Contribution of Chromatography to Virus and TSE Removal in the Factor VIII/VWF Optivate® Process

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INTRODUCTION

Manufacture of Optivate®, a factor VIII concentrate which also contains von Willebrand factor, includes two dedicated virus-inactivation steps: solvent/detergent treatment and terminal severe dry-heat treatment. It is possible that other manufacturing steps may also contribute to pathogen removal.

The present study investigates the removal of viruses and prion protein (believed to be the transmissible causative agent of TSE disease) by a chromatography process step during Optivate® manufacture. Those non-enveloped viruses which are most resistant to virus-inactivation (e.g. hepatitis A and parvovirus B19) were used in this study, along with a scrapie model for prion protein.

METHODS

Chromatographic Model

A small-scale model of the chromatographic process was developed and validated by assessment of the protein concentration, FVIII yield and specific activity of various process fractions.

The chromatography column was loaded with product intermediate spiked with virus (Hepatitis A virus [HAV]; Canine Parvovirus [CPV]; Human Parvovirus [B19]), or prion protein (Scrapie [263K]) and eluate fractions were collected.

Between protein runs, the routine column regeneration procedure was modelled, using 1M NaOH, 1M NaCl and 20% ethanol. The efficacy of this regime was assessed by performing an unspiked protein run after a spiked protein run and testing for residual virus.

Assays

Virus infectivity in the eluted product fraction was determined by infectivity assays in cell-culture. For HAV and CPV a plaque assay on A72 cells or a $TCID_{50}$ on BSC-1 cells respectively, was used.

In the case of parvovirus B19, a quantitative PCR method was used.

For scrapie, an immuno-Western blot assay for detecting the abnormal form of prion protein ie PrP^{SC} was used.

RESULTS

Incubation of the product with CPV or B19 viruses did not interfere with the virus titre. The chromatographic step resulted in a reduction of approximately 2 log_{10} in both cases (Table 1), contributing to the overall virus reduction across the process (Table 2).

The overall reduction of HAV was approximately 2.5 log_{10}. Unexpectedly, most of this was due to inactivation in the solvent-detergent-treated column load material before chromatography. This was not solely attributable to antibody neutralisation, but appears to be potentiated by the presence of solvent/detergent.

The column regeneration procedure used between chromatography runs was shown to be effective. No residual virus was found in the eluted product fraction for any of the 3 viruses tested (Table 3).

The chromatographic process removed approximately 4 log_{10} of scrapie (Table 4). Given that the level of scrapie prion protein is known to correlate with infectivity, this procedure removed about 4 log_{10} of TSE.

Figure 1. Outline of Optivate® Manufacturing Process

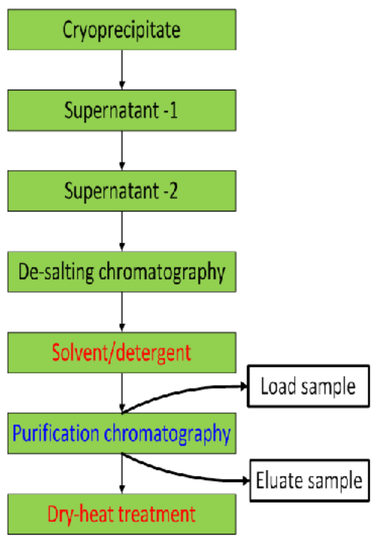

Table 1. Virus reduction by the chromatography step used in the Optivate® process

Virus	Assay	Run No	Total Virus (log)		
			Load	Product Eluate	Reduction
CPV	Infectivity	1	8.0	6.4	1.6
		2	8.2	6.5	1.7
B19	PCR	1	10.6	8.5	2.1
		2	10.8	8.3	2.5
		3	10.9	8.7	2.2
HAV	Infectivity	1	6.8[A]	4.3	2.5
		2	6.8[A]	4.3	2.5
HAV	PCR	1	7.6	7.2	0.4
		2	7.7	7.2	0.5

[A] load titre taken from the medium control, virus was undetectable in the product load

Table 2. Summary of virus removal by multiple steps in Optivate®

Relevant Virus	Model	Virus Reduction (log)			
		Solvent/ Detergent	Dry Heat Treatment	Column	Total
HIV	HIV-1	> 5.9	5.0	nd	> 10.9
HBV	HSV-1	> 4.8	> 3.6	nd	> 8.4
HCV/WNV	Sindbis	> 6.5	> 3.7	nd	> 10.2
HAV	HAV	na	> 4.9	2.5	> 6.5
B19	BPV	na	> 7.1	nd	> 7.1
B19	CPV	na	> 5.7	1.7	> 7.4
B19	B19	na	> 4.7[A]	2.3	> 7.0

[A] estimate based on results with the 8Y process (Transfusion 2006; 46: 1648)
na, not applicable as these are non-enveloped viruses
nd, not determined

Table 3. Effective removal of residual virus during column regeneration

Virus	Total Virus (log)		
	Spiked Run		Blank Run
	Load	Product Eluate	Product Eluate
B19	10.8	8.3	Not detected
CPV	8.2	6.5	Not detected
HAV	6.8	4.3	Not detected

Table 4. Scrapie reduction by Optivate® purification chromatography

Prion Protein	Assay	Run No			
			Load	Product Eluate	Reduction
Scrapie	PrP^{SC}	1	6.7	< 2.6	> 4.1

CONCLUSIONS

The Optivate® chromatographic step has the capacity to remove at least 2 log_{10} of the non-enveloped viruses B19, CPV and HAV.

The column regeneration procedure was effective at preventing the theoretical risk of virus carry-over between batches.

The chromatographic step also effectively removed > 4 log_{10} of the TSE model scrapie.

In summary, this chromatographic step provides pathogen removal during the Optivate® manufacturing process and contributes to the biosafety profile of Optivate®.

REFERENCES

Roberts PL et al. Biologicals 2009; 37: 26-31.
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