Lentiviral vector (LV) and adeno-associated viral vector (AAV) are the most commonly used viral vectors for therapeutic purposes due to their specific functional properties. The first process step after cell culture is the removal of cells, cell debris, and other impurities to reduce biological burden as much as possible. The easiest and most economical technology to clarify the cell culture is filtration. The chosen filter or filter combination should demonstrate high throughput and high yield.

This study not only describes how different filter materials for cell culture clarification influence yield, but it also demonstrates a strategy to define an efficient and scalable method for clarification. The study investigates the feasibility of filters made from cellulose, polyester, inorganic materials such as glass fiber to clarify LV that is produced using HEK290T cells in adherent format, or AAV produced in HEK293 cells grown in suspension. The results that are shown demonstrate the influence of filter materials and construction on throughput and yield during the clarification step, and will help illustrate a strategy to define the most efficient and scalable filtration steps.

### RESULTS & DISCUSSION

#### 1. Lentiviral Vectors

In the first step of evaluation, all filters listed in Table 1 for LV process, except the Supor® EAV filter, were tested. The cell culture feed turbidity was 430 NTU. Figures 8 and 9 show throughputs and viral vector recovery with different filter options.

- GF filter achieved a throughput that was 5–10 times higher than the other filters. The GF filter had an acceptable turbidity reduction, as well as an infectious particle yield close to 100%.
- Since the GF filter is nominally 0.45 μm prefilter, the inclusion of an additional bioburden reduction membrane filter as a second filtration stage can protect the finer membrane filter.

A variety of membrane filters in series with the GF filter were tested. Additionally, a nominally rated 0.2 μm PES was tested.

- The cell culture for the second run had a feed turbidity of 4 NTU and the throughput experiments were performed with a constant pressure of 0.65 bar. Figures 3 and 4 show throughputs and viral vector recovery with different depth filter options.

- GF plus PVDF filter train achieved the highest throughput and highest infectious particle yield. This combination had an acceptable turbidity reduction.

Even though the throughput of the 0.2 μm PES was the lowest, this is a feasible option as well, considering only one filter is being used and it simplifies the process.

#### 2. Cost/Efficiency Analysis for LV Filtration

- Comparing 254 mm (10 in.) capsules, the PES membrane filter with 1.06 m² effective filtration area (EFA) provides a significantly higher surface area than the GF prefilter with 0.68 m² EFA, and the PVDF membrane filter with 0.55 m² EFA.

- To evaluate the influence of surface area per 254 mm (10 in.) capsule, another test was performed. The combination of the GF prefilter and the PVDF membrane filter was tested in parallel with the 0.2 μm PES membrane. The cell culture feed had a turbidity of 14 NTU and the experiment was performed at a constant pressure of 1 bar. Figures 5 and 6 show throughputs and viral vector recovery with different depth filter options.

Normalizing the filters to determine the theoretical volumes that could be processed by a 254 mm (10 in.) filter capsule are shown in Figure 7.

- The difference in throughputs seen in Figure 5 is compensated for by the higher area per 254 mm (10 in.) module for the 0.2 μm PES membrane filter.

- Disposables for a single step filtration can potentially cost less than disposables for a dual step filtration. For this reason, both listed options are viable, but throughput, yield, and cost need to all be considered when making a choice.

#### 3. Adeno-Associated Viral Vectors

The AAV suspension cell cultures in this study required a lysis step to release the viral vector from the cells prior to clarification. The combination of cells in suspension and the lysis step results in a significantly higher turbidity than an adherent cell culture process. For this study, the AAV cell culture used had a turbidity of 430 NTU. Figures 8 and 9 show throughputs and viral vector recovery with different depth filter options.

- The Seitz HP PDH11 depth filter (Saitz K700P in series with Seitz V100P) had a high recovery similar to Seitz Bio 10 10 filter. It also had the highest throughput of all three depth filter options.

- The Seitz HP PDH11 layer retained contaminants in the range of 6 to 15 μm and protected the finer Seitz V100P layer of the filter. This was evident when comparing the throughputs between the Seitz V100P filter alone versus the Seitz HP PDH11 filter.

- The Seitz 10 filter showed the highest yield. Since the retention rating of the Seitz 100 filter ranges from 0.2 to 0.4 μm, a second filtration test was performed to determine if a suitable coarser depth filter could protect the Seitz Bio 10 layer and improve the throughput without reducing the viral vector yield.

For this second test, an AAV cell culture with a feed turbidity of 540 NTU was used. The clarification experiments were stopped when the filter system reached a predetermined terminal differential pressure or no more feed material was available. Figure 10 shows the throughput for each filter combination that was tested, while Figure 11 shows the viral vector yield post-filtration.

### MATERIALS & METHODS

#### Cell Culture Properties

To cover a broad range of processes, two types of cell culture were used:

- LV: The lentiviral vector was produced with HEK293T cells in adherent cell culture bioreactor. The harvested post-transfection collection had a turbidity of up to 20 naphlumeric turbidity units (NTU).

#### AAV

The adeno-associated viral vector was produced using HEK293 cells in a suspension cell culture bioreactor. The suspension cell culture was harvested after the cells were lysed and had a turbidity around 400–500 NTU.

#### Filter Choice

Depth filters, prefilters, and bioburden membrane filters were tested with the described cell cultures.

### CONCLUSION

For the clarification of the adherent LV process, the PES Super EAV 0.2 μm filter and the combination of the Pall Fluorodyne II DBL 0.45 μm PVDF prefilter in series with the Fluorodyne II DBL 0.45 μm PVDF membrane filter performed best, in terms of throughput and yield.

For the clarification of the suspension AAV process, the dual layer, single step filter options of Saltz HP PDH11 and Saltz HP PDK11 filters, as well as the triple layer, dual step combination of the Saltz HP PDPH filter in series with the Saltz 10 filter, can all provide a viable clarification option for these applications.

The method of clarification needs to be evaluated on a case by case basis where throughput, yield, and cost are concerned. Figure 12 shows the filter guide which gives an overview about the appropriate filter choice for each application.

### Acknowledgement

The lentiviral vector work was a collaborative effort of the Institute of Experimental Biology and Technology (iBET, Portugal), and Pall Corporation (USA). Special thanks to Cristina Peixoto and Tiago Faria of iBET.