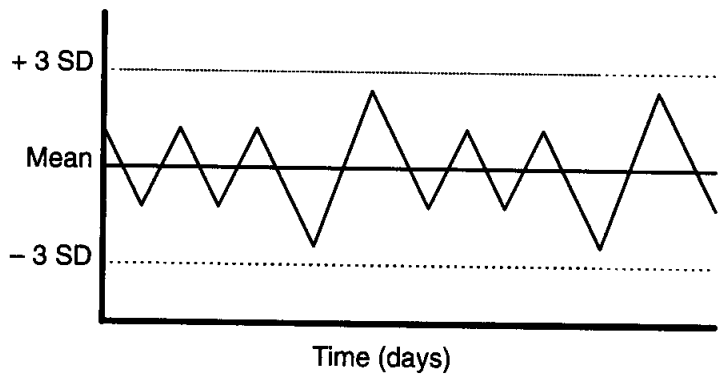


FIGURE A2.4 **Example of an SPC chart which shows a cyclic pattern**

Annex 3
Case histories

Case history 1

Background

Farzia is a country of 5 million people with a rapidly expanding economy due to the large-scale exportation of petroleum products. The health care system has been recently created and has sophisticated facilities, most particularly centred in the capital city. Many expatriate personnel staffing the health care programme occupy responsible positions.

There is no national blood transfusion service. The blood supply was largely dependent upon the importation of blood, but this practice has been banned by legislation. Each hospital now collects the blood it needs. There are no official statistics concerning blood collection or usage. The university hospital in the capital city (700 beds), collects 6000 units per year, from both paid and "family replacement" donors. Cryoprecipitate and platelets are prepared only in this hospital and single-donor platelet concentrates are produced by apheresis. It is known, from a combination of commercial sources and government trade figures that there is heavy importation of plasma fractions (albumin, 2000 kg per year; factor VIII:C, 2 million IU per year; IV Ig, 70 kg per year). No plasma is produced for fractionation. There are plans to build a plasma fractionation plant, so as to avoid the necessity of importing plasma fractions from abroad.

1. How can this country reduce its dependence upon expertise from other countries?
2. Should a national blood programme be established, and should the operation of the BTSs be centralized?
3. How can the dependence upon paid blood donors be eliminated?
4. How much plasma is required to produce sufficient plasma fractions to meet current demand?
5. Will the establishment of a plasma fractionation facility help the country to become self-sufficient in plasma fractions?

Comment

- This country has the size and financial strength to be able to develop its own human resources. Thus, a national training programme for BTS personnel, including medical leadership, laboratory technologists and scientists, specialist phlebotomists, donor

recruitment specialists and BTS managers, is essential. Initiation of this programme will probably call for international assistance.

- Operational centralization is controversial and is, to some extent, a discretionary decision. However, a strong case can be made for the centralization of core functions at a national level, in this country where transfusion services are so fragmented. The key functions would comprise overall regulation of the BTS, training of BTS personnel, donor recruitment, production planning of plasma resources and plasma fractionation, specialized testing and laboratory problem-solving and control over the importation of blood products. It will be difficult to overcome the paid donor problem if donor procurement continues to be in the hands of individual hospitals. Hence the recommendation that donor recruitment becomes the responsibility of a national BTS. Laboratory testing of donors should be restricted to the minimum number of specialized laboratories, in order to pool sparse expertise to better effect and to assure high standards of quality assurance.
- Question 3 has already been discussed in the preceding comment. Essential first steps are: national legislation emphasizing the need for voluntary, nonremunerated donation, as well as the need to phase out the "family replacement" system within a defined time-frame; a national organization for donor recruitment based upon this principle; a specific time-table for conversion to an all-volunteer system.
- Question 4: One can currently expect 1 L (or 1 kg) of good quality plasma to yield about 25 g albumin, 150 to 200 IU factor VIII:C, and 2 IV Ig. If 1 kg of plasma can be produced from approximately four whole blood donations, then plasma feed-stock requirements in this country will be as follows:

Demand	Yield/L	Plasma (L)	Whole blood units	
Albumin	2 000 kg	25 g	80 000	320 000
F VIII:C	2 m IU	150 IU	13 333	53 333
IV Ig	70 kg	2 g	35 000	140 000

Clearly, at least 320 000 units of whole blood (or an equivalent volume from apheresis) must be collected to meet current demand for plasma fractions from domestic sources. It is worth remarking that albumin usage appears to be unduly heavy and should be rationalized, while F VIII:C demand is probably too low to provide for even minimal haemophilia care.

- Building a fractionation plant will do nothing to make this country self-sufficient in plasma fractions. The problem is not so much with fractionation itself, as with the absence of any organized plasma production system. As the BTSs are currently organized in this country, a plasma fractionation plant will be useless, because there

will be no plasma for it to fractionate. It might be more realistic to organize a progressively expanded plasma procurement programme, while using an established fractionator to prepare fractions on contract. Since current regulatory and licensing constraints may cause the fractionator to be reluctant to handle plasma of unknown infectivity, a short-term solution may be to continue importing fractions, while building up central coordination, and the quality assurance system, as well as moderating over-use.

Case history 2

Background

An industrialized country with a population of 21 million possesses a strong economy and a relatively well developed pharmaceutical and biologics industry, served by a highly trained workforce (educated both locally and abroad), of scientists and technologists. The medical sector is sophisticated, and local medical schools and departments of biology are well versed in modern research techniques.

There is an established, centralized national blood service which enjoys considerable public support and collects some 75 (250 mL) donations per 1000 inhabitants from voluntary donors, either at their spacious new headquarters building or at mobile sessions. Quality management and cGMP are being energetically pursued, although they are not fully in place as yet.

Approximately 20% of their blood collection is issued as whole blood and as much as 40% of the plasma produced through this programme (about 78 t) is devoted to clinical FFP. The Ministry of Health and commercial establishments together import over 450 kg per million population of albumin, 1 IU of F VIII:C per inhabitant, and 5 kg per million population of IV Ig per year.

There is an underlying ethic of national self-sufficiency and the government has firm plans to develop a national plasma fractionation plant, which will be managed by a local pharmaceutical firm. It is planned that plasma for fractionation will be harvested from voluntary donors entirely through apheresis, as a separate enterprise bypassing the Blood Service.

How should this country proceed in the realization of its aspirations?

Comment

- Blood donors over 50 kg should be persuaded to give a standard donation volume of 450 to 500 mL.
- Clinicians should be persuaded to use at least 90% to 95% of blood as red cells resuspended in an additive solution such as SAGM. Unprocessed whole blood issues

would then be limited to no more than 5% to 10% of the total blood collected. This would require education of the medical community in the principles of transfusion medicine.

- Clinical utilization of FFP is unjustifiably high in this country. Again, through a progressive education and persuasion process (publication of evidence-based clinical guidelines; the establishment of hospital transfusion committees and a process of clinical audit, etc.), the medical fraternity should greatly reduce FFP consumption to a level of no more than 10% to 20%.
- In the light of current outcome data regarding the clinical value of albumin in cases of hypovolaemic shock, burns and sepsis, the present utilization of this derivative is inappropriately high. Clinicians should be persuaded to be more conservative in their use of albumin, through education and the skilful presentation of scientific evidence.
- At the present levels of consumption for whole blood and clinical FFP, no more than 63 000 kg of plasma would be available for fractionation. This would not provide for national self-sufficiency at prevalent yield levels and would fall short of the volumes of albumin and F VIII:C which are imported. It should be noted that haemophilia care is still at a rudimentary state of development.

  In the future, however, the collection of at least 45 donations per 1000 inhabitants and the implementation of the strategies referred to (complemented as required by some of the techniques discussed in Chapter 11) can potentially provide 210 000 kg of plasma for fractionation.

  This, in turn, should yield: 42 million IU F VIII:C (2 IU per inhabitant) and 250 kg albumin per million population, as well as more than adequate levels of IV Ig. Apheresis may be used as an adjunct to top up the plasma harvest as required, or to provide better control of the quality of plasma destined for coagulant factor manufacture.

Thus, the overall strategy would be:

- To maximize the quantity and quality of plasma derived from the existing BTS whole blood programme.
- To establish a contract fractionation agreement with a reputable commercial or state fractionator abroad, simply as an interim step in the evolution of a local manufacturing capability and as an educational discipline in developing technical skills, a credible quality programme and accredited plasma.
- Progressively to introduce local fractionation, probably starting with the more robust, less demanding derivatives, such as albumin and Ig.

The infrastructural attributes of this country suggest that this development programme would be entirely feasible.

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Plasma fractionation programmes for developing countries

Technical aspects and infrastructural requirements

This book is a response to repeated expression of interest by blood transfusion directors and national health authorities from a variety of countries, mainly but not exclusively in the Eastern Mediterranean Region, in establishing their own national plasma fractionation facilities. It compares the various fractionation methodologies available, addresses the issues which must be considered in making a choice and discusses how to implement a national plasma fractionation programme in a safe, professional and cost-effective manner. The fractionation needs of developing countries and the necessity for radical upgrading of countries' blood systems are also discussed. The importance of having a basic infrastructure and the necessary resources for the establishment of a plasma fractionation programme are emphasized.

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