Development of a 20N Virus Filtration Process for Keyhole Limpet Hemocyanin (KLH) – A High Molecular Weight Protein

Laura Milbrandt, Catherine Brisson, Ph.D., Zully Aguilar, William Bailey
Stellar Biotechnologies, Inc. • Port Hueneme, California USA • www.stellarbiotech.com

Stellar BIOTECHNOLOGIES
Powering and Improving Immunotherapy

Cambridge Healthtech Institute's 15th Annual PEPTALK The Protein Science Week

ABSTRACT

Keyhole Limpet Hemocyanin (KLH) is a very large, complex glycoprotein used extensively in immunotherapy development to stimulate humoral and cellular immune responses. KLH is often used as a carrier molecule active ingredient in new immunotherapies or as an injectable to assess immune effects of new drugs in clinical settings. The expanding use of KLH in the clinical development of new immunotherapies highlights the need for an appropriate and validated method for virus removal during the manufacturing and purification process. Nanofiltration is one of the most effective virus reduction/removal methods in biopharmaceutical manufacturing of protein products. However, removing small viruses by nanofiltration during the purification of large proteins such as KLH is difficult due to the size similarity between the protein and potential viruses. KLH is sold commercially in two forms, native high molecular weight KLH (approximately 4-8 MDa) and subunit KLH (approximately 400-600 kDa). A Planova™ 20N nanofilter was applied as a virus removal step in the biopharmaceutical manufacturing of subunit KLH formulations. Nanofiltration is an ideal choice for this application due to its ability to be performed on a lab scale and effectively scaled up to commercial size batches.
Buffer conditions, protein concentrations, and flow rates were manipulated to determine the optimal parameters for virus filtration of KLH formulations. Effects of variations in pressure and flow rate during filtration on the virus reduction were evaluated. Data was gathered on the reduction rate of Porcine Parvovirus (PPV), Bovine Viral Diarrhea Virus (BVDV) and Hepatitis A Virus (HAV), across the Planova™ 20N nanofiltration step. Successful and robust reduction of viruses from subunit KLH (Log Reduction Values up to >6 log_{10}) was observed. Viral removal rate was determined by quantitative plaque-forming infectivity assay (PPV and BVDV) and quantal calculation of virus via tissue-culture-infectious-dose infectivity assay (HAV).

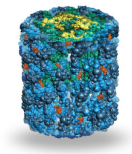
BACKGROUND

According to current regulatory documents, one of the principal approaches to control potential viral contamination of biological products is to test the capacity of the production process to inactivate and/or remove viruses. For this study, the efficiency of a manufacturing step (20N nanofiltration) for viral removal will be evaluated by quantifying the amount of virus at the beginning and end of the scaled-down process step.
The selection of viruses for evaluation of viral clearance of the manufacturing process takes into account the following criteria:

- The level of risk associated with the starting material
- The emerging viruses
- The need to cover a broad spectrum of characteristics in terms of resistance to physicochemical treatment and of viral classification

Three viruses were selected for evaluation, the characteristics of which are described in the table below:

Virus	Abbreviation	Family	Genome	Enveloped/ Non-enveloped	Size (nm)	Physicochemical resistance	Indicator Cells
Porcine Parvovirus	PPV	Parvovirus	DNA	Non-enveloped	18-24	High	PT-1
Bovine Viral Diarrhea Virus	BVDV	Flaviviurs	RNA	Enveloped	40-60	Low	BT
Hepatitis A Virus	HAV	Picornavirus	RNA	Non-enveloped	25-30	Medium	FRhK-4



KLH Molecule (Side View)

About KLH
Keyhole Limpet Hemocyanin (KLH) exists as cylinder-shape didecamer (20-mer), which can dissociate into monomers (KLH subunits or suKLH). The subunit isoforms (approx. 360-400 kDa monomeric molecular weight) are each composed of 7 or 8 functional units. This complex molecular structure can be used to generate multiple product configurations.
The Giant Keyhole Limpet (M. crenulata) is a scarce marine mollusk and the sole source for KLH protein. It naturally lives in the rocky shallows only along a limited stretch of Pacific Ocean coastline. Stellar Biotechnologies is the leader in sustainable manufacture of KLH.

OBJECTIVES

- Develop a viral reduction step suitable for the GMP purification of Subunit KLH (suKLH) protein
- Validate the viral reduction step using a panel of representative viruses according to regulatory agency (FDA and EMA) guidance.

METHODS

1. Development and Optimization of a 20N Filtration Step – Protein concentration, buffer conditions, and pressure were optimized for the 20N viral filtration step. It was determined for filtration of subunit KLH, low protein concentration, slightly alkaline pH, and low pressure yielded optimal results.
2. Preliminary Studies to Determine Validation Protocol
   a) Preliminary Evaluation of Materials for Toxicity/Interference - Toxicity and interference was evaluated to assess the effect of the test material (KLH in virus filtration buffer) on the growth of the indicator cell line and expression of virus.
   b) Unspiked Run at Validation Scale - The 20N nanofiltration step was scaled down from Stellar Biotechnologies' manufacturing scale process. Protein concentration, volume per surface area, pressure, and temperature remained unchanged. Total filtration area and product load were scaled down to 1/1000th of the manufacturing scale. 20N filtration was first performed without virus spike in order to qualify and verify the scaled-down process within the validation facility setting.
3. Viral Clearance Validation – Each of the 3 selected viruses was spiked into the pre 20N KLH material and pre-filtered. The filtered load was loaded onto a Planova 20N nanofilter in duplicate cycles. The amount of virus in the samples collected during the filtration processes evaluating PPV and BVDV was determined by a quantitative plaque-forming infectivity assay. The amount of virus in the samples collected during the filtration processes evaluating HAV was determined by a quantal calculation of virus via a tissue-culture-infectious-dose ($TCID_{50}$) infectivity assay.

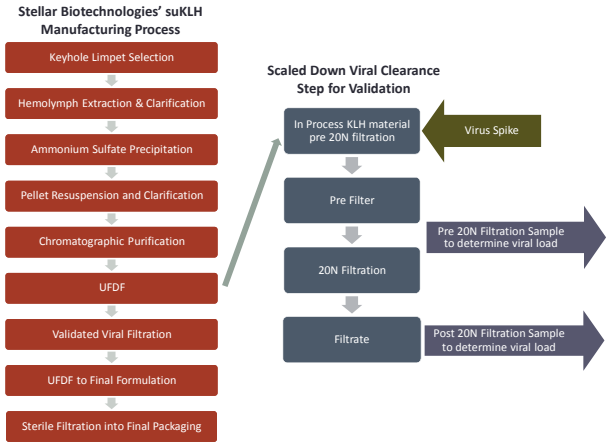


Figure 1: Diagram of validation activities. Stellar Biotechnologies' subunit KLH manufacturing process on the left, validation activities in the center, and analysis on the right. The manufacturing process was carried out at manufacturing scale up to just before the viral clearance step. The product was then spiked with virus, pre-filtered, and passed though a scaled down 20N filtration step. Viral load was determined for samples just before and just after the 20N filtration step for each filtration run.

RESULTS

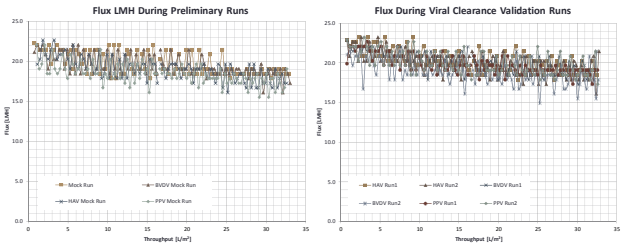


Figure 2: Flux LMH (Liter/m^2/h) over the duration of preliminary and viral clearance 20N filtration runs. A minimal reduction in flux was observed in each run which was reproducible in duplicate runs.

RESULTS

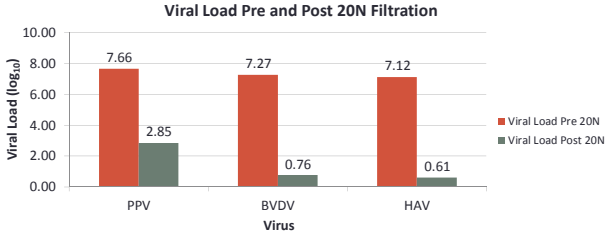


Figure 2: Average viral load pre and post 20N filtration from duplicate runs of scaled down filtration. Values are expressed as log_{10} of virus.

Virus		Log Reduction Value (LRV)
PPV	Cycle 1	4.82
	Cycle 2	4.82
BVDV	Cycle 1	> 6.76
	Cycle 2	> 6.65
HAV	Cycle 1	> 6.83
	Cycle 2	> 6.35
Recommended by regulatory agencies		> 4.00

Figure 3: Viral log reduction values (LRV) for the three viruses evaluated by the Planova 20N nanofiltration. LRV = (Log_{10} viral load pre 20N) – (Log_{10} product post 20N). Values are expressed as log_{10}. EMA and FDA regulatory guidance suggests that log reduction values of 4 log_{10} or more are indicative of a clear effect.

CONCLUSIONS

- The Log Reduction Value (LRV) results from duplicate cycles demonstrate that the nanofiltration step is reproducible for all viruses evaluated.
- The nanofiltration step was most effective in removing BVDV and HAV, as evidenced by LRV values greater than 6 log_{10} for both viruses
- The process was found to be effective even with the smallest of the viruses as evidenced by LRV values of 4.82 log_{10} for PPV.
- The process step using the Asahi Kasei Planova 20N nanofilter proved to not only be effective in virus removal, but also met the FDA and EMA suggested criteria for robustness for all three viruses evaluated.

ACKNOWLEDGEMENTS

- Project sponsored by Stellar Biotechnologies with Araclon Biotech, Zaragoza, Spain
- Viral clearance validation activities took place at Texcell, Frederick, MD in collaboration with Asahi Kasei
- Planova 20N filtration parameters developed in collaboration with Asahi Kasei

CONTACTS & RESOURCES

Stellar Biotechnologies Inc.
332 East Scott Street
Port Hueneme, CA 93041
(805) 488-2800 • www.stellarbiotech.com
Contact:
Catherine Brisson PhD, Chief Operating Officer
cbrisson@stellarbiotech.com

Stellar BIOTECHNOLOGIES
Powering and Improving Immunotherapy

Presented at: PepTalk Protein Science Week 2016
January 18th-22nd, 2016, San Diego, CA