The European Agency for the Evaluation of Medicinal Products
Evaluation of Medicines for Human Use

London, 25 January 2001
CPMP/BWP/269/95 rev. 3

COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP)

NOTE FOR GUIDANCE ON PLASMA-DERIVED MEDICINAL PRODUCTS

DISCUSSION IN THE BIOTECHNOLOGY WORKING PARTY (BWP)	February - March 1999
TRANSMISSION TO THE CPMP	March 1999
RELEASE FOR CONSULTATION AS ADDENDUM TO NOTE FOR GUIDANCE ON PLASMA-DERIVED MEDICINAL PRODUCTS (CPMP/BWP/269/95 rev. 2)	March 1999
DEADLINE FOR COMMENTS	June 1999
DISCUSSION IN THE BIOTECHNOLOGY WORKING PARTY (BWP)	June 2000
TRANSMISSION TO THE CPMP	June 2000
RELEASE FOR CONSULTATION	July 2000
REVISION OF SECTION 3.2.5 OF CPMP/BWP/269/95.rev 2 ALBUMIN AND OTHER PLASMA-DERIVED PRODUCTS USED IN THE MANUFACTURE AND FORMULATION OF MEDICINAL PRODUCTS	July 2000
DEADLINE FOR COMMENTS	September 2000
TRANSMISSION TO CPMP	January 2001
ADOPTED BY CPMP	January 2001
DATE FOR COMING INTO OPERATION	January 2001

Note:

Revision 3 revises section 3.2.5 of the Note for Guidance. In addition, Ph. Eur. references are updated and the CPMP Position Paper on Plasma-derived ALT testing is incorporated.

7 Westferry Circus, Canary Wharf, London E14 4HB, UK
Tel. (+44-20) 74 18 84 00 Fax: (+44-20-) 74 18 85 45
E_Mail: mail@emea.eudra.org http://www.emea.eu.int/

NOTE FOR GUIDANCE ON PLASMA-DERIVED MEDICINAL PRODUCTS

TABLE OF CONTENTS

1. INTRODUCTION

Council Directive 89/381/EEC extends the scope of Directives 65/65/EEC and 75/319/EEC to medicinal products derived from human plasma (hereinafter called "plasma-derived products").

Human plasma contains many proteins, the extraction and purification of which are of great medical importance. Although the therapeutic use of blood transfusion goes back to the turn of the century, it was not until the 1940s that that the technique of plasma fractionation, devised by Cohn and colleagues, enabled the widespread use of medicinal products extracted from human plasma.

Improvements in protein purification and molecular separation technology over recent years have made available a wide variety of products, with medical applications covering a large and growing field, and the therapeutic value of these is unquestioned. However, the potential for viral transmission is well recognised, and because of the large number of donations which are pooled, a single contaminated batch of a plasma-derived product, with the contamination possibly originating from a single donation, can transmit viral disease to a large number of recipients. The recognition in the mid-1980's that plasma-derived products, in particular coagulation factor concentrates, had caused widespread transmission of human immunodeficiency virus (HIV) and non-A non-B hepatitis (now recognised as mainly hepatitis C) resulted in major changes to the manufacturing processes, with the introduction of specific steps to inactivate or remove these and other blood-borne viruses. However, occasional incidents of transmission of these and other blood-borne viruses have still occurred in recent years, and measures designed to minimise contamination of the starting plasma and to maximise the elimination of blood-borne viruses during production are essential to ensure the virological safety of plasma-derived products.

Products derived from human plasma can generally be divided into two groups:

The first group covers products derived from single donations or from small pools of source material (<12 donors). These products, for example, fresh frozen plasma and cryoprecipitates are made and distributed by blood collection establishments and used in transfusion medicine. They are subjected either to one or a few separation procedures. Their quality and safety are almost exclusively dependent on the careful selection and control of donors, on the screening of donations and on measures taken to minimise contamination such as quarantining.

The second group is represented by derivatives of plasma produced on an industrial scale from pools of source material through various manufacturing procedures. They may be used as therapeutic medicines or as excipients. The quality and safety of these products rely both on donor selection and screening of source materials, and on the choice and control of the manufacturing processes, including processes which inactivate or remove microbial contaminants.

Directive 89/381/EEC covers only plasma-derived products belonging to the second group. These include:

- albumin and plasma protein solutions;
- immunoglobulins;
- coagulation factors and antiproteases;
- other isolated plasma fractions or combinations thereof.

This note for guidance covers these medicinal products derived from plasma and focuses on specific aspects relating to the manufacture and control of these products, paying particular attention to the steps taken to minimise the risks of microbial contamination of the finished product. This note for guidance also covers virus-inactivated pooled plasma (large pools, >12 donors) prepared industrially. It does not cover medicinal products derived from cellular components although many parts contained in this document may be pertinent.

The transmission of viruses is of particular concern. Measures taken to prevent infection by the use of plasma-derived products include selection of donors, screening of individual donations and starting materials for markers of infection with known viruses and validation of the production process for the inactivation or removal of viruses. Clinical follow up of recipients is the final proof of the safety of a product.

Throughout this note for guidance it is assumed that the principles of Good Manufacturing Practice are followed as laid down in Directive 91/356/EEC, the Annex to the EC Guide to GMP on the "Manufacture of products derived from human blood or human plasma" and other relevant guidelines.

Requirements and standards for plasma-derived products are stated in the European Pharmacopoeia, Monograph, "Human Plasma for Fractionation", and specific monographs for plasma-derived products (see annex I and annex II).

Recommendations on the suitability of donors and screening of donations are stated in the Council of the European Union Recommendation on the "Suitability of Blood and Plasma Donors and the Screening of Donated Blood in the European Community" (98/463/EC).

Other documents providing additional guidance and recommendations in the field of plasma-derived products are:

1. Council of Europe "Guide to the preparation, use and quality assurance of blood components", 4th edition which addresses the collection, preparation and use of blood. This document deals primarily with the requirements for blood and blood components for use in blood transfusion and in immunohaematology;
2. the forty-third report of the WHO Expert Committee on Biological Standardisation (Technical Report Series 840, 1994) on the requirements for the collection, processing and quality control of blood, blood components and plasma derivatives. This document covers in four parts the requirements for the collection of source materials; single-donor and small-pool products; large-pool products; national control requirements.

These documents are regularly revised and reference should be made to the latest revision for current guidance.

2. SOURCE MATERIALS

2.1 Classification

Two types of source materials currently used in the manufacture of medicinal products derived from human plasma are specified by the European Pharmacopoeia requirements and the Council of the European Union, the Council of Europe and the WHO recommendations. Their origin and means of collection differ in several respects.

a) Whole blood donations are collected at blood collection establishments. This material is used to prepare products made from single donations for direct transfusion while much of the plasma is used for fractionation on an industrial scale;

b) Plasma obtained by plasmapheresis is collected in plasmapheresis centres and some blood collection establishments. It is predominantly used for products manufactured on an industrial scale.

2.2 Risk Factors

Many factors can affect the safety of blood donations in transfusion medicine. However, not all of these are relevant to medicinal products derived from human plasma manufactured on an industrial scale. Those which have implications are blood borne infections and include viruses found in plasma which establish a viraemia such as HBV, HCV, HIV 1 and 2, HAV and parvovirus B19. In many cases such viruses can establish a persistent or latent infection. Other factors of equal importance relate to the quality of the product, for example the integrity

and biological activity of immunoglobulins and the thrombogenicity, immunogenicity and activity of clotting factors, which can be affected by the handling and preparation of the source materials after collection.

2.3 Collection and Control of Source Materials (Plasma Master File)

2.3.1 Introduction

The source materials, the means of collecting source materials and their control are major factors in the quality assurance of the manufacture of biological medicinal products. Measures taken to reduce risks include the meticulous control of source materials and their origin.

Information on the collection and control of source material should be documented in accordance with the "Contribution to Part II of the structure of the dossier for applications for marketing authorisation - control of starting materials for the production of blood derivatives, EC III/5272/94, and can be provided as part of the dossier as a Plasma Master File. This information is required whenever a human plasma-derived product is used in a medicinal product whether as an active ingredient or an excipient. It should provide information on starting materials whether received by the manufacturer of the plasma derivative as plasma or as partially fractionated intermediate and should be updated once a year. A system should be in place which enables the path taken by each donation to be traced from the donor via the blood collection establishment through to finished products and vice versa.

2.3.2 A Quality Assurance System for collection

The recommendations in the Annex to the EU Guide to GMP "Manufacture of products derived from human blood or human plasma" should be followed.

Additional guidance can be found in:

1. Council of Europe "Guide to the preparation, use and quality assurance of blood components", 4th edition;
2. The forty-third report of the WHO Expert Committee on Biological Standardisation (Technical Report Series 840, 1994);

or their subsequent revisions.

Each establishment involved in the collection of blood or plasma used as source material for the preparation of plasma-derived products should establish, document and maintain an effective quality assurance system. The main requirements are summarised below:

- the preparation of standard operating procedures;
- the establishment of records so that donations can be traced, e.g., date of collection, quality control tests undertaken, with results etc., should be included;
- specifications for source plasma for further industrial processing into medicinal products;
- control of labelling, storage and transportation of donations;
- establishment of quality audits/review;
- appropriate premises.

2.3.3 Blood/plasma collection establishments

Information should be provided on the countries where donations are collected and the organisations responsible for collection. An exhaustive list of names and addresses of blood/plasma collection establishments including any subcontractors and any separate sites for testing of individual donations should be provided. Collection establishments should be inspected and approved by a competent authority. Information should be provided on audits by or on behalf of the Marketing Authorisation applicant/holder (name and frequency) and on any certification by other organisations (name and frequency). The standard contract between the manufacturer and the blood/plasma collection establishments or organisations responsible

for collection should be provided. (Financial information can be excluded.) The manufacturer should certify that all blood/plasma collection establishments have signed the contract. An assurance should be given that in the event of a serious failure of a collection establishment being discovered, CPMP and national authorities would be immediately informed.

2.3.4 Suitability of donors and the screening of donations

Recommendations on the suitability of donors and screening of donations are stated in the Council of the European Union Recommendation on the "Suitability of Blood and Plasma Donors and the Screening of Donated Blood in the European Community" (98/463/EC). Furthermore, the criteria of the European Pharmacopoeia monograph on plasma for fractionation shall apply to the suitability of donors of blood and plasma and the screening of their donations. In particular, using a sensitive, specific and validated test, each donation must be tested for:

- HBsAg, using an ELISA or RIA test which detects 0.5 IU per ml of HBs antigen or less;
- antibody to HIV 1 and HIV 2;
- antibody to hepatitis C.

If a repeat-reactive result is found in any of these tests, the donation is not accepted.

The CPMP position paper on Plasma-Derived medicinal Products and ALT testing is included at Annex VI.

2.3.5 Data on epidemiology of infections transmitted by blood

Assurances are required that centres are not collecting blood or plasma from a population with a high prevalence of infections transmitted by blood regarding relevant markers which are tested on a routine basis (see 2.3.4). In addition, it is advisable to avoid collection during known outbreaks of other infectious diseases, in particular hepatitis A. There is a need for continuing evaluation of the epidemiology at blood/plasma collection establishments. Data should be reported as follows:

- Incidence of confirmed positive seroconversions in donors (per number of donors and per number of donations)
- Prevalence of confirmed positives in new donors (i.e. all persons tested for the first time).

These reports should be part of the plasma master file and updated regularly.

2.3.6 Post-collection information system

A standard operating procedure describing the mutual information system between the blood/plasma collection establishment and manufacturing fractionation facility should be set up so that they can inform each other if subsequent[1] to donation:

- it is found that the donor did not meet the relevant donor health criteria;
- a subsequent donation from a donor previously found negative for viral markers is found positive for any of the viral markers;
- it is discovered that testing for viral markers has not been carried out according to agreed procedures;
- the donor develops an infectious disease caused by an agent potentially transmissible by plasma-derived products (see section 2.2);

---

[1] If maximum time limits between donation and identification of post-collection information are set so that information discovered after these maximum time periods does not trigger further action, these limits should be clearly stated and justification given.

- ∞ the donor develops Creutzfeldt-Jakob disease (CJD - see below);
- ∞ the recipient of blood or a blood component develops post transfusion infection which implicates or can be traced back to the donor.

The procedures to be followed in the event of any of the above should be documented in the standard operating procedure. Look-back should consist of tracing back and further testing of previous donations for at least 6 months prior to the last negative donation. Donations which have not been processed should be identified and withdrawn from processing pending further investigation. The operation of an inventory hold may be helpful in this respect. A careful evaluation should be made of whether the new information compromises the safety of batches of product and requires their withdrawal. This evaluation should take account of criteria such as the disease, the type of seroconversion, the results of further testing of the donation, possibly including testing by nucleic acid amplification technology (NAT), the size of the pool, the nature of the product and its manufacturing method. A re-assessment of the batch documentation should be carried out.

Where there are indications that a donation contributing to a plasma pool was infected with HIV or hepatitis A, B or C, the case should be referred to the relevant Medicines Competent Authority(ies)[2] and the Company's view regarding continued manufacture from the implicated pool or of the possibility of withdrawal of the product(s) should be given.

The mutual information system between blood/plasma collection establishments and manufacturing/fractionation centres should include information about any donor who develops Creutzfeldt-Jakob disease (CJD) or is subsequently found to have a risk factor for CJD, e.g. family history or treatment with substances of pituitary origin or recipients of dura mater grafts. Regarding the risk of transmission of CJD (sporadic, familial and iatrogenic) via plasma-derived products, the CPMP issued a Position Statement in 1995 (CPMP/938/95). A CPMP position statement on new variant (nv) CJD and plasma-derived medicinal products was issued in 1998 (CPMP/201/98) which states:

> "There is no evidence that sporadic, familial or iatrogenic Creutzfeldt-Jakob disease (CJD) are transmitted via blood transfusion or via plasma-derived medicinal products. Therefore, the CPMP reaffirms its recommendation that recall of plasma-derived products is not justified where a donor is later confirmed as having CJD.
>
> It is now recognised that nvCJD has different characteristics to sporadic, familial and iatrogenic CJD. Knowledge of other TSE agents suggests that transmission of nvCJD by medicinal products derived from human blood or plasma is very unlikely. Nevertheless, since there is a lack of specific information on nvCJD, the CPMP considers that, as a precautionary measure, it would be prudent to withdraw batches of plasma-derived medicinal products from the market if a donor to a plasma pool is subsequently strongly suspected, by a recognised reference centre, of having nvCJD. However, consequences for essential medicinal products where alternatives are not available will need careful consideration by national authorities."

The issue will be re-evaluated at regular intervals subject to the availability of epidemiological data and their scientific evaluation.

2.3.7 Bags for collection and storage of blood and plasma

Information should be provided on the bags used for the collection of blood and plasma donations. This should include the following: the name of the bag; its manufacturer; the nature and composition of the anticoagulant solution; confirmation of compliance of bag and solution with European Pharmacopoeia requirements; the CE marking and a summary of any other licensing or registration (competent authority and type of licensing/registration). Where

---

[2] National Authorities where the product has been authorised or the Reference and concerned Member States (Mutual Recognition Procedure) or the EMEA (Centralised Procedure)

the bag does not have a CE marking, further information is required, including the composition of the bag and its specification, a description of the sterilisation procedure and the site where sterilisation is performed, and information on the production and quality control of the anticoagulant solution.

2.3.8 Storage and transport of plasma

The procedures for collection, storage and transport of source materials should be described in the Plasma Master File. Maximum storage times should be stated. Information should be provided on how storage conditions are maintained from the collection centre to the manufacturer. Confirmation of compliance with the requirements of the European Pharmacopoeia monograph for Human Plasma for Fractionation should be given.

3. MANUFACTURE

According to Directive 75/318/EEC, the preparation of plasma-derived products shall be defined and justified in terms of strategy, and described with all relevant details regarding procedures, in-process and final controls.

3.1 Risks Arising During Processing

In the manufacture of medicinal products derived from human plasma, consideration should be given to the following factors:

a) microbial contamination may occur and may lead to the accumulation of pyrogens;

b) viruses may be introduced by reagents during manufacture (e.g., enzymes from tissue extracts or monoclonal antibodies used for affinity chromatography);

c) the methods of manufacture may introduce chemical contaminants such as enzymes, solvents, detergents, and antibodies or other ligands from chromatography.

d) methods of manufacture may modify the product resulting in adverse consequences for recipients, for example by the formation of neo-antigens or by compromising the biological activity of the active component, e.g. by activation of coagulation factors leading to enhanced thrombogenicity. This is particularly of concern for steps introduced to inactivate or remove viral contamination which may affect the quality or yield of products.

3.2 The Starting Material

3.2.1 General

The manufacture of plasma-derived products should start from defined pools of source material. For each source material, whether a plasma pool, cryoprecipitate or other intermediate product, records allowing to trace back its origin and the controls to which donor and donation were subjected should be kept and made available upon request to manufacturers and competent authorities. Records as well as samples of each pool should be stored for at least one year after the expiry date of the finished product with the longest shelf-life.

The specification of the plasma and confirmation that it complies with the European Pharmacopoeia monograph for Human Plasma for Fractionation should be provided. Any further in-process tests on the pool should be stated. Where appropriate, compliance with any production requirements of the relevant European Pharmacopoeia monographs should be confirmed.

3.2.2 Virological tests

Testing of plasma pools for viral markers is one of a number of steps which, taken together, provide assurance on the virological safety of plasma-derived products. Individual donations of blood/plasma are tested for viral markers (HBsAg and antibodies to HIV 1 and 2 and

hepatitis C; see Section 2.3.4) and repeat reactive donations are not used. However, because of the possibility of errors in testing and/or pooling, plasma pools should also be tested for these viral markers. A representative sample of the first homogeneous manufacturing pool of plasma or of pooled cryosupernatant should be tested using methods of suitable sensitivity and specificity. Pools should be non-reactive in these tests and confirmed positive pools must be rejected.

The plasma master file section of the dossier should include information on the tests used (brand name, manufacturer, generation of test and countries where licensed) and the criteria for acceptance/rejection of a pool including retest policies. A summary of the information on the validation of the test for screening of plasma pools is required. The sensitivity of the test for each marker in relation to the pool size should be stated.

3.2.3 Nucleic Acid Amplification Technology

The application of nucleic acid amplification technology (NAT) to blood or plasma donations, particularly for hepatitis C and HIV, has the potential to detect infectious donations in the "window period", i.e. before the development of antibodies. NAT may not yet be applicable to single donations for logistical reasons, but it may be useful for testing plasma pools in order to reduce further the virus load with which a manufacturing process is challenged. NAT testing of plasma pools for HCV RNA has been recommended for intramuscular immunoglobulins without an effective validated viral inactivation step in the manufacturing process ("Intramuscular immunoglobulins: nucleic acid amplification tests for HCV RNA detection", CPMP/117/95, and "Implementation of CPMP/117/95 recommendation", CPMP/BWP/391/95)(see Annex III and IV). The accumulation of experience in using NAT for detection of HCV RNA in plasma pools has now reached a stage when the introduction of testing on a routine basis can be proposed (Addendum to this Note for Guidance (CPMP/BWP/390/97), Annex V). The introduction of NAT for the detection of viral nucleic acid is a "state of the art" development which should be considered as another step in the continuously evolving process of assuring the quality of plasma-derived medicinal products.

3.2.4 Manufacture and use of intermediate plasma fractions

An intermediate plasma fraction (intermediate) is partially fractionated material which must undergo further manufacturing steps before it becomes a bulk product or final product. Intermediates, commonly used for further processing into a final product, are fractions recovered from the process for the production of clotting factors (e.g. cryopaste) or from the production process of immunoglobulins or albumin (e.g. fractions II, III, IV, V), and may be prepared and stored by the product manufacturer or obtained from another supplier. The trade in intermediate plasma fractions between a supplier of an intermediate and the manufacturer of the final product is a widespread practice and the use of such intermediates in the production of a final product is a form of contract manufacture.

The collection and control of source materials for the production of an intermediate plasma fraction are important factors in the assurance of its quality. The information on this source material should be provided by the manufacturer of the final product as described in EC/III/5272/94. A contract should be established between the supplier of the intermediate and the manufacturer of the final product. This contract should address information from the manufacturing process, traceability and specifications of the plasma and the intermediate, and the storage and transport of the intermediate.

The introduction of an intermediate in an established production process may have impact on the quality and viral safety of the final product. The suitability of use of an intermediate from a supplier must be demonstrated by the manufacturer. In the assessment of possible impact on quality, the equivalence of the process for production of the intermediate and the validated production process should be demonstrated. If it can be shown that the introduction of an intermediate does not affect subsequent processing, no re-validation will be necessary. Otherwise, a re-validation of the production process is required. The possibility that the viral

safety is not affected should be demonstrated on the basis of equivalence of the process for production of the intermediate and the production process validated by the manufacturer, for those process steps contributing to viral safety. If this equivalence is not demonstrated, a revalidation of the viral safety should be performed.

Storage periods for intermediates should be set and justified by stability data. When releasing a final product produced from a stored intermediate, the manufacturer should ensure that at the time of release the product meets current requirements regarding the risk of transmission of infectious agents. Intermediates produced from plasma or whole blood screened with virus marker methodology which has been superseded may be used during a transitional period, provided that a risk assessment has been performed, possibly supplemented by appropriate testing of manufacturing pools.

3.2.5 Albumin and other plasma-derived products used in the manufacture and formulation of medicinal products

Albumin is widely used as a stabiliser in the formulation of other plasma-derived products, in particular coagulation factor concentrates and some immunoglobulins; it is also extensively used as an excipient for non plasma-derived products such as vaccines and recombinant proteins.

Albumin has an excellent clinical safety record during the last 50 years with regard to transmission of blood-borne viruses. However, the risk of infectious diseases due to the transmission of infective agents cannot be totally excluded when albumin and other plasma-derived products are used in the manufacture and formulation of medicinal products. The development of substitutes for plasma-derived albumin as an excipient for medicinal products is encouraged.

Whenever albumin is used in the manufacture of medicinal products, it should comply with this Note for Guidance, and should have the same documentation, including the origin of donations, the same quality and specifications as that of albumin for therapeutic use. The albumin should always be within its shelf-life and, therefore, within its pharmacopoeial/marketing authorisation specification at the time when it is incorporated into a starting material, intermediate or final product. In these circumstances, the development and testing of the product in which it is incorporated (e.g. pharmaceutical development, in-process and final product testing, and stability studies) will indicate the suitability of the albumin. No specific studies with albumin of different ages are required.

When albumin is used in the manufacture of other medicinal products, the expiry date of the albumin should not be earlier than that of the finished product.

The synchronisation of expiry dates is recommended for the following reasons:

- To help ensure that albumin used in the manufacture of other products complies with current recommendations for donor selection, donation screening and plasma pool testing and that state-of-the-art testing methods are used for these purposes.
- To facilitate maintaining the link, through the manufacturer of the albumin, to post-collection information on the donors and to post-marketing data on the albumin and its starting materials (e.g. plasma pools) until the expiry date of the medicinal product containing albumin. This is to ensure that a manufacturer, using a batch of albumin in his product, and the Competent Authorities would be informed if, in exceptional circumstances, post-collection information would lead to measures regarding the product.

It is recognised that, in some circumstances, it can be difficult for a manufacturer to synchronise the expiry date of a batch of albumin with the expiry date of the formulated product. Any departure from this recommendation should be justified, as part of the Marketing Authorisation. In such cases, an alternative approach should be taken to maintain

the link through the manufacturer of the albumin, to post-collection information. This link to post-donation information may be achieved by:

- A contract between the manufacturers of the albumin and the manufacturers of the finished product[3].

  The contract should describe the period during which the link is maintained to post-collection information on the donors and to post-marketing data on the albumin and its starting materials. The expiry date of the finished product should fall within the period during which the link is maintained to post-collection information on the donors. (The Marketing Authorisation holder for the finished product will have responsibility for traceability of the finished product containing the plasma-derived component.)

- Using albumin from a manufacturer who provides albumin of European Pharmacopoeia quality with a proven longer shelf life.

The recommendation on synchronisation of expiry dates is not intended to apply retrospectively to batches of product where albumin had already been incorporated, during the manufacture of the active substance or the finished product, prior to January 1999.

As a consequence, for a limited time until expiry of stocks, batches may continue to be used when a link to the post-donation information is not established.

After January 1999, for medicinal products evaluated through centralised or mutual recognition procedures a harmonised implementation of this requirement will be achieved. A similar degree of harmonisation should be achieved through national procedures.

Each time the requirements for a plasma derived product or its starting materials are changed, the effect of the change, including impact on safety, will be evaluated, not only for its use as an active substance, but also for its use in the manufacture of medicinal products. This evaluation will determine the action to be taken.

According to the CPMP Position Statement on nvCJD and plasma-derived medicinal products (CPMP/201/98): "Since a recall involving albumin used as an excipient has the potential to cause major supply difficulties for essential products, manufacturers should avoid using, as an excipient, albumin derived from countries where a number of cases of nvCJD have occurred."

The same criteria apply for other plasma products used in the manufacture of medicinal products, e.g. antithrombin added to Factor IX concentrates.

3.3 Manufacturing Procedures

The strategies used in the manufacture of plasma-derived products are critical for product quality and play an essential part in ensuring overall product safety. They vary according to product and manufacturer, and usually include several fractionation/purification procedures, some of which may also contribute to the inactivation and/or removal of potential microbial contaminants. Additionally, procedures specifically designated to inactivate/remove viral contaminants should be a requisite part of the manufacturing strategy for all plasma products.

It should be emphasised that a manufacturing process cannot be considered satisfactory unless it is capable not only of generating a product of high quality but also effectively inactivating and/or removing infectious agents.

Products derived from human plasma have been shown to transmit viruses to recipients even where the starting material has been controlled for viral contamination in accordance with state of the art procedures. This follows in part from the nature of the starting material, which is obtained from a panel of heterogeneous human donors which cannot be virologically characterised as thoroughly as other sources of biological materials, such as cell banks. In addition any contaminating virus is able by definition to infect humans.

---

[3] A contract will not be needed when the albumin user and manufacturer are the same.

While selection of donors and testing of donations are essential safety measures, incidents of viral transmission show that they are insufficient alone to ensure safety of the product. The manufacturing process itself plays a central role and is of great significance for products derived from plasma. Studies of a process for the ability to inactivate or remove virus infectivity will be subject to particularly careful evaluation when products derived from blood or plasma are considered. This will include consideration of the reduction in virus titre achieved, the rates of inactivation and the shape of inactivation curves, how robust the step is to process variables, and whether virus inactivation or removal is selective for a particular kind of virus.

The suitability of the various materials and procedures used in manufacture as well as the selected operating conditions, parameters and tolerances should be validated by correctly designed and interpreted studies.

3.3.1 Fractionation/purification procedures

a) Precipitation methods

Physical methods:

Cryoprecipitation is most often used as the initial step for the production of Factor VIII concentrates. Subsequent purification techniques for FVIII include precipitation, adsorption of other coagulation factors, and chromatographic separation as well as procedures for viral inactivation to obtain the finished products. Cryoprecipitate-depleted plasma is commonly used for the preparation of other coagulation factors by adsorption/elution or chromatographic procedures and the residual plasma can be further processed to yield immunoglobulins and albumins.

Physical/chemical methods:

Among these methods, the ethanol fractionation procedures derived from the Cohn method are the most widely used for albumin and immunoglobulins. They commonly incorporate several steps, in each of which compliance with specific requirements is decisive for product quality; some of these steps may also contribute to effective reduction of potential viral contaminants. Therefore, clear specifications for ethanol and protein concentration, temperature, pH and ionic strength, and time of treatment, with data on acceptable tolerance as well as the means of controlling them should exist.

Appropriate data should also be provided for methods relying on other chemical agents such as ethylacridin-lactate, methanol, ammonium sulphate, polyethylene glycol, cationic detergents, which are sometimes used in the preparation of certain plasma derivatives, as a rule in combination with other purification procedures. Some of these substances may have an impact on viral safety, for others information is still scarce.

b) Chromatographic methods

Three basic types of procedures play an increasing role in the processing of plasma derivatives, as a rule in combination with precipitation procedures and often with each other:

- gel filtration, mainly used for desalination or separation of components with significantly different size;
- ion exchange and hydrophobic interaction chromatography;
- affinity chromatography based on specific interactions with immunological or other ligands immobilised on the matrix.

The selectivity of the procedures and the yields depend critically on the quality of the material as well as on factors like the capacity of the column, nature and concentration of proteins in the product, ionic strength and the pH of buffers, flow rate, contact time and temperature. Therefore, all appropriate specifications and accepted tolerances should be stated, and control data documented.

The conditions of storage of the columns, preservation and elution of preservatives, and methods of regeneration should also be described. Details should be given of clarification and sterile, dia- or ultra-filtration procedures used.

c) Complementary procedures

Immunoglobulins intended for intramuscular administration may cause adverse reactions upon intravenous administration. Therefore, the production process for immunoglobulins intended for intravenous administration includes various procedures which can substantially reduce such reactions. The materials and the procedures used should be described and their suitability justified and documented.

Anticoagulants such as antithrombin and heparin may be added at various stages during the production of coagulation factors to minimise activation. The materials and their use should be fully documented and their concentrations measured in the final product.

Several other compounds like charcoal, bentonite, colloidal silica are sometimes used for clearing various impurities like pigments, lipoproteins etc. Details on the characteristics of the compounds, on their decontamination and on the operating conditions should be provided.

3.3.2 Viral inactivation/removal procedures

Procedures specifically designed to inactivate/remove infectious viruses are included in the manufacturing strategies for most plasma-derived products. The manufacturing process conditions and in-process monitoring for viral inactivation/removal steps should be clearly defined and justified.

It is essential that material that has been subjected to a viral inactivation/removal step should be segregated from untreated material to prevent cross-contamination (as stated in the GMP guideline, Annex 14).

The following is not a comprehensive account of available processes and points to consider but identifies some common criteria that need to be considered for certain processes.

a) Heating in aqueous solution

Heating in aqueous solution at 60°C for 10 hours in the final container is the pharmacopoeial method for viral inactivation for albumin preparations. This method of inactivation is also used for bulk preparations of some other plasma-derived products. The efficacy of such a treatment is dependent upon the composition of the solution. Stabilisation may be necessary to protect proteins and minimise neo-antigen formation but stabilisers can also protect virus from inactivation. Careful validation is, therefore, needed for each product ensuring that the validation includes worst case conditions.

b) Heating of lyophilised products

The effectiveness may vary according to the conditions of lyophilisation. Upper and lower limits of water activity or moisture, whichever is more appropriate, should be set based on viral validation studies. Where such a treatment is applied to the product in its final containers, the variation in water content between vials of product should be within the limits set. Where products are heated under humidified conditions, critical parameters, in particular partial water vapour pressure, temperature and duration of heating should be carefully monitored throughout the process.

c) Solvent/detergent treatment

Treatment with a solvent such as tri-n-butyl-phosphate (TNBP) combined with a non-ionic detergent such as Triton X-100 or Tween 80 can inactivate enveloped viruses. Prior to such treatment, in-process solutions should be free from gross aggregates that may harbour virus and protect it from the treatment. This can be achieved by filtration which should be done prior to addition of the solvent/detergent or if done after, the filters should be demonstrated not to alter the levels of these additives in the incubation solution. Physical validation must

demonstrate that mixing achieves a homogeneous mixture and that the target process temperature is controlled throughout the bulk solution for the duration of the defined incubation time. In-process checks should be carried out to confirm that the correct amounts of solvent and detergent have been added. Validation experiments should investigate the range of key process variables and in-process limits should be set accordingly. Since lipid content can affect the efficacy of inactivation, inactivation should be confirmed under worst case conditions for lipid content. Residual levels of solvent and detergent should be minimised by processing and carefully monitored in the final product. Non-enveloped viruses will not be inactivated by this process.

d) Virus removal by filtration

This is a new and developing area of technology. There may be difficulties with removing the smaller viruses by filtration while maintaining a satisfactory yield of product, especially for material of high molecular weight such as Factor VIII. Certain types of filters may cause activation of coagulation factors; this should be minimised by suitable choice of filter material and activation should be monitored before and after filtration.

The mode of action of the particular filter selected should be described and the parameters critical for virus removal (e.g., volume, ionic strength, flow rate, pressure and loading) should be identified. These critical parameters should be used to define appropriate viral validation studies. Tests to confirm filter integrity are essential in-process controls. In addition, the performance of filters used in virus validation studies must be compared to that of the filters used in routine production.

Aggregation of viruses can affect the level of virus removal by filtration. This should be taken into account when performing validation studies with viruses which will have been propagated and concentrated under laboratory conditions and whose state of aggregation may differ from that expected of a virus present in plasma. Information on the characterisation of the filter material by the manufacturer should also be provided.

e) Low pH

Low pH (approximately 4) can inactivate certain viruses. The reduction factors that have been demonstrated depend on the exact conditions used in manufacturing (e.g., pH value, time and temperature of treatment, composition of the solution, etc.). Each process therefore has to be carefully validated.

3.4 Consistency of production

The manufacturer should demonstrate consistency of the specifications of the product for at least 3 full scale production batches; where different batches are filled from the same bulk, at least three different bulks must be used.

4. QUALITY CONTROL

4.1 In-Process Controls

The procedures for production and equipment monitoring, the production steps where control tests are carried out, the means of sampling and of storing the samples, as well as the testing procedures should be described.

The pooling of source materials should be subject to careful control to avoid contamination and introduction of foreign material.

The testing of samples of starting and bulk material for specific viral markers should be in accordance with up to date methods validated for their intended use.

The monitoring of relevant parameters during fractionation, such as pH, temperature and ethanol concentration where appropriate, as well as the results from bacterial counts and endotoxin should be documented.

4.2 Quality Control of Products

All products must comply with the appropriate European Pharmacopoeia monographs. If methods other than those specified by the European Pharmacopoeia are used, the alternative procedures should be shown to give consistently equivalent results on several batches of product.

All relevant parameters should be measured in each batch of the final product. In addition, measurements should be made of substances used during formulation or during production, e.g. residual solvent/detergent concentrations where these have been used. Appropriate limits for all these parameters should be set after analysis of at least 3 consecutive full-scale production batches.

5. VALIDATION STUDIES

Validation studies should be carried out by each manufacturer for the specific processes used and, unless otherwise justified, for each production site. Moreover, if studies involve modelling the process on a reduced scale, they should be capable of mimicking satisfactorily the conditions of full scale production and the accuracy of the modelling should be demonstrated.

5.1 Process Validation

The effectiveness of a given manufacturing process in consistently yielding a product with expected quality and biological activity should be documented with data based on a broad set of relevant analytical methods. Particular attention should be paid to demonstration of removal of undesirable components, for example chemicals used for, or derived from, fractionation/purification procedures, and naturally occurring substances which may be hazardous, such as blood group substances and activated coagulation factors. Spiking experiments with certain potential contaminants may be necessary to demonstrate the clearing efficiency of the process.

The studies should be designed to justify the selected operating conditions and the acceptable tolerances, including worst case conditions, and to document their adequacy in achieving the expected process performances.

When chromatographic columns are used, conditions leading to overloading as well as leaching from the gels, particularly in the case of affinity chromatography with potentially harmful ligands, should be carefully investigated. Attention should also be paid to the cleaning and regeneration of the columns and to the effective removal of residues from the previous run or of preservatives added for storage, particularly if mild treatments are used.

5.2 Virus Inactivation/Removal

5.2.1 Manufacturing process design

General principles concerning the incorporation of virus inactivation/removal steps in the manufacture of biological products are outlined in the Note for Guidance "Virus Validation Studies (Revised) (CPMP/BWP/268/95)". This section contains further guidance relevant to plasma derivatives. The principles in both guidelines should be taken into account when designing manufacturing processes or modifying processes to give further assurance of viral safety.

a) Incorporation of effective steps for viral inactivation/removal in the manufacturing process.

All production processes should incorporate effective validated steps for the inactivation/removal of viruses. An effective step is defined in the Note for Guidance "Virus Validation Studies (Revised) (CPMP/BWP/268/95)".

For all plasma-derived medicinal products, it is an objective to incorporate effective steps for inactivation/removal of a wide range of viruses of diverse physico-chemical characteristics. In order to achieve this, it will be desirable in many cases to incorporate two distinct effective steps which complement each other in their mode of action such that any virus surviving the first step would be effectively inactivated/removed by the second. At least one of the steps should be effective against non-enveloped viruses. Where a process step is shown to be reliably effective in inactivating/removing a wide range of viruses including enveloped and non-enveloped viruses of diverse physico-chemical characteristics and the process contains additional stages reliably contributing to the inactivation/removal of viruses, a second effective step would not be required.

It is recognised that designing steps which will complement each other and also be effective against a wide range of viruses including enveloped and non-enveloped viruses of diverse physico-chemical characteristics, is not a straightforward task.

Viruses tend to fall into two groups in this respect, those susceptible to a wide range of inactivation/removal procedures and those resistant. Also, there may be viruses potentially present in plasma that are resistant to the inactivation/removal methods that can currently be applied to a class of product, e.g., parvovirus B19 in coagulation concentrates.

Manufacturers should apply their best efforts to develop methods to inactivate/ remove viruses and this should be a continuing process. Previous experience clearly shows that source material may contain unknown viruses and that new viruses may appear. This emphasises the need to design processes to inactivate/remove as wide a range of viruses as possible. Even this may not preclude new or unknown infectious agents breaking through a process.

b) Contribution of partition processes to virus removal.

It is recognised that partitioning through the fractionation process and through purification procedures (e.g. immunoaffinity chromatography) can contribute to the removal of viruses. However, cases of virus transmission have occurred clinically with coagulation factors and intravenous immunoglobulins whose manufacture have relied purely on partition processes. Furthermore, partition processes involve a large number of variables that are difficult to control and are difficult to scale down for validation purposes. Minor differences in physico-chemical properties of viruses can have a major influence on partitioning which makes it difficult to extrapolate from validation studies. Partitioning may also be affected by the presence or absence of antibodies. Consequently, it may be difficult to demonstrate that partition processes are reliably effective.

If a partition process gives reproducible reduction of virus load and if manufacturing parameters influencing the partition can be properly defined and controlled and if the desired fraction can be reliably separated from the putative virus-containing fraction, then it could fit the criteria of an effective step.

Since fractionation can contribute to virus removal, particular attention needs to be given to validation studies and clinical safety if novel manufacturing processes depart from standard fractionation techniques.

c) Selection of specific virus inactivation/removal steps.

The rationale for the choice of specific virus inactivation/removal steps deliberately introduced into the process should be given. Marketing Authorisation holders are expected to keep their processes under review in the light of developments in virus inactivation/removal techniques and of any emergence of relevant, novel contaminants of plasma and plasma-derived products.

d) Effect of virus inactivation/removal steps on the product

It should be established that the virus inactivation/removal steps selected will contribute to the net safety of the product. For example, a solvent/detergent step might break up aggregates and

allow more non-enveloped virus through a subsequent filtration step intended to remove viruses. Consideration should be given to the maintenance of the integrity of the components of the plasma derivative and clinical efficacy, the potential for formation of neo-antigens, the possibility of enhanced thrombogenicity from activated coagulation factors, and the possibility of toxic residues from chemicals used in-process as well as to virological safety. Separate guidance is available on the clinical studies that should be undertaken (Guidelines to Assess Efficacy and Safety of Human Plasma Derived Factor VIII:c and Factor IX:c Products in Clinical Trials in Haemophiliacs Before and After Authorisation (CPMP/BPWP/198/95) and Guidelines to Assess Efficacy and Safety of Normal Intravenous Immunoglobulin Products for Marketing Authorisations (CPMP/BPWP/388/95).

e) Points to consider for specific product classes

i) Coagulation factors: Effective process steps for the inactivation/removal of enveloped viruses are essential. Non-enveloped viruses such as hepatitis A and parvovirus B19 have been transmitted by this class of products. Coagulation factors are the highest priority class for the development of methods to exclude non-enveloped viruses from the product (see Section 5.2.3). However, it is recognised that some viruses are very resistant to physico-chemical methods for viral inactivation, e.g., parvovirus B19, and that development of an effective inactivation/removal step may be difficult. This should be reflected in the Summary of Product Characteristics.

ii) Immunoglobulins: Certain intravenous immunoglobulins, without specific steps for the elimination of enveloped viruses, have recently transmitted hepatitis C. Effective process steps for the elimination of enveloped viruses are essential for intravenous immunoglobulins.

Intramuscular immunoglobulins have a good safety record. However, the reason for this is poorly understood. The virus challenge and the virus/antibody ratio faced by processes are changing as a result of donor screening and changes in the epidemiology of virus infections in donor populations. Processes have been developed to inactivate enveloped viruses in intravenous immunoglobulins which may also be applicable to intramuscular products and these should be introduced. It should also be established that the net safety of the product is not adversely affected by the addition of such a process.

Immunoglobulins are used successfully to treat or prevent infection by non-enveloped viruses, such as parvovirus B19 or hepatitis A. Provided the relevant antibodies in immunoglobulin preparations are maintained at a protective level, it is highly unlikely that infection will result from the presence of such viruses in any immunoglobulin preparation. However, the possible transmission of a novel non-enveloped virus or the decline of antibody to non-protective levels in donor pools cannot be totally excluded. The addition of a specific virus inactivation/removal step for non-enveloped viruses is therefore an objective.

iii) Albumin: Albumin manufactured by an established fractionation process that includes the terminal pasteurisation specified in the European Pharmacopoeia monograph, has an excellent viral safety record. However, further information is needed from validation studies on the elimination of viruses. The effect of albumin concentration on virus elimination should be considered. Since albumin has additional uses as an excipient, attention should be given to the effect of any proposed process change on other products that may include albumin in the formulation. Albumin used as an excipient should be of the same quality as albumin used as an active ingredient.

5.2.2 Choice of viruses for use in validation studies

General guidance on choice of viruses is given in the CPMP guideline "Virus Validation Studies (Revised) (CPMP/BWP/268/95)". Viruses to be used in validation studies on plasma-derived medicinal products should include at least:

i) HIV-1

It is not necessary to carry out additional studies with HIV-2 as it is similarly affected by inactivation procedures.

ii) A model for hepatitis C virus

Biochemical characterisation of HCV classifies it in the Flaviviridae related to both pestiviruses and flaviviruses. Currently, there are no methods available for propagation of the virus. Various models have been used to validate viral inactivation methods including togaviruses, e.g., Sindbis, flaviviruses, e.g., Yellow Fever virus, and pestiviruses, e.g., bovine viral diarrhoea virus. These viruses have properties in common with HCV. Minor differences in physico-chemical characteristics of viruses can have major effects on how they partition. For example, there is evidence that pestiviruses differ in their partition in the Cohn Oncley fractionation process from togaviruses and that HCV resembles the pestiviruses more closely in this respect. Currently there are insufficient data on HCV to identify the most appropriate model virus for validation studies. Therefore, caution is required in the choice of a model virus and in the interpretation of validation data.

iii) Non-enveloped viruses

The package of validation studies on non-enveloped viruses should establish the range of viruses susceptible to the inactivation/removal processes and identify the limits of the process. For example, a heat inactivation step used in the manufacture of a coagulation factor might be effective against hepatitis A virus but ineffective against another non-enveloped virus.

Hepatitis A transmission has been associated with certain coagulation factors. However, there are insufficient data to identify appropriate models for hepatitis A at the present time. HAV should be used for validation studies for coagulation factors as it is thought to be significantly different to other picornaviruses. Consideration should be given to the possible interfering effects of antibodies.

Validation studies for coagulation factors should also include an appropriate model for the parvovirus B19. Models that have been used include canine, porcine, murine and bovine parvoviruses. Studies using HAV and B19 are not required for immunoglobulins if the presence of protective levels of antibodies in the product can be assured. However, studies with non-enveloped viruses for which antibodies are unlikely to be present should be performed to evaluate the ability of the process to inactivate/remove possible unknown non-enveloped viruses.

iv) Enveloped DNA viruses

To date, there have been no recorded transmissions of a herpesvirus associated with the use of non-cellular blood products. However, since novel herpesviruses continue to be discovered, most of which are lymphotropic and some which may result in a viraemia, a validation study should be performed with an appropriate enveloped DNA virus, e.g., a herpesvirus such as pseudorabies.

Currently, there is no practical test system for hepatitis B virus validation.

5.2.3 Strategy for Introduction of Additional Process Steps for Inactivation and Removal of Viruses

Specific virus inactivation/removal steps are now included in the manufacturing processes for most plasma-derived products. However, recent transmissions of both enveloped and non-enveloped viruses by certain products have highlighted the need for a strategy to further increase the assurance of viral safety of plasma derived products.

Manufacturers should validate their processes for the inactivation/removal of both enveloped and non-enveloped viruses where they have not already done so and, where the current process is not effective in inactivation/removal, develop and validate additional virus

inactivation/removal steps in order to improve safety. The priority order is, starting from the highest: coagulation factors, intravenous immunoglobulins, intramuscular immunoglobulins and albumin.

Marketing Authorisation holders and applicants are required to set and justify timetables for such developments; to submit a programme of process/product improvements to Member States for their agreement and to commit themselves to providing regular reports to Member States on their progress. Timescales for introduction of process changes should reflect the manufacturer's best efforts. In the meantime, product literature needs to be looked at critically and, where necessary, amended to provide relevant and specific information to enable clinicians to make an informed choice of product.

5.2.4 Difficulties in the design and execution of virus validation studies.

Reliable experimental demonstration of the effectiveness of virus inactivation and removal during the processing of plasma and the interpretation of data may be rendered difficult for various reasons. The presence of antibodies may affect partition of viruses or their susceptibility to chemical inactivation and may also complicate the design of the study by neutralising infectivity. Furthermore, undiluted plasma or derived fractions are usually toxic for cell cultures used for virus detection as is the presence in intermediary products of chemicals such as ethanol and ethylacridinlactate. Therefore, assays may have to be preceded by procedures designed to counteract these effects, such as dilution, dialysis, etc. In addition, the product itself or chemicals used to prepare or to treat it may change the properties of viruses, for example leading to their coating and/or aggregation, which may result in difficulties in reliable quantification of residual infectivity.

5.3 Revalidation

New validation studies are required when relevant changes in the manufacturing process or in individual steps are being considered.

Validation experiments have many limitations. Any virus transmission seen in clinical use should result in an evaluation of available data by manufacturers and regulatory authorities so that appropriate action can be taken.

ANNEX I

The following monographs and general methods of the European Pharmacopoeia (current edition) are applicable:

PUBLISHED MONOGRAPHS ON BLOOD PRODUCTS

Monograph Title
Human plasma for fractionation
Human coagulation factor VII, freeze dried
Human coagulation factor VIII, freeze dried
Human coagulation factor IX, freeze dried
Human prothrombin complex, freeze dried
Human antithrombin III concentrate, freeze dried
Albumin solution, human
Human normal immunoglobulin
Human normal immunoglobulin for intravenous use
Human anti-D immunoglobulin
Human hepatitis A immunoglobulin
Human hepatitis B immunoglobulin
Human hepatitis B immunoglobulin for intravenous use
Human measles immunoglobulin
Human rabies immunoglobulin
Human rubella immunoglobulin
Human tetanus immunoglobulin
Human varicella immunoglobulin
Human fibrinogen, freeze-dried
Fibrin sealant kit
Human fibrinogen, dried iodinated (^{125}I)
Technetium (99mTc) human albumin injection
Technetium (99mTc) macrosalb injection
Technetium (99mTc) microspheres injection
Human anti-D immunoglobulin,intravenous
Human varicella immunoglobulin, intravenous

ANNEX II

GENERAL METHODS

Assay of blood coagulation factor VIII, 2.7.4
Assay of human coagulation factor VII, 2.7.10
Assay of human coagulation factor IX, 2.7.11
Assay of heparin in coagulation factor concentrates, 2.7.12
Anti-A and anti-B haemagglutinins (indirect method), 2.6.20
Prekallikrein activator, 2.6.15
Test for Fc function of immunoglobulin, 2.7.9
Test for anticomplementary activity of immunoglobulin, 2.6.17
Immunochemical methods, 2.7.1
Nucleic Acid Amplification Techniques, 2.6.21

ANNEX III: CPMP/117/95

London, 6-7 April 1995
CPMP/117/95

Ad hoc CPMP Biotechnology Working Party

INTRAMUSCULAR IMMUNOGLOBULINS: NUCLEIC ACID AMPLIFICATION TESTS FOR HCV RNA DETECTION

In March 1994, the CPMP urged marketing authorisation holders to develop and validate additional virus inactivation/removal steps in order to improve the viral safety of blood products. A priority setting of classes of blood products, which have to be validated for the removal/inactivation of enveloped and non-enveloped viruses, was set. The product classes are, in decreasing order of priority, coagulation factors, IV immunoglobulins, IM immunoglobulins and albumin.

The FDA has recently requested manufacturers to test those intramuscular (IM) immunoglobulins which have not been subject to a virus inactivation/removal step by the polymerase chain reaction (PCR) for Hepatitis C virus RNA, on the final product. Their position is that IM immunoglobulins, positive by PCR for HCV, should not be used, unless there is no alternative material available.

Nucleic acid amplification (e.g., PCR) remains a novel technique, which has exceptional sensitivity and specificity for the detection of viral nucleic acids. However, these techniques have not been fully standardised for diagnostic use or control purposes and differences in sensitivity in the techniques used by different laboratories have been demonstrated in collaborative studies. Whilst a positive result is indicative of viral contamination in the starting material, it is not indicative of infectious virus in the sample tested. There is a need for further discussion on ways of standardising nucleic acid amplification methodology and the adoption of appropriate standards to allow the general application of validated techniques to plasma pools and/or other materials.

Transmission of hepatitis viruses has occurred with intravenous (IV) immunoglobulins, which are prepared by similar but not identical procedures to those for IM immunoglobulins. However, there is no new information to suggest that IM immunoglobulins safety record should be questioned. Also, some specific IM immunoglobulins which cannot be easily replaced are essential for treatment.

There are, on the European market, IM immunoglobulins which currently have not had specific virus inactivation steps added to their method of manufacture. There is a lack of information for some immunoglobulins on whether their production processes have acceptable virus removal/inactivation capacities. Within Europe, there are also suggestions that for these products nucleic acid amplification tests be carried out on plasma pools or final product.

It has been shown that plasma derived products which have been subject to certain recognised virus inactivation processes have remained PCR positive, because viral nucleic acid may not be removed. Material which is PCR positive is not necessarily infectious, but equally, products tested negative by PCR may still be infectious.

Nevertheless, in the absence of a valid virus inactivation/removal step, it is thought that a gene amplification test, especially of plasma pools, would provide useful information for manufacturers and control authorities in deciding whether batches of IM immunoglobulins should be released.

Conclusions and recommendations:

1. Manufacturers are again urged to implement, where necessary, an additional valid virus removal/inactivation step in line with the established priorities and are asked to provide updates on their programme of implementation. Timescales for introduction of process changes should reflect the manufacturers' best efforts. The information on the situation in each Member State should be reported to the CPMP.

2. Although nucleic acid amplification methods for the detection of HCV RNA have not yet been standardised, international efforts towards that aim are in progress and should be encouraged.

3. For products where valid removal/inactivation steps are absent, nucleic acid amplification tests for HCV RNA, preferably in plasma pools, are requested. IM immunoglobulins from a positive pool should not be made available unless warranted by supply requirements.

ANNEX IV: CPMP/BWP/391/95

London, 4 July 1995
CPMP/BWP/391/95

IMPLEMENTATION OF CPMP/117/95 RECOMMENDATION "INTRAMUSCULAR IMMUNOGLOBULINS NUCLEIC ACID AMPLIFICATION TESTS FOR HCV RNA DETECTION."

The group re-iterates the recommendations and conclusions of CPMP/117/95. This paper concerns the progress on the implementation of tests for HCV RNA detection by nucleic acid amplification on plasma pools for intramuscular (IM) immunoglobulins which do not have a validated, effective inactivation/removal step for enveloped viruses.

1. As recognised in CPMP/117/95 final, there are no standard methods for nucleic acid amplification and there are great differences between manufacturers in their experience with these methods. Initial studies suggest that the currently available commercial kits are not automatically suitable for the examination of plasma pools. Before testing is generally introduced, a working reagent is needed to check that assays are being performed to appropriate sensitivity. Preliminary studies by the National Institute of Biological Standards and Control (NIBSC) have identified a preparation which may be suitable and this material will be made available in the second half of 1995 to interested parties for further exploratory work on how the preparation behaves in practice.

2. For IM immunoglobulins without a validated, effective inactivation/removal step for enveloped viruses, CPMP intends to set a final date by which all batches released onto the market by the manufacturer and, where applicable, by the Control Authority, should be derived from pools tested by suitably sensitive nucleic acid amplification tests for HCV RNA and found negative. A target date in early 1996 is currently envisaged but the feasibility of this will be reviewed at the end of 1995 when the results of exploratory studies are known. Manufacturers should initiate this pool testing for HCV RNA as soon as possible.

3. It is recognised that the proposals at 2 will be an interim step and that development of fully validated nucleic acid amplification methods, for use by all manufacturers of these products, will require some time. The work of the 'International Working Group on the Standardisation of Gene Amplification Methods for the Virological Safety Testing of Blood and Blood Products' sponsored by WHO, will play an important role in defining key requirements for this methodology.

ANNEX V: CPMP/BWP/390/97

London, 24 March 1998
CPMP/BWP/390/97

COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP)

THE INTRODUCTION OF NUCLEIC ACID AMPLIFICATION TECHNOLOGY (NAT) FOR THE DETECTION OF HEPATITIS C VIRUS RNA IN PLASMA POOLS (CPMP/BWP/390/97)

ADDENDUM TO NOTE FOR GUIDANCE ON PLASMA DERIVED MEDICINAL PRODUCTS (CPMP/BWP/269/95)

DISCUSSION IN THE BIOTECHNOLOGY WORKING PARTY (BWP)	June 1997
TRANSMISSION TO THE CPMP	October 1997
RELEASE FOR CONSULTATION	October 1997
DEADLINE FOR COMMENTS	January 1998
DISCUSSION IN THE BIOTECHNOLOGY WORKING PARTY	March 1998
TRANSMISSION TO THE CPMP	March 1998
APPROVAL BY THE CPMP	March 1998
DATE FOR COMING INTO OPERATION	July 1999

NOTE FOR GUIDANCE ON THE INTRODUCTION OF NUCLEIC ACID AMPLIFICATION TECHNOLOGY (NAT) FOR THE DETECTION OF HEPATITIS C VIRUS RNA IN PLASMA POOLS

INTRODUCTION

The viral safety of plasma-derived medicinal products, with respect to hepatitis C, is assured by the following measures: selection of donors, screening of individual donations and starting materials for antibodies to hepatitis C, and the incorporation of effective viral inactivation/removal steps into manufacturing processes. Nucleic acid amplification technology (NAT) is a highly sensitive technique capable of detecting viral genomes even when serological tests are negative and it therefore has the potential to reduce further the virus load with which a manufacturing process may be challenged. The accumulation of experience in using NAT for detection of HCV RNA in plasma pools has now reached a stage when the introduction of testing on a routine basis can be proposed. The introduction of NAT for the detection of viral nucleic acid is a "state of the art" development which should be considered as another step in the continuously evolving process of assuring the quality of plasma-derived medicinal products.

RECOMMENDATION

1. Therefore, CPMP recommends that from **1 July 1999** only batches derived from plasma pools tested and found non-reactive for HCV RNA by NAT, using validated test methods of suitable sensitivity and specificity, should be batch released by the Marketing Authorisation holder. In the case of plasma-derived products used as excipients, the date of **1 July 1999** will apply to their incorporation into a manufacturing intermediate or final product. A strategy of pre-testing by manufacturers of mini-pools (of donations or of samples representative of donations) is encouraged in order to avoid the loss of a complete manufacturing pool and to facilitate tracing back to the donor in the event of a positive test result.

2. In line with the principles outlined above, and particularly in view of the established viral safety for HCV of products already on the market, and taking into account that individual donations and plasma pools are already screened for antibody to HCV and the need for continuity of supply, the CPMP confirms that there are no scientific reasons or safety concerns which would justify the recall of batches of products released by the Marketing Authorisation holder before **1 July 1999** and which are manufactured from plasma pools that have not been tested for HCV RNA by NAT. In exceptional circumstances, e.g. in the case of a shortage of an essential product, release of a batch derived from pools not tested by NAT should be evaluated on a case-by-case basis by the national competent authority

3. Information on test methodology and validation data should be submitted to competent authorities for evaluation and approval. The ability of the assays to detect different HCV genotypes should also be addressed through appropriate validation studies.

4. Each run of a validated assay should include a suitable working reagent (i.e. "run control"). The level of HCV RNA in the run control should be equivalent in HCV RNA content to 100 IU/ml. A non-reactive pool is defined as a pool found non-reactive by an assay run which detects this run control.

London, 29 July 1999
CPMP/BWP/385/99
Corrigendum, September 1999

COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP)

PLASMA-DERIVED MEDICINAL PRODUCTS: POSITION PAPER ON ALT TESTING

DISCUSSION IN THE BLOOD PRODUCTS WORKING PARTY	May - June 1999
TRANSMISSION TO THE CPMP	June 1999
RELEASE TO NATIONAL COMPETENT AUTHORITIES FOR CONSULTATION	June 1999
DEADLINE FOR COMMENTS	8 July 1999
DISCUSSION IN THE BIOTECHNOLOGY WORKING PARTY	July 1999
TRANSMISSION TO THE CPMP	July 1999
ADOPTION BY THE CPMP	July 1999

PLASMA-DERIVED MEDICINAL PRODUCTS: POSITION PAPER ON ALT TESTING

INTRODUCTION

The ALT test measures the level of alanine aminotransferase as an indicator of liver cell damage. As such it is a surrogate test for the presence of viruses that cause liver cell damage. ALT screening of donor blood was introduced in several countries (e.g. in Germany in the mid 1960s, France in the mid 1980s) in order to prevent transmission of hepatitis to recipients of plasma derivatives. After introduction of specific testing of hepatitis B virus the ALT test was intended to detect what was then known as non-A, non-B hepatitis (now known to be primarily due to infection with hepatitis C virus (HCV)). After introduction of a specific test for hepatitis C antibodies in 1991, the usefulness of the ALT test can be reconsidered. At present the test is required in the EU in Austria, Belgium, France, Germany, Italy and Portugal while the other EU member states do not require ALT testing[4] (*Commission Staff Working Paper, 1997)*.

PRESENT VALUE OF ALT TESTING

Hepatitis C virus

There is general agreement that the test no longer adds to blood safety as far as hepatitis C is concerned *(Blajchman et al. 1995; Busch et al. 1995; NIH, 1995)*. This was also confirmed by a meta-analysis of prospective clinical European studies on ALT testing for the prevention of non-A, non-B hepatitis following introduction of anti-HCV testing *(van der Poel, 1995)* which showed no beneficial effect for ALT screening after introduction of anti-HCV screening. The development of more sensitive tests for anti-HCV and, in the case of plasma-derived products, the requirement of nucleic acid amplification technology (NAT) testing for HCV in plasma pools as from July 1999 renders ALT testing for HCV even further redundant.

Hepatitis B

ALT testing might have benefit in detecting HBsAg mutants (*Jongerius et al, 1998; Hsu et al, 1997*) that escape the routine screen for HBsAg. However, if HBsAg mutants were considered to present a safety risk for plasma-derived products a more specific test such as NAT testing for hepatitis B virus (HBV) would be appropriate. In this context it must be remembered that the manufacturing processes for all plasma-derived products should include effective inactivation/removal steps for enveloped viruses such as hepatitis B.

Hepatitis A

There are currently no requirements for the screening of donations or plasma pools for hepatitis A virus (HAV). It has been argued that ALT testing will screen out donors with hepatitis A infections. However, an investigation on HAV transmissions caused by factor VIII concentrates several years ago led to the conclusion that the donors involved did not have elevated ALT levels at the time of donation. Also the fact that HAV transmissions by plasma products have happened where plasma is sourced from ALT-testing and non-ALT-testing countries, confirms that the ALT test is not a reliable screening test as far as HAV infection is concerned. This is not unexpected since the brief period of viremia seen with hepatitis A largely precedes the rise in ALT (*NIH, 1995*). Manufacturers have introduced, or are introducing into their manufacturing processes for coagulation factors, effective steps for the inactivation/removal of non-enveloped viruses such as hepatitis A. If it was considered necessary to also introduce a screening test for hepatitis A, a more specific test is needed (e.g. NAT).

---

[4] Finland and Ireland have confirmed to the BWP that ALT testing is not a national requirement.

Parvovirus B19

Human parvovirus B19 may be associated with acute hepatitis with elevated ALT (*Yoto et al., 1996; Hillingsø et al., 1998; Naides et al., 1996; Tsuda, 1993*). Parvovirus B19 is frequently transmitted through blood products and the virus inactivating methods have shown to poorly prevent transmission (*Mauser et al. 1998; Rollag et al., 1998, Santagostino et al., 1997*). ALT testing will only detect parvovirus B19 infections that have associated hepatitis. Therefore, the test will not prevent parvovirus B19 entering plasma pools and potential transmission by coagulation factor concentrates. This is illustrated by a study where B19 seroconversion (IgM) and B19 viremia were observed within 2 weeks of the first concentrate infusion in 8 of 15 susceptible patients treated with Factor VIII and IX from ALT screened donations (*Santagostino et al., 1997*).

Other viruses

Epstein-Barr virus (EBV) and Cytomegalovirus (CMV) are examples of viruses which might be detected by ALT testing. However, these viruses are not transmitted via plasma-derived products.

Hepatitis G virus (HGV) or GB virus C (GBV-C) has not been found to be associated with significant hepatic disease or symptoms and no other disease association has been identified. A recent study (*Blair et al., 1998*) on HGV/GBV-C in Scottish blood donors revealed no elevated ALT levels in infected donors.

It is still unclear whether the recently identified TT virus (TTV) is associated with disease. Recent data suggest that TTV, similar to HGV/GBV-C may be a human virus without clear disease association. Although some cases of transient infection associated with fulminant hepatitis have been reported (*Okamoto et al., 1998*), recent data suggest that there is also no clear correlation between TTV DNA levels and ALT levels (*Simmonds et al., 1998; Naoumov et al., 1998; Cossart 1998*).

ALT testing might be useful in screening out as yet unidentified viruses causing liver damage but this is inherently speculative.

Lack of specificity

ALT levels can rise because of various factors such as age, gender, obesity and alcohol use that are not necessarily related to infectious diseases. As a consequence, donors are deferred and donations are rejected where no transmissible infectious disease exists. Also, non-specificity of ALT may mean that donors could have a transmissible infectious hepatitis with an elevated ALT below the cut-off value.

RECOMMENDATIONS/REQUIREMENTS WITH RESPECT TO ALT TESTING

The Adare Colloquium on Blood Safety and Self-sufficiency held in 1996, under the auspices of the Irish presidency of the EU and with the support of the European Commission, concluded that ALT screening had become redundant *(Colloquium on Blood Safety and Self-Sufficiency, 1996).* The NIH Consensus Panel on Infectious Disease Testing for Blood Transfusions held in January 1995 made a similar recommendation i.e. that ALT testing of volunteer blood donors is no longer scientifically valid and therefore could be discontinued (NIH, 1995), which was confirmed by the FDA's Blood Product Advisory Committee in March 1995 (BPAC, 1995).

There is no core requirement for ALT testing of plasma for production of plasma derivatives in any European Directive, guideline or Pharmacopoeia. In most regulations there is however a statement that competent authorities may require additional screening tests (*Ph.Eur. monograph on human plasma for fractionation, 1998; Council of the European Union Recommendation, 1998*).

On request of the Ph.Eur. Commission, expert group 6B on blood products carried out a review of the current status of ALT testing as applied in the screening of donors of plasma for fractionation during its meeting in October 1998. It was concluded that there is at present an irreconcilable split on ALT testing among Member States.

The CPMP guideline on plasma-derived medicinal products states that in particular each donation must be tested for HBsAg, antibody to HIV1 and HIV2, and antibody to HCV (*CPMP Note for Guidance, 1998*).

Lack of harmonisation on ALT and marketing authorisation of plasma derivatives in the EU

Lack of harmonisation on ALT testing was already identified in 1994 (*Ad Hoc Working Party on Biotechnology/Pharmacy, position paper, 1994*). Due to this lack of harmonisation on ALT testing, barriers for marketing in the EU of plasma derivatives derived from non-ALT tested plasma remain. An example of this barrier has been encountered with one of the first applications for a plasma derivative in the centralised procedure. Since in this case the plasma has been derived from European donations, the principle of self-sufficiency within the European Union also has a bearing. This example clearly illustrates the urgent need for harmonisation on ALT testing for plasma derivatives among EU Member States.

CONCLUSION

Measures taken to prevent infection by the use of plasma-derived products include selection of donors, screening of individual donations and starting materials for markers of infection with known viruses and validation of the production process for the inactivation or removal of viruses. The incorporation into manufacturing processes of effective steps for the inactivation/removal of a wide range of viruses of diverse physico-chemical characteristics provides the best safeguard against transmission of as yet unidentified viruses. Once the causative agent has been identified, sensitive and specific screening tests can be developed.

There is no clear benefit from ALT testing for identified viruses but a possible role for screening out as yet unidentified viruses causing liver damage cannot be excluded. However, the test is non-specific and excludes donations that do not pose any safety risk.

There is no core requirement in any EU legislation for ALT testing of plasma for production of plasma derivatives. The current lack of harmonisation among Member States on ALT as a requirement for testing of donor blood for production of plasma derivatives obstructs Marketing Authorisations in the European procedures of plasma derivatives produced from plasma collected without ALT testing.

RECOMMENDATION

Major improvements in donor selection, specific screening test methods and manufacturing processes have been achieved during the past years. There is no evidence that ALT testing provides any significant increase of safety for plasma-derived medicinal products.

Thus, with the current state of the art of manufacture and control of plasma-derived medicinal products, as defined in the note for guidance CPMP/BWP/269/95, rev.2, there is no scientific basis for objecting to the use of plasma for fractionation collected without ALT testing.

REFERENCES

1) Ad Hoc Working Party on Biotechnology/Pharmacy Sub-Group on Medicinal Products Derived from Human Blood or Plasma, Position paper for CPMP on harmonisation of selection and screening of donors/exclusion criteria (III/5941/94) in Inventory of provisions relating to medicinal products derived from human blood or plasma, European Commission, DGIII: Pharmaceuticals, March 1995.

2) Blair, C.S., Davidson F., Lycett C., McDonald D.M., Haydon G.H., Yap P.L., Hayes P.C., Simmonds P., Gillon J., Prevalence, incidence and clinical characteristics of hepatitis G virus/GB virus C infection in Scottish blood donors. The Journal of Infectious Diseases, 1998, 178, 1779-82.

3) Blajchman M.A., Bull S.B., Feinman S.V., Post-transfusion hepatitis: Impact of non-A, non-B hepatitis surrogate tests. Lancet, 1995, 345, 21-25.

4) Blood Products Advisory Committee, 23-24 March 1995.

5) Busch M.P, Korelitz J.J., Kleinman S.H., Lee S.R., AuBuchon J.P., Schreiber G.B., and the Retrovirus Epidemiology Donor Study, Declining value of alanine aminotransferase in screening of blood donors to prevent posttransfusion hepatitis B and C virus infection. Transfusion,1995, 35, 903-910.

6) Colloquium on Blood safety and self-sufficiency: an agenda for the European Community, conclusions and recommendations. Under the auspices of: Department of Health – Ireland with support from the Commission of the European Communities, Adare, County Limerick, Ireland, September 1996.

7) Commission Staff Working Paper on The suitability of blood and plasma donors and the screening of their donations: a 1997 survey of the regulations and practices in the Member States of the European Community. SEC(97) 2298, Brussels, 10.12.1997.

8) Cossart Y., TTV a common virus, but pathogenic? Lancet, 1998, 352, 164.

9) Council of the European Union Recommendation of 29 June 1998 On the suitability of blood and plasma donors and the screening of donated blood in the European Community (98/463/EC), Official Journal, 1998, L 203, 14-26.

10) CPMP Note for guidance on plasma-derived medicinal products (CPMP/BWP/269/95, rev 2), 1998.

11) Hillingsø J.G., Jensen I.P, Tom-Petersen L., Parvovirus B19 and acute hepatitis in adults. Lancet, 1998, 351, 955-6.

12) Hsu H.Y., Chang M.H., Ni Y. H., Lin H.H., Wang S.M., Chen D.S., Surface gene mutants of hepatitis B virus in infants who develop acute or chronic infections despite immunoprophylaxis. Hepatology, 1997, 26(3), 786-791.

13) Jongerius, J.M., Wester, M., Cuypers H.T., van Oostendorp W.R., Lelie P.N., van der Poel C.L., van Leeuwen E. F., New hepatitis B virus mutant form in a blood donor that is undetectable in several hepatitis B surface antigen screening assays. Transfusion, 1998, 38(1), 56-59.

14) Mauser-Bunschoten E.P., Zaaijer H.L., van Drimmelen A.A.J., de Vries S., Roosendaal G., van den Berg H.M., Lelie P.N., High prevalence of Parvovirus B19 IgG antibodies among Dutch hemophilia patients. Vox Sang, 1998, 74, 225-227.

15) Naides S.J., Karetnyi Y.V., Cooling L.L.W., Mark R.S., Langnas A.N., Human parvovirus B19 infection and hepatitis. Lancet, 1996, 347, 1563-4.

16) Naoumov N.V., Petrova E.P., Thomas M.G., Williams R., Presence of a newly described human DNA virus (TTV) in patients with liver disease. Lancet, 1998, 352, 195-197.

17) National Institutes of Health Consensus Development Conference Statement: Infectious disease testing for blood transfusions. January 1995.

18) Okamoto H., Nishizawa T., Kato N., *et al.,* Molecular cloning and characterization of a novel DNA virus (TTV) associated with posttransfusion hepatitis of unknown etiology. Hepatology Res, 1998, 10, 1-16.

19) Rollag H., Solheim B.G., Svennevig J.L., Viral safety of blood derivatives by immune neutralization, Vox sang, 1998, Suppl 1, 213-217.

20) Santagostino E., Mannucci P.M., Gringeri A., Azzi A., Morfini M., Musso R., Santoro R., Schiavoni M., Transmission of parvovirus B19 by coagulation factor concentrates exposed to 100°C heat after lyophilization. Transfusion, 1997, 37, 517-22.

21) Simmonds P., Davidson F., Lycett C., Prescott L.E., MacDonald D.M., Ellender J., Yap P.L., Ludlam C.A., Haydon G.H., Gillon J., Jarvis L.M., Detection of a novel DNA virus (TTV) in blood donors and blood products, Lancet, 1998, 352, 191-195.

22) Tsuda H. Liver dysfunction caused by parvovirus B19. Am J Gastroenterol, 1993, 88, 1463.

23) Van der Poel C.L., ALT testing in prospective trials: meta-analysis on European studies and Surrogate testing in blood transfusion: concluding remarks. in Proceedings of the ISBT 5th Regional (4th European) Congress, Eds: Rossi, U., Massaro, A.L., Sciorelli, G., 1995, 943-949

24) Yoto Y., Kudoh T., Haseyama K., Suzuki N., Chiba S., Human parvovirus B19 infection associated with acute hepatitis. Lancet, 1996, 347, 868-9