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GUIDELINE ON PLASMA-DERIVED MEDICINAL PRODUCTS

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This guideline replaces Note for Guidance on Plasma-Derived Medicinal Products CPMP/BWP/269/95, rev.3 dated 25 January 2001.

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GUIDELINE ON PLASMA-DERIVED MEDICINAL PRODUCTS

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EXECUTIVE SUMMARY

This guideline lays down the requirements for the collection of starting material, the manufacturing and the quality control of plasma-derived medicinal products. Specific attention will be given to the viral safety of these products.

The current revision 4 has included an update on the legal framework as well as an update on specific guidance in relation to:

- Directive 2002/98/EC and its technical directives 2004/33/EC, 2005/62/EC;
- The collection and testing of starting material, where reference to the PMF guideline is given;
- The detection of HCV RNA by NAT, which became a mandatory requirement for plasma pool testing through introduction in the Ph. Eur. monograph "Human plasma for fractionation" and as a consequence Annexes III – V which provided background information on introduction of HCV RNA NAT have been deleted:
  - Annex III: Intramuscular immunoglobulins: nucleic acid amplification tests for HCV RNA detection (CPMP/117/95);
  - Annex IV: Implementation of CHMP/117/95 recommendation "Intramuscular immunoglobulins: nucleic acid amplification tests for HCV RNA detection" (CPMP/BWP/391/95);
  - Annex V: 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).

  Regarding the content of Annexes III-V, the interested reader is referred to the 3rd revision of this guideline published on the EMEA website http://www.emea.europa.eu/pdfs/human/bwp/026995en.pdf)
- The requirement for ALT testing which has been deleted from the Ph. Eur. Monograph "Human plasma for fractionation":
  - Annex VI "Plasma-derived medicinal products": Position paper on ALT testing (CPMP/BWP/385/99; corrigendum September 1999)"included the scientific rationale for the deletion of the requirement for ALT testing. It has been taken out of this guideline with the 4th revision and is published on the EMEA website http://www.emea.europa.eu/pdfs/human/press/pp/038599en.pdf
- Inclusion of Guideline on Assessing the Risk for Virus Transmission - New Chapter 6 of the Note for Guidance on Plasma-Derived Medicinal Products (CPMP/BWP/5180/03) into the main guideline text
- Reference to the Guideline on the replacement of rabbit pyrogen testing by an alternative test for plasma-derived medicinal products (EMEA/CHMP/BWP/452081/2007)

References to relevant guidelines refer always to the current version of these guidelines.

1. INTRODUCTION (background)

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 beginning of the 20th century, it was not until the 1940s 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 have made available a wide variety of products, with medical applications covering a large 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 hepatitis C (previously identified as non-A non-B hepatitis) resulted in major changes to the manufacturing processes, with the introduction of specific steps to inactivate or remove these and other blood-borne viruses. Infectious non-enveloped viruses were detected in certain plasma-derived products during the 1990's and early 2000's. Therefore, recent process development has been devoted to further reducing non-enveloped viruses such as Hepatitis A (HAV) and B19 virus.

Measures taken to prevent infection include selection of donors, screening of individual donations and plasma pools for markers of infection with known viruses and validation of the production process for inactivation or removal of viruses. From the 1990's on, measures designed to minimise contamination of the starting plasma have been improved by the refinement of serological test kits and the use of nucleic acid amplification technology (NAT) for the testing of viral DNA and RNA, thereby shortening the window period during which infectious donations are not detected.

Recent cases of variant Creutzfeldt-Jakob (vCJD) identified in blood transfusion recipients raised concerns about the risk of vCJD transmission and precautionary measures have been put in place to minimize the risk of prion transmission by plasma-derived products.

The legal basis for EU harmonised requirements for the quality and safety of the starting material for plasma-derived medicinal products has been revised along with the pharmaceutical legislation and specific provisions have been laid down in the pharmaceutical Directive 2001/83/EC as amended. In this legislation the option of a centralised certification of Plasma Master File was established.

Recently the European Parliament and the Council have issued the overarching Directive 2002/98/EC "Setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components…", also known as the "Blood Directive". Thereby, from 8 February 2005, Directive 2002/98/EC amending Directive 2001/83/EC established the requirements for the collection and testing of human blood and blood components whatever the intended purpose. In line with this directive, the technical directives 2004/33/EC, 2005/61/EC and 2005/62/EC have been issued by the Commission. Further guidance is provided by the "Guide to the Preparation, Use and Quality Assurance of Blood Components" of the Council of Europe which contains a compendium of measures designed to ensure the safety, efficacy and quality of blood components.

2. SCOPE

In this guideline specific requirements for plasma-derived medicinal products are described. These include collection and testing of starting materials, manufacturing of the different plasma-derived medicinal products, quality control issues and a special focus on the validation studies and more specifically the viral validation studies.

Medicinal products derived from human blood and human plasma (hereinafter called "plasma-derived products") fall under the definition of Article 1(10) of Directive 2001/83/EC as follows: "Medicinal products based on blood constituents which are prepared industrially by public or private establishments, such medicinal products including, in particular, albumin, coagulating factors and immunoglobulins of human origin." Furthermore, the pharmaceutical legislation also applies to plasma that is prepared by a method involving an industrial process (Article 3(6) of Directive 2001/83/EC). Solvent-detergent treated plasma is an example of this latter category.

This guideline also covers plasma derivatives used as:

- Excipients;
- Ancillary substances in medical devices (Directive 2000/7/EC amending Directive 93/42/EEC);
- Investigational products as such or as excipients.

Many parts of this guideline could also be applicable to active substances extracted from cellular components such as haemoglobin.

In accordance with article 3 (sections 1, 2 and 6) of Directive 2001/83/EC, the scope does not cover blood, blood components or medicinal products prepared on a small scale for individual patients in

accordance with a medical prescription, although many parts contained in this document may be pertinent.

3. LEGAL BASIS

In addition to the general conditions laid down for biological medicinal products, there are specific conditions for medicinal products derived from human blood or human plasma, briefly summarised in Annex I.

This guideline has to be read in conjunction with the requirements laid down in Directive 2001/83/EC of the European Parliament and of the Council, as amended by the Directive 2003/63/EC[1], which in turn refers to Directive 2002/98/EC as concerns regulations for *collection* and *testing* of blood components. In essence, the reference in Directive 2001/83/EC to Directive 2002/98/EC, along with the corresponding Commission directives, should ensure that there is an equivalent level of safety and quality of blood/plasma either used for transfusion or for the manufacture of medicinal products. These requirements refer, where applicable, also to blood/plasma and plasma-derived medicinal products imported from third countries.

Furthermore, it is a legal requirement that before an authorisation to market a plasma-derived medicinal product the manufacturer must demonstrate batch-to batch consistency. In addition, the absence of specific viral contaminants must be demonstrated to the extent the state of technology permits.

European Pharmacopoeia standards for plasma-derived medicinal products are provided in the monograph “Human plasma for fractionation” and specific monographs for plasma-derived medicinal products (Annexes II and III).

Whereas the free movement of goods is applied for medicinal products in general, Member States are allowed to apply more stringent requirements for plasma-derived products, e.g. the Competent Authority may request the MAH to submit samples from each bulk or batch of medicinal product for testing by a State laboratory before being released to the market (EC/EEA Official Control Authority Batch Release).

4. STARTING MATERIAL

The collection and testing of starting material are major factors in the quality assurance of the manufacture of biological medicinal products. Measures taken to reduce risks for transmission of blood born infections from plasma-derived products include the meticulous control of starting material.

Starting material for fractionation is plasma which can be obtained either from whole blood donations or by plasmapheresis and this has to comply with the Ph. Eur. monograph “human plasma for fractionation”.

All information on the starting material should be in accordance with the Guideline on the scientific data requirements for a plasma master file (EMEA/CHMP/BWP/3794/03).

If a MAH decides not to use the PMF certification procedures, it is also possible to provide the same information in Module 3, section 3.2.S. of the documentation for the medicinal product. This information should be updated and re-submitted for approval on an annual basis. Reference to more than one PMF is possible and should be clearly indicated in the dossier.

The immunisation of donors to obtain immunoglobulins with specific activities, should be in compliance with the requirements of the relevant Ph. Eur. Monographs. This also includes testing of donors of erythrocytes used for immunisation of donors for anti-D plasma. This information, which is specific to a particular product (e.g. immunisation scheme used for specific immunoglobulins), should be included in section 3.2 S of the dossier for the relevant product and not in the PMF. Reference is

[1] Introduction and general principles (4) and part I, module 3 and part III 1.1

made to WHO (The forty-third report of the WHO Expert Committee on Biological Standardisation, Technical Report Series 840, current edition.

4.1 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 and 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 B19 virus, or any other emerging infectious viruses or other agents such as (v)CJD. In many cases such viruses can establish a persistent or latent infection.

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.

Other factors of equal importance relate to the quality of the product, for example the integrity and biological activity of clotting factors and of immunoglobulins, which can be affected by the processing of the starting materials after collection (see conditions on storage and transport of plasma in the Guideline EMEA/CHMP/BWP/3794/03). Therefore, the risk of generation of thrombogenicity and immunogenicity should be considered.

4.2 Selection of donors and testing of starting material

Selection of donors and testing of donations and of plasma pools, are important factors in the safety of plasma-derived medicinal products:

Selection and exclusion criteria

For blood/plasma donors should be in compliance with Directive 2002/98/EC and Directive 2004/33/EC. Further guidance is provided in Guideline on scientific data requirements for a plasma master file (EMEA/CHMP/BWP 3794/03). Article 110 of Directive 2001/83/EC states: '*Member States shall take the necessary measures to promote Community self-sufficiency in human blood and plasma. For this purpose, they shall encourage the voluntary unpaid donation of blood and plasma and shall take the necessary measures to develop the production and use of products derived from human blood or human plasma coming from voluntary unpaid donations'*. However, according to the CPMP position statement on non-remunerated and remunerated donors (EMEA/CPMP/BWP/188/02), both non-remunerated and remunerated donors contribute to the supply of safe plasma-derived medicinal products.

Testing

Each donation and plasma pool should be tested in compliance with Directive 2002/98/EC, Directive 2004/33/EC and the Ph. Eur. monographs "human plasma for fractionation", "human plasma (pooled and treated for virus inactivation)", "human anti-D immunoglobulin" and "human anti-D immunoglobulin for intravenous administration). Further guidance is given in the Guideline on scientific data requirements for a plasma master file (EMEA/CHMP/BWP3794/03). These monographs require testing for HBsAg, HIV antibodies, HCV RNA of each fractionation pool and additional testing for B19-DNA for specific products (i.e. virus-inactivated pooled plasma and anti D immunoglobulins).

Parvovirus B19 has been transmitted by plasma-derived medicinal products such as coagulation factors, fibrin sealants, and by solvent-detergent treated plasma. In immuno-competent patients without specific underlying diseases, the infection is usually asymptomatic or mild. However, transient aplastic crisis may be observed in patients with erythropoietic disorders or prolonged anaemia may occur in immuno-compromised patients. Highly viraemic donations occur quite frequently and may lead to high contamination levels of plasma pools with more than 10^8 IU B19 DNA per mL. It is recognised that NAT screening for exclusion of such high titer donations can

significantly reduce the contamination of plasma pools thereby reducing the risk for transmissions and resulting potential complications. Therefore, introduction of high titre screening is encouraged. The appropriate limit for contamination of plasma pools is evaluated based on the B19V reducing capacity of the product-specific manufacturing process. A risk assessment according to chapter 4.6 of this guideline is performed in order to substantiate claims that a product can be considered safe with regard to B19V infections. It is important that the manufacturer clarifies whether all plasma used as starting material for a specific product is subjected to B19- NAT testing in order to allow a correct risk assessment. Testing of plasma pools with regard to parvovirus B19 (B19V) DNA is mandatory for pooled plasma treated by the solvent-detergent method (Ph. Eur monograph "human plasma (pooled and treated for virus inactivation)" and anti D immunoglobulins (Ph. Eur. Monographs "human anti-D immunoglobulin" and "human anti-D immunoglobulin for intravenous administration). If albumin or normal immunoglobulin is used in the manufacture of anti-D immunoglobulins, the parvo B19 titre of the plasma pools used for production of the albumin and normal immunoglobulin should comply with the requirements in Ph. Eur. monograph "Human anti-D Immunoglobulin".

4.3 Traceability

According to directive 2003/63/EC a system has to be in place which enables each donation to be traced from the donor via the blood establishment through to finished products and vice versa. Traceability has to be maintained as described in Directives 2002/98/EC, 2005/61/EC and GMP Annex 14. It is strongly recommended that every time a product is administered to a patient, the name and batch number of the product are recorded in order to maintain a link between the patient and the batch of the product in accordance with the Note for Guidance on the warning on transmissible agents in the Summary of Product Characteristics (SPCs) and Package Leaflets for plasma-derived medicinal products (CPMP/BPWG/BWP/561/03).

In compliance with the requirements laid down in the directives 2002/98/EC and 2005/61/EC, "facilities" to which blood and blood components are delivered, including manufacturers, should retain traceability records for at least 30 years after the time of the donation. To ensure that the duration of traceability is not shorter for batches of medicinal products compared to their raw/starting materials, a link should be maintained by the manufacturer of the plasma-derived product for at least 30 years after the time of the donation. This is to ensure that the MAH for this product or a manufacturer, using a batch of a plasma-derived product in his product, and the Competent Authorities would be informed if, in exceptional circumstances, post-collection information would lead to measures regarding the product.

4.4 Post-collection measures including look back procedures

A post collection information system should be in place describing the measures for reporting serious adverse reactions[2] and events[3]. Reporting to the competent authority of serious adverse reactions and events which may affect the quality and safety of blood and blood components should be made according to procedures laid down in EU GMP requirements including Annex 14 as amended to take account of Directives 2002/98/EC, 2005/61/EC and 2005/62/EC.

The management of post-collection information between the blood/plasma establishment and, in case of a PMF the PMF holder, and the manufacturing/fractionation facility should be described in standard operating procedures. These should be in place at the blood establishment(s), the PMF holder (if applicable) and at the manufacturer(s) of the plasma derived medicinal products and subject to written agreements between parties. If the reliability of a blood establishment/centre or the quality and safety

[2] According to Directive 2002/98/EC a serious adverse reaction "shall mean an unintended response in donor or in patient associated with the collection or transfusion of blood or blood components that is fatal, life-threatening, disabling, incapacitating, or which results in, prolongs, hospitalisation or morbidity."

[3] According to Directive 2002/98/EC a serious adverse event "shall mean any untoward occurrence associated with the collection, testing, processing, storage and distribution, of blood and blood components that might lead to the death or life-threatening, disabling or incapacitating conditions for patients or which results in, or prolongs, hospitalisation or morbidity."

of plasma could be questionable the PMF holder should inform national competent authorities and the EMEA as the PMF certification body. The following information should be communicated by the blood establishment to affected manufacturers of plasma derived medicinal products without delay after receipt of the information, if subsequent[4] to donation:

a) It is found that the donor did not meet the relevant donor health criteria;

b) A subsequent donation from a donor previously found negative for viral markers is found positive for any of the viral markers[5];

c) It is discovered that testing for viral markers has not been carried out according to agreed procedures;

d) The donor develops an infectious disease caused by an agent potentially transmissible by plasma-derived products (HBV, HCV, HAV, other hepatitis viruses, HIV 1 and 2, and other agents in the light of current knowledge) (see section 4.1.1);

e) The donor develops CJD or vCJD (see below);

f) The recipient of blood or of a labile blood component develops post transfusion infection which implicates or can be traced back to the donor.

For these cases a look-back procedure should be initiated which consists of tracing previous donations back for at least 6 months prior to the last negative donation and testing of any retained samples. The following should be considered:

- Donations which have not been processed should be identified and withdrawn from processing pending further investigation. The operation of an appropriate inventory hold (e.g. 60 days) may be helpful in this respect.
- In case the donation has been processed, an urgent 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 sensitivity of the tests performed (on the individual donations, the mini-pools and the plasma fractionation pools) the size of the pool, the cumulative look-back units that might be present in that particular batch and the implicated plasma pool, the nature of the product, its manufacturing method and the virus inactivation removal capacity of the process.
- A system for the compilation of the look-back units for every plasma pool should be in place and the information should be kept together with the batch record of the affected finished product and the respective plasma fractionation pool(s) records to ensure that this information is readily available to the QP(s) responsible for the release of intermediates or finished products.

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 also be referred to the relevant Medicines Competent Authority(ies)[6] together with a risk-based evaluation by the manufacturer regarding continued manufacture from the implicated pool or of the need for withdrawal of batches of product(s).

The mutual information system between blood/plasma establishments and manufacturing/fractionation centres should include information about any donor who develops any form of Creutzfeldt-Jakob disease (CJD), or is subsequently found to have a risk factor for CJD/vCJD. In case of post-donation

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[4] Where traceability data are available, it is expected that information will be communicated whatever the time period between the post-collection information and the donation. Any departure from this should be clearly stated and adequately justified.

[5] Communication of such cases should already be made based on repeat positive results and not await confirmatory testing. The length of time between donation and testing should be minimised in order to increase the likelihood that a seroconversion is detected before processing of previous donations in inventory hold.

[6] National Authorities where the product has been authorised or the Reference and concerned Member States (Mutual Recognition Procedure) or the EMEA (Centralised Procedure) and in addition, if different, to the Competent Authority supervising the manufacturer for batch release in the EEA ,

information regarding vCJD a risk assessment should be performed by the manufacturer in order to reach a decision on product recalls. (Position Statement on Creutzfeldt-Jakob Disease and plasma-derived and urine-derived medicinal products (EMEA/CPMP/2879/02)).

A traceability system (directive 2003/63/EC) and information system in line with the procedures for reporting of serious adverse reactions according to Directive 2001/83/EC and volume 9A of the Rules Governing Medicinal Products in the EU should be established for cases where a plasma derived medicinal product or medical device containing a blood derivative is under suspicion of causing an infection in a recipient.

5. MANUFACTURE

The manufacture of plasma-derived products should be defined and justified in terms of strategy, and described with all relevant details regarding procedures, in-process controls and final controls.

According to Directive 2001/83/EC, Annex I (3.2.1.2. manufacturing process of active substance(s), c)), amended by Directive 2003/63/EC, the conditions for manufacture of active substances for biological medicinal products are applicable: "If the presence of potentially pathogenic adventitious agents is inevitable, the corresponding material shall be used only when further processing ensures their elimination and/or inactivation, and this shall be validated."

5.1 Risk 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 and other adventitious agents 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 process related impurities such as proteins, 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 product related impurities, such as 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. A thorough characterisation, using state of the art methods, should be undertaken to ensure that functional characteristics are maintained and that aggregated, degraded or other modified forms, are appropriately controlled.

5.2 Plasma Pools

The manufacture of plasma-derived medicinal products starts from defined plasma pools. Samples of each plasma pool should be stored for at least one year after the expiry date of the finished product with the longest shelf-life. A description of all relevant procedures for the preparation and the sampling of the plasma pools should be provided according to guideline EMEA/CHMP/BWP/3794/03, in part 3.2.S of the dossier of the medicinal product or a reference to the relevant PMF(s) can be given.

In the dossier of the medicinal product all specifications of the plasma pool(s) should be stated. A clear reference to the PMF(s) is acceptable with respect to the description and testing of the plasma pool for viral markers, which should be performed according to the relevant Ph. Eur. monographs and the guideline EMEA/CHMP/BWP/3794/03. Where appropriate, compliance of the plasma pool with any production requirements of the relevant Ph. Eur. monographs should be confirmed.

5.3 Intermediates

An intermediate plasma fraction (intermediate) is partially fractionated starting 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, a contract manufacturer.

The collection and control of starting materials for the production of an intermediate plasma fraction are important factors in the assurance of its quality. Information up to and including the production of the plasma pool should be provided in the Plasma Master File or in part 3.2. S of the dossier, following the guideline EMEA/CHMP/BWP/3794/03. This information should be provided to the manufacturer of the finished product. A contract should be established between the supplier of the intermediate and the manufacturer of the finished 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 Marketing Authorisation Holder/applicant has final responsibility for the quality and safety of the medicinal product and therefore, should hold all the relevant information described in this section and have a contract with the manufacturer of the intermediate/finished product when different to the MAH.

The use of alternative processes for the production of intermediates is usually not acceptable because, as with biological medicinal products in general, plasma-derived medicinal products are largely defined by reference to their method of manufacture.

However, a variant of an established process may be employed if it concerns an intermediate used at an early stage of the manufacturing process of the medicinal product and if it does not concern the steps validated for viral reduction.

The suitability of use of the alternative intermediate must be demonstrated by the manufacturer. In the assessment of possible impact on quality, the process for production of the alternative intermediate should be validated, as such, and it should be validated that the use of the alternative intermediate does not affect the quality and viral safety of the finished product. Comparability should also be demonstrated (Note for Guidance for Biotechnological/Biological Products Subject to Changes in their Manufacturing Process (CHMP/ICH/5721/03)).

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.

5.4 Manufacturing Procedures

The strategies used in the manufacture of plasma-derived products are critical for product quality, safety and efficacy. Manufacturing strategies 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, specific procedures to inactivate/remove viral contaminants should be a requisite part of the manufacturing strategy for all plasma products. As already emphasised in the previous section (in connection with the use of intermediates), plasma-derived medicinal products are defined largely by reference to their method of manufacture, as with biological medicinal products in general. Therefore, the use of alternative processes is usually not acceptable.

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.

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 (see also 4.5.2 below). 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, caprylic (octanoic) acid, 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 such as caprylic (octanoic) acid, for others information is still scarce.

b) Chromatograpic methods

A number of different chromatographic procedures may be used in the purification and manufacture of plasma derived products. It has to be taken into account that the selectivity of the procedures and the yields depend critically on the quality of the chromatographic resins 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. The chosen procedures should be based on data of process development studies. 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, sanitisation and methods of regeneration should also be described. Details should be given of clarification and sterile, dia- or ultra-filtration procedures used.

c) Additional Considerations

Anticoagulants such as antithrombin and heparin may be added as raw materials/reagents at various stages during the production of coagulation factors to minimise activation. The materials and their use should be documented and their residual 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.

Viral inactivation/removal procedures

Procedures to inactivate/remove infectious viruses are included in the manufacturing strategies for plasma-derived products. The manufacturing process conditions and in-process monitoring for viral inactivation/removal steps should be clearly defined and justified. Careful validation is needed for each inactivation/removal step ensuring that the validation includes worst case conditions. The integrity of the product should be demonstrated under established manufacturing conditions. For further information, reference is made to section 8 Adventitious Agents.

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).

Process validation

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. For the principles of pharmaceutical development of the drug product, reference is made to the guideline CPMP/BWP/328/99.

In the development of the manufacturing process, critical parameters and critical controls should be identified and controlled. This is particularly important for novel process designs, including new designs for products traditionally manufactured using ethanol fractionation. The general principles of the guideline CPMP/QWP/848/96 are useful in this work, although the plasma-derived products are not included in the scope of the guideline. 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 process- and product-related impurities, 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 with particular emphasis on pyrogen elimination and virus carry over. The criteria for the use and re-use of chromatography resins and their life time should be provided. This is also applicable to filters in case of re-use.

For the establishment of release specifications, reference is made to the general principles laid out in the guideline Q6B (CPMP/ICH365/96). The manufacturer should demonstrate consistency at full scale production**,** showing compliance with the established specifications of the product. To this aim, batches should be derived from different bulks. In case that the manufacturing process starts from different amounts of plasma, it should be shown that the process yields a comparable product under the range of conditions applied. If a manufacturer decides to use intermediates from different manufacturing sites it should be shown that comparable products are consistently obtained. In the case of different manufacturing sites used in parallel a detailed validation program should be presented to demonstrate consistency.

Reprocessing should only be performed in case of process failures. The procedures and criteria should be fully described. Validation data should demonstrate that repetition has no negative influence on product quality.

6. QUALITY CONTROL

6.1 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 starting materials should be subject to careful control to avoid contamination and introduction of foreign material.

The monitoring of relevant parameters during manufacture, such as pH, temperature, ethanol concentration, protein and potency where appropriate, as well as the results from bacterial counts and endotoxin should be documented. Identification of critical in-process controls and limits for these parameters should be justified in line with the guideline Q6B (CPMP/ICH365/96).

6.2 Quality Control of Products

All products must comply with the appropriate European Pharmacopoeia monographs.

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 reflecting the capability of the production process in line with Guideline Q6B.

Batches which are used as in-house reference materials should be sufficiently characterised and their intended purpose specified. Any differences in their manufacturing process in comparison to the commercial process should be clear. A procedure for replacement of reference materials should be established.

The variability of the starting material and the heterogeneity of the plasma-derived products are important considerations in the validation of analytical methods used for starting materials, in-process controls, active substances and medicinal products. Validation should be performed according to the CPMP/ICH/381/95-ICH Q2 (R1) guideline. Validation of methods described in specific monographs is also needed to take into account product specific aspects, such as matrix interference. If reference is made to Ph. Eur. methods, which are described in general terms (e.g. immunochemical methods 2.7.1) validation studies should also to be performed. 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.

European Pharmacopoeia monographs for plasma derived-medicinal products (e.g. human albumin, human normal immunoglobulin, human immunoglobulin for intravenous administration and human blood coagulation factor VIII) are revised to encourage the use of alternative tests to the rabbit pyrogen test. Guidance on this aspect of quality control is provided in the Guideline on the replacement of rabbit pyrogen testing by an alternative test for plasma derived medicinal products (EMEA/CHMP/BWP/452081/2007).

7. STABILITY STUDIES

Stability studies should be performed, taking into account ICH guidelines, especially “Quality of Biotechnological Products: Stability Testing of Biotechnolgical/Biological products” (Q5C). Stability studies on the intermediate and, unless otherwise justified, on the finished product should also be performed if an intermediate from a new manufacturing site is introduced.

8. ADVENTITIOUS AGENTS

8.1 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. The rationale for the choice of specific virus inactivation/removal steps deliberately introduced into the process should be given.

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

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. (an effective step is defined in the NfG CPMP/BWP/268/95.) Thus it is desirable in most 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. It is recognised that it is difficult to inactivate or remove all known non-enveloped viruses efficiently using a single process step. Some non-enveloped viruses (such as animal parvoviruses) are stable against a number of heat-treatments while extremely small viruses (such as circoviruses) might penetrate even small filters designed for parvovirus reduction. Manufacturers are encouraged to develop/implement complementary process steps reducing a wide spectrum of viruses. This will enhance confidence in safety including unknown potentially emerging viruses. 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. 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 might not be required.

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 starting 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.

Contribution of partition processes to virus removal

Partition processes such as fractionation or purification procedures (e.g. immunoaffinity chromatography) may contribute to virus removal. 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.

Effect of virus inactivation/removal steps on the product

It should be established that the virus inactivation/removal steps selected will contribute to the overall 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, to the potential for formation of neo-antigens, to the possibility of enhanced thrombogenicity from activated coagulation factors, and to the possibility of toxic residues from chemicals used in the process as well as to virological safety. Separate guidance is available on the clinical studies that should be undertaken.

http://www.emea.europa.eu/htms/human/humanguidelines/efficacy.htm

8.2 Viral inactivation/removal procedures

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

Precipitation with ethanol

Ethanol fractionation may contribute to the viral safety of albumin and immunoglobulins by removing adventitious viruses rather than inactivating them. In fact, the disinfectant effect of ethanol/low pH occurs mostly at room temperature or above, whereas plasma fractionation is carried out at a low temperature to avoid protein denaturation. Where differential partitioning of the plasma components and viruses occurs during the precipitation steps, this results in the removal of viruses with the discarded fraction. Furthermore, precipitated proteins can be separated by centrifugation or, alternatively, by filtration. Filter aids are used to prevent clogging of the filters. As these substances may also adsorb viruses, they can enhance the viral removal capacity of the precipitation process.

Heating in aqueous solution

Heating in aqueous solution at 60^{o}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 several other plasma-derived products. It has been shown that pasteurization is an effective inactivation step for enveloped and some non-enveloped viruses according to CHMP/BWP/268. 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 and therefore have to be chosen carefully.

Heating of lyophilised products

The effectiveness of virus inactivation may vary according to the characteristics of the lyophilisate and the heating conditions. Upper and lower limits of residual moisture should be set based on viral validation studies as well as protein integrity studies and aggregate formation studies. Where such a treatment is applied to the product in its final containers, the variation in residual moisture between vials of product should be within the limits set. Critical parameters, in particular residual moisture, preferably measured on each vial with non-destructive methods (e.g. by near infrared spectroscopy), temperature and duration of heating should be carefully monitored throughout the process.

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.

Filtration

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, pH, 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.

Complex formation with antibodies as well as the protein content of the solution or adsorption of the viruses to membrane surface and the composition of the intermediate (e.g. buffer composition) can have an important impact on the removal of viruses. This should be considered in virus validation studies as well in routine production processes.

Low pH

Low pH (approximately 4) can be effective for immunoglobulins to inactivate enveloped viruses and certain non-enveloped viruses (e.g. B19 has been shown in some studies to be inactivated, whereas HAV and animal parvoviruses are not). Additionally, enveloped viruses may be inactivated at low pH in ethanol-containing intermediates in albumin production.

For both enveloped and non-enveloped viruses, the reduction factors that have been demonstrated depend on the exact conditions (e.g. pH value, time and temperature of treatment, composition of the solution, etc.) and the virus strain used in validation studies.

8.3 Points to consider for specific products classes

Coagulation factors

As for all plasma-derived products, 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. For Factor IX products, steps should be included in the process that are effective for HAV and parvovirus B19. Since steps like heat inactivation may have some limitations regarding certain non-enveloped viruses, companies are encouraged to increase the safety with regards to small, heat-resistant, non-enveloped viruses by use of removal procedures, like nanofiltration, where this is technically feasible.

For Factor VIII (and Factor VIII /von Willebrand) and fibrinogen products, where the large molecular size renders a size-based separation from virus particles less feasible, at least one step in the manufacturing process should be effective for HAV for which inactivation procedures have shown to be applicable. 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 for this type of virus.

Immunoglobulins

Immunoglobulin products have a good safety record for the known non-enveloped viruses due in part to the contribution from neutralising antibodies in the product. However, the possible transmission of unknown or emerging non-enveloped virus or the decline of antibody titres to non-protective levels in donor pools cannot be totally excluded. Thus at least one effective virus inactivation/removal steps for non-enveloped viruses is therefore required. Ethanol fractionation/precipitation steps can be accepted as effective for non-enveloped viruses if adequately controlled and validated (see also section 4.5.1.b). In the case that ethanol fractionation/precipitation steps are not found effective, another effective step for non-enveloped viruses should be introduced. If the process is based solely on chromatographic purification, an additional step(s) shown to be effective for non-enveloped viruses is needed.

Introduction of nanofiltration (small pore size 15-20 nm) into immunoglobulin processes has been shown to be effective for many non-enveloped viruses.

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 required from validation studies on the reduction of viruses during the manufacturing process. The effect of albumin concentration on virus reduction should be considered.

S/D Plasma

SD plasma has good safety measures for enveloped viruses and adequate safety measures are in place for HAV and B19 (Ph. Eur. monograph Human Plasma (Pooled and Treated for Virus Inactivation). The risk from other non-enveloped viruses already circulating in the population is considered low, because it is assumed that neutralising antibodies are present in plasma pools. There remains a theoretical risk from newly emerging non-enveloped viruses. Therefore, manufacturers are encouraged to carefully follow the epidemiology of such viruses in their donor population.

8.4 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:

Enveloped viruses

HIV-1

It is not necessary to carry out additional studies with HIV-2 as it is similarly affected by inactivation procedures. HIV-1 is not required in robustness studies on established virus reduction steps, such as SD treatment, heat treatment and ethanol fractionation steps. For new reduction methods HIV-1 should be considered when there is a lack of evidence that robustness can be covered by other enveloped model viruses.

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. However, 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.

Enveloped DNA viruses

To date, there have been no recorded transmissions of a herpesvirus associated with the use of non-cellular blood components. However, since some herpesviruses 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. An animal virus model, the duck hepatitis B virus (DHBV), may be used as a model of human HBV. However, it requires the use of its natural animal host (duck or primary duck cells) for titration. In consequence, there is no general requirement to include DHBV in the virus panel. However, in some specific situations where the efficacy of new inactivation procedures (e.g. UV illumination) are highly virus strain-dependent among enveloped viruses and for which inactivation/removal efficacy cannot be extrapolated from limited number of model viruses, the use of DHBV could be requested.

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. 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.

Model viruses for virus reduction filtration (nanofiltration)

Our knowledge of the viral clearance efficacy of nanofilters has improved with the increasing use of nanofiltration in manufacturing processes. Clearance efficiency should be demonstrated for each product with a range of virus sizes whatever the nanofiltration system used. Robustness studies may focus on the most difficult viruses to remove with a particular filter. For small pore size filters, HIV and BVDV should still be part of the virus panel, but robustness studies may focus on small non enveloped viruses. For medium pore size filters, large viruses such as herpesviruses should also be included in validation studies, with robustness studies focusing on e.g. BVDV.

8.5 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 (see also guideline CPMP/BWP/268/95 on virus validation studies). 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.

In some situations, NAT can be an alternative to infectivity tests to measure virus load and determine reduction capacities of removal steps. When performing validation studies with NAT, careful characterisation of the virus spike should be carried out in order to ensure the reduction of intact particles is measured and not removal of free nucleic acid or damaged particles. NAT studies may be useful to distinguish removal from inactivation when they occur at the same process step (e.g.

caprylate fractionation steps) or when an infectivity assay is not feasible (e.g. due to neutralising antibody interference).

8.6 Strategy for introduction of additional process steps for inactivation and removal of viruses

Since manufacturers should apply their best efforts to develop methods to inactivate/ remove viruses and this should be a continuing process, manufacturers should keep this under constant review in the light of technological developments. This is particularly important for products where there are currently limitations in what can be achieved in the reduction of non-enveloped viruses. Where it has been identified that specific process/product improvements can be made, Marketing Authorisation holders and applicants should set and justify timetables for such developments; and commit themselves to providing regular reports to the relevant competent authorities on their progress. Timescales for introduction of process changes should reflect the manufacturer's best efforts. In the meantime, product literature should be critically re-evaluated and, where necessary, amended to provide relevant and specific information to enable clinicians to make an informed choice of product (Note for Guidance on the Warning on Transmissible Agents in Summary of product Characteristics (SPCs) and Package Leaflets for Plasma-derived Medicinal Products (CPMP/BPWG/BWP/561/03)).

8.7 Revalidation

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

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.

8.8 Investigation of reduction of TSE agents

All issues concerning reduction of TSE agents are discussed in the respective EMEA documents ("CHMP Position Statement on Creutzfeldt-Jacob Disease and Plasma-Derived and Urine-Derived Medicinal Products" (CHMP/BWP/2879/02) and "Investigation of Manufacturing Processes for Plasma-Derived Medicinal Products with Regard to VCJD risk" (CPMP/BWP/5136/03)). Latest CHMP recommendations should be followed.

9. ASSESSING THE RISK FOR VIRUS TRANSMISSION (FORMER GUIDELINE CPMP/BWP/5180/03)

9.1 Introduction

The aim of this chapter is to outline the general principles that manufacturers should follow in performing risk assessments for virus transmission by plasma-derived medicinal products. These risk assessments are required for the substantiation of statements on virus safety and any remaining potential risk in the product information for these products, as outlined in the Note for Guidance on the Warning on Transmissible Agents in SPCs and Package Leaflets for Plasma-derived Medicinal Products (CPMP/BPWG/BWP/561/03). The risk assessment should, where possible, include a quantitative estimation of the probability of a virus contaminant being present in a defined dose of final product. The principles presented below can be applied to both known and emerging viruses.

9.2 General Principle of the Risk Assessment

The principle of the risk assessment is to consider various factors, such as epidemiology, viraemic titre, testing for viral markers, virus inactivation/removal steps and product yield that influence the potential level of infectious virus particles in a dose of final product. The reliability of the risk assessment will depend on the extent of information available on these factors. Many of the factors may vary and realistic worst case in order to obtain a result which can give greatest assurance for the statements on viral safety.

An estimate of the capacity of the manufacturing process to inactivate or remove the contaminant virus ("overall virus inactivation/removal capacity") versus the potential amount of a given virus that may be present in the starting material ("potential virus input") should also be provided. In addition, by considering the amount of starting material needed to manufacture a single dose of product, the probability of potential virus contamination in a single dose of the final product can be estimated.

Potential virus input

For viruses that are potential contaminants of human plasma, the amount of virus that may contaminate the plasma pool for manufacture ('potential virus input') should be estimated. The 'potential virus input' is determined by the number of viraemic donations that could enter the manufacturing pool, the volume of individual donations and the titre of a viraemic donation that might escape detection in a virus assay.

The number of viraemic donations depends on the epidemiology in the donor population and on the frequency of donations from an individual donor. Donor selection and exclusion criteria, as well as inventory hold measures, should be assessed for their effectiveness in decreasing the number of viraemic donations that may enter the manufacturing pool. Any available information on the specific donor population from the Plasma Master File should be incorporated into the risk assessment. In cases where such data are not available, information should be sought from other sources e.g. general epidemiological surveys or investigational studies on the donor population**.**

The viraemic period should be described with respect to its length and virus titre. With respect to individual screening by specific tests (serological or nucleic acid amplification technologies (NAT)), the titre of viraemic donations that are not recognised by such tests (e.g. donations from the 'window period') has to be considered. A 'minipool' represents a defined number of aliquots of donations that are pooled for testing purposes. Testing of minipools (e.g. by NAT) may be a valuable tool in identifying and excluding highly viraemic donations. In both cases, single donation testing and minipool testing, the 'potential virus input' in the manufacturing pool has to be extrapolated using estimates on the titer and on the number of undetected viraemic donations. Measures that identify and exclude contaminations at the minipool level or at the single donation level will more readily detect a contamination than tests applied to the manufacturing pool. However, a sensitive NAT testing of the manufacturing pool defines a well-controlled upper limit for a potential virus contamination.

Virus inactivation/removal capacity

The principles for determination of the virus inactivating/removal capacity of a production process and for interpretation of these data have been outlined in the CPMP guideline on virus validation (CPMP/BWP/268/95). Virus validation is an approach that has to be interpreted carefully, considering qualitative aspects in addition to quantitative data. For example, the reliability of the data from scaled-down experiments and of the virus reduction factors with respect to variations of manufacturing process parameters, should be carefully considered. Other limitations include the validity of summing-up logarithmic reduction numbers from single steps, the relevance of the viruses used in validation studies (model viruses or specific laboratory strains from the same species), and experimental limitations on the level of inactivation/removal that can be measured.

For emerging viruses, the specific physical characteristics of the emerging virus should be discussed carefully with respect to any model viruses for which data have previously been derived. If it is possible to handle the emerging virus in the laboratory, investigational studies are recommended to evaluate the relevance of previously derived data. If it is not possible to use the emerging virus for investigational studies, and if pre-existing data were derived using viral species that are not adequate models of the emerging virus, investigational studies with a closely related model virus should be considered. Depending on the available data, further validation with the relevant virus or a more specific model virus should be decided on a product-specific basis.

Contribution from specific antibodies to virus safety

Specific antibodies may contribute to virus safety. A specification of the antibody content in the final product and validation of its neutralisation capacity could substantiate the role of specific antibodies in assuring the virus safety of a specific product. The benefit of specific antibodies in the pool for fractionation is difficult to assess as there is no reliable information on viral neutralisation at this manufacturing stage nor on the stability of virus-antibody complexes during further downstream

processing. If claims are made in the risk assessment on removal of virus-antibody complexes from product intermediates, this should be substantiated by appropriate validation data.

Estimation of virus particles in the finished product

As a general principle for a safe product, the virus inactivation/removal capacity should clearly exceed the potential amount of virus that could enter the production process leading to an adequate safety margin of the finished product. However, no specific limit is defined because, as outlined above, the viral reduction factor is subject to various qualitative aspects of interpretation and the potential number of viral particles per vial of product should be discussed in relationship to these and other factors.

The amount of plasma used for production of one vial of final product should be defined considering the product yield from plasma, the batch size, and the number of vials produced from a batch. The relevant data should be provided from process validation. The information on the amount of required plasma should be used along with the data deriving from virus validation studies and the potential viral input to estimate the number of viral particles per vial. The estimated number of viral particles per vial can be calculated from the product of the worst case virus concentration in the starting material and the plasma required to produce one vial, divided by the viral reduction factor obtained from validation studies.[7]

The number of estimated virus particles per vial may also be discussed in respect to what is known about the minimum human infectious dose and the amount of medicinal product typically used in treatment. Any statement about the human infectious dose should be substantiated by data regarding the route of administration. If such data are not available, a conservative approach using viral genomes as an indicator of potentially infectious virus particles in the starting material should be followed. In-vitro infectivity data is generally not acceptable.[8]

Clinical experience and surveillance

The clinical experience with respect to virus transmission from the product, including any reports of virus transmission with the product or any similar product, should be discussed. It should be borne in mind that virus transmissions tend to be related to specific batches of product. The number of investigated patients from clinical studies is usually too low to detect infections, and only a limited number of batches are used. A long and satisfactory clinical experience may be very helpful to support the safety of a product, provided that any factor affecting virus safety (e.g. epidemiology) is not significantly changed. However, an absence of reported transmissions does not prove the viral safety of a product e.g. because undetected transmissions may have occurred or the product may have been used in a non-susceptible population. This is especially the case for emerging viruses or viruses that have not been carefully considered by a surveillance system (such as B19 virus).

9.3 Application of this Chapter

A viral risk assessment for HIV, HBV, HCV, B19 and HAV should be performed for all new marketing applications with the exception of albumin (see below). This will substantiate statements on virus safety and any remaining potential risk in the SPC, as outlined in the Note for Guidance on the Warning on transmissible agents in SPCs and Package Leaflets for plasma-derived medicinal products (CPMP/BPWG/BWP/561/03)**.**

For products for which a marketing authorisation has already been obtained, a risk assessment will be expected for HAV and B19 if claims are made regarding effective measures for these viruses. If no

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[7] $N = c \times V \div R$ where N is the potential number of viral particles per vial of product, c is the potential virus concentration in the plasma pool, V is the volume of plasma required to produce one vial of product, R is the viral reduction factor obtained from validation studies. An example of this type of calculation is given in ICH guideline Q5A: Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin (CPMP/ICH/295/95).

[8] It is usually not clear if the relation between infectious particles and genomes from a virus which has been produced in cell culture reflects the virus which occurs *in vivo*. Further, the sensitivity of the cell culture system may not reflect the efficiency of an *in vivo* transmission event.

claims are made, no risk assessment is required. In either case, risk assessments for HIV, HBV and HCV are not required.

A risk assessment will not be expected for new marketing applications or existing marketing authorisations in the case of albumins manufactured according to European Pharmacopoeia specifications and by established fractionation processes. For such albumins, a general statement on virus safety is foreseen in the core SPC. A risk assessment would be expected if an albumin was manufactured by other methods.

According to Section 4.4 of this guideline , the relevant Medicines Competent Authority(ies) have to be informed when there are indications that a donation contributing to a plasma pool was infected with HIV or hepatitis A, B, or C. A lot-specific risk assessment should be performed whenever post-pooling information indicates that a contaminated donation has entered the manufacturing plasma pool[9]. In such situations, reference can be made to the risk assessment included in the Marketing Authorisation Dossier. A specified NAT limit of the manufacturing pool may be helpful in substantiating such risk assessments.

10. PLASMA-DERIVED PRODUCTS USED IN THE MANUFACTURE AND FORMULATION OF MEDICINAL PRODUCTS OR AS ANCILLARY BLOOD DERIVATIVE IN MEDICAL DEVICES

Plasma derived products are widely used in the manufacture of other medicinal products, as raw materials (e.g. albumin used in cell culture media), as reagents (e.g. antithrombin added during Factor IX concentrate production), as active substances (e.g. radiopharmaceuticals) or as excipients (e.g. albumin added to plasma-derived products, vaccines and recombinant DNA products or antithrombin added to prothrombin complex concentrates). In addition, plasma derived products are used as ancillary blood derivatives incorporated in medical devices and are evaluated in analogy to the medicinal product legislation according to Directive 93/42/EEC as amended.

Link to post collection information

The dossier requirements referred to in this guideline for starting materials and for traceability, from blood/plasma donations through to finished product and vice versa, also apply to plasma derived products used in the manufacture and formulation of other medicinal products or used as ancillary blood derivative incorporated in medical devices. This includes a contract between the manufacturer of the plasma-derived product and the manufacturer of the finished medicinal product or the medical device in which maintenance of traceability records for at least 30 years after the time of donation is specified.

Quality and specifications

Whenever a plasma-derived product is used in the manufacture of a medicinal product or incorporated in a medical device, it should have the same quality and specifications as that of the product for therapeutic use. Full documentation should be provided for the plasma-derived product used. The plasma-derived product used in the manufacture 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, final product or medical device. 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 plasma-derived product used in the manufacture. With regard to stability no specific studies for finished products including excipients/reagents of different ages are required.

Whereas the EC/EEA official control authority batch release of plasma-derived medicinal products may be required by a Member State, for ancillary blood derivatives used in medical devices there is a

[9] Further guidance on the actions to be taken in this situation is provided in Annex 14 to the EU guide to Good Manufacturing Practice.

legal requirement that a sample of each batch of bulk and/or finished product of the blood derivative shall be tested by a State laboratory or a laboratory designated for that purpose by a Member State.

Synchronisation of expiry dates

When a plasma-derived product is used as an excipient or ancillary blood derivative, synchronisation of expiry dates with the finished product or medical device is recommended 1) to help ensure that the plasma-derived product used as excipient of other products or as ancillary blood derivative 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 and 2) to help ensure that the pharmaceutical characteristics comply with the current requirements.

It is recognised that, in some circumstances, it can be difficult for a manufacturer to synchronise the expiry date of a batch of the plasma-derived product used as excipient or ancillary blood derivative with the expiry date of the formulated product or medical device. Any deviation from this recommendation should be justified, as part of the Marketing Authorisation procedure or consultation procedure.

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 or as ancillary blood derivative. This evaluation will determine the action to be taken.

Albumin

Albumin manufactured according to established processes 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 or as ancillary blood derivative in medical devices.

As a single batch of albumin may be used to produce a number of batches of a medicinal product or medical device because of the small amounts that are typically used as an excipient or ancillary blood derivative, a careful selection of the products is recommended to avoid large volume product recalls because of suspicion for vCJD contamination (CHMP Position Statement on Creutzfeldt-Jakob Disease and plasma-derived and urine-derived medicinal products (EMEA/CHMP/BWP/2879/02).

ANNEX I: LEGAL BASIS TABLE

Special regulations for medicinal products derived from human blood or human plasma, in addition to general regulations for biological medicinal products

Major scope	**Legal framework, Definitions, Scope/purpose**		**Documentation**	**Production Quality and safety requirements**			**Quality system**	
	2001/83/EC	2002/98/EC	2003/63/EC, Annex 1	European Pharmacopoea	2004/33/EC	2005/61/EC	2005/62/EC	2003/94/EC GMP
Plasma as raw or starting material								
Collection, testing, including traceability, reporting of adverse events	*Art. 109*: Ref. to 2002/98/EC	x[3]	*Part III. 1.1* PMF format and procedure, incl. AU[10]	See Annex I, II	x	x	x	
Processing, storage, transport								*GMP guide incl. Annex 14*
Medicinal product								
Manufacture	*Art. 115* Supervision of consistency and viral clearance		*3.2.1.1-2* Requirements for plasma as raw and starting materials	See Annex I, II				*GMP guide incl. Annex 14*
MA dossier			*Part III. 1.1* Ref. to PMF[11] in 2nd step, incl. AU					
Record keeping								
Up to and including the facility to which blood/plasma is delivered		x				x	x	
Through complete chain from donation to finished product and vice versa			*Part III. 1.1*					GMP guide incl Annex 14
Wholesale distribution	*Art 83* MS may apply more stringent requirements							
Supervision Including official batch release	*Art. 114.2, 115*							

[10] AU: Annual update
[11] The MA dossier may refer to more than one PMF
[3] "x" indicates that the complete document is an addition to the general regulations for biological medicinal products

ANNEX II – LIST OF PUBLISHED MONOGRAPHS ON BLOOD PRODUCTS

The following monographs and general methods of the

European Pharmacopoeia (current edition) are applicable:

Monograph Title
Fibrin sealant kit
Human albumin injection, iodinated (125I)
Human albumin solution
Human anti-D immunoglobulin
Human anti-D immunoglobulin for intravenous administration
Human antithrombin III concentrate
Human coagulation factor VII
Human coagulation factor VIII
Human coagulation factor IX
Human coagulation factor XI
Human fibrinogen
Human hepatitis A immunoglobulin
Human hepatitis B immunoglobulin
Human hepatitis B immunoglobulin for intravenous administration
Human measles immunoglobulin
Human normal immunoglobulin
Human normal immunoglobulin for intravenous administration
Human plasma for fractionation
Human plasma (pooled and treated for virus inactivation)
Human prothrombin complex
Human rabies immunoglobulin
Human rubella immunoglobulin
Human tetanus immunoglobulin
Human varicella immunoglobulin
Human varicella immunoglobulin for intravenous administration
Human von Willebrand factor
Technetium (99mTc) human albumin injection
Technetium (99mTc) macrosalb injection
Technetium (99mTc) microspheres injection

ANNEX III – LIST OF GENERAL METHODS

Activated coagulation factors (2.6.22)
Anti-A and anti-B haemagglutinins (indirect method) (2.6.20)
Assay of human anti-D immunoglobulin (2.7.13)
Assay of human antithrombin III (2.7.17)
Assay of human coagulation factor II (2.7.18)
Assay of human coagulation factor VII (2.7.10)
Assay of human coagulation factor VIII (2.7.4)
Assay of human coagulation factor IX (2.7.11)
Assay of human coagulation factor X (2.7.19)
Assay of human coagulation factor XI (2.7.22)
Assay of Human von Willebrand factor (2.7.21)
Immunochemical methods (2.7.1)
Nucleic Acid Amplification Techniques (2.6.21)
Prekallikrein activator (2.6.15)
Test for anti-D antibodies in human immunoglobulin for intravenous administration (2.6.26)
Test for anticomplementary activity of immunoglobulin (2.6.17)
Test for Fc function of immunoglobulin (2.7.9)