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Virus reduction in an intravenous immunoglobulin by solvent/detergent treatment, ion-exchange chromatography and terminal low pH incubation

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ABSTRACT

Virus reduction by several steps in the manufacturing process for the intravenous immunoglobulin Vigam®, has been investigated. The solvent/detergent step based on treatment with 0.3% tri-n-butyl phosphate and 1% polysorbate 80 at 37 °C, was confirmed to be effective for a range of enveloped viruses. Virus infectivity was undetectable i.e. >6 log inactivation within 30 min of the standard 6 h process. This was consistent over the range of conditions tested i.e. solvent/detergent and protein concentration, temperature and pH. The ion-exchange chromatography step in the process was also able to remove some viruses. Virus spiked followed by blank column runs confirmed the effectiveness of the sanitisation step for ensuring there was no virus cross contamination between column runs. The terminal low pH incubation step was also able to inactivate enveloped viruses, as well as some non-enveloped viruses. The combination of these three steps ensures a high margin of virus safety for this product.

1. Introduction

The virus safety of plasma products such as immunoglobulins is ensured by a combination of screening tests and the incorporation of virus inactivation/removal steps in the manufacturing processes [1,2]. In the past there have been cases of hepatitis C transmission by immunoglobulins prior to the inclusion of any dedicated virus reduction steps in the manufacturing process [3]. However more recently the incorporation of such procedures has ensured the virus safety of these products. In the case of the intravenous immunoglobulin (IvIg) Vigam® [4], a solvent/detergent step has been included as a specific virus inactivation step in the manufacturing process. This procedure is commonly used with many plasma products for the inactivation of enveloped viruses such as the human immunodeficiency virus, hepatitis B and C viruses. While Vigam® has an extensive safety record in clinical use since its launch in 1996, it is still necessary to confirm the effectiveness of this virus reduction procedure in laboratory studies. In addition to this specific virus inactivation procedure, this IvIg undergoes a terminal microbiological quality control step designed to assess the bacteriological sterility of the product, which involves incubation at pH 5 at an elevated temperature for an extended time period. Viruses are generally considered unstable outside of neutral pH conditions and a low pH of about 4 has been effectively used for inactivating viruses in IvIg products [2]. In view of this, the low pH incubation step was also considered likely to have some virus inactivation capabilities and thus was also tested with regard to its contribution to virus safety. One of the major steps used in the manufacturing process involves ion-exchange chromatography on a CM-Sepharose column used for removing the solvent/detergent reagents and any IgG aggregates. Ion-exchange chromatography procedures in particular have been shown to have some potential for virus removal [5], and thus this step was also evaluated. In the current study the effectiveness of these steps for inactivating or removing viruses during the manufacturing process has been tested. As it is essential to ensure the effectiveness of the critical virus reduction steps in the manufacturing process, the robustness of the solvent/detergent step for virus inactivation was also evaluated in more detail. Furthermore, as the chromatography column is recycled between uses, the effectiveness of the sanitisation procedure for removing any potential viral contamination that may theoretically remain, has also been tested.

2. Materials and methods

2.1. Immunoglobulin

Cryoprecipitate depleted human plasma was used as the starting material for the manufacturing process. This involved fractionation

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by the cold ethanol procedure of Kistler and Nitschmann [6]. The resulting Fraction II precipitate was then subjected to DEAE Sephadex adsorption, solvent/detergent treatment, oil extraction and CM-Sepharose chromatography to remove the solvent/detergent and any aggregated IgG [4]. The product was finally formulated with sucrose (2.4%) and albumin (2.0%) as excipients at pH 5.0. The final formulated liquid product was incubated at 30 °C for 2 weeks before final release.

2.2. Solvent/detergent

The solvent/detergent mixture polysorbate (Tween®)-80 and tri-n-butyl phosphate (TnBP), were prepared as a ×20 concentrated stock solution in water. Where indicated, intermediate that already contained solvent/detergent at final concentration, was obtained from Manufacturing.

2.3. Virus

Sindbis virus (SINV; strain Lister, Lister Institute, UK), herpes simplex virus type 1 (HSV-1; strain HFEM, Leeds University, UK), Semliki Forest virus (SFV; strain 32, Warwick University, UK), vesicular stomatitis virus (VSV; strain Indiana, Lister Institute, UK) and vaccinia virus (VAC; strain Lister, Lister Institute, UK) were propagated in BHK-21 cells grown in minimal essential medium (MEM) with 5% newborn-calf serum. Human immunodeficiency virus type 1 (HIV-1; strain HTLV-IIIb, CLB, The Netherlands) was propagated in H9 cells, and assayed using PD5 cells grown in Iscove's Modified Dulbecco's Medium (IMDM) with 10% foetal-calf serum Pseudorabies (PSR; strain Bartha K61, CLB, The Netherlands) was propagated in PD5 cells grown in IMDM with 5% foetal-calf serum.

Encephalomyocarditis virus (EMC; strain EMC, ATCC, USA) was propagated in Vero cells grown in medium 199 supplemented with 4% newborn-calf serum and 1% foetal-calf serum. Polio virus type 1 (PV-1; Sabin type 1, NIBSC, UK) was propagated in Vero cells grown in medium 199 with 5% foetal-calf serum. Hepatitis A (HAV; Strain HM175A2, NIBSC, UK) was propagated in BSC-1 cells grown in MEM supplemented with 5% foetal-calf serum.

Bovine parvovirus (BPV; strain Haden, Inveresk Research, UK) and Simian virus 40 (SV40; strain A2875) were propagated in MDBK cells grown in MEM with 5% newborn-calf serum. Canine parvovirus (CPV; strain C-780916, ATCC, US) was propagated in A72 cells grown in Dulbecco's Modified Eagles medium supplemented with 5% foetal-calf serum.

2.4. Solvent/detergent treatment

Solvent/detergent was added to the product intermediate from a ×20 stock solution to give a final concentration of 1% polysorbate and 0.3% TnBP. Virus was spiked into the solvent/detergent containing intermediate at a dilution of 1 in 20, mixed and a sample taken as a control. The virus spiked intermediate was then incubated at 37 °C and mixed by inversion at regular intervals. Samples were removed at various time points and diluted in PBS to a stop-dilution of 1/100 before infectivity assay. This dilution had been shown to be the minimum dilution of the solvent/detergent containing intermediate that had no toxic effect on the cells or to interfere with the infectivity assays for any of the viruses being tested.

In some experiments, where indicated, material was obtained from manufacturing that already contained solvent/detergent. In this case inactivation was initiated by the addition of virus to the intermediate. A control was prepared by adding virus to cell culture medium at a similar dilution. All experiments were carried out in duplicate.

2.4.1. Effect of process parameters on inactivation

Batches of intermediate were prepared with a solvent/detergent concentration ranging from 25 to 200% of the standard amount. To test the effect of temperature, experiments were conducted at various temperatures over the range of 17–42 °C. To test the effect of protein concentration, material was prepared by redissolving Fraction II in a smaller volume of buffer before further processing. This was further diluted to obtain samples with a protein concentration ranging from 32 to 137 g/L. For experiments to test the effect of pH, the product intermediate was adjusted to a range of pH values from 5.5 to 7.6 by the addition of 0.1 M NaOH or 0.1 M HCl as appropriate.

2.5. CM-Sepharose chromatography

A small-scale model of the CM-Sepharose chromatographic process [4] used in the manufacturing of Vigam® was used. The column bed height was identical, with a direct linear scale-down of the volume and flow-rates used. The standard production intermediate, which contained solvent/detergent, was used unless otherwise stated. In some cases, where noted, bovine gamma globulin (purity > 97%, Sigma) was used as an alternative to the standard production intermediate and this was treated with solvent/detergent. The intermediate was then spiked with virus at a dilution of 1/20 and applied to the column. After loading and washing the column, the IgG was eluted by the application of buffer at pH 9. The column was then washed with 1 M NaCl, sanitised by treatment with 1 M NaOH and finally stored in 1 M NaCl. During the process, the protein elution profile was continuously monitored by measurement at A_{280}. Flow-through, product peak, post-peak and salt wash fractions were collected, and the protein, pH, conductivity and infectivity determined.

In order to confirm the effectiveness of the column sanitisation procedures, virus carry over experiments were conducted. After every virus spiked column run, the column was subjected to the standard column sanitisation procedure. A subsequent column run was then carried out using product alone i.e. without virus spike, to load the column. Samples of all fractions were diluted to 1/100 in PBS, adjusted to neutral pH where necessary, and the virus infectivity determined. This had been shown to be the minimum dilution of the solvent/detergent containing column samples i.e. load and flow-through that had no toxic effect on the cells or to interfere with the infectivity assays for any of the viruses being tested. For consistence, other less toxic samples were also tested at the same dilution. Experiments were carried out in duplicate.

2.6. Terminal low pH incubation

The final product at pH 5.0 was spiked with virus at a dilution of 1/20 and incubated at 30 °C for 14 days. It was confirmed that spiking had no effect on the pH of the product. Samples were taken at various time points, diluted 1/10 in PBS, adjusted to neutral pH where necessary, and assayed for infectivity. This dilution had been shown to be the minimum dilution of the solvent/detergent containing intermediate that had no toxic effect on the cells or to interfere with the infectivity assays for any of the viruses being tested. All experiment was carried out in duplicate.

2.7. Virus assays

Virus infectivity was determined by plaque assay using monolayers of Vero (HSV-1, SFV, VSV, PV-1, VAC, SV40), BHK-21 (SINV,