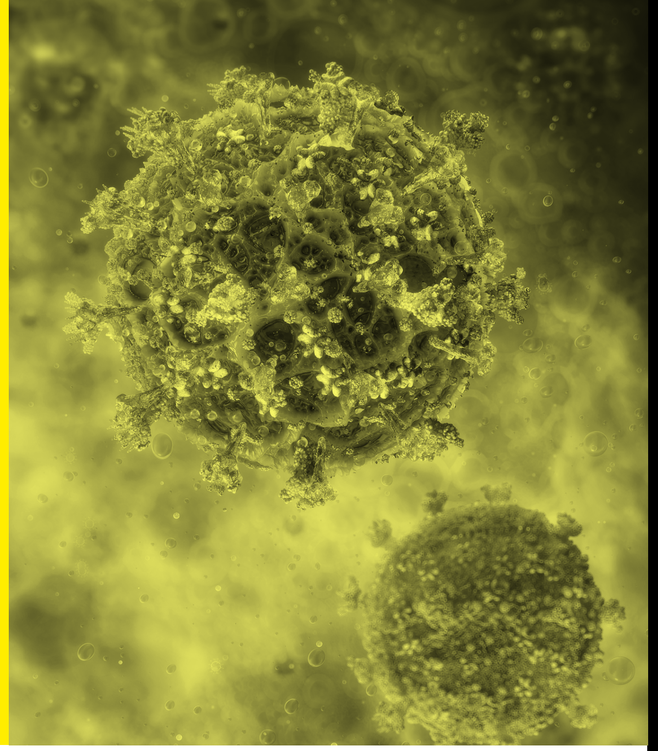
SARTORIUS

A Scalable and GMP-Ready Downstream Process for Lentiviral Vectors



Customer Challenge

As part of its ongoing commitment to advancing lentiviral vector manufacturing, NecstGen was investigating new approaches to further optimise downstream processing performance and efficiency. The objective was to evaluate whether TU recovery above 60% could be achieved, alongside a greater than 100-fold reduction in impurities, while maintaining a cost-efficient process.

Provided Solution

NecstGen collaborated with Sartorius BIA Separations to further optimise its manufacturing platform through the implementation of a monolith-based anion-exchange chromatography (AEX) step.

The work resulted in improved transducing unit recovery, lower host cell protein and residual dsDNA levels, and stronger overall purification performance.

For more information, visit biaseparations.com/lenti

Customer Case Study

Case Profile

Target Molecule:
Lentivirus

Company Name:
NecstGen

Netherlands Center for the Clinical Advancement of Stem Cell & Gene Therapies

Company Location:
The Netherlands

Company Type:
Non-profit CDMO

NecstGen

Ligand Selection and Feed Optimization

The development and optimization of the DSP process were carried out using two Design of Experiments (DOE). The DOE were conducted on CIM® Monolithic Well Plates to select the best-performing ligand and optimize the feed conditions.

The first DOE examined three factors:

- Ligand type (DEAE, QA)
- NaCl concentration in both the feed and wash buffer,
- pH levels in the feed and wash buffer.

Data from NecstGen showcased (Figure 1) higher TU recovery with CIM QA Monolithic Well Plate compared to CIM DEAE Monolithic Well Plate, optimal NaCl concentration at 300 mM and pH of 7.25.

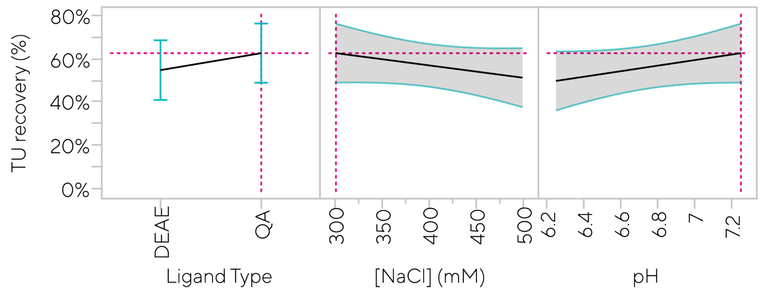


Figure 1: DOE Predictions with Varying Additives and Concentrations: Left: The first graph illustrates that the QA column slightly outperforms the DEAE column. Middle: The optimal salt concentration is identified at 300 mM; beyond this point, TU recovery begins to decline. Right: This graph indicates optimal pH levels at 7.25.

Functional titration of eluate samples was performed to evaluate the impact of these factors on TU recovery. QA was identified as the best-performing ligand, and the optimized feed conditions were selected for the second DOE.

The second DOE focused on the concentration of buffer additives ($MgCl_2$ and Arginine) in the feed and wash buffer, and minor effects from additives were observed. The optimized feed conditions derived from this DOE were used in subsequent experiments.

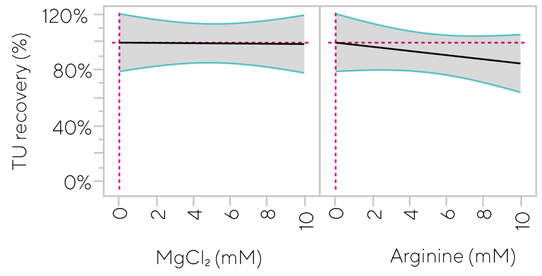


Figure 2: Left: The addition of $MgCl_2$ shows no significant effect on TU recovery. Right: The inclusion of arginine slightly negatively impacts TU recovery.

Gradient Elution

An AEX run was performed on a CIMmultus® QA 1 mL (6 µm) column using the refined feed conditions. This phase, initially carried out by Sartorius BIA Separations and further analyzed by NecstGen, implemented a linear elution gradient with increasing NaCl concentrations to determine the conductivity at which LV was eluted.

Goals

Scalability

Impurity reduction over
100× in host-cell proteins

High yield with
>60% TU recovery

Low cost of goods

CIM® QA Monolithic Well Plates and CIMmultus® QA

Several fractions were sampled and analyzed using a functional titration assay to measure TU recovery per fraction. Additionally, HEK293 HCP ElIa analysis was conducted to evaluate host-cell protein reduction across different phases and fractions.

In the linear gradient elution experiment (Figure 3), the majority of functional LV was eluted in the first few fractions (E1-E3), achieving an overall 85% TU recovery. Most host-cell proteins (HCPs) were removed during the load phase, resulting in a 2.20 HCP log-reduction value in fractions E1-E3.

Step Elution

Following the determination of the optimal salt concentration for LV elution, a step elution was performed. This involved another AEX run on a CIMmultus QA 1 mL column to confirm process performance under the optimized conditions, with samples collected and analyzed using a functional titration assay to determine TU recovery.

The step elution process led to a 92% TU recovery and achieved a 2.42 HCP log-reduction value.

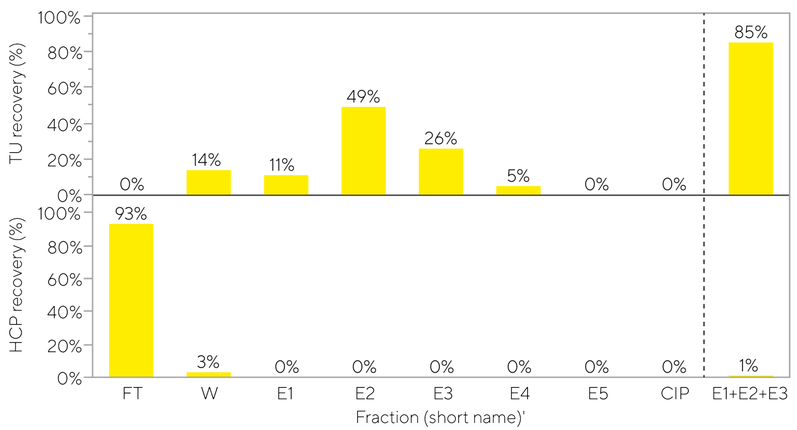
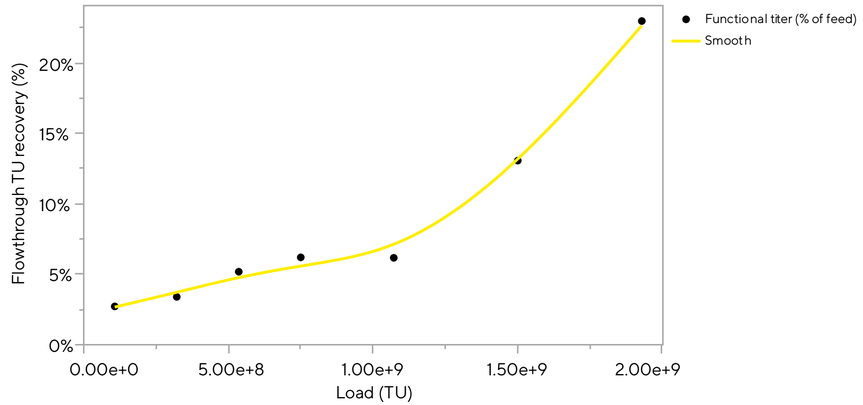


Figure 3: Top: The TU recovery is illustrated using a linear gradient, demonstrating LV elution within fractions E1, E2, and E3. The combined TU recovery for E1-E3 reaches 85%. Bottom: The graph depicts HCP removal within the flowthrough (FT) fraction, with no HCP presence detected in the elution fractions.

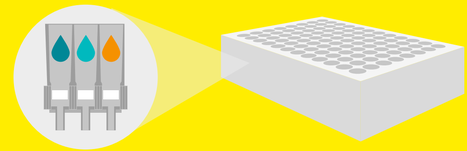
Dynamic Binding Capacity (DBC)

Finally, an AEX run was conducted on a CIMmultus QA 1 mL (6 µm) column with an increased load volume to assess LV binding capacity. The flow-through was fractionated and analyzed using a functional titration assay to evaluate TU recovery per fraction. DBC at 10% breakthrough was determined to be at approximately 300 CV (total 1.3 E+9 TU).

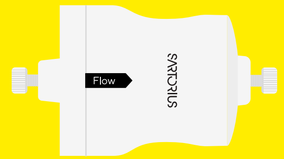


Quick Guide

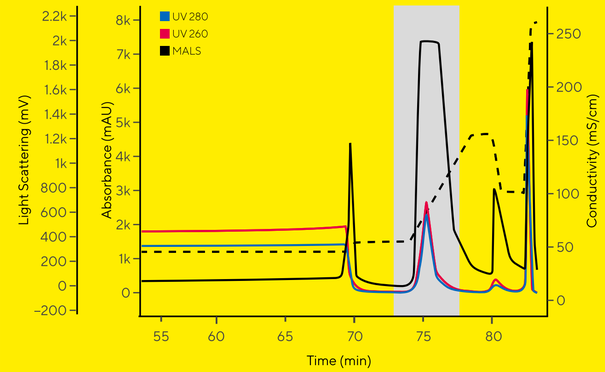
1 Condition screening and DOE performed on a CIM® QA (6µm) Monolithic Well Plate



2 Validation run performed on a CIMmultus® QA (6µm) Column with optimized conditions



3 The optimized chromatogram showcases clear peak definition and effective separation.



The final elution achieves high purity by efficiently removing hcDNA and hc proteins, while enhancing the recovery of infectious LVV.

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Conclusion

The collaboration between NecstGen and Sartorius BIA Separations optimized downstream processing for lentiviral vectors, achieving 92% TU recovery and 2.42 log-reduction in host-cell proteins.

The implementation of CIM QA-based capture chromatography provided an alternative, efficient approach for lentiviral vector purification within the manufacturing platform.

Further Reading

- Poster | Development of a Scalable, GMP-Ready LV DSP Platform using Quality by Design and Design of Experiments
- Poster | Advancements in Lentiviral Purification: From High Throughput Screening to Scalable Solutions

About NecstGen

NecstGen is a mission-driven, nonprofit contract development and manufacturing organization (CDMO) and Center of Excellence for Cell and Gene Therapy. It is owned by Leiden University Medical Center (LUMC) and is located in Leiden, the Netherlands. NecstGen has a state-of-the-art facility for development and manufacturing.

It provides process and analytical development, GMP manufacturing, cleanroom rental and consultancy services for cell, in vivo, and ex vivo gene therapies.

About Sartorius BIA Separations

Sartorius BIA Separations is a leader in developing CIM® monolithic chromatographic columns. The company provides advanced purification and analytical solutions for large biomolecules, such as viruses, plasmids, mRNA, and LNPs. With over 25 years of experience, the company improves the robustness and yield of biopharmaceutical production while ensuring safety. Its technology is essential for manufacturing advanced therapeutics and novel drug candidates, setting industry standards for gene therapy and vaccines, including adeno-associated virus (AAV), adeno virus (Adeno), plasmid DNA (pDNA), viral vector particles (VLP), and viral vaccines.