# Optimizing the Clarification of Viral Vector Culture for Gene Therapy

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## INTRODUCTION

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, polymers, inorganic material such as glass fiber to clarify LV that is produced using HEK293T 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.

## 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 an adherent cell culture bioreactor. The harvested post-transfection solution had a turbidity of up to 20 nephelometric 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.

Table 1
*Filter types and retention ratings of the filters tested in these studies*

| Process | Filter | Media Material of Construction | Retention Ratings (µm) | Filter Type |
|---|---|---|---|---|
| LV | SuporLife® | PES* | 0.45 | Bioburden membrane filter |
| | Fluorodyne® II DBL | PVDF* | 0.45 | |
| | Ultipor® N66 | Nylon 66 | 0.45 | |
| | PreFlow™ UB | Resin-bonded GF | 0.45 | Prefilter |
| | Supor® EAV | PES* | 0.2 | Bioburden membrane filter |
| AAV | Seitz Bio 10 | Cellulose, resin | 0.2 – 0.4 | Depth filter |
| | Seitz V100P | Cellulose, perlite, resin | 2 – 4 | |
| | Seitz HP PDH11 (K700P + V100P) | Cellulose, diatomaceous earth, perlite, resin | 2 – 15 | |
| | Seitz HP PDK11 (K900P + V100P) | | 2 – 20 | |
| | Seitz HP PDP8 (T1500P + 700P) + Bio 10 in series | | 0.2 – 30 | |

** PES = Polyethersulfone; PVDF = Polyvinylidene difluoride*

## RESULTS & DISCUSSION

### 1. Lentiviral Vectors

In the first stage of evaluation, all filters listed in Table 1 for LV process, except the Supor EAV filter, were tested. The cell culture feed turbidity was 7 NTU and the filtration experiments were performed at a constant pressure of 0.5 barg. Figures 1 and 2 show throughputs and viral vector recovery with different depth filter options.

Figure 1
*Throughput (L/m²)*

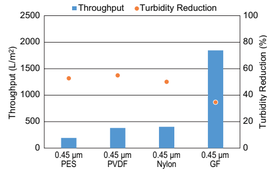
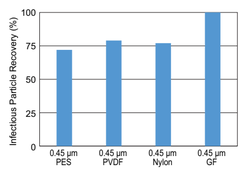



Figure 2
*Infectious particle yield (%)*




- 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 a nominally rated 0.45 µm prefilter, the inclusion of an additional bioburden reduction membrane filter as a second filtration step is required.
- 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 filtration experiments were performed at a constant pressure of 0.5 barg. Figures 3 and 4 show throughputs and viral vector recovery with different depth filter options.

Figure 3
*Throughput (L/m²)*

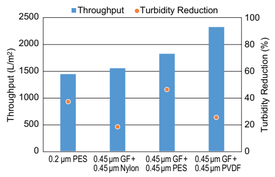
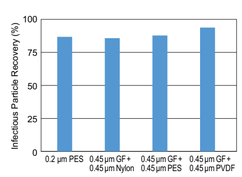



Figure 4
*Infectious particle yield (%)*




- 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 barg. 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.

Figure 5
*Throughput (L/m²)*

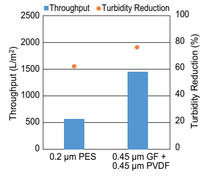



Figure 6
*Infectious particle yield (%)*

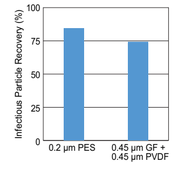
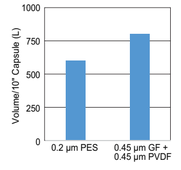



Figure 7
*Volume (L) that can be processed by a 254 mm (10 in.) filter capsule*




- 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 feed 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.

Figure 8
*Throughput (L/m²)*

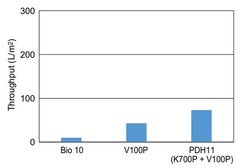
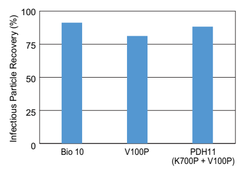



Figure 9
*Viral vector yield (%)*




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

***Acknowledgement***
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Figure 10
*Throughput (L/m²)*

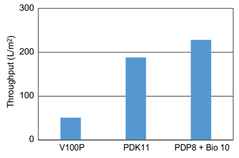
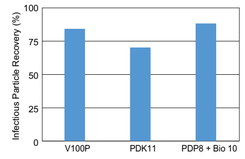



Figure 11
*Viral vector yield (%)*




The Seitz HP PDP8 filter protects the finer Seitz Bio 10 single layer filter and improves the throughput on the Seitz Bio 10 filter. This is evident when comparing the throughput of the Seitz Bio 10 filter in Figure 8 versus the throughput of the Seitz HP PDP8 filter and Seitz Bio 10 filter combination in Figure 10.

The throughput of the Seitz HP PDP8 and Seitz Bio 10 filter combination was approximately 5 times higher than the Seitz V100P filter alone. The capacity of the dual layer Seitz HP PDK11 filter was approximately 4 times higher than the Seitz V100P filter alone.

### 4. Cost/Efficiency Analysis for AAV Filtration

From an economical perspective, the filter area per capsule and the number of filtration steps are used to determine the 'best' filter system. Dual layer and single layer capsules look the same and have identical outer dimensions. Dual layer capsules, such as Seitz HP PDH11, PDK11 and PDP8 media, contain half the EFA compared to the same size single layer depth filters such as Seitz Bio 10 and V100P filters. Dual layer needs to have at least 2x the capacity of a single layer filter to make economic sense.

For the two-step filtration consisting of Seitz HP PDP8 and Seitz Bio 10 filters, the throughput was approximately 5 times greater than the Seitz V100P single step, single layer filter. This filter train also provided the highest yield, meaning this combination provides the best overall performance.

## CONCLUSION

- **For the clarification of the adherent LV process, the PES Supor EAV 0.2 µm filter and the combination of the PreFlow UB 0.45 µm GF 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 Seitz HP PDH11 and Seitz HP PDK11 filters, as well as the triple layer, dual step combination of the Seitz HP PDP8 filter in series with the Seitz Bio 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 all considered. Figure 12 shows the filter guide which gives an overview about the appropriate filter choice for each application.**

Figure 12
*Filter guide for clarification of adherent cell culture producing LV and suspension cell culture producing AAV*