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Robustness of nanofiltration for increasing the viral safety margin of biological products

Santiago Caballero [a,*], José M. Diez [a], Francisco J. Belda [a], Magdalena Otegui [a], Steven Herring [b], Nathan J. Roth [c], Douglas Lee [c], Rodrigo Gajardo [a], Juan I. Jorquera [a]

[a] Research and Development Area, Biologics Industrial Group, Grifols, Parets del Vallès, Barcelona, Spain
[b] Research and Development Area, Biologics Industrial Group, Grifols, Los Angeles, CA, USA
[c] Research and Development Area, Biologics Industrial Group, Grifols, Research Triangle Park, NC, USA

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ABSTRACT

In this study, the virus-removal capacity of nanofiltration was assessed using validated laboratory scale models on a wide range of viruses (pseudorabies virus; human immunodeficiency virus; bovine viral diarrhea virus; West Nile virus; hepatitis A virus; murine encephalomyocarditis virus; and porcine parvovirus) with sizes from 18 nm to 200 nm and applying the different process conditions existing in a number of Grifols' plasma-derived manufacturing processes (thrombin, α1-proteinase inhibitor, Factor IX, antithrombin, plasmin, intravenous immunoglobulin, and fibrinogen). Spiking experiments ($n = 133$) were performed in process intermediate products, and removal was subsequently determined by infectivity titration. Reduction Factor (RF) was calculated by comparing the virus load before and after nanofiltration under each product purification condition. In all experiments, the RFs were close to or greater than 4 $\log_{10}$ (>99.99% of virus elimination). RF values were not significantly affected by the process conditions within the limits assayed (pH, ionic strength, temperature, filtration ratio, and protein concentration). The virus-removal capacity of nanofiltration correlated only with the size of the removed agent. In conclusion, nanofiltration, as used in the manufacturing of several Grifols' products, is consistent, robust, and not significantly affected by process conditions.

1. Introduction

When therapeutic products are obtained from biological sources, the risk of transmission of infectious agents cannot be completely excluded. For several decades, multiple measures have been utilized before and during the manufacturing processes of blood derivatives to increase the safety margin [1,2]. These specific safety measures include selection and monitoring of qualified healthy donors, plasma donation screening for specific agents by serological and nucleic-acid amplification technologies (NAT), as well as a voluntary decision to hold plasma donation for extended periods before manufacturing, allowing the donor to provide more donations that can be subsequently tested before release of the previous plasma units (Inventory Hold) and, finally, specific viral elimination steps.

Serologic and NAT tests are employed to detect the more relevant specific agents with a history of transmission through blood transfusion such as human immunodeficiency virus (HIV), hepatitis B and C viruses (HBV and HCV, respectively), but also hepatitis A virus (HAV) and parvovirus B19, which are not regularly tested for whole blood that is targeted for transfusion. In addition, specific inactivation and removal steps are included in the manufacturing processes of plasma derivatives, such as solvent/detergent treatment [3], caprylate inactivation [4], pasteurization [5] and nanofiltration [6,7] to further increase the safety margin.

Nanofiltration is a process in which a protein solution is filtered through membranes with pores of nanometric size. It is a dedicated pathogen removal method widely employed during biological product purification processes [8–10]. In the early 1990s, nanofiltration was first implemented into the manufacturing process of some plasma-derived products. Asahi Kasei Corporation was one of the first companies to develop filters (the Planova™ filters) specifically designed for virus removal, although nanofilters from other

* Corresponding author. Instituto Grifols S.A., Research and Development Area, Biologics Industrial Group, Grifols, Polígono Levante. Can Guasch, 2 08150 Parets del Vallès, Barcelona, Spain. Tel.: +34 935710934; fax: +34 935710855.
E-mail address: santiago.caballero@grifols.com (S. Caballero).

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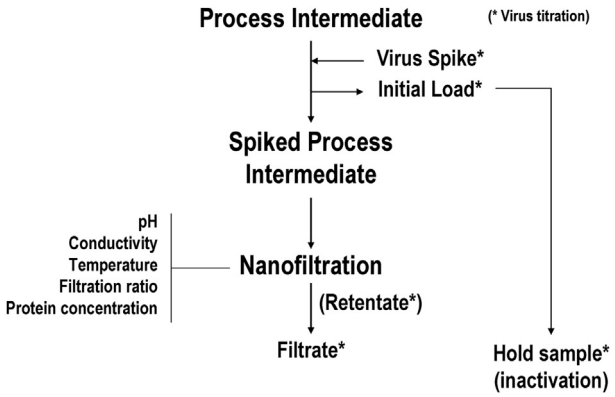

Fig. 1. Flow diagram of nanofiltration experiment for virus validation studies.

companies such as Millipore Corporation, Sartorius AG, and Pall Corporation are now available. Since the nanofiltration removal mechanism is based on size exclusion, this technology is designed to be efficient against a wide range of viruses, but it has a special relevance for small non-enveloped viruses (e.g., HAV and parvovirus B19) which have usually been considered the most difficult to inactivate/remove [11–13].

Fifteen years ago, Grifols began to incorporate nanofiltration technology into its manufacturing processes. Since then, nanofiltration by different filtration platforms has been evaluated by Grifols for the removal of a wide range of viruses in size (from large viruses of 180–200 nm such as of pseudorabies virus [PRV] to very small viruses of 18–24 nm such as porcine parvovirus [PPV]) and under a wide variety of process conditions including pH, ionic strength, temperature, filtration ratio, and protein concentration.

In this study, a high virus-removal capacity of nanofiltration is shown across a wide variety of process conditions existing in several Grifols' plasma-derived product manufacturing processes. In addition, the robustness of nanofiltration is demonstrated by individually evaluating the impact of potentially critical process parameters on the virus clearance capacity.

2. Materials and methods

2.1. Objectives and study design

We have used validated laboratory scale models, representative of industrial scale processes of different Grifols' plasma-derived products, to evaluate the virus removal capacity of Planova 20N and Planova 15N (Asahi Kasei Corp., Osaka, Japan) and Viresolve 70 and Viresolve NFP (Millipore Corp., MA, USA) nanofiltration platforms under the variety of conditions existing in several Grifols' manufacturing processes. The effectiveness of nanofiltration was assessed by spiking the process intermediate product with virus and then determining virus elimination by infectivity titration of residual virus. The general procedure followed in most of the experiments is summarized in Fig. 1. In some cases, an alternative approach to ensure that the filters were challenged with monodispersed viruses (e.g., pre-filtration of virus stocks) was followed.

A total of 133 spiking experiments with at least 2 independent experiments per virus and process conditions were performed. Experiment parameters were adjusted to manufacturing conditions, but in some cases, when appropriate, specific parameters were adjusted to a worst case scenario for virus removal. The experiments were performed according to international guidelines [14,15].

2.2. Viruses

Relevant and model viruses with different physicochemical properties were selected according to the recommendations of international guidelines on virus validation studies for plasma-derived products [14–16]. A list of properties of the selected viruses (PRV, HIV-1, bovine viral diarrhea virus [BVDV], West Nile virus [WNV], HAV, murine encephalomyocarditis virus [EMCV], and PPV) is shown in Table 1. Viruses were grown in cell cultures to obtain suspensions with high infectious titers.

2.3. Plasma proteins and nanofiltration experiments

The list of proteins and the specific filtration system used is shown in Table 2.

The nanofiltration experiments were performed using relevant materials obtained from the corresponding industrial intermediates. Parameters and ranges evaluated in the nanofiltration processes are shown in Table 3. Each parameter was divided into several categories for results interpretation.

Prior to the experiments, optimization of virus spike ratio was performed with mock spikes to confirm that the spike had no undesired effects on nanofiltration kinetics and protein recovery. According to these determinations (data not shown), HIV-1; PRV; BVDV; WNV; EMCV; HAV; and PPV were spiked in the process intermediate at <2% (v/v).

Table 1
Choice of viruses for the study.

Virus	Virus strains/cell lines	Family	Genome	Lipid envelope	Size (nm)	Model for
PRV	- Aujesky/Vero - Bartha/MDBK or Vero - dl tk/Mv 1 Lu	*Herpesviridae*	dsDNA	Yes	180–200	Large enveloped DNA viruses
HIV-1	- Bru, IIIB or RF/C8166	*Retroviridae*	ssRNA	Yes	80–100	HIV-1/2 (relevant viruses)
WNV	- B956 or Uganda/Vero	*Flaviviridae*	ssRNA	Yes	40–50	HCV, WNV (relevant viruses) and other Flavivirus
BVDV	- Kentucky 22/BT - NADL/BT, EBTr, KL-2 or MDBK	*Flaviviridae*	ssRNA	Yes	40–50	HCV, WNV (relevant viruses) and other Flavivirus
HAV	- HM175/FRhK-4	*Picornaviridae*	ssRNA	No	27–32	HAV (relevant virus)
EMCV	- EMC/L929 or Vero	*Picornaviridae*	ssRNA	No	27–32	HAV
PPV	- NADL-2/MPK, PK-13, Sk or ST - Tennessee/PT-1_	*Parvoviridae*	ssDNA	No	18–24	B19

B19: parvovirus B19; BVDV: bovine viral diarrhea virus; dsDNA: double-stranded deoxyribonucleic acid; EMCV: murine encephalomyocarditis virus; HAV: hepatitis A virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; PPV: porcine parvovirus; PRV: pseudorabies virus; ssDNA: single-stranded deoxyribonucleic acid; ssRNA: single-stranded ribonucleic acid; WNV: West Nile virus.