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VIRAL SAFETY AND PROCESS VALIDATION FOR INACTIVATION OR REMOVAL OF VIUSES FROM PLASMA-DERIVED MEDICINAL PRODUCTS

In Seop Kim
Technical Operations Service, Korea Green Cross Corp.

Introduction

Human plasma, the liquid portion of blood, contains many dissolved components, primarily proteins which are important in the humoral immune systems and the coagulation system. Immune proteins are predominantly immunoglobulins, antibodies, which react specifically with antigens. Coagulation proteins contribute to the series of enzyme-substrate reactions and platelet function which are responsible for maintaining hemostasis. Many important therapeutic products such as albumin, immunoglobulins, and coagulation factors, are purified from the plasma. Although the therapeutic use of blood transfusion goes back to the turn of the 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 derived from human plasma (2).

Because plasma-derived proteins are manufactured from human plasma, special precautions must be taken during the production of these proteins to assure against the possibility of the products transmitting infectious diseases to the recipients (4, 5). For a long time the major risk associated with the use of blood products was viral infection such as Hepatitis A, B, C, and G, Human immunodeficiency virus (HIV), Human T-cell lymphotropic viruses (HTLV) I and II and the parvoviruses (1, 3, 6). Recently the transmissible spongiform encephalopathies (TSE's) have drawn particular attention to this risk (7). 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 processes for the inactivation and/or removal of viruses. Clinical follow up of recipients is the final proof of the safety of a product. In this paper, general strategy for validation of the production processes for the inactivation and/or removal of viruses, based on the guidelines of USA and EU (8-12), will be described.

Necessary of Process Validation Studies for Virus Removal/Inactivation

Testing of the starting material and product during the production process can only assure a certain level of safety as it is not feasible to test for all possible contaminants and there may exist variants or as yet unidentified viruses which may not be detected by existing techniques. Testing also suffers from the limitation that only a small percentage of the overall material can be sampled and if virus contamination is present at low concentration then there is a high

probability that the test will fail to reveal the presence of contamination in the total product. Therefore process validation studies for virus removal and/or inactivation play an important role in establishing the safety of plasma-derived medicinal products. Process validation is a documented programme which provides a high degree of assurance that a specific process will consistently manufacture a product meeting its predetermined specifications and quality attributes. Performing process validation studies for virus removal or inactivation involves several basic step.

Design of Process Validation for Virus Removal/Inactivation

Scaling down of the process and validation for comparability with the manufacturing scale process

Table 1 summarizes the methods currently in use for the inactivation and/or removal of viruses in plasma derivatives and also lists the methods which are now in the course of development. It is important that all potential virus clearance or inactivation steps, from initial treatment e.g. precipitation, clarification through to the final steps in the production process, such as freeze-drying are validated for virus removal or inactivation. Down-scaling of the purification process is an essential part in performing process validation studies for virus removal/inactivation. For several reasons, including the scale of the production process and GMP constraints regarding introduction of viruses, it is either impossible or impractical to perform these studies on the full manufacturing scale. Therefore the steps to be studied are scaled-down to laboratory scale which generally means a down-scale factor of 1/100-1/200, or greater for large-scale processes although there are no set guidelines governing the size of a down-scaled steps of the process. The most essential clue is to show validity of the scaling down step. The down-scaled process should mimic as closely as possible the full manufacturing scale process and that the experiments should be performed according to Good Laboratory Practice (GLP). Therefore the down-scaled process should be validated to ensure that product yield, purity, buffer composition etc. are as close as possible to those achieved by the full manufacturing process. This ensures that the virus clearance studies are performed on a process that is as close a representation of the full manufacturing process as possible. For chromatographic equipment, column bed-height, linear flow-rate, flow-rate-to-bed-volume ratio (i.e., contact time), buffer and gel types, pH, temperature, and concentration of protein, salt, and product should all be shown to be representative of commercial-scale manufacturing. A similar elution profile should result. For other procedures, similar considerations apply.

Choosing the appropriate viruses for the study

The viruses that may be transmitted via blood and blood products are HIV-1, HIV-2, HTLV-1,

HTLV-II, Hepatitis viruses (A, B, C, E, G, Delta), Tick-borne encephalitis, Cytomegalovirus (CMV), Epstein-Barr virus, Herpesvirus (HHV-6, HHV-7), Herpes simplex virus (HSV), Parvovirus B19, and JV virus (1, 3, 6). Those of major importance are HIV-1, HIV-2, the hepatitis viruses (HAV, HBV and HCV) and the T-cell lymphotropic viruses (HTLV-1 and HTLV-2). CMV and the parvovirus B19 may also be of concern, especially to immunocompromised recipients. Viruses for clearance evaluation and process characterization studies should be chosen to resemble viruses which may contaminate the product and to represent a wide range of physico-chemical properties in order to test the ability of the system to eliminate viruses in general. The rationale for the choice of specific virus inactivation/removal steps deliberately introduced into the process should be given by the responsible person, organ, or manufacturer.

A major issue in performing a viral clearance study is to determine which viruses should be used. Such viruses fall into three categories: "Relevant" viruses, specific "model" viruses, and nonspecific "model" viruses. "Relevant" viruses are viruses used in process evaluation of viral clearance studies which are either the identified viruses, or of the same species as the viruses that are known, or likely to contaminate the cell substrate or any other reagents or materials used in the production process. The purification and/or inactivation process should demonstrate the capability to remove and/or inactivate such viruses. When a "relevant" virus is not available or when it is not well adapted to process evaluation of viral clearance studies (e.g., it cannot be grown in vitro to sufficiently high titers), a specific "model" virus should be used as a substitute. An appropriate specific "model" virus may be a virus which is closely related to the known or suspected virus (same genus or family), having similar physical and chemical properties to the observed or suspected virus.

Table 1 Methods used for improving the safety of plasma derivatives with respect to transmission of viruses

PARTITION METHODS	INACTIVATION METHODS	METHODS UNDER DEVELOPMENT
•Ethanol fractionation •Precipitation by e.g. PEG, Octanoic Acid •Chromatography •Filtration	•Heat treatment •Sovent/detergent •Beta-propiolactone/UV •Low/High pH •Irradiation	•Photochemical inactivation •Sodium chlorite •Caprylate •Iodine treatment •Microwave heating

Performing the Process Validation Study

Virus spiking studies

Process validation experiments are performed by spiking the starting material for each step of

the process with the relevant viruses. The virus spike is added in a volume that is <10% v/v of the total volume of the material to be spiked. This is to ensure that the nature of the starting material is not affected by adding virus in tissue culture medium. This material is then taken through the purification process and the appropriate fractions are collected for assay of infectious virus.

Titrating the output samples and calculating virus reduction and clearance factors

The titre is expressed in statistical units as 50% tissue culture infected dose ($TCID_{50}$). Once the virus titrations have been performed then the next stage is to calculate the virus reduction factors for each step tested in the validation. The virus reduction factor for an individual purification or inactivation step is defined as the $\log_{10}$ of the ratio of the virus load in the pre-purification material (Spiked starting material – SSM) divided by the virus load in the post purification material (Output Material). The formula takes into account both the titres and volumes of the materials before and after the purification step.

$10^{Ri} = (v^{I})\ (10^{aI})/(v^{II})\ (10^{aII})$

where : Ri =reduction factor for a given stage, v^{I} = volume of the input material, aI = titre of the virus in the input material, v^{II}= volume of the retained output material, aII= titre of the virus in the output material

The virus clearance factor is calculated similarly by substituting the theoretical total virus spiked into the SSM by the total virus input.

Interpretation of Virus Reduction/Clearance

The overall reduction/clearance factor for a complete production process is the sum logarithm of the reduction/clearance factors of the individual steps. It represents the logarithm of the ratio of virus load at the beginning of the first process clearance step and at the end of the last process clearance step. According to CPMP guideline, the overall clearance factor for a production process should be substantially greater than the maximum possible titer that could potentially occur in the source material. Acceptable limits will be considered on a case-by-case basis (11).

Examples of Validation Study

Korea Green Cross Corp. has done the validation studies to ensure the viral safety of plasma derived products (13-15). Five viruses were selected for this study; HIV-1 (as a relevant virus and model virus for HIV-2) , Bovine viral diarrhoea virus (BVDV: as a model virus for hepatitis C or hepatitis G), Bovine herpesvirus (BHV: as a model virus for human herpesvirus such as HHV-6, HHV-7, HHV-8, Epstein Barr virus, or HSV-1), Murine encephalomyocarditis virus (EMC: as a model virus for HAV) and Porcine parvovirus (PPV: as a model virus for human

parvovirus).

Validation of viral clearance during fraction III precipitation and 60 ℃ heat treatment steps of the manufacturing process for ISG (Immuno serum globulin)

Fraction III precipitation step, an alcohol fractionation process, was a very effective step to precipitate all the viruses tested. 60℃ heat treatment step was effective more to lipid enveloped viruses rather than non-enveloped viruses. These results indicate that ISG manufacturing process are capable of removing/inactivating a wide range of relevant and model viruses which show a broad spectrum of physico-chemical properties (Table 2).

Validation of viral clearance during manufacturing process of a highly purified factor VIII (GreenMono II)

S/D treatment was a very effective step to inactivate enveloped viruses such as HIV, BVDV, and BHV, which were inactivated to undetectable level within one minute. Monoclonal antibody (mAb) column chromatography was shown to partition non-enveloped viruses such as HAV, EMC and PPV. Also the Q-Sepharose column chromatography was found to further eliminate viruses. The study also showed that non-enveloped viruses rather than enveloped viruses were effectively inactivated by lyophilization. These results indicate that GreenMono II manufacturing process are capable of removing/inactivating a wide range of relevant and model viruses which show a broad spectrum of physico-chemical properties (Table 3).

Table 2. Virus clearance during ISG process (Log Clearance Factor)

	TARGET VIRUSES				
	Lipid enveloped			**Non-enveloped**	
PROCESS STEP	**HIV**	**BHV**	**BVDV**	**EMC**	**PPV**
Fraction III precipitation	>4.4	6.8	>3.5	4.4	4.8
60℃ heat treatment	>5.3	>7.3	>4.8	2.9	3.2
Cumulative log clearance factor	**>9.7**	**>14.1**	**>8.3**	**7.3**	**8.0**

Table 3. Virus clearance during GreenMono II process (Log Clearance Factor)

	TARGET VIRUSES				
	Lipid enveloped			**Non-enveloped**	
PROCESS STEP	**HIV**	**BHV**	**BVDV**	**EMC**	**PPV**
S/D treatment	>4.1	>4.3	>3.6	-	-
mAb column chromatography	-	-	-	4.8	3.81
Q-Sepharose column chromatography	5.8	2.3	2.4	2.7	1.28
Lyophilization	-	1.5	1.9	4.4	2.16
Cumulative log clearance factor	**>9.9**	**>8.07**	**>7.86**	**11.85**	**7.25**

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